Gamete and Embryo-fetal Origins of Adult Diseases

He-Feng Huang
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Preface

Prenatal and early postnatal events increase the risk of some diseases in later life including diabetes, coronary heart disease and hypertension etc. In 1934, a landmark paper, published in the *Lancet*, found that death rates from all causes in the UK and Sweden decreased with each successive year-of-birth cohort between 1751 and 1930 [1]. The authors concluded that the health of child is determined by the environmental conditions existing during early life (0–15 years), and, the health of the adult is largely determined by the physical constitution of child [1]. In 1977, Forsdahl discovered a significant positive correlation between infant mortality rates and later mortality rates from arteriosclerotic heart disease [2]. Poverty in childhood followed by prosperity in later life is a risk factor for arteriosclerotic heart disease [2, 3].

Studies in the UK a decade later shifted the focus back to prenatal rather than postnatal events. In 1989, Barker et al. examined relationships between postneonatal mortality for the period 1911–1925 and later adult mortality in 1968–1978. They found that regional differences in stroke and coronary heart disease mortality were predicted by birthweight [4]. Barker subsequently showed that lower birthweights, and, weight at 1 year, were associated with an increased risk of death from stroke and coronary heart disease in adults [5]. Barker proposed that the roots of cardiovascular disease lay in the effects of poverty on the mother, and, undernutrition in fetal life and early infancy. Subsequent studies in UK, Europe, USA, and China have confirmed these findings and shown that it is restricted fetal growth rather than preterm delivery which carries the risk of later adult diseases [6]. These observations have been collectively termed the "Barker hypothesis".

Most human physiological systems and organs begin to develop early in gestation but become fully mature only after birth. A relatively long gestation and period of postnatal maturation allows for prolonged pre- and postnatal interactions with the environment. The primary determinants of fetal growth are genes, the integrity of the feto-placental unit, and, the appropriate endocrine environment that is largely represented by insulin action, and, the insulin-like growth factor system [7, 8].

Normal fetal growth and development take place in two phases; the embryonal and fetal phases: The embryonal phase (1–8 weeks) consists of the proliferation, organization, and differentiation of the embryo, whereas the fetal phase (9–38 weeks) describes the continued growth and functional maturation of different

tissues and organs [7, 8]. Embryonic and fetal periods are clearly vulnerable to environmental factors, and acquired changes can persist transgenerationally, despite the lack of continued exposure. One possible explanation is the epigenetic regulation of the human genome where changes in gene expression or cellular phenotype result from mechanisms other than changes in the underlying DNA [9]. In 2010, Motrenko proposed the "embryo-fetal origin of diseases" theory, where proposed abnormal development of gamete and embryo may induce poor health after birth [10].

Adaptive responses of a gamete or embryo reacting with adverse factors, e.g. culture systems and manipulations in ART, toxins, endocrine disrupting chemicals, etc., make it susceptible to permanent damage of organs, congenital abnormality, and, development of chronic adult diseases. Passing such changes to offspring may result in transgenerational, epigenetic re-programming with transmission of adverse traits and characteristics to offspring.

This book systematically introduces the growing body of evidence from epidemiological observations and clinical and experimental, animal studies that support the gamete and embryo-fetal origins of the metabolic syndrome.

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References

1. Kermack WO, McKendrick AG, McKinlay PL. Death rates in Great Britain and Sweden: some general regularities and their significance. Lancet 1934;226:698–703.
2. Forsdahl A. Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? Br J Prev Soc Med. 1977;31:91–5.
3. Forsdahl A. Living conditions in childhood and subsequent development of risk factors for arteriosclerotic heart disease. The cardiovascular survey in Finnmark 1974–75. J Epidemiol Community Health 1978;32:34–7.
4. Barker DJ, Osmond C, Law CM. The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis. J Epidemiol Community Health 1989;43:237–40.
5. Barker DJ, Winter PD, Osmond C et al. Weight in infancy and death from ischaemic heart disease. Lancet 1989;2:577–80.
6. Paneth N, Susser M. Early origin of coronary heart disease (the "Barker hypothesis") BMJ 1995;310:411–2.
7. Gluckman PD, Harding JE. Nutritional and hormonal regulation of fetal growth—evolving concepts. Acta Paediatr. 1994;399: 60–3.
8. Kanaka-Gantenbein Ch, Mastorakos G, Chrousos GP. Endocrine-related causes and consequences of intrauterine growth retardation. Ann NY Acad Sci. 2003;997:150–7.
9. Canani RB, Costanzo MD, Leone L, et al. Epigenetic mechanisms elicited by nutrition in early life. Nutr Res Rev. 2011;24:198–205.
10. Motrenko T. Embryo-fetal origin of diseases – new approach on epigenetic reprogramming. Archiv Perinatal Med. 2010;16:11–5.

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Physiology of Gametogenesis

1

Ying-Hui Ye, Le-Jun Li, Yue-Zhou Chen, He-Feng Huang, and Zhong-Yan Liang

Abstract

Gametogenesis is a biological process by which diploid or haploid precursor cells undergo cell division and differentiation to form mature haploid gametes. The biology of gamete production is different between males and females. In human, gametogenesis is the development of diploid germ cells into either haploid sperm (spermatogenesis) or eggs (oogenesis). Gamete production in females is intimately part of the endocrine responsibility of the ovary. If there are no gametes, then hormone production is drastically curtailed. Depletion of oocytes implies depletion of the major hormones of the ovary. In the male this is not the case. Androgen production will proceed normally without a single spermatozoon in the testes. This chapter reviews some of the basic structural morphology and physiology of the testes and the ovaries.

1.1 Spermatogenesis

The male reproductive tract in human consists of testes, epididymides, ductus deferentes, accessory sex glands, and penis. The differentiation of testes takes place from indifferent gonads after expression of the sex-determining region Y gene (SRY) on the short arm of the Y chromosome. The fetal Leydig cells then secrete androgens that induce differentiation of the mesonephric (or wolffian) duct to form the epididymis, ductus deferens, and, accessory sex glands, as well as the indifferent external genitalia into a penis and a scrotum. There are two components to the testis;

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the interstitial (intertubular) component and the seminiferous tubule. The interstitial component is well vascularized and contains Leydig cells clustered around the vessels. The seminiferous tubules contain epithelium consisting of germ cells which form numerous concentric layers penetrated by a single type of somatic cells first identified by Enrico Sertoli in 1865 [1]. The cytoplasm of Sertoli cells extends around germ cells to provide nutrition and support while the germ cells undergo progressive differentiation and development into mature spermatozoa.

Spermatogenesis is a dynamic process in which stem spermatogonia become mature spermatozoa throughout the reproductive lifetime of the individual [2, 3]. By the process of mitosis, stem spermatogonia produce two types of cells, additional stem cells and differentiating spermatogonia. The differentiating spermatogonia then undergo rapid, successive mitotic divisions to form primary spermatocytes, which are then followed by a lengthy meiotic phase as preleptotene spermatocytes proceed through two cell divisions (meiosis I and II) to give rise to haploid spermatids. These, in turn, produce mature spermatozoa which undergo a complex process of morphological and functional differentiation. To study such complex and lengthy processes, spermatogenesis has been organized into different "stages" and "phases" that include mitosis, meiosis and spermiogenesis.

1.1.1 Spermatogonia and Mitosis

Spermatogenesis can be divided into three phases, each of which involves a class of germ cells [2–4]. The initial phase, spermatocytogenesis, is the proliferative or spermatogonial phase during which stem spermatogonia undergo mitosis and produce two types of cells; additional stem cells and differentiating spermatogonia. In rat, there are three types of spermatogonia; stem cell spermatogonia (Ais or A isolated), proliferative spermatogonia (Apr or A paired and Aal or A aligned), and differentiated spermatogonia (Al, A2, A3, A4 and B). The stem cells, Ais, divide sporadically to replicate themselves and to produce pairs of Apr spermatogonia, which engage in a series of synchronous divisions, resulting in the formation of chains of Aal spermatogonia. The Aal spermatogonia differentiate into A1 spermatogonia which divide to give rise to more differentiated (A2, A3, A4, and B) cells. In man, three different types of spermatogonia [the dark type A (Ad), pale type A (Ap) and B type] have been identified [5]. The Ap cells have the capacity to give rise to new Ap cells as well as to more differentiated B spermatogonia, and, that are the renewing stem cells. The Ad spermatogonia are reserve stem cells which rarely divide under normal circumstances. The precise mechanisms of differentiation, and, renewing their own population of stem spermatogonia remain unknown.

1.1.2 Meiosis

The meiotic, or spermatocyte, phase deals with the formation of haploid spermatids and divides into five sequential stages including leptotene, zygotene, pachytene, diplotene, and diakinesis. DNA synthesis is involved in each of these five stages of

the primary spermatocytes (preleptotene), and, RNA synthesis in the diplotene stage. Elaborate morphological changes occur in the chromosomes as they pair (synapse) and then begin to unpair (desynapse) during the first meiotic prophase. Some of the changes during this stage include: (1) initiation of intimate chromosome synapsis at zygotene stage, when the synaptonemal complex begins to develop between the two sets of sister chromatids in each bivalent; (2) completion of synapsis with fully formed synaptonemal complex and crossing over at pachytene stage; (3) dissipation of the synaptonemal complex and desynapsing at the diplotene stage. Following the long meiotic prophase, the primary spermatocytes rapidly complete their first meiotic division, resulting in formation of two secondary spermatocytes, each contains 22 duplicated autosomal chromosomes and either a duplicated X or a duplicated Y chromosome. These cells, after a short interphase with no DNA synthesis, undergo a second maturation division to produce four spermatids, each of them has a haploid number of single chromosomes.

1.1.3 Spermiogenesis

The spermiogenic phase, also known as spermiogenesis, involves morphological and functional differentiation of newly formed spermatids into mature spermatozoa. During this phase, the spherical, haploid spermatids transform into elongated, highly condensed, mature spermatozoa, which are released into the lumen of seminiferous tubules. The differentiation of spermatids passes through four phases including Golgi, cap, acrosomal, and maturation phases.

Golgi apparatus is important during the early steps of spermiogenesis [1, 6, 7]. The Golgi apparatus creates vesicles and granules containing enzymes that will become the acrosome. During the capping phase, the acrosome covers the developing sperm nucleus. The acrosomic granule touches the nuclear envelope and the vesicle begins to flatten into a small cap over the nuclear surface. Then, the acrosomic vesicle becomes very thin and the granule flattens. Finally, the acrosome flattens over approximately one third of the nuclear surface. The acrosomal phase involves migration of the acrosomal system over the ventral surface of the elongating spermatid nucleus. Maturation shows fewer changes in nuclear shape and acrosomal migration. The nucleus continues to condense and the acrosome matures into a thin periodic Acid-Schiff stain positive (PAS+) structure that protrudes at the apex but covers nearly all the nucleus, except for that portion connected to the tail [1]. After the formation of prominent cytoplasmic lobes and residual bodies, the excess cytoplasm, which contains unused mitochondria, ribosomes, lipids, vesicles and other cytoplasmic components, is removed [1, 8, 9].

1.1.4 Sperm Production

Spermatogenesis has the greatest number of cell divisions compared to other self-renewing cells in the body. Both the kinetics and rate of germ cell loss affect sperm production. The total duration of spermatogenesis based on 4.5 spermatogenic

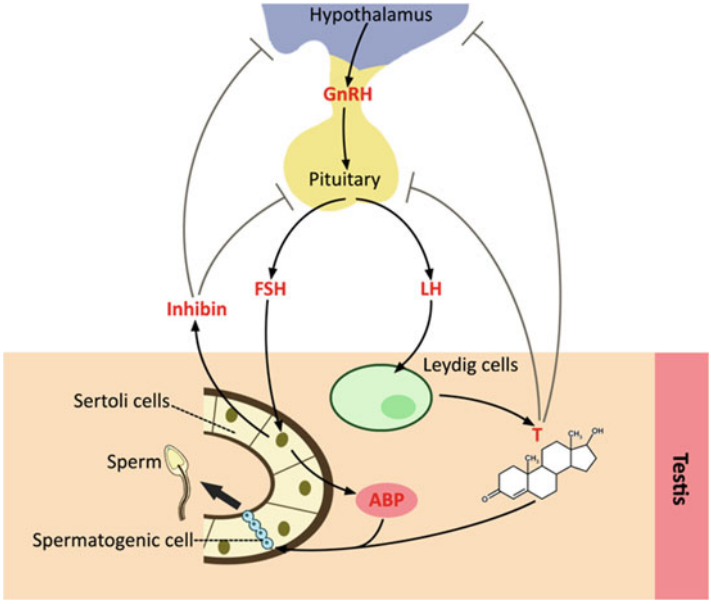

Fig. 1.1 The hypothalamic-pituitary-testicular axis

cycles ranges from approximately 30–78 days in mammals [1, 10–12], and is under the control of the germ cell genotype [13]. Similar results were found utilizing porcine and ovine testis xenografts [14]. However, temperature and some drugs may influence the duration of spermatogenesis [15–17], probably by altering the cell cycle [18, 19]. In humans, the entire spermatogenic process lasts more than 70 days. Germ cell loss (apoptosis) also occurs during spermatogenesis [20], playing a critical role in determining total sperm output. However, the greatest influence on germ cell production is the capacity for mitosis. Significant germ cell apoptosis occurs during the spermatogonial phase, called "density-dependent regulation", primarily during mitotic divisions of spermatogonia. Apoptosis is also frequent during meiosis, especially in humans, and is probably related to chromosomal damage. However, missing generations of spermatocytes and spermatids in the seminiferous epithelium, plus apoptosis, contribute to the low efficiency of human spermatogenesis [20, 21].

1.1.5 Regulation of Spermatogenesis

Androgen production and spermatogenesis are two key functions of the testis that rely on regulation by pituitary gonadotrophins; luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Other hormones contribute to testicular regulation, and there is a plethora of paracrine and autocrine regulatory effects between, and within, different testicular cell compartments. The hypothalamic-pituitary-testicular (HPT) axis plays a key role in the regulation of spermatogenesis (Fig. 1.1) [2, 22–26].

The HPT axis is a classical example of an endocrine regulatory circuit, with cascades of positive and negative regulatory events at multiple functional levels. The highest level is at the hypothalamus, where the cells of specific nuclei synthesize the decapeptide gonadotropin-releasing hormone (GnRH), giving the positive stimulus for gonadotrophin secretion from the anterior pituitary. The same GnRH peptide is responsible for the release of both gonadotrophins, although evidence for a separate FSH-releasing hypothalamic principle also exists [27]. The axon terminals of GnRH neurons make contact with hypophyseal portal vessels, which transport the releasing hormone, secreted in pulses of 1–2 h intervals, to the anterior pituitary gland [28].

In pituitary gonadotroph cells, GnRH stimulates the synthesis and release of LH and FSH. The secretory peaks are more distinct with LH, due to its shorter circulatory half-life compared to FSH [29]. Targets of LH action in the testis are interstitial Leydig cells, whereas FSH regulates Sertoli cells in seminiferous tubuli. LH stimulates steroidogenesis in mature Leydig cells, and is responsible for the supply of testosterone for the maintenance of spermatogenesis, and, for extragonadal androgen effects. FSH maintains the functional capacity of Sertoli cells in the support of spermatogenesis. It is well established that LH stimulates testicular steroidogenesis and maintains the high intratesticular steroid concentration that is necessary for spermatogenesis [30]. In contrast, it is not known how exactly high levels of intratesticular testosterone regulate this process. Androgen receptors are present in Leydig cells, peritubular myoid cells and Sertoli cells, but apparently not in spermatogenic cells [2].

The Sertoli cells are the only target of FSH action in the testis. The FSH stimulates the proliferation of Sertoli cells in the prenatal and prepubertal periods [31]. Sertoli cells become differentiated at puberty, and rarely differentiate thereafter. Thus, FSH determines the spermatogenic capacity before the onset of puberty. Both testicular steroid and peptide hormones regulate FSH secretion. Inhibin and activin originate in Sertoli cells, and, are the main regulators of FSH secretion at the pituitary level. Inhibin is a heterodimer of an α subunit and a PA (inhibin A) or PB (inhibin B) subunit, which inhibits both the synthesis and secretion of FSH. Activin is a homodimer of two P subunits which stimulate FSH. Follistatin, also inhibits FSH by binding and inactivating activin. Androgens and estrogens inhibit FSH action by affecting the functions of hypothalamus. Inhibin B is the product of Sertoli cells and is also important in feedback regulation [32].

The HPT axis plays a role in the negative feedback link between gonadal steroid and peptide hormones [33]. In the male, testosterone mainly controls GnRH secretion, by suppressing GnRH secretion at the hypothalamic level, and, gonadotropin synthesis in the pituitary gland. Testicular steroids also have effects on FSH through the two Sertoli cell proteins, activin and inhibin.

Some part of the steroid feedback is directed to inhibition of gonadotropin synthesis at the pituitary level [34] by both androgenic and oestrogenic components. This feedback occurs mainly at the hypothalamic or pituitary level. Steroids affect GnRH neurons indirectly, and, are mediated by inhibitory inputs from neighboring neurons.

1.1.6 Sertoli Cell

The Sertoli cell also plays an important role in spermatogenesis through (1) support and nutrition of the active germ cells; (2) compartmentalization of the seminiferous tubule by tight junctions; (3) controlled release of mature spermatids into the tubular lumen; (4) secretion of fluid, proteins and several growth factors; (5) phagocytosis of degenerating germ cells and phagocytosis of excess cytoplasm [34]; (6) mediation of the actions of FSH- and LH-stimulated testosterone production in the testis [2]. Some investigations of the Sertoli cell-specific, knockout of androgen receptor (SCARKO) found that spermatogenesis rarely advanced beyond diplotene spermatocytes [35]. Thus, at least in mouse, androgens are crucial for late meiosis and spermiogenesis.

1.1.7 Sperm Maturation in the Human Epididymis

1.1.7.1 Sperm Transit

Based on thymidine labelling of spermatozoa [36], sperm migrate through the human epididymis in 1–21 days. Measurements of extragonadal sperm reserves suggest shorter values of 3–4 days [37, 38]. Faster transit (up to 2 days) may occur in men with large, daily sperm production [38]. However, such rapid transit may result from poor production of spermatozoa, because human epididymal fluid is not viscous [39]. Interestingly, rapid sperm transit associated with large testicular size and high sperm production also occurs in the chimpanzee [40].

1.1.7.2 Sperm Heads and Tails

Morphological analysis of human epididymal sperm following air-drying of smears results in artefactual swelling of the heads of sperm from the caput region, but not those from the caudal epididymidis [41, 42]. These changes demonstrate maturational changes that affect primate spermatozoa [43]. Thus, some spermatozoa with ‘acorn-shaped’ heads in human semen indicate the appearance of immature spermatozoa, because of abnormal epididymal function [44]. The percentage of spermatozoa with normal heads still increases with maturation, not as a result of removal of abnormal cells by epididymal epithelium [45, 46], but by processes of maturation. During transit through the epididymis the sperm increase intramolecular disulphide bonding [47] to withstand the stresses associated with air drying. The dimensions of heads of non-swollen sperm also change with maturation. This dehydration caused by high, intraluminal, osmotic pressures results in cell shrinkage, however, little is known about this in man. The osmotic pressure for fluid obtained from the human vas deferens is 342 mmol/kg [48], while the osmolality of fluid entering the human epididymis is 280 mmol/kg. An increase in compactness of nuclear contents may also explain the decrease in head size. The abnormal morphology of sperm tails is related to human epididymal dysfunction [49], however, the mechanisms are still unclear.

Cytoplasmic Droplets

There is difference between the cytoplasmic droplets of morphologically normal cells and excess residual cytoplasm of abnormal cells [50]. The air-drying procedure of seminal smears routinely is used for the morphological analysis of human spermatozoa. However, this has been changed based on the insufficiency for the preservation of normal cytoplasmic droplets on the majority of spermatozoa in fixed preparations [50]. A neck droplet on human epididymal spermatozoon was shown on the electron micrographs and well fixed seminal preparations display the same [51]. Spermatozoa leaving the human testis present in fluid collected from epididymal spermatocoeles of accumulations of testicular fluid mainly [52], also shows droplets at the neck. The failure of droplet migration along the midpiece in man may indicate a heat stress, since spermatozoa from an inguinal testis and epididymis display neck droplets after exposure of abdominal temperature of cryptorchidism [53]. The failure of migration could results on the low sperm concentration within the epididymal lumen, which reduces the shear forces incumbent upon more highly packed spermatozoa, since the migration of the droplet along the midpiece can be induced by centrifugation of porcine and caprine testicular spermatozoa [54, 55].

1.1.7.3 Motility

Spermatozoa are kept immotile, not by the viscosity of the fluid in the human epididymis [39] but possibly because of low pH [56]. They are activated to become motile after release into the female genital tract or physiological fluids. Sperm obtained from epididymal spermatocoeles and caput epididymidis are weakly motile [52, 57]. Examination of the duration and intensity of motility in vitro of sperm retrieved from epididymides obtained from 21 to 44 year-old men within 1–5 h of death demonstrated that the duration was far higher for sperm from the cauda (18.7 h) than those from the caput (4.6 h) or testis (2.1 h) [58]. The percentage of motile sperm increases as sperm pass through the epididymis. However, in old men, a decrease in motility was observed when recovered from the cauda, which may be associated with reduced ejaculation and sperm storage. Sperm obtained from the testis and caput are only motile in men with ductal occlusion [59] while sperm retrieved by testicular biopsy become motile after incubation in vitro [60], suggesting that the initiation of motility may be time-dependent rather than a result of epididymal secretions. The epididymal region where sperm motility normally develops is shifted proximally after vasectomy [61] or ductal occlusion [62].

1.1.7.4 Sperm Numbers

The small storage capacity of the human epididymis of about 3 days worth of testicular production is demonstrated by the small size of the cauda region [63, 64], and, the rapidity with which sperm reserves can diminish after multiple ejaculation [65]. After epididymal emptying by providing three ejaculates within 4 h, sperm numbers in the first of three subsequent ejaculates is increased from 50 millions after 1 day

abstinence to over 300 millions after 10 days and beyond this time ejaculated sperm numbers remain constant as the epididymis is filled and sperm begin to enter the urine [66].

1.1.7.5 Sperm Protection

Mammals have both adaptive (acquired) immunity, and innate (natural) immunity. Mechanisms of innate immunity include the generation of disinfectants (H_2O_2, NO), large antimicrobial proteins (lysozyme, cathepsins, lactoferrin, phospholipase A2) and small antimicrobial peptides (cathelicidins e.g. glycodelins, and, defensins) [67–69]. A wide range of defensins have been identified in human epididymis. As defensins are more active in the low ionic strength of epididymal fluid, they are situated to prevent the migration of invading micro-organisms into the male tract. A variety of anti-oxidant enzymes are present in human seminal plasma but most are not of epididymal origin [70], although some anti-oxidant activity does originate there [71].

1.1.8 Epigenetic Patterns in Male Germ Cells

Epigenetics refers to non-sequence based mechanisms that control gene expression. The paternal epigenome plays an important role in the developing embryo which is not limited to nucleosome retention data. To date three main mechanisms, DNA methylation, histone modifications and RNA-associated silencing, have been associated with epigenetic silencing of gene expression [72]. The sperm epigenetic program is unique, and, tailored to meet the needs of this highly specialized cell. Chromatin changes in sperm contribute to virtually every function that the male gamete must perform throughout spermatogenesis and in the mature cell [73]. But the requisite replacement of canonical histones with sperm-specific protamine proteins has called into question the utility of the paternal epigenome in embryonic development [74]. The protamination of sperm chromatin provides the compaction necessary for safe delivery to the oocyte, but removes histones which are capable of eliciting gene activation or silencing via tail modifications (methylation, acetylation, etc.) [75]. In effect, protamination removes a potentially informative epigenetic layer from the paternal chromatin, leading to the previously held belief that sperm are incompetent to drive epigenetic changes in the embryo and suggesting that their utility is found only in the delivery of an undamaged DNA blueprint. In fertile patients, histone retention is found at the promoters of genes important in the embryo including developmental gene promoters, microRNA clusters, and imprinted loci, suggesting that the nucleosome retention is programmatic in nature [76].

1.1.8.1 DNA Methylation

DNA methylation, one of the best-characterized DNA modifications associated with the modulation of gene activity is a common regulatory mark found on the 5 carbon of cytosine residues (5-mC) at cytosine–phosphate–guanine dinucleotides

(CpGs), which exert strong epigenetic regulation in many cell types [77]. During gametogenesis, non-imprinted genes acquire their methylation similarly to imprinted genes, however, after fertilization both the maternal and paternal genomes become demethylated while imprinted genes retain their methylation status [78]. Some repeat sequences appear to escape demethylation completely during gametogenesis, and, retain a high proportion of their initial methylation marking during preimplantation development [79]. Founding cells of the germ line, the primordial germ cells (PGCs), are thought to carry full complements of parental methylation profiles when they begin migrating towards the genital ridge [80]. Upon entry into the genital ridge, around 10.5 days of gestation, they undergo extensive genome-wide demethylation [81]. Early studies employing methylation-sensitive, restriction enzymes, Southern blot and PCR approaches indicated that PGCs have completely demethylated genomes by 13.5 days of gestation [82–84]. A number of imprinted genes, including Peg3, Kcnq1ot1 (also known as Lit1), Snrpn, H19, Rasgrf1 and Gtl2, as well as non-imprinted genes such as α-actin, become demethylated between 10.5 and 13.5 days of gestation [85]. However, certain sequences (at least some repetitive elements) appear to be treated differently: IAP, LINE-1 and minor satellite sequences are only subject to partial demethylation, whereas most imprinted and single-copy genes become demethylated [86, 87]. Rapid, and possibly active, genome-wide erasure of methylation patterns takes place between 10.5 and 12.5 days of gestation, leaving PGCs of both sexes in an equivalent epigenetic state by embryonic day 13.5 [88, 89]. Following demethylation in PGCs, male and female gametes acquire sex- and sequence-specific genomic methylation patterns. For nonimprinted genes and repeat sequences, DNA methylation can be assessed directly. For imprinted genes, determination of DNA methylation status and assessment of mono- or biallelic expression of the genes of interest in the resulting embryos, are necessary. A second genome-wide demethylation occurs in the early embryo. Marks established on imprinted genes and some repeat sequences must be faithfully maintained during preimplantation development at a time when the methylation of non-imprinted sequences is lost (Fig. 1.2).

In humans, abnormalities of DNA methylation have been linked to infertility, imprinting disorders in children, and, cancer. Recent studies have suggested that assisted reproductive technologies (ARTs) may be associated with an increased incidence of epigenetic defects in children, and, it is unclear whether the etiology is related to infertility with an underlying epigenetic cause, or, the specific experimental techniques.

1.1.8.2 Retained Histones

Protamination creates a highly condensed nuclear structure that helps to enable proper motility and protects DNA from damage. Although incorporation of this unique, sperm-specific protein results in a quiescent chromatin structure, some regions retain histones and their associated modifications. Recent studies have found this nucleosome retention to be programmatic, and not a result of random distribution [76]. In theory, this selective retention in sperm may allow for targeted gene activation or silencing in the embryo. Multiple histone variants found in

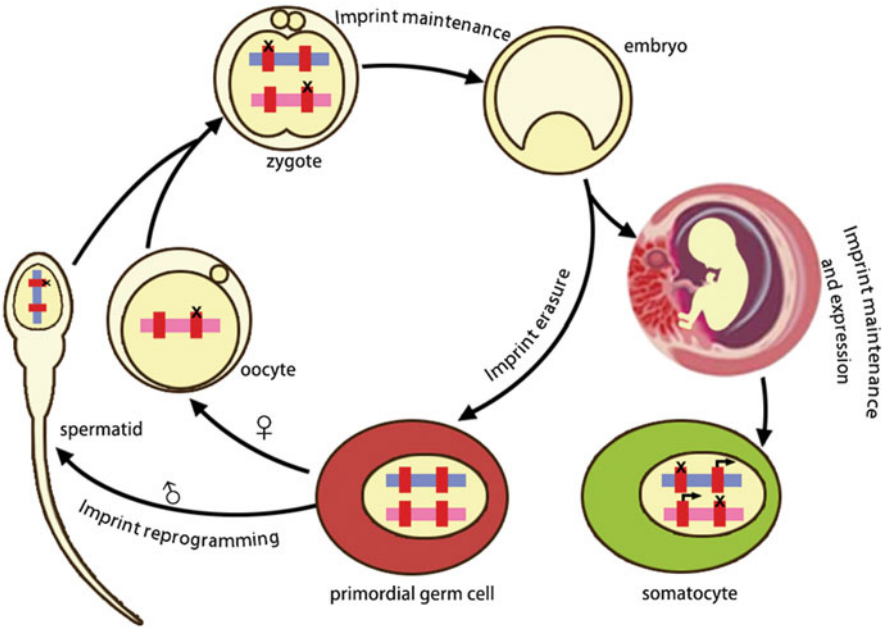

Fig. 1.2 Genes and their imprinting

sperm play an essential role throughout spermatogenesis as well as in the mature spermatozoa. Among these, important nuclear proteins are histone 2A and B (H2A and H2B), histone 3 (H3), histone 4 (H4), and the testes variant (tH2B) [90]. Recent studies implicate aberrant histone methylation and/or acetylation in the mature sperm in various forms of infertility. Loss-of-function mutation of JmjC-domain-containing-histone demethylase 2A (JHDM2A), an enzyme with known H3K9 demethylase activity, reveal decreased transcription of transition protein 1 and P1 during spermatogenesis [91]. Additional studies demonstrate that varying degrees of infertility, including sterility, are correlated with perturbations in histone methylation.

1.1.9 Spermatozoal RNA Transcripts

As a terminally differentiated cell, the ejaculated spermatozoon is exquisitely specialized for delivering the paternal genome to the egg. The presence of RNA in the sperm nucleus is paradoxical if one assumes that it serves no function [92]. The selective retention of mRNAs and siRNAs when most cytoplasmic RNA is lost to the residual body (normally destroyed during sperm preparation) during remodeling argues against passive trapping as does the evidence that sperm RNA can support

protein synthesis de novo during capacitation [92]. Based on a heterologous model system, we know that the spermatozoon delivers its RNA cargo to the oocyte [93]. One study showed that a c-Kit-derived heritable effect on hair color in the mouse was strongly influenced by the presence of aberrant levels of 'scrambled', noncoding c-Kit RNA transferred by the spermatozoa of the affected individual [94]. The concept of active, RNA-dependent translation in capacitating sperm is supported by reports showing that specific sperm RNAs are 'consumed' during manual processing that supports capacitation of normal viable sperm in vitro [95]. An effect of sperm RNA on non-Mendelian inheritance of coat color in mice has also been reported [94]. The mechanism appeared to be through an RNA-mediated epigenetic and heritable paramutation effect.

RNA transcripts co-localize with nucleosome-bound chromatin near the nuclear envelope in the mature sperm, as is the case with the insulin-like growth factor 2 (IGF2) locus [96]. Spermatozoal RNA transcripts are capable of inhibiting the protamination process and maintaining a histone-bound chromatin structure.

1.2 Oogenesis

Oogenesis is a complex and highly orchestrated sequence of differentiation from germ cells to mature oocyte. Human oogenesis is characterized by germ cell migration, differentiation and proliferation during the embryonic period, initiation of meiosis and follicle formation during the fetal period, and oocyte growth and maturation in the adult [97]. To accomplish its role as an oocyte, a PGC must erase the epigenetic program obtained in early embryogenesis and re-establish parental imprints during oogenesis [98]. Erasure and re-establishment of imprinted genes without the alteration of DNA sequence, is termed epigenetic reprogramming. Here, we briefly summarize the development of the oocyte and its epigenetic reprogramming during this process.

1.2.1 Primordial Germ Cells (PGCs)

1.2.1.1 Origin of PGCs

Mammalian development derives from fusion of a highly specialized oocyte with a sperm. The totipotent zygote replicates haploid parental genomes and begins to undergo cleavage divisions. At embryonic day (E) 3.5, the blastocysts include two cell types, the trophectoderm (TE) cells and the inner cell mass (ICM) cells. The trophectoderm can be classified into the polar TE (pTE), which contacts and covers the ICM, and the mural TE (mTE), which delineates the blastocoele cavity [99]. The ICM is the pluripotent cell population and is destined to become the embryo proper, including germ cells. With further development, the primitive endoderm (PE) differentiates from the ICM, located on the inner surface of the ICM at E4.5. The PE layer grows to cover the mTE and becomes the parietal endoderm. The undifferentiated cells in the ICM maintain pluripotency and become the

primitive ectoderm. During the process of implantation, pTE will grow into a thick column of extraembryonic ectoderm. The primitive ectoderm cells eventually will give rise to a cup-liked epithelial sheet, called the epiblast [99]. All the somatic cells including the germ cells derive from these epiblast cells. Although the exact time and place of origin of PGCs in human is unknown, mouse germ cell lineage recruits from pluripotent epiblast cells in response to extraembryonic signals at E6.25 [100]. PGCs in both males and females can be first identified within the wall of the human yolk sac, an extraembryonic membrane, during the 4th week of gestation [101].

1.2.1.2 PGC Migration

PGC migration is a process shared among many species during germ cell development. PGCs migrate from their extragonadal origin to the developing gonad (genital ridge), where they interact with gonadal somatic cells that will support germ cells development and maturation into functional gametes [97]. Between 4th and 6th weeks, PGCs migrate by amoeboid movement from the yolk sac to the wall of the gut tube, and then via the mesentery of the gut to the dorsal body wall. In the dorsal body wall, these cells come to rest on either side of the midline in the loose mesenchymal tissue just deep to the membranous lining of the coelomic cavity. Most of the PGCs populate the region of the body wall at the level that will form the gonads [101].

A series of proteins facilitate PGC migration. SDF1 is expressed along the migratory route of the hindgut and genital ridge, providing a guidance signal for PGC migration by binding to CXCR-4 receptor expressed on PGCs [97, 102]. PGCs also express β1/2 integrin that can interact with extracellular matrix along their journey [103]. Furthermore, the interactions themselves are important for PGC migration and clonization. PGCs express the CX43 gap-junction protein during migration, and PGCs up-regulate E-cadherin on leaving the gut and colonizing the genital ridge. E-cadherin mediated, cell-cell adhesion may also support PGC migration arrest in the developing gonad [104, 105].

During PGC migration there is a reduction in genome-wide, DNA methylation. The process includes loss of methylation marks that control somatic gene expression and also removes DNA methylation imprints from imprinted gene loci [106]. Several imprinted genes exhibit earlier demethylation; for example, *Igf2r* begins demethylation in some PGCs before colonization of the genital ridge [107]. The genome-wide DNA methylation level declines at around E8.0 in the mouse [108]. The DNA methyltransferases (Dnmts) are responsible for the methylation of DNA. Dnmt1 methylates hemimethylated CpG di-nucleotides in the genome, and, is the key maintenance methyltransferase during cell division. Dnmt3 are responsible for de novo methylation by targeting the unmethylated CpG site [109]. Dnmt3 are actively repressed in PGCs at E6.75 to E7.5, suggesting that the loss of Dnmts activities may contribute to genome-wide DNA demethylation in PGCs [108, 110]. From around E7.75 onwards, PGCs undergo genome-wide demethylation of H3K9me2 [108]. All PGCs at E8.75 show low H3K9me2 levels. Levels of H3K9me2 halve their original levels during this period [108]. Following the genome-wide loss of DNA methylation and H3K9me2, genomic-wide H3K27me3

becomes upregulated during PGC migration from around E8.25 onwards [111]. Upregulation of genomic-wide H3K27me3, however, may not depend on de novo transcription of some specific factors. The crosstalk between H3K27 methylation and H3K9 methylation may contribute to this process. And the hypomethylation of H3K9me2 in migrating PGCs may be sensed and rescued by the methylation of H3K27me3 [112].

Taken together, DNA methylation and H3K9me2 play critical roles in the stable maintenance of the repressed state of unused genes during cell fate specification. H3K27me3 may be a more plastic repression of the lineage-specific genes in pluripotent cells [99]. Upregulation of H3K27me3 in PGCs may contribute to the creation of an ES cell-like genome organization. Indeed, it is possible to derive pluripotent mice stem cells from PGCs from E8.5 to E12.5 [113].

1.2.1.3 PGC Proliferation

Classically, once PGCs reach approximately 40 in number in the mouse, they proliferate constantly with a doubling time of about 16 h [114]. PGCs continue to multiply by mitosis during the migration. However, it has been shown that PGCs do not increase their number constantly [111]. From around E7.75 to E9.25, coincident with their migration in the developing hindgut endoderm, their increase in number is relatively slow compared to that after E9.5. Through calculation of DNA contents of PGCs, a majority of PGCs (approximately 60 %) from E7.75 to E8.75 are in the G2-phase of the cell cycle. After E9.75, PGCs exhibit a cell cycle distribution with a clear G1 peak, a wide S-phase, and less prominent G2 peak, which is indicative of a rapidly cycling state. These results indicate that a majority of PGCs migrating in the hidgut endoderm arrest at the G2-phase of the cell cycle [99].

When PGCs arrive in the presumptive gonad region, they stimulate cells of the adjacent coelomic epithelium to proliferate and form somatic support cells. Proliferation of the somatic support cells creates a swelling just medial to each mesonephros on both the right and left sides of the gut mesentery. These swellings, the genital ridges, represent the primitive gonads. In both male and female, PGCs undergo further mitotic division within the gonads. PGCs are usually called gonocytes during this period [115].

1.2.1.4 Imprinted Genes in PGC Development

The vast majority of genes possess a bi-allelic pattern of expression. Imprinting corresponds to specific epigenetic regulation leading to expression of only one parental allele of a gene. Some imprinted genes exhibit paternal expression while others exhibit maternal expression. Imprinting is mediated by epigenetic modifications including DNA methylation and histone modifications imposed on either maternal or paternal alleles [99]. Currently, about 75 imprinted genes have been identified to date in humans, although it is estimated that from 100 to 600 imprinted genes may exist in the human genome [109].

Imprinting marks from their parents generally maintain at somatic lineages through specific mechanisms, however, newly-formed germ cells need to erase the

imprints of the previous generation and established new ones depending on the sex of the new generation [116]. Numerous studies examined DNA methylation states of imprinted genes in migrating and postmigrating PGCs from E9.5 to E12.5 [117, 118]. Allele-specific DNA methylation in nearly all imprinted genes is maintained in migrating PGCs until about E9.5, indicating that imprinted genes are resistant to the global DNA demethylation in migrating PGCs. The erasure of imprinting commences in a part of the migrating PGCs at E9.5, progresses gradually afterward, and completes in PGCs at E12.5 [99].

Despite the mechanism of imprint erasure being unknown, it has been suggested that erasure is an active process involving the direct removal of DNA methylation [119]. A recent study showed that some imprinted loci, *H19* and *Lit1*, are resistant to the demethylation process in *Aid*-deficient PGCs [120]. Another study showed that several imprinted regions undergo complete demethylation in the Dnmt3a and 3b knockout ES cells [121]. The results indicated that the absence of Dnmt3a and 3b may be a critical requirement for erasure of imprints.

1.2.2 From Gonocytes to Primary Oocytes

In the primitive gonads, PGCs (now called gonocytes) undergo more mitotic divisions after they are invested by the somatic support cells and then differentiate into oogonia. By 12 weeks development, oogonia in the genital ridges enter the first meiotic prophase and then almost immediately become dormant.

1.2.2.1 Meiotic Arrest

Meiosis is a specialized process of cell division that occurs only in germ cell lines. In mitosis, a diploid cell replicates its DNA and undergoes a single division to yield two diploid cells. In meiosis, a diploid germ cell replicates its DNA and undergoes two successive cell divisions to yield four haploid gametes [101]. In females, the meiotic divisions of oogonia are unequal and yield a single, massive, haploid definitive oocyte and three, small, nonfunctional, haploid, polar bodies. Oocytes enter meiosis prenatally but do not finish the second meiotic division until fertilization, thus the process can take several decades to complete [122].

The preliminary step in meiosis, as in mitosis, is the replication of each chromosomal DNA molecule; thus, the diploid cell converts from 2N to 4N. This event marks the beginning of gametogenesis. The oogonium is now called a primary oocyte. Once the DNA replicates, each consists of two chromatids joined together at the centromere. In the next step, prophase, the chromosomes condense into compact, double-stranded structures. During the later stages of prophase, the double-stranded chromosomes of each homologous pair match up to form a joint structure, or, chiasma. Chiasma formation makes it possible for two homologous chromosomes to exchange large segments of DNA by "crossing over". The resulting recombination of genetic material on homologous maternal and paternal chromosomes is largely random, and, increases the genetic variability of the future gametes. Once primary oocytes enter meiotic "arrest" during the first meiotic prophase, they

enter a state of dormancy and remain in meiotic arrest as primary oocytes until sexual maturity [123].

1.2.2.2 Meiosis and Epigenetic Transition

In PGCs, the genomes are wiped clean of most of their DNA methylation and other chromatin modifications, so that germ cells can acquire the capacity to support postfertilization development. This process also prepares germ cells for meiosis, during which homologous chromosomes become aligned to allow synapsis and recombination [106].

A functional link between DNA methylation and meiosis has been discovered in female germ cells. Specifically, ablation of ATP-dependent chromatin remodeling factor *Lsh* (lymphoid specific helicase) gives rise to DNA demethylation and activation of transposable elements in mouse female germ cells. Reduced methylation occurs at tandem repeats and pericentric heterochromatin. These changes are associated with incomplete synapsis of homologous chromosomes and developmental arrest at the pachytene stage [106]. The use of antibodies against methylated cytosines (5mC) has shown low methylation levels in both nuclei and chromosomes of proliferating oogonia in female mice. In early oocytes at both late zygotene and late pachytene/diplotene stages of meiosis, overall DNA methylation remains low [107].

1.2.3 Folliculogenesis

1.2.3.1 Primordial Follicle

By the 5th month of human fetal development all oogonia begin meiosis, after which they are called primary oocytes [101]. The nucleus of each of these dormant primary oocytes, containing the partially condensed prophase chromosomes, becomes very large and watery and is referred to as a germinal vesicle. The swollen condition of the germinal vesicle may protect the oocyte's DNA during the long period of meiotic arrest [101].

A single-layered, squamous capsule of epithelial follicle cells derived from the somatic support cells tightly encloses each primary oocyte. This capsule and its enclosed primary oocyte constitute a primordial follicle. The first primordial follicles appear in the human fetus as early as 15th week of gestation, and, are complete by 6 months after birth [124]. Studies in human and rodents identify a variety of genes involved in primordial follicle assembly, such as transcription factors, zona proteins, meiosis-specific enzymes and nerve growth factors [125].

Initiation of meiosis in oogonia with investment of granulosa cells to form the primordial follicle appear to protect from atresia, as they cannot persist beyond the 7th month of gestation without entering meiosis. Therefore, ovaries in the newborn are usually devoid of oogonia [125, 126]. By 5 months, the number of primordial follicles in the ovaries peaks at about seven million. Most of these follicles subsequently degenerate. By birth only 700,000 to 2 million remain, and by puberty, only about 400,000 [101].

1.2.3.2 Primordial-to-Primary Follicle Transition

Primordial follicles remain in a dormant phase until being recruited into the primary stage for growth. The transition process is an orchestrated multi-directional communication among the oocytes and somatic cells (granulosa cells and thecal cells), and certain matrix components and growth factors play roles in this process and subsequent growth of follicles [127].

Recent studies on genetically modified mice confirm inhibitory signals that maintain primordial follicles in the dormant state. Loss of function of any of the inhibitory molecules for follicular activation, including tumor suppressor tuberous sclerosis complex 1 (Tsc-1), phosphatase and tensin homolog deleted on chromosome 10 (PTEN), Foxo3a, p27 and Fox12, leads to premature activation of the primordial follicle pool [128–130]. Global activation of primordial follicles inevitably causes early exhaustion of the follicle pool and premature ovarian failure (POF) though only the Fox12 mutation has been linked to POF in human [131]. The Foxo3a knockout mouse is infertile owing to total depletion of follicles, however, mutation or common single nucleotide polymorphisms in the Foxo3a gene are not associated with POF in human [132, 133].

Anti-Mullerian hormone (AMH) is a member of the transforming growth factor-beta (TGF-β) family, produced by granulosa cells of growing preantral and small antral follicles as a dimeric glycoprotein [125]. In fetal ovaries, AMH is first detected at 36 weeks of gestation in granulosa cells of developing preantral follicles and reaches its highest levels in puberty, becoming undetectable after menopause [134, 135]. Increased recruitment of primordial follicles into the growing pool in AMH null mice suggests a negative effect of AMH on the primordial-to-primary follicle transition [136, 137]. Flattened granulosa cells of primordial follicles become cuboidal during transition into their primary stages along with an increase in oocyte diameters and acquisition of zona pellucidae [138]. The transcription factor Fox12 appears to play a critical role in this transition. Although mice with interruption of Fox12 gene had normal formation of primordial follicles, the granulosa cells did not complete their squamous-to-cuboidal transition, which led to the absence of secondary follicles [139].

Other signals in the ovary promote transition of primordial follicles to primary follicles. Studies on animal models and the human ovary confirm that BMP-4, BMP-7 and GDF-9 are involved in these processes [140, 141]. Mice with the GDF-9 null gene are infertile owing to arrested follicle development at the primary stage with no further growth beyond that stage [142]. Furthermore, FSH is not required for this transition as primordial follicles do not express FSH receptors [143]. Other growth factors and cytokines, such as kit-ligand (KL) and leukemia inhibitory factor (LIF) also act at a paracrine level in the formation of primary follicles [144, 145].

1.2.3.3 Preantral and Antral Follicle

With mitotic expansion of granulosa cells, single-layer primary follicles are transformed into multi-layered ones. An increase in oocyte diameter, and formation of basal lamina, zona pellucida and theca cell layer are among other changes that characterize this developmental stage [146]. During this phase follicle diameter

increases from 40–60 μm at the primary stage to 120–150 μm at the preantral stage. With further growth, follicles reach a diameter of 200 μm, and, enter the antral stage. It is during this stage that the follicle begins to develop fluid-filled spaces within the granulosa cell layers, which will coalesce to form the antral cavity. Development of a multi-layered secondary follicle from a primary follicle with a single layer of granulosa cells is a lengthy process that takes months in humans. This process appears to be independent of the actions of gonadotrophins. Even though preantral follicles may express FSH receptors, FSH may have a permissive role rather than being essential to preantral follicle growth [143].

A series of growth factors and cytokines, such as GDF-9, BMP-15, BMP-4, BMP-7 and TGF-β play crucial roles in the growth of primary follicles into preantral and antral stages. In vitro experiments show that GDF-9 increases the number of primary and secondary follicles in human and rodents [147]. Mice null for the GDF-9 gene show arrested follicle growth at the primary stage, which further confirms the growth-promoting effects of GDF-9 on follicles beyond the primary stage [148]. Another positive regulator of follicle growth into preantral and antral stage is BMP-15. This oocyte-derived growth factor stimulates the proliferation of granulosa cell mitosis in pre-antral follicles during the FSH-independent stages of early follicular growth [149]. Theca cells play an important role in follicle growth. Theca-derived BMP-4 and -7 promote follicle growth beyond the primary stage in rodents [150]. During rapid growth of preantral-antral follicles, BMP-4 and -7 also modulate FSH signaling in a way that promotes estradiol production while inhibiting progesterone synthesis, and acting as a luteinization inhibitor. BMP-4 and -7 do not affect granulosa cell steroidogenesis in the absence of FSH [151]. The actions of TGF-β include proliferation of granulosa cells, progesterone production and FSH-induced estradiol production [152].

In contrast to the positive effects of these growth factors and cytokines, AMH appears to have a negative effect on preantral follicle development beyond primordial-primary transition [153]. Its expression is first detected on granulosa cells of primary follicles and continues until mid-antral stages of follicle development in humans. The highest level of expression occurs in granulosa cells of secondary, preantral and small antral follicles less than 4 mm in diameter [154]. These findings suggest a negative effect of AMH on preantral follicle development beyond the primordial-primary transition.

1.2.3.4 Premature Follicle and the Selection of Dominant Follicle

Follicle growth after the antral stage is characterized by further proliferation of granulosa and theca cells, increased vascularization, oocyte growth and formation of the antral space. FSH becomes a critical determinant of further follicle growth and survival at this stage. Cyclical recruitment of a cohort of antral follicles for further growth and selection of a dominant follicle from among this cohort are the characteristic features of this phase. When a selected cohort of antral follicles grow, modulation of their steroidogenetic activity and their response to gonadotrophins are necessary to sensitize certain follicles for further growth and select a dominant

follicle [125]. Current evidence suggests that these goals are accomplished by locally produced growth factors, such as activin, GDF-9 and BMPs.

Activin A promotes FSH receptor expression in rat granulosa cells and suppresses the growth of primary follicles while inducing follicular growth at later stages [155, 156]. Activin A also involves regulation of estrogen synthesis, LH receptor expression and oocyte maturation [157]. When activin signalling is disrupted, follicle development arrests [158]. However, AMH negatively affects cyclic recruitment and dominant follicle selection by reducing the responsiveness of preantral and small antral follicles to FSH [159].

Another characteristic of follicular development is a switch from an activin A-dominant to inhibin A-dominant environment. Small follicles tend to produce more activin A relative to inhibin A, whereas large, selected, antral follicles secrete more inhibin A [160]. Activin A can attenuate LH-dependent androgen production by theca cells of small preantral follicles [161]. On the other hand, inhibin A released in large quantities by selected antral follicles counteracts the inhibitory effects of activin A and increases LH-induced androgen secretion from theca cells. This provides a sufficient supply of androgen for conversion into estrogens in granulosa cells as there will be a great demand for estrogen synthesis during the peri-ovulatory period. The expression pattern of activin A subunits and its receptors suggests a role for activin in acquisition of maturational competence in oocytes of growing pre-antral follicles [161]. By contrast inhibin A can function as a meiotic inhibitor and therefore impair oocyte maturation and developmental competence [162].

1.2.3.5 Folliculogenesis and Epigenetic Modification

During follicle maturation, appropriate epigenetic modifications are necessary for normal oocyte growth and development [163]. Methylation marks are re-established with oocyte growth and follicle development. The methylation pattern is related to oocyte size and increases throughout the entire nucleus until it reaches its maximal level in the fully grown GV-stage oocyte [107]. Several studies show that DNA methylation levels increase with oocyte growth, with a slight increase on day 10 (post-partum) and marked increases thereafter until the later GV stage in mice [163]. DNA methylation of imprinting genes establishes between days 10 and 15 in oocytes [164]. In addition, de-novo expression of Dnmt3a and Dnmt3b mRNA was observed in late GV-stage and 15 day-old oocytes [163]. These enzymes may catalyze DNA methylation in regions of the genome other than the imprinted genes until the late GV stage. The role of global DNA methylation is still unclear, but it may involve transiently repressing transposon mobilization to maintain genome stability during oocyte growth [165].

Maternal imprinting is also reset during oocyte growth and maturation. This modification may be dependent on the development stage of the oocyte. Various imprinted genes receive an imprinting mark asynchronously at particular stages during the primordial to antral follicle transition [166]. These imprints are not established at the same time, and each imprinted gene is methylated at a specific time. Meanwhile, the imprinting pattern of the oocyte is not fully laid down until the oocyte is within a mature follicle ready to ovulate [167]. Remethylation of

maternally expressed imprinted genes starts at the secondary follicle stage and completes at the antral follicle stage [166]. Another study shows that *Snrpn*, *Znf127*, and *Ndn* genes are imprinted during the process of primordial-to-primary follicle transition, whereas imprinting of *Peg3*, *Igf2r*, and $p57^{KIP2}$ occurs at the secondary follicle stage [167]. Additionally, there is species-specific difference in the establishment of methylation imprinting. For example, *Snrp* is methylated in late GV-stage, metaphaseI(MI), and metaphaseII (MII) human oocytes [168]. The establishment of maternal methylation imprints for some genes may be later in humans than in mice during oocyte development.

1.2.4 Oocyte Maturation

To become competent for normal fertilization and embryonic development, oocytes in preovulatory follicles undergo both nuclear and cytoplasmic maturation. Nuclear maturation includes at least two steps: oocytes resume meiosis, undergo GVBD and progress to metaphase I; the first polar body is extruded and the oocytes proceed to metaphase II. In addition to nuclear maturation, oocytes also undergo cytoplasmic maturation characterized by cytoplasmic changes essential for monospermic fertilization, processing of the sperm, and preparation for development to preimplantation embryos [169].

1.2.4.1 Meiotic Arrest Maintenance

Before reproductive maturity, oocytes arrest in prophase I of meiosis. Maintenance of oocyte meiotic arrest is dependent upon follicular somatic cells, because oocytes or cumulus-oocyte-complexes released from preovulatory follicles resume meiosis spontaneously [170].

Meiotic arrest of oocytes is maintained by high concentrations of the second messenger cyclic AMP (cAMP) [171]. cAMP levels within the oocyte affect the activity of maturation promoting factor (MPF), a complex of Cdc2 and cyclin B, via cAMP-dependent protein kinase A (PKA). High cAMP levels in oocytes result in phosphorylation of Cdc2 on Thr14 and Tyr15, keeping the MPF complex inactive. A decrease of cAMP levels within oocytes leads to dephosphorylation of Thr14 and Tyr15, and MPF becomes active so that oocytes can resume meiosis. The phosphorylation is catalyzed by Wee1 kinases, whereas dephosphorylation is dependent on Cdc25 phosphatases. The activity of Wee1 kinases and Cdc25 phosphatases is directly regulated by PKA [172, 173].

Because oocyte meiotic arrest is dependent on the interaction between oocyte and follicular somatic cells, cAMP in oocytes may derive from somatic cells through gap junctions which are present between cumulus cells and oocytes. However, cAMP levels remain constant when gap junctions are closed, suggesting that cAMP can be produced by the oocyte. Recent studies found that G-protein coupled receptors (GPCRs), GPR3 and GPR12, are expressed by rodent oocytes and these receptors play a role in maintaining high cAMP levels within oocyte and meiotic arrest. Other intracellular components required for cAMP generation such as Gs protein

and adenylyl cyclases are present in oocytes and are functional [173]. Thus, cAMP may also be generated autonomously by the oocyte at levels sufficient to maintain meiotic arrest.

Cyclic GMP is also important for maintaining meiotic arrest. Increasing cGMP levels in the oocyte maintains meiotic arrest. Phosphodiesterase 3A (PDE3A), an oocyte-specific phosphodiesterase, is crucial for meiotic resumption. This enzyme decreases cAMP levels within oocyte after LH surge, and, initiates pathways leading to meiotic resumption. Before the LH surge, the activity of PDE3A is inhibited by cGMP [174]. Recent studies show that mural granulosa cells express natriuretic peptide precursor type C (NPPC), whereas cumulus cells surrounding oocytes express NPR2, the receptor of NPPC. NPPC can increase cGMP levels in cumulus cells and oocytes in cultured cumulus-oocyte-complexes. And meiotic arrest is not sustained in Graafian follicles of Nppc or Npr2 mutant mice [175]. It is likely that NPPC produced by mural granulosa cells activates NPR2 in cumulus cells, and activated NPR2 increases cGMP levels in cumulus cells. Since NPR2 is not detectable in oocytes, high levels of cGMP within oocytes may be a result of transferring cGMP from cumulus cells to oocytes through gap junctions. High, cGMP levels in oocytes suppress PDE3A activity, resulting in elevated cAMP levels within oocytes that block meiotic progression.

1.2.4.2 Meiotic Resumption Triggered by LH

The preovulatory LH surge triggers a cascade of events in ovarian follicles, including resumption of meiotic maturation, luteinization, expansion or maturation of the cumulus cells, and follicle rupture. The LH receptor belongs to the group of GPCRs that depend on interaction with Gsα and activation of adenylyl cyclase to produce cAMP. It is accepted that cAMP is the primary signal mediating LH stimulation, but numerous signals activate after the LH surge, and, are involved in the resuming oocyte meiosis.

Regulation of meiotic maturation may include mitogen activated protein kinase (MAPK), also termed extracellular regulation kinase (ERK). The most widely studied MAPKs in oocytes are ERK1 and ERK2, which can be phosphorylated by upstream MEK (MAPK kinase). Artificial activation of MAPK re-initiates meiosis. LH-dependent ERK 1/2 is activated downstream of cAMP and dependent on PKA activation. Recent studies show that MAPK in cumulus cells plays a critical role in resumption of oocyte meiosis [176]. Multiple signaling cascades, including PKC, gap junctions, epidermal growth factor (EGF) and endothelin-1 pathways may also mediate ERK 1/2 activation.

Phosphatidylinositol 3-kinase (PI3K) phosphorylates the D-3 position of the inositol ring of phosphatidylinositol to produce phosphatidylinositol-3-phosphate and its analogues. The role of PI3K in resuming meiosis in mouse, rat, porcine and bovine includes regulating oocyte maturation through gap junctions between cumulus cells and oocytes [177]. PI3K may also regulate oocyte maturation by activation of RAS since PI3K related enzyme is crucial for RAS-induced, MPF activation [178].

Mammalian oocytes express few LH receptors and are insensitive to direct LH stimulation [179]. Thus, factors released by somatic follicular cells convey LH

stimuli to oocytes. Recently, the discovery that LH surge activates EGF signaling network has provided new insights on how the LH signal is conveyed from mural granulosa cells to the cumulus-oocyte-complexes to induce oocyte maturation. LH induces a rapid and transient expression of EGF-like factors, amphiregulin (AREG), epiregulin (EREG) and betacellulin (BTC), in somatic cells of preovulatory murine follicles. In vitro culture of follicles with EGF-like factors promotes cumulus expansion and oocyte maturation [180]. EGF-like factor influences LH induction of oocyte maturation in several other species, including pig, horse, monkey and human [181]. In addition to EGF-like factors, insulin-like 3 (INSL3) and endothelin-1 also mediate the actions of LH in controlling GVBD of the oocyte. These factors transmit ovulatory LH signals from theca or granulosa cells to cumulus cells or oocytes via their receptors in a paracrine manner. Binding to their receptors in cumulus cells or oocytes, activates downstream signaling cascades to resume meiosis. EGF-like factors activate EGF receptors on cumulus cells and induce resumption of oocyte meiosis via PI3K and ERK 1/2 signal pathways, probably by disrupting gap junctions [182–184]. LH stimulates INSL3 transcripts in ovarian theca cells and INSL3 binds a GPCR, LGR8 (leucine-rich repeat-containing G protein-coupled receptor 8), expressed in oocyte to activate the inhibitory G protein, thus leading to decreases in cAMP production [185]. Endothelin-1 promotion of GVBD is mediated by cumulus cells through EDNRA, involving MAPK/ERK pathway [186].

Intercellular communications are essential for oocyte responses to the LH surge. Gap junctions are responsible for transmitting signals between somatic cells of the follicle, as well as the cumulus cells and oocytes. Gap junctions consisting of intercellular channels are located at points of very close cell-cell contact. The junctional channel is composed of two end-to-end hemichannels, each of which is a hexamer of connexin subunits [187]. Different types of gap junctions are distinguishable on connexins and permeable to molecules of different sizes and charge. Of the 20 connexins encoded in the mammalian genome, connexin 43 (CX43) and connexin 37 (CX37) play critical roles in oocyte maturation. CX43 is the primary connexin in the follicular somatic cell junctions, by contrast, CX37 is found at the oocyte surface in oocyte-somatic cell gap junctions. LH causes a decrease in the gap junction permeability between the somatic cells, resulting from MAP kinase-dependent phosphorylation of CX43 [188]. CX43 phosphorylation induces gap junction closure sufficient for initiating resumption of meiosis. However, a parallel meiosis-stimulatory mechanism may be present, as the inhibition of MAP kinase activation, which prevents the LH-induced channel closure, does not prevent the meiotic progression in response to LH. Oocytes of CX37 mutant mice fail to undergo GVBD when released from the follicles and granulosa cells prematurely became luteal cells, suggesting CX37 is responsible for transmitting signals from oocyte to granulosa cells to maintain the differentiated status of granulosa cells [187].

1.2.4.3 Cytoplasmic Oocyte Maturation

The processes of oocyte nuclear maturation and cytoplasmic maturation are usually coordinated. However, some oocytes competent to complete nuclear maturation are unable to develop to blastocyst stage, which is indicative of deficient

or defective cytoplasmic maturation [169]. Cytoplasmic maturation involves organelle redistribution and storage of mRNA, proteins and transcription factors which are crucial for completion of meiosis, fertilization and early embryo development [189].

Ultrastructural changes and organelle redistribution occur during oocyte maturation. Cytoskeletal microfilaments and microtubules present in the cytoplasm promote the movements of these organelles and act on chromosome segregation. Ultrastructural examination shows that mitochondria in bovine oocytes move from a peripheral distribution to a more dispersed distribution after LH surge. Upon reaching metaphase II, the mitochondria move to the central area of oocytes and produce ATP necessary for synthesis of proteins supporting oocyte maturation and subsequent embryonic development [189]. Mouse oocytes from small antral follicles, matured in vitro, have reduced number of mitochondria, reduced amounts of ATP and abnormal mitochondrial distributions, suggesting compromised developmental potential [189]. Ca^{2+} oscillation, triggered by a fertilizing spermatozoon, is essential for activation of the embryonic development. The ability to generate Ca^{2+} oscillation develops during the oocyte maturation process and involves several cytoplasmic changes [190]. Endoplasmic reticulum (ER) plays an important role in regulation of Ca^{2+} gradients. Storing and releasing Ca^{2+} are mediated by inositol 1,4,5 tri-phosphate receptor (IP3R), which is located on the ER membrane [190]. Thus, biochemical and structural changes in the ER during maturation are critical for generation of Ca^{2+} oscillation. In GV stage oocytes, the ER is uniformly distributed in the ooplasm. However, following maturation to MII, the ER moves to cortical regions and accumulates in 1~2 μm wide clusters. Morphology of the ER also characteristically changes during oocyte maturation [191]. The exocytosis of cortical granules is an important mechanism used by the oocyte to prevent polyspermy. Cortical granules of GV oocytes are distributed in cluster throughout the cytoplasm. When the oocytes reach MII stage, cortical granules aggregate at the inner surface close to the plasma membrane.

To acquire developmental competence, oocytes complete “molecular maturation” consisting of transcription, storage and processing of maternal mRNA. The ribosomes further translate these mRNAs into proteins involved in maturation, fertilization and embryogenesis. Since global transcriptional silencing in fully grown oocytes is a critical event during mammalian oogenesis, these mRNAs will be stored in an inactive form and sustain the very early stages of preimplantation development through timely translation. Altered expression of maternal genes such as Zar1, Npm2, stella, Smarca4 and Oct4 leads to developmental arrest at the time of zygotic genome activation [192].

1.2.5 Epigenetic Regulation During Oogenesis and Oocyte Maturation

The molecular changes that occur during oogenesis are important for oocytes to acquire its developmental competence. In addition to transcription factor

binding to promoters, regulation of transcription may be achieved through epigenetic mechanisms. Epigenetic mechanisms in oocyte and early embryo include chromatin remodeling, DNA methylation, histone modification and non-coding RNAs [193].

1.2.5.1 Chromatin Remodeling

During mammalian oocyte growth, the nucleus of oocyte arrests at diplotene stage, termed the germinal vesicle (GV), and undergoes chromatin remodeling for control of gene expression. GV oocytes from murine antral follicles divide into two groups according to the chromatin distribution in the nucleus. (I) surrounded nucleolus (SN) oocytes, with rather condensed chromatin surrounding the nucleolus; (II) non-surrounded nucleolus (NSN) oocytes, with more dispersed chromatin not surrounding the nucleolus [194]. Other mammalian GV oocytes, including rats, monkeys, pigs and humans also possess similar SN/NSN chromatin organization.

During oocyte growth and maturation, GV chromatin configuration varies in different species. In mice, oocytes in preantral follicles with a diameter between 10 and 40 μm have NSN configuration. SN oocytes can only be found in antral follicles and the proportion of SN configuration increases as the size of the oocytes becomes larger. SN oocytes are silent during transcription, while NSN oocytes are actively transcribing. Transcriptional repression is associated with meiotic competence in fully grown GV oocytes [195]. The percentage of oocytes that resume meiosis is higher in SN oocytes than in NSN oocytes. After fertilization, NSN oocytes cannot develop beyond the 2-cell stage while a proportion of SN oocytes can develop to blastocyst [196]. Thus, the chromatin configuration is highly related to oocyte developmental competence.

1.2.5.2 DNA Methylation

In mammals, maternal and paternal alleles of most genes are expressed at similar levels, but some genes behave differently depending on their parent of origin. These genes are called imprinted genes and regulated by DNA methylation in differentially methylated region (DMR) during gametogenesis. Up to now, more than 100 imprinted genes are identified in mammals and most of them are maternally imprinted. In female germlines, DNA methylation is erased during the differentiation of primordial germ cells, and de novo DNA methylation initiates asynchronously during the growth phase of diplotene-arrested oocyte [197]. DNA methylation is established in an oocyte size-dependent manner, and the maternal methylation imprints become fully established by the fully grown oocyte stage [198].

DNA methylation occurs at the C5 position of cytosine, mostly within CpG dinucleotides. CpG methylation regulates the expression of imprinted and non-imprinted genes. DNA methyltransferases are a family of enzymes that catalyze the transfer of a methyl group to DNA. Dnmt3a and Dnmt3b are responsible for establishing de novo CpG methylation, while Dnmt1 maintains the methylation pattern during chromosome replication. The activity of Dnmt3a and Dnmt3b is catalyzed by a related protein, Dnmt3L. Dnmt3L is highly expressed in germ cells and forms a complex with Dnmt3a and Dnmt3b [197]. Although Dnmt3b is dispensable for

the establishment of maternal imprints, Dnmt3a and Dnmt3L are both necessary to establish maternal imprints in growing oocytes. Dnmt1o, the oocyte-specific isoform of Dnmt1s methyltransferase, is produced in oocytes and maintains the CpG methylation in oocytes and preimplantation embryos [192].

1.2.5.3 Histone Modification

In addition to DNA methylation, histone modification plays an important role in controlling gene expression in gametes and early embryos. Nucleosome is the fundamental building component of chromatin and it is composed of 147 base pairs of genomic DNA and an octamer of two subunits of each of the core histones H2A, H2B, H3 and H4. The amino-terminal portion of the histone protein contains a flexible and highly-basic tail region, which is subject to various post-translational modifications, including acetylation, phosphorylation, methylation, ADP-ribosylation, ubiquitination, sumoylation, biotinylation and proline isomerization [199].

Histone acetylation is associated with enhanced transcriptional activity, whereas histone deacetylation is correlated with repression of gene expression. Acetylation of H3 and H4 is more extensively studied than H2A and H2B. The level of acetylation on histone H3 and H4 increases during oocyte growth and generally all the lysine residues are acetylated in fully grown GV oocytes. However, with the resumption of meiosis, deacetylation will take place in several lysine residues and reaches its peak in MII oocytes. Histone (de)acetylation is related to chromatin remodeling during oocyte growth and is necessary for the binding of a chromatin remodeling protein to the centromeric heterochromatin, an essential step for the correct alignment of the chromosomes [192].

Although all core histones contain phosphor-acceptor sites, the phosphorylation of serine 10 and 28 residue on histone H3 (H3/Ser10ph and H3/Ser28ph) is the most extensively characterized. However, studies on distribution and expression of H3/Ser10ph and H3/Ser28ph during oocyte maturation are discordant. The phosphorylation level of both H3/Ser10ph and H3/Ser28ph increases as oocytes proceed to MI stage, but the distribution pattern are different between them. Although phosphorylation of H3/Ser10ph correlates with chromosome condensation in mitotic cells, recent studies demonstrate that there are no relationships between H3/Ser10ph phosphorylation and chromosome condensation. However, H3/Ser28ph may be associated with chromosome condensation in oocytes [199].

In contrast to acetylation and phosphorylation, histone methylation is relatively stable during oocyte maturation. The main methylation sites are the basic amino acid side chains of lysine (K) and arginine (R) residues. Histone methylation may contribute to establishment and maintenance of an imprinted pattern of gene expression together with DNA methylation [192, 199].

1.2.5.4 Small RNAs Regulation of Oocyte Maturation

From the fully grown oocyte stage until zygotic genome activation (ZGA), the genome is transcriptionally silent. During this period, all mRNA regulation must occur post-transcriptionally. Transcripts expressed by oocytes will support its

maturation, fertilization and early stages of embryonic development [200]. By MII stage, more than half of the mRNA stored in oocytes will be degraded. Small, non-coding RNAs are implicated in the elimination of maternal mRNAs [201].

Small, non-coding RNAs range in size from 18 to 32 nucleotides (nt) in length and play a critical role in post-transcriptional regulation. Three major classes of small, non-coding RNAs have been identified in mammals: microRNAs (miRNAs), endogenous small interfering (endo-siRNAs) and Piwi-interacting RNAs (piRNAs) [34]. There are two subclasses of miRNAs, canonical and non-canonical miRNAs. Canonical miRNAs will be processed by Drosha-Dgcr8 complex to form pre-miRNAs transported from the nucleus into cytoplasm while non-canonical miRNAs can bypass the Drosha-Dgcr8 step [202]. Both miRNAs and endo-siRNAs involve RNase III enzyme Dicer processing with Dicer products being assembled into ribonucleoprotein complexes called RNA-induced silencing complexes (RISC). RISC binds the target RNA and silences gene expression by cleaving the target RNA [33]. The key components of RISC are proteins of Argonaute (Ago) family. In mammals, four Ago proteins function in miRNA repression but only Ago2 functions in siRNA repression. Ago2 is maternally expressed and plays an essential role in degradation of maternal mRNAs. In contrast, piRNAs don't require Dicer processing and are expressed predominantly in germ lines in mammals. They are able to interact with the piwi proteins, a distinct family of Argonaute family [202].

Previous studies demonstrate that miRNAs, such as Let-7, Mir22, Mir16-1 and Mir29, are present in oocytes. Furthermore, mRNA profiling and bioinformatic analyses show that many targets for the expressed miRNAs are also present in oocytes. However, there is little miRNA function in mature oocytes [203] and the mRNA levels of oocytes from Dgcr8 mutant mice are unchanged [204]. These results suggest that miRNA function is downregulated during oocyte development. In contrast, endo-siRNAs may play an essential role in oocyte meiosis. Dicer loss in oocytes show hundreds of misregulated transcripts and results in meiosis arrest with abnormal spindles and severe chromosome congression defects [205]. Ago2-deficient oocytes have similar phenotypes [206]. These results suggest a role for endo-siRNA in regulating oocyte meiosis. piRNAs have been identified in growing oocytes, but deletion of Piwi proteins have no oocyte phenotype [202]. The role of piRNAs in oogenesis remains to be defined.

1.3 Fertilization

Fertilization is the union of a humanoid egg and sperm, that usually occurs in the ampulla of the uterine tube. Fertilization is more a chain of events than a single, isolated phenomenon. Indeed, interruption of any step in the chain will almost certainly cause fertilization failure. Successful fertilization requires not only that a sperm and egg fuse, but that only one sperm fuses with the egg. Fertilization by more than one sperm, polyspermy, almost inevitably leads to early embryonic death. The eggs of many species adopt different strategies to avoid polyspermy.

1.3.1 Sperm Capacitation

Mammalian sperm are unable to fertilize eggs immediately after ejaculation. They acquire fertilization capacity after residing in the female tract for a finite period of time that varies depending on the species. The sperm of many mammals, including humans, can also be capacitated by incubation in certain fertilization media. Molecular events implicated in the initiation of capacitation include lipid rearrangements in the sperm plasma membrane, ion fluxes resulting in alteration of sperm membrane potential [207], and increased tyrosine (tyr) phosphorylation [208] of proteins involved in induction of hyperactivation and the acrosome reaction. Sperm that have undergone capacitation are said to become hyperactivated, and, display hyperactivated motility. Most importantly however, capacitation appears to destabilize the sperm's membrane to prepare it for the acrosome reaction.

1.3.2 Sperm-Zona Pellucida Binding

The egg is encased within a zona and surrounded by layers of cells of the cumulus. Sperm must penetrate these vestments to fuse with the egg. The cumulus acts as a selective barrier, arresting both uncapacitated sperm as well as sperm that have completed the acrosome reaction, while permitting capacitated, acrosome-intact sperm to penetrate and engage in the later steps of fertilization [209]. Exposure of sperm to either cumulus cells or to cumulus-conditioned medium increase the duration of motility, the forward velocity, and the force generated by flagellar beating. Binding of sperm to the zona pellucida is a receptor-ligand interaction with a high degree of species specificity [210]. The carbohydrate groups on the zona pellucida glycoproteins function as sperm receptors. The sperm molecule that binds this receptor is not known with certainty, and indeed, there may be several proteins that can serve this function. In addition, cumulus cells may release a sperm chemo-attractant [211]. To reach the egg plasma membrane, sperm adhere to the outer edge of the zona, undergo acrosomal exocytosis that is regulated by the zona, and then move into, and through, the zona matrix.

1.3.3 The Acrosome Reaction

The acrosome is a Golgi-derived, exocytotic organelle that covers the tip of the sperm head. Acrosomal exocytosis, the so-called acrosome reaction, happens only in capacitated sperm and is a prerequisite for a sperm to fuse with an egg [212]. The acrosome reaction provides the sperm with an enzymatic “drill” to get through the zona pellucida. The same zona pellucida protein that serves as a sperm receptor also stimulates a series of events that lead to many areas of fusion between the plasma membrane and outer acrosomal membrane. Membrane fusion (actually an

exocytosis) and vesiculation expose the acrosomal contents, leading to leakage of acrosomal enzymes from the sperm's head [213].

The mechanism of the acrosome reaction itself has been well characterized. In brief, transient calcium influx leads to activation of phospholipase C (PLC), and activated PLC generates IP3 and diacylglycerol (DAG) from PIP2. IP3 releases calcium from intracellular stores, and DAG mediates PKC activation and phosphorylation of substrate proteins [214]. These early events promote a subsequent calcium influx via transient receptor potential cation channels (TRPCs), which induces the complete acrosome reaction. Disruption of Plcd4 impairs the in vitro ZP-induced acrosome reaction, while the Ca^{2+} ionophore A23187-induced acrosome reaction occurs normally [215].

Sperm that lose their acrosomes before encountering the oocyte are unable to bind to the zona pellucida and thereby unable to fertilize. Assessment of acrosomal integrity of ejaculated sperm is commonly used in semen analysis.

1.3.4 Penetration of the Zona Pellucida

After passing through the cumulus oophorus, sperm encounter the ZP, their last hurdle before meeting the egg. The major components of the ZP are three glycosylated proteins, Zp1, Zp2, and Zp3. Zp3 functions as the primary sperm receptor and can induce the acrosome reaction [216].

The ZP not only functions as a receptor for sperm but also acts as a species-specific barrier. One study replaced mouse Zp2 and Zp3 with their human homologs and examined the fertilization potential of eggs surrounded with a mouse Zp1/human ZP2/human ZP3 chimeric ZP [217]. Mouse sperm, but not human sperm, were able to bind to the chimeric ZP and fertilize the eggs. As Zp3 is thought to be the primary sperm receptor, these results suggest that oligosaccharides attached to the ZP proteins, rather than the peptide sequences themselves, are critical for species-specific sperm binding.

Five knockout mouse strains (Clgn-, Ace-, Adam1a-, Adam2-, and Adam3-knockout mice) have sperms that show defective sperm-ZP binding [218–222]. And sperm share another notable phenotype: they are unable to migrate into the oviduct. This suggests that oviduct migration and ZP binding might share a common mechanism.

1.3.5 Sperm-Oocyte Binding

After penetration of the ZP, sperm immediately meet and fuse with the egg plasma membrane. Once a sperm penetrates the zona pellucida, it binds to and fuses with the plasma membrane of the oocyte. Binding occurs at the posterior (post-acrosomal) region of the sperm head.

The molecular nature of sperm-oocyte binding is not completely resolved. A leading candidate in some species is a dimeric sperm glycoprotein called

fertilin, which binds to a protein in the oocyte plasma membrane and may also induce fusion. Interestingly, humans and apes have inactivating mutations in the gene encoding one of the subunits of fertilin, suggesting that they use a different molecule to bind oocytes. Izumo1$^{-/-}$ male mice are completely sterile, even though the mutant sperm can penetrate the ZP and contact the egg plasma membrane. When the fusion step was bypassed by intracytoplasmic sperm injection into unfertilized eggs, Izumo1$^{-/-}$ sperm activated eggs, and the fertilized eggs developed to term normally when transferred to the uterus of female mice. Therefore, the Izumo1 protein is essential for sperm-egg fusion [223]. And the Cd9-knockout mouse shows a defect restricted to eggs, in which the protein was found to be essential for sperm-egg fusion [224]. If these proteins do indeed interact, it is likely that they both require associating proteins on the sperm and egg cell surface, and the identity of these putative factors is being intensively investigated.

1.3.6 Egg Activation and the Cortical Reaction

Prior to fertilization, the egg is in a quiescent state, arrested in metaphase of the second meiotic division. The transition of an unfertilized, MII-arrested egg into a developing embryo requires completion of a sequence of events known as egg "activation". These events include cortical granule (CG) exocytosis, modifications of the zona pellucida and plasma membrane that prevent polyspermy, completion of meiosis, recruitment of maternal mRNAs into polysomes for translation, and formation of male and female pronuclei [225].

Upon binding of a sperm, the egg rapidly undergoes a number of metabolic and physical changes. Prominent effects include a rise in the intracellular concentration of calcium, completion of the second meiotic division and the so-called cortical reaction.

The cortical reaction refers to a massive exocytosis of cortical granules seen shortly after sperm-oocyte fusion [226]. Cortical granules contain a mixture of enzymes, including several proteases, which diffuse into the zona pellucida following exocytosis from the egg. These proteases alter the structure of the zona pellucida, inducing what is known as the zona reaction. Components of cortical granules may also interact with the oocyte plasma membrane.

1.3.7 The Zona Reaction

The zona reaction refers to an alteration in the structure of the zona pellucida catalyzed by proteases from cortical granules. The critical importance of the zona reaction is that it represents the major block to polyspermy in most mammals [227]. This effect is the result of hardening of the zona pellucida. "Runner-up" sperm that have not finished traversing the zona pellucida by the time the hardening occurs, cannot proceed further.

1.3.8 Post-fertilization Events

Following fusion of the fertilizing sperm with the oocyte, the sperm head incorporates into the egg cytoplasm. The nuclear envelope of the sperm disperses, and the chromatin rapidly loosens from its tightly packed state in a process called de-condensation. In vertebrates, other sperm components, including mitochondria, are degraded rather than incorporated into the embryo.

Chromatin from both the sperm and egg become encapsulated in a nuclear membrane, forming pronuclei. Each pronucleus contains a haploid genome [228]. They migrate together, their membranes break down, and the two genomes condense into chromosomes, thereby reconstituting a diploid organism.

References

1. Hess RA, Franca LR. History of the Sertoli cell discovery. In: Griswold M, Skinner M, editors. Sertoli cell biology. New York: Academic; 2005. p. 3–14.
2. Russell LD, Etllin RA, Sinha Hikim AP, et al. Histological and histopathological evaluation of the testis. Clear Water: Cache River Press; 1990. p. 1–40.
3. Sharpe RM. Regulation of spermatogenesis. In: Knobil E, Neil JD, editors. The physiology of reproduction. Raven Press: New York; 1994. p. 1363–434.
4. Kerr JB. Functional cytology of the human testis. Bailliere's Clin Endocrinol Metab. 1992;6:235–50.
5. Heller CG, Clermont Y. Kinetics of the germinal epithelium in man. Recent Prog Horm Res. 1964;20:545–75.
6. Hess RA. Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic-embedded testes. Biol Reprod. 1990;43:525–42.
7. Leblond CP, Clermont Y. Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-fuchsin sulfurous acid" technique. Am J Anat. 1952;90: 167–215.
8. Hess RA, Miller LA, Kirby JD, et al. Immunoelectron microscopic localization of testicular and somatic cytochromes c in the seminiferous epithelium of the rat. Biol Reprod. 1993;48:1299–308.
9. Franca LR, Ye SJ, Ying L, et al. Morphometry of rat germ cells during spermatogenesis. Anat Rec. 1995;241:181–204.
10. Franca LR, Avelar GF, Almeida FF. Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. Theriogenology. 2005;63:300–18.
11. Franca LR, Russell LD. The testis of domestic mammals. In: Martinez-Garcia F, Regadera J, editors. Male reproduction: a multidisciplinary overview. Madrid: Churchill Communications Europe Espa; 1998. p. 197–219.
12. Hess RA, Schaeffer DJ, Eroschenko VP, et al. Frequency of the stages in the cycle of the seminiferous epithelium in the rat. Biol Reprod. 1990;43:517–24.
13. Franca LR, Ogawa T, Avarbock MR, et al. Germ cell genotype controls cell cycle during spermatogenesis in the rat. Biol Reprod. 1998;59:1371–7.
14. Zeng W, Avelar GF, Rathi R, et al. The length of the spermatogenic cycle is conserved in porcine and ovine testis xenografts. J Androl. 2006;27:527–33.
15. Hess RA, Chen P. Computer tracking of germ cells in the cycle of the seminiferous epithelium and prediction of changes in cycle duration in animals commonly used in reproductive biology and toxicology. J Androl. 1992;13:185–90.

16. Creasy DM. Evaluation of testicular toxicity in safety evaluation studies: the appropriate use of spermatogenic staging. Toxicol Pathol. 1997;25:119–31.
17. Creasy DM. Evaluation of testicular toxicology: a synopsis and discussion of the recommendations proposed by the Society of Toxicologic Pathology. Birth Defects Res B Dev Reprod Toxicol. 2003;68:408–15.
18. Liu Y, Nusrat A, Schnell FJ, et al. Human junction adhesion molecule regulates tight junction resealing in epithelia. J Cell Sci. 2000;113:2363–74.
19. Vilela DAR, Silva SGB, Peixoto MTD, et al. Spermatogenesis in teleost; Insights from the Nile tilapia (*Oreochromis niloticus*) model. Fish Physiol Biochem. 2003;28:187–90.
20. Russell LD, Chiarini-Garcia H, Korsmeyer SJ, et al. Bax-dependent spermatogonia apoptosis is required for testicular development and spermatogenesis. Biol Reprod. 2002;66:950–8.
21. Johnson L, Chaturvedi PK, Williams JD. Missing generations of spermatocytes and spermatids in seminiferous epithelium contribute to low efficiency of spermatogenesis in humans. Biol Reprod. 1992;47:1091–8.
22. Saez JM. Leydig cells: endocrine, paracrine, and autocrine regulation. Endocr Rev. 1994;5: 574–611.
23. Huhtaniemi I, Toppari J. Endocrine, paracrine and autocrine regulation of testicular steroidogenesis. Adv Exp Med Biol. 1995;377:33–54.
24. Jégou B, Pineau C. Current aspects of autocrine and paracrine regulation of spermatogenesis. Adv Exp Med Biol. 1995;377:67–86.
25. Weinbauer GF, Gromoll J, Simoni M, et al. Physiology of testicular function. In: Nieschlag E, Behre H, editors. Andrology. Berlin/Heidelberg: Springer; 1997. p. 23–57.
26. Griffin JE, Wilson JD. Disorders of the testis and the male reproductive tract. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, editors. Williams textbook of endocrinology. Philadelphia: Saunders; 1998. p. 19–875.
27. Yu WH, Karanth S, Walczewska A, et al. A hypothalamic follicle-stimulating hormone-releasing decapeptide in the rat. Proc Natl Acad Sci U S A. 1997;94:9499–503.
28. Hotchkiss J, Knobil E. The hypothalamic pulse generator: the reproductive core. In: Adashi EY, Rock JA, Rosenwaks Z, editors. Reproductive endocrinology, surgery, and technology. Philadelphia: Lippincott-Raven; 1996. p. 123–62.
29. Thorner MO, Vance ML, Laws Jr ER, et al. The anterior pituitary. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, editors. Williams textbook of endocrinology. Philadelphia: Saunders; 1998. p. 249–340.
30. Rao CV. The beginning of a new era in reproductive biology and medicine: expression of low levels of functional luteinizing hormone/human chorionic gonadotropin receptors in nongonadal tissues. J Physiol Pharmacol. 1996;47:41–53.
31. Orth JM. The role of follicle-stimulating hormone in controlling Sertoli cell proliferation in testes of fetal rats. Endocrinology. 1984;115:1248–55.
32. Burger HG, Robertson DM. Editorial: inhibin in the male – progress at last. J Clin Endocr Metab. 1997;138:1361–2.
33. Evans WS, Griffin ML, Yankov VI. The pituitary gonadotroph: dyna111ics of gonadotropin release. In: Adashi EY, Rock JA, Rosenwaks Z, editors. Reproductive endocrinology, surgery, and technology. Philadelphia: Lippincott-Raven; 1996. p. 181–210.
34. Russell LD, Griswold MD, editors. The Sertoli cell. Clearwater: Cache River Press; 1993.
35. De Gendt K, Atanassova N, Tan KA, et al. Development and function of the adult generation of Leydig cells in mice with Sertoli cell-selective or total ablation of the androgen receptor. Endocrinology. 2005;146:4117–26.
36. Rowley MJ, Teshima F, Heller CG. Duration of transit of spermatozoa through the human male ductular system. Fertil Steril. 1970;21:390–6.
37. Amann RP, Howards SS. Daily spermatozoal production and epididymal spermatozoal reserves of the human male. J Urol. 1980;124:211–15.
38. Johnson L, Varner DD. Effect of daily sperm production but not age on transit time of spermatozoa through the human epididymis. Biol Reprod. 1988;39:812–17.

39. Turner TT, Reich GW. Cauda epididymal sperm motility: a comparison among five species. Biol Reprod. 1985;32:120–8.
40. Smithwick EB, Gould KG, Young LG. Estimate of epididymal transit time in the chimpanzee. Tissue Cell. 1996;28:485–93.
41. Soler C, Pérez-Sánchez F, Schulze H, et al. Objective evaluation of the morphology of human epididymal sperm heads. Int J Androl. 2000;23:77–84.
42. Yeung CH, Perez-Sanchez F, Soler C, et al. Maturation of human spermatozoa (from selected epididymides of prostatic carcinoma patients) with respect to their morphology and ability to undergo the acrosome reaction. Hum Reprod Update. 1997;3:205–13.
43. Gago C, Soler C, Perez-Sanchez F, et al. Effect of cetrorelix on sperm morphology during migration through the epididymis in the cynomolgus macaque (*Macaca fascicularis*). Am J Primatol. 2000;51:103–17.
44. Ludwig G, Frick J. Spermatology: atlas and manual. Berlin: Springer; 1987.
45. Cooper TG, Yeung CH, Jones R, et al. Rebuttal of a role for the epididymis in sperm quality control by phagocytosis of defective sperm. J Cell Sci. 2002;115:5–7.
46. Sutovsky P, Moreno R, Ramalho-Santos J, et al. A putative, ubiquitin-dependent mechanism for the recognition and elimination of defective spermatozoa in the mammalian epididymis. J Cell Sci. 2001;114:1665–75.
47. Bedford JM, Calvin H, Cooper GW. The maturation of spermatozoa in the human epididymis. J Reprod Fertil. 1973;l18:199–213.
48. Hinton BT, Pryor JP, Hirsch AV, et al. The concentration of some inorganic ions and organic compounds in the luminal fluid of the human ductus deferens. Int J Androl. 1981;4:457–61.
49. Haidl G, Badura B, Hinsch K-D, et al. Disturbances of sperm flagella due to failure of epididymal maturation and their possible relationship to phospholipids. Hum Reprod. 1993;8:1070–3.
50. Cooper TG, Yeung CH, Fetic S, et al. Cytoplasmic droplets are nor-mal structures of human sperm but are not well preserved by routine procedures for assessing sperm morphology. Hum Reprod. 2004;19:2283–8.
51. Holstein AF, Roosen-Runge EC. In: Holstein AF, Roosen-Runge EC, editors. Atlas of human spermatogenesis. Berlin: Grosse Verlag; 1981.
52. Cooper TG, Raczek S, Yeung CH, et al. Composition of fluids obtained from human epididymal cysts. Urol Res. 1992;20:275–80.
53. Bedford JM, Berrios M, Dryden GL. Biology of the scrotum. Testis location and temperature sensitivity. J Exp Zool. 1982;224:379–88.
54. Kato S, Yasui T, Nano I, et al. Migration of cytoplasmic droplet in boar spermatozoa by centrifugation. Jpn J Anim AI Res. 1984;6:15–8.
55. Kato S, Yasui T, Kanda S. Migration of cytoplasmic droplet in goat testicular spermatozoa by centrifugation. Jpn J Anim Reprod. 1983;29:214–16.
56. Carr DW, Usselman MC, Acott TS. Effects of pH, lactate, and viscoelastic drag on sperm motility: a species comparison. Biol Reprod. 1985;33:588–95.
57. Yeung CH, Cooper TG, Oberpenning F, et al. Changes in movement characteristics of human spermatozoa along the length of the epididymis. Biol Reprod. 1993;49:274–80.
58. Belonoschkin B. Biologie der Spermatozoe n im menschlichen Hoden und Nebenhoden. Arch M Gynakoel. 1943;174:357–68.
59. Jow WW, Steckel J, Schlegel PN, et al. Motile sperm in human testis biopsy specimens. J Androl. 1993;14:194–8.
60. Emiliani S, Van den Bergh M, Vannin AS, et al. Increased sperm motility after in-vitro culture of testicular biopsies from obstructive azoospermic patients results in better post-thaw recovery rate. Hum Reprod. 2000;15:2371–4.
61. Mooney JK, Horan AH, Lattimer JK. Motility of spermatozoa in the human epididymis. J Urol. 1972;108:443–5.
62. Mathieu C, Guerin JF, Cognat M, et al. Motility and fertilizing capacity of epididymal human spermatozoa in normal and pathological cases. Fertil Steril. 1992;57:871–6.

63. Bedford JM. Sperm dynamics in the epididymis. In: Asch RA, Balmaceda JP, Johnston I, editors. Gamete physiology. Norwell: Serono Symposia; 1990. p. 53–67.
64. Schoysman RJ, Bedford JM. The role of the human epididymis in sperm maturation and sperm storage as reflected in the consequences of epididymovasostomy. Fertil Steril. 1986;l46:293–9.
65. Cooper TG, Keck C, Oberdieck U, et al. Effects of multiple ejaculations after extended periods of sexual abstinence on total, motile and normal sperm numbers, as well as accessory gland secretions, from healthy normal and oligozoospermic men. Hum Reprod. 1993;8:1251–8.
66. Barratt CLR, Cooke ID. Sperm loss in the urine of sexually rested men. Int J Androl. 1988;11:201–7.
67. Bourgeon F, Evrard B, Brillard-Bourdet M, et al. Involvement of semenogelin-derived peptides in the antibacterial activity of human seminal plasma. Biol Reprod. 2004;70:768–74.
68. Com E, Bourgeon F, Evrard B, et al. Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. Biol Reprod. 2003;68:95–104.
69. Yang D, Biragyn A, Kwak LW, et al. Mammalian defensins in immunity: more than just microbicidal. Trend Immunol. 2002;23:291–6.
70. Yeung CH, Cooper TG, DeGeyter M, et al. Studies on the origin of redox enzymes in seminal plasma and their relationship with results of in-vitro fertilisation. Mol Hum Reprod. 1998;4:835–9.
71. Perey B, Clermont Y, Leblond CP. The wave of the seminiferous epithelium in the rat. Am J Anat. 1961;108:47–77.
72. Egger G, Liang G, Aparicio A, et al. Epigenetics in human disease and prospects for epigenetic therapy. Nature. 2004;429:457–63.
73. Jenkins TG, Carrell DT. The sperm epigenome and potential implications for the developing embryo. Reproduction. 2012;143:727–34.
74. Orsi GA, Couble P, Loppin B. Epigenetic and replacement roles of histone variant H3.3 in reproduction and development. Int J Dev Biol. 2009;53:231–43.
75. Strahl BD, Allis CD. The language of covalent histone modifications. Nature. 2000;403: 41–5.
76. Hammoud SS, Nix DA, Zhang H, et al. Distinctive chromatin in human sperm packages genes for embryo development. Nature. 2009;460:473–8.
77. Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotech. 2010;28: 1057–68.
78. Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. Nature. 2007;447:425–32.
79. Lucifero D, Chaillet JR, Trasler JM. Potential significance of genomic imprinting defects for reproduction and assisted reproductive technology. Hum Reprod Update. 2004;10:3–18.
80. Shiota K. DNA methylation profiles of CpG islands for cellular differentiation and development in mammals. Cytogenet Genome Res. 2004;105:325–34.
81. Kafri T, Gao X, Razin A. Mechanistic aspects of genome-wide demethylation in the pre-implantation mouse embryo. Proc Natl Acad Sci U S A. 1993;90:10558–62.
82. Brandeis M, Ariel M, Cedar H. Dynamics of DNA methylation during development. BioEssays. 1993;15:709–13.
83. Chaillet JR, Vogt TF, Beier DR, et al. Parental-specific methylation of an imprinted transgene is established during gametogenesis and progressively changes during embryogenesis. Cell. 1991;66:77–83.
84. Kafri T, Ariel M, Brandeis M, et al. Developmental pattern of gene-specific DNA methylation in the mouse embryo and germ line. Genes Dev. 1992;6:705–14.
85. Li E. Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet. 2002;3:662–73.
86. Hajkova P, Erhardt S, Lane N, et al. Epigenetic reprogramming in mouse primordial germ cells. Mech Dev. 2002;117:15–23.
87. Lane N, Dean W, Erhardt S, et al. Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. Genesis. 2003;35:88–93.

88. Szabo PE, Mann JR. Biallelic expression of imprinted genes in the mouse germ line: implications for erasure, establishment, and mechanisms of genomic imprinting. Genes Dev. 1995;9: 1857–68.
89. Szabo PE, Hubner K, Scholer H, et al. Allele-specific expression of imprinted genes in mouse migratory primordial germ cells. Mech Dev. 2002;115:157–60.
90. Gatewood JM, Cook GR, Balhorn R, et al. Isolation of four core histones from human sperm chromatin representing a minor subset of somatic histones. J Biol Chem. 1990;265:20662–6.
91. Okada Y, Scott G, Ray MK, et al. Histone demethylase JHDM2A is critical for Tnp1 and Prm1 transcription and spermatogenesis. Nature. 2007;450:119–23.
92. Millar MR, Sharpe RM, Maguire SM, et al. Localization of mRNAs by in-situ hybridization to the residual body at stages IX-X of the cycle of the rat seminiferous epithelium: fact or artefact? Int J Androl. 1994;17:149–60.
93. Ostermeier GC, Dix DJ, Miller D, et al. Spermatozoal RNA profiles of normal fertile men. Lancet. 2002;360:772–7.
94. Rassoulzadegan M, Grandjean V, Gounon P, et al. RNA-mediated non-Mendelian inheritance of an epigenetic change in the mouse. Nature. 2006;441:469–74.
95. Lambard S, Galeraud-Denis I, Martin G, et al. Analysis and significance of mRNA in human ejaculated sperm from normozoospermic donors: relationship to sperm motility and capacitation. Mol Hum Reprod. 2004;10:535–41.
96. Boissonnas CC, Abdalaoui HE, Haelewyn V, et al. Specific epigenetic alterations of IGF2-H19 locus in spermatozoa from infertile men. Eur J Hum Genet. 2010;18:73–80.
97. Nicholas CR, Chavez SL, Baker VL, et al. Instructing an embryonic stem cell-derived oocyte fate: lessons from endogenous oogenesis. Endocr Rev. 2009;30:264–83.
98. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. Science. 2001;293:1089–93.
99. Rousseaux S, Khochbin S. Epigenetic reprogramming associated with primordial germ cell development. Epigenetics and human reproduction. Berlin/Heidelberg: Springer; 2011. p. 99–117.
100. Ohinata Y, Payer B, O'Carroll D, et al. Blimp1 is a critical determinant of the germ cell lineage in mice. Nature. 2005;436:207–13.
101. Schoenwolf GC, Bleyl SB, Brauer PR, et al. Ganetogenesis, fertilization, and first week. Larsen's human embryology. 4th ed. Philadelphia: Churchill Livingstone; 2009. p. 15–50.
102. Ara T, Nakamura Y, Egawa T, et al. Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (SDF-1). Proc Natl Acad Sci U S A. 2003;100:5319–23.
103. Anderson R, Fassler R, Georges-Labouesse E, et al. Mouse primordial germ cells lacking beta1 integrins enter the germline but fail to migrate normally to the gonads. Development. 1999;126:1655–64.
104. Bendel-Stenzel MR, Gomperts M, Anderson R, et al. The role of cadherins during primordial germ cell migration and early gonad formation in the mouse. Mech Dev. 2000;91:143–52.
105. Juneja SC, Barr KJ, Enders GC, et al. Defects in the germ line and gonads of mice lacking connexin43. Biol Reprod. 1999;60:1263–70.
106. Kota SK, Feil R. Epigenetic transitions in germ cell development and meiosis. Dev Cell. 2010;19(5):675–86.
107. Pan Z, Zhang J, Li Q, et al. Current advances in epigenetic modification and alteration during mammalian ovarian folliculogenesis. J Genet Genomics. 2012;39(3):111–23.
108. Seki Y, Hayashi K, Itoh K, et al. Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. Dev Biol. 2005;278(2):440–58.
109. Paoloni-Giacobino A. Epigenetics in reproductive medicine. Pediatr Res. 2007;61(5 Pt 2): 51R–7.
110. Yabuta Y, Kurimoto K, Ohinata Y, et al. Gene expression dynamics during germline specification in mice identified by quantitative single-cell gene expression profiling. Biol Reprod. 2006;75(5):705–16.

111. Seki Y, Yamaji M, Yabuta Y, et al. Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. Development. 2007; 134:2627–38.
112. Peters AH, Kubicek S, Mechtler K, et al. Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. Mol Cell. 2003;12:1577–89.
113. Matsui Y, Zsebo K, Hogan BL. Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. Cell. 1992;70:841–7.
114. Tam PP, Zhou SX, Tan SS. X-chromosome activity of the mouse primordial germ cells revealed by the expression of an X-linked lacZ transgene. Development. 1994;120:2925–32.
115. Raz E. Primordial germ-cell development: the zebrafish perspective. Nat Rev Genet. 2003;4: 690–700.
116. Surani MA. Reprogramming of genome function through epigenetic inheritance. Nature. 2001;414:122–8.
117. Yamazaki Y, Low EW, Marikawa Y, et al. Adult mice cloned from migrating primordial germ cells. Proc Natl Acad Sci U S A. 2005;102:11361–6.
118. Sato S, Yoshimizu T, Sato E, et al. Erasure of methylation imprinting of Igf2r during mouse primordial germ-cell development. Mol Reprod Dev. 2003;65:41–50.
119. Hajkova P, Erhardt S, Lane N, et al. Epigenetic reprogramming in mouse primordial germ cells. Mech Dev. 2002;117:15–23.
120. Popp C, Dean W, Feng S, et al. Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. Nature. 2010;463:1101–5.
121. Chen T, Ueda Y, Dodge JE, et al. Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. Mol Cell Biol. 2003;23: 5594–605.
122. Lynn A, Ashley T, Hassold T. Variation in human meiotic recombination. Annu Rev Genomics Hum Genet. 2004;5:317–49.
123. Strich R. Meiotic DNA, replication. Curr Top Dev Biol. 2004;61:29–60.
124. Maheshwari A, Fowler PA. Primordial follicular assembly in humans–revisited. Zygote. 2008;16:285–96.
125. Oktem O, Urman B. Understanding follicle growth in vivo. Hum Reprod. 2010;25:2944–54.
126. Abir R, Orvieto R, Dicker D, et al. Preliminary studies on apoptosis in human fetal ovaries. Fertil Steril. 2002;78:259–64.
127. Oktay K, Briggs D, Gosden RG. Ontogeny of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. J Clin Endocrinol Metab. 1997;82:3748–51.
128. Rajareddy S, Reddy P, Du C, et al. p27kip1 (cyclin-dependent kinase inhibitor 1B) controls ovarian development by suppressing follicle endowment and activation and promoting follicle atresia in mice. Mol Endocrinol. 2007;21:2189–202.
129. Reddy P, Liu L, Adhikari D, et al. Oocyte-specific deletion of Pten causes premature activation of the primordial follicle pool. Science. 2008;319:611–13.
130. Adhikari D, Zheng W, Shen Y, et al. Tsc/mTORC1 signaling in oocytes governs the quiescence and activation of primordial follicles. Hum Mol Genet. 2010;19:397–410.
131. De Baere E, Beysen D, Oley C, et al. FOXL2 and BPES: mutational hotspots, phenotypic variability, and revision of the genotype-phenotype correlation. Am J Hum Genet. 2003;72: 478–87.
132. Castrillon DH, Miao L, Kollipara R, et al. Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. Science. 2003;301:215–18.
133. Gallardo TD, John GB, Bradshaw K, et al. Sequence variation at the human FOXO3 locus: a study of premature ovarian failure and primary amenorrhea. Hum Reprod. 2008;23: 216–21.
134. Rajpert-De ME, Jorgensen N, Graem N, et al. Expression of anti-Mullerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. J Clin Endocrinol Metab. 1999;84:3836–44.
135. De Vet A, Laven JS, de Jong FH, et al. Antimullerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril. 2002;77:357–62.

136. Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology. 2001;142:4891–9.
137. Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology. 2002;143:1076–84.
138. Rankin T, Familari M, Lee E, et al. Mice homozygous for an insertional mutation in the Zp3 gene lack a zona pellucida and are infertile. Development. 1996;122:2903–10.
139. Schmidt D, Ovitt CE, Anlag K, et al. The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. Development. 2004;131: 933–42.
140. Nilsson E, Skinner MK. Bone morphogenetic protein-4 acts as an ovarian follicle survival factor and promotes primordial follicle development. Biol Reprod. 2003;69:1265–72.
141. Vitt UA, McGee EA, Hayashi M, et al. In vivo treatment with GDF-9 stimulates primordial and primary follicle progression and theca cell marker CYP17 in ovaries of immature rats. Endocrinology. 2000;141:3814–20.
142. Carabatsos MJ, Elvin J, Matzuk MM, et al. Characterization of oocyte and follicle development in growth differentiation factor-9-deficient mice. Dev Biol. 1998;204:373–84.
143. Oktay K, Briggs D, Gosden RG. Ontogeny of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. J Clin Endocrinol Metab. 1997;82:3748–51.
144. Nilsson EE, Skinner MK. Kit ligand and basic fibroblast growth factor interactions in the induction of ovarian primordial to primary follicle transition. Mol Cell Endocrinol. 2004;214: 19–25.
145. Nilsson EE, Kezele P, Skinner MK. Leukemia inhibitory factor (LIF) promotes the primordial to primary follicle transition in rat ovaries. Mol Cell Endocrinol. 2002;188:65–73.
146. Knight PG, Glister C. TGF-beta superfamily members and ovarian follicle development. Reproduction. 2006;132:191–206.
147. Wang J, Roy SK. Growth differentiation factor-9 and stem cell factor promote primordial follicle formation in the hamster: modulation by follicle-stimulating hormone. Biol Reprod. 2004;70:577–85.
148. Hanrahan JP, Gregan SM, Mulsant P, et al. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). Biol Reprod. 2004;70:900–9.
149. Otsuka F, Yao Z, Lee T, et al. Bone morphogenetic protein-15. Identification of target cells and biological functions. J Biol Chem. 2000;275:39523–8.
150. Lee WS, Yoon SJ, Yoon TK, et al. Effects of bone morphogenetic protein-7 (BMP-7) on primordial follicular growth in the mouse ovary. Mol Reprod Dev. 2004;69:159–63.
151. Shimasaki S, Zachow RJ, Li D, et al. A functional bone morphogenetic protein system in the ovary. Proc Natl Acad Sci U S A. 1999;96:7282–7.
152. Saragueta PE, Lanuza GM, Baranao JL. Autocrine role of transforming growth factor beta1 on rat granulosa cell proliferation. Biol Reprod. 2002;66:1862–8.
153. Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology. 2001;142:4891–9.
154. Visser JA, Themmen AP. Anti-Mullerian hormone and folliculogenesis. Mol Cell Endocrinol. 2005;234:81–6.
155. Xiao S, Robertson DM, Findlay JK. Effects of activin and follicle-stimulating hormone (FSH)-suppressing protein/follistatin on FSH receptors and differentiation of cultured rat granulosa cells. Endocrinology. 1992;131:1009–16.
156. Oktem O, Oktay K. The role of extracellular matrix and activin-A in in vitro growth and survival of murine preantral follicles. Reprod Sci. 2007;14:358–66.
157. Findlay JK. An update on the roles of inhibin, activin, and follistatin as local regulators of folliculogenesis. Biol Reprod. 1993;48:15–23.
158. Matzuk MM, Kumar TR, Bradley A. Different phenotypes for mice deficient in either activins or activin receptor type II. Nature. 1995;374:356–60.
159. Visser JA, Themmen AP. Anti-Mullerian hormone and folliculogenesis. Mol Cell Endocrinol. 2005;234:81–6.

160. Yamoto M, Minami S, Nakano R, et al. Immunohistochemical localization of inhibin/activin subunits in human ovarian follicles during the menstrual cycle. J Clin Endocrinol Metab. 1992;74:989–93.
161. Hsueh AJ, Dahl KD, Vaughan J, et al. Heterodimers and homodimers of inhibin subunits have different paracrine action in the modulation of luteinizing hormone-stimulated androgen biosynthesis. Proc Natl Acad Sci U S A. 1987;84:5082–6.
162. Silva CC, Groome NP, Knight PG. Demonstration of a suppressive effect of inhibin alpha-subunit on the developmental competence of in vitro matured bovine oocytes. J Reprod Fertil. 1999;115:381–8.
163. Kageyama S, Liu H, Kaneko N, et al. Alterations in epigenetic modifications during oocyte growth in mice. Reproduction. 2007;133:85–94.
164. Lucifero D, Mann MR, Bartolomei MS, et al. Gene-specific timing and epigenetic memory in oocyte imprinting. Hum Mol Genet. 2004;13:839–49.
165. Kato M, Miura A, Bender J, et al. Role of CG and non-CG methylation in immobilization of transposons in Arabidopsis. Curr Biol. 2003;13:421–6.
166. Hiura H, Obata Y, Komiyama J, et al. Oocyte growth-dependent progression of maternal imprinting in mice. Genes Cells. 2006;11:353–61.
167. Obata Y, Kono T. Maternal primary imprinting is established at a specific time for each gene throughout oocyte growth. J Biol Chem. 2002;277:5285–9.
168. Geuns E, De Rycke M, Van Steirteghem A, et al. Methylation imprints of the imprint control region of the SNRPN-gene in human gametes and preimplantation embryos. Hum Mol Genet. 2003;12:2873–9.
169. Eppig JJ. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. Reprod Fertil Dev. 1996;8:485–9.
170. Pincus G, Enzmann EV. Can mammalian eggs undergo normal development in vitro? Proc Natl Acad Sci U S A. 1934;20:121–2.
171. Conti M, Andersen CB, Richard F, et al. Role of cyclic nucleotide signaling in oocyte maturation. Mol Cell Endocrinol. 2002;187:153–9.
172. Mehlmann LM. Stops and starts in mammalian oocytes: recent advances in understanding the regulation of meiotic arrest and oocyte maturation. Reproduction. 2005;130:791–9.
173. Conti M, Hsieh M, Zamah AM, et al. Novel signaling mechanisms in the ovary during oocyte maturation and ovulation. Mol Cell Endocrinol. 2012;356:65–73.
174. Norris RP, Ratzan WJ, Freudzon M, et al. Cyclic GMP from the surrounding somatic cells regulates cyclic AMP and meiosis in the mouse oocyte. Development. 2009;136:1869–78.
175. Zhang M, Su YQ, Sugiura K, et al. Granulosa cell ligand NPPC and its receptor NPR2 maintain meiotic arrest in mouse oocytes. Science. 2010;330:366–9.
176. Liang CG, Su YQ, Fan HY, et al. Mechanisms regulating oocyte meiotic resumption: roles of mitogen-activated protein kinase. Mol Endocrinol. 2007;21:2037–55.
177. Shimada M, Terada T. Phosphatidylinositol 3-kinase in cumulus cells and oocytes is responsible for activation of oocyte mitogen-activated protein kinase during meiotic progression beyond the meiosis I stage in pigs. Biol Reprod. 2001;64:1106–14.
178. Gaffre M, Dupre A, Valuckaite R, et al. Deciphering the H-Ras pathway in Xenopus oocyte. Oncogene. 2006;25:5155–62.
179. Peng XR, Hsueh AJ, LaPolt PS, et al. Localization of luteinizing hormone receptor messenger ribonucleic acid expression in ovarian cell types during follicle development and ovulation. Endocrinology. 1991;129:3200–7.
180. Park JY, Su YQ, Ariga M, et al. EGF-like growth factors as mediators of LH action in the ovulatory follicle. Science. 2004;303:682–4.
181. Zamah AM, Hsieh M, Chen J, et al. Human oocyte maturation is dependent on LH-stimulated accumulation of the epidermal growth factor-like growth factor, amphiregulin. Hum Reprod. 2010;25:2569–78.
182. Hsieh M, Lee D, Panigone S, et al. Luteinizing hormone-dependent activation of the epidermal growth factor network is essential for ovulation. Mol Cell Biol. 2007;27:1914–24.

183. Gilchrist RB. Recent insights into oocyte-follicle cell interactions provide opportunities for the development of new approaches to in vitro maturation. Reprod Fertil Dev. 2011;23: 23–31.
184. Li M, Liang CG, Xiong B, et al. PI3-kinase and mitogen-activated protein kinase in cumulus cells mediate EGF-induced meiotic resumption of porcine oocyte. Domest Anim Endocrinol. 2008;34:360–71.
185. Kawamura K, Kumagai J, Sudo S, et al. Paracrine regulation of mammalian oocyte maturation and male germ cell survival. Proc Natl Acad Sci U S A. 2004;101:7323–8.
186. Kawamura K, Ye Y, Liang CG, et al. Paracrine regulation of the resumption of oocyte meiosis by endothelin-1. Dev Biol. 2009;327:62–70.
187. Kidder GM, Vanderhyden BC. Bidirectional communication between oocytes and follicle cells: ensuring oocyte developmental competence. Can J Physiol Pharmacol. 2010;88:399–413.
188. Norris RP, Freudzon M, Mehlmann LM, et al. Luteinizing hormone causes MAP kinase-dependent phosphorylation and closure of connexin 43 gap junctions in mouse ovarian follicles: one of two paths to meiotic resumption. Development. 2008;135:3229–38.
189. Ferreira EM, Vireque AA, Adona PR, et al. Cytoplasmic maturation of bovine oocytes: structural and biochemical modifications and acquisition of developmental competence. Theriogenology. 2009;71:836–48.
190. Ajduk A, Malagocki A, Maleszewski M. Cytoplasmic maturation of mammalian oocytes: development of a mechanism responsible for sperm-induced Ca^{2+} oscillations. Reprod Biol. 2008;8:3–22.
191. Stricker SA. Structural reorganizations of the endoplasmic reticulum during egg maturation and fertilization. Semin Cell Dev Biol. 2006;17:303–13.
192. Zuccotti M, Merico V, Cecconi S, et al. What does it take to make a developmentally competent mammalian egg? Hum Reprod Update. 2011;17:525–40.
193. Prather RS, Ross JW, Isom SC, et al. Transcriptional, post-transcriptional and epigenetic control of porcine oocyte maturation and embryogenesis. Soc Reprod Fertil Suppl. 2009;66: 165–76.
194. Tan JH, Wang HL, Sun XS, et al. Chromatin configurations in the germinal vesicle of mammalian oocytes. Mol Hum Reprod. 2009;15:1–9.
195. Liu H, Aoki F. Transcriptional activity associated with meiotic competence in fully grown mouse GV oocytes. Zygote. 2002;10:327–32.
196. Zuccotti M, Ponce RH, Boiani M, et al. The analysis of chromatin organisation allows selection of mouse antral oocytes competent for development to blastocyst. Zygote. 2002;10:73–8.
197. Li Y, Sasaki H. Genomic imprinting in mammals: its life cycle, molecular mechanisms and reprogramming. Cell Res. 2011;21:466–73.
198. Hiura H, Obata Y, Komiyama J, et al. Oocyte growth-dependent progression of maternal imprinting in mice. Genes Cells. 2006;11:353–61.
199. Gu L, Wang Q, Sun QY. Histone modifications during mammalian oocyte maturation: dynamics, regulation and functions. Cell Cycle. 2010;9:1942–50.
200. Paynton BV, Rempel R, Bachvarova R. Changes in state of adenylation and time course of degradation of maternal mRNAs during oocyte maturation and early embryonic development in the mouse. Dev Biol. 1988;129:304–14.
201. Rana TM. Illuminating the silence: understanding the structure and function of small RNAs. Nat Rev Mol Cell Biol. 2007;8:23–36.
202. Suh N, Blelloch R. Small RNAs in early mammalian development: from gametes to gastrulation. Development. 2011;138:1653–61.
203. Ma J, Flemr M, Stein P, et al. MicroRNA activity is suppressed in mouse oocytes. Curr Biol. 2010;20:265–70.
204. Suh N, Baehner L, Moltzahn F, et al. MicroRNA function is globally suppressed in mouse oocytes and early embryos. Curr Biol. 2010;20:271–7.
205. Murchison EP, Stein P, Xuan Z, et al. Critical roles for Dicer in the female germline. Genes Dev. 2007;21:682–93.

206. Kaneda M, Tang F, O'Carroll D, et al. Essential role for Argonaute2 protein in mouse oogenesis. Epigenet Chromatin. 2009;2:9.
207. Flesch FM, Gadella BM. Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochim Biophys Acta. 2000;1469:197–235.
208. Ficarro S, Chertihin O, Westbrook VA, et al. Phosphoproteome analysis of capacitated human sperm. Evidence of tyrosine phosphorylation of a kinase-anchoring protein 3 and valosin-containing protein/p97 during capacitation. J Biol Chem. 2003;278:11579–89.
209. Legendre LM, Stewart-Savage J. Effect of cumulus maturity on sperm penetration in the golden hamster. Biol Reprod. 1993;49:82–8.
210. Fetterolf PM, Jurisicova A, Tyson JE, et al. Conditioned medium from human cumulus oophorus cells stimulates human sperm velocity. Biol Reprod. 1994;51:184–92.
211. Sun F, Bahat A, Gakamsky A, et al. Human sperm chemotaxis: both the oocyte and its surrounding cumulus cells secrete sperm chemoattractants. Hum Reprod. 2005;20:761–7.
212. Chapman N, Kessopoulou E, Andrews P, et al. The polypeptide backbone of recombinant human zona pellucida glycoprotein-3 initiates acrosomal exocytosis in human spermatozoa in vitro. Biochem J. 1998;330(Pt 2):839–45.
213. Tesarik J, Drahorad J, Peknicova J. Subcellular immunochemical localization of acrosin in human spermatozoa during the acrosome reaction and zona pellucida penetration. Fertil Steril. 1988;50:133–41.
214. Roldan ER, Shi QX. Sperm phospholipases and acrosomal exocytosis. Front Biosci. 2007;12:89–104.
215. Fukami K, Nakao K, Inoue T, et al. Requirement of phospholipase Cdelta4 for the zona pellucida-induced acrosome reaction. Science. 2001;292:920–3.
216. Beebe SJ, Leyton L, Burks D, et al. Recombinant mouse ZP3 inhibits sperm binding and induces the acrosome reaction. Dev Biol. 1992;151:48–54.
217. Rankin TL, Coleman JS, Epifano O, et al. Fertility and taxon-specific sperm binding persist after replacement of mouse sperm receptors with human homologs. Dev Cell. 2003;5:33–43.
218. Ikawa M, Inoue N, Benham AM, et al. Fertilization: a sperm's journey to and interaction with the oocyte. J Clin Invest. 2010;120:984–94.
219. Hagaman JR, Moyer JS, Bachman ES, et al. Angiotensin-converting enzyme and male fertility. Proc Natl Acad Sci U S A. 1998;95:2552–7.
220. Nishimura H, Kim E, Nakanishi T, et al. Possible function of the ADAM1a/ADAM2 Fertilin complex in the appearance of ADAM3 on the sperm surface. J Biol Chem. 2004;279:34957–62.
221. Yamaguchi R, Muro Y, Isotani A, et al. Disruption of ADAM3 impairs the migration of sperm into oviduct in mouse. Biol Reprod. 2009;81:142–6.
222. Ikawa M, Wada I, Kominami K, et al. The putative chaperone calmegin is required for sperm fertility. Nature. 1997;387:607–11.
223. Inoue N, Ikawa M, Isotani A, et al. The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. Nature. 2005;434:234–8.
224. Le Naour F, Rubinstein E, Jasmin C, et al. Severely reduced female fertility in CD9-deficient mice. Science. 2000;287:319–21.
225. Ducibella T, Kurasawa S, Duffy P, et al. Regulation of the polyspermy block in the mouse egg: maturation-dependent differences in cortical granule exocytosis and zona pellucida modifications induced by inositol 1,4,5-trisphosphate and an activator of protein kinase C. Biol Reprod. 1993;48:1251–7.
226. Lee SH, Ahuja KK, Gilburt DJ, et al. The appearance of glycoconjugates associated with cortical granule release during mouse fertilization. Development. 1988;102:595–604.
227. Sato K. Polyspermy-preventing mechanisms in mouse eggs fertilized in vitro. J Exp Zool. 1979;210:353–9.
228. McGrath J, Solter D. Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell. 1984;37:179–83.

Physiology of Embryonic Development

2

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Abstract

Human embryo development is a complex process. The life of an embryo begins when a male's spermatozoa makes contact with a woman's egg. A zygote cell, the very first representation of the fetus, is the result of this fertilization process. Contained within this one cell is the DNA of both the male and female, as well as the blueprint from which the fetus will develop. This chapter reviews some of the basic physiology of embryonic development.

2.1 Early Development of the Embryo

The embryonic period ranges from fertilization up to the 8th week of development. It is divided into a pre-embryonic period (from the 1rst to the 3rd week,) and the embryonic period (from 3rd to the 8th week). Pre-embryonic development includes cleavage of the zygote, formation of the morula, and implantation. The embryonic period extends from implantation to the end of the eighth developmental week. During the embryonic period the three germ layers arise and most of the organ systems are established.

2.1.1 Cleavage of the Zygote (0–3 Days)

Cleavage describes repeated mitotic divisions of the zygote within the zona pellucida. It results in a rapid increase in the number of cells where the enormous volume of

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egg cytoplasm is divided into numerous small, nucleated cells. These cleavage-stage cells are called blastomeres. Cleavage takes place in the uterine (fallopian) tube up to 3 days after fertilization and includes several stages; (1) 2-cell stage; the first cleavage division produces two cells of unequal size about 30 h after fertilization; (2) 3-cell stage; the large cell continues to divide and form 3-cells; (3) 4-cell stage; the smaller cell also divides into two. In this way two large and two small cells form at about 40–50 h of fertilization; (4) 8-cell stage is reached after further cleavage; (5) 12-cell stage is reached about 72 h after fertilization [1].

Firstly, cleavages in mammalian zygotes are among the slowest in the animal kingdom. Human cleavage is relatively slow. For instance, first cleavage is not completed for 24 h in humans and subsequent early divisions take about 10–12 h each. Secondly, the first cleavage is a normal meridianal division, and the second cleavage division may not occur simultaneously in both blastomeres; one of the two blastomeres divides meridianally and the other divides equatorially. In many mammals, the mitotic spindle of one of the blastomeres rotates 90° during the second cleavage division, which yields a crosswise arrangement of blastomeres at the 4-cell stage. This type of cleavage is called rotational cleavage [2]. Thirdly, the marked asynchrony of early cell division is the important feature of human cleavage [3–5]. All blastomeres do not divide at same time. Thus, human embryos frequently contain odd numbers of cells instead of typical doublings of cells from 2- to 4-to 8-cell stages. Fourthly, unlike almost all other animal genomes, the mammalian genome is activated during early cleavage, and produces the proteins necessary for cleavage to occur [6, 7].

Finally, the most important feature of human zygote cleavage involves compaction where changes take place to form a “compact” ball of cells [8–10]. Until the 8-cell stage mouse blastomeres form a loose arrangement with plenty of space between them, where accumulation of fluid takes place inside the embryo. During compacting process, the blastomeres undergo a spectacular change, they flatten and become tightly joined, maximizing their contact with one another and forming a compact ball of cells [11–13]. The cells within the compacted sphere form stabilized tight junctions, which prevent the free exchange of fluid between the inside and the outside of the embryo, and, enable small molecules and ions to pass between cells [14].

2.1.2 Formation of Morula and the Transition from Morula to Blastocyst

The cells of the compacted 8-cell embryo divide to produce a 16-cell morula (mulberry), consisting of a small group of internal cells surrounded by a larger group of external cells [15]. In this process, blastomeres tightly align themselves against each other to form a compact ball of cells, enclosed within the zona pellucia. In morula, two distinct populations of cells begin to appear; inner cells which form inner cell mass are distinguishable from the surrounding cells which form the external cell mass [16, 17]. Most of the descendants of the external cells constitute

the outer wall of the blastocyst, collectively called the trophoblast, which assume the configuration and many of the properties of epithelial cells. Trophoblast covering the embryonic pole of the blastocyst is called polar trophoblast formed by 30 cells, while that occupying the rest of the wall including the embryonic pole is called mural trophoblast and is comprised of about 60 cells [18], and they later contributes to extra-embryonic structures and the embryonic part of placenta. Thus, the embryo derives from the inner cell mass of the morula at the 16-cells stage, a group of clustered blastomeres, formed by only 8 cells and also called embryoblast, supplemented by cells dividing from the trophoblast during the transition to the 32-cell stage [19]. The resulting structure, called the blastocyst, is another hallmark of mammalian cleavage.

Some embryologists conclude that the position of a blastomere in the morula determines whether it will become part of the trophoblast or the inner cell mass [20–22]. According to the "inside-outside" hypothesis, the external cells create a unique microenvironment and enable cells of the morula which have no contact with the exterior to develop into the inner cell mass, whereas cells located at the surface of the morula, presumably because of physiological functions required by their superficial position, are channelled into becoming trophoblastic epithelium. Tight junctions between outer cells are thought to preserve the environmental differences between outer and inner cells. But an alternate hypothesis, the "polarization" or "cytoplasmic segregation" hypothesis, relates the differentiation of the two cell types to gradients of cytoplasmic determinants which become segregated into internal or external blastomeres as cleavage divisions proceeds.

External cells produce no embryonic structures. Rather, they form the tissue of the chorion, the embryonic portion of the placenta that enables transfer of oxygen and nutrients from the mother. The chorion also produces hormones to enable the mother's uterus to retain the fetus, and, regulators of the immune response so that the mother will not reject the embryos as it would be an organ graft. An unusual feature of trophoblastic cells is that maternal X-chromosomal genes are preferentially expressed and paternal genes are inactivated [23, 24].

Cells in the inner cell mass join to one another with communicating gap junctions, and, they retain the ability to reaggregate if separated or mixed with cells of other embryos. Cells in the inner cell mass can neither pump fluid nor evoke the decidual reaction, as can be seen in the cells of the trophoblastic layer. They are destined to form the embryo and its associated yolk sac, allantois, and amnion. By the 64-cell stage, the inner cell mass (approximately 13 cells) and the trophoblast cells have become separate cell layers, neither contributing cells to the other group [25]. Thus, the distinction between trophoblast and inner cell mass blastomeres represents the first differentiation event in mammalian development. This differentiation is required for the early mammalian embryo to adhere to the uterus. The inner cell mass actively supports the trophoblast, secreting proteins that cause trophoblast cells to divide.

Initially, the morula does not have an internal cavity. However, during "cavitation" the trophoblast cells secrete fluid into the morula to create a blastocele, or, the blastocyst cavity. There are two obvious changes during the transition from morula

to blastocyst, one is a rapid enlargement of blastocyst cavity and the second is the emergence of "inside-outside" cell mass within the embryo. During this time, the zona pellucida covering the outer blastocyst avoid adhering to the oviduct walls. When the embryo reaches the uterus, however, it must "hatch" from the zona so that it can adhere to the uterine wall.

2.1.3 Regulation During Cleavage and Blastulation

Early in the 1880s, Weismann put forward a model of development in which the nucleus of the zygote contains a number of special factors, or, determinants. These determinants distribute unequally to the daughter cells from cleavage, and, control the future development of cells. This type of model was termed 'mosaic', as the egg could be considered to be a mosaic of discrete, localized determinants [26–28]. In the late 1880s, the germen embryologist, Wilhelm Roux, destroyed one of the two cells in frog embryos with a hot needle and found that the undamaged cell developed into a well-formed half-larva, but in the damaged half of the embryos, no cells formed, thus providing the initial support for Weismann's ideas [29]. However Hans Driesch demonstrated the developmental process known as "regulation", and illustrated that an embryo is able to develop normally even when some portions are removed, or rearranged [30]. Later, Hans Spemann and his assistant Hilde Mangold assembled the famous theory, known as the Spemann-Mangold "organizer" [31]. They showed that a partial second embryo may be induced by grafting one small region of an early new embryo onto another at the same stage. This small region they called the organizer, since it seemed to be ultimately responsible for controlling the organization of a complete embryonic body. In fact, early development is controlled by the genotype, and, environmental factors interacting with the genotype to influence the phenotype. Despite having identical genotypes, identical twins can develop considerable differences in their phenotypes as they grow up and these tend to become more evident with age.

In summary, the potential for development of cells in an early embryo is usually much greater than their normal fate, but this potential becomes restricted as development proceeds. Inductive interactions, involving signals from one cell to another, are one of the primary ways of leading development. Asymmetric cell divisions, in which cytoplasmic components are unequally distributed to daughter cells, can also make cells into different fates. The embryo contains a generative, not a descriptive, program—it is more like the instructions for making a structure by paper folding than a blueprint [32].

A number of investigations of early development in hybrid embryos, particularly sea urchin embryos, show that many features such as the rate of cleavage, follow the instructions of the maternal, rather than the paternal, genome [33]. The pattern of molecular activities of early development in mammals differs from that in the sea urchin and in other vertebrates that have been studied. In the mouse, enough informational macromolecules accumulate in the developing oocyte to guide the zygote through the first cleavage division, but a rapid switching over from control

by the maternal to the embryonic genome begins at the two-cell stage [34, 35]. Demonstrations of high rates of degradation of maternal RNAs, a burst of synthesis of new RNAs, blockage of development past the two-cell stage by the transcriptional inhibitors actinomycin D and alpha-amanitin, changing patterns of polypeptide synthesis, and results of crosses of parents with differing variants of key enzymes confirm this change of control [36].

Patterns of protein synthesis in the early mammalian embryo are complex. During the first cleavage division there is evidence for protein synthesis that takes place whether or not fertilization has occurred, and, the synthesis of other proteins that occurs only as a result of fertilization. Most of these early proteins are made from maternal mRNAs [37]. As might be expected, the synthesis of histones is prominent during cleavage. There is little evidence that any maternal mRNAs guide protein synthesis after the four-cell stage [38]. The processes of cavitation, compaction, and formation of the blastocyst are under the control of the embryonic genome. Overall, the rates of protein synthesis are low until about the 8-cell stage. Thereafter there is a sharp increase in the production of ribosomes and protein synthesis.

2.2 Dynamic Epigenetic Modification—Parental Imprinting

In mammals, the contributions of gametes, both the egg and the sperm, to the developing diploid embryo are unequal. Functional differences exist between the two sets of parental chromosomes due to "genomic imprinting". Imprinting is a particularly important genetic mechanism in mammals, and may influence the transfer of nutrients to the fetus and the newborn from the mother. The effect of the imprint on gene activity is to allow the expression of some imprinted genes from either the maternally-inherited chromosome, or, others from the paternally-inherited chromosome. Imprinting is involved in the process of development and aberrant imprinting may cause various disease syndromes in mammals. It is not known why such a process evolved, nor are the precise mechanisms involved in the regulation of imprinted genes fully understood. However, it follows that the dosage of an imprinted gene can either be doubled or lost completely if there is uniparental duplication or deficiency involving the gene or chromosomal region in which it resides. Such aberrations can have profound effects on mammalian embryonic development and can cause human disease.

Parental imprinting may be a functional reflection of different patterns of DNA methylation during gametogenesis in males and females. The methylation pattern that a zygote inherits is transmitted to all daughter cell after mitosis, even in postnatal life. When gametes enter meiosis the original imprints on the chromosomes are erased and new imprints are made. Sperm chromosomes receive a paternal imprint and egg chromosomes receive a maternal imprint. Imprints are 'established' during the development of germ cells into sperm or eggs, and, are necessary for normal fetal development [39, 40]. It is common that the established imprints are 'maintained' following with the duplication of chromosomes and segregate in the developing

organism. The competed imprints cycle contains, (1) imprints are 'erased' at an early stage, and (2) new imprints must be re-established at a later stage of germ-cell development. But in somatic cells, imprints are maintained and modified during development.

The resetting of imprint is a critical portion of epigenetic re-programming in germ cells. For most imprints, current evidence indicates that there might be two stages for this resetting process—the step of "erasure" and the later step of "establishment". Several studies have now demonstrated that the erasure of at least methylation imprints occurs in the germline and it is completed by embryonic day 12–13 (E12–13) in both sexes, after the PGCs enter the gonadal ridge [40]. Indeed, in female embryo germ cells-somatic hybrid cells there were striking changes in methylation of the somatic nucleus, resulting in demethylation of several imprinted and non-imprinted genes, whether this demethylation is active or passive is not known [41]. The evidence so far indicates that all imprints are unlikely to sustain unchanged as the developing embryo, whatever imprints inheriting from a parent with the same sex, and it is obvious that methylation and demethylation of imprinted genes are not persisting in stationary condition, and may be functionally intact before the erasure stage. In addition to methylation imprints, differential replication of DNA is also apparently erased in both germ lines [42]. After erasure of germline methylation imprints, differentiating germ cell genomes must become maternalised or paternalised depending on germ cell sex, and this must occur before the onset of meiosis. In the female germline, imprints are re-established in growing oocytes. During the transition from primordial to antral follicles, the erasure and establishment of imprinted genes are not synchronized to oocyte [43, 44]. In the process of imprinting establishment, de novo methylation begins in both germ lines at late fetal stages, and continues after birth. It is not fully understood which enzymes are responsible for de novo methylation in germ cells. Dnmt1 (DNA methyltransferase 1) and its germ-cell specific isoforms, Dnmt3a or Dnmt3b are candidates, which are required for de novo methylation in postimplantation embryos [45]. DNA methylation in mammals occurs in the dinucleotide CpG. Allele-specific methylation patterns are maintained due to presence of repetitive sequence regions near DMRs. Methyl groups can be introduced into unmethylated DNA by the de novo methylation enzymes Dnmt3a and Dnmt3b (and perhaps others). Once the imprints cycle are completed in the early embryo, and, fully matured during differentiation, it begins to the shift from the resetting of imprints into differential gene expression. Differential gene expression occurs largely at the level of transcription, although post-transcriptional mechanisms may also exist.

2.3 Implantation of the Blastocyst

While the embryo is passing through the oviduct to the uterus, the blastocyst expands within the zona pellucida. After the blastocyst enters the uterus cavity, the zona pellucida exposing the trophoblast layer in blastocyst disappears, so that the blastocyst can adhere to the uterine wall. Fluid accumulation in the blastocyst cavity

is the product of a sodium pump (a N^+/K^+ ATPase) system in the plasma membranes of the trophoblast cells [46, 47]. This sodium pump faces the blastocoele, and pumps sodium ions, accompanied by water molecules, among the inner blastomeres in exchange for intra-embryonic H^+, which is transported out of the embryo. Accordingly, by the accumulation of sodium ions into the central cavity, the blastocoele draw in water osmotically and become enlarged. The early mammalian blastocyst remains enclosed within the zona pellucida, but the overall size of the embryo increases because of this accumulation of fluid. Thus, the blastocyst is able to "hatch" from the zona pellucida. The mouse blastocyst hatches from the zona by lysing a small hole in it and squeezing through that hole as the blastocyst expands. This small hole is due to the effort of strypsin a trypsin-like protease on the trophoblast cell membranes [48, 49].

Once out of the zona pellucida, the blastocyst makes direct contact with the uterus. Decidualisation of the uterine endometrium takes place by enlargement of stromal cells which become vacuolated and filled with glycogen and lipids. The uterine epithelium matrix contains collagen, laminin, fibronectin, hyaluronic acid, and heparin sulfate receptors [50, 51]. So, the trophoblast cells contain integrins that will bind to the uterine collagen, fibronectin, and laminin, and they synthesize heparan sulfate proteoglycan precisely prior to implantation. Once in contact with the endometrium, the trophoblast secrets another set of proteases, including collagenase, stromelysin, and plasminogen activator. These protein-digesting enzymes digest the extracellular matrix of the uterine tissue, enabling the blastocyst to bury itself within the uterine wall. Thus, sticky and polar trophoblast adheres with the hormonally-prepared endometrium (now called decidua).

The adhesion process is assisted by the interaction of substance, pentasaccharide lacto-N-fucopentose-1 on the epithelium and its receptors on the trophoblasts [52]. The blastocysts then erode and burrow into the decidua with the help of proteolytic enzymes secreted by the trophoblast. Eventually it is completely buried under the mucosa. The implantation of the blastocyst takes place on 6th or 7th day after fertilization. The site of implantation normally is the dorsal wall near the junction of fundus and body of uterus. But implantation may take place anywhere in the upper part of uterine cavity. Sources of nutrition for the embedded embryo are glycogen and lipid filled decidual cells until the placenta becomes functional.

2.4 Formation and Differentiation of Three Germinal Layers

In the course of the second week the embryoblast differentiates into two germinal layers: the epiblast and the hypoblast. The tissue of the embryo as well as the amniotic epithelium will arise out of the epiblast. And the hypoblast forms the umbilical vesicle. During the 3rd week of its development, the epiblast experiences a number of complex changes that lead to the differentiation of three embryonic germinal layers: ectoderm, mesoderm, and endoderm [1]. The hypoblast, or primitive endoderm, will generate extraembryonic tissues only, such as the lining of the primary (primitive)

yolk sac (exocoelomic cavity). Each of the three germ layers gives rise to different structures and organs. As the result of the organogenesis, the appearance of the embryo alters considerably [14].

These three germ layers take part in establishing the functional organs and organ systems [28]. Each germ layer has particular characteristics, but each can constitute extra structures under certain natural or experimental effects (e.g., transplantation). Therefore, the germ layers are no longer believed entirely specific to different groups of tissues. During this important period, the exposure of the embryo to certain drugs or reagents may result in major congenital malformations because the precursors of the major organ systems are growing and developing at a considerable rate.

2.4.1 Formation of Endoderm

Cells migrating inward along the archenteron constitute the inner layer of the gastrula, and leads to the formation the endoderm [53]. Development of the blastodermic vesicle is followed by the separation of cells from the inner cell mass. The detached cells thrust into the blastocoele to constitute the embryonal endoderm or hypoblast. These cells speedily increase in number to construct the second complete layer inside the original outer layer of the blastodermic vesicle [54].

A section of the embryo during this stage displays a tube enclosed within a tube. This inner tube is surrounded by endoderm, and, constitutes the primitive gut. The primitive gut differentiates into two parts; the embryonal part constitutes the gut tract, and, the distal sac constitutes the yolk sac that communicates with the gut of the embryo. After the construction of the endoderm, the leftover cells of the inner cell mass gets coalesce by the steady organisation of cells to form the embryonic disc [54].

Ectoderm gives rise to the epidermis and epidermal derivatives of the integumentary system, including hair follicles, nails, and glands that make contact with the skin surface (i.e., the sudoriferous, mammary, and sebaceous glands). The lining of the mouth, salivary glands, nasal passageways, and anus come from ectoderm, as does portions of the skull, pharyngeal arches, teeth, and endocrine system (pituitary and parts of adrenal glands) [55].

2.4.2 Formation of Mesoderm

During the gastrulation period, some of the cells shift inward to the mesoderm, a supplementary layer between the endoderm and the ectoderm. Developments within the mesoderm give rise to a coelom containing organs that can move, grow, and develop freely, independent of the body wall while fluid cushions and protects them from shocks [56]. Mesoderm gives rise to the lining of the pleural, pericardial, and peritoneal cavities, the muscular, skeletal, cardiovascular, and lymphatic systems, the kidneys and portions of the urinary tract, the gonads and most of the reproductive

tracts, and the connective tissues that support all organ systems. Portions of the endocrine system (parts of the adrenal glands and endocrine tissues of the reproductive system) also originate from mesoderm [55].

2.4.3 Formation of Ectoderm

After the construction of the mesoderm, the leftover cells of the embryonic disc organize themselves outside the mesoderm to constitute the ectoderm. The ectoderm is the beginning of a structure that covers the body surfaces [57]. It gives rise to most of the epithelium of the digestive system (except the mouth and anus), the exocrine glands (except salivary glands), and the liver and pancreas. Most of the respiratory system, including the epithelium (except that of the nasal passageways) and mucous glands originates from endoderm, as does portions of the urinary and reproductive systems (ducts and the stem cells that produce gametes). Portions of the endocrine system (thymus, parathyroid, and pancreas) also come from endoderm [58].

2.4.4 Neurulation and Organogenesis

Neurulation follows gastrulation. During this period, the primordium of nervous system, also known as the neural plate is laid down., The neural crest is often regarded as a fourth germ layer, because of its great significance however, it is produced from the ectoderm. Neurulation is followed by organogenesis. During this stage, diverse organs of the foetus develop and begin to function [59].

2.4.5 Amnion and Allantois

Amnion surrounds the embryo. It protects the embryo from injury because the amniotic cavity is filled with amniotic fluid. The amniotic cavity uses a thick umbilical cord to hang the embryo. The cord is attached to the midgut region of the embryo. The umbilical cord provides the main blood vessels between fetus and placenta [60]. Another cavity, the allantois develops with the amniotic cavity. It removes waste and provides nutrients for embryo [61].

2.4.6 Chorion

The outermost layer that encircles the embryo is known as chorion. It consists of an outer layer of trophoblast and an inner layer of mesoderm. It dissolves with the lining of the allantois to establish allanto–chorion. Chorion helps to exchange gases at his stage, and, develops into the placenta with important roles in nutrition, excretion and respiration.

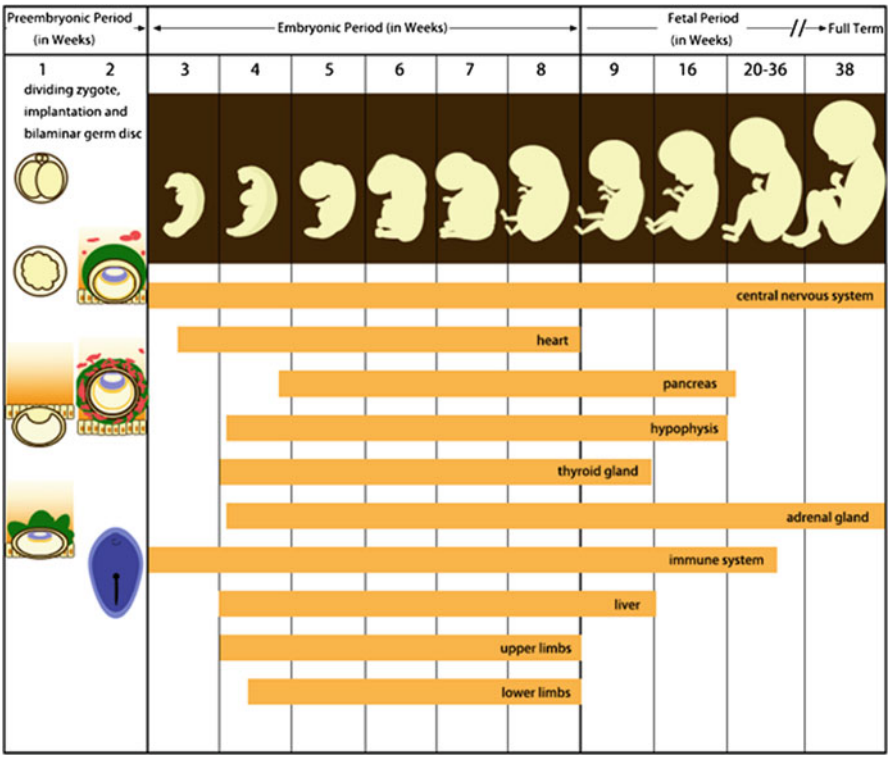

Fig. 2.1 Development of organs from embryo to fetus

2.5 Intrauterine Development of the Fetus

The fetal period refers to the time period following the embryonic period (9th week of development) until birth, about ten lunar months after fertilization (usually about 38 weeks). Development from this period consists of the growth, maturation and refinement of organ systems formed during the embryonic period. A small amount of tissue differentiation and organ development still occurs during the fetal stage. During weeks 10–20, the fetus grows primarily in length. During weeks 21–40, the fetus grows primarily in weight. The stages of development of organs from embryo to fetus are illustrated below (Fig. 2.1).

2.5.1 Fetal Circulation

Structural differentiation of the heart takes place during the embryonic period. The myocardium grows by cell division until birth, and growth beyond birth is due to cell enlargement. The density of myofibrils increases particularly in early

pregnancy and the contractility continues to improve during the second half of pregnancy. The blood volume in the fetus is 10–12 % of the body weight, compared with 7–8 % in adults. The main reason for this difference is the large volume of blood contained in the placenta; the volume reduces as gestation progresses. Compared with adults, the fetus is able to regulate and restore the blood volume faster due to high diffusion rates between fetal compartments.

Oxygen and nutrient materials for fetal development and maturation are delivered from the placenta by umbilical vein. The umbilical vein divides into the ductus venosus and the portal sinus. The former is the main branch of the umbilical vein and passes the liver to enter the inferior vena cava, carrying oxygenated blood to the heart. The portal sinus carries blood to the hepatic veins primarily on the left side of the liver. Relatively deoxygenated blood from the liver then flows to the inferior vena cava. The inferior vena cava receives less oxygenated blood returning from the lower body. Blood flowing to the fetal heart from the inferior vena cava is made up of blood that passes directly through the ductus venosus and less oxygenated blood that returns from most of the veins. The oxygen concentration in blood to the heart from the inferior vena cava is lower than that leaving the placenta.

2.5.2 Fetal Haemopoiesis

In early embryo, haemopoiesis first appears in the yolk sac followed by the liver, and finally, the bone marrow. The first erythrocytes released into the fetal circulation are nucleated and macrocytic [62]. During fetal growth, more and more circulating erythrocytes become non-nucleated and smaller. With fetal development, both the volume of blood in the fetoplacental circulation and hemoglobin level increase. Fetal erythrocytes have a short life span about 90 days at term [63]. Therefore, red blood cell production increases. The fetus produces the liver enzymes and other plasma proteins [64]. The levels of plasma protein, albumin, lactic dehydrogenase, aspartate aminotransferase, and alanine transferase are all increased with fetal growth [65]. At birth, average total plasma protein and albumin level in fetal blood are similar to maternal levels [66].

2.5.3 Gastrointestinal System

Swallowing starts from the 10th week, as does peristalsis in the small intestine along with the ability to transport glucose actively [67, 68]. Much of the swallowed fluid is absorbed, and other unabsorbed material is propelled to the lower colon. Fetal swallowing has little effect on amniotic fluid volume early in pregnancy. Fetuses at term swallow between 200 and 760 mL/day [69]. Digestive enzymes and hydrochloric acid are present in the stomach and small intestine in small amounts in the early fetus. Intrinsic factors can be detected at 11 weeks, and pepsinogen at 16 weeks [70]. The emptying ability of the stomach appears to be stimulated primarily by volume. Amniotic fluid movement through the gastrointestinal system may have an important role in the development of the alimentary canal.

2.5.4 Liver

The fetal liver converts free unconjugated bilirubin into conjugated bilirubin which varies with gestational age. The fetal liver conjugates only a small fraction that is excreted into the intestinal tube and oxidized to biliverdin. Much of the unconjugated bilirubin is excreted to the amniotic fluid after 12 weeks and is transferred through the placenta [71]. Most fetal cholesterol is synthesized in liver to meet the demand for LDL cholesterol by the fetal adrenal glands. Serum liver enzyme concentrations increase with fetal growth. Hepatic glycogen begins to appear during the second trimester and increase near term. After birth, glycogen content falls again.

2.5.5 Pancreas

Insulin in fetal plasma can be detected at 12 weeks, and insulin-containing granules can be identified by 9–10 weeks [72]. The pancreas responds to hyperglycemia by secreting insulin [73]. Glucagon has been identified in the pancreas at 8-week fetus. Most pancreatic enzymes are present from 16 weeks. Trypsin, chymotrypsin, phospholipase A, and lipase are found at 14 weeks fetus at low levels, and begin to increase with gestation [74]. Amylase has been identified in amniotic fluid at 14 weeks [75].

2.5.6 Urinary System

Organogenesis of kidney is the result of interactions between the undifferentiated metanephric blastema and the ureteric bud, a branching epithelial structure emerging from the Wolffian duct. As the tips of the ureteric bud interact with the metanephric blastema, they induce local mesenchymal cells to condense and undergo a mesenchymal-to-epithelial conversion process, which forms the nephron [76]. At the end of nephrogenesis, a permanent number of nephrons have formed.

The pronephros and the mesonephros precede the development of the metanephros. The mesonephros begins to produce urine from 5 weeks and degenerates at 11–12 weeks. Between 9 and 12 weeks, the ureteric bud and the nephrogenic blastema produce the metanephros [77]. New nephrons continue to be formed until 36 weeks. The urethra develops from the urogenital sinus, and the bladder develops from the urogenital sinus and allantois. At week 14, the ability to reabsorb occurs and the loop of Henle begins to function [78]. Renal vascular resistance is high, and the filtration fraction is low [78]. The fetal kidneys start to produce urine at 12 weeks producing 7–14 mL/day at 18 weeks increasing to 650 mL/day at term [79]. Fetal renal blood flow and thus urine production are influenced by several systems, such as the atrial natriuretic peptide, sympathetic nervous system, prostaglandins, and kallikrein.

2.5.7 Lungs

There are three essential stages of lung development [59]: The pseudoglandular stage when the lung looks like a gland microscopically between 5 and 17 weeks. The canalicular stage is from 16 to 25 weeks when each terminal bronchiole divides into several respiratory bronchioles, and these respiratory bronchioles divide into several saccular ducts. The terminal sac stage starts at 25 weeks when alveoli form terminal sacs. At the same time, the extracellular matrix develops from lung segments and type II pneumonocytes start to produce surfactant.

2.5.8 Endocrine Glands

2.5.8.1 Pituitary Gland

The pituitary adenohypophysis derives from Rathke's pouch, and the neurohypophysis develops from neuroectoderm. The adenohypophysis, or anterior pituitary, differentiates into five different cell types which secrete six important protein hormones; lactotropes produce prolactin, somatotropes produce growth hormone, corticotropes produce corticotrophin, thyrotropes produce thyroid-stimulating hormone; and gonadotropes produce luteinizing hormone and follicle-stimulating hormone. The posterior pituitary gland develops at 10–12 weeks, and oxytocin and arginine vasopressin conserve water actively at the lung and placenta [80, 81].

2.5.8.2 Thyroid Gland

The thyroid gland begins to synthesize hormones from 10 to 12 weeks, and thyroid-stimulating hormone, thyroxine, and thyroid-binding globulin can be detected in fetal serum from 11 weeks [82]. The placenta actively concentrates iodide on the fetal side. From 12 weeks to term, the fetal thyroid concentrates iodide avidly. Normal fetal levels of free thyroxine (T4), free triiodothyronine (T3), and thyroxin-binding globulin increase with fetal growth [82]. By 36 weeks, total and free T3 concentrations are lower; fetal TSH concentration is higher, and T4 is similar when compared with adult levels [83, 84]. Thyroid hormone plays an important role in fetal development, especially the fetal brain. The placenta prevents maternal thyroid hormones passing to the fetus by rapidly de-iodinating maternal T4 and T3 to form reverse T3, a relatively inactive thyroid hormone [85].

2.5.8.3 Adrenal Glands

The bulk of adrenal glands consist of the inner or fetal zone of the adrenal cortex and involutes rapidly after birth. The fetal adrenal glands play an important role, mainly through steroidogenesis, in the regulation of intrauterine homeostasis and in fetal development and maturation. The steroidogenesis is characterized by transient cortisol biosynthesis during early gestational period, followed by its limited synthesis until late gestation. The dehydroepiandrosterone and precursors of placental estrogen are produced throughout gestation.

2.6 Development of Genitalia

2.6.1 Embryology of Uterus and Oviducts

The uterus and tubes arise from the Müllerian ducts, then appear near the upper region of the urogenital ridge at 5 weeks. This urogenital ridge consists of the mesonephros, gonad, and related ducts. The first indication of Müllerian duct development is a thickening of the coelomic epithelium at the level of the 4th thoracic segment. At 6 weeks, the growing tips of two Müllerian ducts move to each other in the midline region to reach the urogenital sinus by 7 weeks. At the same time, the two Müllerian ducts fuse to form a single canal at the level of the inguinal crest. The upper pole of the Müllerian ducts give rise to the fallopian tube, and the fused parts produce the uterus. The vaginal canal extends throughout its entire length from 24 weeks [86].

2.6.2 Embryology of the Ovaries

Gonads form on the ventral surface of the embryonic kidney between the 8th thoracic and 4th lumbar segments at about 4 weeks. At 4–6 weeks, the primordial germ cells are characterized by their large size and special morphology and cytochemical features. These germ cells have migrated into the body of the embryo from the yolk sac. After the primordial germ cells move to the genital area, some enter the germinal epithelium and others mingle with the cells in the mesenchyme. From 6 weeks, all the cells begin to rapidly differentiate to form a prominent genital ridge which projects into the body cavity medially to Müllerian ducts. At 7 weeks, it is separated from the mesonephros and the sexes can be distinguished at this time.

In the female embryo, the germinal epithelium proliferates for a longer time and cells lie at first in the region of the hilum. The sex cords appear as connective tissue develops between them and form the medullary cords [87]. The bulk of the ovary is made up of cortex. Strands of cells extend from the germinal epithelium to the cortical mass and mitoses are remarkable. The rapid pace of mitoses reduces the size of the germ cells to differentiate from the neighboring cells. These germ cells are now called oogonia.

At 16 weeks, some germ cells in the medullary region start to enlarge. These primary oocytes are at early phase of development and many oocytes undergo degeneration before and after birth. A single layer of flattened follicular cells are called primordial follicles and first seen in the medulla, then in the cortex. Some follicles begin to grow even before birth, and some would persist in the cortex until menopause. At 32 weeks, the ovary has become a long, narrow and lobulated structure. The germinal epithelium has been separated by tunica albuginea. Underlying the cortex, there are two distinct zones; in the outer zone there are nests of germ cells in meiotic synapsis, interspersed with Pflüger cords and strands of connective tissue, whereas, in the inner zone, there are many germ cells in synapsis, primary oocytes, follicular cells, and a few primordial follicles.

2.7 Fetal Nutrition

Nutrition is the major intrauterine environmental factor that affects expression of the fetal genome and may have lifelong consequences. This phenomenon, termed "fetal programming," has led to the recent theory of "fetal origins of adult disease." Namely, alterations in endocrine status and fetal nutrition may affect fetal development adaptations that permanently change the fetal structure, physiology, and metabolism, thereby predisposing individuals to endocrine, metabolic, and cardiovascular diseases in later life time.

2.7.1 Glucose and Fetal Growth

The fetal depends on the mother for nutrition. At the same time, the fetus also participates to provide for its own nutrition. Glucose is the major nutrient for fetal growth and energy. At second trimester, fetal glucose levels are independent of maternal levels [88]. Some mechanisms exist to allow the maternal supply satisfy the fetal needs.

2.7.2 Leptin

During pregnancy, mother, fetus, and placenta produce leptin. Syncytiotrophoblasts and fetal vascular endothelial cells express leptin. Five per cent of leptin from the placenta enters the fetal circulation, whereas the remainder is transferred to the mother [89]. Thus, the placenta greatly contributes to maternal leptin concentrations. Fetal concentrations begin to increase at 34 weeks and correlate with fetal weight [90].

2.7.3 Triglycerides and Free Fatty Acids

The mechanism of uptake and use of low-density lipoprotein (LDL) in the placenta is for fetal assimilation of amino acids and essential fatty acids. Maternal LDLs bind to LDL receptors on the syncytiotrophoblasts of the placenta. Larger LDLs are taken up by the procedure of receptor-mediated endocytosis. The apoprotein and cholesterol esters of LDL are hydrolyzed by lysosomal enzymes in the syncytium. Arachidonic acid and linoleic acid must be absorbed from maternal intake. The fetus at term has a large proportion of fat indicating a substantial part of the substrate to the fetus is formed as fat [91]. Glycerol but neutral fat does not cross the placenta, and fatty acids are synthesized in the placenta. Lipoprotein lipase is present on the maternal side but not on the fetal side of the placenta. Fatty acids are transformed into triacylglycerols in the fetal liver.

2.7.4 Amino Acids

The placenta synthesizes a large number of amino acids [92]. Neutral amino acids from maternal plasma are taken up by trophoblasts by at least three processes. Amino acids are concentrated in syncytiotrophoblasts and transferred to the fetal side by diffusion. The concentration of amino acids in umbilical cord plasma is greater than in maternal venous or arterial plasma [93]. Ability of the transport systems is influenced by gestational age and environmental factors such as under- and over-nutrition, heat stress, growth hormone, glucocorticoids, and leptin [94]. It is demonstrated that amino acids transport is associated with fetal growth [95].

2.7.5 Proteins

Larger proteins are limited to transfer to placenta except for IgG which is present in approximately the same concentrations in maternal sera and cord, but IgM and IgA of maternal origin are effectively excluded from the fetus [96].

2.7.6 Ions and Trace Metals

Transportation for iodide is a carrier-mediated, energy-requiring, active process. The placenta concentrates iodide. The zinc levels in the fetal plasma are greater than those in maternal plasma. While the copper concentrations in fetal plasma are less than those in maternal plasma because copper-requiring enzymes are necessary for fetal development.

2.7.7 Calcium and Phosphorus

These maternal minerals are transported to the fetus actively. A calcium-binding protein is present in placenta. Parathyroid hormone-related protein (PTH-rP) acts as a surrogate PTH, including the movement of calcium ions and the activation of adenylate cyclase. PTH-rP is produced by the placenta and parathyroid glands, kidney, and other fetal tissues. Additionally, PTH is not detectable in fetal plasma, but PTH-rP is present [97]. The amount of cytotrophoblastic PTH-rP is affected by the level of extracellular Ca^{2+} [98]. The PTH-rP in placenta, decidua, and other fetal tissues is important for Ca^{2+} transfer and homeostasis in the fetus.

2.7.8 Vitamins

The fetal level of vitamin A is greater than that of the mother. Vitamin A binds to retinol-binding protein and prealbumin. The vitamin C transport from mother to fetus is an energy-dependent, carrier-mediated process. The levels of the principal

vitamin D metabolites are greater in maternal plasma than those in fetal plasma. The 1-hydroxylation of 25-hydroxyvitamin D3 takes place in decidua and placenta.

2.7.9 Amnionic Fluid

In early pregnancy, amniotic fluid comes from the ultrafiltrate of maternal plasma. At the early part of second trimester, amniotic fluid is made of extracellular fluid through the fetal skin and reflects the composition of fetal plasma [99]. After 20 gestational weeks, amniotic fluid is composed largely of fetal urine, and the cornification of fetal skin prevents this diffusion. Fetal kidneys start producing urine from 12 weeks, and by 18 weeks, they are producing 7–14 mL/day. Fetal urine contains of higher levels of urea, creatinine, and uric acid than those of fetal plasma. Amniotic fluid also contains desquamated fetal cells, lanugo, vernix, and other secretions. Pulmonary fluid contributes only a small proportion of the amniotic fluid volume. The volume of amniotic fluid at each week is quite variable. In general, the volume increases by 10 mL/week at 8 weeks and increases up to 60 mL/week at 21 weeks, then declines gradually back to a steady state by 33 weeks [100].

Amniotic fluid plays a very important role in the fetal development. Amniotic fluid helps to cushion the fetus, improves musculoskeletal development and protects it from trauma. It also keeps temperature and has a minimal nutritive function. Ingestion of fluid into the gastrointestinal tract and inhalation may promote the pulmonary growth and differentiation of these tissues.

References

1. Arushi, Khurana I. Human embryology. 1st ed. New Delhi: CBS Publisher & Distributors Pvt Ltd.; 2010.
2. Gulyas BJ. A reexamination of cleavage patterns in eutherian mammalian eggs: rotation of blastomere pairs during second cleavage in the rabbit. J Exp Zool. 1975;193:235–48.
3. Gardner RL. The early blastocyst is bilaterally symmetrical and its axis of symmetry is aligned with the animal-vegetal axis of the zygote in the mouse. Development. 1997;124:289–301.
4. Garner W, McLaren A. Cell distribution in chimaeric mouse embryos before implantation. J Embryol Exp Morphol. 1974;32:495–503.
5. Beddington RS, Robertson EJ. Axis development and early asymmetry in mammals. Cell. 1999;96:195–209.
6. Gross PR, Cousineau GH. Synthesis of spindle-associated proteins in early cleavage. J Cell Biol. 1963;19:260–5.
7. Crosby IM, Gandolfi F, Moor RM. Control of protein synthesis during early cleavage of sheep embryos. J Reprod Fertil. 1988;82:769–75.
8. Lee S, Gilula NB, Warner AE. Gap junctional communication and compaction during preimplantation stages of mouse development. Cell. 1987;51:851–60.
9. Levy JB, Johnson MH, Goodall H, et al. The timing of compaction: control of a major developmental transition in mouse early embryogenesis. J Embryol Exp Morphol. 1986;95:213–37.

10. Handyside AH. Distribution of antibody- and lectin-binding sites on dissociated blastomeres from mouse morulae: evidence for polarization at compaction. J Embryol Exp Morphol. 1980;60:99–116.
11. Pratt HP, Ziomek CA, Reeve WJ, et al. Compaction of the mouse embryo: an analysis of its components. J Embryol Exp Morphol. 1982;70:113–32.
12. Reeve WJ, Ziomek CA. Distribution of microvilli on dissociated blastomeres from mouse embryos: evidence for surface polarization at compaction. J Embryol Exp Morphol. 1981;62:339–50.
13. Sutherland AE, Speed TP, Calarco PG. Inner cell allocation in the mouse morula: the role of oriented division during fourth cleavage. Dev Biol. 1990;137:13–25.
14. Carlson BM. Foundations of embryology. 6th ed. New York: McGraw-Hill; 1996.
15. Barlow PW, Sherman MI. The biochemistry of differentiation of mouse trophoblast: studies on polyploidy. J Embryol Exp Morphol. 1972;27:447–65.
16. Johnson MH, McConnell JM. Lineage allocation and cell polarity during mouse embryogenesis. Semin Cell Dev Biol. 2004;15:583–97.
17. Marikawa Y, Alarcón VB. Establishment of trophectoderm and inner cell mass lineages in the mouse embryo. Mol Reprod Dev. 2009;76:1019–32.
18. Müntener M, Hsu YC. Development of trophoblast and placenta of the mouse. A reinvestigation with regard to the in vitro culture of mouse trophoblast and placenta. Acta Anat (Basel). 1977;98:241–52.
19. Fleming TP. A quantitative analysis of cell allocation to trophectoderm and inner cell mass in the mouse blastocyst. Dev Biol. 1987;119:520–31.
20. Pijnenborg R, Robertson WB, Brosens I, et al. Review article: trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. Placenta. 1981;2:71–91.
21. Ziomek CA, Johnson MH. The roles of phenotype and position in guiding the fate of 16-cell mouse blastomeres. Dev Biol. 1982;91:440–7.
22. Yamanaka Y, Ralston A, Stephenson RO, et al. Cell and molecular regulation of the mouse blastocyst. Dev Dyn. 2006;235:2301–14.
23. Goto T, Monk M. Regulation of X-chromosome inactivation in development in mice and humans. Microbiol Mol Biol Rev. 1998;62:362–78.
24. McGrath J, Solter D. Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell. 1984;37:179–83.
25. Garbutt CL, Johnson MH, George MA. When and how does cell division order influence cell allocation to the inner cell mass of the mouse blastocyst? Development. 1987;100:325–32.
26. Gurdon JB, Byrne JA. The first half-century of nuclear transplantation. Proc Natl Acad Sci U S A. 2003;100:8048–52.
27. Smith JM. The theory of evolution. Cambridge: Cambridge University Press; 1993.
28. Gibert SF. Developmental biology. Sunderland: Sinauer Associates, Inc.; 2000.
29. Williams GC. Adaptation and natural selection. Princeton: Princeton University Press; 1996.
30. Gurdon JB, Hopwood N. The introduction of *Xenopus laevis* into developmental biology: of empire, pregnancy testing and ribosomal genes. Int J Dev Biol. 2000;44:43–50.
31. Sander K, Faessler PE. Introducing the Spemann-Mangold organizer: experiments and insights that generated a key concept in developmental biology insights that generated a key concept in developmental biology. Int J Dev Biol. 2001;45:1–11.
32. Wolpert L, Jessell T, Lawrence P, et al. Principles of development. 3rd ed. Oxford: Oxford University Press; 2007.
33. Davidson EH. Gene activity in early development. 2nd ed. New York: Academic; 1976. p. 452.
34. Gandolfi TA, Gandolfi F. The maternal legacy to the embryo: cytoplasmic components and their effects on early development. Theriogenology. 2001;55:1255–76.
35. Memili E, First NL. Zygotic and embryonic gene expression in cow: a review of timing and mechanisms of early gene expression as compared with other species. Zygote. 2000;8:87–96.

36. Biggers JD, Borland RM. Physiological aspects of growth and development of the preimplantation mammalian embryo. Annu Rev Physiol. 1976;38:95–119.
37. Dworkin MB, Dworkin-Rastl E. Functions of maternal mRNA in early development. Mol Reprod Dev. 1990;26:261–97.
38. Wang Q, Chung YG, deVries WN, Struwe M, Latham KE. Role of protein synthesis in the development of a transcriptionally permissive state in one-cell stage mouse embryos. Biol Reprod. 2001;65:748–54.
39. Bao S, Obata Y, Carroll J, et al. Epigenetic modifications necessary for normal development are established during oocyte growth in mice. Biol Reprod. 2000;62:616–21.
40. Allegrucci C, Thurston A, Lucas E, et al. Epigenetics and the germline. Reproduction. 2005;129:137–49.
41. Tada M, Tada T, Lefebvre L, et al. Embryonic germ cells induce epigenetic reprogramming of somatic nucleus in hybrid cells. EMBO J. 1997;16:6510–20.
42. Simon I, Tenzen T, Reubinoff BE, et al. Asynchronous replication of imprinted genes is established in the gametes and maintained during development. Nature. 1999;401:929–32.
43. Hajkova P, Erhardt S, Lane N, et al. Epigenetic reprogramming in mouse primordial germ cells. Mech Dev. 2002;117:15–23.
44. Obata Y, Kono T. Maternal primary imprinting is established at a specific time for each gene throughout oocyte growth. J Biol Chem. 2002;277:5285–9.
45. Okano M, Bell DW, Haber DA, et al. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999;99:247–57.
46. DiZio SM, Tasca RJ. Sodium-dependent amino acid transport in preimplantation mouse embryos: III. Na^+-K^+-ATPase-linked mechanism in blastocysts. Dev Biol. 1977;59:198–205.
47. Johansson M, Jansson T, Powell TL. Na(+)-K(+)-ATPase is distributed to microvillous and basal membrane of the syncytiotrophoblast in human placenta. Am J Physiol Regul Integr Comp Physiol. 2000;279:R287–94.
48. Vu TK, Liu RW, Haaksma CJ, Tomasek JJ, et al. Identification and cloning of the membrane-associated serine protease, hepsin, from mouse preimplantation embryos. J Biol Chem. 1997;272:31315–20.
49. Perona RM, Wassarman PM. Mouse blastocysts hatch in vitro by using a trypsin-like proteinase associated with cells of mural trophectoderm. Dev Biol. 1986;114:42–52.
50. Das SK, Wang XN, Paria BC, et al. Heparin-binding EGF-like growth factor gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition: a possible ligand for interaction with blastocyst EGF-receptor in implantation. Development. 1994;120:1071–83.
51. Aplin JD, Seif MW, Graham RA, et al. The endometrial cell surface and implantation. Expression of the polymorphic mucin MUC-1 and adhesion molecules during the endometrial cycle. Ann N Y Acad Sci. 1994;734:103–21.
52. Sidhu SS, Kimber SJ. Hormonal control of H-type alpha(1–2)fucosyltransferase messenger ribonucleic acid in the mouse uterus. Biol Reprod. 1999;60(1):147–57.
53. Gilbert SF. The epidermis and the origin of cutaneous structures. In: Developmental biology. 6th ed. Sunderland: Sinauer Associates; 2000.
54. Gilbert SF. Comparative embryology. In: Developmental biology. 6th ed. Sunderland: Sinauer Associates; 2000.
55. Gilbert SF. Early mammalian development. In: Developmental biology. 6th ed. Sunderland: Sinauer Associates; 2000.
56. Hall BK. The neural crest as a fourth germ layer and vertebrates as quadroblastic not triploblastic. Evol Dev. 2000;2:3–5.
57. Moore KL. The developing human. 2nd ed. Philadelphia: Saunders; 1977.
58. Moore KL. The developing human: clinically oriented embryology. 4th ed. Philadelphia: Saunders; 1988.
59. Moore KL. Before we are born. Basic embryology and birth defects. Philadelphia: Saunders; 1983.

60. Usher R, Shephard M, Lind J. The blood volume of the newborn infant and placental transfusion. Acta Paediatr. 1963;52:497–512.
61. Jansson T, Powell TL. Human placental transport in altered fetal growth: does the placenta function as a nutrient sensor? A review. Placenta. 2006;27:S91.
62. Sipes SL, Weiner CP, Wenstrom KD, et al. The association between fetal karyotype and mean corpuscular volume. Am J Obstet Gynecol. 1991;165:1371–6.
63. Pearson HA. Recent advances in hematology. J Pediatr. 1966;69:466–79.
64. Weiner CP, Sipes SL, Wenstrom K. The effect of fetal age upon normal fetal laboratory values and venous pressure. Obstet Gynecol. 1992;79:713–18.
65. Fryer AA, Jones P, Strange R, et al. Plasma protein levels in normal human fetuses: 13 to 41 weeks' gestation. Br J Obstet Gynaecol. 1993;100:850–5.
66. Foley ME, Isherwood DM, McNicol GP. Viscosity, hematocrit, fibrinogen and plasma proteins in maternal and cord blood. Br J Obstet Gynaecol. 1978;85:500–4.
67. Koldovsky O, Heringova A, Jirsova V, et al. Transport of glucose against a concentration gradient in everted sacs of jejunum and ileum of human fetuses. Gastroenterology. 1965;48: 185–7.
68. Miller AJ. Deglutition. Physiol Rev. 1982;62:129–84.
69. Pritchard JA. Fetal swallowing and amniotic fluid volume. Obstet Gynecol. 1966;28:606–10.
70. Lebenthal E, Lee PC. Review article. Interactions of determinants of the ontogeny of the gastrointestinal tract: a unified concept. Pediatr Res. 1983;1:19–24.
71. Bashore RA, Smith F, Schenker S. Placental transfer and disposition of bilirubin in the pregnant monkey. Am J Obstet Gynecol. 1969;103:950–8.
72. Adam PAJ, Teramo K, Raiha N, et al. Human fetal insulin metabolism early in gestation: response to acute elevation of the fetal glucose concentration and placental transfer of human insulin-I-131. Diabetes. 1969;18:409–16.
73. Obenshain SS, Adam PAJ, King KC, et al. Human fetal insulin response to sustained maternal hyperglycemia. N Engl J Med. 1970;283:566–70.
74. Werlin SL. Exocrine pancreas. In: Polin RA, Fox WW, editors. Fetal and neonatal physiology. Philadelphia: Saunders; 1992. p. 1047.
75. Davis MM, Hodes ME, Munsick RA, et al. Pancreatic amylase expression in human pancreatic development. Hybridoma. 1986;5:137–45.
76. Saxén L, Sariola H. Early organogenesis of the kidney. Pediatr Nephrol. 1987;1:385–92.
77. Geelhoed JJ, Verburg BO, Nauta J, et al. Tracking and determinants of kidney size from fetal life until the age of 2 years: the Generation R Study. Am J Kidney. 2009;53(2):248–58.
78. Smith FG, Nakamura KT, Segar JL et al. In: Polin RA, Fox WW, editors. Fetal and neonatal physiology, vol 2, Chap. 114. Philadelphia: Saunders; 1992. p. 1187.
79. Wladimiroff JW, Campbell S. Fetal urine-production rates in normal and complicated pregnancy. Lancet. 1974;1:151–4.
80. Chard T, Hudson CN, Edwards CRW, et al. Release of oxytocin and vasopressin by the human foetus during labour. Nature. 1971;234:352–4.
81. Polin RA, Husain MK, James LS, et al. High vasopressin concentrations in human umbilical cord blood—lack of correlation with stress. J Perinat Med. 1977;5:114–19.
82. Ballabio M, Nicolini U, Jowett T, et al. Maturation of thyroid function in normal human foetuses. Clin Endocrinol. 1989;31:565–71.
83. Thorpe-Beeston JG, Nicolaides KH, Felton CV, et al. Maturation of the secretion of thyroid hormone and thyroid-stimulating hormone in the fetus. N Engl J Med. 1991;324:532–6.
84. Wenstrom KD, Weiner CP, Williamson RA, et al. Prenatal diagnosis of fetal hyperthyroidism using funipuncture. Obstet Gynecol. 1990;76:513–17.
85. Vulsma T, Gons MH, De Vijlder JJ. Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect or thyroid agenesis. N Engl J Med. 1989;321:13–6.
86. Koff AK. Development of the vagina in the human fetus. Contrib Embryol. 1933;24:59–91.
87. Konishi I, Fujii S, Okamura H, et al. Development of interstitial cells and ovigerous cords in the human fetal ovary: an ultrastructural study. J Anat. 1986;148:121–35.

88. Bozzetti P, Ferrari MM, Marconi AM, et al. The relationship of maternal and fetal glucose concentrations in the human from midgestation until term. Metabolism. 1988;37:358–63.
89. Hauguel-de Mouzon S, Lepercq J, Catalano P. The known and unknown of leptin in pregnancy. Am J Obstet Gynecol. 2006;193(6):1537–45.
90. Grisaru-Granovsy S, Samueloff A, Elstein D. The role of leptin in fetal growth: a short review from conception to delivery. Eur J Obstet Gynecol Reprod Biol. 2008;136(2):146–50.
91. Kimura RE. Lipid metabolism in the fetal-placental unit. In: Cowett RM, editor. Principles of perinatal-neonatal metabolism. New York: Springer; 1991. p. 291.
92. Lemons JA. Fetal placental nitrogen metabolism. Semin Perinatol. 1979;3:177–90.
93. Morriss FH Jr, Boyd RDH, Manhendren D. Placental transport. In: Knobil E, Neill J, editors. The physiology of reproduction, vol II. New York: Raven; 1994. p. 813.
94. Fowden AL, Ward JW, Wooding FP, et al. Programming placental nutrient transport capacity. J Physiol. 2006;572(1):5–15.
95. Jansson T, Powell TL. IFPA 2005 Award in Placentology Lecture. Human placental transport in altered fetal growth: does the placenta function as a nutrient sensor? – a review. Placenta. 2006;27:S91–7.
96. Gitlin D, Kumate J, Morales C, et al. The turnover of amniotic fluid protein in the human conceptus. Am J Obstet Gynecol. 1972;113:632–45.
97. Abbas SK, Pickard DW, Illingworth D, et al. Measurement of PTH-rP protein in extracts of fetal parathyroid glands and placental membranes. J Endocrinol. 1990;124:319–25.
98. Hellman P, Ridefelt P, Juhlin C, et al. Parathyroid-like regulation of parathyroid hormone related protein release and cytoplasmic calcium in cytotrophoblast cells of human placenta. Arch Biochem Biophys. 1992;293:174–80.
99. Gilbert WM, Brace RA. Amniotic fluid volume and normal flows to and from the amniotic cavity. Semin Perinatol. 1993;17:150–7.
100. Brace RA, Wolf EJ. Normal amniotic fluid volume changes throughout pregnancy. Am Obstet Gynecol. 1989;161:382–8.

Adverse Intrauterine Environment and Gamete/Embryo-Fetal Origins of Diseases

3

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Abstract

The 'fetal origins of adult disease (FOAD)' hypothesis proposes that developmental programming during gestation may influence adult health and disease [1]. It suggests a process where events occurring at critical, or sensitive, periods of fetal development, permanently alter structure, physiology, or metabolism. These changes predispose affected individuals to diseases in later life.

Barker and his colleagues were the first to develop the concept of FOAD based on significant associations between low birthweight and the risk of chronic diseases in adulthood, including coronary artery disease, hypertension and stroke, type 2 diabetes, and osteoporosis. Several other groups confirmed associations between birthweight and adult health in other populations. These adverse intrauterine environments include gestational diabetes mellitus (GDM), intrauterine undernutrition and pre-eclampsia, which are common and severe gestational complications. Furthermore, certain antenatal nutritional disturbances can increase the risk of diseases later in life without affecting fetal growth. In this chapter, we will discuss the evidence related to adverse intrauterine environment and embryo-fetal origins of diseases.

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3.1 Gestational Diabetes Mellitus and Adult Disease

GDM is a common medical complication of pregnancy. It is defined as any degree of glucose intolerance that was not present, or recognized, prior to pregnancy. GDM affects between 2 and 5 % of pregnant women [2]. Intrauterine hyperglycemia has detrimental effects on both mother and fetus. There is increasing evidence that women with GDM are at increased risk of cardiovascular disease, type 2 diabetes and other metabolic diseases of their offspring compared with women without GDM [3, 4]. The FOAD hypothesis proposes that although occurring in response to a transient phenomenon, these adaptations become permanent if they occur during critical periods of early development. Since the fetus is dependent on nutrients from the mother, offspring of GDM adapting to an increased nutrient supply, may be at risk of metabolic diseases in later life. For the same reason, intrauterine undernutrition and intrauterine growth restriction (IUGR) may permanently alter the endocrine and metabolic status of the fetus, thus interfering with physiological functions in later life.

3.2 Intrauterine Undernutrition/Intrauterine Growth Restriction

Undernutrition is still a considerable public health problem in developing countries. Maternal undernutrition can affect several physiological functions of the newborn. Normal fetal growth takes place in two different stages: the first stage, embryonic life, consists of the proliferation, organization, and differentiation of the embryo, whereas the second stage, fetal life, consists of the continued growing and functional maturation of different tissues and organs of the fetus. Clinically, intrauterine growth retardation (IUGR) describes newborns with a birthweight below the 10th percentile for their gestational age with pathological restriction of fetal growth due to adverse genetic or environmental influences. The offspring develop type 2 diabetes mellitus, cardiovascular diseases and metabolic syndrome in adult life, especially when followed by rapid postnatal ‘catch-up’ growth. Fetal malnutrition not only induces adaptations necessary for fetal survival and health, but also undermines future health if the postnatal environment is unfavourable.

3.2.1 Studies in Human

Many epidemiological studies show that impaired fetal growth resulting from IUGR was associated with the development of arterial hypertension and cardiovascular disease, dyslipidemia, glucose intolerance or even type 2 diabetes and visceral adiposity during adult life [5–11]. All are features of the metabolic syndrome and contribute to morbidity and mortality in later life. These studies were undertaken in the UK, and subsequently confirmed in other countries such as Holland, South

Africa, India and other developing countries [5, 12–15]. Short stature, premature adrenarche and polycystic ovarian syndrome (PCOS) are also endocrinological associations of IUGR [16].

3.2.2 Epidemiological Studies

Intrauterine growth restriction is associated with increased risks of ischaemic heart disease and hypertension in later life. Vascular endothelial dysfunction has been implicated in hypertension, insulin resistance, type 2 diabetes and atherosclerosis. An increase in stiffness of large arteries causes a rise in systolic blood pressure and isolated systolic hypertension [17, 18]. Altered structure and numbers of small arteries and capillaries may also play a significant role in the development of increased peripheral resistance.

Leptin, a major adipokine secreted by adipose tissue, regulates food intake and energy expenditure. Tzschoppe et al. [19] compared placental leptin synthesis and leptin-binding capability in venous cord blood between IUGR newborns (<10th percentile), and, appropriate-for-gestational age neonates (AGA, 10–90th percentile). They found that placental leptin synthesis was significantly higher in IUGR infants compared to AGA infants, and, leptin-binding capability in venous cord blood was increased in IUGR newborns. Reduced biologically-active leptin levels may contribute to perturbed regulation of appetite. IUGR may also affect the development of adipocytes. Development of obesity is associated with increased adipocyte differentiation, adipocyte hypertrophy, and/or upregulation of lipogenic genes. As an adipogenic transcription factor, PPARG2 promotes adipocyte differentiation and lipid storage [20]. Therefore, IUGR individuals may demonstrate dysregulation of appetite, and abnormal activation of adipocytes, contributing to development of obesity.

Ten per cent of children born small for gestational age (SGA) remain short, which constitutes a significant proportion of adults with short stature. Growth hormone (GH) trials in the USA and in Europe led to the approval of GH for treatment of short stature in SGA infants. GH treatment reduces body fat while promoting lean body mass, with a 40 % drop in circulating high molecular weight adiponectin and a 30 % lowering of follistatin. The change in adiponectin may explain the decrease in insulin sensitivity when GH is given. Follistatin inhibits myostatin, so a decline in follistatin might be expected to reduce muscle mass. On the other hand, it promotes adipogenesis and thus the decline contributes to the reduction in fat mass that occurs in response to GH [16].

Reproductive endocrinology is also disturbed in IUGR infants. Young women are at increased risk of polycystic ovarian disease and early menarche. Leptin has effects upon GnRH secretion and is a risk factor for early development of puberty and potentially menarche. Young women who are overweight are more likely to suffer early puberty. The insulin-resistance and dyslipidaemia that follows SGA birth in young women may yield a hyperandrogenic state, resulting in premature pubarche followed by PCOS in adolescence. Controlled trials of metformin to

determine its effect on the clinical course of premature pubarche also demonstrate that early metformin can prevent or delay manifestations of hyperandrogenism, including PCOS [16].

3.2.3 Experimental Studies

Insulin resistance is one of the most common adult outcomes associated with IUGR infants. Preterm SGA infants have lower insulin sensitivity than preterm, AGA infants in the first few months of life [21]. Epidemiological studies demonstrate that IUGR infants were more likely to suffer from glucose intolerance as adults. A key fetal adaptation to poor fetal nutrition is upregulation of insulin receptors without upregulation of insulin signalling in fetal skeletal muscle [21]. However, postnatally in IUGR infants there is upregulation of both insulin receptors and insulin signalling pathways. At birth, SGA infants have low concentrations of circulating insulin and insulin-like growth factor-1 (IGF1). In 48 h after birth, they are more insulin-sensitive, and, have high plasma non-esterified fatty acid (FFA). They then undergo a period of accelerated postnatal growth associated with increased insulin sensitivity. This early period of increased insulin sensitivity and accelerated growth precedes subsequent development of insulin-resistance later in life [21].

Endothelial dysfunction in adults is another common outcome in IUGR infants. Leeson et al. [22] found that low birthweight was associated with reduced, endothelium-dependent dilatation in children aged 9–11 years. Cardiovascular risk factors showed no relationship to flow-mediated dilation, but an inverse relationship with HDL cholesterol levels. The inverse relationship with blood HDL cholesterol concentrations might imply a role for the lipid environment in the origin of the defect; endothelial dysfunction being a consequence of a primary defect in lipid metabolism. Flow-related, vasodilatation was impaired in low birthweight children relative to normal birthweight at age x years [23]. Endothelium-dependent vasodilation of normal birthweight children was 1.5 times that of low birthweight children at the same age [24]. In low birthweight children, the maximal hyperaemic response was reduced, whereas the acetylcholine response was unaffected [24]. Although Goodfellow [23] found no evidence of a correlation between birthweight and serum concentrations of von Willebrand factor, a marker of endothelial cell activation, in fit young adults, a smaller study found higher concentrations in low birthweight subjects [25].

Martin [24] reported that low birthweight children showed increased carotid artery stiffness compared with normal birthweight controls at age 9 years. Another study of 281 young adults demonstrated that low birthweight was related to increased carotid, femoral and brachial artery stiffness [26]. Changes in the architecture or the number of peripheral arterioles and capillaries have been implicated in the aetiology of increased peripheral vascular resistance in hypertension, which could partially contribute to the observed relationship between the resistance index (RI) and birthweight [27, 28].

3.2.4 Animal Studies

Several animal models demonstrate that maternal undernutrition during the neonatal period can affect offspring. Among these various models, many similarities of adult offspring phenotypes are observed including raised blood pressure, insulin resistance and increased adiposity, which are hallmarks of the metabolic syndrome [11, 29].

Intrauterine growth retardation in animals can be induced by both prolonged modest changes in maternal diet, and, by more severe changes in uterine blood supply. The effect of maternal protein restriction in rodents on the phenotypes of offspring has been assessed, including insulin resistance, dyslipidaemia and hypertension. In both rats and sheep, low newborn weight has been associated with an increased risk for type 2 diabetes with abnormal insulin secretion and glucose intolerance [30, 31]. Nevertheless, the effect of IUGR on whole body insulin sensitivity and metabolic activity in adult rats which were fed either a normal protein diet or a low protein diet during pregnancy and 2 weeks of lactation, suggests that IUGR results in improved insulin sensitivity without 'catch-up' growth. Animal models also suggest that leptin deficiency, or leptin resistance, may result in the pathogenesis of the metabolic syndrome in IUGR offspring [32, 33]. IUGR rat offspring showed significantly increased expression of PPARG both as newborns and as adults. Further, the expression of adipogenic transcription factors regulating PPARG was also upregulated in both groups. Maternal protein restriction also leads to endothelial dysfunction in isolated small arteries from adult rat offspring [34, 35]. Small resistance arteries of adult offspring exposed prenatally to a 50 % reduction in maternal protein intake appeared to demonstrate endothelial dysfunction, although dilatation in the aorta was normal. Reduced endothelium-dependent vasodilatation of cerebral microvessels in offspring of dams fed a low-protein diet [36].

Hypoxia-induced IUGR has long-term effects on cardiac susceptibility to ischaemic-reperfusion injury that are independent of sex and age [37]. This group also identified a mismatch in glucose metabolism, resulting in proton accumulation in the post-ischaemic myocardium of IUGR offspring as a potential mechanism [37]. There have been few studies of arterial distensibility in models of maternal nutrient restriction. However, loss of diurnal variation in heart rate and blood pressure in adulthood has resulted from maternal undernutrition followed by postnatal overnutrition [38].

Are adverse outcomes in IUGR infants gender-specific? Food-restricted (FR) male rats develop increased hepatic triglyceride and cholesterol content with elevated sterol regulatory element-binding protein-1c, fatty acid synthase, and lipoprotein lipase expression [39]. However, FR females have decreased hepatic cholesterol levels., and, plasma lipid levels in FR males and females did not differ significantly. These data suggest that intrauterine events may result in sex-dependent, altered lipid metabolism with an increased risk in male rats.

3.2.5 Underlying Mechanisms

Lifelong programming of the ACTH/hypothalamic–pituitary–adrenal (HPA) axis has been proposed as a mechanism to explain the association between low-birthweight infants and later development of metabolic syndrome and hypertension in adult life. Increased cortisol levels due to alterations in the regulation of the ACTH/glucocorticoid axis may be one mediating mechanism. Indeed, infants born after significant exposure to stressful conditions are often SGA and have blunted HPA axis responses to stressors compared to AGA infants. These findings are consistent with animal models showing that adverse intrauterine conditions can result in blunted cortisol responses to acute stressors and may provide a mechanism for adult susceptibility to disease for SGA infants [40].

The predictive-adaptive response (thrifty phenotype) hypothesis proposes that the fetus makes adaptations in the early developmental period based on the predicted postnatal environment. If prenatal and postnatal environments match, the physiological settings achieved through the processes of developmental plasticity will leave the organism well prepared for the postnatal environment and the organism will cope adequately with postnatal cues. A mismatch between prenatal and postnatal environments renders the organism more susceptible to later disease [41]. Any evidence to support this hypothesis?

If adverse events take place during intrauterine life, especially during a specific crucial window of developmental plasticity, epigenetic modifications may alter the expression of genes. This may drive metabolic pathways towards survival in the short term, but has detrimental long-term impact during adult life [41, 42]. Non-genomic changes may take place during the crucial window of developmental plasticity [43]. Shifts in the transcriptional activity of DNA may produce sustained metabolic adaptations. Within tissues and organs that control metabolic homeostasis, a range of phenotypes can be induced by sustained changes in maternal diet via modulation of genes that control DNA methylation or histone acetylation, or through small non-coding RNAs activity [44, 45]. The phenotypic effects of epigenetic modifications during development may not manifest until later life, especially if they affect genes modulating responses to later environmental challenges, such as high-fat diet [41].

Lambertini et al. [46] showed widespread epigenetic changes in IUGR infants, and, suggested the possibility that a specific signature in the epigenome may characterise IUGR infants. However, the relationship between the programming of specific genes and alterations in subsequent growth and metabolism is obscure. Einstein's group [47] identified epigenetic alterations that may provide a mechanism linking IUGR with T2DM later in life. They identified 56 candidate loci near genes controlling growth such as those involved in the cell cycle. Reductions in DNA methylation in the HNF4A promoter region may be responsible for maturity onset diabetes while, epigenetic changes in the H19/IGF2 locus may be a site related to human IUGR [47].

In recent years, it became clear that the onset of puberty is also regulated by genes that underlie epigenetic modification. The onset of puberty is accompanied by

alteration of DNA methylation and histone modification of transcriptional repressors, contributing to activation of genes that are known to be critically involved in the onset of puberty. Genome-wide analysis of hypothalamic DNA methylation reveals profound changes in methylation patterns associated with the onset of female puberty [48].

DNA methylation and histone acetylation are potential explanations for the mechanisms involved in fetal origins of adult diseases. Micro-RNA's also represent a possible mechanism. miR-16 and miR-21 expression are markedly reduced in the placenta of growth-restricted infants [49]. The potential role of miR-21 is intriguing because it targets genes that affect apoptosis and the cell cycle. Traditional genetic inheritance may also play a role. Genetic variations affecting the insulin axis might influence both birthweight and subsequent development of T2DM, and, explain transgenerational effects. Finally, epigenetic modifications are frequently tissue-specific, so findings in the placenta may not apply to muscle, adipose tissue or pancreatic cells [16].

3.3 Preeclampsia

Preeclampsia is a syndrome defined by the hypertension and proteinuria, which typically occurs after 20 weeks of gestation and resolves after delivery. Depending on ethnicity, the incidence of preeclampsia ranges from 3 to 7 % in nulliparae and 1 % to 3 % in multiparae. Overall, 10–15 % of maternal deaths are directly associated with preeclampsia and eclampsia. Although this multisystem disorder constitutes a major cause of maternal mortality as well as perinatal morbidity and mortality worldwide, the mechanisms underlying the development of preeclampsia are not yet understood [50].

Pre-eclampsia is not just an isolated disease of pregnancy. It also increases the vulnerability of offspring to adult diseases, including cardiovascular diseases, obesity and cancer. Offspring of pregnancy complicated by preeclampsia tend to be thinner, have higher blood pressures [51–53], and, they are more likely to suffer stroke and epilepsy [54, 55]. However, the daughters of women with preeclampsia during pregnancy have higher risks of breast cancer [56]. Explaining these disparate associations has been speculative rather than derived from a consistent, unifying hypothesis.

3.3.1 Studies in Human

Pre-eclampsia is associated with an increased risk of hospitalization for a number of diseases among non-SGA children born at term including infectious and parasitic diseases, diseases of the blood and blood-forming organs, endocrine, nutritional, and metabolic diseases, diseases of respiratory system, and, congenital malformations. SGA is associated with an increased risk of several diseases in adult life [57].

Davis et al., using the available BP data, found that children exposed to preeclampsia in utero have approximately 2–3 mmHg higher SBP (systolic BP) during childhood and young adult life [58]. Long-term follow-up studies demonstrate a doubling of risk from stroke [54]. Debbie et al. also confirmed that conclusion, and supported the view that preeclampsia and gestational hypertension were risk factors specific for higher blood pressures in offspring [59]. The key biological pathways relevant to the cardiovascular health of offspring are therefore likely to have additional effects, on other features of stroke risk, beyond clinic blood pressure measures alone.

Male and female offspring have similar outcomes except that males exposed to mild or severe preeclampsia have an increased prevalence of congenital malformations of the genital organs [57]. Males, born at term exposed to severe preeclampsia, had an increased risk of diseases of the blood and blood-forming organs, and, disorders of the immune system. While females exposed to severe preeclampsia or eclampsia had an increased risk of cerebral palsy, and, diseases of the musculoskeletal system and connective tissue [57].

3.3.2 Epidemiological Studies

Pre-eclampsia and IUGR result from inadequate formation of spiral arteries that may compromise the flow of nutrients and oxygen to the fetus, and, similarly reduce transportation of waste from the fetus. Offspring of preeclampsia pregnancies do not have higher risks of obesity. Moreover, after adjusting for parental BMI, there is an inverse association between preeclampsia and the BMI of offspring, as mothers experiencing preeclampsia are more likely to have higher BMI [51, 60]. However, in preeclampsia pregnancy, there is no difference in height between offspring of preeclampsia and normotensive pregnancies [57, 60, 61].

Offspring of preeclampsia pregnancies have lower risks of developing breast or prostate cancer, possibly due to abnormal intra-uterine exposure to sex hormone. Trichopoulos [62] hypothesized that the developing breast is influenced by the fetal environment, particularly variations in hormone concentrations, which could mediate subsequent breast cancer development. Trichopoulos [62] also proposed that increased concentrations of oestrogens in pregnancy increase the probability of future occurrence of breast cancer in daughters. Maternal, urinary estriol excretion declines late in preeclamptic pregnancies [63]. However, circulating maternal estrogens near delivery do not seem to be lower in preeclampsia [64, 65]. The limited data are not consistent with lower umbilical cord blood estriol, estradiol, and estrone concentrations in preeclampsia. Vatten et al. [65] found higher concentration of AFP in cord blood in pregnancies complicated by severe preeclampsia, and, they indicated that elevated AFP levels are associated with reduced breast cancer risk among female offspring. The authors attributed their findings to an anti-oestrogenic effects of AFP [65]. It has also been suggested that exposure to elevated androgen concentrations mediate the associations of preeclampsia with lower breast cancer risk [66]. Low expression of the aromatase gene, or a small or impaired placenta, as

found in preeclampsia, increases the release of androgens from the placenta late in pregnancy when the fetal adrenal gland, the source of dehydroepiandrosterone (DHEA)-sulfate, undergoes rapid growth. Elevation of androgen, accompanied by low sex-hormone binding globulin in fetal blood, might confer long-term protection against breast carcinogenesis by antagonizing the effect of estrogens on ductal development in the fetal breast. Other factors, such as some angiogenic factors, growth factors and endocrine factors may contribute to these associations [56, 67, 68].

3.3.3 Experimental Studies

Women with a history of preeclampsia are at increased risk of type 2 diabetes in later life. However, some studies demonstrate that birthweight is more important than preeclampsia in the development of type 2 diabetes. There is also a strong relationship between babies who have been small for gestational age and subsequent development of type 2 diabetes [69]. Tenhola et al. [70] measured mean concentrations of serum total LDL, HDL, cholesterol, triglycerides, fasting insulin, blood glucose, serum cortisol and dehydroepiandrosterone sulfate in offspring of pre-eclampsia pregnancies and offspring of non-preeclamptic pregnancies. There was no difference between these two groups, however, SGA children of preeclampsia pregnancies had the highest concentrations of serum total LDL and cholesterol. The concentrations of LDL and cholesterol are higher than AGA children of preeclamptic pregnancies, or, in SGA or AGA children of non-preeclamptic pregnancies - even though the differences were not significant [70]. Pre-eclampsia is positively associated with offspring BP after adjustment for family adiposity, suggesting IUGR may mediate this association. Another perinatal cohort study examined whether IUGR and childhood growth trajectory may mediate the association between pre-eclampsia and childhood SBP at 7 years of age. Results demonstrate that preeclampsia-eclampsia was significantly associated with higher SBP, independent of IUGR [52]. This finding suggests that other mechanisms may be involved, such as genetic transmission and placental-fetal vascular impairment [51, 52]. Jayet et al. [53] showed that offspring of mothers with preeclampsia displayed marked vascular dysfunction in the pulmonary and systemic circulations, as evidenced by 30 % higher pulmonary artery pressure, and, 30 % lower flow-mediated dilation (FMD) of the brachial artery. Moreover, oxidative stress was increased in offspring of women with preeclampsia, and, suggests that it might represent an underlying mechanism. Finally, pulmonary artery pressure during nitric oxide inhalation remained significantly higher in offspring of mothers with preeclampsia than in control subjects, suggesting that a structural defect, possibly related to remodelling of the pulmonary vascular wall, also contributes to pulmonary hypertension. Thus, preeclampsia leaves persistent defects in the systemic and pulmonary circulation of the offspring. This predisposes offspring to exaggerated hypoxic pulmonary hypertension during childhood and may contribute to premature cardiovascular disease later in life.

Eero Kajantie et al. [54] found that children from pregnancies complicated by severe pre-eclampsia were thin at the age of 2 years. This is consistent with the

reported association between thinness at 2 years of age, and, later hemorrhagic and thrombotic stroke. This association was not the result of the children's living conditions after birth as assessed by the father's occupation. This led to the suggestion that the association was a consequence of fetal undernutrition leading to thinness at birth that persisted through infancy. Statistical analysis showed the association between people exposed to pre-eclampsia with increased risk of hemorrhagic or thrombotic stroke was independent of the babies' birthweight or gestational age at birth. Babies from pregnancies complicated by pre-eclampsia had reduced brain circumferences, probably as a consequence of impaired brain growth in uterus[54]. The investigators speculate that stroke may originate through reduced brain growth in utero as a consequence of fetal undernutrition. Given the redistribution of cardiac output in favor of the brain, one of the fetal brain-sparing responses, may permanently change the structure of the cerebral arteries. They speculated that stroke might originate in two ways in pre-eclampsia, either through reduced brain growth or impaired brain growth leading to "brain sparing" responses.

Wu et al. [55] reported that prenatal exposure to pre-eclampsia was associated with an increased risk of epilepsy in children born after 37 weeks of gestation. The mechanisms underlying the associations between them were unclear, but probably because pre-eclampsia may cause fetal brain ischemia and vascular fetal brain lesions. Pre-eclampsia has been shown to be an important risk factor for neonatal encephalopathy [71]. The association may be mediated by placental dysfunction. The fact that preeclampsia was associated with an increased risk only in children who were born after 37 weeks of gestation reflects that other causes of preterm birth outweigh the effect of preeclampsia or indicates that the pathology related to preeclampsia needs gestational time to increase the susceptibility to epilepsy, or, the fetal brain may be more susceptible later in pregnancy [54].

3.3.4 Animal Studies

In pre-eclampsia, the spiral arteries inadequately remodel so that uterine flow is reduced by 50 % and there is chronic placental ischaemia or, at best, intermittent flow that induces an ischaemia/reperfusion phenomenon [72]. Reactive oxygen species (ROS) and cytokines released from the ischaemic placenta trigger systemic oxidative and inflammatory reactions. The placenta also overexpresses anti-angiogenic factors that inhibit the normal function of pregnancy-related proangiogenic factors, including VEGF (vascular endothelial growth factor) and PlGF (placental growth factor). The combination of these factors may stimulate systemic endothelial dysfunction that is consistently found during symptomatic pre-eclampsia in the mother.

Offspring of pre-eclamptic pregnancies develop from the first trimester onwards within an environment of placental insufficiency and restricted oxygen supply. By the start of the second trimester the offspring are confronted with elevated circulating anti-angiogenic factors, which antedate the later emergence of the clinical pre-eclamptic syndrome and systematic maternal inflammatory, oxidative

and dysfunctional endothelial states [5]. To mimic the human syndrome an animal model needs to develop the cardinal features of pre-eclampsia, which include pregnancy-specific hypertension, proteinuria and associated alterations in vascular function and biomarkers. Although a variety of models exist that are based on different aspects of the pathophysiology of pre-eclampsia, no model has been able to successfully mimic all the pathophysiological features of pre-eclampsia, or, to accurately replicate the first trimester origin of the human condition.

Mechanical reduction in maternal uterine artery blood flow by unilateral or bilateral uterine artery ligation in animal models induces a pregnancy-specific increase in maternal blood pressure [73]. If uterine perfusion I reduced at 14 days of gestation, changes in resting BP are consistently pronounced in the offspring throughout life. When uterine perfusion takes place later in gestation, despite resulting in growth restriction, either no increase in BP or modest changes in later life was observed [73]. However, early ligation of uterine vessels has been associated with the full range of features of preeclapsia in dams, including disordered antiangiogenic factors and vascular dysfunction.

The pathogenesis of pre-eclampsia may be described in two stages. The initial stage indicates abnormal placental implantation, followed by transition to the second stage of endothelial dysfunction. Placental insufficiency is likely to lead to offspring of pre-eclamptic pregnancies developing in an environment of significant hypoxia from early in the pregnancy [74]. Early placental development takes place under low oxygen environment as this predates the formation of an effective maternal-fetal circulation, while abnormal placentation in pre-eclampsia is likely to result in significantly lower oxygen delivery to the fetus for the majority of gestation [75]. In mice, exposure to anoxic circumstances induces hypertension, proteinuria, IUGR, renal pathology and elevations in maternal soluble endoglin (sEng). When the data from models of pregnancy hypoxia are collected, there is a striking lack of consistent reports of elevated BP in the offspring, suggesting that hypoxia alone is insufficient to induce the long-term effects of pre-eclampsia, or, that the levels of hypoxia currently used are not representative of the levels offspring are exposed to during development [76].

Many studies show an increase in levels of circulating anti-angiogenic factors including sFlt-1 and sEng [77, 78]. sFlt-1 inhibits the normal function of pregnancy-related angiogenic factors, including VEGF and PlGF [77]. Overexpression of sFlt-1 in rats induces hypertension, proteinuria and glomerular endotheliosis, even in non-pregnant rats. Lu et al. [79] found gender-specific impacts on weight and BP in offspring. Male offspring born to sFlt-1–treated pregnant mice have significantly lower birthweights than male offspring of the control group, while there was no significant difference in the post-weaning weight in female offspring. Furthermore, mean, systolic, and diastolic BP were significantly higher in male offspring born to sFlt-1–treated mothers, while no differences existed in female [80]. In maternal under-nutrition models, the increase of BP is more pronounced in male offspring than female.

The specific role of systemic maternal endothelial dysfunction is relatively easier to study in animal models. These models are based either on systemic

inhibition of eNOS (endothelial NO synthase) by administration of L-NAME (NG-nitro-larginine methyl ester) or by eNOS knockout [81]. The mice consistently show abnormal endothelial function and, through selective breeding of an eNOS-knockout mother with a wild-type male, an experimental scenario can be produced to study the effect of maternal endothelial dysfunction on the in utero development of an eNOS-heterozygous offspring. These offspring have higher BP during adulthood than the genetically similar offspring bred from a wild-type mother and an eNOS-knockout father, who differ only in that their in uterine development was in a wild-type mother with normal endothelial responses [81]. The study indicates that the maternal eNOS genotype influences both blood pressure and behavior of offspring, possibly because developmental programming associated with intrauterine growth retardation. In addition, prenatal exposure to glucocorticoids can induce adult cardiovascular and metabolic physiological dysfunction in gender-specific patterns [82, 83]. Male embryos may be more susceptible to maternal environment and cannot adapt successfully. This gender sensitivity may be attributable to differences in the hormonal status between the two different genders where females may have a protective effect in relation to hypertension and epigenetic factors.

3.3.5 Underlying Mechanisms

The mechanisms underlying pre-eclampsia or eclampsia include chronic uterine ischemia, dysfunction of the nitric oxide system, insulin resistance, hypersensitivities of the autonomic nervous and rennin-angiotensin systems, activation of a systemic inflammatory response, and activation of circulating proteins that interfere with angiogenesis. In pre-eclampsia, exposure to the abnormal intra-uterine circumstances leads to vascular structural remodeling, that persists into post-natal life. In animal models, there is also evidence of increased aortic stiffness, as well as greater elastic fibre content in the vessel wall [84]. By adult life, 16-month-old offspring of hypoxic dams exhibit distinct vascular structural changes with oedematous and necrotic aortic endothelium and disarranged proliferative smooth muscle cells [85]. Other studies of rat model offspring have also identified an increased propensity to develop arterial internal elastic lamina lesions, an early atherosclerotic process, at 8 and 16 weeks of age [86]. These observations underline development of an early atherogenic phenotype, as a potential link between pre-eclampsia exposure and later cardiovascular disease. Consistent with the observations in above animal studies, offspring of pre-eclamptic pregnancies also show an increased intima-media thickness with aortic arterial thickening already evident at birth [87]. In an sFlt-1 overexpressing model, sFlt-1 as a splice variant of the VEGF receptor Flt-1 seems to be involved centrally in the pathogenesis of preeclampsia. The high levels of circulating sFlt-1 in early pregnancy predict later onset of preeclampsia. These increased levels of sFlt-1 are accompanied by reduced levels of free VEGF and PlGF in the maternal circulation, suggesting that sFlt-1 inhibits VEGF and PlGF. This prevents them from binding their endothelial cell receptor, resulting in

abnormal angiogenesis and altered circulation at the utero-placental interface, and consequently poor perfusion of the placenta-fetal unit [79].

Exposed to hypoxia during late gestation, rat offspring manifest increased cardiac size, reduced left ventricular wall thickness, reduced cardiomyocte proliferation as well as epicardial detachment [88]. Since the epicardium is a major source of growth factors during cardiac development, this detachment of the epicardium in fetal life may be one potential mechanism of the myocardiac changes. Higher levels of apoptotic proteins and induction of HIF -1α are also involved in this process [89, 90]. In addition, maternal hypoxia can change cardiac collagen content of offspring, via a potential link between in utero hypoxia and later cardiovascular disease via alterations in cardiac ischaemia/reperfusion mechanisms. Rats exposed to hypoxia in late gestation had alterations in cardiac proton production and increased myocardial production of acetyl-CoA during reperfusion [37, 90]. In fetal life, such animals also have significant reductions in cardiac PKCε (protein kinase Cε), which may be secondary to increased methylation at the PKCε promoter site [91]. PKCε plays a critical role in cardioprotection during ischaemia [92].

Endothelial dysfunction is an early biological factor in the development of atherosclerotic vascular disease and predisposes to the development of left ventricular hypertrophy. Model offspring have enhanced vascular contraction in response to phenylephrine and a reduction in endothelium-dependent relaxation during early life. Experimental studies imply several potential mechanisms underlying vascular abnormality. In model offspring, both basal and acetylcholine-induced NO production is reduced in arterial segments. A characteristic feature of pre-eclampsia is a derangement of circulating anti-angiogenic factors. Persistent abnormalities in anti-angiogenic factors in the offspring may lead to persistent endothelial dysfunction in later life. Nevertheless, study of anti-angiogenic factors in children of 5–8 years of age has demonstrated no long term difference [93]. On one hand, it is possible that intrauterine exposure to anti-angiogenic factors causes altered in utero endothelial development and physiology. Consistently, sFlt-1 administered in vitro inhibits endothelial cell proliferation and tubule formation [94]. Additionally, HUVECs from pre-eclamptic donors appear to differ from normal in their response to oxygen levels. Under normal oxygen conditions, they develop a higher numbers of connections and shorter tubule lengths, creating networks similar to those seen when control cells were grown under hypoxic conditions [95]. These data indicate that endothelial cells from pre-eclamptic pregnancies may be fixed in a 'hypoxic' phenotype. On the other hand, it is possible that endothelial dysfunction represents a biomarker of other underlying metabolic abnormalities related to cardiovascular disease. Alternatively, the vascular changes may be inherited between mother and child. Polymorphisms in certain genes, such as those encoding eNOS, angiotensin converting enzyme and angiotensin, have been proposed as potential links underlying the development of the condition [96, 97]. During fetal life, adverse exposures may lead to heritable characteristics through programming of the epigenome, as the greatest level of active programming of the epigenome occurs during fetal life [98]. Hypoxia is a promising stimulus for epigenetic programming since it has been shown to induce a global decrease in transcriptional activity in the vascular endothelium [99].

3.4 Conclusions

Adverse intrauterine environments, including GDM, intrauterine undernutrition/IUGR and pre-eclampsia, adversely influence the responses of offspring to later challenges. Obesogenic diets or physical inactivity increase the risk of future disease predisposing to insulin resistance, type 2 diabetes, obesity and cardiovascular disease. Prevention of these disorders must begin in the uterus and continue throughout the life course. Special emphasis should therefore be given to optimal intrauterine milieu and to the avoidance of an obesogenic postnatal environment to reduce poor adult health outcomes.

References

1. Barker DJ. The fetal and infant origins of disease. Eur J Clin Invest. 1995;25:457–63.
2. King H. Epidemiology of glucose intolerance and gestational diabetes in women of childbearing age. Diabetes Care. 1998;21 Suppl 2:B9–13.
3. Shah BR, Retnakaran R, Booth GL. Increased risk of cardiovascular disease in young women following gestational diabetes mellitus. Diabetes Care. 2008;31:1668–9.
4. Carr DB, Utzschneider KM, Hull RL, et al. Gestational diabetes mellitus increases the risk of cardiovascular disease in women with a family history of type 2 diabetes. Diabetes Care. 2006;29:2078–83.
5. Barker DJ, Winter PD, Osmond C, et al. Weight in infancy and death from ischaemic heart disease. Lancet. 1989;2:577–80.
6. Barker DJ, Gluckman PD, Godfrey KM, et al. Fetal nutrition and cardiovascular disease in adult life. Lancet. 1993;341:938–41.
7. Stein CE, Fall CH, Kumaran K, et al. Fetal growth and coronary heart disease in south India. Lancet. 1996;348:1269–73.
8. Barker DJ, Hales CN, Fall CH, et al. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia. 1993;36:62–7.
9. Vickers MH, Breier BH, Cutfield WS, et al. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. Am J Physiol Endocrinol Metab. 2000;279:E83–7.
10. Phillips DI. Insulin resistance as a programmed response to fetal undernutrition. Diabetologia. 1996;39:1119–22.
11. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev. 2005;85:571–633.
12. Leon DA, Lithell HO, Vagero D, et al. Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915–29. BMJ. 1998;317:241–5.
13. de Rooij SR, Painter RC, Holleman F, et al. The metabolic syndrome in adults prenatally exposed to the Dutch famine. Am J Clin Nutr. 2007;86:1219–24.
14. Levitt NS, Lambert EV, Woods D, et al. Impaired glucose tolerance and elevated blood pressure in low birth weight, nonobese, young South African adults: early programming of cortisol axis. J Clin Endocrinol Metab. 2000;85:4611–18.
15. Yajnik CS. Early life origins of insulin resistance and type 2 diabetes in India and other Asian countries. J Nutr. 2004;134:205–10.
16. Chernausek SD. Update: consequences of abnormal fetal growth. J Clin Endocrinol Metab. 2012;97:689–95.
17. Black HR. The paradigm has shifted to systolic blood pressure. J Hum Hypertens. 2004;18 Suppl 2:S3–7.

18. Kingwell BA, Gatzka CD. Arterial stiffness and prediction of cardiovascular risk. J Hypertens. 2002;20:2337–40.
19. Tzschoppe A, Struwe E, Rascher W, et al. Intrauterine growth restriction (IUGR) is associated with increased leptin synthesis and binding capability in neonates. Clin Endocrinol (Oxf). 2011;74:459–66.
20. Spiegelman BM, Choy L, Hotamisligil GS, et al. Regulation of adipocyte gene expression in differentiation and syndromes of obesity/diabetes. J Biol Chem. 1993;268:6823–6.
21. Tsubahara M, Shoji H, Mori M, et al. Glucose metabolism soon after birth in very premature infants with small- and appropriate-for-gestational-age birth weights. Early Hum Dev. 2012;88:735–8.
22. Leeson CP, Whincup PH, Cook DG, et al. Flow-mediated dilation in 9- to 11-year-old children: the influence of intrauterine and childhood factors. Circulation. 1997;96:2233–8.
23. Goodfellow J, Bellamy MF, Gorman ST, et al. Endothelial function is impaired in fit young adults of low birth weight. Cardiovasc Res. 1998;40:600–6.
24. Martin H, Hu J, Gennser G, et al. Impaired endothelial function and increased carotid stiffness in 9-year-old children with low birthweight. Circulation. 2000;102:2739–44.
25. McAllister AS, Atkinson AB, Johnston GD, et al. Relationship of endothelial function to birth weight in humans. Diabetes Care. 1999;22:2061–6.
26. Wilkinson IB, Cockcroft JR. Commentary: birthweight arterial stiffness and blood pressure: in search of a unifying hypothesis. Int J Epidemiol. 2004;33:161–2.
27. Antonios TF, Singer DR, Markandu ND, et al. Rarefaction of skin capillaries in borderline essential hypertension suggests an early structural abnormality. Hypertension. 1999;34:655–8.
28. Broyd C, Harrison E, Raja M, et al. Association of pulse waveform characteristics with birth weight in young adults. J Hypertens. 2005;23:1391–6.
29. Armitage JA, Khan IY, Taylor PD, et al. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? J Physiol. 2004;561:355–77.
30. Limesand SW, Rozance PJ, Zerbe GO, et al. Attenuated insulin release and storage in fetal sheep pancreatic islets with intrauterine growth restriction. Endocrinology. 2006;147:1488–97.
31. Hales CN, Ozanne SE. For debate: Fetal and early postnatal growth restriction lead to diabetes, the metabolic syndrome and renal failure. Diabetologia. 2003;46:1013–19.
32. Coupe B, Grit I, Hulin P, et al. Postnatal growth after intrauterine growth restriction alters central leptin signal and energy homeostasis. PLoS One. 2012;7:e30616.
33. Bar-El Dadon S, Shahar R, Katalan V, et al. Leptin administration affects growth and skeletal development in a rat intrauterine growth restriction model: preliminary study. Nutrition. 2011;27:973–7.
34. Brawley L, Itoh S, Torrens C, et al. Dietary protein restriction in pregnancy induces hypertension and vascular defects in rat male offspring. Pediatr Res. 2003;54:83–90.
35. Torrens C, Brawley L, Barker AC, et al. Maternal protein restriction in the rat impairs resistance artery but not conduit artery function in pregnant offspring. J Physiol. 2003;547: 77–84.
36. Lamireau D, Nuyt AM, Hou X, et al. Altered vascular function in fetal programming of hypertension. Stroke. 2002;33:2992–8.
37. Rueda-Clausen CF, Morton JS, Lopaschuk GD, et al. Long-term effects of intrauterine growth restriction on cardiac metabolism and susceptibility to ischaemia/reperfusion. Cardiovasc Res. 2011;90:285–94.
38. Remacle C, Bieswal F, Bol V, et al. Developmental programming of adult obesity and cardiovascular disease in rodents by maternal nutrition imbalance. Am J Clin Nutr. 2011;94: 1846S–52.
39. Choi GY, Tosh DN, Garg A, et al. Gender-specific programmed hepatic lipid dysregulation in intrauterine growth-restricted offspring. Am J Obstet Gynecol. 2007;196:477 e471–7.
40. Osterholm EA, Hostinar CE, Gunnar MR. Alterations in stress responses of the hypothalamic-pituitary-adrenal axis in small for gestational age infants. Psychoneuroendocrinology. 2012;37: 1719–25.

41. Gluckman PD, Hanson MA, Cooper C, et al. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359:61–73.
42. Gluckman PD, Lillycrop KA, Vickers MH, et al. Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. Proc Natl Acad Sci U S A. 2007;104:12796–800.
43. Godfrey KM, Gluckman PD, Hanson MA. Developmental origins of metabolic disease: life course and intergenerational perspectives. Trends Endocrinol Metab. 2010;21:199–205.
44. Goldberg AD, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. Cell. 2007;128: 635–8.
45. Gluckman PD, Hanson MA, Buklijas T, et al. Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. Nat Rev Endocrinol. 2009;5:401–8.
46. Diplas AI, Lambertini L, Lee MJ, et al. Differential expression of imprinted genes in normal and IUGR human placentas. Epigenetics. 2009;4:235–40.
47. Einstein F, Thompson RF, Bhagat TD, et al. Cytosine methylation dysregulation in neonates following intrauterine growth restriction. PLoS One. 2010;5:e8887.
48. Roth CL, Sathyanarayana S. Mechanisms affecting neuroendocrine and epigenetic regulation of body weight and onset of puberty: potential implications in the child born small for gestational age (SGA). Rev Endocr Metab Disord. 2012;13:129–40.
49. Banister CE, Koestler DC, Maccani MA, et al. Infant growth restriction is associated with distinct patterns of DNA methylation in human placentas. Epigenetics. 2011;6:920–7.
50. Uzan J, Carbonnel M, Piconne O, et al. Pre-eclampsia: pathophysiology, diagnosis, and management. Vasc Health Risk Manag. 2011;7:467–74.
51. Geelhoed JJ, Fraser A, Tilling K, et al. Preeclampsia and gestational hypertension are associated with childhood blood pressure independently of family adiposity measures: the Avon Longitudinal Study of Parents and Children. Circulation. 2010;122:1192–9.
52. Wen X, Triche EW, Hogan JW, et al. Prenatal factors for childhood blood pressure mediated by intrauterine and/or childhood growth? Pediatrics. 2011;127:e713–21.
53. Jayet PY, Rimoldi SF, Stuber T, et al. Pulmonary and systemic vascular dysfunction in young offspring of mothers with preeclampsia. Circulation. 2010;122:488–94.
54. Kajantie E, Eriksson JG, Osmond C, et al. Pre-eclampsia is associated with increased risk of stroke in the adult offspring: the Helsinki birth cohort study. Stroke. 2009;40:1176–80.
55. Wu CS, Sun Y, Vestergaard M, et al. Preeclampsia and risk for epilepsy in offspring. Pediatrics. 2008;122:1072–8.
56. Tamimi R, Lagiou P, Vatten LJ, et al. Pregnancy hormones, pre-eclampsia, and implications for breast cancer risk in the offspring. Cancer Epidemiol Biomarkers Prev. 2003;12:647–50.
57. Wu CS, Nohr EA, Bech BH, et al. Health of children born to mothers who had preeclampsia: a population-based cohort study. Am J Obstet Gynecol. 2009;201:269 e1–10.
58. Davis EF, Lazdam M, Lewandowski AJ, et al. Cardiovascular risk factors in children and young adults born to preeclamptic pregnancies: a systematic review. Pediatrics. 2012;129: e1552–61.
59. Lawlor DA, Macdonald-Wallis C, Fraser A, et al. Cardiovascular biomarkers and vascular function during childhood in the offspring of mothers with hypertensive disorders of pregnancy: findings from the Avon Longitudinal Study of Parents and Children. Eur Heart J. 2012;33:335–45.
60. Ogland B, Vatten LJ, Romundstad PR, et al. Pubertal anthropometry in sons and daughters of women with preeclamptic or normotensive pregnancies. Arch Dis Child. 2009;94:855–9.
61. Ros HS, Lichtenstein P, Ekbom A, et al. Tall or short? Twenty years after preeclampsia exposure in utero: comparisons of final height, body mass index, waist-to-hip ratio, and age at menarche among women, exposed and unexposed to preeclampsia during fetal life. Pediatr Res. 2001;49:763–9.
62. Trichopoulos D. Hypothesis: does breast cancer originate in utero? Lancet. 1990;335:939–40.
63. Garoff L, Seppala M. Toxemia of pregnancy: assessment of fetal distress by urinary estriol and circulating human placental lactogen and alpha-fetoprotein levels. Am J Obstet Gynecol. 1976;126:1027–33.

64. Troisi R, Potischman N, Roberts JM, et al. Maternal serum oestrogen and androgen concentrations in preeclamptic and uncomplicated pregnancies. Int J Epidemiol. 2003;32:455–60.
65. Vatten LJ, Romundstad PR, Odegard RA, et al. Alpha-foetoprotein in umbilical cord in relation to severe pre-eclampsia, birth weight and future breast cancer risk. Br J Cancer. 2002;86: 728–31.
66. Acromite MT, Mantzoros CS, Leach RE, et al. Androgens in preeclampsia. Am J Obstet Gynecol. 1999;180:60–3.
67. Ekbom A, Wuu J, Adami HO, et al. Duration of gestation and prostate cancer risk in offspring. Cancer Epidemiol Biomarkers Prev. 2000;9:221–3.
68. Troisi R, Potischman N, Hoover RN. Exploring the underlying hormonal mechanisms of prenatal risk factors for breast cancer: a review and commentary. Cancer Epidemiol Biomarkers Prev. 2007;16:1700–12.
69. Libby G, Murphy DJ, McEwan NF, et al. Pre-eclampsia and the later development of type 2 diabetes in mothers and their children: an intergenerational study from the Walker cohort. Diabetologia. 2007;50:523–30.
70. Tenhola S, Rahiala E, Martikainen A, et al. Blood pressure, serum lipids, fasting insulin, and adrenal hormones in 12-year-old children born with maternal preeclampsia. J Clin Endocrinol Metab. 2003;88:1217–22.
71. Badawi N, Kurinczuk JJ, Keogh JM, et al. Antepartum risk factors for newborn encephalopathy: the Western Australian case–control study. BMJ. 1998;317:1549–53.
72. McCarthy FP, Kingdom JC, Kenny LC, et al. Animal models of preeclampsia; uses and limitations. Placenta. 2011;32:413–19.
73. Huizinga CT, Engelbregt MJ, Rekers-Mombarg LT, et al. Ligation of the uterine artery and early postnatal food restriction – animal models for growth retardation. Horm Res. 2004;62: 233–40.
74. Soleymanlou N, Jurisica I, Nevo O, et al. Molecular evidence of placental hypoxia in pre-eclampsia. J Clin Endocrinol Metab. 2005;90:4299–308.
75. Cheng MH, Wang PH. Placentation abnormalities in the pathophysiology of preeclampsia. Expert Rev Mol Diagn. 2009;9:37–49.
76. Lai Z, Kalkunte S, Sharma S. A critical role of interleukin-10 in modulating hypoxia-induced preeclampsia-like disease in mice. Hypertension. 2011;57:505–14.
77. Luttun A, Carmeliet P. Soluble VEGF receptor Flt1: the elusive preeclampsia factor discovered? J Clin Invest. 2003;111:600–2.
78. Venkatesha S, Toporsian M, Lam C, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med. 2006;12:642–9.
79. Lu F, Bytautiene E, Tamayo E, et al. Gender-specific effect of overexpression of sFlt-1 in pregnant mice on fetal programming of blood pressure in the offspring later in life. Am J Obstet Gynecol. 2007;197:418 e411–5.
80. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest. 2003;111:649–58.
81. Van Vliet BN, Chafe LL. Maternal endothelial nitric oxide synthase genotype influences offspring blood pressure and activity in mice. Hypertension. 2007;49:556–62.
82. McMullen S, Langley-Evans SC. Sex-specific effects of prenatal low-protein and carbenoxolone exposure on renal angiotensin receptor expression in rats. Hypertension. 2005;46:1374–80.
83. O'Regan D, Kenyon CJ, Seckl JR, et al. Glucocorticoid exposure in late gestation in the rat permanently programs gender-specific differences in adult cardiovascular and metabolic physiology. Am J Physiol Endocrinol Metab. 2004;287:E863–70.
84. Mazzuca MQ, Wlodek ME, Dragomir NM, et al. Uteroplacental insufficiency programs regional vascular dysfunction and alters arterial stiffness in female offspring. J Physiol. 2010;588:1997–2010.
85. Wang Z, Huang Z, Lu G, et al. Hypoxia during pregnancy in rats leads to early morphological changes of atherosclerosis in adult offspring. Am J Physiol Heart Circ Physiol. 2009;296: H1321–8.

86. Pascoe KC, Wlodek ME, Jones GT. Increased elastic tissue defect formation in the growth restricted Brown Norway rat: a potential link between in utero condition and cardiovascular disease. Pediatr Res. 2008;64:125–30.
87. Akcakus M, Altunay L, Yikilmaz A, et al. The relationship between abdominal aortic intima-media thickness and lipid profile in neonates born to mothers with preeclampsia. J Pediatr Endocrinol Metab. 2010;23:1143–9.
88. Davis EF, Newton L, Lewandowski AJ, et al. Pre-eclampsia and offspring cardiovascular health: mechanistic insights from experimental studies. Clin Sci (Lond). 2012;123:53–72.
89. Bae S, Xiao Y, Li G, et al. Effect of maternal chronic hypoxic exposure during gestation on apoptosis in fetal rat heart. Am J Physiol Heart Circ Physiol. 2003;285:H983–90.
90. Li G, Xiao Y, Estrella JL, et al. Effect of fetal hypoxia on heart susceptibility to ischemia and reperfusion injury in the adult rat. J Soc Gynecol Investig. 2003;10:265–74.
91. Patterson AJ, Chen M, Xue Q, et al. Chronic prenatal hypoxia induces epigenetic programming of PKC{epsilon} gene repression in rat hearts. Circ Res. 2010;107:365–73.
92. Budas GR, Mochly-Rosen D. Mitochondrial protein kinase Cepsilon (PKCepsilon): emerging role in cardiac protection from ischaemic damage. Biochem Soc Trans. 2007;35:1052–4.
93. Kvehaugen AS, Dechend R, Ramstad HB, et al. Endothelial function and circulating biomarkers are disturbed in women and children after preeclampsia. Hypertension. 2011;58:63–9.
94. Wang Y, Zhou Y, He L, et al. Gene delivery of soluble vascular endothelial growth factor receptor-1 (sFlt-1) inhibits intra-plaque angiogenesis and suppresses development of atherosclerotic plaque. Clin Exp Med. 2011;11:113–21.
95. Moyes AJ, Maldonado-Perez D, Gray GA, et al. Enhanced angiogenic capacity of human umbilical vein endothelial cells from women with preeclampsia. Reprod Sci. 2011;18:374–82.
96. Medica I, Kastrin A, Peterlin B. Genetic polymorphisms in vasoactive genes and preeclampsia: a meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2007;131:115–26.
97. Yu CK, Casas JP, Savvidou MD, et al. Endothelial nitric oxide synthase gene polymorphism (Glu298Asp) and development of pre-eclampsia: a case–control study and a meta-analysis. BMC Pregnancy Childbirth. 2006;6:7.
98. Odom LN, Taylor HS. Environmental induction of the fetal epigenome. Expert Rev Obstet Gynecol. 2010;5:657–64.
99. von Zglinicki T. Oxidative stress shortens telomeres. Trends Biochem Sci. 2002;27:339–44.

Gamete/Embryo-Fetal Origins of Diabetes

4

He-Feng Huang, Guo-Dian Ding, Shen Tian, and Qiong Luo

Abstract

Epidemiological evidence suggests that environmental exposures in early life can profoundly influence long-term health, particularly adult risks of metabolic diseases such as type 2 diabetes mellitus (T2DM). At present there are three primary hypotheses with some theoretical basis and supporting evidence; the "thrifty phenotype", the "thrifty genotype" and the "fetal insulin" hypotheses. In this chapter, we will discuss the evidence related to embryo-fetal origins of diabetes.

4.1 Three Hypotheses

Metabolic syndrome is a combination of medical disorders that, when occurring together, increase the risk of developing cardiovascular disease and diabetes. Insulin resistance, which is present in the majority of people with the metabolic syndrome, has been recognized as the fundamental "defect". Hales and Barker coined the term the "thrifty phenotype" hypothesis, suggesting the fetal origin of insulin resistance. The hypothesis proposes that an undernourished baby maintains high levels of sugar in the bloodstream that benefits the brain, but less sugar is stored in muscles; muscle growth may be "traded" to protect the brain. This "thrifty" behavior becomes permanent and, combines with adiposity to produce type 2 diabetes in later life [1, 2].

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The "thrifty genotype" hypothesis was originally proposed to explain the tendency of certain ethnic groups such as the Pima to tend towards obesity and diabetes [3]. He proposed that native Indians might have accumulated "thrifty" genes, which were selected during evolution at a time when food resources were scarce. These genes are beneficial for survival under famine conditions by resulting in a "fast insulin trigger" and thus an enhanced capacity to store fat. However, they may place the individual at risk of insulin resistance and type 2 diabetes in affluent societies.

The "fetal insulin" hypothesis of Hattersley proposed that genetically determined insulin resistance results in impaired insulin-mediated growth in the fetus as well as insulin resistance in adult life. The hypothesis emphasizes that insulin secreted by the fetal pancreas in response to high maternal glucose concentrations is a key contributing factor [4]. Monogenic disorders that impair glucose sensing, such as glucokinase gene mutations, lower insulin secretion, or increase insulin resistance, as in IGF-1 gene polymorphism, are often associated with impaired fetal growth [5]. On the other hand, polygenic disorders resulting in insulin resistance in the normal population are therefore likely to result in lower birthweight.

4.2 "Embryo-Fetal Origin of Diseases" Theory

Embryonic and fetal periods are clearly vulnerable to environmental factors, and acquired changes can persist transgenerationally, despite the lack of continuing exposure. In 2010, Motrenko set out the "embryo-fetal origin of diseases" theory, proposing that abnormal development of gamete and embryo may induce poor health after birth [6]. In similar vein, adaptive responses of a gamete/embryo reacting with adverse factors, like culture systems and manipulations in ART, may make them susceptible to permanent damage to organ function and structure, inducing later chronic adult diseases. Altered epigenetic re-programming causing transgenerational effects may be a cause for considerable concern.

4.3 Epigenetic Mechanisms During Preimplantation Development

Epigenetic "re-programming" may occur by both active and passive mechanisms, interfering and shaping imprinting mechanisms in the early embryo [7].

4.3.1 Post-fertilization

Paternal genome is actively demethylated within a few hours after fertilization to achieve formation of the male pronucleus, following a protamine-to-histone exchange. Oocyte methylation appears to be tied to Lys4 of histone H3 (H3K4) with absence of replication or transcription in pronucleus. Zygotic genome activation (ZGA) is the critical nuclear reprogramming event that governs the transition from

maternal to embryonic control of development. The process of ZGA includes (1) degradation of maternal transcript with iRNA; (2) replacement of maternally derived transcript with zygotically expressed ones; and (3) transcriptional upregulation of zygote genes that will function to reset and reprogram gene expression pattern in the developing embryos. A "zygotic clock", combined with the nuclear environment, ensures that zygotic gene expression occurs in an ordered and temporally regulated way. The zygotic genome gradually becomes demethylated through a passive mechanism, that DNMT1 is exported from the nucleus, resulting in newly replicated DNA strands being unable to be methylated by DNMT1. This process will increase unmethylated DNAs [8].

4.3.2 Cleavage Stage

Embryo genome activation (EGA) includes two stages, weak transcriptional activity at the end of the zygote stage and weak activity at the four-cell stage. This implies a longer reliance on maternally inherited information and a shortened delay between EGA and cell differentiation. However, no studies have yet been conducted [8].

4.3.3 Blastocyst Formation

The studies suggest that mechanisms of cellular adhesion and epigenetic modification involving inner cell mass (ICM) and trophectoderm (TE) differentiation, may mean that TE and ICM differentially express several, lineage-specific transcription factors. Cdx2 becomes restricted to the trophectoderm cell line and is required for its formation; Oct4 and Nanog become restricted, and, may influence the inner cell mass [8].

4.4 Developmental Regulation of Somatic Imprints

Epidemiological and animal studies demonstrate that nutrition and other environmental factors could influence prenatal developmental pathways, thereby inducing permanent changes in metabolism and susceptibility of chronic disease. Epigenetic changes may partly explain the diversity of disease phenotype and other traits. Since somatic methylome establishment consists of multiple developmental steps, the causal relationship between the intrauterine environment and epigenetic dysregulation is still unclear. It has been revealed that, during pregnancy, nutritional stimuli from maternal intake (such as genistein, bisphenol A, or methyl donors) could alter the fetal epigenome in utero, change the methylation levels of the A^{vy} allele. These somatic epigenetic changes are thought to have occurred before germ layer differentiation in the early embryo. Therefore, it is important to elucidate the effects of environmental modification on the fetal epigenome, particularly in this early developmental stage. Sato et al. found genistein perturbed the methylation pattern of differentiated ES

cells after de novo methylation, suggesting that, for a subset of genes, regulation after de novo DNA methylation in the early embryo may be sensitive to genistein [9].

By virtue of the repeated action of DNMT1 in replicating DNA methylation through cell division, both gametic and somatic differentially methylated regions (DMRs) can be maintained in perpetuity. Where these DMRs directly span the promoter of a gene expressed in the adult, imprinted expression will also be maintained. There are also genes where imprinting expression is known to be present in the adult in the absence of direct promoter DNA methylation, but there is the potential for transcriptional interference with a second transcript that does originate at a DMR. Thus initial studies on the genes marked by somatic imprints support a role for genomic imprinting in modifying metabolic efficiency and behavior in mammals [10]. Imprinting may provide a mechanism to modify reproductive strategies, social behavior and metabolism such that mammals flourish under a wide variety of environmental conditions.

4.5 Animal and Human Evidence

Experimental studies on laboratory animals, and, epidemiological studies in human populations both clearly demonstrate that fetal development in an unfavorable intra-uterine milieu that can induce persistent alterations in the metabolism of offspring throughout life. Not only maternal diabetes, maternal/fetal malnutrition also could induce a diabetogenic tendency in the offspring [11].

A number of studies illustrate a latent tendency for impairment of glucose tolerance in populations subject to poor nutrition during fetal and postnatal life. If they remain on a low calorie diet, the glucose tolerance will maintain normal. Otherwise, their glucose metabolism will be challenged with higher caloric intake and lower physical activity, increasing the incidence of impaired glucose tolerance and type-2 diabetes. The effect is clearest in those individuals that gain most weight. Examples of this phenomenon have been described in the Pima Indians in Arizona [12]. The transition from a hunter-gatherer life style to a more modern way of life has dramatically increased the occurrence of type-2 diabetes and gestational diabetes. A similar effect occurs in Aboriginal populations in Australia [13]. The arrival of a supermarket in a remote country increases the prevalence of abnormal glucose tolerance.

4.6 Trans-generational Propagation

Offspring of both mild and severely hyperglycemic mothers (second generation) may develop gestational diabetes. Their offspring, the third generation, display the same disorders as offspring of mildly hyperglycemic mothers: (1) fetuses are macrosomic, hyperinsulinemic with islet hyperplasia; and (2) adults have abnormal glucose tolerance associated with an insulin secretion defect [14].

A study showed that female F2 rats procreated by F1 pre- and postnatally nutrient and growth restricted (IUGR) mothers but embryo-transferred to gestate in control mothers on d1 had increased hepatic weight, fasting hyperglycemia, hyperinsulinemia, and unsuppressed hepatic glucose production, with no change in glucose futile cycling or clearance, compared with age- and sex-matched control F2 progeny that gestated in a similar manner [15]. These hormonal and metabolic aberrations were associated with increased skeletal muscle total GLUT4 and pAkt concentrations but decreased plasma membrane-associated GLUT4, pPKCζ, and PKCζ enzyme activity in IUGR F2 compared with F2 controls. This observation supports the trans-generational presence of aberrant glucose/insulin metabolism and skeletal muscle insulin signaling in the adult F2 IUGR female offspring that is independent of the immediate intrauterine environment.

Investigations into the F3 generation will differentiate between this possibility and epigenetic DNA modifications being responsible for the trans-generational propagation of type 2 diabetes mellitus. Investigations in the protein-restricted, IUGR rat demonstrate persistence of abnormal glucose homeostasis in the F3 generation lending credence to a role for epigenetic regulation [16].

Zambrano et al. fed female rats with a normal control 20 % casein diet (C) or a protein-restricted isocaloric diet (R) containing 10 % casein during pregnancy and lactation. After delivery, according to the diet of F0 female rats, F1 offspring were divided into four groups: CC (first letter pregnancy diet and second lactation diet), RR, CR and RC. All F1 female offspring were fed *ad libitum* with C diet after weaning and during their first pregnancy and lactation. They found that F1 female offspring of RR and CR mothers exhibited low body weight and food intake with increased sensitivity to insulin during a glucose tolerance test at 110 days of postnatal life. F2 male offspring of CR showed evidence of insulin resistance. In contrast, in RC group, F2 female offspring were insulin resistant. Sex differences were also observed in F2 offspring in the resting glucose and insulin. Therefore, maternal protein restriction adversely affects glucose and insulin metabolism of F2 offspring in a manner specific to sex and developmental time window during their mother's (the F1) fetal and neonatal development [17].

The studies on the mice found that maternal undernutrition during pregnancy programs reduced birth weight, glucose intolerance, and obesity in first- and second-generation offspring, despite *ad libitum* feeding during second pregnancy. Different aspects of these phenotypes are transmitted via the maternal lineage (obesity), the paternal lineage (reduced birth weight), or both (glucose intolerance) [18]. Sex differences in transmission of phenotypes implicate complex mechanisms: (1) matrilineal inheritance of disease is multifactorial and includes metabolic, epigenetic, and mitochondrial mechanisms; and (2) patrilineal inheritance is primarily due to epigenetic mechanisms. Such studies may be significant in the design of future therapeutic interventions that aim to prevent, and/or modulate, adult phenotypes, not only in LBW humans but also in their children and grandchildren.

4.7 Modulation of Genomic Expression and Embryo-Fetal Origins of Diabetes

4.7.1 Epigenetic Alteration of Embryo and Fetus by Environment

Separate from the immediate in utero environment, epigenetic changes that include aberrant DNA methylation of CpG clusters and histone N-tail posttranslational modifications affect downstream gene expression, thereby creating the basis for trans-generational propagation. This, in turn, may contribute to the present epidemic of diabetes mellitus in both developing and developed countries [19].

Several imprinted genes have been identified as potential causes of impaired, glucose-regulated, metabolic processes and some have also been associated with metabolic disorders in humans. Alterations in fetal growth related to imprinting of H19/Igf2, Gnas, Dlk1, Grb10, Plagl1, are associated with metabolic disorders in adulthood, including obesity and diabetes [20]. The epigenetic, regulatory mechanisms that define imprinted genes render them uniquely susceptible to changes in expression that can be set during embryogenesis, and, may persist into adulthood. This, together with their regulatory roles in both fetal growth and adult metabolism means that the imprinted genes may be strong candidates for involvement in fetal "programming" of adult health status.

In IUGR rat, it is found that epigenetic modifications of key genes regulating β-cell development were induced by abnormal intrauterine environment, which directly link chromatin remodeling and suppression of transcription. Dietary protein restriction of pregnant rats induces hypomethylation of the glucocorticoid receptor and peroxisome proliferator-activated, receptor g genes in liver of the offspring. These epigenetic changes may result in observed increases in expression of these genes [21].

4.7.2 The Roles of Epigenetic Alteration in Trans-generational propagation

Animal studies support the role of environmental epigenetics in T2DM susceptibility. In the female Agouti mouse, a genomic region called the Agouti locus could be modified by epigenetic alterations by diet, to promote changes in coat colour, and, obesity and diabetes in the offspring [22]. Human epidemiological studies also provide evidence that maternal factors influence the adult risk of developing various chronic diseases, such as cancer, cardiovascular disease and T2DM. The concept of 'maternal effects', where environmental influences on one generation may have significant impact on the next generation, and, potentially on a third generation, is well recognized. A range of nutritional hormonal and behavioural cues affecting parents (the F0 generation) can have consequences for the next generation (F1) and in some instances for subsequent generations (F2 onwards), even if they did not experience the same cue. Exposure to several environmental factors during early

embryonic development has been shown to increase disease susceptibility in the F1 generation. Some of the well-studied environmental disruptors are abnormal nutrition (for example, caloric restriction) causing diabetic and uterine defects, endocrine disruptors causing reproductive and endocrine effects, and, also some chemical exposures like heavy metals. Giving low-calorie food to female mice during puberty and then feeding them with normal caloric food during their pregnancy, produced abnormal insulin and glucose metabolism in the F1 offspring. Nutritional depletion may affect the development of F0 oocytes, though not the intrauterine development of F1 embryo because the mother was normally fed during the fetal growth period and the influence of attenuated maternal germ line development could pass to the next generation and cause impaired health situation of F1, especially metabolic diseases. Furthermore, both human and animal studies demonstrate that exposure of an F0 generation mother can produce an F2 generation phenotype, and these kinds of effects of environment on the F0 germ line have been observed in species ranging from insects to mammals [23–25]. In rodents, nutritional deficiency of mother during gestation affects the incidence of diabetes and growth defects in the F2 generation [26]. On the other hand, the analysis on the F3 generation is now conducted in some studies [27].

We established a GDM mouse model of intrauterine hyperglycemia. The female (♀) and male (♂) F1 adults of control and GDM mice were intercrossed to obtain F2 offspring of four groups: (1) C♂-C♀, (2) C♂-GDM♀, (3) GDM♂-C♀ and (4) GDM♂-GDM♀. After exposure to intrauterine hyperglycemia, parental characteristics were transgenerational transmitted to F2 offspring. For F2 offspring, paternal line factors are more prone to transmission compared to maternal line. Further experiments showed that both F1 and F2 offspring demonstrated dysregulated expression of the imprinting gene *Igf2* and abnormal methylation status. Additionally, altered *imprinting* gene expression was also found in sperm of adult F1-GDM with, or without, IGT, suggesting transgenerational transmission of epigenetic changes in male germ cells [28].

4.7.3 Environmental Causes of Altered Gene Expression in Embryo and Fetus

Cagnone et al. studied the impact of hyperglycemia on embryo development using different glucose concentrations (0.2 [control], 1, 2, 5, and 10 mM) during early cleavage stages of development (until the 8- to 16-cell stage), and, then cultured all embryos in control media until the blastocyst stage. They found that although adding 1–5 mM of glucose did not affect the developmental rate of in vitro culture (IVC) to 8–16 cells, a 10 mM concentration of glucose decreased the rate of embryos reaching the 8–16 cell stage and prevented blastocyst development, indicating that high glucose concentrations might impair embryo development [29]. Exposure to 5 mM glucose during early cleavage stages impacts subsequent gene expression at blastocyst stage. Statistical analysis of microarray comparisons reveal a significantly different expression for 490 transcript sequences between control and treated blastocysts, among which 63 had more than 1.5 fold-change differences.

Moreover, 57 sequences among these 63 were upregulated in treated blastocysts. The differential gene expression profile included sex-related genes, stage-related genes, and, genes for cellular and molecular functions. These functions are associated with cell motility, defense against external wounding, and calcium management. The canonical pathways with significant value are high-mobility group box 1 (HMGB1) signaling, integrin-linked kinase (ILK) signaling, hypoxia inducible factor 1 alpha subunit basic helix loop-helix transcription factor (HIF1A) signaling, transforming growth factor beta (TGF-B) signaling, oxidative stress response, tumor necrosis factor receptor superfamily member 1A (TNFRSF1A) signaling, tight junction signaling, interferon signaling, glutathione metabolism, and peroxisome proliferator-activated receptor alpha (PPARA) signaling. Ingenuity-generated networks reflect cellular processes of tissue development, metabolic disease and tumor morphology. On a pathophysiological level, higher glucose treatment associates with diabetes and tumorigenesis through genes controlling the Warburg effect, that is, emphasis on use of anaerobic glycolysis rather than oxidative phosphorylation.

4.7.4 Gene Mutation of Embryo and Fetus by Environment

As for T2DM, there is a special and rare type of infant diabetes. For the vast majority of infant diabetes cases, their parents do not have the similar disease and may result from a new mutation or chromosomal abnormalities. The most common cause of infant diabetes is mutation of ATP-sensitive potassium (KATP) channel genes, and, the prepro-insulin (INS) gene expressed by islet β-cells. These two gene mutations explain more than half the incidence of neonatal diabetes less than 6 months of age [30].

Maturity-onset diabetes of the young (MODY) is defined as early-onset, non-autoimmune diabetes that occurs in childhood, adolescence or young adulthood. It is caused by a primary defect in pancreatic β-cell function [4]. However, residual insulin secretion may be maintained for some years after diagnosis and exogenous insulin is generally not required at the time of diagnosis. MODY may account for 1–2 % of all T2D cases. Heterozygous mutations or partial/whole gene deletions in seven susceptibility genes may cause MODY. The MODY genes encode the enzyme glucokinase (GCK), the transcription factors HNF-1a (HNF1A), −1b (HNF1B), −4a (HNF4A), PDX1 and NEUROD1 or the preproinsulin (INS) each having a crucial role in the development and function of pancreatic b-cells. Mutations in GCK, HNF1A and HNF4A are the most common causes of MODY [4].

4.8 Gametogenesis and Embryo-Fetal Origins of Diabetes

4.8.1 Gametogenesis Phase

Formation and maturation of gametes, especially oocytes, may last for decades, exposing them to much longer periods for potential damage, both in-vivo and in-vitro. Gametogenesis and fertilization take place during the period of epigenetic

re-programming. Modulation of genomic imprints for imprinted genes begins when migratory primordial germ cells (PGCs) enter the embryonic genital ridge and concludes at the point of fertilization. In this period, egg and sperm-derived imprints, and some other germline sequences, may undergo extensive imprinting erasure and re-establishment while non-imprinting genes retain their methylation state. The critical time for epigenetic modification of the germ line is the period of primordial germ cell migration and gonadal sex determination. The permanent alteration in the epigenetic programming of the germ line appears to be the mechanism involved in the transgenerational phenotype.

4.8.2 Aberrant Oogenesis and Adult Diabetes

Epidemiological evidence shows that alteration of the maternal environment can impact the development of oocyte, and, influence the offspring's risk for type 2 diabetes and obesity over the life course. In the Chicago Diabetes in Pregnancy study, 12-year-old offspring of mothers with type 1 diabetes before their pregnancy, had a significantly higher prevalence of impaired glucose tolerance than a non-concurrent age and sex-matched control group [31]. Similarly, a study in Pima Indian women showed that at every age before 20 years, offspring of women with type 2 diabetes had more chance of having type 2 diabetes than those of pre-diabetic and non-diabetic ones. Over-nutrition has been less studied than fetal undernutrition in this context. However, several studies suggest that high energy intakes resulting in maternal obesity, and diets high in fat, cholesterol, and carbohydrates may influence the early embryo development, and have programming consequences. In humans, limited evidence suggests that maternal nutritional intake influences glucose metabolism in offspring. Clapp et al. demonstrated that compared with the offspring conceived by mothers on normal glycemix index diets, those offspring of high glucose mothers had higher birth weight and skinfold thickness [32].

One general mechanism by which parental exposure may be linked to phenotypic changes later in their offspring's life is the alteration of epigenetic marks. The discovery of genomic imprinting, in which expression of certain genes is determined by the gender of the parent that contributes the allele, establishes that patterns of gene expression can be inherited without changes in the sequence of genomic DNA through silencing of one set of alleles. Widespread reprogramming of epigenetic marks, involving both active and passive demethylation and reorganization of histone modification, occurs in early post-fertilization mammalian development to ensure totipotency of the developing zygote.

During oogenesis, the imprinting genes undergo a dramatic process of demethylation from the maternal allele while the non-imprinting genes maintain their imprinting status. Between fertilization and embryo development, the imprints of non-imprinting genes are erased and imprinting genes are reestablished. The imprinting genes may be involved in the development of the placenta and the advent of viviparity, but they are also important to the outcome of the embryo.

In the mouse, demethylation occurs at about E12 in both sexes and remethylation of the male genome begins at about E16 and is complete shortly after birth. In contrast, in the female, remethylation occurs during postnatal oogenesis, with some imprints being acquired relatively late, though the process is complete before metaphase Certain proteins such as TRIM28 can protect imprinting genes like H19 from demethylation in early embryo development, maternal TRIM28 knock-out mice demonstrate loss of imprinting in H19 DMR [33]. Because of the dramatic epigenetic change in the phase of oogenesis, maternal exposure to nutritional, chemical and physical factors have the potential influences to alter gene modification, and, may increase adult disease susceptibility in various ways through changes in the epigenome, and lead to adverse consequences such as T2DM in later life. Animal studies have characterized epigenetic modifications that contribute to permanent alterations in genes involved in the regulation of energy homeostasis, such as leptin [34], SOCS3 [35] and glucose transporter [36]. When an F0 gestating female is exposed, both the F1 and F2 generations might show the same phenotype as their parents. Epigenetic modification in the F0 germ line may be a plausible mechanism. In some cases, the phenotypic changes are maintained in the F3 generation, which can absolutely rule out the toxicology of direct exposure to any environmental factors.

Some genomic targets are likely to be susceptible to gene-expression changes owing to environmental perturbations of epigenetic marks include the promoter regions of some important genes, transposable elements that lie adjacent to genes with metastable epialleles, which are defined as loci that can be epigenetically modified in a variable and reversible manners. Only a few genes with metastable epialleles have been identified, including the mouse A^{vy}, $Axin^{fu}$, and $Cabp^{IAP}$ genes [37]. These variable epigenetic modulations can affect the expression of neighbouring genes, causing epigenetic mosaicism between cells and phenotypic variability. However, what role does this gene play in the development of adult T2DM? The Agouti gene encodes a paracrine signal molecule. A cryptic promoter in the proximal end of the A^{vy} IAP promotes constitutive ectopic agouti transcription, leading to different fur colour, with diabetes and obesity.

DNA copy number variation is also a pattern by which the maternal response to environment can be transmitted to the offspring. Mitochondrial DNA copy number is inherited through the maternal line, its synthesis during maternal germ cells development and preimplantation is likely susceptible to environment. For example, offspring of rat fed with abnormal fat/protein diet was found of changes in mitochondrial copy number [38]. Transmission of mitochondrial dysfunction could therefore involve maternal epigenetic processes [39]. For instance, the frequency and reproducibility of the vinclozolin (a former mentioned endocrine disruptor) induced transgenerational pathologies, and because of the rareness of DNA-sequence mutation (even with ionizing radiation, is normally less than 0.01 %), DNA-sequence mutation may not be a main cause of dramatic changes in mitochondrial copy number and dysfunction. The plausible explanation for these findings is therefore that the vinclozolin-induced, epigenetic reprogramming of the parental germ line at the stage of gonadal sex determination and gametogenesis.

4.8.3 IVF and Embryo-Fetal Origins of Diabetes

In-vitro fertilization (IVF) involves hyper-ovulation and in vitro manipulation of gametes and early embryos. These non-physiological procedures can disturb the normal development of oocyte and lead to transgenerational adverse effects on the next generation. This period is vital for maternal epigenetics and so any interference may lead to alteration of the offspring's epigenome and adult diseases. The presence of altered fetal growth, methylation alterations in the placenta, and transgenerational inheritance of imprinting errors in animals raises concern for global epigenetic changes following ART procedures. Several studies show that superovulation is associated with methylation changes in maternal and paternal alleles in both oocytes and blastocysts [31, 34]. Stouder demonstrated that imprinting errors after superovulation might have transgenerational effects on offspring [35]. In human research, effects of ART on a single maternal imprinting control region has been demonstrated in normal children. Gomes et al. found that 3 of 12 ART-conceived children demonstrated KvDMR1 hypomethylation [36] . All three children had a dizygotic twin who expressed a discordant (normal) KvDMR1 methylation pattern. This discordance was hypothesized to result from either differential vulnerability to imprinting of the embryos, or, epigenetic alterations that occurred during gametogenesis.

4.8.4 Aberrant Spermatogenesis and Adult Diabetes

In human, adverse paternal conditions like obesity are associated with low birth weight in offspring, which is an independent risk factor for adult T2DM [40]. Exposure to endogenous, or exogenous, endocrine disruptors during the formation of male reproductive organs interferes with normal endocrine signaling. For example, the plastic component bisphenol A (BPA) acts as an estrogenic compound causing numerous pathologies including prostate cancer in low doses [41]. Abnormalities in mouse testicular Leydig cells are induced by chronic low dose exposure to arsenic [42].

But more importantly, disruption during the critical time window of spermatogenesis may have transgenerational effects promoting adult-onset disease to the next generations. An epigenetic change in the male germ line may be involved in the transgenerational transmission of some induced phenotypes, like impaired glucose intolerance and islet β-cell dysfunction. Guerrero-Bosagna et al. [43] reported that exposing to exogenous endocrine disruptors during mid-gestation, produces transmission defects from F1 to F4, and the phenotype includes adult-onset diseases. They ascribed this phenomenon to the abnormal development of the sperm of F1 male rats for several reasons. Firstly, the embryonic testis has a subset of genes that have their expression altered in a consistent manner in males from the F1 through the F3 generation. Secondly, DNA sequence mutations occur at a very low frequency. Russell and colleagues found some heritable deletions and

mutations in mouse germ cells induced by endocrine disruptors [44]. However, most environmental disruptors cannot cause as much damage as altering the DNA sequence and nucleotide composition.

DNA methylation is an important part of epigenetic modulation during the period of sperm development. Environment is an equally important consideration in adult metabolic disease as because of its ability to regulate DNA modification [45]. When identical twins develop in different geographic regions, they also have different disease frequencies, including diabetes [46]. There are also a large number of environmental compounds and toxicants shown to promote disease, but most do not alter the DNA sequence [47]. Several studies have now shown marked effects of environmental toxic agents on the F3 generation through germline alterations in the epigenome. The endocrine disruptor vinclozolin, which is an anti-androgenic compound resulting in spermatogenic defects, causes male infertility at frequencies ranging from 20 to 90 %. The transgenerational pathogenesis phenotypes are transmitted to the majority of progeny for four generations and, although the transgenerational effect is transmitted through only the male line, both males and females are affected.

Meanwhile, histone modification plays a role in epigenetic programming during gametogenesis. During spermiogenesis, most of the histones are replaced by protamines, leading to a loss of the epigenetic component. The sperm is, therefore, viewed as a passive carrier of the paternal genome with a disproportionately lower epigenetic contribution, except for DNA methylation, to the next generation. A small fraction of histones are retained in sperm, which include H2A, H2AX and H4 [48]. Further, centromeric heterochromatin characterized by the presence of H4K8ac and H4K12ac, is inherited from the father [49]. Recent studies have also indicated the presence of testis-specific histone variants [50]. But, a recent study overturns this view by demonstrating a locus-specific retention of histones, with specific modifications in the sperm chromatin at the promoters of developmentally important genes [51]. The authors used high-throughput genomic techniques such as ChIP-on-chip and ChIP-sequencing, to identify regions in the sperm chromatin which are associated with histones. This provides evidence of a programmed retention of histones and locus-specific histone modifications. The locus-specific retention of epigenetic marks in sperm chromatin may play a role in the development of embryo-origin adult T2DM. Environmental factors may also cause retention in the sperm chromatin, which can be transmitted to the offspring and lead to abnormal gene expression, increasing the disease susceptibility.

Male mice, whose mothers consumed a high-fat-diet (HFD), were heavier, diabetic and insulin resistant, and, produced second-generation offspring who were insulin resistant, though not obese [52, 53]. In fact, HFD, as a well-known in-vitro endocrine disruptors, can increase sperm DNA damage in humans [54]. Ng and colleagues [55] focused on the paternal contribution to the onset of diabetes in next generation. They demonstrated that father's HFD exposure programs β-cell 'dysfunction' in rat F1 female offspring, particularly the role of non-genetic factors in the causal pathway. Moreover, they performed genome-wide microarray analysis of isolated islets to explore mechanisms of impaired insulin secretion. Paternal HFD

altered the expression of 642 pancreatic islet genes in adult female offspring, including calcium-, MAPK- and Wnt-signalling pathways, apoptosis and the cell cycle. Impaired glucose tolerance and insulin secretion, in the absence of obesity, in these female offspring indicate that a paternal HFD targets the endocrine pancreas and β-cells. Other scientists had already shown that paternal lifestyle and particular environmental factors can affect spermatogenesis, and, may also interfere with Sertoli-cell proliferation, and the integrity of the blood–testis barrier, thus affecting DNA reprogramming of the gamete [56]. These findings extend the concept of developmental and adaptive plasticity to include a paternal role in the early origins of diabetes, and, the underlying mechanisms seem to include epigenetic modifications.

References

1. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 1992;35:595–601.
2. Barker DJ. Developmental origins of adult health and disease. J Epidemiol Community Health. 2004;58:114–15.
3. Neel JV. Diabetes mellitus: a “thrifty” genotype rendered detrimental by “progress”? Am J Hum Genet. 1962;14:353–62.
4. Velho G, Hattersley AT, Froguel P. Maternal diabetes alters birth weight in glucokinase-deficient (MODY2) kindred but has no influence on adult weight, height, insulin secretion or insulin sensitivity. Diabetologia. 2000;43:1060–3.
5. Arends N, Johnston L, Hokken-Koelega A, et al. Polymorphism in the IGF-I gene: clinical relevance for short children born small for gestational age (SGA). J Clin Endocrinol Metab. 2002;87:2720–4.
6. Motrenko T. Embryo-fetal origin of diseases – new approach on epigenetic reprogramming. Arch Perinat Med. 2010;16:11–5.
7. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. Science. 2001;293:1089–93.
8. Palini S, De Stefani S, Scala V, et al. Epigenetic regulatory mechanisms during preimplantation embryo development. Ann N Y Acad Sci. 2011;1221:54–60.
9. Sato N, Yamakawa N, Masuda M, et al. Genome-wide DNA methylation analysis reveals phytoestrogen modification of promoter methylation patterns during embryonic stem cell differentiation. PLoS One. 2011;29:6 :e19278.
10. John RM, Lefebvre L. Developmental regulation of somatic imprints. Differentiation. 2011;81:270–80.
11. Aerts L, Van Assche FA. Animal evidence for the transgenerational development of diabetes mellitus. Int J Biochem Cell Biol. 2006;38:894–903.
12. Pettitt DJ. Diabetes in subsequent generations. In: Dornhorst A, Hadden DR, editors. Diabetes and pregnancy. An international approach international approach to diagnosis and management. Chichester: Wiley; 1996. p. 367–76.
13. Gault A, O’Dea K, Rowley KG, et al. Abnormal glucose tolerance and other coronary heart disease risk factors in an isolated aboriginal community in central Australia. Diabetes Care. 1996;19:1269–73.
14. Fetita LS, Sobngwi E, Serradas P, et al. Consequences of fetal exposure to maternal diabetes in offspring. J Clin Endocrinol Metab. 2006;91:3718–24.
15. Thamotharan M, Garg M, Oak S, et al. Transgenerational inheritance of the insulin-resistant phenotype in embryo-transferred intrauterine growth-restricted adult female rat offspring. Am J Physiol Endocrinol Metab. 2007;292:E1270–9.

16. Benyshek DC, Johnston CS, Martin JF. Glucose metabolism is altered in the adequately-nourished grand-offspring (F3 generation) of rats malnourished during gestation and perinatal life. Diabetologia. 2006;49:1117–19.
17. Zambrano E, Martínez-Samayoa PM, Bautista CJ, et al. Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. J Physiol. 2005;566:225–36.
18. Jimenez-Chillaron JC, Isganaitis E, Charalambous M, et al. Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. Diabetes. 2009;58:460–8.
19. Devaskar SU, Thamotharan M. Metabolic programming in the pathogenesis of insulin resistance. Rev Endocr Metab Disord. 2007;8:105–13.
20. Smith FM, Garfield AS, Ward A. Regulation of growth and metabolism by imprinted genes. Cytogenet Genome Res. 2006;113:279–91.
21. Simmons RA. Developmental origins of diabetes: the role of epigenetic mechanisms. Curr Opin Endocrinol Diabetes Obes. 2007;14:13–6.
22. Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet. 2007;8:253–62.
23. Ruden DM, Xiao L, Garfinkel MD, et al. Hsp90 and environmental impacts on epigenetic states: a model for the trans-generational effects of diethylstibesterol on uterine development and cancer. Hum Mol Genet. 2005;14:R149–55.
24. Omholt SW, Amdam GV. Epigenetic regulation of aging in honeybee workers. Sci Aging Knowledge Environ. 2004;26:pe28.
25. Seidl MD, Paul RJ, Pirow R. Effects of hypoxia acclimation on morpho-physiological traits over three generations of *Daphnia magna*. J Exp Biol. 2005;208:2165–75.
26. Zambrano E, Rodríguez-González GL, Guzmán C, et al. A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. J Physiol. 2005;563:275–84.
27. Blatt J, Van Le L, Weiner T, et al. Ovarian carcinoma in an adolescent with transgenerational exposure to diethylstilbestrol. J Pediatr Hematol Oncol. 2003;25:635–6.
28. Ding GL, Wang FF, Shu J, et al. Transgenerational glucose intolerance with Igf2/H19 epigenetic alterations in mouse islet induced by intrauterine hyperglycemia. Diabetes. 2012;61:1133–42.
29. Cagnone GL, Dufort I, Vigneault C, et al. Differential gene expression profile in bovine blastocysts resulting from hyperglycemia exposure during early cleavage stages. Biol Reprod. 2012;86:50,1–12
30. Vaxillaire M, Bonnefond A, Froguel P. The lessons of early-onset monogenic diabetes for the understanding of diabetes pathogenesis. Best Pract Res Clin Endocrinol Metab. 2012;26:171–87.
31. Fauque P, Jouannet P, Lesaffre C, et al. Assisted Reproductive Technology affects developmental kinetics, H19 Imprinting Control Region methylation and H19 gene expression in individual mouse embryos. BMC Dev Biol. 2007;7:116.
32. Clapp JF. Effects of diet and exercise on insulin resistance during pregnancy. Metab Syndr Relat Disord. 2006;4:84–90.
33. Messerschmidt DM, de Vries W, Ito M, et al. Trim28 Is Required for Epigenetic Stability During Mouse Oocyte to Embryo Transition. Science. 2012;335:1499–502.
34. Sato A, Otsu E, Negishi H, et al. Aberrant DNA methylation of imprinted loci in superovulated oocytes. Hum Reprod. 2007;22:26–35.
35. Stouder C, Deutsch S, Paoloni-Giacobino A. Superovulation in mice alters the methylation pattern of imprinted genes in the sperm of the offspring. Reprod Toxicol. 2009;28(4):536–41.
36. Gomes MV, Huber J, Ferriani RA, et al. Abnormal methylation at the KvDMR1 imprinting control region in clinically normal children conceived by assisted reproductive technologies. Mol Hum Reprod. 2009;15:471–7.
37. Duhl DM, Vrieling H, Miller KA, et al. Neomorphic agouti mutations in obese yellow mice. Nat Genet. 1994;8:59–65.

38. McConnell JML. A mitochondrial component of developmental programming. In: Gluckman PD, Hanson MA, editors. Developmental origins of health and disease. Cambridge: Cambridge University Press; 2006. p. 75–81.
39. Choi YS, Kim S, Kyu Lee H, et al. In vitro methylation of nuclear respiratory factor-1 binding site suppresses the promoter activity of mitochondrial transcription factor A. Biochem Biophys Res Commun. 2004;314:118–22.
40. Power C, Li L, Manor O, et al. Combination of low birth weight and high adult body mass index: at what age is it established and what are its determinants. J Epidemiol Community Health. 2003;57:969–73.
41. Ho SM, Tang WY, Belmonte de Frausto J, et al. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res. 2006;66:5624–32.
42. Singh KP, DuMond Jr JW. Genetic and epigenetic changes induced by chronic low dose exposure to arsenic of mouse testicular Leydig cells. Int J Oncol. 2007;30:253–60.
43. Guerrero-Bosagna CM, Skinner MK. Epigenetic transgenerational effects of endocrine disruptors on male reproduction. Semin Reprod Med. 2009;27:403–8.
44. Russell LB, Hunsicker PR, Shelby MD. Melphalan, a second chemical for which specific-locus mutation induction in the mouse is maximum in early spermatids. Mutat Res. 1992;282:151–8.
45. Yajnik CS. Early life origins of insulin resistance and type 2 diabetes in India and other Asian countries. J Nutr. 2004;134:205–10.
46. Kukreja A, Maclaren NK. NKT cells and type-1 diabetes and the “hygiene hypothesis” to explain the rising incidence rates. Diabetes Technol Ther. 2002;4:323–33.
47. Williamson DM. Studies of multiple sclerosis in communities concerned about environmental exposures. J Womens Health (Larchmt). 2006;15:810–14.
48. Gatewood JM, Cook GR, Balhorn R, et al. Isolation of four core histones from human sperm chromatin representing a minor subset of somatic histones. J Biol Chem. 1990;265:20662–6.
49. van der Heijden GW, Derijck AA, Ramos L, et al. Transmission of modified nucleosomes from the mouse male germline to the zygote and subsequent remodeling of paternal chromatin. Dev Biol. 2006;298:458–69.
50. Wu F, Caron C, De Robertis C, et al. Testis-specific histone variants H2AL1/2 rapidly disappear from paternal heterochromatin after fertilization. J Reprod Dev. 2008;54:413–17.
51. Hammoud SS, Nix DA, Zhang H, et al. Distinctive chromatin in human sperm packages genes for embryo development. Nature. 2009;460:473–8.
52. Dunn GA, Bale TL. Maternal high-fat diet effects on third-generation female body size via the paternal lineage. Endocrinology. 2011;152:2228–36.
53. Ghanayem BI, Bai R, Kissling GE, et al. Diet-induced obesity in male mice is associated with reduced fertility and potentiation of acrylamide-induced reproductive toxicity. Biol Reprod. 2010;82:96–104.
54. Kasturi SS, Tannir J, Brannigan RE. The metabolic syndrome and male infertility. J Androl. 2008;29:251–9.
55. Ng SF, Lin RC, Laybutt DR, et al. Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. Nature. 2010;467:963–6.
56. Aitken RJ, Koopman P, Lewis SE. Seeds of concern. Nature. 2004;432:48–52.

Gamete/Embryo-Fetal Origins of Cardiovascular Diseases

5

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Abstract

Cardiovascular-related diseases are the leading cause of death in the world. The embryo-fetal origins of disease hypothesis asserts that pre-natal and early postnatal life exposures are critical in determining adult health because they are said to program organ function for life [1]. Embryo-fetal origins are an important potential explanation of cardiovascular diseases, which proposes that a stimulus or insult acting during critical periods of growth and development may permanently alter tissue structure and function [2]. Epidemiological investigations clearly demonstrate close relationships between the intrauterine environment, and, adult cardiovascular diseases of the offspring. Animal experiments confirm this relationship and suggest some possible mechanisms. In this chapter, we will discuss the evidence related to embryo-fetal origins of cardiovascular diseases.

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5.1 Common Cardiovascular Diseases

5.1.1 Hypertension

Hypertension is a major risk factor for stroke, myocardial infarction, heart failure, aneurysms, peripheral artery disease and is a cause of chronic kidney disease. There is a substantial literature showing that birthweight is associated with differences in blood pressure within normal range [3, 4]. The effects of early life exposure to famine on subsequent cardiovascular functions in childhood and adulthood have been derived from the Dutch Famine in World War II [5–7], and, the Chinese Great Famine in 1959–1961 [8]. These studies show that low birthweight is associated with high blood pressure both in children and adults, however, the differences in blood pressure in children and young adults are small. These small differences may progress to larger differences as vulnerable individual age. The contrast between this small effect of birthweight on blood pressure in children and the larger effect on later hypertension may reflect amplification of the low birth weight effect with aging.

Several hypotheses of hypertension originate from alterations in embryo-fetal nutrition. Unbalanced maternal nutrition might lead to premature activation of the maternal-fetal hypothalamic-pituitary-adrenal axis (HPA axis) and increased fetal cortisol levels that may alter the expression of the glucocorticoid receptor [9]. These alterations may influence growth and maturation of fetal organs, preterm delivery, the long-term functions of many organs, and may increase cardiovascular disease risk in adult [10]. Another hypothesis suggests that hypertension may result from the reduced number of nephrons in the kidneys of people who were small at birth [11]. In humans, fetal growth restriction is associated with reduced nephron numbers, reduced renal volume, and fewer glomeruli with increased glomerular volume [12]. Loss of glomeruli accompanying normal aging occurs in people born with a relatively low number of nephrons; accelerated glomerular loss occurs, along with a self-perpetuating cycle of rising blood pressure. Reduced glomerular numbers were also observed in animal models where fetal growth was constrained [13].

5.1.2 Coronary Heart Disease

Coronary heart disease (CHD) is the narrowing or blockage of coronary arteries, usually caused by atherosclerosis. Atherosclerosis is the buildup of cholesterol and fatty deposits on the inner walls of the arteries. These plaques can restrict blood flow to the heart muscle by physically clogging the artery or by causing abnormal arterial tone and function.

Accumulating evidence suggests that CHD may originate during embryo-fetal development [1, 14–17]. In 1986, Barker et al. found that the geographic pattern of death rates among babies in Britain during the early 1900s was similar to the pattern of death rates from coronary heart disease in 1980s [14]. They showed that those, who had low birthweight and weight at 1 year, had increased risk of later

cardiovascular disease [1], especially in those who became obese [16]. On the other hand, a cohort study of 4,630 men who were born in Helsinki, Finland, suggests that those who were smaller at birth, remained small in infancy, but had accelerated weight gain and BMI thereafter (catch-up growth), were more likely to develop coronary heart disease later in life [17, 18].

5.2 Pathophysiological Alteration

5.2.1 Endothelial Dysfunction

Endothelial function describes the ability of flow-regulating arterioles to dilate under the stimulation of endothelial-cell-derived vasoactive mechanisms. These include release of nitric oxide and vasodilatory prostaglandins, as well as functional hyperpolarising factors. Endothelial dysfunction, or the loss of proper endothelial function, is a hallmark of vascular diseases, and is often regarded as a key early event in the development of atherosclerosis. Impaired endothelial function, causing hypertension and thrombosis, is often seen in patients with CHD, diabetes mellitus, hypertension. Endothelial dysfunction is predictive of future adverse cardiovascular events. Several studies have been designed to determine whether adults that were undergrown as fetuses have persistently impaired endothelial function. Using forearm plethysomography techniques, McAllister et al. [19] found evidence of endothelial injury in adults that were undergrown at birth based upon increased plasma von Willebrand factor, though did not find a deficit in the vasodilatory acetylcholine response. Studies in adult rats have also shown an association of either maternal protein restriction or reduced uterine blood flow during prenatal life with endothelial dysfunction in offspring [20]. However, in the rat model of nutritional imbalance, the offspring of rat fed an imbalanced diet during pregnancy later had elevated blood pressure, increased responses to salt loading, as well as reduced vasodilator function in the systemic arteries [21].

Martin and colleagues [22] tested endothelial function in nine small babies (birth weight less than 2.5 kg) at 1 week of age. They showed a 2.4-fold increase in flow above baseline compared to a 6.5-fold increase for 10 babies of normal weight after administration of acetylcholine through the skin by iontophoresis, suggesting that low birth weight babies show signs of endothelial dysfunction. These studies indicate that global endothelial function is compromised in humans and animals that were stressed during fetal life, or, undergrown at birth, and, compromised endothelial function may lead to increased risk for coronary disease [23].

5.2.2 Alteration in the Number of Cardiomyocytes

The number of cardiomyocytes is important because it influences the final architecture of the coronary arterial tree. If the number of cardiomyocytes is greatly reduced, the size of the myocytes will be enhanced to accommodate the size of heart that is

needed to discharge the pump function of the adult heart. However, fewer large cells mean fewer capillaries and thus a myocardium that is potentially more vulnerable to ischaemic damage than normal.

Li et al. [24] reported that rat pups, subject to hypoxic conditions during gestation, have fewer but larger cardiomyocytes than pups exposed to normal oxygen levels, and, are more susceptible to infarction during periods of ischaemia and reperfusion as adults. These data indicate that prenatal hypoxic stress potently alters the growth and maturation of cardiomyocytes and reduces cardiomyocyte number, making the offspring more vulnerable to cardiovascular heart disease.

In severe cases of intrauterine growth restriction, placental vascular resistance increases with gestational age rather than decreasing as in normal development. Thus babies born under intrauterine stress may develop under the influence of an increased systolic and or diastolic pressure load. The studies based on human and animal models suggest that the number of fetal cardiomyocytes may decrease when they are shocked during fetal development, which may influence heart development in infants and adults.

5.3 Embryonic and Fetal Exposures

5.3.1 Periconceptional or Perimplantational Undernutrition

A rat model where a maternal low-protein diet (LPD) was administered exclusively during the 4 days of preimplantation followed by a control diet thereafter, is often used to study the effects of intrauterine undernutrition on the cardiovascular functions of offspring [25]. With this rat model, Kwong et al. compared LPD rats with an isocaloric normal protein diet (NPD), and found that LPD-induced hyperglycaemia and altered numbers of blastocyst cells, led to long-term changes in peri- and post-natal growth. There was increased risk of cardiovascular disease in offspring, notably hypertension. In a similar mouse model, another study demonstrated that LPD caused adult hypertension along with alterations in postnatal growth [26]. The effect of LPD before or after mating, specifically during the 3.5-day period of oocyte maturation period, resulted in a similar pattern of postnatal cardio-metabolic abnormalities [27].

The rise in systolic blood pressure, evident in both male and female offspring following maternal LPD in rat or mouse during periconception, or oocyte maturation, is approximately 3 % [27]. Such an increase at population level is quite substantive; if manifest, for instance, within the human population in the UK, it would be equivalent to a 9 % rise in cardiovascular disease and about two million more people diagnosed as clinically hypertensive in the population [28]. The mechanisms which might link early protein-restriction nutritional deficit with such a significant adverse cardiovascular outcome may include attenuated vasodilatation and increased lung angiotensin-converting enzyme activity [29].

The sheep model has also emerged as a critical model of the mechanisms underlying developmental programming that have clear correlation with adult health

while assessing the effect of maternal periconceptional undernutrition [30]. This dietary regimen usually leads to adult cardiovascular dysfunction. Targeting maternal undernutrition to the post-fertilization period (Days 1–30) resulted in elevated pulse pressure and altered baroreflex response to angiotensin II in young adults, indicating that periconceptional undernutrition may program long-term cardiovascular dysfunction that ultimately increases the risk of hypertension in later life [30]. Pre- or peri-conceptional undernutrition also displayed increased vasoconstriction, or attenuated vasodilatation in the offspring of adult sheep, with the challenge affecting formation of vascular beds [31].

Maternal, LPD-fed during the pre-implantation period (0–4.25 days after mating, before return to control diet for the remainder of gestation) induced programming of altered birth weight, postnatal growth rate, hypertension, and organ/body weight ratios in either male or female offspring up to 12 weeks of age [25]. Further study found that the methylation of imprinted genes was involved in these outcomes [32]. The conclusion was confirmed by other researchers who found maternal LPD-fed leads to offspring with increased weight from birth, sustained hypertension, and abnormal anxiety-related behavior, especially in females [26]. A further study found this diet induced vascular dysfunction, and, elevated serum and lung angiotensin-converting enzyme (ACE) activity in female and male offspring. Elevated systolic blood pressure in offspring is associated with impaired arterial vasodilatation and elevated serum and lung ACE activity [29]. Another study in sheep also found elevated offspring blood pressure after maternal restricted periconceptional nutrition [33]. Moreover, studies on pre- and peri-conceptional maternal undernutrition show greater vasoconstrictor responses in adult offspring. Endothelium-dependent and -independent vasodilatation was attenuated in pre- and periconceptional groups compared with controls [31].

Maternal obesity also contributes to cardiovascular alterations in offspring. Embryo culture from two-cell to blastocyst stages in T6 medium led to increased systolic blood pressure at 21 weeks compared with in vivo development [34]. Moreover, activity of some regulators of cardiovascular and metabolic physiology was significantly elevated in response to embryo culture and/or ET in female offspring at 27 weeks [34].

5.3.2 Low Birth Weight

Other observations support the fetal origins hypothesis of cardiovascular disease. The associations between birthweight and later disease extend across the whole range of birthweight, and, are not confined to neonates with pathologically low birth weight [1, 3, 4]. This observation suggests that normal variations in the supply of nutrients to the fetus may have important effects on the health of the next generation. Underlying this finding is the idea that increased risks for low birthweight are not the long-term consequences of reduced total body size but rather the effects of reduced allocation of resources on the development of key systems including the vasculature, and, key organs including heart and kidney.

Martin et al. reported that, at 3 days of age, forearm skin vasodilation responses to local application of Ach in SGA newborns, was significantly attenuated compared to normal birthweight babies (240 % compared with a 650 % increase respectively, in blood flow measured by laser Doppler) [35]. Former low-birthweight children at 9 years of age had impaired endothelium-dependent vasodilation (in response to local application of ACh), but not in response to the NO donor, sodium nitroprusside, (an endothelium-independent vasodilator) [35].

5.3.3 Hypoxia

Hypoxia is critical for proper myocardial formation. There are several causes for hypoxia, such as high altitude, pre-existing maternal illness, pre-eclampsia, cord compression, smoking, pollution, hemoglobinopathy, and aberrant placental development. Prenatal hypoxia may alter myocardial structure and causes a decline in cardiac performance. Not only are the effects of hypoxia apparent during the perinatal period, but prolonged hypoxia in utero, also causes fetal programming of abnormality in cardiac development. Altered expression of cardioprotective genes such as protein kinase C epsilon, heart shock protein 70, and endothelial nitric oxide synthase, may predispose the developing heart to increased vulnerability to ischaemia and reperfusion injury later in life [36].

Furthermore, tissue hypoxia alters gene expression. Hypoxia stabilizes HIF-1, -2 and -3, a family of transcription factors that play central roles in cellular adaptation to insufficient oxygen, which sheds new light on the role of hypoxia in fetal heart maturation. Hypoxia promotes HIF-induced, up-regulation of erythropoietin (EPO), which stimulates erythropoiesis, inhibits apoptosis, and mobilizes endothelial progenitors for vessel growth by binding to EPO receptors [37]. Although it is not fully understood how HIF-1 dependent mechanisms coordinate in cardiogenesis, it is known that HIF-1 expression is vital for proper myocardial remodeling and coronary vessel formation. Intrauterine hypoxia increases HIF-1 expression, which was also found in the nuclei of avian cardiomyocytes undergoing apoptosis [37–39] implying that HIF-1 activity is involved in orchestrating the fate of avian cardiomyocytes.

Hypoxic stress increases vessel formation in fetal heart tissue, while hypoxia delays vessel growth [40]. Though fetal heart shows remarkable ability to survive and function under low oxygen tensions, chronic pathophysiological hypoxia is associated with numerous complications that have both short and long-term effects. Epidemiological studies indicate that pregnancies at high altitudes increase the risk of intrauterine growth restriction and low birth weight [41]. These factors are known to cause preterm birth, infant mortality, and an increased risk of developing cardiovascular-related diseases [42].

Increasing evidence indicates that the adverse effects of prolonged hypoxia in utero are substantial. Studies have found that insufficient oxygen in utero produces myocardial thinning, ventricular dilatation, and epicardial detachment [43]. Other studies demonstrate cardiomyocyte hypertrophy and myocardial hypoplasia in fetal hearts subject to chronic hypoxia.

Intrauterine stress via hypoxia induces changes in fetal heart morphology, and function. In a study examining the significance of catecholamines in development, researchers reported hypoxia decreases the heart rate of fetal mice by 35–40 % in culture and by 20 % in utero when compared to wild-type hearts [44]. Possible explanations include decreased Mg^{2+}-activated myofibrillar ATPase activity [45].

Epigenetics has been implicated in fetal development as well as tumorigenesis. Epigenetic modifications alter gene expression patterns in the long-term. Zhang et al. showed that cocaine exposure in utero increases hypermethylation of CpG dinucleotides of SP1 binding sites for PKCξgene in left ventricles of 3-month-old rats [46]. Meyer et al. used DNA methylation inhibitors 5-aza-2-dexycytine and procainamide, to block cocaine-mediated down-regulation of PKCξ [47]. These findings link epigenetics via DNA methylation in utero with an increased susceptibility to coronary heart disease in later life. The similarities between prenatal hypoxia and cocaine exposure are striking, and it is possible that the underlying mechanisms are concurrent [48].

5.3.4 Prenatal Nutrient Imbalance

Prenatal nutrient imbalance can be induced by reducing overall maternal food intake or by protein restriction in an isocaloric diet, or, glucocorticoid exposure without any change in diet. In offspring of rats subject to a 50 % caloric reduction, blood pressure is significantly increased at 4 weeks of age [49]. In female adult offspring of rats fed an low protein diet for 14 days prior to mating and for the whole of gestation, systolic BP appears to decrease progressively from 9 to 21 weeks of age when the difference between the low protein and the control groups was minimal [49].

Much attention has been focused on fetal undernutrition, as a facilitator of predisposition to later disease, but there is evidence that excessive energy supply to the fetus or infant also has adverse consequences. Maternal hyperglycemia, for example, may lead to fetal hyperinsulinemia. Offspring of obese women, or, women with diabetes are at greater risk of developing metabolic disorders themselves, including hypertension and cardiovascular heart disease in adults [50].

5.3.5 Maternal Smoking

Maternal smoking is one of the most important modifiable risk factors for low birth weight [51]. Several studies have shown that blood pressure is higher among children of mothers who smoked during pregnancy [52]. Smoking during pregnancy will decrease oxygenation of the maternal-fetal unit, associated with complications of pregnancy such as fetal apnea, increased carboxyhemoglobin levels, increased placenta-to-birthweight ratios, placenta previa and abruptio placentae, and reduced uterine and placental blood flow [53]. The association between smoking during pregnancy and elevated childhood blood pressure suggests an increase in peripheral vascular resistance which leads to decreased blood flow and oxygen delivery.

Stopping smoking during early pregnancy may prevent the adverse effects on the blood pressure of offspring [52].

A long term follow-up study showed that fetal exposure to maternal smoking is associated with an increased rise in total cholesterol levels and appears to lead to an adverse lipoprotein profile [54]. Fetal exposure to maternal smoking may also negatively affect the vascular wall, and, eventually lead to atherosclerosis [55].

5.3.6 Preterm Delivery

Siewert-Delle and Ljungman investigated 430, 49-year-old Swedish men and found that adult blood pressure appears to be related to different variables at different stages of gestation [56]. In preterm subjects, gestational age appeared to have a significant impact on adult blood pressure, and every additional week of gestational at birth was associated with a decrease in adult systolic BP of 7.2 mmHg. However, in subjects born at term or later, adult blood pressure was not correlated with birthweight, but only with adult BMI [56]. In women, former preterm adolescents and young adults have higher BP, an increased resistance in the vascular tree and abnormal retinal vascularization, after puberty [57]. These findings may have implications for future cardiovascular risk in the growing adult population surviving preterm birth.

5.3.7 Exposure to Glucocorticoids

Glucocorticoids are associated with long-term organizational effects; glucocorticoid treatment during pregnancy has been shown to reduce birthweight in animals and humans [58]. In rats, antenatal dexamethasone treatment leads to hypertension, elevated hepatic gluconeogenic enzymes and impaired glucose tolerance in adult offspring [59]. In humans, glucocorticoids are widely used in the management of women at risk of preterm delivery to enhance fetal lung maturation, and, in the antenatal management of fetuses at risk of congenital adrenal hyperplasia. Human studies have confirmed that antenatal glucocorticoid administration is associated with a reduction in birth weight [60], which also is linked with high blood pressure in adolescence [61].

5.3.8 Exposure to Endocrine-Disrupting, Environmental Pollutants

Prenatal exposure to common environmental pollutants which have endocrine activity (‘endocrine disruptors’) has been shown in animal studies to result in cardiovascular disease in later life [62]. Chemicals shown to have this effect include oestrogenic compounds such as diethylstilbestrol (DES, used in the past to prevent miscarriage), bisphenol A (BPA, a weak oestrogenic component of

polycarbonate plastic used in food containers), polychlorinated biphenyls (PCBs, used in electrical equipment), dichlorodiphenyl dichloroethene (DDE, a breakdown product of the pesticide DDT) and phytoestrogens (derived from soya products) [62].

5.4 Mechanisms by Which Cardiovascular Disease Is Programmed in Utero

Fetal origins of cardiovascular disease may help to understand the underlying mechanisms for chronic diseases, such as hypertension, coronary heart disease, etc. Studies have indicated that the pathogenesis of cardiovascular disease and related disorders depends on a series of interactions that occur at different stages of development. To begin with, the effect of the genes acquired at conception may be conditioned by early intrauterine environment [63, 64].

5.4.1 Hypothalamo-Pituitary-Adrenal Axis Regulation

Pregnancy results in major changes in the hypothalamo-pituitary-adrenal axis, which, in turn, influences fetal growth and the timing of labor [65]. From the beginning of the second trimester maternal cortisol secretion increases, and in late pregnancy the placenta, in large part mediated through corticotroph-releasing hormone, plays a crucial role in the regulation of the fetal HPA axis to ensure the synchronization of different processes involved in parturition.

5.4.2 Molecular Changes

Fetal undernutrition and fetal glucocorticoid overexposure may be linked by common mechanisms. The enzyme, 11β-hydroxysteroid dehydrogenase type 2, converts active glucocorticoids to inactive products, and is present in the placenta [66]. This enzyme may act as a barrier to protect the fetus from maternal glucocorticoids. Reduced placental 11β-hydroxysteroid dehydrogenase type 2 gene expression has been reported in human pregnancies complicated by intrauterine growth retardation [66]. Thus, maternal malnutrition potentially exerts programming effects by inducing fetal overexposure to the effects of maternal glucocorticoids.

5.4.3 Mitochondrial Dysfunction

Mitochondria are double-membrane organelles that perform multiple intracellular functions, most notably the production of ATP as an energy source, and the generation of reactive oxygen species [67]. Exposure to maternal undernutrition is associated with a decrease in mitochondrial respiration in kidneys of adult

rats, and leads to increased oxidative stress and/or a dysfunctional fuel supply in mitochondria [68]. These findings may explain the long-term impact of the maternal diet in offspring.

5.4.4 Sex-Specific Effects

A number of sex-specific effects have been described in animal models of fetal programming [69], with females appearing to be more sensitive to some programming effects. Human studies have revealed some sex differences in long-term disease risks associated with low birth weight or exposure to famine prenatally [70]. Such sex-specific effects may represent the differential sensitivity of males and females to programming phenomena with stronger programming in females amplifying the matrilineal pattern of intergenerational inheritance.

5.4.5 Epigenetic Changes

Several studies indicate that impaired fetal growth and in utero exposure to risk factors may be relevant for the early onset of cardiovascular damage. Epigenetics may represent a possible explanation of the impact of such intrauterine risk factors for the subsequent development of cardiovascular diseases during adulthood. Different epigenetic effects that change in utero environment depend on the gestational age.

Examples of epigenetic mechanisms include coordinated changes in the methylation of cytidine-guanosine (CpG) nucleotides in the promoter regions of specific genes, changes in chromatin structure through histone acetylation and methylation, and post-transcriptional control by microRNA [71]. Epigenetic modifications are gene-specific and cell-type-specific. Since only a small set of enzymes is involved in making these modifications, it is likely that this specificity is directed by interactions between DNA and small RNA molecules. Developmentally-induced epigenetic modifications of DNA are generally stable during the mitotic cell divisions that continue throughout a lifetime. Challenges during pregnancy or neonatal life in experimental models of programming result in changes in promoter methylation and thus directly, or indirectly, affect gene expression in pathways associated with a range of physiologic processes. The effects of maternal nutrition and behaviour clearly target the promoter regions of specific genes rather than being associated with global changes in DNA methylation.

5.4.6 Intergenerational Influences

Increasing evidence shows that the programmes targeting adult life, that relate to the health, growth and development, in fetuses exposed to adverse environments can be transmitted from one generation to the next generation [72]. The developmental cue is not limited to the nutritional environment during the period of gestation; rather,

the information passed to the fetus or neonate from conception to weaning is a summation of maternal nutritional experience, integrating a lifetime of signals from the mother, and perhaps even the grandmother [73]. For example, in rat models, exposure during pregnancy to a low-protein diet results in elevated blood pressure and endothelial dysfunction in first generation (F1). These changes can be transmitted to the secondary generation (F2) without further challenge to members of the F1 generation [74]. The mechanism of intergenerational transfer is unclear. Potential explanation for intergenerational effects include: (1) that genetic attributes may manifest themselves similarly in mother and offspring; (2) that adverse extrinsic environmental conditions may persist across generations; and (3) that adverse in utero experiences may permanently affect maternal growth and development, altering her metabolism in such a way as to provide an adverse environment for her fetus. This last hypothesis suggests a mechanism by which programming effects may be self-perpetuating through several generations.

5.5 Conclusions

There is evidence from animal and human research that nutrient imbalance, hypoxia and other exposures during pre-natal development have lifelong effects on the pathways that regulate endothelial function, and, numbers of myocardiocytes. These may induce cardiovascular diseases. Both human models and animal models, maternal nutrient imbalance, hypoxia, chemical exposure, fetal gluco-corticoid exposure, have all been shown to cause increased cardiovascular diseases in the offspring, with transmission to the F2 offspring. These are examples of "programming", whereby transient exposures occurring during critical periods of early development, permanently alter gene expression and the lifelong structural and metabolic phenotype. They represent non-genetic mechanisms for inter-generational (mother-offspring) pathways of disease risk.

References

1. Barker DJ, Gluckman PD, Godfrey KM, et al. Fetal nutrition and cardiovascular disease in adult life. Lancet. 1993;341:938–41.
2. McCance RA. Food, growth, and time. Lancet. 1962;2:671–6.
3. Gamborg M, Byberg L, Rasmussen F, et al. Birth weight and systolic blood pressure in adolescence and adulthood: meta-regression analysis of sex- and age-specific results from 20 Nordic studies. Am J Epidemiol. 2007;166:634–45.
4. Hardy R, Kuh D, Langenberg C, et al. Birthweight, childhood social class, and change in adult blood pressure in the 1946 British birth cohort. Lancet. 2003;362:1178–83.
5. Roseboom TJ, van der Meulen JH, Ravelli AC, et al. Blood pressure in adults after prenatal exposure to famine. J Hypertens. 1999;17:325–30.
6. Painter RC, de Rooij SR, Bossuyt PM, et al. Early onset of coronary artery disease after prenatal exposure to the Dutch famine. Am J Clin Nutr. 2006;84:322–7.
7. Stein AD, Zybert PA, van der Pal-de Bruin K, et al. Exposure to famine during gestation, size at birth, and blood pressure at age 59 y: evidence from the Dutch Famine. Eur J Epidemiol. 2006;21:759–65.

8. Wang PX, Wang JJ, Lei YX, et al. Impact of fetal and infant exposure to the Chinese great famine on the risk of hypertension in adulthood. PLoS One. 2012;7:e49720.
9. Kapoor A, Dunn E, Kostaki A, et al. Fetal programming of hypothalamo-pituitary-adrenal function: prenatal stress and glucocorticoids. J Physiol. 2006;572(Pt 1):31–44.
10. Kajantie E. Fetal origins of stress-related adult disease. Ann N Y Acad Sci. 2006;1083:11–27.
11. Brenner BM, Chertow GM. Congenital oligonephropathy: an inborn cause of adult hypertension and progressive renal injury? Curr Opin Nephrol Hypertens. 1993;2:691–5.
12. Spencer J, Wang Z, Hoy W. Low birth weight and reduced renal volume in Aboriginal children. Am J Kidney Dis. 2001;37:915–20.
13. Briscoe TA, Rehn AE, Dieni S, et al. Cardiovascular and renal disease in the adolescent guinea pig after chronic placental insufficiency. Am J Obstet Gynecol. 2004;191:847–55.
14. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet. 1986;1:1077–81.
15. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet. 2004;363:1642–5.
16. Frankel S, Elwood P, Sweetnam P, et al. Birthweight, body-mass index in middle age, and incident coronary heart disease. Lancet. 1996;348:1478–80.
17. Eriksson JG, Forsén T, Tuomilehto J, et al. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ. 1999;318:427–31.
18. Eriksson JG, Forsén T, Tuomilehto J, et al. Early growth and coronary heart disease in later life: longitudinal study. BMJ. 2001;322:949–53.
19. McAllister AS, Atkinson AB, Johnston GD, et al. Relationship of endothelial function to birth weight in humans. Diabetes Care. 1999;22:2061–6.
20. Payne JA, Alexander BT, Khalil RA. Reduced endothelial vascular relaxation in growth-restricted offspring of pregnant rats with reduced uterine perfusion. Hypertension. 2003;42:768–74.
21. Woods LL, Ingelfinger JR, Nyengaard JR, et al. Maternal protein restriction suppresses the newborn renin-angiotensin system and programs adult hypertension in rats. Pediatr Res. 2001;49:460–7.
22. Martin H, Gazelius B, Norman M. Impaired acetylcholine-induced vascular relaxation in low birth weight infants: implications for adult hypertension? Pediatr Res. 2000;47:457–62.
23. Takase B, Uehata A, Akima T, et al. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. Am J Cardiol. 1998;82: 1535–9. A7-8.
24. Li G, Xiao Y, Estrella JL, et al. Effect of fetal hypoxia on heart susceptibility to ischemia and reperfusion injury in the adult rat. J Soc Gynecol Investig. 2003;10:265–74.
25. Kwong WY, Wild AE, Roberts P, et al. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. Development. 2000;127:4195–202.
26. Watkins AJ, Ursell E, Panton R, et al. Adaptive responses by mouse early embryos to maternal diet protect fetal growth but predispose to adult onset disease. Biol Reprod. 2008;78:299–306.
27. Watkins AJ, Wilkins A, Cunningham C, et al. Low protein diet fed exclusively during mouse oocyte maturation leads to behavioural and cardiovascular abnormalities in offspring. J Physiol. 2008;586:2231–44.
28. Lenfant C, Chobanian AV, Jones DW, et al. Seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7): resetting the hypertension sails. Hypertension. 2003;41:1178–9.
29. Watkins AJ, Lucas ES, Torrens C, et al. Maternal low-protein diet during mouse pre-implantation development induces vascular dysfunction and altered renin-angiotensin-system homeostasis in the offspring. Br J Nutr. 2010;103:1762–70.
30. Gardner DS, Pearce S, Dandrea J, et al. Peri-implantation undernutrition programs blunted angiotensin II evoked baroreflex responses in young adult sheep. Hypertension. 2004;43:1290–6.
31. Torrens C, Snelling TH, Chau R, et al. Effects of pre- and periconceptional undernutrition on arterial function in adult female sheep are vascular bed dependent. Exp Physiol. 2009;94: 1024–33.

32. Kwong WY, Miller DJ, Ursell E, et al. Imprinted gene expression in the rat embryo-fetal axis is altered in response to periconceptional maternal low protein diet. Reproduction. 2006;132:265–77.
33. Edwards LJ, McMillen IC. Periconceptional nutrition programs development of the cardiovascular system in the fetal sheep. Am J Physiol Regul Integr Comp Physiol. 2002;283:R669–79.
34. Watkins AJ, Platt D, Papenbrock T, et al. Mouse embryo culture induces changes in postnatal phenotype including raised systolic blood pressure. Proc Natl Acad Sci U S A. 2007;104:5449–54.
35. Martin H, Hu J, Gennser G, et al. Impaired endothelial function and increased carotid stiffness in 9-year-old children with low birthweight. Circulation. 2000;102:2739–44.
36. Patterson AJ, Zhang L. Hypoxia and fetal heart development. Curr Mol Med. 2010;10:653–66.
37. Jiang BH, Rue E, Wang GL, et al. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J Biol Chem. 1996;271:17771–8.
38. Arcasoy MO. The non-haematopoietic biological effects of erythropoietin. Br J Haematol. 2008;141:14–31.
39. Sugishita Y, Leifer DW, Agani F, et al. Hypoxia-responsive signaling regulates the apoptosis-dependent remodeling of the embryonic avian cardiac outflow tract. Dev Biol. 2004;273:285–96.
40. Yue X, Tomanek RJ. Stimulation of coronary vasculogenesis/angiogenesis by hypoxia in cultured embryonic hearts. Dev Dyn. 1999;216:28–36.
41. Moore LG. Fetal growth restriction and maternal oxygen transport during high altitude pregnancy. High Alt Med Biol. 2003;4:141–56.
42. Giussani DA, Camm EJ, Niu Y, et al. Developmental programming of cardiovascular dysfunction by prenatal hypoxia and oxidative stress. PLoS One. 2012;7:e31017.
43. Ream M, Ray AM, Chandra R, et al. Early fetal hypoxia leads to growth restriction and myocardial thinning. Am J Physiol Regul Integr Comp Physiol. 2008;295:R583–95.
44. Portbury AL, Chandra R, Groelle M, et al. Catecholamines act via a beta-adrenergic receptor to maintain fetal heart rate and survival. Am J Physiol Heart Circ Physiol. 2003;284:H2069–77.
45. Onishi J, Browne VA, Kono S, et al. Effects of long-term high-altitude hypoxia and troponin I phosphorylation on cardiac myofilament calcium responses in fetal and nonpregnant sheep. J Soc Gynecol Investig. 2004;11:1–8.
46. Zhang H, Meyer KD, Zhang L. Fetal exposure to cocaine causes programming of Prkce gene repression in the left ventricle of adult rat offspring. Biol Reprod. 2009;80:440–8.
47. Meyer K, Zhang H, Zhang L. Direct effect of cocaine on epigenetic regulation of PKCepsilon gene repression in the fetal rat heart. J Mol Cell Cardiol. 2009;47:504–11.
48. Franco Mdo C, Dantas AP, Akamine EH, et al. Enhanced oxidative stress as a potential mechanism underlying the programming of hypertension in utero. J Cardiovasc Pharmacol. 2002;40:501–9.
49. Langley SC, Jackson AA. Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. Clin Sci (Lond). 1994;86:217–22.
50. Hillier TA, Pedula KL, Schmidt MM, et al. Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. Diabetes Care. 2007;30:2287–92.
51. Suter MA, Anders AM, Aagaard KM. Maternal smoking as a model for environmental epigenetic changes affecting birthweight and fetal programming. Mol Hum Reprod. 2013;19:1–6.
52. Brion MJ, Leary SD, Lawlor DA, et al. Modifiable maternal exposures and offspring blood pressure: a review of epidemiological studies of maternal age, diet, and smoking. Pediatr Res. 2008;63:593–8.
53. Abel EL. Smoking and pregnancy. J Psychoactive Drugs. 1984;16:327–38.
54. Jaddoe VW, de Ridder MA, van den Elzen AP, et al. Maternal smoking in pregnancy is associated with cholesterol development in the offspring: a 27-years follow-up study. Atherosclerosis. 2008;196:42–8.
55. Geerts CC, Bots ML, Grobbee DE, et al. Parental smoking and vascular damage in young adult offspring: is early life exposure critical? The atherosclerosis risk in young adults study. Arterioscler Thromb Vasc Biol. 2008;28:2296–302.

56. Siewert-Delle A, Ljungman S. The impact of birth weight and gestational age on blood pressure in adult life: a population-based study of 49-year-old men. Am J Hypertens. 1998;11(8 Pt 1):946–53.
57. Bonamy AK, Bendito A, Martin H, et al. Preterm birth contributes to increased vascular resistance and higher blood pressure in adolescent girls. Pediatr Res. 2005;58:845–9.
58. Newnham JP. Is prenatal glucocorticoid administration another origin of adult disease? Clin Exp Pharmacol Physiol. 2001;28:957–61.
59. Cleasby ME, Kelly PA, Walker BR, et al. Programming of rat muscle and fat metabolism by in utero overexposure to glucocorticoids. Endocrinology. 2003;144:999–1007.
60. French NP, Hagan R, Evans SF, et al. Repeated antenatal corticosteroids: size at birth and subsequent development. Am J Obstet Gynecol. 1999;180(1 Pt 1):114–21.
61. Doyle LW, Ford GW, Davis NM, et al. Antenatal corticosteroid therapy and blood pressure at 14 years of age in preterm children. Clin Sci (Lond). 2000;98:137–42.
62. Lubick N. Cardiovascular health: exploring a potential link between BPA and heart disease. Environ Health Perspect. 2010;118:A 116.
63. Sun C, Burgner DP, Ponsonby AL, et al. Effects of early life environment and epigenetics on cardiovascular disease risk in children: highlighting the role of twin studies. Pediatr Res. 2013;73:523–30.
64. Abitbol CL, Rodriguez MM. The long-term renal and cardiovascular consequences of prematurity. Nat Rev Nephrol. 2012;8:265–74.
65. Bertram C, Khan O, Ohri S, et al. Transgenerational effects of prenatal nutrient restriction on cardiovascular and hypothalamic-pituitary-adrenal function. J Physiol. 2008;586:2217–29.
66. McTernan CL, Draper N, Nicholson H, et al. Reduced placental 11beta-hydroxysteroid dehydrogenase type 2 mRNA levels in human pregnancies complicated by intrauterine growth restriction: an analysis of possible mechanisms. J Clin Endocrinol Metab. 2001;86:4979–83.
67. Patti ME, Corvera S. The role of mitochondria in the pathogenesis of type 2 diabetes. Endocr Rev. 2010;31:364–95.
68. Engeham S, Mdaki K, Jewell K, et al. Mitochondrial respiration is decreased in rat kidney following fetal exposure to a maternal low-protein diet. J Nutr Metab. 2012;2012:989037.
69. Liu L, Li A, Matthews SG. Maternal glucocorticoid treatment programs HPA regulation in adult offspring: sex-specific effects. Am J Physiol Endocrinol Metab. 2001;280:E729–39.
70. Walker BR, Irving RJ, Andrew R, et al. Contrasting effects of intrauterine growth retardation and premature delivery on adult cortisol secretion and metabolism in man. Clin Endocrinol (Oxf). 2002;57:351–5.
71. Goldberg AD, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. Cell. 2007;128:635–8.
72. Vaag AA, Grunnet LG, Arora GP, et al. The thrifty phenotype hypothesis revisited. Diabetologia. 2012;55:2085–8.
73. Kuzawa CW. Fetal origins of developmental plasticity: are fetal cues reliable predictors of future nutritional environments? Am J Hum Biol. 2005;17:5–21.
74. Burdge GC, Slater-Jefferies J, Torrens C, et al. Dietary protein restriction of pregnant rats in the F0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F1 and F2 generations. Br J Nutr. 2007;97:435–9.

Gamete/Embryo-Fetal Origins of Tumours

6

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Abstract

Epidemiological and animal studies indicate that carcinogenesis may start as early as the prenatal period. Modifying the prenatal environment may alter genes through the epigenetic route, and, these alterations may be inherited by the offspring. Epigenetic factors like nutritional factors, endocrine disruptors, infection and lifestyle may affect tumour development, or, target cell differentiation to increase susceptibility to cancer. In this chapter, we discuss the evidence related to embryo-fetal origins of tumours.

6.1 Breast Cancer

The mammary gland has three phases for growth when developmental events take place that are sensitive to exogenous factors: (a) prenatal period (mammary bud development), (b) peripuberty period (exponential mammary growth), and (c) pregnancy period (differentiating breast) [1]. Oestrogen plays a critical role in breast cancer development across the female lifespan. In 1990, Trichopoulos [2] postulated that breast cancer may originate in utero because the developing fetus is exposed to

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oestrogen levels up to tenfold higher than at any other time in the individual's lifetime. He proposed that exposure of the fetus to high concentrations of oestrogens, or other growth factors, may increase the susceptibility of mammary tissue to undergo tumorigenesis later in life. Since then, researchers have studied many proxy measures of the oestrogenic environment in utero and other perinatal factors associated with risks of breast cancer, including maternal characteristics, maternal nutrients intake, birth characteristics, endocrine-disrupting chemicals (EDC) and other pregnancy-related complications. In utero exposure to those factors may affect breast cancer risk by altering the hormonal environment of the fetus, and, the morphological and functional development of the mammary gland by determining the number of breast progenitor cells [1, 3, 4]. Data is limited about the influence of intrauterine factors on male breast cancer incidence. Reports show no, or at best a weak, association between birth weight and male breast cancer risk [5].

6.1.1 Maternal Age

Older pregnant women have higher serum concentrations of oestrogen [6]. That means fetuses of older mothers face increased oestrogen exposures and may have increasing risk of breast cancer [6]. A 15-year increase in maternal age is associated with a 29 % increase in the risk of breast cancer in daughters [7], and a 25 % increase in the risk adjust for the daughter's age, reproductive history, and other possible confounding factors [7].

Two recent meta-analyses also show that being born to an older mother is associated with higher breast cancer risk [8, 9]. Findings from studies that separately assessed premenopausal and postmenopausal breast cancer also suggest that maternal age is associated with increased risks of breast cancer in daughters. The cut-off above which there is an increased risk of breast cancer ranges from a maternal age of 25–35 [9]. However, findings from the Western New York Exposures and Breast Cancer (WEB) Study indicate that maternal age at delivery is not associated with risk in either pre- or postmenopausal women [10].

6.1.2 Pre-pregnancy BMI and Weight Gain

Increased weight gain during pregnancy is associated with higher levels of oestrogen [11]. Higher, pre-pregnancy, body mass index (BMI) is associated with lower levels of estradiol (E_2), and, with higher insulin and lower insulin like growth factor binding protein 1 (IGFBP1) [12], that may interact with E_2 to promote oncogenesis.

Sanderson et al. [13] reported that women whose mothers gaining 25–34 lb during pregnancy have an increased risk of breast cancer, while there was no indication of increased risk for mothers who gained 35 or more pounds, or, less than 15 lb. However, another study reported that weight gain during pregnancy showed no correlation with increasing a daughters' risk of breast cancer [14].

6.1.3 Maternal Dietary Intake

6.1.3.1 Polyunsaturated Fatty Acid (PUFA)

N-3 and n-6 PUFA are specific dietary factors which play pivotal roles in fatty acid metabolism; n-3 PUFA may have protective effects on tumour development, while n-6 PUFA has a tumour promoting effect [15, 16]. Pregnant women taking n-3 PUFA have elevated estrogen levels that are linked to reduced, rather than increased, breast cancer risk among their offspring [15, 16] suggesting that other effects of n-3 PUFA may counteract the effects of high fetal oestrogenicity on the mammary gland [16]. Studies also show a preventive effect of fish oil supplementation during the perinatal period on subsequent, carcinogen-induced, mammary tumour risk [17].

6.1.3.2 Methyl Nutrients

Maternal, high-dose methyl nutrients, such as lipotropes, intake during pregnancy decrease both expression of DNA methylation-related gene histone deacetylase 1 (Hdac1), and, the risk of developing carcinogen-induced, mammary tumors in female offspring [18]. Studies also show that pups from folic acid supplemented dams showed a lower number of terminal end buds (TEB) than controls; terminal end buds are may relate to increased mammary tumour risk in offspring [19]. On the contrary, in a dimethylbenz[a]anthracene (DMBA) induced breast cancer model, high intrauterine dietary exposure to folic acid may increase the risk of mammary tumours in the offspring through altered DNA methylation and methyltransferase activity [20].

6.1.3.3 Soy Products and Fibre

Soy products contain isoflavones including genistein and daidzein, which have weak oestrogenic effects. Intake during adult life seems to reduce breast cancer risk [21], though exposure to isoflavones or genistein during fetal periods may have opposite effects. The carcinogen-induced, mammary tumorigenesis was increasing in maternal exposures to genistein in female rat offspring [22]. Maternal intake of milk containing high levels of isoflavones also increase mammary carcinogen-induced mammary carcinogenesis in female rat offspring [23]. Maternal exposure to whole wheat during pregnancy may decrease offspring's breast cancer risk by improving DNA damage repair mechanisms [24].

6.1.4 Maternal Lifestyle

6.1.4.1 Smoking

Maternal cigarette smoking causes small reductions in total serum oestrogen levels [25, 26], as well as increased alpha fetoprotein (AFP) and reduced human chorionic gonadatropin (hCG) levels [26, 27]. That means decreased oestrogen exposure for the fetus, and possibly, a decreased breast cancer risk. Data from the National Cooperative DES Adenosis (DESAD) Project suggests fetal exposure to maternal smoking appears to be inversely associated to breast cancer incidence,

especially for women whose mothers smoked 15 cigarettes or fewer per day [28]. Several studies report no significant association between breast cancer risk and maternal smoking [8, 29].

6.1.4.2 Alcohol

Alcohol intake may increase serum estrogen levels by stimulating aromatase activity [30]. An early report indicated no association between alcohol intake and risk of breast cancer in daughters [13]. However, in animal studies, maternal alcohol intake increased female offspring's mammary tumorigenesis by affecting persistently in morphology and gene expression of the fetal mammary gland programming [31–33]. In addition, those animals may develop more tumours with phenotypic characteristics of poor-prognosis breast cancer [31], and, they suggested that alterations in the IGF and E_2 systems may take part in the underlying mechanisms [31–33].

6.1.5 Maternal Illness and Medication Use

Women, who had been exposed to famine in utero, were found to have a higher incidence of breast cancer compared to unexposed women [34]. Daughters, whose mothers had a lifetime history of diabetes, were showed with a decreased risk of breast cancer, and also with the strongest negative association of breast cancer on premenopause being among premenopausal breast cancer [35].

Pregnant women with pre-eclampsia have shorter gestational ages and higher levels of androgens and lower oestrogen levels than women in normal pregnancies due to placental dysfunction and deficient aromatisation of androgens in the placenta [36]. All those factors may play a role in decreasing fetal estrogen exposure, and, may explain decreased risks of breast cancer in offspring [37].

6.1.6 Gestational Age

Oestrogen exposure of the fetus in utero is determined by maternal oestrogen concentrations and gestational age. Data from epidemiological studies suggest that maternal hormones are higher in pregnancies that end prematurely [38, 39]. Meta-analyses found no association between prematurity and breast cancer risk [8, 9]. Protective effect of prematurity for breast cancer risk was reported in one study [40]. However, others have found that women born at $\leq$33 weeks gestation were almost 4 times more likely to develop breast cancer compared with those born at >33 weeks [36]. One possible mechanism is that differentiation of stem cells in human breast tissue cells is inhibited in pre-term fetuses, and, the risk of breast cancer is increased in later life [40, 41]. Low survival rates of pre-term infants may differentiate pre-term infants who survived to adulthood and showed a risk of developing breast cancer [42].

6.1.7 Twin Membership

Dizygotic twins have separate placentae and may be exposed to increased levels of pregnancy-related hormones compared to monozygotic twins or singletons who only have one placenta. However, twins may be delivered preterm to avoid pregnancy-related complications, and, thus be exposed to hormones for shorter times than singletons. Monozygotic twins typically have a shorter gestation than dizygotic twins [43]. Although conflicting results exist, twins, especially dizygotic twins, may represent an increased risk for breast cancer. One recent meta- analysis noted no association between monozygotic twins and breast cancer. Separate analysis of dizygotic twins suggests a significant increased risk of breast cancer. Combined studies of both types of twins show decreased risks of breast cancer, though with marginal statistical significance [9]. Elevated levels of Oestrogens and hCG have been proposed as one possible reason for the association between dizygotic twins and risks of breast cancer [44].

6.1.8 Birth Size

Body size is measured by birth weight, birth length, or head circumference. Birth length is strongly associated with birthweight [45]. Birthweight and birth length are both positively associated with oestrogen concentrations in maternal blood [46]. And both may have similar associations with increased risks of breast cancer [9, 45] . One recent meta-analysis [47] showed a significant effect of breast cancer risk with increased birth weight (>4,000 g) compared with the lowest category of birth weight (<2,500 g) among women of all ages. The results indicate that breast cancer risk increased approximately 7 % per kilogram increase in birthweight, similar to the WCRF report which indicated that breast cancer risk might increase 8 % per kilogram increase in birthweight [48]. The mechanisms underlying the association may include exposure to high levels of oestrogens and elevated levels of growth hormones that may increase the number of susceptible stem cells in the mammary gland [45, 49].

6.1.9 Endocrine-Disrupting Chemicals

Environmental endocrine disruptors are defined by the US Environmental Protection Agency as ‘exogenous agents that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and behaviour’. Studies show a positive correlation between breast cancer incidence and endocrine disruptor exposure in utero such as DES, BPA, dioxin and Vinclozolin.

6.1.9.1 DES

Diethylstilbestrol (DES) is an orally active synthetic estrogen that was first synthesized in 1938. This potent synthetic estrogen was administered to women during spontaneous miscarriage between the years 1948 and 1971 in the US, Europe and Australia. Subsequent, adverse health outcomes halted its usage.

The first prospective study on the influence of DES suggested that women with prenatal exposure to DES showed an increased risk of breast cancer after age 40 years [50]. National Cancer Institute (NCI) DES follow-up study also revealed a similar influence on breast cancer incidence after age 40 [51]. The excess risk among exposed women over 40 years of age, was found to be 1.7 % for breast cancer diagnosis [52].

European studies, a large cohort of 12,091 DES-daughters, found no increased risk of cancer, but its median age at the conclusion of follow-up was only 44.0 years [53].

6.1.9.2 Bisphenol A (BPA)

BPA is a component of polycarbonate plastics and has been used in a wide range of products including baby bottles, food beverage containers and dental sealants. Fetal bisphenol A exposure increases the number of terminal end buds (TEBs), and, the susceptibility of the mammary gland to transformation. Pre-neoplastic and neoplastic lesions in the mammary gland may also be induced by BPA exposure alone [54, 55].

Prenatal exposure to BPA increases carcinogenic susceptibility in a dose-dependent manner [56, 57]. Tamoxifen may reverse the effects of BPA [57], which suggested oestrogenic effects may be a possible explanation for BPA's effect.

6.1.9.3 TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin)

TCDD is an endocrine disrupter with anti-oestrogenic effects. Prenatal TCDD exposure predisposes the offspring of rats to developing mammary cancer later in life in a DMBA-model of rodent mammary carcinogenesis [58]. Epigenetic mechanisms may alter the mammary proteome include a decrease in superoxide dismutase 1 (SOD1) and mammary gland differentiation [58, 59].

6.1.9.4 Vinclozolin

Vinclozolin is a fungicide used in agricultural crops and is an anti-androgenic compound. Transient embryonic exposure to vinclozolin at the time of gonadal sex determination in rats increased incidence of tumours including breast cancer. It may result from epigenetic alterations in the male germ line [60].

6.1.10 Growth and Endocrine Factors

6.1.10.1 Oestrogen

Trichopoulos [2] proposed that prenatal exposure to estrogen may have a relationship to breast cancer. Oestrogen concentrations and secretion vary widely between individuals and this variability was partly accounted for by exogenous factors.

Researchers have made great efforts to search for the ideal proxy for oestrogen exposure like gestational age, birth size and twins. And the changed estrogen concentrations caused by exogenous factors like alcohol, smoking, soy products and endocrine-disrupting chemicals is considered the possible explanation for their efforts. Increased exposure to oestrogens does not explain the varying incidence of breast cancer. Circulating estrogen concentrations are higher in Asian pregnancies and in Asian-American neonates where the breast cancer incidence is low [61, 62].

Oestrogen receptors (ERs) including ERα and ERβ are nuclear transcription factors that regulate the expression of certain genes. ERs have been localized in breast epithelial cells as early as 30 weeks gestation [63]. Oestrogen binds to oestrogen receptors to exert its effect, and, exogenous factors during the fetal period may have effects on ER expression and subsequent gene expressions. Maternal alcohol and flax seed intake during pregnancy increase oestrogen receptor-α expression [32, 64].

6.1.10.2 Androgens

Elevated androgen concentrations and sex-hormone binding globulin (SHBG) levels have been observed in Asian and African-American (low incidence of breast cancer) compared to Caucasian pregnancies (high incidence of breast cancer [65]). SHBG may inhibit oestradiol-induced, breast cancer cell growth and proliferation and is associated with a reduced risk after oestrogen exposure [66].

Although epidemiological data are limited, observations also show a protective effect of prenatal androgens on breast cancer risk [67]. Pre-eclamptic pregnancies may be a protective factor for breast cancer, but may also increase the release of androgens by reducing aromatase gene expression [68]. While antiandrogenic compound, vinclozolin, increases tumour incidence including breast cancer after exposure during the embryonic period [60].

6.1.10.3 Insulin-Like Growth Factors

Insulin-like growth factors (IGF) are an important regulatory pathway in breast cancer. The IGF system comprises of ligands (IGF-I and IGF-II), their cognate receptors, IGF-binding proteins (IGFBP-1–6), and IGFBP proteases. They may act synergistically with oestrogens system to exert effects on breast cancer cells [69–71]. IGF may play a role in associations of prenatal factors with breast cancer risk. And the balance of IGF-1 and IGF-2 in cord blood may be a cruial role of early life determinant of later, breast cancer risk [65]. Alcohol exposure in utero enhanced IGF and E2 which also increases breast cancer risk in adulthood [31, 33]. In normal pregnancies, birth size has a strong relationship with cord IGF-I concentrations [72], which is associated with breast cancer risk.

6.1.10.4 α-Fetoprotein (AFP) and Human Chorionic Gonadotropin (hCG)

Levels of AFP and hCG during pregnancy may be related to maternal breast cancer risk [73, 74]. High levels of hCG occur in dizygotic twins who also have increased risks of breast cancer compared to singletons. Pregnant women smokers have

increased alpha fetoprotein (AFP) levels and reduced hCG levels [26, 75], and, reduced risks of breast cancer in their daughters [28]. Reduced placental function with reduced transfer of maternal blood components to the fetus, may explain this protective effect [26].

6.1.11 Possible Mechanisms

A number of attempts were carried on to develop hypotheses about mechanisms that might explain the prenatal risk factors on breast cancer or other disease.

6.1.11.1 Mammary Gland Morphology

Mammary gland morphology is usually evaluated by (a) ductal development, by measuring the distance from the lymph node to the end of the epithelial tree, and, distance from the tip of the epithelial tree to the end of fat pad. (b) the amount of terminal ductal lobular units (TDLUs) in human and terminal end buds (TEBs) in rats, and, (c) the density of mammary tissue. TDLUs and TEBs located at the distal end of the mammary epithelial tree are epithelial targets for malignant transformation and initiation site of breast cancer [76]. Mammographic density is one of the strongest risk factors for breast cancer [77, 78]. Birthweight was positively associated with increased mammographic breast density in postmenopausal women [77].

Epigenetic factors in the fetal period may influence programming in utero and breast cancer risk in adulthood. Rats exposed to alcohol in utero had an increased number of TEBs [32], and folic acid-supplemented dams had a significantly lower number of terminal end buds in daughters [19] suggesting that exposure to exogenous factors during fetal life modify development of the mammary gland. Perinatal treatment with oestrogen and EDCs with oestrogenic properties also promote mammary gland development in size, density and number of TEBs [1, 54].

6.1.11.2 Mammary Stem Cells

Rudland and colleagues first isolated possible candidates for stem cells in human breast immortalized epithelial cell lines more than two decades ago [79]. Mammary gland may be the only organ that is not fully developed until lactation. During the different periods of prenatal, early childhood, puberty, pregnancy and lactation, primitive mammary gland structure evolves to different types of breast [76]. Although mammary stem cells are thought to arise primarily during the fetal/perinatal period, it is hard to identify the stem cells in fetal breast tissues for technical reasons [4]. Recently, measurable breast and hematopoietic stem cells were isolated in human umbilical cord blood which provided a plausible mechanisms for a prenatal influence on breast cancer risk [80]. It also provided an alternative way to verify the role of stem cells in the intrauterine and perinatal influences like estrogen on stem-cell burden and susceptibility to breast cancer.

Epigenetic factors during fetal life may have an influence on breast cancer risk. These studies will help us clarify the mechanism of breast cancer occurrence.

And since epigenetic alterations are considered to be more easily reversible compared to genetic changes, epigenetic therapy could facilitate new methods for the treatment of breast cancer [81].

6.2 Cancer of Vagina and Cervix

Exposure of developing tissues to an adverse stimulus during crucial periods of development may increase the risk of many diseases, including cancer, later in life [82, 83]. Diethylstilbestrol (DES) is the most common one that was administered to several millions of pregnant women worldwide from the late 1940s to the early 1970s, to prevent miscarriages and other pregnancy complications [84, 85]. Among women exposed prenatally, adverse health effects include cancers of the vagina, cervix and uterine and reproductive tract abnormalities before age 30 years. DES exposure increases excess risks for cancer of the vagina and cervix (CCA) [51, 53, 86, 87] . The daughters of women who took DES during pregnancy (DES daughters) were frequently diagnosed with cervical intraepithelial neoplasia, and with a significantly increased rate of a rare type of vaginal clear cell adenocarcinoma [86, 87]. Of 12,091 DES-exposed women, 348 verified cancers occurred at a median age at end of 44.0 years. The risk of clear cell adenocarcinoma of the vagina and cervix (CCA) increased, and, the elevated risk persisted beyond 40 years of age [53].

Animal studies suggest that neonatal DES exposure causes increased expression of EGF and TGF-a mRNA, possibly resulting in the induction of persistent proliferation and cornification of vaginal epithelium in mice [51]. DES exposure in utero leads to imbalances in expression of c-jun, c-fos, c-myc, bax, bcl-2, and bcl-x protooncogenes [53]. Other oestrogen responsive genes, e.g. lactoferrin gene, cause demethylation following DES exposure. Abnormal gene imprinting may also be involved in tumour induction and other cellular alterations in the reproductive tract [88].

6.3 Uterine Leiomyoma

Uterine leiomyoma arise from uterine smooth muscle and are the most common benign tumour in women [89]. Early life exposure to DES causes uterine leiomyomata. Baird et al. [90] reported all five black women reporting DES exposure had leiomyomata whereas 76 % of white women reporting prenatal DES exposure had leiomyomata compared with 52 % of unexposed women. Experimental animal data also support the view that DES exposure play an important role in the etiology of uterine leiomyoma [91].

DES exposure imparts an endocrine imprint on developing myometrium, causing increased expression of oestrogen-responsive genes. Early-life exposure to DES in rats, carrying a genetic defect in the tuberous sclerosis 2 (Tsc2) tumour suppressor gene, causes the tumour suppressor defect to become fully penetrant, increasing tumour incidence from 65 % to more than 90 % [92]. Increased penetrance is

associated with re-programming of oestrogen-responsive genes in uterine leiomyomas. These include calbindin D9k and Dio2, normally induced by oestrogen, exhibiting increased expression in DES-exposed animals during both phases of the oestrus cycle. Gdf10, Car8, Gria2, and Mmp3, are genes normally suppressed by oestrogen, that exhibit increased expression in DES-exposed animals during the proliferative phase, when oestrogen levels peak. The re-programming leads to over-expression of these genes in the myometrium of exposed animals, promoting the development of hormone-dependent uterine leiomyomas [93].

6.4 Endometrial Hyperplasia and Uterine Adenocarcinoma

Progression from normal endometrium to carcinoma may take place by an intermediate stage of endometrial hyperplasia. At 5 months of age, 36/60 (60 %) of rats exposed to DES on days 3–5 after birth developed endometrial hyperplasia, while none in vehicle-treated controls [94].

Uterine adenocarcinoma occurs in a time- and dose-related manner after DES exposure. At 18 months, neoplastic lesions occurred in 90 % of mice exposed neonatally to 2 μg/pup of DES/day, while none was observed in the DES-treated mice which were ovariectomized before puberty, indicating that DES-induced uterine tumours might be oestrogen dependent [95]. It is proposed that DES acts as an inducer to affect uterine cells in early development phase and that ovarian estrogens act as promoters thereafter, to stimulate proliferation of DES-transformed cells in the adult mouse.

IGF-I signaling pathway was reported to be involved in endometrial hyperplasia and tumorigenesis. Ki67-positive cells increased in DES-exposed endometrium, that is consistent with activation of a mitogenic signaling pathway. IGF-II and insulin receptor substrate-1(IRS-1) were over-expressed in the endometrium of DES-exposed rats. Negative feedback to IRS-1, which was observed in control, was deficient in DES-exposed endometrium. Activation of IGF-IR signaling and abrogation of negative feedback to IRS-1 seems to be re-programmed by DES in endometrial hyperplasia, indicating loss of negative feedback to IRS-1 might affect the development of a preneoplastic lesion for the first time.

Early studies indicated that neonatal DES exposure induced abnormal methylation of specific CpG sites in Ltf [96] and Fos [97] in the mouse uterus. Exposure to DES in utero resulted in increased promoter methylation and decreased HOXA10 expression [98]. Similarly, neonatal exposure of mice to DES or genistein also induces low methylation of the Hmgn5 promoter and aberrant high expression of this gene in the uterus throughout life, and, exposure might lead to a potential increased risk of developing uterine tumours in adult female mice [99]. Additional studies show that exposure to environmental oestrogens of the developing uterus might re-programmes many oestrogen-responsive genes, including S100 calcium-binding protein G (S100G; also known as CALB3), glutamate receptor ionotropic AMPA2 (GRIA2), growth differentiation factor 10 (GDF10) and matrix

metalloproteinase 3 (MMP3), and affect them to become hyper-responsive to oestrogen [92]. Increased gene expression may be triggered by later-life events such as the ovarian steroid hormones during puberty. In these settings, ovariectomy before puberty completely decreases the epigenetic re-programming effect on gene expression and uterine tumour development.

6.5 Prostate Cancer

Prostate cancer is the sixth leading cause of cancer death among men worldwide. The highest incidences have been reported in United States and Western Europe and the lowest in South East Asia [100]. Approximately one in six men in USA will develop prostate cancer during their lifetime, while the risk of death from prostate cancer is 1 in 35 [101]. Risk factors for prostate cancer include increasing age, African American Ethnicity, family history, and diet [102].

The prostate develops from endodermal origin and arises by the 9th week of embryonic life [103]. Epidemiological studies show that African-American women have higher concentrations of testosterone and oestradiol during the first-trimester pregnancy, compared to white women [104, 105]. These hormonal patterns, acting in utero, may influence the development of prostate cancer in male children [106]. Meanwhile, birthweight, as a marker of the pre- and perinatal environment, predicted prostate cancer incidence of 366 men born in Sweden in 1913 and in which 21 prostate cancer cases were found since 1963 [107]. Subsequently, another Swedish case control study found a modest positive association between birth weight and incidence of prostate cancer (per 500 g, RR = 1.04, 95 % Cl 0.88–1.23) or death from the disease (per 500 g, RR = 1.22, 95 % Cl 0.87–1.70) [108]. Maternal smoking is not associated with any risk [109].

Rodent studies have associated maternal exposure to BPA with higher body weight, increased prostate cancer as well as breast cancer risk. Maternal BPA exposure may change the offspring phenotype by altering the epigenome through decreasing CpG methylation, and can be counteracted by maternal dietary supplements with folic acid [110].

6.6 Testicular Cancer

Testicular malignancy is the most common cancer among men aged 20–39 years. Although the incidence of testicular cancer is rising, the 5-year survival rate has increased from 63 % in 1963 to 96 % today [111]. Epidemiological data suggest that patients with a family history of testicular cancer, cryptorchidism, or testicular atrophy have an increased risk [112, 113]. A number of different early-life factors have been identified that predispose men to testicular cancer, such as subfertility, exposure to exogenous estrogens, levels of hormones during gestation, birth weight, birth order etc. [114].

6.6.1 Exposure to Exogenous Estrogens

A pooled analysis of cohort studies including 1,700 men exposed to DES found a relative risk of testicular cancer of 3.05 (95 % CI: 0.65–22.0), based on seven testicular cancer cases [115]. Meanwhile, a case-control study of 108 cases of testicular cancer in men suggested that exposure of the mother to exogenous estrogen during pregnancy developed a significant risk in the son (RR = 8.0) [116].

6.6.2 Levels of Hormones During Gestation

Higher levels of oestriol occur in twin pregnancy, ovarian stimulation in ART, obesity during pregnancy, as well as hyperemesis gravidarum and first pregnancy. In Akre et al. the point estimates [OR = 0.51 (0.11–2.32)] indicate a lower risk of testicular cancer for lower prenatal estrogen exposures [116], while some studies found a higher risk for testicular cancer [RR = 1.9 (0.7–5.0)] [117, 118]. All studies eliminate differential misclassification, either by use of registries, or by using a control group of young men suffering from other cancers.

6.6.3 Birthweight and Birth Order

Moller and Skakkebaek observed that birth weights below 3,000 g or above 4,000 g were associated with increased risks of testicular cancer, with OR up to 2.6 (CI = 1.1–5.9) for birth weight below 2,500 g [119], but a later meta-analysis of 12 studies showed no definite conclusion of the association between low birth weight and testicular cancer [120].

6.6.4 Maternal Smoking

An association between maternal smoking and testicular cancer has been suggested by two correlation studies, whereas a case–control study with prospective measurement of maternal smoking including 192 Swedish men with testicular cancer and 494 controls found no association [121].

6.7 Colorectal Cancer

In the United States, Colorectal cancer (CRC) is the second leading cause of cancer death [122, 123]. There is at least a 25-fold variation in the occurrence of CRC across different regions of the world, including developed areas such as Australia, Western Europe, Japan (in males), and North America—having the highest incidence rates. In different regions estimated 5-year survival rates also vary widely from 30 % in India to 65 % in North America. environmental factors, which are believed

to play an important role in CRC etiology may attribute to the large differences in incidence. For more than three decades, epidemiologists have described strong international relationship between CRC and various dietary constituents, including the consumption of fiber, animal fats, and meat; recent evidence seems to suggest that the consumption of red meat and physical inactivity may be significant [124].

6.7.1 Maternal Nutrients Intake

Supplementation of folic acid has played a controversial role in the prevention of colorectal cancer, which is depending on the time of intervention and the dose of supplementation [125, 126]. Animal studies and randomised controlled trials have shown that the supplementation of folic acid appears to prevent the development of new colorectal cancer while it may promote the progression of existing preneoplastic lesions to colorectal cancer [127–130].

Owing to widespread use of supplements and periconceptional folic acid supplementation, intrauterine and early life exposure to folic acid has significantly increased in North America [131]. Furthermore, women of childbearing age are routinely advised to take 0.4–1.0 mg of folic acid to prevent neural tube defects [132].

It was reported that maternal supplementation of folic acid significantly increased ($p=0.007$) colorectal global DNA methylation while reduced the odds of colorectal adenocarcinoma by 64 % in the offspring (OR 0.36; 95 % CI 0.18–0.71; $p=0.003$) [133]. However, another study reported that postweaning supplementation of folic acid significantly increased the number of small intestinal adenom in the offspring [134].

These data suggest that maternal folic acid supplementation at a proper level at reproductive age protects against the development of colorectal cancer in the offspring. This protective effect may be partly mediated by increased global DNA methylation and decreased epithelial proliferation and DNA damage in the colorectum [133].

6.7.2 Birth Size

Relatively small birth size has been involved with a higher risk of cardiovascular disease and diabetes in men [135, 136]. In adulthood, colorectal cancer risk has been affected by the presence of diabetes [137, 138], hyperglycaemia [139, 140], hyperinsulinaemia [139, 141, 142], and high concentrations of IGF-I [142]. The evidence that metabolic aberrations involve with the development of type 2 diabetes may have an intrauterine origin, and their positive associations with colorectal cancer, suggest that the long term effects of intrauterine growth restriction may also be count for colorectal carcinogenesis.

Sandhu et al. has explored the risk of colorectal cancer related to birth dimensions and found a J-shaped association with birth weight [143] and found that babies born with macrosomia appear to have the greatest risk. However, the study was

based on 52 cases only. A larger study by McCormack et al. [144] showed a 16 % elevated risk per one standard deviation increase in birth weight. Another advance cohort research of Norwegian men and women with long term cancer, which follow up through the Norwegian Cancer Registry, found that men with birth length of less than 51 cm had a nearly twofold higher risk of colorectal cancer (RR 1.9 (95 % CI 1.0–3.7)) compared with men who were 53 cm or more, after adjustment for other gestational factors. Similar associations were found for birth weight and head circumference among men, suggesting that among men, relatively short birth length is associated with increased risk of colorectal cancer in adulthood, indicating that intrauterine growth could be important for colorectal carcinogenesis [145].

6.7.3 Possible Mechanisms

Several biological mechanisms may attribute to the dual effects of folate on colorectal carcinogenesis [125]. In normal tissues, folic acid supplementation provides nucleotide precursors for DNA synthesis and replication, therefore ensuring DNA fidelity, DNA integrity and optimal DNA repair, which would reduce the risk of neoplastic transformation [125]. However, folic acid supplementation promotes the progression of (pre)neoplastic lesions though providing nucleotide precursors to the rapidly replicating transformed cells, adding to lowing accelerated proliferation [125].

Global DNA hypomethylation contributes to colorectal cancer development through several mechanisms including chromosomal instability. Folate modulates DNA methylation of cytosine within the cytosineeguanine (CpG) sequences for it plays a role in the provision of S-adenosylmethionine, which is the primary methyl group donor for most biological methylation reactions [146]. DNA methylation at promoter CpG islands silences transcription and hence inactivates the function of a wide array of tumour suppressor and cancer-related genes [146]. Folic acid supplementation can reverse pre-existing global DNA hypomethylation and increase the level of global DNA methylation based on the pre-existing level [146], thereby reducing the risk of neoplastic transformation. In contrast, folic acid supplementation may cause de novo methylation of CpG islands in tumour suppressor genes, which inactive those genes and lead to tumour development and progression [125].

6.8 Lung Cancer

Nikonova studied transplacental and direct effects of benzo(a)pyrene (BP) and pyrene on A and C57BL mice and their offspring, and 76.8 % of offsprings of A mice developed lung tumours against 12.3 % in controls ($P < 0.001$) [147]. Dibenzo(a,l)pyrene (DBP) is one of the most potent carcinogenic polycyclic aromatic hydrocarbons. Castro et al. demonstrated that exposure to DBP during late gestation shortly presents a greater risk to offspring than that following 3 weeks of nursing [148]. Transplacental exposure of BALB/c mice to urethane on day 17 of

gestation resulted in the appearance of two distinct crops of primary lung adenomas [149]. A single subcutaneous injection of 200 mu Ci [^{3}H] thymidine into pregnant BALD/c mice, followed by intraperitoneal injections of phorbol, twice weekly for 25 weeks, resulted in higher rate of tumorigenesis in the offsprings' lungs and livers [150]. Genetically nonresponsive (Ahd/Ahd) offspring had a higher incidence of nodules, adenomas, and diffuse bronchiolar hyperplasia in lung than responsive offspring within the same treatment group. Thus genetic differences in Ah genotype may influence susceptibility to transplacental carcinogenesis to 3-MC [151]. Similarly, Wessner et al. reported that after maternal exposure to 3-MC, offspring with Ki-ras-2 mutations developed lung cancer early, suggesting that the type of mutation produced by environmental chemicals can influence the carcinogenic potential of the tumour [152].

In mice exposed transplacentally to environmental carcinogens, the combination of mutated Ki-ras-2 and alterations in the Rb regulatory gene locus may be the preferred pathway for lung tumour pathogenesis [152–157]. One study using the transplacental carcinogenesis model shows that alterations in the Ink4a locus occur in only 15 and 27 % of the lung and liver tumours, respectively, which imply that damage to the Ink4a gene is not a frequent pathway to malignant progression in mouse lung and liver tumours [158].

The role of nitroso compounds as possible causative agents for human childhood cancers has been reported by epidemiological studies. Published evidence from animal models indicates that capacity for metabolic activation of nitrosamines is limited in rodent fetuses, so nitrosamines are correspondingly weak transplacental carcinogens. But NDMA has significant transplacental carcinogenic effects, when treatment of pregnant mice took place on day 19 [159]. The incidence of lung tumours in 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-exposed progeny was significantly higher than controls [160].

Arsenic is a known human carcinogen, but development of rodent models of inorganic arsenic carcinogenesis has been problematic. A transplacental carcinogenicity study in mice using inorganic arsenic demonstrated that oral inorganic arsenic exposure, as a single agent, induced tumour formation in rodents, thus establishing inorganic arsenic as a complete transplacental carcinogen in mice [161, 162]. ER-alpha activation was specifically involved with arsenic-induced lung adenocarcinoma and adenoma, suggesting that arsenic-induced, aberrant ER signaling may disrupt early-life, genetic programming in the lung which eventually lead to lung tumour formation much later in adulthood [163]. The biomethylation product of arsenic, dimethylarsinic acid (DMA), which is a multi-site tumour promoter. also increased lung adenocarcinomas and adrenal adenomas compared to controls [164].

There are some protective chemical agents for lung cancer during gestation. Indole-3-carbinol in the maternal diet protected the fetus from transplacental carcinogenesis by the polycyclic aromatic hydrocarbon dibenzo[a,l]pyrene [165], and reduced lung tumours in mice by approximately 50 % . CHL can provide potent chemoprotection in a transplacental carcinogenesis model and support a mechanism involved with complex-mediated reductions of carcinogen uptake [166].

6.9 Wilms Tumour

Wilms tumor is an embryonal malignancy of the kidney. Most cases are sporadic, although 1–2 % of patients have a familial predisposition. Goel et al. examined the effects of pre-conception or in utero maternal medical radiation exposure on Wilms tumour, using data from a large population-based case-control study, which shows no significant association between the risk of Wilms tumour and maternal X-ray exposure [167]. No meaningful associations were seen from analysis of gonadal or non-gonadal radiation exposure [167].

Stjernfeldt et al. found a dose-dependent relationship between the cigarettes smoked per day by the pregnancy mother and the risk of cancer in the offspring. When taking all tumour sites into consideration the cancer risk was 50 % higher compared to controls [168].

Olshan et al. also found that certain paternal occupations have an elevated odds ratio (OR) of Wilms' tumor, including vehicle mechanics, auto body repairmen, and welders. Further more, offspring of fathers who with a occupation of auto mechanics had a four- to sevenfold increased risk of Wilms' tumor [169].

6.10 Renal Cancer

In 2009, it was estimated that there were 58,000 newly diagnosed renal cell and renal pelvic cancer patient in the United States and that almost 13,000 people would die of the disease [170]. The incidence varies widely depends on different countries, with the highest rates seen in Northern Europe and North America [171].

Numerous environmental and clinical factors have been involved with the etiology of renal cell carcinoma [172, 173]. These include tobacco use; toxic compounds exposure such as cadmium, asbestos, and petroleum by-products; obesity; acquired polycystic disease of the kidney; and analgesic abuse nephropathy. Cigarette smoking doubles the likelihood of renal cell carcinoma and accounts for as many as one third of all cases [173–175].

Ohaki et al. studied the renal lesions induced by transplacental administration of n-ethylnitrosourea (ENU) in the rat, which induced a wide variety of renal tumors and tumor-like conditions in the offspring, comparable to the various types of renal lesions observed in children, and may provide a useful model to study the pathogenesis of embryonal tumors in man [176].

Bergstrom et al. found a weak association of birth weight with kidney cancer only in males when comparing those with birth weight above 3,500 g with 3,000–3,499 g [177]. However, another study showed that there were no association between birth weight and malignant neoplasm in the urinary system [144]. Several studies have indicated an higher risk of Wilms' tumour in children aged less than 2 years at diagnosis with a high birth weight. The largest study with 1,800 cases pinpointed that the strength of the association was different for the various subtypes [178].

6.11 Leukemia and Lymphoma

The leukemias are the most common malignant neoplasms in childhood. However, the precise etiologies of leukemia are unknown. Known risk factors include several congenital/genetic disorders, ionizing radiation, and certain drug or toxin exposures [179]. In utero exposure to inhibitors of topoisomerase II such as dietary flavonoids may increase the risk of MLL-rearranged infant AML [180]. Children with radiation exposure from the atomic bombs in Japan, had an increased incidence of leukemia, thus several studies have addressed the question whether exposure to low-frequency, non-ionizing radiation (e.g., electromagnetic fields) is leukemogenic [181]. Recent reports did not find an increased risk of acute lymphoblastic leukemia in children exposed to residential magnetic fields [182, 183].

As so many environmental factors may contribute to the development of leukemia, and, the embryo is sensitive to toxicity from environmental chemicals, it is believed that fetal basis of adult leukemia may be important. Such factors include maternal diet, occupation exposure, parental age, intrauterine virus infection, birthweight, etc.

6.11.1 Maternal Nutritional Intake

Bailey et al. explored whether maternal dietary folate intake during pregnancy is involved with risk of acute lymphoblastic leukemia (ALL) in offspring. Results show higher levels of dietary folate and B12 appear to add to a decreased risk of ALL. When mothers consumed alcohol in pregnancy the associations were strengthened [184].

Milne et al. performed a case-control study recruiting 416 cases and 1,361 controls between 2003 and 2007. There was evidence of a weak protective effect of maternal supplementation of folate before pregnancy from risk of childhood ALL [185]. Kwan et al. reported that the risk of ALL was inversely associated with maternal vegetable consumption; protein sources; fruit; and legume food groups. The risk reduction was most significant for consumption of the protein sources and vegetable food groups [186].

6.11.2 In-Vitro Fertilization

Petridou et al. investigated the risk of leukemia and lymphoma results from IVF using two nationwide datasets. These studies showed similar-size excess risk of leukemia following IVF, but no significant association between IVF and lymphoma. The proportion of leukemia cases conceived through IVF was 2.7 % in Sweden and 3 % in Greece; prevalence of IVF in matched controls was 1.8 % and 1.6 % respectively. The higher risk of leukemia was restricted to age below 3.8 years and to acute lymphoblastic leukemia (ALL). Following IVF, OR for ALL was 2.58 before age 3.8 and 4.29 before age 2 years [187].

6.11.3 Parental Age

Hemminki et al. analyzed the effect of parental age on childhood leukemia and brain cancer, which suggests a parental age effect for both leukemia and brain cancer, with the former (of about 50 % excess in those over 35 years) being mediated by maternal age and the latter (of about 25 % excess) by paternal age. One possible mechanism may be accumulation of chromosomal aberrations and mutations during the maturation of germ cells which can help to explain the secular trends of these malignancies, and, the high risks offspring of the higher social classes [188]. Hori, M et al. reported intrauterine transmission of human T-cell leukemia virus type I in rats, which may add to the risk of ALL in offspring [189]. Similar results were found in rabbits [190].

6.11.4 Parental Occupational Exposure

Reid et al. reported that exposure to moderate or substantial levels of exhausts by mothers or fathers during pregnancy increased the risk of ALL in their offspring. Exposure to paints, pigments, glues, and resins was similar in case and control parents [191]. DBP administration to pregnant mice leading to high mortality of offspring result from an aggressive T-cell lymphoma [192].

Wigle et al. reported there was no overall association between childhood leukemia and any paternal occupational pesticide exposure. However, the relationship between childhood leukemia and prenatal maternal occupational exposure of pesticide and insecticides and herbicides were has been demonstrated by other researchers [193]. Monge et al. showed maternal exposures to any pesticides during the year before conception and the first and second trimesters were involved with the risk [194]. Yu et al. demonstrated that transplacental PAH exposure can induce a highly aggressive lymphoma in mice and raises the possibility that PAH exposures of pregnant women could contribute to similar cancers in children and young adults [195]. Perez-Saldivar et al. did a case-control study with 193 children and reported that children whose fathers have been exposed to a high level of carcinogenic agents seem to have a higher risk of developing acute leukemia [196].

Infante-Rivard, C's study report an increased risk of childhood leukemia among children whose mothers were exposed to the highest levels of ELF-MF during pregnancy [197]. Stjernfeldt et al. also report maternal smoking and irradiation during pregnancy as risk factors for child leukemia [198]. Little et al. found that exposure to diagnostic X-rays in utero is associated with leukaemia. While for lymphoma, no factor in utero has been found to be consistently associated [199].

6.11.5 Maternal Illness and Medication Use

Lehtinen et al. report that reactivation of maternal EBV infection is probably associated with childhood acute lymphoblastic leukemia (ALL) [200]. Caballero et al.

later evaluated whether maternal illness and drug/medication use (prescription, over-the-counter, and illicit) during pregnancy may be related to childhood leukemia risk using data (1995–2002) from the Northern California Childhood Leukemia Study [201]. The results suggested that maternal infection might contribute to the etiology of leukemia. While iron supplements use was indicative of decreased ALL risk [201].

Shaw et al. also report a significantly increased risk of childhood ALL in the offspring of mothers who had a history of using any medication or any teratogenic medication during pregnancy. However, only central nervous system depressants were involved with a significantly increased risk, which was higher with increased dose and in children diagnosed before 2 years of age [202]. Wen et al. did a similar study, which suggests that certain parental medication use immediately before and during the index pregnancy may affects risk of ALL in offspring [203].

6.11.6 Birthweight

McCormack et al. found a positive association between birthweight and cancer in the lymphatic and haematopoietic tissue in adult persons, and a similar tendency was seen for multiple myeloma and non-Hodgkin lymphoma [144]. A meta analysis of more than 10,000 children, concluded that there was a higher risk of acute lymphoblastic leukaemia for those with birth weight above 4,000 g compared to those below 4,000 g [204]. Similarly, a positive linear relation was found between gender-adjusted birth weight and all leukemias and AML by Paltiel et al., which was especially notable among infants but was also observed among subjects ages >14 years at diagnosis. The relation was particularly strong among females ($P=0.001$). Other risk factors for AML risk on univariate analysis include maternal origin, socioeconomic status, birth weight of sibling >3,500 g, and family size [205].

6.12 Tumors in Brain and Nervous System

An estimated 43,800 newly diagnosed cases of primary CNS system tumours were reported in the United States in 2005, in which approximately 18,500 were malignant, representing 1.35 % of all cancers diagnosed that year.

Deorah and colleagues [206] found that the incidence of brain cancer increased until 1987, when the annual percentage of change reversed direction. A relationship has been shown between high birthweight (above 4,000 g) and the incidence of astrocytoma [199]. Samuelsen et al. showed that there is association between risk of brain cancer and head circumference in the age group 0–15 years, suggesting that brain pathology originates during foetal life [207]. On the other hand, a study showed that exposure to diagnostic X-rays in utero is classified as “associated”, and maternal consumption of cured meats, maternal use of vitamin supplements and high birth weight (astrocytoma) during pregnancy have been classified as associated with some degree of consistency [199].

6.13 Conclusions

The fetus and neonate are sensitive to environmental chemicals, and, changes in the intrauterine environment including the intrauterine nutritional milieu due to epigenetic and metabolic programming. These phenomena collectively contribute to the embryo-fetal basis of tumours.

References

1. Mandrup KR, Hass U, Christiansen S, et al. Perinatal ethinyl oestradiol alters mammary gland development in male and female Wistar rats. Int J Androl. 2012;35:385–96.
2. Trichopoulos D. Hypothesis: does breast cancer originate in utero? Lancet. 1990;335:939–40.
3. De Assis S, Hilakivi-Clarke L. Timing of dietary estrogenic exposures and breast cancer risk. Ann N Y Acad Sci. 2006;1089:14–35.
4. Savarese TM, Low HP, Baik I, et al. Normal breast stem cells, malignant breast stem cells, and the perinatal origin of breast cancer. Stem Cell Rev. 2006;2:103–10.
5. Sorensen HT, Olsen ML, Mellemkjaer L, et al. The intrauterine origin of male breast cancer: a birth order study in Denmark. Eur J Cancer Prev. 2005;14:185–6.
6. Panagiotopoulou K, Katsouyanni K, Petridou E, et al. Maternal age, parity, and pregnancy estrogens. Cancer Causes Control. 1990;1:119–24.
7. Thompson WD, Janerich DT. Maternal age at birth and risk of breast cancer in daughters. Epidemiology. 1990;1:101–6.
8. Park SK, Kang D, McGlynn KA, et al. Intrauterine environments and breast cancer risk: meta-analysis and systematic review. Breast Cancer Res. 2008;10:R8.
9. Xue F, Michels KB. Intrauterine factors and risk of breast cancer: a systematic review and meta-analysis of current evidence. Lancet Oncol. 2007;8:1088–100.
10. Barba M, McCann SE, Nie J, et al. Perinatal exposures and breast cancer risk in the Western New York Exposures and Breast Cancer (WEB) Study. Cancer Causes Control. 2006;17:395–401.
11. Petridou E, Katsouyanni K, Hsieh CC, et al. Diet, pregnancy estrogens and their possible relevance to cancer risk in the offspring. Oncology. 1992;49:127–32.
12. Jansson N, Nilsfelt A, Gellerstedt M, et al. Maternal hormones linking maternal body mass index and dietary intake to birth weight. Am J Clin Nutr. 2008;87:1743–9.
13. Sanderson M, Williams MA, Daling JR, et al. Maternal factors and breast cancer risk among young women. Paediatr Perinat Epidemiol. 1998;12:397–407.
14. Wilson KM, Willett WC, Michels KB, et al. Mothers' pre-pregnancy BMI and weight gain during pregnancy and risk of breast cancer in daughters. Breast Cancer Res Treat. 2011;130:273–9.
15. Wu AH, Pike MC, Stram DO, et al. Meta-analysis: dietary fat intake, serum estrogen levels, and the risk of breast cancer. J Natl Cancer Inst. 1999;91:529–34.
16. Hilakivi-Clarke L, Cho E, Cabanes A, et al. Dietary modulation of pregnancy estrogen levels and breast cancer risk among female rat offspring. Clin Cancer Res. 2002;8:3601–10.
17. Su HM, Hsieh PH, Chen HF. A maternal high n-6 fat diet with fish oil supplementation during pregnancy and lactation in rats decreases breast cancer risk in the female offspring. J Nutr Biochem. 2010;21:1033–7.
18. Cho K, Mabasa L, Bae S, et al. Maternal high-methyl diet suppresses mammary carcinogenesis in female rat offspring. Carcinogenesis. 2012;33:1106–12.
19. Sie KK, Chen J, Sohn KJ, et al. Folic acid supplementation provided in utero and during lactation reduces the number of terminal end buds of the developing mammary glands in the offspring. Cancer Lett. 2009;280:72–7.

20. Ly A, Lee H, Chen J, et al. Effect of maternal and postweaning folic acid supplementation on mammary tumor risk in the offspring. Cancer Res. 2011;71:988–97.
21. Trock BJ, Hilakivi-Clarke L, Clarke R. Meta-analysis of soy intake and breast cancer risk. J Natl Cancer Inst. 2006;98:459–71.
22. Hilakivi-Clarke L, Cho E, Onojafe I, et al. Maternal exposure to genistein during pregnancy increases carcinogen-induced mammary tumorigenesis in female rat offspring. Oncol Rep. 1999;6:1089–95.
23. Nielsen TS, Purup S, Warri A, et al. Effects of maternal exposure to cow's milk high or low in isoflavones on carcinogen-induced mammary tumorigenesis among rat offspring. Cancer Prev Res (Phila). 2011;4:694–701.
24. Yu B, Khan G, Foxworth A, et al. Maternal dietary exposure to fiber during pregnancy and mammary tumorigenesis among rat offspring. Int J Cancer. 2006;119:2279–86.
25. Petridou E, Panagiotopoulou K, Katsouyanni K, et al. Tobacco smoking, pregnancy estrogens, and birth weight. Epidemiology. 1990;1:247–50.
26. Bernstein L, Pike MC, Lobo RA, et al. Cigarette smoking in pregnancy results in marked decrease in maternal hCG and oestradiol levels. Br J Obstet Gynaecol. 1989;96:92–6.
27. Waller DK, Lustig LS, Smith AH, et al. Alpha-fetoprotein: a biomarker for pregnancy outcome. Epidemiology. 1993;4:471–6.
28. Strohsnitter WC, Noller KL, Titus-Ernstoff L, et al. Breast cancer incidence in women prenatally exposed to maternal cigarette smoke. Epidemiology. 2005;16:342–5.
29. Park SK, Garcia-Closas M, Lissowska J, et al. Intrauterine environment and breast cancer risk in a population-based case–control study in Poland. Int J Cancer. 2006;119:2136–41.
30. Purohit V. Can alcohol promote aromatization of androgens to estrogens? A review. Alcohol. 2000;22:123–7.
31. Polanco TA, Crismale-Gann C, Reuhl KR, et al. Fetal alcohol exposure increases mammary tumor susceptibility and alters tumor phenotype in rats. Alcohol Clin Exp Res. 2010;34:1879–87.
32. Hilakivi-Clarke L, Cabanes A, de Assis S, et al. In utero alcohol exposure increases mammary tumorigenesis in rats. Br J Cancer. 2004;90:2225–31.
33. Polanco TA, Crismale-Gann C, Cohick WS. Alcohol exposure in utero leads to enhanced prepubertal mammary development and alterations in mammary IGF and estradiol systems. Horm Cancer. 2011;2:239–48.
34. Painter RC, De Rooij SR, Bossuyt PM, et al. A possible link between prenatal exposure to famine and breast cancer: a preliminary study. Am J Hum Biol. 2006;18:853–6.
35. Stephansson O, Granath F, Ekbom A, et al. Risk of breast cancer among daughters of mothers with diabetes: a population-based cohort study. Breast Cancer Res. 2010;12:R14.
36. Ekbom A, Hsieh CC, Lipworth L, et al. Intrauterine environment and breast cancer risk in women: a population-based study. J Natl Cancer Inst. 1997;89:71–6.
37. Vatten LJ, Forman MR, Nilsen TI, et al. The negative association between pre-eclampsia and breast cancer risk may depend on the offspring's gender. Br J Cancer. 2007;96:1436–8.
38. Wuu J, Hellerstein S, Lipworth L, et al. Correlates of pregnancy oestrogen, progesterone and sex hormone-binding globulin in the USA and China. Eur J Cancer Prev. 2002;11:283–93.
39. Mazor M, Hershkovitz R, Chaim W, et al. Human preterm birth is associated with systemic and local changes in progesterone/17 beta-estradiol ratios. Am J Obstet Gynecol. 1994;171:231–6.
40. Innes K, Byers T, Schymura M. Birth characteristics and subsequent risk for breast cancer in very young women. Am J Epidemiol. 2000;152:1121–8.
41. Ekbom A, Erlandsson G, Hsieh C, et al. Risk of breast cancer in prematurely born women. J Natl Cancer Inst. 2000;92:840–1.
42. Ruder EH, Dorgan JF, Kranz S, et al. Examining breast cancer growth and lifestyle risk factors: early life, childhood, and adolescence. Clin Breast Cancer. 2008;8:334–42.
43. Carroll SG, Tyfield L, Reeve L, et al. Is zygosity or chorionicity the main determinant of fetal outcome in twin pregnancies? Am J Obstet Gynecol. 2005;193(3 Pt 1):757–61.

44. Wald N, Cuckle H, Wu TS, et al. Maternal serum unconjugated oestriol and human chorionic gonadotrophin levels in twin pregnancies: implications for screening for Down's syndrome. Br J Obstet Gynaecol. 1991;98:905–8.
45. Michels KB, Xue F. Role of birthweight in the etiology of breast cancer. Int J Cancer. 2006;119:2007–25.
46. Mucci LA, Lagiou P, Tamimi RM, et al. Pregnancy estriol, estradiol, progesterone and prolactin in relation to birth weight and other birth size variables (United States). Cancer Causes Control. 2003;14:311–18.
47. Xu X, Dailey AB, Peoples-Sheps M, et al. Birth weight as a risk factor for breast cancer: a meta-analysis of 18 epidemiological studies. J Womens Health (Larchmt). 2009;18:1169–78.
48. Wiseman M. The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Proc Nutr Soc. 2008;67:253–6.
49. Nagata C, Iwasa S, Shiraki M, et al. Estrogen and alpha-fetoprotein levels in maternal and umbilical cord blood samples in relation to birth weight. Cancer Epidemiol Biomarkers Prev. 2006;15:1469–72.
50. Palmer JR, Wise LA, Hatch EE, et al. Prenatal diethylstilbestrol exposure and risk of breast cancer. Cancer Epidemiol Biomarkers Prev. 2006;15:1509–14.
51. Troisi R, Hatch EE, Titus-Ernstoff L, et al. Cancer risk in women prenatally exposed to diethylstilbestrol. Int J Cancer. 2007;121:356–60.
52. Hoover RN, Hyer M, Pfeiffer RM, et al. Adverse health outcomes in women exposed in utero to diethylstilbestrol. N Engl J Med. 2011;365:1304–14.
53. Verloop J, van Leeuwen FE, Helmerhorst TJ, et al. Cancer risk in DES daughters. Cancer Causes Control. 2010;21:999–1007.
54. Murray TJ, Maffini MV, Ucci AA, et al. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. Reprod Toxicol. 2007;23:383–90.
55. Moral R, Wang R, Russo IH, et al. Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. J Endocrinol. 2008;196(1):101–12.
56. Durando M, Kass L, Piva J, et al. Prenatal bisphenol A exposure induces preneoplastic lesions in the mammary gland in Wistar rats. Environ Health Perspect. 2007;115:80–6.
57. Weber Lozada K, Keri RA. Bisphenol A increases mammary cancer risk in two distinct mouse models of breast cancer. Biol Reprod. 2011;85:490–7.
58. Brown NM, Manzolillo PA, Zhang JX, et al. Prenatal TCDD and predisposition to mammary cancer in the rat. Carcinogenesis. 1998;19:1623–9.
59. Jenkins S, Rowell C, Wang J, et al. Prenatal TCDD exposure predisposes for mammary cancer in rats. Reprod Toxicol. 2007;23:391–6.
60. Anway MD, Leathers C, Skinner MK. Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease. Endocrinology. 2006;147:5515–23.
61. Lipworth L, Hsieh CC, Wide L, et al. Maternal pregnancy hormone levels in an area with a high incidence (Boston, USA) and in an area with a low incidence (Shanghai, China) of breast cancer. Br J Cancer. 1999;79:7–12.
62. Shibata A, Harris DT, Billings PR. Concentrations of estrogens and IGFs in umbilical cord blood plasma: a comparison among Caucasian, Hispanic, and Asian-American females. J Clin Endocrinol Metab. 2002;87:810–15.
63. Friedrichs N, Steiner S, Buettner R, et al. Immunohistochemical expression patterns of AP2alpha and AP2gamma in the developing fetal human breast. Histopathology. 2007;51:814–23.
64. Khan G, Penttinen P, Cabanes A, et al. Maternal flaxseed diet during pregnancy or lactation increases female rat offspring's susceptibility to carcinogen-induced mammary tumorigenesis. Reprod Toxicol. 2007;23:397–406.
65. Lagiou P, Samoli E, Okulicz W, et al. Maternal and cord blood hormone levels in the United States and China and the intrauterine origin of breast cancer. Ann Oncol. 2011;22:1102–8.

66. Fortunati N, Catalano MG, Boccuzzi G, et al. Sex Hormone-Binding Globulin (SHBG), estradiol and breast cancer. Mol Cell Endocrinol. 2010;316:86–92.
67. Cohn BA, Cirillo PM, Christianson RE, et al. Placental characteristics and reduced risk of maternal breast cancer. J Natl Cancer Inst. 2001;93:1133–40.
68. Troisi R, Potischman N, Roberts JM, et al. Maternal serum oestrogen and androgen concentrations in preeclamptic and uncomplicated pregnancies. Int J Epidemiol. 2003;32:455–60.
69. Key TJ, Appleby PN, Reeves GK, et al. Insulin-like growth factor 1 (IGF1), IGF binding protein 3 (IGFBP3), and breast cancer risk: pooled individual data analysis of 17 prospective studies. Lancet Oncol. 2010;11:530–42.
70. Cullen KJ, Yee D, Sly WS, et al. Insulin-like growth factor receptor expression and function in human breast cancer. Cancer Res. 1990;50:48–53.
71. Savarese TM, Strohsnitter WC, Low HP, et al. Correlation of umbilical cord blood hormones and growth factors with stem cell potential: implications for the prenatal origin of breast cancer hypothesis. Breast Cancer Res. 2007;9:R29.
72. Boyne MS, Thame M, Bennett FI, et al. The relationship among circulating insulin-like growth factor (IGF)-I, IGF-binding proteins-1 and -2, and birth anthropometry: a prospective study. J Clin Endocrinol Metab. 2003;88:1687–91.
73. Toniolo P, Grankvist K, Wulff M, et al. Human chorionic gonadotropin in pregnancy and maternal risk of breast cancer. Cancer Res. 2010;70:6779–86.
74. Troisi R, Potischman N, Hoover RN. Exploring the underlying hormonal mechanisms of prenatal risk factors for breast cancer: a review and commentary. Cancer Epidemiol Biomarkers Prev. 2007;16:1700–12.
75. Palomaki GE, Knight GJ, Haddow JE, et al. Cigarette smoking and levels of maternal serum alpha-fetoprotein, unconjugated estriol, and hCG: impact on Down syndrome screening. Obstet Gynecol. 1993;81(Pt 1):675–8.
76. Russo J, Russo IH. Biological and molecular bases of mammary carcinogenesis. Lab Invest. 1987;57:112–37.
77. Cerhan JR, Sellers TA, Janney CA, et al. Prenatal and perinatal correlates of adult mammographic breast density. Cancer Epidemiol Biomarkers Prev. 2005;14:1502–8.
78. Boyd NF, Rommens JM, Vogt K, et al. Mammographic breast density as an intermediate phenotype for breast cancer. Lancet Oncol. 2005;6:798–808.
79. Rudland PS. Stem cells and the development of mammary cancers in experimental rats and in humans. Cancer Metastasis Rev. 1987;6:55–83.
80. Qiu L, Low HP, Chang CI, et al. Novel measurements of mammary stem cells in human umbilical cord blood as prospective predictors of breast cancer susceptibility in later life. Ann Oncol. 2012;23:245–50.
81. Khan SI, Aumsuwan P, Khan IA, et al. Epigenetic events associated with breast cancer and their prevention by dietary components targeting the epigenome. Chem Res Toxicol. 2012;25:61–73.
82. Gillman MW. Developmental origins of health and disease. N Engl J Med. 2005;353:1848–50.
83. Barker DJ. Sir Richard Doll Lecture. Developmental origins of chronic disease. Public Health. 2012;126:185–9.
84. Heinonen OP. Diethylstilbestrol in pregnancy. Frequency of exposure and usage patterns. Cancer. 1973;31:573–7.
85. Noller KL, Fish CR. Diethylstilbestrol usage: its interesting past, important present, and questionable future. Med Clin North Am. 1974;58:793–810.
86. Herbst AL, Scully RE. Adenocarcinoma of the vagina in adolescence. A report of 7 cases including 6 clear-cell carcinomas (so-called mesonephromas). Cancer. 1970;25:745–57.
87. Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. N Engl J Med. 1971;284:878–81.
88. Sato T, Fukazawa Y, Ohta Y, et al. Involvement of growth factors in induction of persistent proliferation of vaginal epithelium of mice exposed neonatally to diethylstilbestrol. Reprod Toxicol. 2004;19:43–51.

89. Walker CL, Stewart EA. Uterine fibroids: the elephant in the room. Science. 2005;308:1589–92.
90. Baird DD, Newbold R. Prenatal diethylstilbestrol (DES) exposure is associated with uterine leiomyoma development. Reprod Toxicol. 2005;20:81–4.
91. Brody JR, Cunha GR. Histologic, morphometric, and immunocytochemical analysis of myometrial development in rats and mice: II. Effects of DES on development. Am J Anat. 1989;186:21–42.
92. Cook JD, Davis BJ, Cai SL, et al. Interaction between genetic susceptibility and early-life environmental exposure determines tumor-suppressor-gene penetrance. Proc Natl Acad Sci U S A. 2005;102:8644–9.
93. Greathouse KL, Cook JD, Lin K, et al. Identification of uterine leiomyoma genes developmentally reprogrammed by neonatal exposure to diethylstilbestrol. Reprod Sci. 2008;15:765–78.
94. McCampbell AS, Walker CL, Broaddus RR, et al. Developmental reprogramming of IGF signaling and susceptibility to endometrial hyperplasia in the rat. Lab Invest. 2008;88:615–26.
95. Newbold RR, Bullock BC, McLachlan JA. Uterine adenocarcinoma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis. Cancer Res. 1990;50:7677–81.
96. Li S, Washburn KA, Moore R, et al. Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. Cancer Res. 1997;57:4356–9.
97. Li S, Hansman R, Newbold R, et al. Neonatal diethylstilbestrol exposure induces persistent elevation of c-fos expression and hypomethylation in its exon-4 in mouse uterus. Mol Carcinog. 2003;38:78–84.
98. Bromer JG, Wu J, Zhou Y, et al. Hypermethylation of homeobox A10 by in utero diethylstilbestrol exposure: an epigenetic mechanism for altered developmental programming. Endocrinology. 2009;150:3376–82.
99. Tang WY, Newbold R, Mardilovich K, et al. Persistent hypomethylation in the promoter of nucleosomal binding protein 1 (Nsbp1) correlates with overexpression of Nsbp1 in mouse uteri neonatally exposed to diethylstilbestrol or genistein. Endocrinology. 2008;149:5922–31.
100. Center MM, Jemal A, Lortet-Tieulent J, et al. International variation in prostate cancer incidence and mortality rates. Eur Urol. 2012;61:1079–92.
101. Brawley OW. Prostate cancer epidemiology in the United States. World J Urol. 2012;30:195–200.
102. Gronberg H. Prostate cancer epidemiology. Lancet. 2003;361:859–64.
103. Moore KL, Persaud TVN, Torchia MG. Before we are born: essentials of embryology and birth defects. 7th ed. Philadelphia: Saunders/Elsevier; 2008. x, 353.
104. Ahluwalia B, Jackson MA, Jones GW, et al. Blood hormone profiles in prostate cancer patients in high-risk and low-risk populations. Cancer. 1981;48:2267–73.
105. Henderson BE, Bernstein L, Ross RK, et al. The early in utero oestrogen and testosterone environment of blacks and whites: potential effects on male offspring. Br J Cancer. 1988;57:216–18.
106. Ross RK, Henderson BE. Do diet and androgens alter prostate cancer risk via a common etiologic pathway? J Natl Cancer Inst. 1994;86:252–4.
107. Tibblin G, Eriksson M, Cnattingius S. High birthweight as a predictor of prostate cancer risk. Epidemiology. 1995;6:423–4.
108. Ekbom A, Hsieh CC, Lipworth L, et al. Perinatal characteristics in relation to incidence of and mortality from prostate cancer. BMJ. 1996;313:337–41.
109. Sandler DP, Everson RB, Wilcox AJ, et al. Cancer risk in adulthood from early life exposure to parents' smoking. Am J Public Health. 1985;75:487–92.
110. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci U S A. 2007;104:13056–61.

111. Rosen A, Jayram G, Drazer M, et al. Global trends in testicular cancer incidence and mortality. Eur Urol. 2011;60:374–9.
112. Bosl GJ, Motzer RJ. Testicular germ-cell cancer. N Engl J Med. 1997;337:242–53.
113. Horwich A, Shipley J, Huddart R. Testicular germ-cell cancer. Lancet. 2006;367:754–65.
114. Henderson BE, Benton B, Jing J, et al. Risk factors for cancer of the testis in young men. Int J Cancer. 1979;23:598–602.
115. Leary FJ, Resseguie LJ, Kurland LT, et al. Males exposed in utero to diethylstilbestrol. JAMA. 1984;252:2984–9.
116. Storgaard L, Bonde JP, Olsen J. Male reproductive disorders in humans and prenatal indicators of estrogen exposure. A review of published epidemiological studies. Reprod Toxicol. 2006;21:4–15.
117. Brown LM, Pottern LM, Hoover RN. Prenatal and perinatal risk factors for testicular cancer. Cancer Res. 1986;46:4812–16.
118. Sonke GS, Chang S, Strom SS, et al. Prenatal and perinatal risk factors and testicular cancer: a hospital-based case–control study. Oncol Res. 2007;16:383–7.
119. Moller H, Skakkebaek NE. Testicular cancer and cryptorchidism in relation to prenatal factors: case–control studies in Denmark. Cancer Causes Control. 1997;8:904–12.
120. Michos A, Xue F, Michels KB. Birth weight and the risk of testicular cancer: a meta-analysis. Int J Cancer. 2007;121:1123–31.
121. Chen Z, Robison L, Giller R, et al. Risk of childhood germ cell tumors in association with parental smoking and drinking. Cancer. 2005;103:1064–71.
122. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. CA Cancer J Clin. 2007;57:43–66.
123. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55:74–108.
124. Willett WC. Diet and cancer: one view at the start of the millennium. Cancer Epidemiol Biomarkers Prev. 2001;10:3–8.
125. Kim YI. Folate and colorectal cancer: an evidence-based critical review. Mol Nutr Food Res. 2007;51:267–92.
126. Kim YI. Folic acid supplementation and cancer risk: point. Cancer Epidemiol Biomarkers Prev. 2008;17:2220–5.
127. Song J, Sohn KJ, Medline A, et al. Chemopreventive effects of dietary folate on intestinal polyps in Apc+/−Msh2−/− mice. Cancer Res. 2000;60:3191–9.
128. Song J, Medline A, Mason JB, et al. Effects of dietary folate on intestinal tumorigenesis in the apcMin mouse. Cancer Res. 2000;60:5434–40.
129. Lindzon GM, Medline A, Sohn KJ, et al. Effect of folic acid supplementation on the progression of colorectal aberrant crypt foci. Carcinogenesis. 2009;30:1536–43.
130. Gluckman PD, Hanson MA, Cooper C, et al. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359:61–73.
131. Pfeiffer CM, Johnson CL, Jain RB, et al. Trends in blood folate and vitamin B-12 concentrations in the United States, 1988 2004. Am J Clin Nutr. 2007;86:718–27.
132. Wilson RD. Pre-conceptional vitamin/folic acid supplementation 2007: the use of folic acid in combination with a multivitamin supplement for the prevention of neural tube defects and other congenital anomalies. J Obstet Gynaecol Can. 2007;29:1003–26.
133. Sie KK, Medline A, van Weel J, et al. Effect of maternal and postweaning folic acid supplementation on colorectal cancer risk in the offspring. Gut. 2011;60:1687–94.
134. Lawrance AK, Deng L, Rozen R. Methylenetetrahydrofolate reductase deficiency and low dietary folate reduce tumorigenesis in Apc min/+ mice. Gut. 2009;58:805–11.
135. Barker DJ, Winter PD, Osmond C, et al. Weight in infancy and death from ischaemic heart disease. Lancet. 1989;2:577–80.
136. Leon DA, Lithell HO, Vagero D, et al. Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915–29. BMJ. 1998;317:241–5.
137. Will JC. Colorectal cancer: another complication of diabetes mellitus? Am J Epidemiol. 1998;147:816–25.

138. Hu FB, Manson JE, Liu S, et al. Prospective study of adult onset diabetes mellitus (type 2) and risk of colorectal cancer in women. J Natl Cancer Inst. 1999;91:542–7.
139. Schoen RE, Tangen CM, Kuller LH, et al. Increased blood glucose and insulin, body size, and incident colorectal cancer. J Natl Cancer Inst. 1999;91:1147–54.
140. Yamada K, Araki S, Tamura M, et al. Relation of serum total cholesterol, serum triglycerides and fasting plasma glucose to colorectal carcinoma in situ. Int J Epidemiol. 1998;27:794–8.
141. Ma J, Giovannucci E, Pollak M, et al. A prospective study of plasma C-peptide and colorectal cancer risk in men. J Natl Cancer Inst. 2004;96:546–53.
142. Kaaks R. Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women. J Natl Cancer Inst. 2000;92:1592–600.
143. Sandhu MS, Luben R, Day NE, et al. Self-reported birth weight and subsequent risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2002;11:935–8.
144. McCormack VA, dos Santos SI, Koupil I, et al. Birth characteristics and adult cancer incidence: Swedish cohort of over 11,000 men and women. Int J Cancer. 2005;115:611–17.
145. Nilsen TI, Romundstad PR, Troisi R, et al. Birth size and colorectal cancer risk: a prospective population based study. Gut. 2005;54:1728–32.
146. Kim YI. Nutritional epigenetics: impact of folate deficiency on DNA methylation and colon cancer susceptibility. J Nutr. 2005;135:2703–9.
147. Shabad LM, Sorokina JD, Golub NI, et al. Transplacental effect of some chemical compounds on organ cultures of embryonic kidney tissue. Cancer Res. 1972;32:617–27.
148. Castro DJ, Lohr CV, Fischer KA, et al. Lymphoma and lung cancer in offspring born to pregnant mice dosed with dibenzo[a, l]pyrene: the importance of in utero vs. lactational exposure. Toxicol Appl Pharmacol. 2008;233:454–8.
149. Anderson LM. Two crops of primary lung tumors in BALB/c mice after a single transplacental exposure to urethane. Cancer Lett. 1978;5:55–9.
150. Armuth V, Berenblum I. Tritiated thymidine as a broad spectrum initiator in transplacental two-stage carcinogenesis, with phorbol as promoter. Int J Cancer. 1979;24:355–8.
151. York RG, Stemmer K, Manson JM. Lung tumorigenesis and hyperplasia in offspring associated with the Ahd allele following in utero exposure to 3-methylcholanthrene. Toxicol Appl Pharmacol. 1984;72:427–39.
152. Wessner LL, Fan M, Schaeffer DO, et al. Mouse lung tumors exhibit specific Ki-ras mutations following transplacental exposure to 3-methylcholanthrene. Carcinogenesis. 1996;17:1519–26.
153. Leone-Kabler S, Wessner LL, McEntee MF, et al. Ki-ras mutations are an early event and correlate with tumor stage in transplacentally-induced murine lung tumors. Carcinogenesis. 1997;18:1163–8.
154. Miller MS, Leone-Kabler S, Rollins LA, et al. Molecular pathogenesis of transplacentally induced mouse lung tumors. Exp Lung Res. 1998;24:557–77.
155. Miller MS, Gressani KM, Leone-Kabler S, et al. Differential sensitivity to lung tumorigenesis following transplacental exposure of mice to polycyclic hydrocarbons, heterocyclic amines, and lung tumor promoters. Exp Lung Res. 2000;26:709–30.
156. Jennings-Gee JE, Moore JE, Xu M, et al. Strain-specific induction of murine lung tumors following in utero exposure to 3-methylcholanthrene. Mol Carcinog. 2006;45:676–84.
157. Koujitani T, Ton TV, Lahousse SA, et al. K-ras cancer gene mutations in lung tumors from female Swiss (CD-1) mice exposed transplacentally to 3′-azido-3′-deoxythymidine. Environ Mol Mutagen. 2008;49:720–6.
158. Mizesko MC. Alterations at the Ink4a locus in transplacentally induced murine lung tumors. Cancer Lett. 2001;172:59–66.
159. Anderson LM, Hagiwara A, Kovatch RM, et al. Transplacental initiation of liver, lung, neurogenic, and connective tissue tumors by N-nitroso compounds in mice. Fundam Appl Toxicol. 1989;12:604–20.
160. Anderson LM, Hecht SS, Dixon DE, et al. Evaluation of the transplacental tumorigenicity of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in mice. Cancer Res. 1989;49:3770–5.

161. Waalkes MP, Ward JM, Liu J, et al. Transplacental carcinogenicity of inorganic arsenic in the drinking water: induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. Toxicol Appl Pharmacol. 2003;186:7–17.
162. Waalkes MP, Ward JM, Diwan BA. Induction of tumors of the liver, lung, ovary and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers. Carcinogenesis. 2004;25:133–41.
163. Shen J, Liu J, Xie Y, et al. Fetal onset of aberrant gene expression relevant to pulmonary carcinogenesis in lung adenocarcinoma development induced by in utero arsenic exposure. Toxicol Sci. 2007;95:313–20.
164. Tokar EJ, Diwan BA, Waalkes MP. Renal, hepatic, pulmonary and adrenal tumors induced by prenatal inorganic arsenic followed by dimethylarsinic acid in adulthood in CD1 mice. Toxicol Lett. 2012;209:179–85.
165. Yu Z, Mahadevan B, Lohr CV, et al. Indole-3-carbinol in the maternal diet provides chemoprotection for the fetus against transplacental carcinogenesis by the polycyclic aromatic hydrocarbon dibenzo[a, l]pyrene. Carcinogenesis. 2006;27:2116–23.
166. Castro DJ, Lohr CV, Fischer KA, et al. Identifying efficacious approaches to chemoprevention with chlorophyllin, purified chlorophylls and freeze-dried spinach in a mouse model of transplacental carcinogenesis. Carcinogenesis. 2009;30:315–20.
167. Goel R, Olshan AF, Ross JA, et al. Maternal exposure to medical radiation and Wilms tumor in the offspring: a report from the Children's Oncology Group. Cancer Causes Control. 2009;20:957–63.
168. Stjernfeldt M, Berglund K, Lindsten J, et al. Maternal smoking during pregnancy and risk of childhood cancer. Lancet. 1986;1:1350–2.
169. Olshan AF, Breslow NE, Daling JR, et al. Wilms' tumor and paternal occupation. Cancer Res. 1990;50:3212–17.
170. Jemal A, Siegel R, Ward E, et al. Cancer statistics. CA Cancer J Clin. 2009;l59:225–49.
171. Parkin DM, Bray F, Ferlay J, et al. Estimating the world cancer burden: Globocan 2000. Int J Cancer. 2001;94:153–6.
172. Mandel JS, McLaughlin JK, Schlehofer B, et al. International renal-cell cancer study. IV. Occupation. Int J Cancer. 1995;61:601–5.
173. La Vecchia C, Negri E, D'Avanzo B, et al. Smoking and renal cell carcinoma. Cancer Res. 1990;50:5231–3.
174. Hunt JD, van der Hel OL, McMillan GP, et al. Renal cell carcinoma in relation to cigarette smoking: meta-analysis of 24 studies. Int J Cancer. 2005;114:101–8.
175. Yu MC. Cigarette smoking, obesity, diuretic use, and coffee consumption as risk factors for renal cell carcinoma. J Natl Cancer Inst. 1986;77:351–6.
176. Byrne J. Reproductive problems and birth defects in survivors of Wilms' tumor and their relatives. Med Pediatr Oncol. 1988;16:233–40.
177. Bergstrom A, Lindblad P, Wolk A. Birth weight and risk of renal cell cancer. Kidney Int. 2001;59:1110–13.
178. Leisenring WM, Breslow NE, Evans IE, et al. Increased birth weights of National Wilms' Tumor Study patients suggest a growth factor excess. Cancer Res. 1994;54:4680–3.
179. Greaves M. A natural history for pediatric acute leukemia. Blood. 1993;82:1043–51.
180. Ross JA, Potter JD, Robison LL. Infant leukemia, topoisomerase II inhibitors, and the MLL gene. J Natl Cancer Inst. 1994;86:1678–80.
181. Feychting M, Ahlbom A. Magnetic fields and cancer in children residing near Swedish high-voltage power lines. Am J Epidemiol. 1993;138:467–81.
182. Mezei G, Kheifets L. Selection bias and its implications for case–control studies: a case study of magnetic field exposure and childhood leukaemia. Int J Epidemiol. 2006;35: 397–406.
183. Kheifets L, Swanson J, Greenland S. Childhood leukemia, electric and magnetic fields, and temporal trends. Bioelectromagnetics. 2006;27:545–52.

184. Bailey HD, Miller M, Langridge A, et al. Maternal dietary intake of folate and vitamins b6 and B12 during pregnancy and the risk of childhood acute lymphoblastic leukemia. Nutr Cancer. 2012;64:1122–30.
185. Bailey HD, Armstrong BK, de Klerk NH, et al. Exposure to diagnostic radiological procedures and the risk of childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomarkers Prev. 2010;19:2897–909.
186. Kwan ML, Jensen CD, Block G, et al. Maternal diet and risk of childhood acute lymphoblastic leukemia. Public Health Rep. 2009;124:503–14.
187. Petridou ET, Sergentanis TN, Panagopoulou P, et al. In vitro fertilization and risk of childhood leukemia in Greece and Sweden. Pediatr Blood Cancer. 2012;58:930–6.
188. Hemminki K, Kyyronen P, Vaittinen P. Parental age as a risk factor of childhood leukemia and brain cancer in offspring. Epidemiology. 1999;10:271–5.
189. Hori M, Ami Y, Kushida S, et al. Intrauterine transmission of human T-cell leukemia virus type I in rats. J Virol. 1995;69:1302–5.
190. Uemura Y, Kotani S, Yoshimoto S, et al. Mother-to-offspring transmission of human T cell leukemia virus type I in rabbits. Blood. 1987;69:1255–8.
191. Reid A, Glass DC, Bailey HD, et al. Parental occupational exposure to exhausts, solvents, glues and paints, and risk of childhood leukemia. Cancer Causes Control. 2011;22:1575–85.
192. Castro DJ, Baird WM, Pereira CB, et al. Fetal mouse Cyp1b1 and transplacental carcinogenesis from maternal exposure to dibenzo(a, l)pyrene. Cancer Prev Res (Phila). 2008;1:128–34.
193. Wigle DT, Turner MC, Krewski D. A systematic review and meta-analysis of childhood leukemia and parental occupational pesticide exposure. Environ Health Perspect. 2009;117:1505–13.
194. Monge P, Wesseling C, Guardado J, et al. Parental occupational exposure to pesticides and the risk of childhood leukemia in Costa Rica. Scand J Work Environ Health. 2007;33:293–303.
195. Yu Z, Loehr CV, Fischer KA, et al. In utero exposure of mice to dibenzo[a, l]pyrene produces lymphoma in the offspring: role of the aryl hydrocarbon receptor. Cancer Res. 2006;66:755–62.
196. Perez-Saldivar ML, Ortega-Alvarez MC, Fajardo-Gutierrez A, et al. Father's occupational exposure to carcinogenic agents and childhood acute leukemia: a new method to assess exposure (a case–control study). BMC Cancer. 2008;8:7.
197. Infante-Rivard C, Deadman JE. Maternal occupational exposure to extremely low frequency magnetic fields during pregnancy and childhood leukemia. Epidemiology. 2003;14:437–41.
198. Stjernfeldt M. Maternal smoking and irradiation during pregnancy as risk factors for child leukemia. Cancer Detect Prev. 1992;16:129–35.
199. Little J. Epidemiology of childhood cancer. IARC Sci Publ. 1999;149:1–386.
200. Lehtinen M, Koskela P, Ogmundsdottir HM, et al. Maternal herpesvirus infections and risk of acute lymphoblastic leukemia in the offspring. Am J Epidemiol. 2003;158:207–13.
201. Caballero OL. Maternal illness and drug/medication use during the period surrounding pregnancy and risk of childhood leukemia among offspring. Am J Epidemiol. 2007;165:27–35.
202. Shaw AK, Infante-Rivard C, Morrison HI. Use of medication during pregnancy and risk of childhood leukemia (Canada). Cancer Causes Control. 2004;15:931–7.
203. Wen W. Parental medication use and risk of childhood acute lymphoblastic leukemia. Cancer. 2002;95:1786–94.
204. Hjalgrim LL. Birth weight as a risk factor for childhood leukemia: a meta-analysis of 18 epidemiologic studies. Am J Epidemiol. 2003;158:724–35.
205. Paltiel O, Harlap S, Deutsch L, et al. Birth weight and other risk factors for acute leukemia in the Jerusalem Perinatal Study cohort. Cancer Epidemiol Biomarkers Prev. 2004;13:1057–64.
206. Deorah S, Lynch CF, Sibenaller ZA, et al. Trends in brain cancer incidence and survival in the United States: Surveillance, Epidemiology, and End Results Program, 1973 to 2001. Neurosurg Focus. 2006;20:E1.
207. Samuelsen SO. Head circumference at birth and risk of brain cancer in childhood: a population-based study. Lancet Oncol. 2006;7:39–42.

Gamete/Embryo-Fetal Origins of Obesity

7

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Abstract

Obesity is defined as abnormal or accumulation of excessive fat accumulation. More than 1.4 billion adults were overweight in 2008, of these over 200 million men and nearly 300 million women were obese. Obesity has become one of the most important risk factors contributing to the overall burden of diseases worldwide, so much so that the World Health Organization (WHO) has called obesity an epidemic.

Origins of obesity and metabolic dysfunction can be traced back to the embryonic and fetal stages of life, when the developing fetus is acted upon by, and responds to, sub-optimal, intrauterine environments during critical periods of cellular proliferation, differentiation, and maturation. It produces structural and functional changes in cells, tissues and organ systems. These changes may have long-term consequences increasing an individual's risk for developing complex common disorders including obesity, diabetes, cardiovascular disease and tumours. In this chapter, we will discuss the evidence related to embryo-fetal origins of obesity.

7.1 Risks of Obesity

7.1.1 Birthweight and Later Obesity

Birthweight is an indicator of fetal growth and long-term health. High birth weights are associated with increased risks of adverse adult health outcomes, such as obesity [1, 2] and type 1 diabetes [3], which are important determinants of adult

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mortality [4, 5]. High birthweights are also associated with higher risks of some adult cancers [6, 7].

Individuals with intrauterine growth retardation (IUGR) are at higher risk of neonatal morbidity and of developing metabolic diseases later in life, including type-2 diabetes, obesity and hypertension [8, 9]. Both human epidemiological and experimental evidence indicate that IUGR contributes to low birthweight, and, higher incidence of obesity in adults [10].

7.1.2 Maternal Undernutrition and Later Obesity

Nutrition during the intrauterine phase may be particularly important for the development of obesity. In October 2012, 868 million people worldwide including a large proportion of women of reproductive age were reported by the United Nations Food and Agriculture Organization to suffer from hunger; nearly all of the undernourished reside in low- and middle-income countries [11]. Deficiencies of protein, vitamin A, iron, zinc, folate, and other micronutrients remain major nutritional problems in poor regions of the world [11].

The Dutch Famine studies have demonstrated that there are different consequences of exposure to undernutrition in different trimesters of pregnancy [12]. Exposure in early pregnancy can increase the risks of adult obesity significantly [13]. Much epidemiological evidence shows that fetal exposure to maternal undernutrition is associated with increased risks of adult obesity. Vignini et al. put forward the viewpoint that maternal undernutrition during pregnancy may permanently change or 'programme' the offspring [14]. Barker concluded that coronary heart disease, and, other associated conditions, such as hypertension, stroke, and, non-insulin dependent diabetes, may be the results of 'programming' [15–17]. At a critical, sensitive period of early life, a stimulus or insult promotes long-term changes in physiology or metabolism in offspring's later life. Some studies showed that nutrient restriction during the first half of pregnancy in humans, mice and rats is associated with postnatal metabolic and endocrine disorders, as well as cardiovascular disorders [17–19].

7.1.3 Animal Studies

Mouse models of low birthweight produced by maternal caloric undernutrition during late gestation found reduced birth weight, IGT, and obesity in both first- and second-generation offspring [18]. Ikenasio-Thorpe et al., used a model of nutrient-restricted rats were randomly assigned to receive 30 % of the ad libitum amount during gestation exhibited fetal growth retardation compared with controls [19]. When these offspring were fed a high-fat diet (45 % kcal as fat) for 20 weeks from weaning, there were significant alterations in POMC, NPY, AgRP and OBRb gene expression together with elevations in circulating levels of both plasma leptin and

insulin [19]. There appeared to be interactions between prenatal undernutrition and postnatal high-fat nutrition on the development of postnatal obesity [19].

Long et al. offered cows 70 % of a control diet during early and mid gestation to evaluate effects of maternal nutrient restriction on the morphology of offspring adipose tissue at standard production endpoints [20]. In nutritionally-restricted offspring adipocyte size altered, showing that nutritional restriction during gestation increased adipose tissue depots in finished calves [20]. Multiparous ewes fed 50 % (nutrient-restricted) of their nutrient requirements between 28 and 78 days of gestation demonstrated that maternal undernutrition during early to midgestation increased body weight and fat deposition during adolescence [21].

7.1.4 The 'Thrifty Phenotype' Hypothesis

The "thrifty phenotype" hypothesis proposes that undernutrition during development leads to reallocation of nutrients to favour development of organs; these organs then go on to break down and fail in adulthood [22]. Human epidemiological studies have associated maternal undernutrition and fetal growth restriction during gestation with development of a "thrifty phenotype" in the later lives of offspring. As an extension of this hypothesis, the DOHaD hypothesis describes the origins of adult disease in terms of fetal developmental 'plasticity', or, the ability of the fetus to respond to poor in-utero conditions.

Inappropriate hyperphagia in adult life induced by fetal undernutrition is an example of an adaptive response, where postnatal hypercaloric nutrition can further amplify the metabolic abnormalities [23]. Similarly, rats undernourished in utero exhibit rapid catch-up growth and are more susceptible to developing obesity [24].

Lifestyle choices that exacerbate obesity, may also have a prenatal origin. Offspring of mothers who were undernourished in pregnancy, are significantly more sedentary in postnatal life compared to those born to well-fed mothers [25]. Postnatal hypercaloric nutrition may exacerbate this sedentary behavior, implying that "programmed" adults may be more resistant to public health policies and interventions aimed at increasing physical exercise and reducing food intake.

7.1.5 Neural Plasticity

Neural plasticity may be another important contributor to the continuation of obesity between early development and adulthood. Early pre- and postnatal metabolic conditions such as undernutrition may lead genetically-predisposed offspring to become even more obese as adults. Maternal metabolic consequences of prenatal undernutrition may modify the developing neural systems that control energy homeostasis in the fetus [26].

Increased hypothalamus-pituitary-adrenal axis activity and decreased sympatho-adrenal system activity in adult male rats [27] was induced by 50 % food restriction

during the last third of pregnancy and lactation. Another rat model fed half of the daily intake during the last week of gestation until weaning, produced similar effects on the hypothalamo-pituitary-adrenal (HPA) axis of offspring [28]. These data suggest that there may be chronic hyperactivity of the HPA axis leading to high glucocorticoid levels in adulthood.

7.1.6 Epigenetic Modifications

Epigenetic mechanisms, such as DNA methylation and nucleoprotein acetylation or methylation, are important to the physiological development of several tissues in mammals, and, they involve several mechanisms to guarantee fluctuations of enzymes and other proteins that regulate metabolism [29–31]. Alterations in nutrition during development can alter epigenetic marks, including DNA methylation and histone modifications in rodents [32–35]. Epidemiological and controlled studies in humans and animals demonstrate that undernutrition can result in DNA hypomethylation in offspring, implicating epigenetics as a potential mechanism through which maternal diet may affect the health of offspring [36, 37]. In humans, emerging data suggests that severe maternal undernutrition may result in persistent epigenetic changes in the offspring [38]. Recent data suggests that gene methylation changes in DNA extracted from umbilical cord tissue are associated with later childhood obesity [39]. Hypomethylation of glucocorticoid receptor (GR) promoter in fetal sheep leads to increasing GR expression in the undernourished group which may, in turn, contribute to fetal programming of a predisposition to obesity [40]. Another animal study showing similar results suggests that hypomethylation of GR promoter associated hypothalamic neuropeptide mRNA expression may lead to obesity in the next generation [41].

7.1.7 Leptin Resistance

Offspring of undernourished mothers have higher concentrations of fasting plasma leptin and insulin which decrease appetite. On the other hand, exposure to a postnatal hypercaloric diet will increase hyperphagia, suggesting an inappropriate response due to insulin and leptin resistance induced by early programming. Ikenasio-Thorpe et al. showed significant alterations in POMC, NPY, AgRP and OBRb gene expression together with elevations in circulating levels of both plasma leptin and insulin when exposed to prenatal undernutrition, suggesting that central leptin resistance possibly increased food intake, and, results in adult dysregulation of appetite homeostasis and reduced AgRP mRNA expression [19]. Leptin-deficient ob/ob mouse offspring exposed to intrauterine undernutrition show that premature leptin surges during neonatal growth promoted lifelong changes in energy-regulating circuitry in the hypothalamus on a high-fat diet, thus playing an important role in accelerating obesity [42].

7.1.8 Hyperinsulinemia and Peripheral Insulin Resistance

Restricting the supply of food to fetus and infant may cause obesity, hyperinsulinemia and peripheral insulin resistance in adult life [43]. This insulin resistance occurs in conjunction with altered glucose uptake in adipose tissue but not in skeletal muscle, and, there is an accompanying increase in adipose tissue insulin receptors in nutrient-restricted offspring [43]. Circulating hormones, including IGF-I and leptin, are important in the regulation of fetal adipose tissue development. Maternal nutrient restriction during this period results in increased expression of both IGF-I and IGF-II receptors, in conjunction with enhanced adipose tissue deposition [44]. A study demonstrated that offspring of sheep mothers who were nutrient-restricted in late gestation went on to have greater adiposity as young adults, along with glucose intolerance and insulin resistance [45]. Adipose tissue deposition in offspring can also be reduced by manipulating the maternal metabolic and hormonal environment by increasing food intake in late gestation [46].

7.1.9 Abnormal Response of Glucose Transporter

Increased glucose transporter 1 (GLUT1) in nutrient-restricted fetuses may enhance responsiveness to IGF and promote the anabolic effects of glucose on fetal adipose tissue growth [47]. Therefore, maternal nutrient restriction in mid-gestation, results in enhanced fetal fat deposition combined with increased numbers of IGF receptors and glucose supply, that may exacerbate the deposition of fat following the restoration of the maternal diet [48]. GLUT4 decreased significantly in adipose tissue of offspring from nutrient-restricted mothers suggesting that impaired glucose tolerance may be related to the ability of adipose tissue to take up glucose in an insulin-responsive manner with reductions closely associated with insulin resistance [49].

7.1.10 Maternal Overnutrition and Later Obesity

Traditionally, the increasing prevalence of childhood obesity is related to life styles with less physical activity and changing dietary habits. Predisposition to obesity may be “programmed” in utero [50]. Studies in rodents show that exposure to maternal obesity or overnutrition during both pregnancy and lactation is associated with development of obesity in the offspring [51, 52]. LaCoursiere et al. demonstrated that the incidence of women being overweight or obese at the start of pregnancy increased from 25 to 35 % between 1991 and 2001, and that the incidence of maternal obesity at delivery increased from 29 to 39 % across the same period [8]. A high maternal BMI increases the risk of developing hypertension, preeclampsia and gestational diabetes mellitus, and, giving birth to a macrosomic infant [53]. These findings may suggest that fetal undernutrition may increase susceptibility to diseases

that occur later in life. Evidence from animal studies suggests that the fetus may adapt to an adverse intrauterine environment by slowing growth and metabolism [18, 20, 21]. This adaptive strategy appears to increase short-term survival, but perhaps with adverse long-term consequences on health [18, 21]. Alternatively, common genetic factors may influence birth size and adult disease or a combination of genetic, and non-genetic, factors may interact throughout the life course to determine disease susceptibility [19].

7.1.11 Animal Studies

Rodent dams, fed a fat-rich or cholesterol-rich diets during the preconception period, pregnancy, or lactation, confirm that maternal peripartum overnutrition can program lifelong offspring with excess adiposity and being overweight [54–56]. Similar results are also observed in sheep experiments [57]. Interestingly, a fat-rich diet during pregnancy may program offspring' obesity even though the mothers themselves are not overweight, indicating that maternal diet may program offspring phenotype even without the relevant maternal phenotype [58]. Maternal fat-rich diets before conception or during lactation only, do not confer similar risks, suggesting that pregnancy itself is the critical time for exposure [59].

Maternal overnutrition during pregnancy in rodents results in offspring phenotypes that almost resemble the metabolic syndrome in humans, such as abnormal glucose homeostasis and serum lipid profiles, increased blood pressure and adiposity [56, 60, 61]. Overweight offspring of overfed mothers had higher glucose, insulin, leptin, and triglyceride levels, and demonstrated the increase in fat mass, reduced muscle mass, and, lower adiponectin secretion [62–65].

7.1.12 The Regulation of Appetite In Utero

The fetus obtains its nutrition entirely from the maternal circulation through transplacental transfer and so it has a limited capacity to respond to alterations in nutrient supply by altering nutrient intake [66]. However appetite-regulating neural networks appear before birth in humans [67] and higher-order mammals, such as sheep [68].

Control areas for appetite and energy balance are expressed principally in the arcuate nucleus (ARC) of the hypothalamus (Fig. 7.1). The hypothalamic neural network integrates pathways relating to energy supply, utilization and total energy reserves, in order to appropriately regulate food intake and energy expenditure to maintain energy balance [69]. Several studies have identified numbers of appetite-stimulating (and suppressing) hormones and neurotransmitters, which bind and activate their CNS receptors, triggering downstream pathways or regulators, resulting in appropriate changes in behaviour of ingestion. Appetite-stimulating neurohormones include neuropeptide Y (NPY), melanin concentrating hormone (MCH),

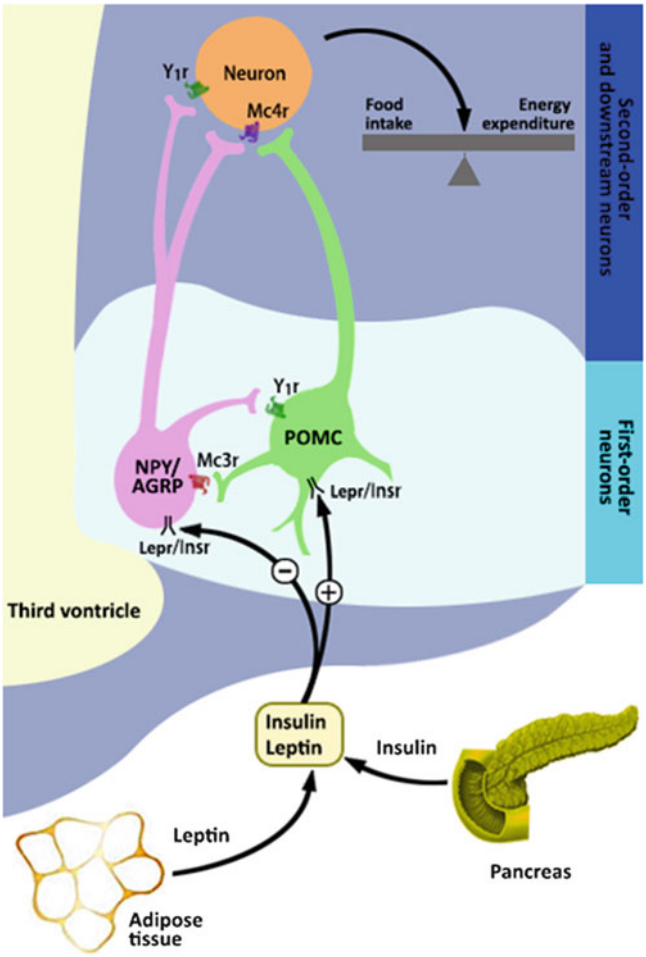

Fig. 7.1 A schematic overview of the appetite regulatory pathways in the adult hypothalamus

the orexins, endorphins, galanin, glucocorticoids, γ-amino butyric acid (GABA), and agouti gene-related protein (AGRP). Negative regulators of appetite include leptin, bombesin, glucagon-like peptide-1 (GLP-1), corticotropin releasing hormone (CRH), cholecystokinin (CCK), cocaine and amphetamine-regulated transcript (CART), and α-melanocyte-stimulating hormone (α-MSH).

Many animal studies suggest that overnutrition before birth may cause reduced enhance sensitivity to satiety signals, or, an exaggerated response to signals of hunger and negative energy balance, in the early postnatal period [70, 71]. The disturbance of appropriately-upregulated, appetite-inhibitory pathways may result in the development of obese phenotypes in animal experiments (Fig. 7.2).

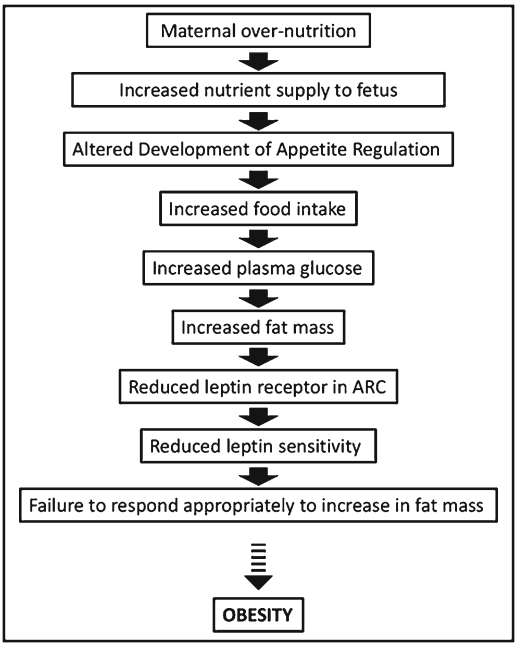

Fig. 7.2 Overview of our current working hypothesis on the pathway through which maternal overnutrition results in the programming of obesity in postnatal life

7.1.13 Altered Glucose Intolerance and Glucose/Insulin Homeostasis

Animal studies support the view that maternal overnutrition can cause later obesity and glucose intolerance of offspring in humans [55]. Offspring of obese women are more likely to be overweight, and may develop insulin resistance in later life [72]. Besides, it is also related with the development of metabolic dysfunction in offspring, including hyperglycaemia, hyperinsulinaemia, and increased plasma levels of triglycerides, cholesterol and leptin [56, 60–65].

7.1.14 Altered Adiposity and Adipocyte Metabolism

Adipogenesis is important in the developmental programming which begins in utero and accelerates in neonatal life. For humans, it will accelerate rapidly again at about age of 6 years old [73]. Premature onset of such adipose tissue mass (before 5.5 years of age) in childhood may be related to adult obesity [73]. It is still unclear how maternal overnutrition influences adipogenesis in offspring, and, how it may determine the critical timing of the 'adiposity rebound'. Unlike many tissues, adipose tissue has potential for unlimited growth, and, diet-induced increases in fat cell number are normally irreversible [74]. Maternal over-nutrition has a direct influence on adipocyte hypertrophy in offspring because glucose is the primary metabolic precursor in lipid synthesis. Increased glucose supply

may increase fat mass in ovine fetus [75]. Altered expression of proteins which influence adipocyte metabolism, such as peroxisome proliferator-activated receptor- γ (PPAR-γ), may have a permanent influence on adipocyte proliferation and hypertrophy [76].

7.1.15 Altered Methylation Status

The methylation status of nuclear DNA (nDNA) has effects on persistent epigenetic changes. Dolinoy et al. suggested that considering the critical roles that genomically imprinted genes play in mammalian growth and development, early nutritional influences on these genomic components may have a substantial impact on human health [32]. The genome of the pre-implantation mammalian embryo undergoes extensive demethylation, and appropriate patterns of cytosine methylation are reestablished after implantation [77, 78]. These DNA methylation patterns must then be maintained over many rounds of rapid cellular proliferation during fetal and early postnatal development. Availability of dietary methyl donors and cofactors during critical ontogenic periods may therefore influence DNA methylation patterns [77, 78]. Dietary methionine and choline are the most important sources for one-carbon units, and folic acid, vitamin B12, pyridoxal phosphate are major cofactors in methyl metabolism. Early methyl donor malnutrition (i.e., overnutrition) could effectively lead to premature "epigenetic aging," [79], thereby contributing to enhanced susceptibility to chronic disease in later life. Besides, the function of leptin, which may primarily programme appetite regulatory centres in the developing hypothalamus may be changed by the alteration in methylation [80]. Altered methylation status in very early embryonic development can contribute to the obese phenotype through embryo transfer and cloning [81].

7.1.16 Parental Factors

Offspring with two obese parents may have higher risks of being overweight in childhood, and, also reveal a stronger pattern of tracking from childhood to adulthood [82]. A cohort study in Washington State found that without obese parents, obese children under 3 years of age were at low risk of becoming obese in adulthood [83]. In this study parental obesity doubled the risk of adult obesity among children less than 10 years of age irrespective of whether the child was obese or not [83]. In another cohort study, they quantified the individual and combined effects of maternal and paternal obesity on childhood obesity [84]. They found that the association between parental weight status and risk of childhood obesity was strong and graded, and, significantly stronger for maternal weight [84].

It has been shown that there is no strong difference between the maternal-offspring and paternal-offspring associations of BMI [82–84]. A large population-based study showed similar parental-offspring BMI associations when the offspring were 3 years old, which indicates that the maternal-offspring association may be explained

by shared familial risk factors including environmental and genetic risk factors rather than by the intrauterine environment [83].

Can obesity be "transmitted" to subsequent generations by fathers? Sperm transmit solely genetic and epigenetic factors. In order to separate environmental from genetic factors, Perez-Pastor et al. extended the analysis of BMI relationships to gender-assorted pairings of mother-daughter and father-son, comparing them with mother-son and father-daughter [85]. They succeeded in finding a same-sex association of body mass index (BMI), which might imply shared environment rather than shared genes because selective mother-daughter and father-son gene transmission is not a common Mendelian trait [85]. However, in 2010, a large UK birth cohort found no evidence of significant differences in mother-daughter and father-son body mass index concordance [86].

To explore the contribution of obese fathers to adiposity and metabolism in offspring, Ng and his colleagues established paternal high-fat-diet (HFD) rat model and found that chronic HFD consumption in Sprague-Dawley fathers induced increased body weight, adiposity, impaired glucose tolerance and insulin sensitivity in female offspring [87]. Carone et al. fed male rats with reduced-protein diets and bred them with chow-fed females and found that both male and female offspring had increased hepatic expression of lipid and cholesterol synthesis genes [88]. Overall DNA methylation in offspring was found unchanged; however, the methylation in an intergenic CpG island between PPAR-α and Wnt7-β modestly increased. These data strongly support the idea that what fathers eat affects the metabolism of their offspring.

7.1.17 Endocrine-Disrupting Chemicals

Over the years, humans have evolved to tolerate and metabolize natural products encountered in diet, however, they may be unable to handle the molecules not usually found in nature. There are a subset of synthetic chemicals referred to as endocrine-disrupting chemicals (EDC), which are environmental pollutants with hormone-like activity that may disrupt programming of endocrine signaling pathways during development and result in adverse effects including obesity, diabetes etc.

Recent epidemiology reports suggest links between exposure to EDCs during development, and, overweight or obesity later in life. If exposed to polychlorinated bisphenyl (PCB) in prenatal and early life, both boys and girls will be heavier at puberty [89]. Children with higher levels of hexachlorobenzene (HCB) in their cord blood weighed more, and, had higher BMI at the age of 6.5 years [90]. Also, children in the higher exposure group of HCB were more likely to be overweight and obese [90].

Numerous animal studies also demonstrate the association between obesity and exposure to various environmental chemicals during development [91]. For example, mice treated with a low dose of diethylstilbestrol (DES) on days 1–5 of neonatal life did not affect body weight during treatment but was associated with a significant increase in body weight at 4–6 months of age [92]. High prenatal DES

doses caused lower birthweight compared to controls, followed by a catch-up growth at puberty, and then resulted in obesity in the DES-treated mice after 2 months of age [92]. In both mouse and rat, there are associations between low doses of BPA during prenatal and neonatal periods and increased body weight [91, 92].

These toxic chemicals can initiate or exacerbate the development of obesity by targeting nuclear hormone receptor, including sex steroid receptor, glucocorticoid receptor, and RXR-PPARγ (retinoic X receptor-peroxisome proliferate activated receptor gamma) [91, 92]. By perturbing these signaling pathways, EDCs alter adipocyte proliferation, differentiation or mediate systemic homeostatic controls and result in long-term consequences. The fetus and newborn, whose detoxification and metabolic mechanisms are still immature, are particularly vulnerable to the effects of EDCs. These adverse effects may be magnified if perturbation occurs during fetal or early childhood development [91, 92]. Newbold and his colleagues analyzed gene expression in uterine samples from DES-treated mice and found alteration in genes involved in fat distribution, including down-regulation of Thbd and Nr2f1 and up-regulation of Sfrp2 [91, 92]. These findings suggest that EDCs may modulate the development of obesity by regulating expression of these genes.

7.1.18 Effect of Prenatal Smoking

A series of studies suggest an association between prenatal maternal smoking and offspring's obesity. Oken and his colleagues performed a meta-analysis of results of 84,563 children reported in 14 studies, and, concluded that offspring of mothers who smoked during pregnancy had higher risk for overweight at ages 3–33 years [93]. A meta-analyses of the effects of maternal environmental tobacco smoke exposure (ETS) during pregnancy on birth outcomes found a small reduction in mean birth weight, and, an increased pooled risk of babies being small for gestational age at birth [94]. A systemic review further demonstrated that exposure of non-smoking pregnant women to ETS reduces mean birth weight by 33 g or more and increases the risk of higher morbidity, low birth weight births by 22 % [95].

In a Spanish cohort study, the authors used longitudinal ultrasound measurements to assess the effects of in utero tobacco exposure on fetal growth. They found that active smoking during pregnancy was associated with a reduction in abdominal circumference, femur length and estimated fetal weight from mid-gestation, and, environmental tobacco smoke adversely affected biparietal diameter from early pregnancy [96]. How maternal smoking programs affect child weight is not well understood. In both humans and animals, nicotine acts both centrally and peripherally to reduce appetite and body weight, and nicotine withdrawal results in hyperphagia and weight gain [97, 98]. In animal studies, exposure of pregnant mothers to nicotine resulted in offspring that were smaller at birth but had increased body fat, and, rats prenatally exposed to low doses of nicotine were normal sizes at birth but became heavier by 5–10 weeks of age [99, 100]. Maternal smoking throughout

pregnancy may be associated with lower cord blood leptin [101]. However, Helland et al. could not confirm that lower birthweights of neonates among smoking mothers is not due to altered plasma leptin concentrations [102].

7.1.19 Maternal PCOS

Polycystic ovary syndrome (PCOS) is characterized by irregular menses, chronic anovulation, hyperandrogenism and infertility and is strongly associated with obesity, increased risk of developing type 2 diabetes (T2D), and cardiovascular disease [103]. Forty to eighty percent of women with PCOS are overweight or obese, implicating BMI as an important determinant in the manifestation of the syndrome [104, 105]. These observations suggest that obesity and PCOS are linked co-morbidities, and, PCOS may be one of the most important causes of obesity in women and their offspring.

7.1.20 Genes Determining the Obesity in Offspring of PCOS Patients

Family-based studies suggest that brothers of PCOS women have decreased insulin sensitivity and glucose tolerance, as well as hypercoagulability, that is independent of obesity [106]. Therefore, brothers of PCOS women may have inherited the speculative genotype for insulin resistance and metabolic syndrome that is characteristic of PCOS [106]. Possible candidate genes predisposing to PCOS include those involved in the regulation of ovarian steroidogenesis but also those genes that influence BMI and adiposity. A likely explanation for the mechanisms underlying the development of obesity in women with PCOS is the combined effect of a genetic predisposition in the context of an obesogenic environment [107]. Recent technological and computational advances in genome-wide association studies (GWAS) have identified variations in or near FTO, INSIG2, GNPDA2, MC4R, NEGR1, SH2B1 MTCH2, KCTD15, and TMEM18 as susceptibility loci for obesity [108–112]. PCOS patients carrying these obesity-susceptible genes, and, may pass on these pathogenic genes to their offspring through oocytes. However, few genetic studies on PCOS have focused on obesity to date, consequently, the contribution of genes that influence body composition in PCOS remains to be clarified.

7.1.21 Insulin Resistance

Abnormal insulin action influences both the ovarian production of androgens by theca cells and their bioavailability by reducing hepatic SHBG production [113–115]. Up to 50 % of PCOS women are obese that additionally contributes to insulin resistance, which, in turn, increases the risk for development of glucose intolerance, T2D, as well as dyslipidemia and hypertension [116]. The D19S884 allele 8 (A8)

which is the susceptibility locus of PCOS is associated with insulin resistance, β-cell dysfunction, and other metabolic phenotypes in PCOS families [117]. D19S884 maps to chromosome 19p13.2 within the FBN3 gene. FBN3 encodes fibrillin-3, one of the three members of the fibrillin family of extracellular matrix proteins [118]. Fibrillins provide structural integrity to connective tissues and regulate the activity of members of the TGF-β superfamily [119], which have been implicated in PCOS, insulin resistance, T2D, and glucose homeostasis [120, 121]. If members of the TGF-β pathway are implicated in various states of insulin resistance, then FBN3, a potential extracellular regulator of this pathway, may also play a role in regulating maternal glycemia [122, 123].

GYS2 gene is a new susceptibility gene that significantly impacts the risk for PCOS through obesity-related conditions [124]. The human GYS2 is located at 12p12.2. It is an enzyme responsible for the synthesis of 1, 4-linked glucose chains in glycogen, and, encodes for rate-limiting liver glycogen synthesis. Its activity is highly regulated through phosphorylation at multiple sites and by allosteric effectors, mainly glucose 6-phosphate. Some studies report that defects in the GYS2 gene cause inherited monogenic disease glycogen storage disease [125, 126]. In addition, GYS2 gene is one of the adipose tissue-enriched genes contributing to obesity from a stratified transcriptomics analysis [127]. GYS2 gene on chromosome 12p12.2 was identified in a PCOS GWAS for obesity-related conditions, and confirmed further associations in an independent childhood obesity study and a gestational diabetes study [124].

7.1.22 Genes for Adiposity

Accumulating evidence suggests a role for the blood coagulation factor gene F13A1 in obesity [128]. Schweighofer et al. found an association of the G allele of F13A1 SNP rs7766109 in PCOS patients with higher BMI, raised FAI, decreased levels of SHBG, and HDL, elevated levels of free testosterone and TG, and higher systolic blood pressure [128]. Some of the associations were more pronounced in obese PCOS women including FAI, free testosterone, SHBG, AUCins, while some in lean PCOS women included BMI, TG, HDL. F13A1 SNP rs7766109 did not contribute to PCOS susceptibility. They also found an association of the G allele of the F13A1 SNP rs7766109 with lower HDL levels in PCOS women [128]. These findings are of particular interest because dyslipidemia is a common feature of PCOS, and, HDL levels are inversely correlated with androgen levels and body fat. Billings et al. investigated the association between F13A1 SNPs and HDL cholesterol levels in a Finnish EUFAM population, and, identified 10 SNPs within the introns 3–5 that were associated with serum lipid levels [129].

Expression of 11β-hydroxysteroid dehydrogenase type 1(11β-HSD1) in visceral and subcutaneous adipose tissues of patients with PCOS is associated with adiposity. PCOS is not associated with increased 11β-HSD1 expression. Increased expression of this gene correlates with markers of adiposity, and, predicts insulin resistance and an unfavorable metabolic profile, independently of PCOS [130]. In the development

of insulin resistance increased cortisol activity in adipose tissue may be important [131]. The enzyme 11β-HSD1 interconverts glucocorticoids cortisone and cortisol in adipose and other tissues [131]. In vivo, the reductase activity prevails, generating cortisol by both autocrine and paracrine functions [131]. Various studies report increased expression of 11β-HSD1 gene (HSD11B1) [130, 131] and activity of this enzyme in subcutaneous adipose tissue of obese people [132]. Several studies demonstrate a positive correlation between HSD11B1 expression with obesity [133, 134], while others found exclusive associations of subcutaneous adipose HSD11B1 expression with obesity and IR [135, 136].

PCOS of women has profound effects on their offspring, and obesity is one of the most important influences. Although the mechanisms responsible are not clearly elucidated, there is consistent evidence that parents may influence the risk of adiposity in their offspring through genetics, the intrauterine environment, and behavioral and environmental factors. The prevalence of PCOS is likely to increase in parallel with the obesity epidemic. The complex aetiology of PCOS is influenced by genetic and environmental – particularly dietary factors. Both factors contribute to adiposity, which in turn influences the severity and expression of PCOS. Given the complexity of adipocyte physiology and pathophysiology, it is likely that we have only just begun to understand the mechanisms linking PCOS with adiposity, and, obesity of their offspring.

References

1. Gluckman PD, Hanson MA, Cooper C, et al. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359:61–73.
2. Rugholm S, Baker JL, Olsen LW, et al. Stability of the association between birth weight and childhood overweight during the development of the obesity epidemic. Obes Res. 2005;13:2187–94.
3. Harder T, Roepke K, Diller N, et al. Birth weight, early weight gain, and subsequent risk of type 1 diabetes: systematic review and meta-analysis. Am J Epidemiol. 2009;169:1428–36.
4. Batty GD, Shipley MJ, Jarrett RJ, et al. Obesity and overweight in relation to disease-specific mortality in men with and without existing coronary heart disease in London: the original Whitehall study. Heart. 2006;92:886–92.
5. Whitlock G, Lewington S, Sherliker P, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet. 2009;373:1083–96.
6. Wilcox AJ. On the importance – and the unimportance – of birthweight. Int J Epidemiol. 2001;30:1233–41.
7. Galtier-Dereure F, Boegner C, Bringer J. Obesity and pregnancy: complications and cost. Am J Clin Nutr. 2000;71:1242S–8.
8. LaCoursiere DY, Bloebaum L, Douncan JD, et al. Population-based trends and correlates of maternal overweight and obesity, Utah 1991–2001. Am J Obstet Gynecol. 2005;192:832–9.
9. Barker DJ, Forsén T, Eriksson JG, et al. Growth and living conditions in childhood and hypertension in adult life: a longitudinal study. J Hypertens. 2002;20:1951–6.
10. Kajantie E, Osmond C, Barker DJ, et al. Size at birth as a predictor of mortality in adulthood: a follow-up of 350,000 person-years. Int J Epidemiol. 2005;34:655–63.
11. McGuire S. WHO, World Food Programme, and International Fund for Agricultural Development. 2012. The State of Food Insecurity in the World 2012. Economic growth is necessary but not sufficient to accelerate reduction of hunger and malnutrition. Rome, FAO. Adv Nutr 2013;4:126–127

12. Roseboom TJ, van der Meulen JH, Ravelli AC, et al. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. Mol Cell Endocrinol. 2001;185:93–8.
13. Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. N Engl J Med. 1976;295:349–53.
14. Vignini A, Raffaelli F, Cester A, et al. Environmental and genetical aspects of the link between pregnancy, birth size, and type 2 diabetes. Curr Diabetes Rev. 2012;8:155–61.
15. Barker DJ, Osmond C, Simmonds SJ, et al. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. BMJ. 1993;306:422–6.
16. Eriksson JG, Forsen T, Tuomilehto J, et al. Early growth, adult income, and risk of stroke. Stroke. 2000;31:869–74.
17. Huxley R, Owen CG, Whincup PH, et al. Is birth weight a risk factor for ischemic heart disease in later life? Am J Clin Nutr. 2007;85:1244–50.
18. Jimenez-Chillaron JC, Isganaitis E, Charalambous M, et al. Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. Diabetes. 2009;58: 460–8.
19. Ikenasio-Thorpe BA, Breier BH, Vickers MH, et al. Prenatal influences on susceptibility to diet-induced obesity are mediated by altered neuroendocrine gene expression. J Endocrinol. 2007;193:31–7.
20. Long NM, Tousley CB, Underwood KR, et al. Effects of early- to mid-gestational undernutrition with or without protein supplementation on offspring growth, carcass characteristics, and adipocyte size in beef cattle. J Anim Sci. 2012;90:197–206.
21. Ford SP, Hess BW, Schwope MM, et al. Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. J Anim Sci. 2007;85:1285–94.
22. Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull. 2001;60:5–20.
23. Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. Am J Physiol Endocrinol Metab. 2000;279:E83–7.
24. Levin BE. The obesity epidemic: metabolic imprinting on genetically susceptible neural circuits. Obes Res. 2000;8:342–7.
25. Vickers MH, Breier BH, McCarthy D, et al. Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. Am J Physiol Regul Integr Comp Physiol. 2003;285:R271–3.
26. Sebaai N, Lesage J, Breton C, et al. Perinatal food deprivation induces marked alterations of the hypothalamo-pituitary-adrenal axis in 8-month-old male rats both under basal conditions and after a dehydration period. Neuroendocrinology. 2004;79:163–73.
27. Anouar Y, Vieau D. Maternal perinatal undernutrition has long-term consequences on morphology, function and gene expression of the adrenal medulla in the adult male rat. J Neuroendocrinol. 2011;23:711–24.
28. Vieau D, Sebaai N, Leonhardt M, et al. HPA axis programming by maternal undernutrition in the male rat offspring. Psychoneuroendocrinology. 2007;32 Suppl 1:S16–20.
29. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002;16:6–21.
30. Burdge GC, Hanson MA, Slater-Jefferies JL, et al. Epigenetic regulation of transcription: a mechanism for inducing variations in phenotype (fetal programming) by differences in nutrition during early life? Br J Nutr. 2007;97:1036–46.
31. Maclennan NK, James SJ, Melnyk S, et al. Uteroplacental insufficiency alters DNA methylation, one-carbon metabolism, and histone acetylation in IUGR rats. Physiol Genomics. 2004;18: 43–50.
32. Dolinoy DC, Weidman JR, Waterland RA, et al. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect. 2006;114:567–72.
33. Fu Q, McKnight RA, Yu X, et al. Uteroplacental insufficiency induces site-specific changes in histone H3 covalent modifications and affects DNA-histone H3 positioning in day 0 IUGR rat liver. Physiol Genomics. 2004;20:108–16.

34. Chong S, Vickaryous N, Ashe A, et al. Modifiers of epigenetic reprogramming show paternal effects in the mouse. Nat Genet. 2007;39:614–22.
35. Morgan HD, Sutherland HG, Martin DI, et al. Epigenetic inheritance at the agouti locus in the mouse. Nat Genet. 1999;23:314–18.
36. Anway MD, Cupp AS, Uzumcu M, et al. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science. 2005;308:1466–9.
37. Stevens A, Begum G, Cook A, et al. Epigenetic changes in the hypothalamic proopiomelanocortin and glucocorticoid receptor genes in the ovine fetus after periconceptional undernutrition. Endocrinology. 2010;151:3652–64.
38. Heijmans BT, Tobi EW, Stein AD, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A. 2008;105:17046–9.
39. Godfrey KM, Sheppard A, Gluckman PD, et al. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. Diabetes. 2011;60:1528–34.
40. Begum G, Stevens A, Smith EB, et al. Epigenetic changes in fetal hypothalamic energy regulating pathways are associated with maternal undernutrition and twinning. FASEB J. 2012;26:1694–703.
41. Lillycrop KA, Slater-Jefferies JL, Hanson MA, et al. Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. Br J Nutr. 2007;97:1064–73.
42. Tamashiro KL, Wakayama T, Akutsu H, et al. Cloned mice have an obese phenotype not transmitted to their offspring. Nat Med. 2002;8(3):262–7.
43. Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ. 1991;303:1019–22.
44. Randhawa RS. The insulin-like growth factor system and fetal growth restriction. Pediatr Endocrinol Rev. 2008;6:235–40.
45. Hyatt MA, Keisler DH, Budge H, et al. Maternal parity and its effect on adipose tissue deposition and endocrine sensitivity in the postnatal sheep. J Endocrinol. 2010;204:173–9.
46. Hocquette JF, Sauerwein H, Higashiyama Y, et al. Prenatal developmental changes in glucose transporters, intermediary metabolism and hormonal receptors related to the IGF/insulin-glucose axis in the heart and adipose tissue of bovines. Reprod Nutr Dev. 2006;46:257–72.
47. Sohlström A, Katsman A, Kind KL, et al. Food restriction alters pregnancy-associated changes in IGF and IGFBP in the guinea pig. Am J Physiol. 1998;274(3 Pt 1):E410–16.
48. Thamotharan M, Shin BC, Suddirikku DT, et al. GLUT4 expression and subcellular localization in the intrauterine growth-restricted adult rat female offspring. Am J Physiol Endocrinol Metab. 2005;288:E935–47.
49. Armitage JA, Khan IY, Taylor PD, et al. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? J Physiol. 2004;561(Pt 2):355–77.
50. Shankar K, Harrell A, Liu X, et al. Maternal obesity at conception programs obesity in the offspring. Am J Physiol Regul Integr Comp Physiol. 2008;294:R528–38.
51. Guo F, Jen KL. High-fat feeding during pregnancy and lactation affects offspring metabolism in rats. Physiol Behav. 1995;57:681–6.
52. Levin BE, Govek E. Gestational obesity accentuates obesity in obesity-prone progeny. Am J Physiol. 1998;275(4 Pt 2):R1374–9.
53. Salihu HM, Weldeselasse HE, Rao K, et al. The impact of obesity on maternal morbidity and feto-infant outcomes among macrosomic infants. J Matern Fetal Neonatal Med. 2011;24:1088–94.
54. Bayol SA, Farrington SJ, Stickland NC. A maternal 'junk food' diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring. Br J Nutr. 2007;98:843–51.
55. Bayol SA, Simbi BH, Bertrand JA, et al. Offspring from mothers fed a 'junk food' diet in pregnancy and lactation exhibit exacerbated adiposity that is more pronounced in females. J Physiol. 2008;586:3219–30.

56. Samuelsson AM, Matthews PA, Argenton M, et al. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. Hypertension. 2008;51:383–92.
57. Yan X, Zhu MJ, Xu W, et al. Up-regulation of Toll-like receptor 4/nuclear factor-kB signaling is associated with enhanced adipogenesis and insulin resistance in fetal skeletal muscle of obese sheep at late gestation. Endocrinology. 2010;151:380–7.
58. Liang C, Oest ME, Prater MR. Intrauterine exposure to high saturated fat diet elevates risk of adult-onset chronic diseases in C57BL/6 mice. Birth Defects Res B Dev Reprod Toxicol. 2009;86:377–84.
59. Howie GJ, Sloboda DM, Kamal T, et al. Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. J Physiol. 2009;587(Pt 4):905–15.
60. Nivoit P, Morens C, Van Assche FA, et al. Established diet-induced obesity in female rats leads to offspring hyperphagia, adiposity and insulin resistance. Diabetologia. 2009;52: 1133–42.
61. Tamashiro KL, Terrillion CE, Hyun J, et al. Prenatal stress or high-fat diet increases susceptibility to diet-induced obesity in rat offspring. Diabetes. 2009;58:1116–25.
62. Taylor PD, McConnell J, Khan IY, et al. Impaired glucose homeostasis and mitochondrial abnormalities in offspring of rats fed a fat-rich diet in pregnancy. Am J Physiol Regul Integr Comp Physiol. 2005;288:R134–9.
63. Khan IY, Taylor PD, Dekou V, et al. Gender-linked hypertension in offspring of lard-fed pregnant rats. Hypertension. 2003;41:168–75.
64. Palinski W, D'Armiento FP, Witztum JL, et al. Maternal hypercholesterolemia and treatment during pregnancy influence the long-term progression of atherosclerosis in offspring of rabbits. Circ Res. 2001;89:991–6.
65. Bayol SA, Simbi BH, Stickland NC. A maternal cafeteria diet during gestation and lactation promotes adiposity and impairs skeletal muscle development and metabolism in rat offspring at weaning. J Physiol. 2005;567(Pt 3):951–61.
66. Hay Jr WW. Placental transport of nutrients to the fetus. Horm Res. 1994;42:215–22.
67. Mühlhäusler BS. Programming of the appetite-regulating neural network: a link between maternal overnutrition and the programming of obesity? J Neuroendocrinol. 2007;19:67–72.
68. Muhlhausler BS, Adam CL, Findlay PA, et al. Increased maternal nutrition alters development of the appetite-regulating network in the brain. FASEB J. 2006;20:1257–9.
69. Kalra SP, Dube MG, Pu S, et al. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr Rev. 1999;20:68–100.
70. Warnes KE, Morris MJ, Symonds ME, et al. Effects of increasing gestation, cortisol and maternal undernutrition on hypothalamic neuropeptide Y expression in the sheep fetus. J Neuroendocrinol. 1998;10:51–7.
71. Muhlhausler BS, McMillen IC, Rouzaud G, et al. Appetite regulatory neuropeptides are expressed in the sheep hypothalamus before birth. J Neuroendocrinol. 2004;16:502–7.
72. O'Reilly JR, Reynolds RM. The risk of maternal obesity to the long-term health of the offspring. Clin Endocrinol (Oxf). 2013;78:9–16.
73. Smink A, Ribas-Fiton N, Garcia R, et al. Exposure to hexachlorobenzene during pregnancy increases the risk of overweight in children aged 6 years. Acta Paediatr. 2008;97:1465–9.
74. Faust IM, Johnson PR, Stern JS, et al. Diet-induced adipocyte number increase in adult rats: a new model of obesity. Am J Physiol. 1978;235:E279–86.
75. Adam CL, Bake T, Findlay PA, et al. Effects of altered glucose supply and adiposity on expression of hypothalamic energy balance regulatory genes in late gestation growth restricted ovine fetuses. Int J Dev Neurosci. 2011;29:775–81.
76. Fajas L, Debril MB, Auwerx J. Peroxisome proliferator-activated receptor-gamma: from adipogenesis to carcinogenesis. J Mol Endocrinol. 2001;27:1–9.
77. Bao S, Obata Y, Carroll J, et al. Epigenetic modifications necessary for normal development are established during oocyte growth in mice. Biol Reprod. 2000;62:616–21.
78. Allegrucci C, Thurston A, Lucas E, et al. Epigenetics and the germline. Reproduction. 2005;129:137–49.

79. Vickers MH. Developmental programming of the metabolic syndrome – critical windows for intervention. World J Diabetes. 2011;2:137–48.
80. Davidowa H, Plagemann A. Decreased inhibition by leptin of hypothalamic arcuate neurons in neonatally overfed young rats. Neuroreport. 2000;11:2795–8.
81. Lawlor DA, Relton C, Sattar N, et al. Maternal adiposity – a determinant of perinatal and offspring outcomes? Nat Rev Endocrinol. 2012;8:679–88.
82. Cooper R, Pinto Pereira SM, Power C, et al. Parental obesity and risk factors for cardiovascular disease among their offspring in mid-life: findings from the 1958 British Birth Cohort Study. Int J Obes (Lond). 2013. doi:10.1038/ijo.2013.40.
83. Whitaker RC, Wright JA, Pepe MS, et al. Predicting obesity in young adulthood from childhood and parental obesity. N Engl J Med. 1997;337:869–73.
84. Whitaker KL, Jarvis MJ, Beeken RJ, et al. Comparing maternal and paternal intergenerational transmission of obesity risk in a large population-based sample. Am J Clin Nutr. 2010;91:1560–7.
85. Perez-Pastor EM, Metcalf BS, Hosking J, et al. Assortative weight gain in mother-daughter and father-son pairs: an emerging source of childhood obesity. Longitudinal study of trios (EarlyBird 43). Int J Obes (Lond). 2009;33:727–35.
86. Leary S, Davey Smith G, Ness A. No evidence of large differences in mother-daughter and father-son body mass index concordance in a large UK birth cohort. Int J Obes (Lond). 2010;34:1191–2.
87. Ng SF, Lin RC, Laybutt DR, et al. Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. Nature. 2010;467:963–6.
88. Carone BR, Fauquier L, Habib N, et al. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell. 2010;143:1084–96.
89. Gladen BC, Ragan NB, Rogan WJ. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. J Pediatr. 2000;136:490–6.
90. Newbold RR, Padilla-Banks E, Jefferson WN, et al. Effects of endocrine disruptors on obesity. Int J Androl. 2008;31:201–8.
91. Newbold RR. Developmental exposure to endocrine-disrupting chemicals programs for reproductive tract alterations and obesity later in life. Am J Clin Nutr. 2011;94:1939S–42.
92. Newbold RR, Padilla-Banks E, Snyder RJ, et al. Perinatal exposure to environmental estrogens and the development of obesity. Mol Nutr Food Res. 2007;51:912–17.
93. Oken E, Levitan EB, Gillman MW. Maternal smoking during pregnancy and child overweight: systematic review and meta-analysis. Int J Obes (Lond). 2008;32:201–10.
94. Windham GC, Eaton A, Hopkins B. Evidence for an association between environmental tobacco smoke exposure and birthweight: a meta-analysis and new data. Paediatr Perinat Epidemiol. 1999;13:35–57.
95. Leonardi-Bee J, Smyth A, Britton J, et al. Environmental tobacco smoke and fetal health: systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed. 2008;93:F351–61.
96. Iñiguez C, Ballester F, Amorós R, et al. Active and passive smoking during pregnancy and ultrasound measures of fetal growth in a cohort of pregnant women. J Epidemiol Community Health. 2012;66:563–70.
97. Grove KL, Sekhon HS, Brogan RS, et al. Chronic maternal nicotine exposure alters neuronal systems in the arcuate nucleus that regulate feeding behavior in the newborn rhesus macaque. J Clin Endocrinol Metab. 2001;86:5420–6.
98. Gao YJ, Holloway AC, Zeng Z, et al. Prenatal exposure to nicotine causes postnatal obesity and altered perivascular adipose tissue function. Obes Res. 2005;13:1–6.
99. Holloway AC, Lim GE, Petrik JJ, et al. Fetal and neonatal exposure to nicotine in Wistar rats results in increased beta cell apoptosis at birth and postnatal endocrine and metabolic changes associated with type 2 diabetes. Diabetologia. 2005;48:2661–6.
100. Li MD, Parker SL, Kane JK. Regulation of feeding-associated peptides and receptors by nicotine. Mol Neurobiol. 2000;22:143–65.

101. Mantzoros CS, Varvarigou A, Kaklamani VG, et al. Effect of birth weight and maternal smoking on cord blood leptin concentrations of full-term and preterm newborns. J Clin Endocrinol Metab. 1997;82:2856–61.
102. Helland IB, Reseland JE, Saugstad OD, et al. Smoking related to plasma leptin concentration in pregnant women and their newborn infants. Acta Paediatr. 2001;90:282–7.
103. Diamanti-Kandarakis E, Piperi C. Genetics of polycystic ovary syndrome: searching for the way out of the labyrinth. Hum Reprod Update. 2005;11:631–43.
104. Carmina E, Bucchieri S, Esposito A, et al. Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance. J Clin Endocrinol Metab. 2007;92:2500–5.
105. Barber TM, McCarthy MI, Wass JA, et al. Obesity and polycystic ovary syndrome. Clin Endocrinol (Oxf). 2006;65:137–45.
106. Baillargeon JP, Carpentier AC. Brothers of women with polycystic ovary syndrome are characterised by impaired glucose tolerance, reduced insulin sensitivity and related metabolic defects. Diabetologia. 2007;50:2424–32.
107. Herbert A, Gerry NP, McQueen MB, et al. A common genetic variant is associated with adult and childhood obesity. Science. 2006;312:279–83.
108. Escobar-Morreale HF, Samino S, Insenser M, et al. Metabolic heterogeneity in polycystic ovary syndrome is determined by obesity: plasma metabolomic approach using GC-MS. Clin Chem. 2012;58:999–1009.
109. Loos RJ, Lindgren CM, Li S, et al. Common variants near MC4R are associated with fat mass, weight and risk of obesity. Nat Genet. 2008;40:768–75.
110. Renstrom F, Payne F, Nordstrom A, et al. Replication and extension of genome-wide association study results for obesity in 4923 adults from northern Sweden. Hum Mol Genet. 2009;18:1489–96.
111. Willer C. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet. 2009;41:25–34.
112. Scuteri A, Sanna S, Chen WM, et al. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet. 2007;3:1200–10.
113. Barbieri RL, Makris A, Ryan KJ. Insulin stimulates androgen accumulation in incubations of human ovarian stroma and theca. Obstet Gynecol. 1984;64:S73–80.
114. Bremer AA, Miller WL. The serine phosphorylation hypothesis of polycystic ovary syndrome: a unifying mechanism for hyperandrogenemia and insulin resistance. Fertil Steril. 2008;89:1039–48.
115. Diamanti-Kandarakis E, Argyrakopoulou G, Economou F, et al. Defects in insulin signaling pathways in ovarian steroidogenesis and other tissues in polycystic ovary syndrome (PCOS). J Steroid Biochem Mol Biol. 2008;109:242–6.
116. Dunaif A, Segal KR, Futterweit W, et al. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes. 1989;38:1165–74.
117. Ackerman CM, Lowe LP, Lee H, et al. The role of the polycystic ovary syndrome susceptibility locus D19S884 allele 8 in maternal glycemia and fetal size. J Clin Endocrinol Metab. 2010;95:3242–50.
118. Urbanek M, Woodroffe A, Ewens KG, et al. Candidate gene region for polycystic ovary syndrome on chromosome 19p13.2. J Clin Endocrinol Metab. 2005;90:6623–9.
119. Neptune ER, Frischmeyer PA, Arking DE, et al. Dysregulation of TGF-β activation contributes to pathogenesis in Marfan syndrome. Nat Genet. 2003;33:407–11.
120. Herder C, Zierer A, Koenig W, et al. Transforming growth factor-beta1 and incident type 2 diabetes: results from the MONICA/KORA case-cohort study, 1984–2002. Diabetes Care. 2009;32:1921–3.
121. Mukherjee A, Sidis Y, Mahan A, et al. FSTL3 deletion reveals roles for TGF-β family ligands in glucose and fat homeostasis in adults. Proc Natl Acad Sci U S A. 2007;104:1348–53.
122. Pfeiffer A, Middelberg-Bisping K, Drewes C. Elevated plasma levels of transforming growth factor-β1 in NIDDM. Diabetes Care. 1996;19:1113–17.

123. Urbanek M, Sam S, Legro RS, et al. Identification of a polycystic ovary syndrome susceptibility variant in fibrillin-3 and association with a metabolic phenotype. J Clin Endocrinol Metab. 2007;92:4191–8.
124. Hwang JY, Lee EJ, Jin Go M, et al. Genome-wide association study identifies GYS2 as a novel genetic factor for polycystic ovary syndrome through obesity-related condition. J Hum Genet. 2012;57:660–4.
125. Huo J, Xu S, Lam KP. Fas apoptosis inhibitory molecule regulates T cell receptor mediated apoptosis of thymocytes by modulating Akt activation and Nur77 expression. J Biol Chem. 2010;285:11827–35.
126. Soggia AP, Correa-Giannella ML, Fortes MA, et al. A novel mutation in the glycogen synthase 2 gene in a child with glycogen storage disease type 0. BMC Med Genet. 2010;11:3.
127. Morton NM, Nelson YB, Michailidou Z, et al. A stratified transcriptomics analysis of polygenic fat and lean mouse adipose tissues identifies novel candidate obesity genes. PLoS One. 2011;6(9):e23944.
128. Schweighofer N, Lerchbaum E, Trummer O, et al. Androgen levels and metabolic parameters are associated with a genetic variant of F13A1 in women with polycystic ovary syndrome. Gene. 2012;504(1):133–9.
129. Billings LK, Hsu YH, Ackerman RJ, et al. Impact of common variation in bone-related genes on type 2 diabetes and related traits. Diabetes. 2012;61:2176–86.
130. Mlinar B, Marc J, Jensterle M, et al. Expression of 11β-hydroxysteroid dehydrogenase type 1 in visceral and subcutaneous adipose tissues of patients with polycystic ovary syndrome is associated with adiposity. J Steroid Biochem Mol Biol. 2011;123:127–32.
131. Draper N, Stewart PM. 11beta-hydroxysteroid dehydrogenase and the prereceptor regulation of corticosteroid hormone action. J Endocrinol. 2005;186:251–71.
132. Macfarlane DP, Forbes S, Walker BR. Glucocorticoids and fatty acid metabolism in humans: fuelling fat redistribution in the metabolic syndrome. J Endocrinol. 2008;197:189–204.
133. Paulsen SK, Pedersen SB, Fisker S. 11beta-HSD type 1 expression in human adipose tissue: impact of gender, obesity, and fat localization. Obesity. 2007;15:1954–60.
134. Desbriere R, Vuaroqueaux V, Achard V, et al. 11beta-Hydroxysteroid dehydrogenase type 1 mRNA is increased in both visceral and subcutaneous adipose tissue of obese patients. Obesity. 2006;14:794–8.
135. Li X, Lindquist S, Chen R, et al. Depotspecific messenger RNA expression of 11 beta-hydroxysteroid dehydrogenase type 1 and leptin in adipose tissue of children and adults. Int J Obes. 2007;31:820–8.
136. Engeli S, Bohnke J, Feldpausch M, et al. Regulation of 11beta-HSD genes in human adipose tissue: influence of central obesity and weight loss. Obes Res. 2004;12:9–17.

Gamete/Embryo-Fetal Origins of Mental Disorders

8

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Abstract

The mechanisms of normal development of the central nervous system could be modulated by a variety of spatial and temporal factors. Neurodevelopmental disorders may originate during fetal life because the feto-maternal environment is vulnerable to negative intrauterine and extrauterine factors, such as maternal smoking, alcohol, nutrition, endocrine disruptors, exposure to pesticides such as chlorpyrifos, exposure to drugs such as terbutaline, maternal teratogenic alleles, psychosocial stress, and infection during pregnancy, as well as preterm birth. As a consequence, the process of fetal neurodevelopment, which involves cell programs, developmental trajectories, synaptic plasticity, and oligodendrocyte maturation, could be adversely affected [1, 2]. Possible mechanisms include changes in neurodevelopment, changes in the set points of neuroendocrine systems caused disruption to placental function, environmental toxin cross the placental barrier and early programming effects, and, there is evidence that prenatal adversity interacts with intrinsic genetic factors, and, extrinsic, postnatal, environmental factors [3]. Meanwhile there is also accumulating evidence suggesting that epigenetic changes may mediate programming effects at a molecular level during embryonic and fetal development [4]. In this chapter, we will discuss the evidence related to Embryo-fetal origins of mental disorders.

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8.1 Prenatal Stress

If a pregnant mother suffers from stress or anxiety, her child is more likely to show a range of mental disorder symptoms such as attention deficit hyperactivity disorder, conduct disorder, aggression or anxiety [5]. Prenatal stress is associated with increased risks for a range of neurodevelopmental, behavioural and cognitive changes in the child. Not all children are affected in the same way and most are not affected at all. In monkeys offspring of prenatally-stressed mothers have both increased anxiety and reduced attention span [6]. Many different, human studies show that the child of a mother experiencing stress during pregnancy is at increased risk of anxiety [7, 8], attention deficit/hyperactivity disorder (ADHD) [7, 9] and conduct disorder [7, 10] as well as altered function of the hypothalamo-pituitary axis (HPA) [11]. Cognitive deficits [12–14], increased levels of mixed handedness [12, 15, 16], dermatoglyphic asymmetry [17] have also been reported in these studies. Severe stress in the first trimester has been associated with an increased risk of schizophrenia [18]. The Stress later in pregnancy has been reported to be associated with increased risks of autism in one study [19], but not another [10]. Effects of prenatal stress are often different in male and female [20, 21]. A recent study has demonstrated, using structural magnetic resonance imaging, decreased grey matter in several brain regions in children of mothers with anxiety during pregnancy.

Fetal programming may cause long-lasting changes in brain structure and function, particularly in low birth weight babies who are at risk for a range of later mental health and behavioural problems too [3]. Prenatal stress is linked to a wide variety of stressors, both acute and chronic, with altered outcomes in childhood. The effects of acute disasters such as 9/11 [22], Chernobyl [9], and a Canadian ice storm [14] have been studied. Other studies have found correlations with much milder stress, such as daily hassles, or pregnancy-specific anxiety [23, 24]. Statistical data in healthy populations show that mild to moderate levels of psychological distress may be beneficial for fetal maturation and, the "dose-response curve" may be an inverted U shape, which means that optimal outcomes are associated with mild or moderate prenatal stress rather than less or more stress.

In humans there is evidence that fetal behavior is related to the emotional state of the pregnant mother [25, 26], and, that there is continuity between fetal and infant behaviour [27]. Maternal stress in pregnancy has been found to be associated with a range of altered outcomes for the child, independent of any effect on birth weight [12, 16]. Increased symptoms of ADHD is one of the outcomes most commonly associated with prenatal stress [28, 29]. An increase in symptoms of hyperactivity, independently of attention problems, has also been found to be associated with increased prenatal anxiety and depression [30]. Several studies have found that prenatal stress or anxiety is associated with increased levels of anxiety [7, 8, 12] or internalizing problems in the child [31].

Externalising problems [8, 31] and conduct disorder are associated with prenatal stress or anxiety, independent of postnatal maternal mood [10, 32], or, genetic factors [33]. Prenatal anxiety is especially related to conduct disorder that persists

into adolescence, rather than that limits to childhood [10]. Oppositional defiant behaviour, associated with hyperactivity, is also increased in children of mothers who were anxious or depressed in pregnancy [30]. Prenatal stresses including life events [12], exposure to a Canadian ice storm [14], increasing anxiety [34], the exposure to pregnancy-specific anxiety and increased daily hassles [35], are associated with reduced cognitive function in childhood.

8.2 Nutrition

Adequate maternal nutrition is vital for the neurodevelopment of the fetus. The Dutch famine, not only influenced the first generation, it also triggered a wave of physical and mental problems in later generations [36], especially an increased risk of schizophrenia in adult life. Similarly, the evidence from Chinese population samples also suggests that in utero nutritional deficiency, as indicated by maternal exposure to severe famine during pregnancy, is significantly correlated to adult schizophrenia [20, 37, 38]. The precise mechanisms linking prenatal nutritional deficiency and adult schizophrenia are unknown, but considerable evidence supports a role for epigenetic changes [39, 40]. Methyl donors, such as folate, methionine, choline, vitamin B12,vitamin B6, and vitamin B2, usually contained in the diet, are required for the formation of S-adenosylmethionine (SAM),which acts as a methyl donor for the methylation of cytosine DNA residues [41, 42]. Prenatal exposure to a diet lacking such components at specific developmental time points may cause incorrect pattern of gene expression, which may result in detrimental phenotypic effects. For example, Prenatal exposures in the Dutch famine were relatively hypomethylated at the imprinted IGF2 gene when compared with their unexposed, same sex, siblings six decades after the period of nutritional deficiency, and meanwhile this association is specific for periconceptional deficiency [22].

Epidemiological studies identify overlapping patterns in the incidence of schizophrenia with neural tube defects, suggesting that there are one, or more, shared aetiological risk factors between these disorders. Among these risk factors, nutritional factors, especially folate, play an important role in linking epigenetic variation, early development, and the risk of major psychotic disorders [43]. Elevated homocysteine levels in the third trimester of pregnancy are associated with an increased risk of schizophrenia of the offspring [23]. Furthermore, since postpartum restoration to normal maternal blood folate levels may take about 1 year after giving birth to a baby, birth interval could be used as an indirect indication of folate levels during pregnancy, which also shows an association between folate and schizophrenia [24].

Methylene tetrahydrofolate reductase (encoded by the gene MTHFR) is a key enzymatic molecule in the one carbon metabolic pathway, acting to catalyze the conversion of 5,10-methylenetetrahydrogolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. Genetic association studies of two, single-nucleotide polymorphisms in the MTHFR gene has supported the correlation with both schizophrenia and bipolar disorder [25, 44, 45].

Of course, these negative effects of famine may not be solely attributed to impaired nutrient intake during pregnancy. Famine may increase the level of psychological stress, which is also a risk factor for schizophrenia, and perhaps associated with epigenetic changes.

Some studies have been demonstrated the association of birth weight with a wide range of psychopathology, including depression, schizophrenia and ADHD, suggesting that low birth weight induced by intrauterine undernutrition may increase risks for later psychiatric disorders [33].

8.3 Drugs

Although prenatal cocaine exposure has not been shown to have any detrimental effect on cognition, except as mediated through cocaine-mediated effects on head size, prenatal cocaine exposure has been proved to be associated with numerous abnormalities in arousal, attention, and neurologic and neurophysiological function. However, most of the above negative effects are perhaps self-limited and restricted to early infancy and childhood [46]. Opiate exposure, especially exposure to methadone elicits a well-described withdrawal syndrome affecting central nervous, autonomic, and gastrointestinal systems [47]. While children exposed in utero to drugs tend to fair less well developmentally than unexposed children, distinguishing an independent contribution of the drug under study from other risk factors associated with drug usage, such as chaotic parenting and exposure to other drugs of abuse, is difficult.

The fetus development could be adversely affected by drugs used during pregnancy in both direct way (passage of the drug through the placenta), and indirect way, (poor maternal health habits and environmental conditions) [48]. As a consequence, specific learning and behavior problems may be evoked and even persist into later childhood and adolescence. Even the CNS symptoms which could remit over the early years of life may still be precursors to later adverse developmental outcomes [46, 47].

Exposure to industrial chemicals e.g. lead, methylmercury, polychlorinated biphenyls [PCBs], arsenic, and toluene during embryonic and early fetal development may cause nervous system injury, which could result in neurodevelopmental disorders and subclinical brain dysfunction [48]. More than 200 chemicals have been known to cause clinical neurotoxic effects in adults [48]. With recognition of these risks, prevention programmes, such as elimination of lead additives in petrol has been initiated successfully. However, there are still gaps between testing chemicals for developmental neurotoxicity and the high level of proof required by regulatory authorities, which cause delays of prevention [48]. According to the recognition of the unique vulnerability of the neuro system development, improved, precautionary approaches of testing and control of the dangerous chemicals are needed.

Other drugs, such as anaesthetic agents, may also influence the fetal neurodevelopment [55]. Animal studies demonstrate that commonly used anesthetic agents could adversely affect early brain development both histologically and functionally.

With human epidemiological evidence suggesting an association between anaesthesia and surgery in early life, and, later-onset learning disabilities, attention has focused on the subtle long-term effects of anaesthesia exposure [55]. Evidence is limited but at present there is no scientific justification to prefer the use of one anaesthetic agent to the other.

8.4 Alcohol and Cigarette Smoking

It is clear that prenatal exposure to alcohol could impose a range of negative effects on offspring neuroendocrine and behavioural functions. Alcohol could directly affect developing fetal endocrine organs through easy passage through the placenta. In addition, alcohol may indirectly disrupt the normal hormonal function in fetus and affect fetal development through negative impact on maternal endocrine functions [49]. The HPA axis is highly susceptible to programming during fetal and neonatal development. Alcohol exposure in utero can reprogram the fetal HPA axis which is highly vulnerable to programming during fetal and neonatal development. As a consequence, HPA "tone" is increased throughout life [50]. Furthermore, many studies show the sexually dimorphic effects of alcohol, although alterations in HPA responsiveness and regulation occur in both male and female offspring [50].

Maternal smoking during pregnancy has strong associations with not a few adverse developmental outcomes in offspring, which these include spontaneous abortion, growth restriction, preterm delivery, increased risk of sudden infant death syndrome (SIDS), as well as long-term behavioural and psychiatric disorders [51]. However, the underlying physiological mechanisms for these adverse effects are not fully understood. Nicotine from cigarette smoking, could affect placental vasculature, and function thorough nicotinic acetylcholine receptor binding in fetal membranes, which could also result in dysregulation of catecholaminergic and serotonergic neurotransmitter systems. Both nicotine and CO caused by cigarette smoking could decrease uterine blood flow, and then evoke a state of hypoxia and malnutrition in the fetus. Experimental evidence shows that nicotine can cross the placenta, and bind to nAChRs, which are expressed at high levels during development of the human brain [52]. Considering the role of the nAChRs in differentiation, axonal path finding, and synapse formation, further stimulation related to maternal nicotine usage may alter normal development of the fetal nervous system. So it is important to warn pregnant women of the detrimental effects of smoking, and to encourage them to abstain for healthy fetal development.

Maternal smoking in pregnancy also induce a range of risks associated with psychopathology. Studies of the children of twins [53] and also children who are genetically unrelated to the woman undergoing the pregnancy as a result of conception through assisted reproductive technologies allow separation of prenatal effects from maternal-child genome sharing [52]. These studies have suggested that the exposure to maternal smoking in pregnancy may be linked to lower offspring birth weight [4]. In contrast, for attention-deficit hyperactivity disorder (ADHD) and antisocial behaviour, the association may be explained by inherited factors

transmitted from mother to child [8, 17, 53]. Sibling studies show that when one sibling is exposed to maternal smoking in pregnancy and the other is not, the unexposed sibling also shows increased attentional and behavioural problems [54]. Animal studies consistently show that exposure to cigarette smoke and nicotine in utero reduces birth weight; for ADHD-like behaviours the evidence is mixed. These findings suggest that maternal smoking in pregnancy causing ADHD in offspring may be unfounded, or, the effects may be smaller than currently believed.

8.5 Intrauterine Infection

Maternal or intrauterine infection may play a critical role in perinatal brain damage and result in long-term disabilities including cerebral palsy [56]. Increased levels of the proinflammatory cytokine interleukin (IL)-6 have been detected in amniotic fluid and umbilical cord plasma, and, are associated with the development of white matter lesions. Furthermore, high levels of tumour necrosis factor (TNF) alpha, IL-6 and interferon gamma are expressed in macrophages and astrocytes in regions of white matter damage in the developing brain. The source of the circulating cytokines is thought to be invading microorganisms accessing the amniotic cavity and the fetus by ascending infection from the vagina and cervix. This induces an innate immune response with inflammation of the chorioamniotic membranes and the production of proinflammatory cytokines. The cytokines and other inflammatory mediators then gain access to the fetus and impose adverse effects to the offspring via swallowed amniotic fluid, or, through fetal lungs, eyes or nasal membranes. These agents may increase the permeability of the blood—brain barrier with the enhanced leukocyte infiltration of the brain mediated by brain chemokines; brain microglia and astrocytes upregulate the production of cytokines and brain injury will ensue. Elucidating the mechanisms involved in inflammatory-induced brain injury is made more complex by the observation that inflammation may interrupt hemodynamic stability, and, that activation of inflammatory pathways is involved in the neural response to hypoxia/ischaemia. In human pregnancies where intrauterine inflammation is accompanied by fetal asphyxia, there is a dramatic increase in the risk of cerebral palsy [57]. The synergistic pathways between hypoxia and infection may exist, which has potential to develop into brain damage [57].

Fetal exposure to endotoxin lipopolysaccharide (LPS), a component of gram negative bacteria, in sheep and rats show that LPS binds to the CD14 receptor on the membrane of myeloid cells and, in concert with Toll-like receptors (TLRs) including TLR-4, activates a transmembrane signaling pathway [58]. This pathway involves the activation of nuclear factor (NF)-κB which regulates the transcription of several genes including the proinflammatory cytokines, IL-1, IL-6 and TNF-a [59]. A study shows that, in sheep model, repeated bolus injections of LPS to the fetus over 5 days at 0.7 gestation results in brain injury within 10 days of the first exposure [60]. The injury ranges from focal cystic infarction in the periventricular region to diffuse damage, characterized by reactive gliosis, in the surrounding and subcortical white matter [60]. The pattern of injury is similar to that described in the premature infant.

Blood flow is not significantly altered in the fetal ovine brain during LPS exposure, but as there was significant hypoxemia due to reduced umbilical—placental blood flow, oxygen delivery was reduced by 30–40 % in the fetal brain including the white matter [60].

8.6 Cerebral Hypoxic—Ischaemic Injury

Exposure to cerebral hypoxia—ischaemia leads to different neuropathologies at differing gestational ages [61]. In term infants, neuronal injury predominates; neurons of the CA1 region of the hippocampus, the deeper layers of the cerebral cortex and cerebellar Purkinje cells are injured most frequently [62]. By contrast, in premature infants the cerebral white matter is the major site of injury, leading to the classic neuropathology of periventricular leukomalacia (PVL). PVL includes both the focal cystic infarcts adjacent to the lateral ventricles and more diffuse gliosis extending throughout the cerebral white matter [63]. Magnetic resonance imaging (MRI) studies demonstrate that the diffuse component is very common in premature infants, while focal necrosis now occurs in less than 5 % of preterm infants [64]. In preterm infants, it is increasingly recognized that there is evidence of primary or secondary injury to cortical or deep grey matter, including the cerebral cortex, hippocampus and cerebellum [65]. These alterations in grey matter structures may occur as a result of white matter injury with axonal deafferentation.

In relation to neuronal injury the principal pathways leading to cell death after hypoxia—ischaemia are initiated by energy depletion followed by activation of glutamate receptors, accumulation of cytosolic calcium, activation of a variety of calcium-mediated deleterious events including the generation of reactive oxygen species (ROS) such as superoxide anion, hydroxyl radicals and nitric oxide derivatives [65]. ROS interact with lipid components of cellular membranes, initiating lipid peroxidation, resulting in the breakdown of lipid constituents into highly reactive by-products including lipid aldehydes, for example hydroxynonenal and malondialdehyde. These reactive aldehydes then bind and modify protein creating protein adducts. Stress from nitric oxide (NO) released from reactive microglia reacts with superoxide anions to form peroxynitrite which targets tyrosine residues of proteins to form nitrotyrosine residues. Both processes are highly damaging to cell membranes [2]. These events can result in mitochondrial disruption, and, immediate or delayed cell death, with the cascade of damaging events unfolding over hours to days after the primary insult. In the preterm infant, the vulnerability of the cerebral white matter appears to be related to the exquisite sensitivity of the immature oligo-dendrocytes to ROS. Although the underlying mechanisms have not yet been proven, there is an association between high levels of lipid peroxidation products, such as 8-isoprostane, in the cerebrospinal fluid and white matter injury in preterm infants [64].

The most frequently used model of hypoxemia-ischaemia is that developed in the neonatal rat at postnatal days 7–10, involving unilateral carotid artery ligation and exposure to low oxygen levels. This results in regional damage to the white and

grey matter but the paucity of white matter in rodents does not allow for a close replication to the human lesion; neither does the paradigm result in neurological deficits [65]. More recently, investigators have applied this injury at an earlier stage of development (postnatal day 3), producing a significant impact on cortical development. Despite the differences from the human lesion, the model has been valuable in developing an understanding of mechanisms involved in hypoxic—ischaemic brain injury.

8.7 Preterm Birth

Advances in perinatal care have led to a significant improvement in the survival of very preterm (<30 weeks gestational age) infants [66]. However, up to 10 % of these infants develop spastic motor deficits and at least another 20 % suffer developmental or behavioural disabilities. As indicated above, the most common cerebral neuropathology observed in preterm infants is white matter injury. Such cerebral white matter injury may have subsequent consequences on the overlying cerebral cortex, with alterations in grey matter development detected by advanced MRI techniques in preterm infants. In this regard it is relevant that Marin-Padilla has demonstrated subtle neuropathological abnormalities in the developing neocortex adjacent to perinatally acquired white matter lesions which were thought to result from sensory deafferentation or axotomy [65]. His study emphasizes the importance that progressive post-injury reorganization of the undamaged cortex plays in the underlying mechanisms of ensuing neurological sequelae.

Periventricular leukomalacia is a disorder of developing white matter, and is of particular importance for the preterm infant. The peak incidence for periventricular leukomalacia occurs in preterm infants born between 24 and 36 weeks of gestation [1]. In preterm births with very low birth weight (<1,500 g), > 90 % survive because of technical improvements in neonatal intensive care, 10 % develop cerebral palsy, and 20–50 % develop cognitive and learning deficits [66]. In considering periventricular leukomalacia as a neurodevelopmental brain disorder, it is important to relate its critical period of onset to the developmental milestones of the human brain. At 30 weeks of gestation, human brain weight is barely >50 % that of a full-term infant. The formation of gyri and sulci, the maturation and migration of neurons, neuronal lamination, and the development of blood-vessel regulation, oligodendrocytes, astrocytes, and microglia all occur during the second half of gestation. The study of periventricular leukomalacia focuses on developing oligodendrocytes and the mechanisms leading to cerebral palsy and developmental disabilities. Another national follow-up study shows that the risk of not completing basic school increased with decreasing gestational age [67]. The risk at ≥31 weeks' gestation was moderate and increased steeply at <31 weeks' gestation; the increase at <31 weeks' gestation could not be explained in isolation by cerebral palsy [67].

Research in the guinea pig model of chronic placental insufficiency/IUGR found enlargement of the lateral cerebral ventricles, most likely resulting from reduced growth of neural processes, and reduced neuronal numbers in some brain regions [68]. Ventriculomegaly is one of the most consistent findings in the brains of patients

with schizophrenia. This study demonstrates experimentally for the first time that such alterations can originate from an insult in utero and persist into adolescence. Furthermore it also demonstrates reduced brain weight, reduced basal ganglia volume, absence of astrogliosis, and, the presence of sensorimotor gating deficits at adolescence, paralleling the situation in some patients with schizophrenia [68].

White matter damage may also occur as a result of reverse operation of the Na^+-Ca^{2+} exchanger in axons. Energy depletion causes failure of Na^+-K^+ ATPase which allows an unopposed inward leakage of Na^+ and membrane depolarization acts to drive the Na^+-Ca^{2+}exchanger in the Ca^{2+} import mode, leading to intracellular Ca^{2+} overload. Excess Ca^{2+} disrupts mitochondrial function, leading to axonal damage. If the insult is less severe, a sublethal influx of Ca^{2+} may significantly retard the growth of axons [69].

8.8 Epigenetics

The major psychotic disorders, schizophrenia and bipolar disorder, are aetiologically complex involving both heritable and non-heritable factors. Since there is less evidence of consistently-replicated, genetic effects, and lasting changes in gene expression after environmental exposures, it is assumed that the biological underpinnings of these disorders are epigenetic in form rather than DNA sequence based [70]. Environmental exposures which are related to mental disorders, particularly happening at certain key developmental stages, may evoke long-lasting epigenetic alterations that impose negative impact on the neurobiological processes [70]. Given, The understanding of the dynamic nature of the epigenetic mechanisms and potential reversibility of epigenetic modifications is of key significance of clinical psychiatry and identifying new targets for prevention.

Exposure to environmental factors could influence epigenetic processes, either globally or at specific loci [70]. For example, DNA methylation could be modified by nutritional, chemical, physical, and even psychosocial factors. During the mitotical process, epigenetic changes could be passed down in different generations of somatic cells so that the effects of environmental factors at several specific stages could be propagated through the whole development, resulting in long-lasting negative phenotypic changes [70]. The epigenome seems to be more vulnerable to disruption during several key developmental periods, especially the time point when standard epigenetic signals driving development are being established [70]. The epigenome-environment interactions may provide a promising mechanism of psychiatric disorders, including psychosis [71, 72]. The pathogenic effect of a polymorphism associated with disrupted gene function is likely to be dependent upon the degree to which that particular variant is actually expressed. Therefore the potential risk may be exaggerated, or suppressed. Interestingly the "metastable epialleles," loci that can be epigenetically modified so that to produce a range of phenotypes in genetically identical cells [73]. Many of these loci are environmentally vulnerable to prenatal external factors during the development of fetus [74, 75]. According to the study of agouti model, enriching the maternal diet with methyl donor supplements which increases offspring DNA methylation, could lead

to gene expression changes associated with brown fur and metabolic health [76]. In addition, genetic polymorphisms could alter the ability to which extent a specific region of the genome is able to be epigenetically altered in response to an environmental pathogen.

Another interesting example of gene-environment interaction is the study of monozygotic (MZ) twins. Of particular note is the high degree of discordance between MZ twins for bipolar disorders. Such phenotypic discordance between MZ twins is often attributed to non-shared, environmental factors, although the empirical evidence for such a large environmental contribution to the disorder is still lacking, with no specific environmental risk factors being conclusively linked to aetiology [77]. Interestingly, the phenotypic discordance between MZ twins may be explained by the partial stability of epigenetic signals. MZ twin methylation differences have been reported for CpG sites in certain genes previously implicated in schizophrenia, including the dopamine D2 receptor gene [78] and the catechol-O-methyltransferase (COMT) gene [79]. This evidence suggests that even between genetically-identical individuals, the expression and function of some specific genes is not always the same, which also implies an important role for epigenetics in mental disorders [80–84]. Other characteristics of major psychotic disorders, including sex differences in prevalence, parent-of-origin effects, and evidence for abnormal levels of folate and homocysteine in the plasma of affected individuals [44] (a marker indicative of dysregulated DNA methylation) also support the previous assumption. Furthermore, cortical r-aminobutyric acid-mediated (GABAergic) neurons in schizophrenia have been shown to express increased levels of DNA-methyltransferase-1 that is associated with altered expression of both RLN and GAD67 [82, 85]. Two recent studies have employed genome-wide approaches to identify DNA methylation changes associated with major psychotic disorder. The first investigated DNA methylation differences between MZ twins discordant for bipolar disorder [86]. In this study, affected twins showed increased methylation in the upstream of the spermine synthase gene (SMS) and lower methylation upstream of the peptidylprolyl isomerase E-like gene (PPIEL). Moreover, a strong inverse correlation between PPIEL gene expression and DNA methylation was observed. Mill et al utilized frontal cortex brain tissue from patients with schizophrenia and bipolar disorder to assess DNA methylation across approximately 12,000 regulatory regions of the genome using CpG island microarrays [84]. This study identified epigenetic changes in loci associated with both glutamatergic and GABAergic neurotransmitter pathways, which are consistent with previous evidence. Glutamate is the most abundant fast excitatory neurotransmitter in the mammalian nervous system, with a critical role in synaptic plasticity. Several studies observed that glutamate receptor agonists may cause psychotic symptoms in unaffected individuals, which link the glutamate system to psychosis.

8.9 Conclusions

Many, varied factors contribute to the mental health of newborns during the period of embryo-fetal development. Quantitative, genetic studies and epidemiological data highlight an important role of environmental factors in the aetiology of major

psychotic disorders [72]. Notwithstanding methodological concerns regarding the validity of environmental measures, some have consistently been associated with both schizophrenia and bipolar disorder [87]. The mechanism through which these environmental factors act upon molecular and cellular biological machineries in the human brain, and ultimately give rise to psychosis-related phenotypes and pathology, remains poorly understood. It is clear that genes and environment act together to increase susceptibility of psychopathology [88]. These findings, however, represent purely statistical interactions and provide little information about precise aetiological mechanisms. Taking this into consideration, it may be attractive to argue that environmental risk factors for major mental dysfunction act, at least in part, via epigenomic alterations. The epigenetic machinery of the cell, which acts to directly control gene expression, may be influenced by adverse environmental exposures so that modify the effects of pathogenic DNA sequence polymorphisms. Evidence remains limited though it is a continuing avenue of inquiry.

References

1. Connors SL, Levitt P, Matthews SG, et al. Fetal mechanisms in neurodevelopmental disorders. Pediatr Neurol. 2008;38:163–76.
2. Rees S, Inder T. Fetal and neonatal origins of altered brain development. Early Hum Dev. 2005;81:753–61.
3. Schlotz W, Phillips DI. Fetal origins of mental health: evidence and mechanisms. Brain Behav Immun. 2009;23:905–16.
4. Swanson JD, Wadhwa PM. Developmental origins of child mental health disorders. J Child Psychol Psychiatry. 2008;49:1009–19.
5. Class QA, Abel KM, Khashan AS et al. Offspring psychopathology following preconception, prenatal and postnatal maternal bereavement stress. Psychol Med. 2013:1–14.
6. Schneider ML, Moore CF, Kraemer GW, et al. The impact of prenatal stress, fetal alcohol exposure, or both on development: perspectives from a primate model. Psychoneuroendocrinology. 2002;27:285–98.
7. O'Connor TG, Heron J, Golding J, et al. Maternal antenatal anxiety and behavioural/emotional problems in children: a test of a programming hypothesis. J Child Psychol Psychiatry. 2003;44:1025–36.
8. Van den Bergh BR, Marcoen A. High antenatal maternal anxiety is related to ADHD symptoms, externalizing problems, and anxiety in 8- and 9-year-olds. Child Dev. 2004;75:1085–97.
9. Huizink AC, Dick DM, Sihvola E, et al. Chernobyl exposure as stressor during pregnancy and behaviour in adolescent offspring. Acta Psychiatr Scand. 2007;116:438–46.
10. Barker ED, Maughan B. Differentiating early-onset persistent versus childhood-limited conduct problem youth. Am J Psychiatry. 2009;166:900–8.
11. O'Donnell K, O'Connor TG, Glover V. Prenatal stress and neurodevelopment of the child: focus on the HPA axis and role of the placenta. Dev Neurosci. 2009;31:285–92.
12. Bergman K, Sarkar P, O'Connor TG, et al. Maternal stress during pregnancy predicts cognitive ability and fearfulness in infancy. J Am Acad Child Adolesc Psychiatry. 2007;46:1454–63.
13. Entringer S, Buss C, Kumsta R, et al. Prenatal psychosocial stress exposure is associated with subsequent working memory performance in young women. Behav Neurosci. 2009;123:886–93.
14. Laplante DP, Brunet A, Schmitz N, et al. Project Ice Storm: prenatal maternal stress affects cognitive and linguistic functioning in 5 1/2-year-old children. J Am Acad Child Adolesc Psychiatry. 2008;47:1063–72.

15. Glover V, O'Connor TG, Heron J, et al. Antenatal maternal anxiety is linked with atypical handedness in the child. Early Hum Dev. 2004;79:107–18.
16. O'Connor TG, Heron J, Golding J, et al. Maternal antenatal anxiety and children's behavioural/emotional problems at 4 years. Report from the Avon Longitudinal Study of Parents and Children. Br J Psychiatry. 2002;180:502–8.
17. Rodriguez A, Bohlin G. Are maternal smoking and stress during pregnancy related to ADHD symptoms in children? J Child Psychol Psychiatry. 2005;46:246–54.
18. Khashan AS, Abel KM, McNamee R, et al. Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events,". Arch Gen Psychiatry. 2008;65: 146–52.
19. Goldstein LH, Harvey EA, Friedman-Weieneth JL. Examining subtypes of behavior problems among 3-year-old children, Part III: Investigating differences in parenting practices and parenting stress. J Abnorm Child Psychol. 2007;35:125–36.
20. Susser E, Neugebauer R, Hoek HW, et al. Schizophrenia after prenatal famine. Further evidence. Arch Gen Psychiatry. 1996;53:25–31.
21. Clair S. Why is Te Tiriti o Waitangi still important? Nurs N Z. 2005;11:28.
22. Heijmans BT, Tobi EW, Stein AD, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A. 2008;105:17046–9.
23. Brown AS, Bottiglieri T, Schaefer CA, et al. Elevated prenatal homocysteine levels as a risk factor for schizophrenia. Arch Gen Psychiatry. 2007;64:31–9.
24. Smits L, Pedersen C, Mortensen P, et al. Association between short birth intervals and schizophrenia in the offspring. Schizophr Res. 2004;70:49–56.
25. Allen NC, Bagade S, McQueen MB, et al. Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat Genet. 2008;40:827–34.
26. Saheed SA, Cierlik I, Larsson KA, et al. Stronger induction of callose deposition in barley by Russian wheat aphid than bird cherry-oat aphid is not associated with differences in callose synthase or beta-1,3-glucanase transcript abundance. Physiol Plant. 2009;135:150–61.
27. Lundback B, Ronmark E, Lindberg A, et al. Asthma control over 3 years in a real-life study. Respir Med. 2009;103:348–55.
28. Obel C, Hedegaard M, Henriksen TB, et al. Psychological factors in pregnancy and mixed-handedness in the offspring. Dev Med Child Neurol. 2003;45:557–61.
29. Huizink AC, de Medina PG, Mulder EJ, et al. Psychological measures of prenatal stress as predictors of infant temperament. J Am Acad Child Adolesc Psychiatry. 2002;41:1078–85.
30. Harvey EA, Friedman-Weieneth JL, Goldstein LH, et al. Examining subtypes of behavior problems among 3-year-old children, Part I: Investigating validity of subtypes and biological risk-factors. J Abnorm Child Psychol. 2007;35:97–110.
31. de Bruijn AT, van Bakel HJ, van Baar AL. Sex differences in the relation between prenatal maternal emotional complaints and child outcome. Early Hum Dev. 2009;85:319–24.
32. O'Connor TG, Heron J, Glover V. Antenatal anxiety predicts child behavioral/emotional problems independently of postnatal depression. J Am Acad Child Adolesc Psychiatry. 2002;41:1470–7.
33. Rice F, Harold GT, Boivin J, et al. The links between prenatal stress and offspring development and psychopathology: disentangling environmental and inherited influences. Psychol Med. 2010;40:335–45.
34. Mennes M, Stiers P, Lagae L, et al. Long-term cognitive sequelae of antenatal maternal anxiety: involvement of the orbitofrontal cortex. Neurosci Biobehav Rev. 2006;30:1078–86.
35. Huizink AC, Robles de Medina PG, Mulder EJ, et al. Stress during pregnancy is associated with developmental outcome in infancy. J Child Psychol Psychiatry. 2003;44:810–8.
36. Susser E, St Clair D. Prenatal famine and adult mental illness: Interpreting concordant and discordant results from the Dutch and Chinese Famines. Soc Sci Med. 2013;pii: S0277-9536(13)00152-4. doi:10.1016/j.socscimed.2013.02.049.
37. Brown AS, Susser ES. Prenatal nutritional deficiency and risk of adult schizophrenia. Schizophr Bull. 2008;34:1054–63.
38. Susser ES, Lin SP. Schizophrenia after prenatal exposure to the Dutch Hunger Winter of 1944–1945. Arch Gen Psychiatry. 1992;49:983–8.

39. Jonsson EG, Larsson K, Vares M, et al. Two methylenetetrahydrofolate reductase gene (MTHFR) polymorphisms, schizophrenia and bipolar disorder: an association study. Am J Med Genet B Neuropsychiatr Genet. 2008;147B:976–82.
40. Roffman JL, Weiss P, Purcell S, et al. Contribution of methylenetetrahydrofolate reductase (MTHFR) polymorphisms to negative symptoms in schizophrenia. Biol Psychiatry. 2008; 63:42–8.
41. Van den Veyver IB. Genetic effects of methylation diets. Annu Rev Nutr. 2002;22:255–82.
42. Haidemenos A, Kontis D, Gazi A, et al. Plasma homocysteine, folate and B12 in chronic schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2007;31:1289–96.
43. Rutten BP, Mill J. Epigenetic mediation of environmental influences in major psychotic disorders. Schizophr Bull. 2009;35:1045–56.
44. Abdolmaleky HM, Cheng KH, Faraone SV, et al. Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. Hum Mol Genet. 2006;15: 3132–45.
45. Grayson DR, Jia X, Chen Y, et al. Reelin promoter hypermethylation in schizophrenia. Proc Natl Acad Sci U S A. 2005;102:9341–6.
46. Lambert BL, Bauer CR. Developmental and behavioral consequences of prenatal cocaine exposure: a review. J Perinatol. 2012;32:819–28.
47. Minnes S, Lang A, Singer L. Prenatal tobacco, marijuana, stimulant, and opiate exposure: outcomes and practice implications. Addict Sci Clin Pract. 2011;6:57–70.
48. Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet. 2006;368:2167–78.
49. Weinberg J, Sliwowska JH, Lan N, et al. Prenatal alcohol exposure: foetal programming, the hypothalamic-pituitary-adrenal axis and sex differences in outcome. J Neuroendocrinol. 2008;20:470–88.
50. Zhang X, Sliwowska JH, Weinberg J. Prenatal alcohol exposure and fetal programming: effects on neuroendocrine and immune function. Exp Biol Med (Maywood). 2005;230:376–88.
51. Huizink AC, Mulder EJ. Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. Neurosci Biobehav Rev. 2006;30:24–41.
52. D'Onofrio BM, Van Hulle CA, Waldman ID, et al. Smoking during pregnancy and offspring externalizing problems: an exploration of genetic and environmental confounds. Dev Psychopathol. 2008;20:139–64.
53. Knopik VS, Heath AC, Jacob T, et al. Maternal alcohol use disorder and offspring ADHD: disentangling genetic and environmental effects using a children-of-twins design. Psychol Med. 2006;36:1461–71.
54. Thapar A, Rutter M. Do prenatal risk factors cause psychiatric disorder? Be wary of causal claims. Br J Psychiatry. 2009;195:100–1.
55. Palanisamy A. Maternal anesthesia and fetal neurodevelopment. Int J Obstet Anesth. 2012;21:152–62.
56. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. Pediatr Res. 1997;42:1–8.
57. Nelson KB, Grether JK. Causes of cerebral palsy. Curr Opin Pediatr. 1999;11:487–91.
58. Yoon BH, Kim CJ, Romero R, et al. Experimentally induced intrauterine infection causes fetal brain white matter lesions in rabbits. Am J Obstet Gynecol. 1997;177:797–802.
59. Cai Z, Pan ZL, Pang Y, et al. Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. Pediatr Res. 2000;47:64–72.
60. Keller M, Enot DP, Hodson MP, et al. Inflammatory-induced hibernation in the fetus: priming of fetal sheep metabolism correlates with developmental brain injury. PLoS One. 2011;6:e29503.
61. Inder TE, Anderson NJ, Spencer C, et al. White matter injury in the premature infant: a comparison between serial cranial sonographic and MR findings at term. AJNR Am J Neuroradiol. 2003;24:805–9.
62. Volpe JJ. Neurology of the newborn. Major Probl Clin Pediatr. 1981;22:1–648.

63. Sizonenko SV, Kiss JZ, Inder T, et al. Distinctive neuropathologic alterations in the deep layers of the parietal cortex after moderate ischemic-hypoxic injury in the P3 immature rat brain. Pediatr Res. 2005;57:865–72.
64. Selip DB, Jantzie LL, Chang M, et al. Regional differences in susceptibility to hypoxic-ischemic injury in the preterm brain: exploring the spectrum from white matter loss to selective grey matter injury in a rat model. Neurol Res Int. 2012;2012:725184.
65. Marin-Padilla M. Developmental neuropathology and impact of perinatal brain damage. III: Gray matter lesions of the neocortex. J Neuropathol Exp Neurol. 1999;58:407–29.
66. Spencer R. Long-term visual outcomes in extremely low-birth-weight children (an American Ophthalmological Society thesis). Trans Am Ophthalmol Soc. 2006;104:493–516.
67. Mathiasen R, Hansen BM, Andersen AM, et al. Gestational age and basic school achievements: a national follow-up study in Denmark. Pediatrics. 2010;126:e1553–1561611561.
68. Mallard EC, Rehn A, Rees S, et al. Ventriculomegaly and reduced hippocampal volume following intrauterine growth-restriction: implications for the aetiology of schizophrenia. Schizophr Res. 1999;40:11–21.
69. Loeliger M, Watson CS, Reynolds JD, et al. Extracellular glutamate levels and neuropathology in cerebral white matter following repeated umbilical cord occlusion in the near term fetal sheep. Neuroscience. 2003;116:705–14.
70. Sutherland JE, Costa M. Epigenetics and the environment. Ann N Y Acad Sci. 2003;983: 151–60.
71. Dolinoy DC, Weidman JR, Jirtle RL. Epigenetic gene regulation: linking early developmental environment to adult disease. Reprod Toxicol. 2007;23:297–307.
72. Caspi A, Moffitt TE. Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci. 2006;7:583–90.
73. van Os J, Rutten BP, Poulton R. Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. Schizophr Bull. 2008;34:1066–82.
74. Rakyan VK, Blewitt ME, Druker R, et al. Metastable epialleles in mammals. Trends Genet. 2002;18:348–51.
75. Dolinoy DC, Weidman JR, Waterland RA, et al. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect. 2006;114:567–72.
76. Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr. 2002;132:2393S–400S.
77. Fraga MF, Ballestar E, Paz MF, et al. Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci U S A. 2005;102:10604–9.
78. Petronis A, Gottesman II, Kan P, et al. Monozygotic twins exhibit numerous epigenetic differences: clues to twin discordance? Schizophr Bull. 2003;29:169–78.
79. Mill J, Dempster E, Caspi A, et al. Evidence for monozygotic twin (MZ) discordance in methylation level at two CpG sites in the promoter region of the catechol-O-methyltransferase (COMT) gene. Am J Med Genet B Neuropsychiatr Genet. 2006;141B:421–5.
80. Dempster EL, Mill J, Craig IW, et al. The quantification of COMT mRNA in post mortem cerebellum tissue: diagnosis, genotype, methylation and expression. BMC Med Genet. 2006;7:10.
81. Tochigi M, Iwamoto K, Bundo M, et al. Methylation status of the reelin promoter region in the brain of schizophrenic patients. Biol Psychiatry. 2008;63:530–3.
82. Veldic M, Guidotti A, Maloku E, et al. In psychosis, cortical interneurons overexpress DNA-methyltransferase 1. Proc Natl Acad Sci U S A. 2005;102:2152–7.
83. Kuratomi G, Iwamoto K, Bundo M, et al. Aberrant DNA methylation associated with bipolar disorder identified from discordant monozygotic twins. Mol Psychiatry. 2008;13:429–41.
84. Mill J, Tang T, Kaminsky Z, et al. Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. Am J Hum Genet. 2008;82:696–711.
85. Benes FM, Berretta S. GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. Neuropsychopharmacology. 2001;25:1–27.

86. Rosa A, Picchioni MM, Kalidindi S, et al. Differential methylation of the X-chromosome is a possible source of discordance for bipolar disorder female monozygotic twins. Am J Med Genet B Neuropsychiatr Genet. 2008;147B:459–62.
87. van Os J, Krabbendam L, Myin-Germeys I, et al. The schizophrenia envirome. Curr Opin Psychiatry. 2005;18:141–5.
88. Oh G, Petronis A. Environmental studies of schizophrenia through the prism of epigenetics. Schizophr Bull. 2008;34:1122–9.

Gamete/Embryo-Fetal Origins of Infertility

9

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Abstract

Infertility is defined as the inability of a couple to conceive despite trying for 1 year. Genetically, infertility is considered a lethal factor, because the family lineage stops at that individual with no further offspring [1]. Traditionally, male infertile factors include azoospermia or oligozoospermia, and, abnormal sperm morphology or motility. Genetic pathogenesis may include Y chromosome microdeletions, chromosomal abnormalities, a single gene mutation, or, rearrangements of sperm mitochondrial DNA (mtDNA). Female infertile factors include congenital malformations, or, dysfunction of female reproductive system including abnormal folliculogenesis and sexual dysfunction. Accumulating data suggests that adverse exposures, or interventions, during the period of gametogenesis and embryo-fetal development may result in infertility [2–7]. Concerns about the effects of development on reproductive health are not new; previous studies in animal models and human epidemiological data indicate that early life events may initiate long term changes that increase the risk of diseases, such as the reproductive disorders [7–76].

Studies in animal models and basic research underscore the vulnerability of the reproductive system at different times of development and across the whole life-cycle. We review data implicating select developmental factors in compromising reproductive capacity in animals. We also review epidemiological and basic research in humans that suggests roles for developmental factors in reproductive dysfunction and infertility. Finally, we summarize the epigenetic modifications and currently-available countermeasures in prevention and treatment.

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9.1 Infertility Originating from Embryo-Fetal Development

The classic studies that show unhealthy, transgenerational effects are observations of the Dutch famine, where it was clearly shown that alterations in fetal nutrition, especially in early pregnancy, can permanently change development across generations. Not only did starved mothers deliver small babies; their daughters also had high rates of small for gestational age (SGA) babies, even though the daughters' diet during pregnancy was normal [77, 78].

In one recent, transgenerational study, researchers intercrossed male and female adult controls with first-generation offspring of gestational diabetes mellitus (F1-GDM) mice to obtain second-generation (F2) offspring. They found that birth weight significantly increased in F2 offspring through the paternal line suffered impaired glucose tolerance (IGT). Regardless of whether they were born from F1-GDM with, or without IGT, there was a high risk of IGT appearing as early as 3 weeks in the F2 offspring that progressed through both parental lineages, especially the paternal line. In both F1 and F2 offspring of GDM, the expression of imprinted genes Igf2 and H19 was downregulated in pancreatic islets, and, this resulted from abnormal methylation status of the differentially methylated region. Furthermore, altered Igf2 and H19 gene expression occurred in sperm of adult F1-GDM, regardless of the presence of IGT, indicating that epigenetic changes in germ cells may have contributed to transgenerational transmission [79].

Related studies have described the ability of environmental factors (e.g. endocrine disruptors), physiological or pathological stress (e.g. malnutrition, excess steroid hormones, etc.), and therapeutic treatments (e.g. ordinary drug, chemotherapy, radiotherapy, assisted reproductive technology, etc.) to disrupt gametogenesis, and, embryo-fetal development to promote transmissible, adult-onset disease, such as the reproductive disorders [7–76] and others [77–119].

As a consequence, concerns emerged regarding adult infertility from the same origins. At present, there is little experimental data on humans originating from gametogenesis and embryo-fetal development; there are some studies in animals.

9.2 Animal Studies

Exposure of gestating animals to adverse environment factors, such as endocrine disruptors, malnutrition or excess steroid hormones, may lead to lots of disorders, including infertility [8–35].

9.2.1 Exposure to Endocrine Disruptors

Studies on transient exposure of gestating maternal rats to endocrine disruptors, such as bisphenol A, vinclozolin, methoxychlor, TCDD (a kind of dioxin), find that there is induction of an adult phenotype in the F1 generation with decreased spermatogenic capacity (cell number and viability), and, an increased incidence of male

infertility [8–15]. These effects may be transferred through the male germ line to subsequent generations. The effects on reproduction correlate with altered DNA methylation patterns in the germ line. The alteration in epigenome may interfere with protein expression profiles of testicular steroid receptor co-regulators, relative hormonal signaling, and, development of male reproductive organs, including spermatogenesis. It has been considered that the environmental agents had the ability to induce epigenetic transgenerational changes in the sperm epigenome and the testes proteome, leading to male infertility [8–15].

In female offspring, transient exposure of gestating maternal rats to adverse endocrine disruptors may induce transgenerational, adult phenotypes of ovarian diseases, including infertility [16–19]. Transient exposure to several environmental toxins of F0 gestating female rats during the embryonic period, gives rise to offspring's ovarian cysts resembling human polycystic ovarian syndrome (PCOS) [120–122], and, a decrease in the size of the ovarian primordial follicle pool resembling primary ovarian insufficiency [123, 124]. There was evidence of transgenerational effects that demonstrated differential DNA methylation in the F3 generation granulosa cells in both the transcriptome and epigenome [19].

9.2.2 Malnutrition

Exposure of gestating animals to a suboptimal nutritional environment, such as maternal diabetes, obesity, intrauterine protein restriction, high-fat diet, etc., may also result in alterations to male and female fertility [20–22].

In a rat study of growth restriction caused by maternal diabetes, body weight and blood glucose levels of male pups, on the third postnatal day, were lower in the offspring of diabetic dams compared to controls. Testicular descent and preputial separation were also delayed in these offspring, as well as lower weights of reproductive organs at 40, 60 and 90 days-old [20].

Exposure of gestating rats to in utero protein restriction, overall fetal growth and development was impaired with altered reproductive phenotypes in the offspring [21, 22]. In female pups, alterations include increased ano-genital distance, delayed vaginal opening and timing of first oestrus, lower levels of serum oestradiol, progesterone, luteinizing hormone, increased cycle length, declining fertility rates, and, reduced survival of the pups. These data show that in utero protein restriction results in delayed sexual maturation and premature ageing of reproductive function in female progeny [21]. In male pups, alterations in reproductive phenotype include decreased sperm reserves, short sperm transit time through the epididymis which is important to sperm maturation, etc. [22].

9.2.3 Steroid Hormones

Many studies provide support for the role of androgens and estrogens during development in relation to various dysfunctions that contribute to the infertile

phenotype [23–35]. Animals exposed to high levels of androgen in utero (by giving very large doses of androgen to the mothers) may develop reproductive and metabolic dysfunctions that mimic those observed in women with PCOS [23–35]. In ewes, androgen-treated female progeny demonstrate abnormalities of early follicle development, increased ovarian follicular recruitment, reduced ovarian reserve and disrupted ovarian cycles [23–27]. All these findings are potential contributors to early reproductive failure. Similar results were also found in successive studies of prenatally androgenized monkeys, including elevated circulating luteinizing hormone (LH) levels and hyperandrogenism in late gestation and early infant life [28].

Development of PCOS-like phenotypes in prenatally androgenized rhesus monkeys may result from perturbation of the epigenome, through altered DNA methylation [125–129]. The effects of maternal androgen exposure on ovarian development may be enhanced by the synergetic actions of estrogens by altering fetal ovarian steroidogenic gene and microRNA expression [29]. The disruptive impact of prenatal testosterone on adult pathology may partially depend on its conversion to estrogen, and, consequent changes on maternal and fetal endocrine environments [27–31].

In a sheep model, prenatal testosterone exposure is associated with changes in the expression of anti-Müllerian hormone (AMH) in pre-antral and antral follicles in adult ovaries, similar to findings in women with PCOS. Changes of AMH expression in adult ovaries may mediate abnormal folliculogenesis in PCOS [32].

9.3 Human Studies

In humans, infertility is usually demonstrated as a primary or secondary manifestation of adult disease [7, 36–49, 81, 82, 90, 128, 130–133]. Infertility may also manifest as impaired fertility owing to malnutrition or, a side-effect of therapeutic treatment such as cytotoxic chemotherapy, radiotherapy or assisted reproductive technology (ART) [7, 57–63, 72–76, 100, 101, 134, 135]. All of the infertility mentioned above could be in the origin of embryo-fetal development.

Classical adult diseases with infertility derived from gametogenesis and embryo-fetal development include prostate disease, congenital hypospadias, cryptorchidism and gonadal dysfunction in males, and, endometriosis, uterine abnormalities, early menarche, PCOS and early menopause in females [7, 37–49, 90, 127–129]. All are associated with impaired intrauterine growth [37–49, 90]. Impaired intrauterine growth in males increases the risk of congenital hypospadias, cryptorchidism and reduced gonadal function, along with smaller testicular volumes and decreased testosterone levels after puberty, and, increased serum follicle-stimulating hormone (FSH) levels in infancy [37–40, 90]. Impaired intrauterine growth in females is associated with increased risks of higher FSH/AMH secretion, premature adrenarche, early puberty, PCOS [41–44]. In adolescent girls born SGA, anomalies included poor ovarian response to FSH, subclinical hyperandrogenism (adrenal or ovarian origin), reduced ovulation rates, and, reduced uterine and ovarian sizes [45–49]. All of these anomalies may contribute to subfertility in either sex.

Infertility also occurs secondary to diabetes, cardiovascular disease, cancers, obesity and some other adult-onset diseases [36, 57–59, 132, 133, 136–138]. For example, obesity is associated with anovulation, and, negatively affects the outcomes of ovarian stimulation and in vitro fertilization [36, 136, 138]. Obesity may affect reproduction and impair ovarian response through fat cell metabolism, steroids and secretion of proteins, such as leptin and adiponectin. It may also affect pancreatic secretion of insulin, androgen synthesis by the ovary and sex hormone-binding globulin (SHBG) production from the liver [36, 136, 138].

One of the major factors proposed to explain embryo-fetal development diseases is maternal malnutrition [7, 20–22, 36–55, 77, 78, 80–90, 130, 131]. There is no doubt that in developing countries, maternal diet can affect birth weight, and may be extremely important in mediating adult disease. Such effects may also be mediated, or indeed amplified, by adverse environmental conditions persisting across a number of generations [88, 89].

At the present time, there is no convincing direct epidemiological data to suggest a role for alterations in maternal nutrition as a developmental factor in reproductive dysfunction and infertility [139]. Also, in young adults from the general population, fertility is not reduced in those born SGA, compared to those born as appropriate-for-gestational age (AGA) [140]. In a case-control study on ovarian reserve in young women with low birth weight and normal puberty, researchers did not found a significant difference between young women born SGA and AGA in follicular phase LH, FSH, E2 and AMH levels. They did not found a significant difference in the response to endogenous GnRH either. They concluded that it seems unlikely that limited ovarian reserve is a significant problem in adolescent SGA [141].

Yet, there are some cohort investigations and experiment studies that provide indirect data in humans implying roles for maternal malnutrition as an embryo-fetal developmental factor in reproductive dysfunction and, possibly, infertility. Infants born SGA demonstrated a hypersecretion of FSH in both boys and girls, and an increase in AMH and GnRH agonist-stimulated estradiol serum concentrations in girls [41, 42]. A lighter and longer girl at birth will have an earlier menarche [50–54]. Women who were born as relatively fat babies do not exhibit normal ovarian suppression in response to physical activity at adulthood. That means fetal programming of reproductive function may result in a developmentally adaptive shift in ovarian response. This adaptive shift in ovarian response may be a subfertility state originating from maternal malnutrition [55].

Chemotherapy, radiotherapy and assisted reproductive technique may also cause relevant abnormalities in fertility of their offspring through the alteration of genetic and epigenetic modifications [57–63, 72, 76, 92, 100].

Gonadal dysfunction occurs after treatment with alkylating agents or pelvic radiotherapy [69–72]. Improved survival rates for childhood and young adult cancer patients, mean that the side-effects of chemo/radiotherapy, like infertility and other gonadal dysfunction, are increasingly recognized [57–72]. Furthermore, treatment of certain precancerous and benign conditions, such as myelodysplasia and systemic lupus erythematosus, may necessitate administration of high-dose chemotherapeutic agents [142, 143]. Thus, it is necessary to consider the risk of

infertility as a side-effect of these treatments based on the origin of embryo-fetal development [57–72].

Infertility usually presents as a long-term consequence of combination chemotherapy and radiotherapy not only in the individual exposed, but also in subsequent progeny [57–72]. This is, in large part, because of the exquisite sensitivity of germ cells to injury by cytotoxic drugs and radiation therapy. It is estimated that, among the population receiving gonadotoxic cancer treatments, approximately 10–30 % patients suffer permanent infertility – that is more frequent in males [57–72]. Resulting testicular damage can affect rapidly dividing cells during spermatogenesis, and, the functions of testis somatic cells, including the Sertoli cells' function of nurturing germ cell development, or, Leydig cells' function of testosterone production. Germ cells that produce spermatozoa are more sensitive to chemotherapy and radiation than Leydig cells that generate testosterone [144]. Adverse effects are associated with depletion of the proliferating germ-cell pool by killing cells at the stage of differentiating spermatogonia [145]. Additionally, those stem spermatogonia that do survive fail to differentiate any further [146]. Thus, a common late effect of cancer therapy for male patients is infertility rather than impaired sexual function [61]. In female patients, chemotherapy and radiation treatment destroy ovarian follicles and predispose treated females to premature ovarian failure [58–71]. Chemo- and radiotherapy cause dose-dependent, oocyte depletion, ovarian fibrosis and blood-vessel damage leading to premature menopause, or, even, acute ovarian failure [58–71].

Epidemiological data have indicated that children conceived through IVF have a greater relative risk of birth defects, genetic abnormalities and imprinting disorders, including the higher risk of infertility [7, 73–76, 94–111].

Iatrogenic risks of infertility of IVF offspring may reflect not only ART, but also factors associated with subfertility in their parents, such as a microdeletion of Yq chromosome, an imprinting error in a defective/deficient sperm or obesity [7, 15, 73–75, 98–101, 134, 147–149]. It is the ability of ART to bypass nature's protective mechanisms that makes the risk of infertility particularly significant. The resulting offspring conceived with ART may transmit heritable traits associated with infertility [7, 15, 73–75, 98–101, 134, 147–149].

Sons conceived by ART inherit similar deletions in the azoospermia factor region of the Y chromosome as their fathers [73]. Infant boys conceived by ICSI demonstrate reduced serum testosterone levels, implying possible inheritance of impaired Leydig cell function from their fathers [74]. The same paternal origin of DNA methylation errors at imprinted loci were found in 7 out of 17 offspring born by ART [75]. All of these directly inherited from infertile parents through ART are the high risks of embryo-fetal development origin of infertility.

Oocytes from ovarian stimulation with gonadotrophins have different global gene expression, including DNA methylation and some imprinting expression compared to normal M II oocytes [150]. So was the global gene expression of oocytes matured in vitro compared with oocytes matured in vivo [151, 152]. In vitro conception is associated with lower genomic methylation in placenta and higher genomic methylation in cord blood [153, 154] . These IVF-associated DNA

methylation differences, including those in the differentially methylated regions (DMRs) of imprinting gene loci such as IGF2, H19, IGF2R, KvDMR1, etc., are associated with differences in gene expression at both imprinted and non-imprinted genes [153–156]. Similar reports also include animal [15, 93, 157–173] and human studies [15, 74–76, 106–111, 174–179]. In mice, superovulation alters methylation patterns of imprinted genes in sperm of offspring [172]. In vitro culture of preimplantation embryos influenced the somatometric parameters of the resulting progeny, some of which were maintained across a generation [15, 93, 173].

In humans, there are variations in the DNA sequence in the gene encoding DNMT3L, which is involved in DNA methylation. Deficiency of this gene in mice is associated with oligospermia [75, 180]. ART may increase clinical mutation detection in male offspring [76]. In that study, the Yq genetic status of 37 babies conceived through ART and their fathers according to 13 Y-specific markers covering four subregions of azoospermia factors (AZF) were investigated. Another 60 babies conceived naturally and their fathers were recruited as controls. The investigators found a 10.8 % (4/37) incidence of de novo Y chromosome microdeletions in boys conceived through ICSI or IVF, including one complicated with hypospadias, and, none in boys conceived naturally. These observations indicate a higher risk of infertility in ART male offspring [181].

9.4 Mechanisms of Infertility

9.4.1 Epigenetics and Developmental Origins of Infertility

Epigenetics provides an interface between environment and the genome for environmental epigenetic modifications in a more plastic molecular process (Fig. 9.1) [6, 7, 182–190]. In developmental biology, the series of responses and consequences

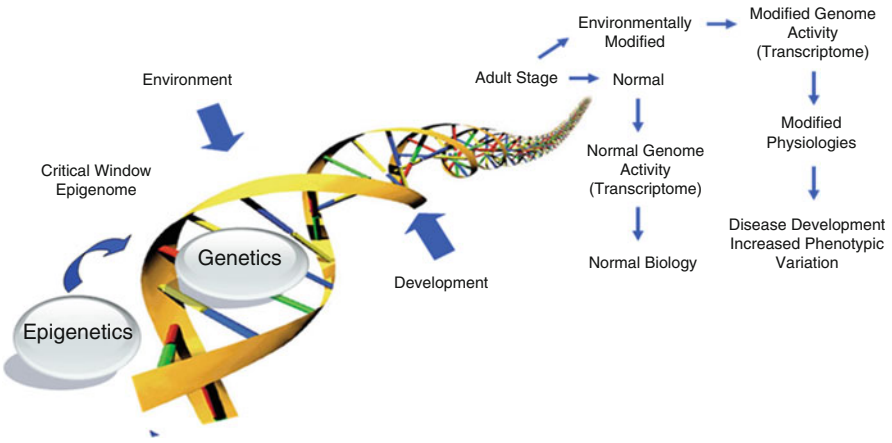

Fig. 9.1 Integrated epigenetics and genetics in development and disease etiology

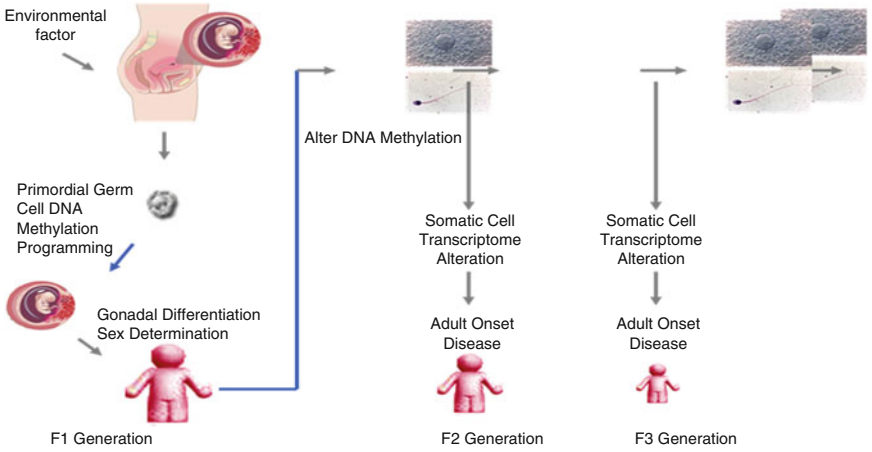

Fig. 9.2 Scheme for epigenetic transgenerational inheritance

controlled by epigenetics may result from developmental plasticity, where one genotype can give rise to a range of different phenotypical states in response to a variety of environmental conditions [2–6]. The resulting responses and consequences may include rapid maturation, growth limitation, or abortion [2–6].

If the germ line, egg or sperm, are affected during their critical development window, i.e. the period of embryonic gonadal development and germ-line differentiation, environmentally-induced epigenetic modifications may become permanent in the germline epigenome, and, may potentially have an impact on subsequent generations by way of epigenetic transgenerational inheritance (Fig. 9.2) [2–13, 15–19, 79, 127, 182–191].

Through these epigenetic modifications, DNA methylation in germ lines is altered like an imprint, and, the transcriptome in somatic cells, including the cells of testis or ovary, is altered as well. The resulting diseases may include adult-onset diseases, including male or female infertility, both within individuals and across generations (Fig. 9.2) [2–13, 15–19, 79, 127, 182–191].

The most sensitive phase for epigenetic modification is the period of intrauterine gametogenesis and embryo-fetal development. The dynamic state of the genome in the periconceptional and perinatal periods make it susceptible for adverse effects through epigenetic modification whose end result may be infertility [2–55, 77–79, 84, 88–90, 125–129, 182–191].

PCOS is one of the most common endocrine disorders in women of reproductive age. Patients with PCOS often present anovulation and infertility. Although the etiology of PCOS remains uncertain, its developmental origins are now well-recognized [19, 23–35, 125–129]. Exposure of a female fetus to hyperandrogenism in utero may result in PCOS phenotype after birth, so, PCOS may be one example of infertility originating from embryo-fetal development [23–35]. Based on these epigenetic modifications, some have described them as “epigenetic abnormalities underlying the fetal origin of PCOS” to attempt to explain the mechanisms of PCOS,

including infertility [125–129]. The hypothesis states that in utero, hyperandrogenism may disturb the epigenetic re-programming in fetal reproductive tissue, resulting in postnatal PCOS phenotypes in women of reproductive age. Meanwhile, the incomplete erasure of such epigenetic abnormality in germ cells after fertilization may promote transgenerational inherence of PCOS.

9.4.2 Epigenetic Transgenerational Inheritance

Epigenetic transgenerational inheritance is defined as a transmission of a modified epigenome, from one generation to the next [6, 7, 182–190]. This effect may span several generations in "epigenetic multi-generational inheritance". The two types of transmission of the epigenome are often integrated [6, 7, 182–190].

Epigenetic transgenerational modification will require some involvement of germ line for transmission of epigenetic information across generations. The characteristics of unequivocal transgenerational transmission of an adult-onset disease phenotype (for example, infertility) through the germ-line make it necessary to assess an epigenetic transgenerational modification through the F3 generation for a F1 generation's embryonic exposure, or though the F2 generation for a F1 generation's postnatal exposure [6, 7, 182–190].

Owing to the general involvement of reprogramming of germ-line epigenome and the ability of environmental factors (e.g. endocrine disruptors) to promote this reprogramming, the way of epigenetic transgenerational modification is the main one responsible to the environmentally induced reproductive toxicology, including developmental origins of infertility [6, 7, 182–191].

9.4.3 Malnutrition-Induced Epigenetic Mechanism

Embryonic nutrition is vital for survival and development [36]. Embryos benefit not only from adequate access to nutritional substrates, but also from proper programming, including epigenetic regulation [190]. Nutritional changes affects longevity of subsequent generations in a human investigation [88]. Maternal methyl supplementation affects DNA methylation and gene expression of offspring, and prevents transgenerational amplification of some phenotypes in mice [86, 192, 193]. All of these data indicate a risk of development origins of infertility and possibly through a malnutrition-induced epigenetic mechanism [7, 85–87, 93, 194].

Reduced amounts of methyl donors in maternal food, such as folates, affect the enzymatic activity of DNMT (an enzyme related to methylation) and are associated with genomic hypomethylation and genomic instability [195, 196]. Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (Igf2) locus and may increase risks of developing disease [197].

Poor fetal nutrition is thought to produce adaptive changes in human developmental trajectories. Differences in nutritional status at birth are associated with adaptive differences in the sensitivity of adult ovarian function to energetic stress [55].

It has been supposed that fetal programming of reproductive function may result in a developmental transgenerational effects or predisposition in a space-time-dependent manner, such that women who were gestated under malnutrition would show infertility in adulthood [7, 85–87, 93]. The involved transgenerational responses in humans may be by way of sex-specificity and mediated by the sex chromosomes, X and Y [87].

9.4.4 Chemotherapy-Induced Epigenetic Mechanism

Chemotherapy-induced infertility is a manifestation of drug toxicity [58, 61–63, 198–202]. Drug-induced epigenetic regulation operate through a combination of chromatin remodeling, DNA methylation, histone acetylation as well as nucleosomal subunit exchange, such that reproduction-related genes are activated or repressed, leading to the infertility [61–63, 198–200].

Alterations in the epigenome may involve direct or indirect mechanisms. The direct effects may be demonstrated by direct alterations of chromatin architecture or DNA methylation. The indirect effects may occur in two-tier stages: a stage of acute exposure and a stage of chronic exposure. The acute exposure to chemotherapy may alter signaling pathways, leading to alterations of transcription factor activity at reproduction-related gene promoters, and, related reproductive toxicology. Chronic exposure to chemotherapy promotes a cellular adaptation to a drug's modifications of the epigenome, leading to possible endurance to the alteration of a given epigenetic state, possible persistence of adverse epigenetic state after the drug is discontinued, and, a possible multigenerational or transgenerational involvement [58, 61–63, 198–202].

High turnover of germ cells (oocyte and sperm) makes the gonads more vulnerable to the cytotoxic effects of chemo- or radiotherapy. Permanent alteration in the germ cell epigenome and a resulted epigenetic transgenerational process may be particularly significant in this context [2–6, 19, 182–190, 198, 199].

9.4.5 ART-Induced Epigenetic Mechanisms

Epigenetic modification of fertility-related gene expression, mainly in the form of alteration of methylation pattern, is an attractive hypothesis that may account for the onset of ART-induced infertility [7, 101, 157, 190].

ART may affect gametogenesis and development of the preimplantation embryo. These periods are also the critical periods for epigenetic modification in mammals, in which paternal and maternal imprints are established, and, the majority of male and female germ line–derived methylation patterns are erased, followed by de novo remethylation of the genome into somatic patterns [190, 203]. Because ART deals mainly with gametes and early embryos, not with primordial germ cells, it is less likely that ART affect the erasure of imprinting marks but are more likely to alter their establishment or maintenance [7].

Owing to the vulnerability of germline (both sperm and oocyte) and preimplantational embryos to adverse environmental factors, a cellular stress or a complex stimulus may influence the relative epigenetic modification of these key periods and result in some modification alterations different from those that naturally occur [76, 93, 153–156, 174–177].

ART-induced, epigenetic modification alterations during gametogenesis and early embryonic development may result in skewing of the phenotype, and, contribute to long-term health consequences [7, 15, 73–76, 93–111, 119, 134, 150–179, 190, 204], and, further, in a transgenerational way [7, 160, 172, 173, 185, 205].

ART may induce widespread epigenetic alterations in gametes and ART-conceived offspring [76, 150–157, 174–177]. These alterations may increase the risk of adverse pathophysiology later in life, including infertility and some other adult onset diseases that may cause infertility, including type 2 diabetes, obesity and cardiovascular diseases [7, 15, 73–76, 93–111, 119, 134, 150–179, 190, 204]. Although there is no direct evidence of the transgenerational effects of ART, these concerns persist because transgenerational imprinting effects of ART have been observed in animal studies [7, 15, 134, 157, 160, 172, 173].

9.5 Prevention and Treatment of Embryo-Fetus-Originated Infertility

As aforementioned, embryo-fetus-originated infertility may be resulted from the negative exposure to peri-conception factors (e.g. ART and the related infertile pathophysiology), prenatal factors (e.g. malnutrition) or even pre-conception factors (e.g. chemo/radiotherapy during their childhood). So, the prevention and treatment of embryo-fetus-originated infertility should be done during all the periods involved and for all the factors involved [206].

9.5.1 Pre-conception Prevention and Treatment

Practically, pre-conception preventions and treatments of embryo-fetus- originated infertility are issues of parent's healthy lifestyle and behaviors from their own conception until ahead of their offspring's conception. If infertility is in a transgenerational way, the pre-conception prevention and treatment should be done as early as up to the period of offspring's grandparents [7–19, 79, 85–88, 160, 172, 173, 183–191, 205].

Adverse exposure of external environmental factors (e.g. endocrine disruptors, chemotherapy, radiotherapy, etc.) is the first for parents or grandparents to be prevented ahead of their offspring's conception for the purpose of pre-conception preventions and treatments of embryo-fetus- originated infertility [207, 208]. If chemotherapy or radiotherapy is mandatory for patients previous to or during their reproductive lifespan, fertility preservation and parenthood should be considered and planned as an essential component of treatment [57–72, 200, 201, 207–213].

For example, prior to radiotherapy, through ovarian transposition operation, ovarian function can be preserved. In patients requiring chemotherapy, ovarian suppression and cryopreservation of gametes, embryo or gonad tissue can be used before treatment to preserve or restore fertility in later life [61, 200, 201, 207, 208, 214–220].

Adverse exposure of internal environments (e.g. the maternal pathophysiology of endometriosis, PCOS or obesity) is another one that is needed for parents or grandparents to be treated ahead of their offspring's conception. Documents have shown that maternal endometriosis, PCOS and obesity affect gametogenesis and embryonic development [36, 120, 136, 138, 221, 222]; these adverse impacts are all high risk factors of embryo-fetus- originated infertility. Pre-conception pre-treatments and ameliorations of maternal ill status, with surgery and/or medical therapy, would be beneficial to preventions of embryo-fetus- originated infertility [36, 120, 122, 138, 222].

9.5.2 Peri-conception Prevention and Treatment

Generally, peri-conception is defined as the period from 3 months ahead of conception to the time of embryo period at 2 weeks post fertilization. Both peri-conception and early pregnancy (the 1st trimester of pregnancy) are critical periods susceptible to adverse influences [2–55, 77–79, 84, 88–90, 125–129, 182–191, 223].

Nutrients that contribute to methyl-group one-carbon metabolism, such as folate, can significantly influence growth and development through epigenetic modification [189, 194–196, 224–230]. Folate deficiency may affect female reproductive function through interrupting folliculogenesis, oocyte fertilization, embryonic implantation and fetal viability [231]. It has been demonstrated in prospective human studies that, high daily doses of supplementary folic acid (5 mg/day) in early pregnancy can be protective for preterm birth, low birth weight, small for gestational age neonates and beneficial to child neurodevelopment at 18 months of age [227, 228]. In animal experiments, maternal methyl supplementation can affect epigenetic variation and DNA methylation of offspring, and prevent transgenerational effects of some phenotypes [86, 192, 193, 232, 233]. Therefore, maternal methyl supplementation (such as folic acid) during peri-conception and early pregnancy, may have positive effects in prevention and treatment of embryo-fetus- originated infertility [189, 194, 223].

Embryo-fetus-originated infertility may be in the form of iatrogenic consequence induced by ART during peri-conception. The ART-induced iatrogenic infertility may be genetic infertile defects directly transmitted from infertile parents [7, 15, 73–75, 98–101, 134, 147–149], or, a de novo infertile status during the process of ART [7, 73–76, 93, 101, 107, 147, 153, 154, 157, 174, 190]. As to the ART-transmitted genetic infertile defects, best prevention and treatment of embryo-fetus-originated infertility is conceived naturally. The indication of ART should be strictly selected. If ART was mandatory, preconception genetic screening and preimplantational genetic diagnosis (PGD) are recommended [234, 235]. For example, paternal microdeletions in the Y chromosome of an infertile couple could be used as a predictive marker of

infertility in male offspring, and a PGD technique could be used as a eugenic measure for selecting female offspring or genetically normal male offspring [147]. As to the ART-induced de novo infertility, the less in ART intervention, the better in prevention and treatment. Therefore, natural or mild ART are preferred to [236].

9.5.3 Gestational Prevention and Treatment

Gestational prevention and treatment of embryo-fetus- originated infertility are mainly designed to deal with possible causes that may result in developmental adult diseases during gestation. Nutritional imbalance and adverse exposure of environmental factors (including endocrine disruptors, drugs. infections and other types of stresses) are the major causes that should be prevented and treated during gestation [7, 20–56, 77–91, 112–118, 130, 131, 237, 238]. Measures to improve nutrition and reduce environmental chemical exposures, from all environmental compartments (air, water, soil) and in food and consumer products, are likely to have positive effects in gestational prevention and treatment of embryo-fetus- originated infertility [206].

9.5.4 Postnatal Prevention and Treatment

Postnatal prevention and treatment of embryo-fetus- originated infertility are mainly based the accumulated evidence that developmental adult diseases may be resulted as well from postnatal adverse factors, such as malnutrition [36, 86–88, 192, 193, 197, 206, 223]. The postnatal prevention and treatment are also based the accruing evidence that lifestyle interventions in endocrine or nutrition during early postnatal life can reverse or ameliorate adverse changes in epigenetics and phenotype induced during pregnancy [28, 36, 86–88, 192, 193, 223, 239]. Developmental plasticity in epigenetic mechanisms provides opportunities of postnatal prevention and treatment of embryo-fetus- originated infertility [206, 223, 240]. Relevant testing progresses in developmental safety make susceptibility assessment and disease diagnosis of embryo-fetus-origin possible and feasible in the near future [199, 234, 241, 242]. Relevant gene therapies can be used as well for those diseases whose etiology in genetics and epigenetics has been identifies. Postnatal prevention and treatment have now been considered as rescuing measures or new strategies to reduce risks of development-originated diseases, including infertility [206, 223, 240].

9.6 Conclusions

Contemporary orthodox views believe that infertility results from adult pathology together with contributions from genetic inheritance. The hypothesis that infertility originates, in part, from embryo-fetal development offers us a new framework for some aspects of the condition. Direct evidence for epigenetics in infertility is

limited though indirect evidence, in both humans and animals, are accumulating to implicate these processes in infertility [2–7].

Nutritional imbalance and adverse exposure of environmental factors have the capacity to alter the epigenome of gametes and embryos though we are still some distance from understanding their precise effects in infertility, and, their possible transgenerational transmission [3–6, 182–187, 189, 190, 206, 240].

Despite advances in prevention and treatment of embryo-fetal originated infertility, many questions remain unanswered. Further research is urgently needed to develop evidence-based measures to reduce the embryo-fetal originated consequence [206].

References

1. Hwang K, Yatsenko AN, Jorgez CJ, et al. Mendelian genetics of male infertility. Ann N Y Acad Sci. 2010;1214:E1–17.
2. Hardy DB. The developmental origins of health and disease: today's perspectives and tomorrow's challenges. PREFACE. Semin Reprod Med. 2011;29:171–2.
3. Nicoletto SF, Rinaldi A. In the womb's shadow the theory of prenatal programming as the fetal origin of various adult diseases is increasingly supported by a wealth of evidence. EMBO Rep. 2011;12:30–4.
4. Barker DJP. The origins of the developmental origins theory. J Intern Med. 2007;261:412–17.
5. Langley-Evans SC, McMullen S. Developmental origins of adult disease. Med Princ Pract. 2010;19:87–98.
6. Motrenko T. Embryo-fetal origin of diseases – new approach on epigenetic reprogramming. Arch Perinat Med. 2010;16:11–5.
7. Diaz-Garcia C, Estella C, Perales-Puchalt A, et al. Reproductive medicine and inheritance of infertility by offspring: the role of fetal programming. Fertil Steril. 2011;96:536–45.
8. Guerrero-Bosagna CM, Skinner MK. Epigenetic transgenerational effects of endocrine disruptors on male reproduction. Semin Reprod Med. 2009;27:403–8.
9. Anway MD, Cupp AS, Uzumcu M, et al. Epigenetic transgenerational actions of endocrine disruptors and mate fertility. Science. 2005;308:1466–9.
10. Anway MD, Leathers C, Skinner MK. Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease. Endocrinology. 2006;147:5515–23.
11. Guerrero-Bosagna C, Settles M, Lucker B, et al. Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. PLoS ONE. 2010;5(9):e13100.
12. Manikkam M, Tracey R, Guerrero-Bosagna C, et al. Dioxin (TCDD) induces epigenetic transgenerational inheritance of adult onset disease and sperm epimutations. PLoS ONE. 2012;7(9):e46249.
13. Anway MD, Memon MA, Uzumcu M, et al. Transgenerational effect of the endocrine disruptor vinclozolin on male spermatogenesis. J Androl. 2006;27:868–79.
14. Salian S, Doshi T, Vanage G. Impairment in protein expression profile of testicular steroid receptor coregulators in male rat offspring perinatally exposed to Bisphenol A. Life Sci. 2009;85:11–8.
15. Price TM, Murphy SK, Younglai EV. Perspectives: the possible influence of assisted reproductive technologies on transgenerational reproductive effects of environmental endocrine disruptors. Toxicol Sci. 2007;96:218–26.
16. Bruner-Tran KL, Osteen KG. Developmental exposure to TCDD reduces fertility and negatively affects pregnancy outcomes across multiple generations. Reprod Toxicol. 2011;31:344–50.
17. Manikkam M, Guerrero-Bosagna C, Tracey R, et al. Transgenerational actions of environmental compounds on reproductive disease and identification of epigenetic biomarkers of ancestral exposures. PLoS ONE. 2012;7(2):e31901.

18. Nilsson EE, Anway MD, Stanfield J, et al. Transgenerational epigenetic effects of the endocrine disruptor vinclozolin on pregnancies and female adult onset disease. Reproduction. 2008;135:713–21.
19. Nilsson E, Larsen G, Manikkam M, et al. Environmentally induced epigenetic transgenerational inheritance of ovarian disease. PLoS ONE. 2012;7(5):e36129.
20. Amorim EMP, Damasceno DC, Perobelli JE, et al. Short- and long-term reproductive effects of prenatal and lactational growth restriction caused by maternal diabetes in male rats. Reprod Biol Endocrinol. 2011;9:154.
21. Guzman C, Cabrera R, Cardenas M, et al. Protein restriction during fetal and neonatal development in the rat alters reproductive function and accelerates reproductive ageing in female progeny. J Physiol. 2006;572:97–108.
22. Toledo FC, Perobelli JE, Pedrosa FPC, et al. In utero protein restriction causes growth delay and alters sperm parameters in adult male rats. Reprod Biol Endocrinol. 2011;9:94.
23. Padmanabhan V, Smith P, Veiga-Lopez A. Developmental programming: impact of prenatal testosterone treatment and postnatal obesity on ovarian follicular dynamics. J Dev Orig Health Dis. 2012;3:276–86.
24. Steckler T, Wang JR, Bartol FF, et al. Fetal programming: prenatal testosterone treatment causes intrauterine growth retardation, reduces ovarian reserve and increases ovarian follicular recruitment. Endocrinology. 2005;146:3185–93.
25. Birch RA, Padmanabhan V, Foster DL, et al. Prenatal programming of reproductive neuroendocrine function: fetal androgen exposure produces progressive disruption of reproductive cycles in sheep. Endocrinology. 2003;144:1426–34.
26. Forsdike RA, Hardy K, Bull L, et al. Disordered follicle development in ovaries of prenatally androgenized ewes. J Endocrinol. 2007;192:421–8.
27. Veiga-Lopez A, Steckler TL, Abbott DH, et al. Developmental programming: impact of excess prenatal testosterone on intrauterine fetal endocrine milieu and growth in sheep. Biol Reprod. 2011;84:87–96.
28. Abbott DH, Tarantal AF, Dumesic DA. Fetal, infant, adolescent and adult phenotypes of polycystic ovary syndrome in prenatally androgenized female rhesus monkeys. Am J Primatol. 2009;71:776–84.
29. Padmanabhan V, Veiga-Lopez A. Developmental origin of reproductive and metabolic dysfunctions: androgenic versus estrogenic reprogramming. Semin Reprod Med. 2011;29:173–86.
30. Luense LJ, Veiga-Lopez A, Padmanabhan V, et al. Developmental programming: gestational testosterone treatment alters fetal ovarian gene expression. Endocrinology. 2011;152:4974–83.
31. Abbott DH, Padmanabhan V, Dumesic DA. Contributions of androgen and estrogen to fetal programming of ovarian dysfunction. Reprod Biol Endocrinol. 2006;4:17.
32. Veiga-Lopez A, Ye W, Padmanabhan V. Developmental programming: prenatal testosterone excess disrupts anti-Mullerian hormone expression in preantral and antral follicles. Fertil Steril. 2012;97:748–56.
33. Franks S. Animal models and the developmental origins of polycystic ovary syndrome: increasing evidence for the role of androgens in programming reproductive and metabolic dysfunction. Endocrinology. 2012;153:2536–8.
34. Tyndall V, Broyde M, Sharpe R, et al. Effect of androgen treatment during foetal and/or neonatal life on ovarian function in prepubertal and adult rats. Reproduction. 2012;143:21–33.
35. Xita N, Tsatsoulis A. Review: Fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. J Clin Endocrinol Metab. 2006;91:1660–6.
36. Baird DT, Cnattingius S, Collins J, et al. Nutrition and reproduction in women. Hum Reprod Update. 2006;12:193–207.
37. Gatti JM, Kirsch AJ, Troyer WA, et al. Increased incidence of hypospadias in small-for-gestational age infants in a neonatal intensive-care unit. BJU Int. 2001;87:548–50.
38. Cicognani A, Alessandroni R, Pasini A, et al. Low birth weight for gestational age and subsequent male gonadal function. J Pediatr. 2002;141:376–80.

39. Main KM, Jensen RB, Asklund C, et al. Low birth weight and male reproductive function. Horm Res. 2006;65:116–22.
40. Fujimoto T, Suwa T, Kabe K, et al. Placental insufficiency in early gestation is associated, with hypospadias. J Pediatr Surg. 2008;43:358–61.
41. Ibanez L, Valls C, Cols M, et al. Hypersecretion of FSH in infant boys and girls born small for gestational age. J Clin Endocrinol Metab. 2002;87:1986–8.
42. Sir-Petermann T, Hitchsfeld C, Codner E, et al. Gonadal function in low birth weight infants: a pilot study. J Pediatr Endocrinol Metab. 2007;20:405–14.
43. Ibanez L, Jaramillo A, Enriquez G, et al. Polycystic ovaries after precocious pubarche: relation to prenatal growth. Hum Reprod. 2007;22:395–400.
44. van Weissenbruch MM. Premature adrenarche, polycystic ovary syndrome and intrauterine growth retardation: does a relationship exist? Curr Opin Endocrinol Diabetes Obes. 2007;14:35–40.
45. Ibanez L, Potau N, de Zegher F. Ovarian hyporesponsiveness to follicle stimulating hormone in adolescent girls born small for gestational age. J Clin Endocrinol Metab. 2000;85:2624–6.
46. Ibanez L, Potau N, Enriquez G, et al. Reduced uterine and ovarian size in adolescent girls born small for gestational age. Pediatr Res. 2000;47:575–7.
47. Ibanez L, Potau N, Ferrer A, et al. Anovulation in eumenorrheic, nonobese adolescent girls born small for gestational age: insulin sensitization induces ovulation, increases lean body mass, and reduces abdominal fat excess, dyslipidemia, and subclinical hyperandrogenism. J Clin Endocrinol Metab. 2002;87:5702–5.
48. Ibanez L, Potau N, Ferrer A, et al. Reduced ovulation rate in adolescent girls born small for gestational age. J Clin Endocrinol Metab. 2002;87:3391–3.
49. Ibanez L, Potau N, Enriquez G, et al. Hypergonadotrophinaemia with reduced uterine and ovarian size in women born small-for-gestational-age. Hum Reprod. 2003;18:1565–9.
50. Adair LS. Size at birth predicts age at menarche. Pediatrics. 2001;107(4):E59.
51. Koziel S, Jankowska EA. Effect of low versus normal birthweight on menarche in 14-year-old Polish girls. J Paediatr Child Health. 2002;38:268–71.
52. Romundstad PR, Vatten LJ, Nilsen TIL, et al. Birth size in relation to age at menarche and adolescent body size: implications for breast cancer risk. Int J Cancer. 2003;105:400–3.
53. Ibanez L, Jimenez R, de Zegher F. Early puberty-menarche after precocious pubarche: relation to prenatal growth. Pediatrics. 2006;117:117–21.
54. Tam CS, de Zegher F, Garnett SP, et al. Opposing influences of prenatal and postnatal growth on the timing of menarche. J Clin Endocrinol Metab. 2006;91:4369–73.
55. Jasienska G, Thune I, Ellison PT. Fatness at birth predicts adult susceptibility to ovarian suppression: an empirical test of the Predictive Adaptive Response hypothesis. Proc Natl Acad Sci U S A. 2006;103:12759–62.
56. Hoover RN, Hyer M, Pfeiffer RM, et al. Adverse health outcomes in women exposed in utero to diethylstilbestrol. N Engl J Med. 2011;365:1304–14.
57. Green DM, Kawashima T, Stovall M, et al. Fertility of male survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. J Clin Oncol. 2010;28:332–9.
58. Wallace WHB. Oncofertility and preservation of reproductive capacity in children and young adults. Cancer. 2011;117:2301–10.
59. Green DM, Kawashima T, Stovall M, et al. Fertility of female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. J Clin Oncol. 2009;27:2677–85.
60. Green DM, Sklar CA, Boice JD, et al. Ovarian failure and reproductive outcomes after childhood cancer treatment: results from the Childhood Cancer Survivor Study. J Clin Oncol. 2009;27:2374–81.
61. Ginsberg JP. The effect of cancer therapy on fertility, the assessment of fertility and fertility preservation options for pediatric patients. Eur J Pediatr. 2011;170:703–8.
62. Blumenfeld Z. Chemotherapy and fertility. Best Pract Res Clin Obstet Gynaecol. 2012;26:379–90.
63. Fleischer RT, Vollenhoven BJ, Weston GC. The effects of chemotherapy and radiotherapy on fertility in premenopausal women. Obstet Gynecol Surv. 2011;66:248–54.

64. Meirow D, Nugent D. The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update. 2001;7:535–43.
65. Sklar CA, Mertens AC, Mitby P, et al. Premature menopause in survivors of childhood cancer: a report from the childhood cancer survivor study. J Natl Cancer Inst. 2006;98:890–6.
66. Thomas-Teinturier C, El Fayech C, Oberlin O, et al. Age at menopause and its influencing factors in a cohort of survivors of childhood cancer: earlier but rarely premature. Hum Reprod. 2013;28:488–95.
67. Gnaneswaran S, Deans R, Cohn RJ. Reproductive late effects in female survivors of childhood cancer. Obstet Gynecol Int. 2012;2012:564794.
68. Hamre H, Kiserud CE, Ruud E, et al. Gonadal function and parenthood 20 years after treatment for childhood lymphoma: a cross-sectional study. Pediatr Blood Cancer. 2012;59:271–7.
69. van Dorp W, van Beek RD, Laven JSE, et al. Long-term endocrine side effects of childhood Hodgkin's lymphoma treatment: a review. Hum Reprod Update. 2012;18:12–28.
70. Hudson MM. Reproductive outcomes for survivors of childhood cancer. Obstet Gynecol. 2010;116:1171–83.
71. Sudour H, Chastagner P, Claude L, et al. Fertility and pregnancy outcome after abdominal irradiation that included or excluded the pelvis in childhood tumor survivors. Int J Radiat Oncol Biol Phys. 2010;76:867–73.
72. Rendtorff R, Hohmann C, Reinmuth S, et al. Hormone and sperm analyses after chemo- and radiotherapy in childhood and adolescence. Klin Padiatr. 2010;222:145–9.
73. Kai CM, Juul A, McElreavey K, et al. Sons conceived by assisted reproduction techniques inherit deletions in the azoospermia factor (AZF) region of the Y chromosome and the DAZ gene copy number. Hum Reprod. 2008;23:1669–78.
74. Kai CM, Main KM, Andersen AN, et al. Reduced serum testosterone levels in infant boys conceived by intracytoplasmic sperm injection. J Clin Endocrinol Metab. 2007;92:2598–603.
75. Kobayashi H, Hiura H, John RM, et al. DNA methylation errors at imprinted loci after assisted conception originate in the parental sperm. Eur J Hum Genet. 2009;17:1582–91.
76. Feng C, Wang LQ, Dong MY, et al. Assisted reproductive technology may increase clinical mutation detection in male offspring. Fertil Steril. 2008;90:92–6.
77. Lumey LH, Stein AD, Ravelli AC. Timing of prenatal starvation in women and birth weight in their first and second born offspring: the Dutch Famine Birth Cohort study. Eur J Obstet Gynecol Reprod Biol. 1995;61:23–30.
78. Lumey LH, Stein AD, Ravelli ACJ. Timing of prenatal starvation in women and offspring birth weight: an update. Eur J Obstet Gynecol Reprod Biol. 1995;63:197.
79. Ding GL, Wang FF, Shu J, et al. Transgenerational glucose intolerance with Igf2/H19 epigenetic alterations in mouse islet induced by intrauterine hyperglycemia. Diabetes. 2012;61:1133–42.
80. Bygren LO, Edvinsson S, Brostrom G. Change in food availability during pregnancy: is it related to adult sudden death from cerebro- and cardiovascular disease in offspring? Am J Hum Biol. 2000;12:447–53.
81. Kanaka-Gantenbein C. Fetal origins of adult diabetes. Ann N Y Acad Sci. 2010;1205:99–105.
82. Dessi A, Ottonello G, Fanos V. Physiopathology of intrauterine growth retardation: from classic data to metabolomics. J Matern Fetal Neonatal Med. 2012;25:13–8.
83. Yuan QX, Chen L, Liu CP, et al. Postnatal pancreatic islet beta cell function and insulin sensitivity at different stages of lifetime in rats born with intrauterine growth retardation. PLoS ONE. 2011;6(10):e25167.
84. Chmurzynska A. Fetal programming: link between early nutrition, DNA methylation, and complex diseases. Nutr Rev. 2010;68:87–98.
85. Aagaard-Tillery KM, Grove K, Bishop J, et al. Developmental origins of disease and determinants of chromatin structure: maternal diet modifies the primate fetal epigenome. J Mol Endocrinol. 2008;41:91–102.
86. Waterland RA, Travisano M, Tahiliani KG, et al. Methyl donor supplementation prevents transgenerational amplification of obesity. Int J Obes. 2008;32:1373–9.
87. Pembrey ME, Bygren LO, Kaati G, et al. Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet. 2006;14:159–66.

88. Kaati G, Bygren LO, Pembrey M, et al. Transgenerational response to nutrition, early life circumstances and longevity. Eur J Hum Genet. 2007;15:784–90.
89. Drake AJ, Walker BR. The intergenerational effects of fetal programming: non-genomic mechanisms for the inheritance of low birth weight and cardiovascular risk. J Endocrinol. 2004;180:1–16.
90. Hall JG. Review and hypothesis: syndromes with severe intrauterine growth restriction and very short stature – are they related to the epigenetic mechanism(s) of fetal survival involved in the developmental origins of adult health and disease? Am J Med Genet A. 2010;152A:512–27.
91. French NP, Hagan R, Evans SF, et al. Repeated antenatal corticosteroids: effects on cerebral palsy and childhood behavior. Am J Obstet Gynecol. 2004;190:588–95.
92. Martinez-Frias ML. The thalidomide experience: review of its effects 50 years later. Med Clin. 2012;139:25–32.
93. Thompson JG, Mitchell M, Kind KL. Embryo culture and long-term consequences. Reprod Fertil Dev. 2007;19:43–52.
94. Sakka SD, Malamitsi-Puchner A, Loutradis D, et al. Euthyroid hyperthyrotropinemia in children born after in vitro fertilization. J Clin Endocrinol Metab. 2009;94:1338–41.
95. Sakka SD, Loutradis D, Kanaka-Gantenbein C, et al. Absence of insulin resistance and low-grade inflammation despite early metabolic syndrome manifestations in children born after in vitro fertilization. Fertil Steril. 2010;94:1693–9.
96. Ceelen M, van Weissenbruch MM, Roos JC, et al. Body composition in children and adolescents born after in vitro fertilization or spontaneous conception. J Clin Endocrinol Metab. 2007;92:3417–23.
97. Ceelen M, van Weissenbruch MM, Vermeiden JPW, et al. Cardiometabolic differences in children born after in vitro fertilization: follow-up study. J Clin Endocrinol Metab. 2008;93:1682–8.
98. Bukulmez O. Does assisted reproductive technology cause birth defects? Curr Opin Obstet Gynecol. 2009;21:260–4.
99. Farhi J, Fisch B. Risk of major congenital malformations associated with infertility and its treatment by extent of iatrogenic intervention. Pediatr Endocrinol Rev. 2007;4:352–7.
100. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012;366:1803–13.
101. Grace KS, Sinclair KD. Assisted reproductive technology, epigenetics, and long-term health: a developmental time bomb still ticking. Semin Reprod Med. 2009;27:409–16.
102. Hansen M, Bower C, Milne E, et al. Assisted reproductive technologies and the risk of birth defects – a systematic review. Hum Reprod. 2005;20:328–38.
103. Halliday JL, Ukoumunne OC, Baker HWG, et al. Increased risk of blastogenesis birth defects, arising in the first 4 weeks of pregnancy, after assisted reproductive technologies. Hum Reprod. 2010;25:59–65.
104. Schieve LA, Rasmussen SA, Reefhuis J. Risk of birth defects among children conceived with assisted reproductive technology: providing an epidemiologic context to the data. Fertil Steril. 2005;84:1320–4.
105. Kanaka-Gantenbein C, Sakka S, Chrousos GP. Assisted reproduction and its neuroendocrine impact on the offspring. Prog Brain Res. 2010;182:161–74.
106. Odom LN, Segars J. Imprinting disorders and assisted reproductive technology. Curr Opin Endocrinol Diabetes Obes. 2010;17:517–22.
107. Manipalviratn S, DeCherney A, Segars J. Imprinting disorders and assisted reproductive technology. Fertil Steril. 2009;91:305–15.
108. Bowdin S, Allen C, Kirby G, et al. A survey of assisted reproductive technology births and imprinting disorders. Hum Reprod. 2007;22:3237–40.
109. Gosden R, Trasler J, Lucifero D, et al. Rare congenital disorders, imprinted genes, and assisted reproductive technology. Lancet. 2003;361:1975–7.
110. Yoon G, Beischel LS, Johnson JP, et al. Dizygotic twin pregnancy conceived with assisted reproductive technology associated with chromosomal anomaly, imprinting disorder, and monochorionic placentation. J Pediatr. 2005;146:565–7.

111. Halliday J, Oke K, Breheny S, et al. Beckwith-Wiedemann syndrome and IVF: a case–control study. Am J Hum Genet. 2004;75:526–8.
112. Xn D, Wu Y, Liu F, et al. A hypothalamic-pituitary-adrenal axis-associated neuroendocrine metabolic programmed alteration in offspring rats of IUGR induced by prenatal caffeine ingestion. Toxicol Appl Pharmacol. 2012;264:395–403.
113. Rueda-Clausen CF, Morton JS, Lopaschuk GD, et al. Long-term effects of intrauterine growth restriction on cardiac metabolism and susceptibility to ischaemia/reperfusion. Cardiovasc Res. 2011;90:285–94.
114. Rueda-Clausen CF, Morton JS, Davidge ST. The early origins of cardiovascular health and disease: who, when, and how. Semin Reprod Med. 2011;29:197–210.
115. Rueda-Clausen CF, Morton JS, Oudit GY, et al. Effects of hypoxia-induced intrauterine growth restriction on cardiac siderosis and oxidative stress. J Dev Orig Health Dis. 2012;3:350–7.
116. Rueda-Clausen CF, Dolinsky VW, Morton JS, et al. Hypoxia-induced intrauterine growth restriction increases the susceptibility of rats to high-fat diet-induced metabolic syndrome. Diabetes. 2011;60:507–16.
117. Rueda-Clausen CF, Morton JS, Davidge ST. Effects of hypoxia-induced intrauterine growth restriction on cardiopulmonary structure and function during adulthood. Cardiovasc Res. 2009;81:713–22.
118. Wojtyla A, Kapka-Skrzypczak L, Diatczyk J, et al. Alcohol-related developmental origin of adult health – population studies in Poland among mothers and newborns (2010–2012). Ann Agric Environ Med. 2012;19:365–77.
119. Mestan KK, Steinhorn RH. Fetal origins of neonatal lung disease: understanding the pathogenesis of bronchopulmonary dysplasia. Am J Physiol Lung Cell Mol Physiol. 2011;301:L858–9.
120. Fauser B, Tarlatzis BC, Rebar RW, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 2012;97:28-U84.
121. Baird DT, Balen A, Escobar-Morreale HF, et al. Health and fertility in World Health Organization group 2 anovulatory women. Hum Reprod Update. 2012;18:586–99.
122. Group TEA-SPCW. Consensus on infertility treatment related to polycystic ovary syndrome. Fertil Steril. 2008;89:505–22.
123. Jin M, Yu YQ, Huang HF. An update on primary ovarian insufficiency. Sci China Life Sci. 2012;55:677–86.
124. Welt CK. Primary ovarian insufficiency: a more accurate term for premature ovarian failure. Clin Endocrinol. 2008;68:499–509.
125. Xu N, Kwon S, Abbott DH, et al. Epigenetic mechanism underlying the development of polycystic ovary syndrome (PCOS)-like phenotypes in prenatally androgenized rhesus monkeys. PLoS ONE. 2011;6(11):e27286.
126. Dumesic DA, Abbott DH, Padmanabhan V. Polycystic ovary syndrome and its developmental origins. Rev Endocr Metab Disord. 2007;8:127–41.
127. Li ZX, Huang HF. Epigenetic abnormality: a possible mechanism underlying the fetal origin of polycystic ovary syndrome. Med Hypotheses. 2008;70:638–42.
128. de Zegher F, Ibáñez L. Early origins of polycystic ovary syndrome: hypotheses may change without notice. J Clin Endocrinol Metab. 2009;94:3682–5.
129. Abbott DH, Dumesic DA, Franks S. Developmental origin of polycystic ovary syndrome – a hypothesis. J Endocrinol. 2002;174:1–5.
130. Jaddoe VWV. Developmental origins of type 2 diabetes and obesity. Acta Paediatr. 2009;98:29.
131. Barker DJP, Hales CN, Fall CHD, et al. Type 2 (non-insulin-dependent) diabetes-mellitus, hypertension and hyperlipemia (syndrome-x) – relation to reduced fetal growth. Diabetologia. 1993;36:62–7.
132. Alves MG, Martins AD, Rato L, et al. Molecular mechanisms beyond glucose transport in diabetes-related male infertility. Biochim Biophys Acta. 1832;2013:626–35.

133. Mallidis C, Agbaje I, McClure N, et al. The influence of diabetes mellitus on male reproductive function. A poorly investigated aspect of male infertility. Urologe. 2011;50:33–7.
134. Batcheller A, Cardozo E, Maguire M, et al. Are there subtle genome-wide epigenetic alterations in normal offspring conceived by assisted reproductive technologies? Fertil Steril. 2011;96:1306–11.
135. Serour GI, Re FCEAH. Ethical guidelines on iatrogenic and self-induced infertility. Int J Gynaecol Obstet. 2006;94:172–3.
136. Zhang D, Zhu YM, Gao HJ, et al. Overweight and obesity negatively affect the outcomes of ovarian stimulation and in vitro fertilisation: a cohort study of 2628 Chinese women. Gynecol Endocrinol. 2010;26:325–32.
137. Du Plessis SS, Cabler S, McAlister DA, et al. The effect of obesity on sperm disorders and male infertility. Nat Rev Urol. 2010;7:153–61.
138. deMola J. Obesity and its relationship to infertility in men and women. Obstet Gynecol Clin North Am. 2009;36:333+.
139. Lumey LH, Stein AD. In utero exposure to famine and subsequent fertility: the Dutch Famine Birth Cohort Study. Am J Public Health. 1997;87:1962–6.
140. Meas T, Deghmoun S, Levy-Marchal C, et al. Fertility is not altered in young adults born small for gestational age. Hum Reprod. 2010;25:2354–9.
141. Sadrzadeh-Broer S, Kuijper EAM, Van Weissenbruch MM, et al. Ovarian reserve in young women with low birth weight and normal puberty: a pilot case–control study. Gynecol Endocrinol. 2011;27:641–4.
142. Luo XQ, Mo Y, Ke ZY, et al. High-dose chemotherapy without stem cell transplantation for refractory childhood systemic lupus erythematosus. Chemotherapy. 2008;54:331–5.
143. Mok CC, Lau CS, Wong RWS. Risk factors for ovarian failure in patients with systemic lupus erythematosus receiving cyclophosphamide therapy. Arthritis Rheum. 1998;41:831–7.
144. Shalet SM, Tsatsoulis A, Whitehead E, et al. Vulnerability of the human Leydig-cell to radiation-damage is dependent upon age. J Endocrinol. 1989;120:161–5.
145. Meistrich ML, Finch M, Dacunha MF, et al. Damaging effects of 14 chemotherapeutic drugs on mouse testis cells. Cancer Res. 1982;42:122–31.
146. Kangasniemi M, Huhtaniemi I, Meistrich ML. Failure of spermatogenesis to recover despite the presence of a spermatogonia in the irradiated LBNF(1), rat. Biol Reprod. 1996;54:1200–8.
147. Tyrkus MY, Makukh GV, Zastavna DV, et al. Microdeletions in the Y chromosome as a predictive marker of infertility in males. Cytol Genet. 2008;42:111–15.
148. Houshdaran S, Cortessis VK, Siegmund K, et al. Widespread epigenetic abnormalities suggest a broad DNA methylation erasure defect in abnormal human sperm. PLoS ONE. 2007;2:e1289.
149. Marques CJ, Francisco T, Sousa S, et al. Methylation defects of imprinted genes in human testicular spermatozoa. Fertil Steril. 2010;94:585–94.
150. Sato A, Otsu E, Negishi H, et al. Aberrant DNA methylation of imprinted loci in superovulated oocytes. Hum Reprod. 2007;22:26–35.
151. Borghol N, Lornage J, Blachere T, et al. Epigenetic status of the H19 locus in human oocytes following in vitro maturation. Genomics. 2006;87:417–26.
152. Jones GM, Cram DS, Song B, et al. Gene expression profiling of human oocytes following in vivo or in vitro maturation. Hum Reprod. 2008;23:1138–44.
153. Katari S, Turan N, Bibikova M, et al. DNA methylation and gene expression differences in children conceived in vitro or in vivo. Hum Mol Genet. 2009;18:3769–78.
154. Turan N, Katari S, Gerson LF, et al. Inter- and intra-individual variation in allele-specific DNA methylation and gene expression in children conceived using assisted reproductive technology. PLoS Genet. 2010;6(7):e1001033.
155. Zechner U, Pliushch G, Schneider E, et al. Quantitative methylation analysis of developmentally important genes in human pregnancy losses after ART and spontaneous conception. Mol Hum Reprod. 2010;16:704–13.
156. Gomes MV, Huber J, Ferriani RA, et al. Abnormal methylation at the KvDMR1 imprinting control region in clinically normal children conceived by assisted reproductive technologies. Mol Hum Reprod. 2009;15:471–7.

157. Thompson JG, Kind KL, Roberts CT, et al. Epigenetic risks related to assisted reproductive technologies – short- and long-term consequences for the health of children conceived through assisted reproduction technology: more reason for caution? Hum Reprod. 2002;17:2783–6.
158. Wang N, Wang LY, Le F, et al. Altered expression of Armet and Mrlp51 in the oocyte, preimplantation embryo, and brain of mice following oocyte in vitro maturation but postnatal brain development and cognitive function are normal. Reproduction. 2011;142:401–8.
159. Wang N, Le F, Liu XZ, et al. Altered expressions and DNA methylation of imprinted genes in chromosome 7 in brain of mouse offspring conceived from in vitro maturation. Reprod Toxicol. 2012;34:420–8.
160. Li L, Wang L, Xu XR, et al. Genome-wide DNA methylation patterns in IVF-conceived mice and their progeny: a putative model for ART-conceived humans. Reprod Toxicol. 2011;32:98–105.
161. Demars J, Le Bouc Y, El-Osta A, et al. Epigenetic and genetic mechanisms of abnormal 11p15 genomic imprinting in Silver-Russell and Beckwith-Wiedemann Syndromes. Curr Med Chem. 2011;18:1740–50.
162. Fauque P, Jouannet P, Lesaffre C, et al. Assisted reproductive technology affects developmental kinetics, H19 imprinting control region methylation and H19 gene expression in individual mouse. BMC Dev Biol. 2007;7:116.
163. Fortier AL, Lopes FL, Darricarrere N, et al. Superovulation alters the expression of imprinted genes in the midgestation mouse placenta. Hum Mol Genet. 2008;17:1653–65.
164. Rivera RM, Stein P, Weaver JR, et al. Manipulations of mouse embryos prior to implantation result in aberrant expression of imprinted genes on day 9.5 of development. Hum Mol Genet. 2008;17:1–14.
165. Doherty AS, Mann MRW, Tremblay KD, et al. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. Biol Reprod. 2000;62:1526–35.
166. Wang ZY, Xu L, He FF. Embryo vitrification affects the methylation of the H19/Igf2 differentially methylated domain and the expression of H19 and Igf2. Fertil Steril. 2010;93:2729–33.
167. Li T, Vu TH, Ulaner GA, et al. IVF results in de novo DNA methylation and histone methylation at an Igf2-H19 imprinting epigenetic switch. Mol Hum Reprod. 2005;11:631–40.
168. Zaitseva I, Zaitsev S, Alenina N, et al. Dynamics of DNA-demethylation in early mouse and rat embryos developed in vivo and in vitro. Mol Reprod Dev. 2007;74:1255–61.
169. Khosla S, Dean W, Brown D, et al. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. Biol Reprod. 2001;64:918–26.
170. Mann MRW, Lee SS, Doherty AS, et al. Selective loss of imprinting in the placenta following preimplantation development in culture. Development. 2004;131:3727–35.
171. Lewis A, Mitsuya K, Umlauf D, et al. Imprinting on distal chromosome 7 in the placenta involves repressive histone methylation independent of DNA methylation. Nat Genet. 2004;36:1291–5.
172. Stouder C, Deutsch S, Paoloni-Giacobino A. Superovulation in mice alters the methylation pattern of imprinted genes in the sperm of the offspring. Reprod Toxicol. 2009;28:536–41.
173. Mahsoudi B, Li A, O'Neill C. Assessment of the long-term and transgenerational consequences of perturbing preimplantation embryo development in mice. Biol Reprod. 2007;77:889–96.
174. Zhang Y, Zhang YL, Feng C, et al. Comparative proteomic analysis of human placenta derived from assisted reproductive technology. Proteomics. 2008;8:4344–56.
175. Kuentz P, Bailly A, Faure AC, et al. Child with Beckwith-Wiedemann syndrome born after assisted reproductive techniques to an human immunodeficiency virus serodiscordant couple. Fertil Steril. 2011;96:E35–8.
176. Shi X, Chen S, Zheng H, et al. Aberrant DNA methylation of imprinted loci in human in vitro matured oocytes after long agonist stimulation. Eur J Obstet Gynecol Reprod Biol. 2012;167(1):64–8.
177. Katagiri Y, Aoki C, Tamaki-Ishihara Y et al. Effects of assisted reproduction technology on placental imprinted gene expression. Obstet Gynecol Int 2010;2010. pii:437528
178. Kallen B, Finnstrom O, Nygren KG, et al. In vitro fertilization (IVF) in Sweden: risk for congenital malformations after different IVF methods. Birth Defects Res A Clin Mol Teratol. 2005;73:162–9.

179. Dumoulin JC, Land JA, Van Montfoort AP, et al. Effect of in vitro culture of human embryos on birthweight of newborns. Hum Reprod. 2010;25:605–12.
180. Kaneda M, Okano M, Hata K, et al. Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. Nature. 2004;429:900–3.
181. McLachlan RI, O'Bryan MK. State of the art for genetic testing of infertile men. J Clin Endocrinol Metab. 2010;95:1013–24.
182. Cortessis VK, Thomas DC, Levine AJ, et al. Environmental epigenetics: prospects for studying epigenetic mediation of exposure-response relationships. Hum Genet. 2012;131:1565–89.
183. Skinner MK. Environmental epigenetic transgenerational inheritance and somatic epigenetic mitotic stability. Epigenetics. 2011;6:838–42.
184. Skinner MK, Manikkam M, Guerrero-Bosagna C. Epigenetic transgenerational actions of environmental factors in disease etiology. Trends Endocrinol Metab. 2010;21:214–22.
185. Skinner MK. Role of epigenetics in developmental biology and transgenerational inheritance. Birth Defects Res C Embryo Today. 2011;93:51–5.
186. Skinner MK. Environmental epigenomics and disease susceptibility. EMBO Rep. 2011;12: 620–2.
187. Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet. 2007;8:253–62.
188. Skinner MK. What is an epigenetic transgenerational phenotype? F3 or F2. Reprod Toxicol. 2008;25:2–6.
189. Hussain N. Epigenetic influences that modulate infant growth, development, and disease. Antioxid Redox Signal. 2012;17:224–36.
190. Chason RJ, Csokmay J, Segars JH, et al. Environmental and epigenetic effects upon preimplantation embryo metabolism and development. Trends Endocrinol Metab. 2011;22: 412–20.
191. Godmann M, Lambrot R, Kimmins S. The dynamic epigenetic program in male germ cells: its role in spermatogenesis, testis cancer, and its response to the environment. Microsc Res Tech. 2009;72:603–19.
192. Wolff GL, Kodell RL, Moore SR, et al. Maternal epigenetics and methyl supplements affect agouti gene expression in A(vy)/a mice. FASEB J. 1998;12:949–57.
193. Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr. 2002;132:2393S–400.
194. Zeisel SH. Epigenetic mechanisms for nutrition determinants of later health outcomes. Am J Clin Nutr. 2009;89:S1488–93.
195. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A. 1997;94:3290–5.
196. Friso S, Choi SW, Girelli D, et al. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. Proc Natl Acad Sci U S A. 2002;99:5606–11.
197. Waterland RA, Lin JR, Smith CA, et al. Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (Igf2) locus. Hum Mol Genet. 2006;15:705–16.
198. Bilinski P, Wojtyla A, Kapka-Skrzypczak L, et al. Epigenetic regulation in drug addiction. Ann Agric Environ Med. 2012;19:491–6.
199. Csoka AB, Szyf M. Epigenetic side-effects of common pharmaceuticals: a potential new field in medicine and pharmacology. Med Hypotheses. 2009;73:770–80.
200. Chuai YH, Xu XB, Wang AM. Preservation of fertility in females treated for cancer. Int J Biol Sci. 2012;8:1005–12.
201. Wallace WHB, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol. 2005;6:209–18.
202. Leung W, Hudson MM, Strickland DK, et al. Late effects of treatment in survivors of childhood acute myeloid leukemia. J Clin Oncol. 2000;18:3273–9.
203. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. Science. 2001;293:1089–93.

204. Rinaudo PF, Giritharan G, Talbi S, et al. Effects of oxygen tension on gene expression in preimplantation mouse embryos. Fertil Steril. 2006;86:1252–65.
205. Dunn GA, Morgan CP, Bale TL. Sex-specificity in transgenerational epigenetic programming. Horm Behav. 2011;59:290–5.
206. Barouki R, Gluckman PD, Grandjean P, et al. Developmental origins of non-communicable disease: implications for research and public health. Environ Health. 2012;11:42.
207. Metzger ML, Meacham LR, Patterson B, et al. Female reproductive health after childhood, adolescent, and young adult cancers: guidelines for the assessment and management of female reproductive complications. J Clin Oncol. 2013;31(9):1239–47.
208. Hancke K, Isachenko V, Isachenko E, et al. Prevention of ovarian damage and infertility in young female cancer patients awaiting chemotherapy-clinical approach and unsolved issues. Support Care Cancer. 2011;19:1909–19.
209. Green DM, Whitton JA, Stovall M, et al. Pregnancy outcome of partners of male survivors of childhood cancer: a report from the childhood cancer survivor study. J Clin Oncol. 2003;21:716–21.
210. Green DN, Whitton JA, Stovall M, et al. Pregnancy outcome of female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. Am J Obstet Gynecol. 2002;187: 1070–80.
211. van Dorp W, van den Heuvel-Eibrink MM, Stolk L, et al. Genetic variation may modify ovarian reserve in female childhood cancer survivors. Hum Reprod. 2013;28(4):1069–76.
212. Knopman JM, Papadopoulos EB, Grifo JA, et al. Surviving childhood and reproductive-age malignancy: effects on fertility and future parenthood. Lancet Oncol. 2010;11:490–8.
213. Wallace WHB, Anderson RA, Irvine DA. Fertility preservation for young patients with cancer: who is at risk and what can be offered? (vol 6, pg 209, 2005). Lancet Oncol. 2005;6:922.
214. Maclaran K, Panay N. Premature ovarian failure. J Fam Plann Reprod Health Care. 2011;37: 35–42.
215. Oktem O, Urman B. Options of fertility preservation in female cancer patients. Obstet Gynecol Surv. 2010;65:531–42.
216. Ben-Aharon I, Gafter-Gvili A, Leibovici L, et al. Pharmacological interventions for fertility preservation during chemotherapy: a systematic review and meta-analysis. Breast Cancer Res Treat. 2010;122:803–11.
217. Blumenfeld Z, von Wolff M. GnRH-analogues and oral contraceptives for fertility preservation in women during chemotherapy. Hum Reprod Update. 2008;14:543–52.
218. Jadoul P, Dolmans MM, Donnez J. Fertility preservation in girls during childhood: is it feasible, efficient and safe and to whom should it be proposed? Hum Reprod Update. 2010;16:617–30.
219. Smitz J, Dolmans MM, Donnez J, et al. Current achievements and future research directions in ovarian tissue culture, in vitro follicle development and transplantation: implications for fertility preservation. Hum Reprod Update. 2010;16:395–414.
220. Wyns C, Curaba M, Vanabelle B, et al. Options for fertility preservation in prepubertal boys. Hum Reprod Update. 2010;16:312–28.
221. Ding GL, Chen XJ, Luo Q, et al. Attenuated oocyte fertilization and embryo development associated with altered growth factor/signal transduction induced by endometriotic peritoneal fluid. Fertil Steril. 2010;93:2538–44.
222. Macer ML, Taylor HS. Endometriosis and infertility: a review of the pathogenesis and treatment of endometriosis-associated infertility. Obstet Gynecol Clin North Am. 2012;39:535+.
223. Hanson M, Godfrey KM, Lillycrop KA, et al. Developmental plasticity and developmental origins of non-communicable disease: theoretical considerations and epigenetic mechanisms. Prog Biophys Mol Biol. 2011;106:272–80.
224. Dominguez-Salas P, Cox SE, Prentice AM, et al. Maternal nutritional status, C-1 metabolism and offspring DNA methylation: a review of current evidence in human subjects. Proc Nutr Soc. 2012;71:154–65.
225. Papadopoulou E, Stratakis N, Roumeliotaki T, et al. The effect of high doses of folic acid and iron supplementation in early-to-mid pregnancy on prematurity and fetal growth retardation: the mother-child cohort study in Crete, Greece (Rhea study). Eur J Nutr. 2013;52:327–36.

226. Chatzi L, Papadopoulou E, Koutra K, et al. Effect of high doses of folic acid supplementation in early pregnancy on child neurodevelopment at 18 months of age: the mother-child cohort 'Rhea' study in Crete. Greece Public Health Nutr. 2012;15:1728–36.
227. Matok I, Gorodischer R, Koren G, et al. Exposure to folic acid antagonists during the first trimester of pregnancy and the risk of major malformations. Br J Clin Pharmacol. 2009;68: 956–62.
228. Yazdy MM, Honein MA, Xing J. Reduction in orofacial clefts following folic acid fortification of the US grain supply. Birth Defects Res A Clin Mol Teratol. 2007;79:16–23.
229. Julvez J, Fortuny J, Mendez M, et al. Maternal use of folic acid supplements during pregnancy and four-year-old neurodevelopment in a population-based birth cohort. Paediatr Perinat Epidemiol. 2009;23:199–206.
230. Schlotz W, Jones A, Phillips DIW, et al. Lower maternal folate status in early pregnancy is associated with childhood hyperactivity and peer problems in offspring. J Child Psychol Psychiatry. 2010;51:594–602.
231. Laanpere M, Altmae S, Stavreus-Evers A, et al. Folate-mediated one-carbon metabolism and its effect on female fertility and pregnancy viability. Nutr Rev. 2010;68:99–113.
232. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci U S A. 2007;104: 13056–61.
233. Cropley JE, Suter CM, Beckman KB, et al. Germ-line epigenetic modification of the murine A(vy) allele by nutritional supplementation. Proc Natl Acad Sci U S A. 2006;103: 17308–12.
234. Martin J, Cervero A, Mir P, et al. The impact of next-generation sequencing technology on preimplantation genetic diagnosis and screening. Fertil Steril. 2013;99:1054-U1225.
235. Harper JC, Wilton L, Traeger-Synodinos J, et al. The ESHRE PGD Consortium: 10 years of data collection. Hum Reprod Update. 2012;18:234–47.
236. Nargund G, Chian RC. ISMAAR: Leading the global agenda for a more physiological, patient-centred, accessible and safer approaches in ART. J Assist Reprod Genet. 2013;30: 155–6.
237. Klip H, Verloop J, van Gool JD, et al. Hypospadias in sons of women exposed to diethylstilbestrol in utero: a cohort study. Lancet. 2002;359:1102–7.
238. Hernandez-Diaz S. Iatrogenic legacy from diethylstilbestrol exposure. Lancet. 2002;359: 1081–2.
239. Wojtyla A. Application of the hypothesis of Developmental Origin of Health and Diseases (DOHaD) in epidemiological studies of women at reproductive age and pregnant women in Poland. Ann Agric Environ Med. 2011;18:355–64.
240. Lillycrop KA, Burdge GC. The effect of nutrition during early life on the epigenetic regulation of transcription and implications for human diseases. J Nutrigenet Nutrigenomics. 2011;4:248–60.
241. Simpson JL. Cell-free fetal DNA and maternal serum analytes for monitoring embryonic and fetal status. Fertil Steril. 2013;99:1124–34.
242. Scala I, Parenti G, Andria G. Universal screening for inherited metabolic diseases in the neonate (and the fetus). J Matern Fetal Neonatal Med. 2012;25:4–6.

Assisted Reproductive Technology and Gamete/Embryo-Fetal Origins of Diseases

10

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Abstract

Suboptimal intrauterine conditions may produce small for gestational age (SGA), and low birth weight (LBW) babies, that are predisposed to develop cardiovascular and metabolic disease in later life [1]. During assisted reproductive technology (ART) treatment, gametes and zygotes are exposed to a series of non-physiological processes and culture media with increasing evidence that offspring of ART have increasing chances of being low birth weight. An increasing incidence of early-onset hypertension has also been reported in the population of ART offspring. It is therefore important to understand whether ART plays any specific role in disadvantaging a fetus, and, whether any such disadvantage carries long-term consequences.

Methylation of imprinted genes is erased and reestablished during gametogenesis, and, maintained throughout pre- and post-implantation development. Sequence-specific DNA hypomethylation frequently occurs in human sperm in compromised spermatogenesis. Transmission of sperm and oocyte DNA methylation defects occurs, though may be prevented by selection of gametes for ART, or, non-viability of the resulting embryos. In vitro fertilization (IVF), oocyte in vitro maturation (IVM), intracytoplasmic sperm injection (ICSI) and preimplantation genetic diagnosis (PGD) manipulate gametes and embryo at a time that is important for methylation reprogramming, and, may influence epigenetic stability leading to increased risks of adult diseases. Aspects of subfertility may also pose a risk factor for imprinting diseases. In this chapter, we will discuss the evidence related to assisted reproductive technology and embryo-fetal origins of diseases.

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10.1 In Vitro Fertilization

Worldwide, it is estimated that more than five million babies have been born after ART. Children conceived by ART comprise 1–4 % of the newborn population [2]. Although the majority of children born after ART are healthy, some concerns remain regarding the safety of this technology. Multiple pregnancies occur more commonly in ART pregnancies, and, their high frequency increases the risk of low birthweight and preterm delivery [3–5]. Several studies indicate that ART may disrupt epigenetic programming [1]. Animal studies and long-term follow-up studies of ART children suggest there may be an increased incidence of genetic, physical, or developmental abnormalities [4, 5], although there are also observations that contradict these findings [6].

10.1.1 Perinatal Outcome of Assisted Reproduction

Multiple births continue to be the major risk for couples embarking on fertility treatment. Their frequency in different countries ranges from 25 % to nearly 50 %, with the rate of high order pregnancies as high as 40 % (compared with only 3 % of infants among the general birth population) [7]. Transferring two embryos is associated with a threefold increase in the birth rate, and, a 16-fold increase in the twin birth rate [8]. Neonatal complications include low birth weight, preterm delivery, placental dysfunction and congenital malformations [9]. In the general population, the risk of preterm delivery is fivefold in twins compared to singletons, and 50 % of twins are born at low birth weights (<2,500 g) compared to 6 % of singletons [10]. The perinatal mortality is 5–6 times higher among twins compared to singletons [9].

In recent years there has been a developing trend to limit the number of embryos transferred into the uterine cavity per cycle of IVF/ICSI [10]. Single embryo transfer developed remarkably, with the proportion increasing from 10.5 to 12.4 %, and now it is highest in Finland (38.5 %), Sweden (30.5 %) and Australia (25.0 %). Since 2000 the proportions of twin and triplet pregnancies decreased from 26.5 to 25.7 % and from 2.9 to 2.5 % respectively [11]. However, reducing multiple pregnancies will not totally eliminate the increased risk of adverse perinatal outcomes in ART pregnancies. Singleton, ART conceptions appear to be at greater risk than unassisted, singleton conceptions for a number of adverse outcomes. Meta-analyses show that singleton children born after ART (IVF, ICSI, or gamete intra fallopian transfer) have a twofold risk of being born preterm, are at increased risk of having a low or very low birthweight (OR 1.70–1.77 and 2.70–3.00, respectively), and, are most usually born small for gestational age (OR 1.40–1.60) [7]. In addition, risks of preeclampsia, placental abruption, and placenta praevia also appear to increase after ART techniques [12, 13]. Despite a number of evidences indicate increased risks of perinatal complications following ART, its causes are still of no consensus. It is not clear whether ART is culpable, or, whether the adverse outcomes are attributable to factors related to the infertile couple.

The biology of subfertile couples may pose health risks to ART children. Singleton pregnancies in infertile couples after ovarian stimulation with, or without, artificial insemination, have a higher risk of low birth weight when compared with naturally conceived offspring [14]. However, after controlling for the origin of the semen, any differences between IUI and natural conception disappeared [15]. The time to-pregnancy interval has a direct link with the risk of preterm birth, and low birthweight. If the time-to-pregnancy interval was longer than 12 months but no fertility treatment had been used, the risk of low birthweight and preterm delivery increased [7]. Romundstad et al. compared the outcomes of two, consecutive singleton pregnancies, including one child conceived through ART and the other conceived spontaneously. In the sibling-relationship comparisons, there are no significant differences in birthweight, risks of small for gestational age babies, gestational age, and preterm delivery among infants conceived both spontaneously and after ART [16]. These results suggest that adverse outcomes after ART compared to those in the general population, may be attributable to the factors leading to infertility, rather than to factors related to the reproductive technology.

However, studies among subfertile women also show that adverse perinatal risks after IVF cannot be explained completely by subfertility; preterm delivery and lower mean birthweight were found among children born from subfertile women conceived after IVF as compared with children born from subfertile women after spontaneous conception [5]. Imprinted genes are major contributors to fetal growth, maternally expressed imprinted genes act as growth suppressors, whereas paternally expressed imprinted genes are growth promoters [4]. Lower birth weight may reflect increased suppression of paternally expressed (maternally imprinted) genes or conversely, overexpression of maternally expressed genes. According to the Barker hypothesis, babies born preterm and low birthweight after ART may be at risk of cardiovascular disease and diabetes in later life [5]. Given the increased risk of low birthweight in ART babies, specific investigation of the long-term consequences of IVF will be necessary.

10.1.2 Neurological Sequelae

Large, registry-based cohort studies report increased risks of cerebral palsy (CP, OR 1.6–3.7) in IVF children compared to naturally conceived children. The increased risk is largely due to the higher frequency of multiple pregnancies, low birth weight, and preterm delivery in IVF children [17]. The incidence of CP increases in multiple births: twins had risks approximately five times higher and triplets 17 times higher than singletons [9]. Many studies have investigated the neurodevelopmental wellbeing of singleton children born after ART. The reports indicate consistently that there are no neurodevelopmental differences between term singleton children conceived by ART and their naturally-conceived peers. A large European study in five countries assessed neurological outcomes of singleton children age 5 years, born after ICSI, IVF alone, or natural conception, with about 500 infants in each group; the results were reassuring [7]. However, Lidegaard et al., compared 442,349 singleton infants

conceived spontaneously with 6,052 children born after IVF and noted an 80 % increased risk of cerebral palsy in the IVF group [18]. A population-based retrospective cohort study in Sweden showed that children born after IVF singletons had increased risks of neurological sequelae risk (OR 2.8) [19].

The surviving co-twin may account for the increased risk of cerebral palsy in singleton births after IVF – singletons born from IVF originating from twin pregnancies [14]. IVF/ICSI is related to an increased risk of cerebral palsy through the association with preterm birth and plurality [20]. Relatively little evidence is available concerning the outcome of these children when they are older, thus larger, long-term, follow-up studies will be necessary to assess the impact of ART on children through puberty into adult life.

10.1.3 Cancer

Several case control studies suggested a possibly increased incidence of embryonal tumors in children who were prenatally exposed to sex hormones. In 1987, Kramer et al. reported that the risk of neuroblastoma increased following exposure to fertility drugs [21]. Michalek et al. noted that children with neuroblastoma were ten times more likely to have mothers with infertility treatment [22]. In addition, a report from the Netherlands identified a fivefold increased risk for retinoblastoma among children born after IVF [23]. However, a number of published cohort studies do not show increased risk of childhood cancer in ART children when compared with the general population. In an Australian cohort study, no significantly-increased risk of cancer occurred in an IVF group (N = 5,249) as compared to the general population [24]. Klip et al. found no increase in childhood cancer after 6 years when an IVF group was compared to the general population, and, spontaneously conceived children born from subfertile parents [22]. In Denmark, Brinton et al. found no significant cancer risk in the children born after ovulation-stimulation after 10.1 years follow-up [14].

Although these cohort studies appear reassuring, there is a continuing need to monitor IVF children to establish the true prevalence of post-IVF childhood cancer, preferably in registry-based studies.

10.1.4 Congenital Malformations

Between 3 and 5 % of all infants are diagnosed with a congenital anomaly soon after birth [25]. A number of meta-analyses indicate a 30 % increased risk of major malformations after IVF or ICSI compared with spontaneous conception, and, there is no difference between the two techniques [2, 7, 10, 26]. The major defects included urogenital cardiovascular, musculoskeletal, and chromosomal anomalies. The increased risk of major malformations may, at least partly, be explained by the higher proportion of multiple births in the IVF group [9]. Mothers of ART children were older and less parous than control mothers [27]. Increasing evidence suggests that parental subfertility may be an important factor, as increasing ‘time to pregnancy’

was associated with a greater risk of congenital abnormalities [2, 25]. After adjustment for parental factors, there was no significant association between increased risk of birth defects and IVF. However, the risk of birth defects associated with ICSI remains increased after multivariate adjustment [28].

10.1.5 Epigenetic Mechanisms in ART

Genomic imprinting occurs during gametogenesis and embryogenesis, and, it is possible that techniques used in ART could cause aberrant genomic imprinting and thus imprinting disorders [29, 30]. Since 2002, there is growing evidence that ART procedures may perturb the important epigenetic processes during the preimplantation period and lead to imprinting syndromes [5]. The most extensively studied imprinting disorders related to ART are Beckwith-Weidemann syndrome (BWS). BWS is a congenital overgrowth syndrome characterized by macroglossia, visceromegaly, macrosomia, umbilical and abdominal wall abnormalities including exomphalos, neonatal hypoglycemia and an increased risk of developing embryonal tumors in childhood [31]. BWS has an estimated incidence of 1 in 13,700 live births in the general population [32], a 6–9 times increase among ART offspring as compared with naturally conceived children [33]. While this would represent a dramatic increase in relative risk, the actual incidence of BWS after ART is 1 in 4,000–5,500 [33]. Methylation errors account for 50–60 % of sporadic cases of BWS, and almost 100 % of cases born after ART [31]. These findings are not confirmed by Lidegaard et al. [18], who assessed the incidence rate of imprinting diseases and related disorders in IVF singletons and spontaneously conceived children born in Denmark between1995 and 2001.

Another classic imprinting defect is Angelman's syndrome (AS). AS is a neurological disease affecting 1 in 15,000 live births that is associated with mental retardation, unusual gait, and seizures. AS is caused by loss of maternal UBE3A [22]. However, fewer than 5 % of AS cases are associated with an imprinting defect [29]. The numbers associated with ART are too low to characterize the effects of ART on AS. The first cases of ART-related Angelman syndrome were reported in 2002 and 2003 [19, 20]. Three patients conceived by intracytoplasmic sperm injection were diagnosed with AS, and, found to have aberrant loss of methylation on chromosome xy [2]. The reason for the increased risk of imprinting disorders in ART children is unknown.

Imprints of oocytes are established during the primordial to antral follicle transition and are not completed until just prior to ovulation [34]. For this reason, gonadotrophic hormones used during superovulation may affect imprint acquisition in oocytes. Ovarian stimulation is associated with an increased risk of aneuploidy in artificially matured oocytes [2] and may alter genomic imprinting of both maternally and paternally-expressed genes [35, 36]. Moreover, superovulation can transgenerationally affect the offspring sperm methylation pattern [37].

Preimplantation represents another developmental stage in which changes in DNA methylation take place. As early as 4 h after fertilization, marked demethylation occurs as the developing embryo initiates epigenetic changes, which will lead to

delineation of cell specificity [38]. Animal models indicate that media and other environmental components may cause epigenetic modifications. In-vitro media alterations during the preimplantation period may result in a particular overgrowth syndrome, known as large offspring syndrome (LOS). LOS has substantial phenotypical similarities with Beckwith-Wiedeman syndrome. LOS in sheep is associated with reduced expression of Igf2r through loss of methylation [38, 39]. In addition, alterations in the culture media of in vitro-conceived embryos may also alter methylation of both H19 and Igf2 [38].

10.2 Oocyte in Vitro Maturation (IVM)

10.2.1 Application of IVM

IVM is a technique where immature oocytes are collected from mid-sized, antral follicles, usually from unstimulated ovaries, and cultured, matured and fertilized in vitro to produce embryos [40]. IVM of oocytes was first demonstrated in animals by Pincus and Enzmann in 1934, and later, in humans by Edwards in 1969. The first birth after IVM of immature oocytes occurred from oocytes collected during gynaecological surgery for oocyte donation in 1991. The first reported IVM with patient's own oocytes was used as a treatment for women with polycystic ovaries or polycystic ovarian syndrome (PCOS) in 1994 [41]. Over the past 30 years there has been 1,300 IVM babies [42].

Advantages of IVM include relative ease of treatment, minimal use of fertility drugs, avoidance of ovarian hyperstimulation syndrome, and, low cost. However, various challenges hinder the selection of oocyte IVM as an established means of assisted reproduction. Although good results are reported in some clinics, IVM has not become a mainstream fertility treatment yet. The most important reasons are:

1. *Technical difficulties in retrieving immature oocytes from unstimulated ovaries and their cultivation.* Antral follicles between 2 and 12 mm are usually aspirated though studies show that follicles greater than 6 mm have a greater capacity for yielding immature oocytes that mature in vitro. In unstimulated ovaries, the follicles are small and often widespread throughout the ovarian stroma. Within large antral follicles, the cumulus oocyte complex (COC) is bathed in follicular fluid (FF) until the time of ovulation. FF itself is a serum transudate modified by the thecal and granulosa cell layers that make up the follicle [43]. FF contains undefined factors as well as known proteins, growth factors, steroids, and metabolites, many of which are present in blood plasma [43]. Not surprisingly, the addition of additives such as serum and FSH to maturation media usually results in improvements in maturation and embryo development [44–47]. However, the use of serum in culture systems may be undesirable because of the unknown nature of its contents and the potential for variability [43]. IVM of oocytes is limited by the currently used culture systems, including the doses and duration of hormones and other factors added to the culture media to initiate and coordinate the events of oocyte maturation.

2. *Lower chances of live birth per treatment compared with conventional in vitro fertilization.* In human the maturation rate of IVM is 30–50 %, which is much lower than other species, and, the pregnancies resulting from IVM are limited [48], and, in vitro matured human oocytes have reduced developmental competence. Recent reports document better fertility outcomes after IVM, with live birth rates of 30 % in donor oocyte programmes and clinical pregnancy rates of 21.9 % in patients with PCOS, for whom IVM may be a pivotal treatment strategy [49, 50].
3. *The quality of in-vitro-matured oocytes is suboptimal since immature human oocytes display increased meiotic spindle and chromosome abnormalities.* This observation may account for lower developmental competence of oocytes matured in vitro compared to those matured in vivo. Whether these abnormalities are due to damage induced during the procedure or whether attributable to factors intrinsic to the ovaries of PCOS patients are not known. Alterations in an oocyte's internal structure, in particular spindles and chromosomes, are important in the ability to be fertilized, develop into a normal embryo, and ultimately produce a healthy live birth. Spindles controlled the movement of chromosomes within the oocyte; specialized components of the oocyte infrastructure that are composed of microtubules. This internal infrastructure is a group of moving materials, scaffolding and structures within the oocyte. Li et al. [51] analyzed the appearance of spindles and chromosomes in IVM oocytes of PCOS patients by confocal microscopy and fluorescent immunocytologic staining and compared their results with in vivo matured oocytes of PCOS patients. It is reported that in IVM oocytes chromosome configurations and disorganized meiotic spindle microtubules were more likely to be abnormal. Another significant concern about clinical use of IVM is the potential for perturbations in subsequent fetal and neonatal development that may influence long-term health. Some concern has been expressed regarding the safety of the method, though, chromosomal abnormalities, obstetric and neonatal outcomes were similar for IVM and conventional ART populations [49, 51, 52]. Cha et al. reported obstetric outcomes after IVM in women with PCOS; they reported 38 pregnancies, of which three had congenital anomalies; one case of hydrops fetalis, one omphalocele, and one cleft palate, and, a spontaneous miscarriage rate of 36 % [49]. There are insufficient evidence is available for women with PCOS yet, so IVM can't be the preferred first line treatment for subfertile women. Little is known about long-term outcomes after IVM, as the few children derived from IVM oocytes are not yet of adult age.

10.2.2 Women Suitable for IVM

IVM is a therapeutic option for women with PCOS or PCO in whom there are significant risks of ovarian hyperstimulation syndrome (OHSS) [53]. Also, more women of reproductive age undergo treatment for malignant diseases that are potentially gonadotoxic, and, may wish to preserve their fertility before such treatment.

For survivors from pediatric cancer this is especially important. In vitro culture of primordial follicles contained in cryopreserved ovarian tissue represents another potential method for restoring female fertility [54].

10.2.3 Future Considerations

IVM, with its numerous benefits and pregnancy rates comparable to those in IVF in most centres, is a safe and effective treatment. Progress requires improved culture conditions to further increase implantation rates, and, predictors of embryo competence may help increase implantation rates. Detailed follow-up and assessment of children conceived through IVM will be necessary to establish the safety of this treatment.

10.3 Intracytoplasmic Sperm Injection (ICSI)

ICSI bypasses the normal fertilization process where a single sperm is injected directly into an egg. It involves the introduction of the entire sperm, including the acrosome and its digestive enzymes, directly into the oocyte. This injection of spermatozoa into the oocyte may potentially disrupt the oocyte's cytoskeleton [55]. Introduction of exogenous material is also possible, which may damage other intracellular structures [56]. Imprinting defects resulting from the ICSI technique may have transgenerational effects [57]. There are increasing concerns that manipulations during ICSI may lead to adult diseases in later life. There are many studies that weigh on both sides of these concerns.

Most of the studies giving reassuring data recommend more thorough investigations because the level of congenital defects is statically higher in babies born through ICSI, and, imprinting defects may be preponderant [58]. Evaluation of epigenetic marks and DNA methylation patterns in recent publications show no ill effects stemming from this method of fertilization [59]. Fulka et al. examined differences. in DNA methylation patterns in mouse zygotes produced either by natural fertilization, IVF, or ICSI by looking at global patterns of DNA methylation in paternal pronuclei after labeling with anti-5-methylcytosine (5-MeC) antibody, and, histone methylation patterns in both pronuclei after labeling with dimethylated H3/K9. No differences in global methylation patterns were found among in vivo, in vitro, and ICSI-produced zygotes in mice [59].

Santos et al. evaluated the potential deregulation of DNA methylation in ART-derived human embryos. They evaluated genome-wide DNA methylation together with chromatin organization in human embryos derived by either IVF or ICSI. DNA methylation was assessed using an antibody against 5-methyl-cytidine, and, chromatin organization by DNA staining. Irrespective of the ART procedure, They observed similar errors in both groups, and, there was positive correlation between abnormal chromatin and inappropriate DNA methylation. The ability to develop to the blastocyst stage accompanied with normal chromatin organization

and DNA methylation, reinforcing the pivotal role of epigenetic regulation forming the early lineages of blastocyst. They found blastocysts derived by ICSI and IVF were affected similarly [60].

Tierling et al. examined the effects of ART on the stability of DNA methylation imprints in phenotypically normal children (ICSI, IVF, and spontaneous conceptions) in ten differentially methylated regions (DMRs), including H19, KvDMR1, MEST, SNRPN, GRB10, GNAS NESP55, DLK1/MEG3 IG-DMR, GNAS XL-alpha-s, GNAS NESPas and GNAS Exon1A [61].

Children conceived through ART do not express a higher degree of imprint defect. Feng et al. investigated expression profiles of imprinted genes in IVF-conceived, ICSI-conceived and naturally-conceived children, by microarray and real-time RT-PCR [62]. Hierarchical clustering demonstrated no obvious clustering between the different groups, suggesting similar genomic imprinting expression between the groups. In the majority of the children conceived after ART and spontaneously, the differentially-expressed, imprinted genes remained allele-specific expression. Monoallelic expression of L3MBTL was disrupted in one ICSI case where all CpGs were completely unmethylated [62].

De Waal et al. demonstrated that ICSI can induce epimutations in somatic tissues of adult mice produced by this method. They compared the occurrence of epimutations in mice produced by natural conception, ICSI and somatic cell nuclear transfer. Surprisingly, they observed more epimutations in somatic tissues from ICSI-derived mice. They also observed a delay in reprogramming of the maternal allele of the imprinted H19 gene in spermatogonia from juvenile ICSI-derived male mice. These observations indicated that exposure to exogenous gonadotropins of maternal gametic genome during superovulation may disrupt the normal epigenetic programming of imprinted loci in somatic tissues and/or epigenetic reprogramming in the germ line of ensuing offspring. To prove this hypothesis, they uncoupled superovulation from ICSI through subjecting female mice to gonadotropin stimulation and then let them produce offspring by natural mating. It was found that mice conceived in this way also showed epimutations and/or epimutant phenotypes in somatic tissues and delayed epigenetic reprogramming in spermatogenic cells, providing evidence that gonadotropin stimulation contributes to the induction of epimutations during ART procedures rather than the ICSI manipulation [63].

Imprinting has also been proposed as the link between IVF/ICSI and a possible increased risk for retinoblastoma. No hypermethylation of the RB1 promoter was found in examination of retinoblastoma tumors in seven IVF or ICSI conceived children. This demonstrates that an association between IVF or ICSI and retinoblastoma through this epigenetic mechanism is unlikely [64].

However, plenty of evidence concerning the risks of ICSI indicate that children born following this procedure are at a higher absolute risk of congenital defects (in particular genitourinary defects) and epigenetic syndromes (such as Beckwith Wiedemann) [65]. Bowen et al. compared outcomes of ICSI-conceived, IVF-conceived, and naturally-conceived children at 12 months of age, and, observed a significantly lower mental development index (MDI) in the ICSI group [66]. Sutcliffe et al. compared 123 ICSI-conceived children with naturally-conceived

children at 12–24 months of age. The only difference was a lower eye–hand coordination in the ICSI group [67]. Bonduelle et al. comparing general health status of ICSI-conceived, IVF-conceived and naturally-conceived children at 5 years of age found that ICSI-conceived children presented with increased numbers of major congenital malformations, the most frequent being urogenital [68].

There are several, possible explanations of increased risks associated with ART procedures. Firstly, the use of ICSI suggests the possibility of imprinting defects in paternal sperm cells. ICSI and round spermatid injection (ROSI) may increase the risk of imprinting disorders and disrupt embryonic development through using immature spermatozoa improper imprints or global methylation [69]. Paternal genomic alterations can compromise fertilization rates and embryo viability that may result in increased rates of spontaneous miscarriage and birth defects [70]. In the ejaculate of infertile men the percentage of spermatozoa with damaged DNA increased compared to healthy fertile donors [71]. DNA fragmentation of morphologically normal sperm reduces embryo quality and probability of pregnancy in ICSI cycles [72]. Using spermatozoa with damaged DNA in ICSI suggests that genetic and epigenetic changes during pre-implantation may occur, leading to altered fetal development and, as a consequence, offspring with aberrant growth, behaviour, early aging and tumours [73]. Functional bovine sperm with damaged DNA can normally fertilize oocytes and no significant effects are observed during the first cleavages of the fertilized oocyte. However, embryo fragmentation, apoptosis and aberrant or no signs of mitotic spindle formation may also occur [74].

Sperm with both fragmented and unfragmented DNA can fertilize oocytes with equal efficiency [72]. However, the activation of the paternal genome on day 3 can show imperfections within the paternal genome that were not evident upon fertilization. More often than not, spermatozoa for ICSI originate in men with abnormal semen parameters. Sperm from these men may contain an increased number of chromosomal abnormalities, contributing to the overall risk of ICSI procedures [75, 76]. One such example stemming from recent investigation demonstrates that children conceived via ICSI procedures have significantly shorter fingers, possibly attributable to perturbations in the paternal genome [77].

Secondly, the injection of a spermatozoa into the oocyte can disrupt the oocyte's cytoskeleton and bring about epigenetic defects. The pattern of histone methylation differs between ART and ICSI in mouse [78] and even in human [79]. In rats, demethylation dynamics of the paternal genome at pronuclear-stage are impaired when ART is used [80]. Sperm chromatin remodeling after ICSI is more asynchronous than ART in mouse embryos [81].

Van der Heijden et al. stained monopronuclear zygotes (MPZ) derived from ICSI or IVF with H3K9me3 antibodies, which allows unambiguous identification of parental origin, and, found they originate through fusion of parental chromatin after sperm penetration. Monopronuclear zygotes derived from ICSI contain uni-parental chromatin, and, after both IVF and ICSI androgenic monopronuclear zygotes were found [78].

Aberrant methylation status lead to further embryonic abnormalities, eventually arrest in development, or embryo degenerate. Moreover, A high frequency of

aneuploidy and embryo death is induced by the inhibition of histone deacetylation during meiosis. Manipulative procedures, such as ICSI, may deteriorate the capability of the oocyte to conduct these epigenetic processes correctly, causing abnormal embryo development from nuclear–cytoplasmic interactions [82]. Qiao et al. collected tripronuclear and normally fertilized embryos acquired from patients undergoing ART. Poor morphological appearances of ICSI-derived embryos that were more likely to display H3K9 demethylation than their IVF counterparts [79].

Yoshizawa et al. studied the timing and extent of the active demethylation of paternal genomes among the pronuclear-stage rat zygotes produced in vitro, produced in vivo/cultured in vitro, and produced in vivo. They found that demethylation dynamics of the paternal genome in pronuclear-stage rat zygotes were impaired by routine protocols for in vitro embryo production such as IVF and ICSI [80].

Ajduk et al. compared remodeling of sperm chromatin, the potential of embryos to develop in vitro, synthesis of DNA in mouse embryos gained from IVF and ICSI, and, tested whether pretreatment of sperm prior to ICSI facilitates chromatin remodeling and deteriorates embryo development. The dynamics of sperm chromatin remodeling in ICSI and IVF embryos changed. In ICSI, chromatin remodeling was not as synchronous as in IVF. Sperm capacitation before injection enhanced remodeling asynchrony and delayed pronuclei formation and DNA synthesis [81].

Furthermore, the use of ICSI for achieving fertilization may introduce exogenous materials and create transgenic offspring. The term transgenic refers to any organism whose genome has been altered by the stable integration of a recombinant DNA sequence, regardless of the impact that this integration may have on phenotypic expression. Animal models have been used to introduce exogenous genetic material into the genome via a sperm carrier with the ICSI technique; this has resulted in the creation of transgenic mammals [83], as well as transgenic primates [84]. It is possible for a foreign gene, either DNA or RNA, from protein supplements in culture media to be introduced into the oocyte genome by binding to the sperm surface, or, by needle-carried medium during the ICSI procedure, resulting in the creation of transgenetic embryos. Although verification of transgenesis is lacking in human IVF, the possibility of this occurring inadvertently remains a viable concern, especially following confirmation of unwanted transgenesis in the mouse model [85]. Prostasomal DNA transfer into human sperm confirms this is indeed a cause for concern [86].

Anecdotal reports also suggest that animals conceived through ART that show apparent epigenetic defects do not pass these epimutations to following generations breeding naturally. De Waal et al. analyzed allele-specific DNA methylation and expression at three imprinted genes, H19, Snrpn, and Peg3, in somatic cells from adult mice generated through ICSI. The results confirm that ICSI can result in the formation of epimutations. While such epimutations may persist indefinitely in somatic cells of the ICSI-derived individuals, they are usually rectified in the germ line through epigenetic reprogramming and not transmitted to subsequent generations [87].

Ciapa et al. propose the probability that pathways such as those involving tyrosine kinases, G-proteins or integrins may be activated besides sperm factor

injection, and, there may be upstream mechanisms involved in later embryonic development. Although most reports are reassuring, some recent data suggest a greater incidence of abnormalities in children conceived by ART compared to those conceived normally. Because of the avoidance of membrane fusion and signalling events during ICSI, Spatio-temporal signals may be missing or abnormal. They discuss the hypothesis that potential perturbations during ICSI may have repercussions for epigenetic processes, inducing not only alterations of embryonic development, but also diseases in young children and, perhaps, in adults [88].

10.4 Preimplantation Genetic Diagnosis (PGD)

PGD is a form of prenatal diagnosis in early stage, in which embryos created in vitro are analyzed for a certain kind of genetic defects and only embryos free of the defects are transferred into the womb. The required disruption of cell-cell contacts for biopsy is reported to disrupt its role in embryonic growth [89]. Suboptimal techniques used for biopsy and fixation are believed to interfere with the clinical success of these techniques. Also, research has shown that biopsies of one or two cells from the whole can disrupt the 50 %/50 % ratio of the random X chromosome inactivation balance, an epigenetic function [90]. Disruption of the embryo at any of these preimplantation stages may have a negative impact on the subsequent development of the remaining cells in the embryo.

Logic tells the educated individual that PGD should make for healthier infants born from IVF in conjunction with fewer embryos needed to be transferred to achieve success in ART. However, studies from the human perspective have revealed a range of negative associations and lack of success when associating these techniques with live-birth delivery rates. The rate of live-birth delivery in women <36 years failed to increase following PGD and single-embryo transfer [91]. In fact, another report, this time using women of advanced maternal age, established that PGD not only failed to increase the ongoing pregnancy and live-delivery rates but found that it significantly decreased them [92]. Nevertheless, encouraging results could be found in a report assessing the mental development of 2-year-old children born following these types of biopsies; these children displayed no ill effects of any type of treatment when compared with children conceived naturally [89].

As with all other topics, PGD research applications have been extended to the mouse model. Very recent publication expressed that neither day 3 biopsy nor blastomere biopsy correlates with alterations in preimplantation development or global gene expression [93], although blastomere biopsy is significantly associated with premature and sometimes abnormal hatching due to infringement on the zona [93]. However, in a similar time frame, a report surfaced about the association of a blastomere biopsy with the increased potential for neurodegenerative disorders in the offspring [94]. Again, this research demonstrates the need for greater knowledge concerning this debatable topic before coming to a well-informed conclusion on the risks for adult diseases of its application.

10.5 Cryopreserved/Thawed Gametes and Embryos

Early life events (prenatal and early postnatal events) can initiate long term changes in individual's life, increasing the risk or producing some diseases that could be apparent in adulthood. It was described in Barker's hypothesis "Fetal origin of adult diseases", based on epidemiological studies from birth records in UK, comparing birth weight and physical characteristics at birth and subsequent health status later in the life of the same individuals. Suboptimal condition for fetal development, producing small for age and low birth weight babies, like maternal undernutrition, or disproportionately large babies at birth can change physiology and metabolism predisposing such organism to cardiovascular and metabolic disease later in the life [95].

The methylation of imprinted genes is erased and reestablished during gametogenesis and is maintained throughout pre- and post-implantation development. ICSI and PGD manipulate gametes during the phase which is crucial for methylation reprogramming, may influence the epigenetic stability, and lead to the increased risks of adult diseases with fetal origin.

10.5.1 Effect of Cryopreservation on the Offspring of ART

Cryopreservation of embryos and zygotes is widely used in assisted reproduction technology (ART), and frozen embryo transfer has become an essential part of ART programmes. Although the studies on perinatal and postnatal outcomes of children born after frozen embryo transfer are limited, previous findings have confirmed the short-term safety of FET.

10.5.2 Different Methods Used to Cryopreservation

Slow cooling is the oldest method using for cryopreservation of embryos, and it is far-ranging used recently. During slow cooling, embryos are usually pre-equilibrated in either 1.5M dimethyl sulfoxide or 1.5M propylene glycol at room temperature. During the next steps, the concentration of cryoprotectants inside and outside the embryos will gradually increase. When the concentration is high enough to support glasslike solidification of the cells and the outside solution, at temperatures approaching −33 to −40 °C, the cells could then be rapidly exposed to much lower temperatures such as −150 °C or lower. To complete these, it costs more than 2 h [95]. Vitrification is a new method which is now commonly applied in cryopreservation of embryos in ART. Vitrification is an ultrarapid method of cryopreservation whereby the embryo transits from 37 to −196 °C in <1 s, resulting in extremely fast rates of cooling [96]. For cryopreserving embryos in a vitrified, glass-like state, high concentrations of cryoprotectants and rapid cooling rates are essential. During vitrification, higher rates of cooling and rewarming are key points to successes.

A research proved that comparing to slow cooling, vitrification using DMSO/sucrose had a higher survival rate, while implantation rates were almost identical [97]. But another study showed that the post-thaw survival of vitrified embryos was significantly better than those of embryos resulting from slow freezing process. And a better pregnancy rate per thawed embryo cycle was observed following vitrification [97]. Using vitrification, the process of embryo freezing is simplified as well as the mechanical injury to embryos during the formation of intra- and extracellular ice crystals can be avoided. So a successful vitrification can significantly improve the embryo recovery rate and integrity.

10.5.3 The Birth Weight

It was postulated that infants born after frozen-thawed embryo transfer (FET) have a higher mean birth weight (BW) than those born after transfer of fresh embryos [98, 99]. A study about obstetric outcome in singletons after IVF with cryopreserved/thawed embryos [100] found that the rates of large for gestational age (LGA) and birth weight >4,500 g were significantly increased for the singletons from the cryopreservation cycles, both in comparison with the general population and in comparison with singletons from fresh cycles.

A large cohort study of infant outcome of 957 singletons born after frozen embryo replacement from 1995 to 2006 was done by Pinborg A. from Denmark [101]. This study showed that the mean BW was 205 g higher in Cryo (3,578 ± 625 g) as compared with the fresh group (3,373 ± 648 g). But in first-born children, the mean BW difference between the Cryo and fresh group was 187 g, and it was 86 g when compared with the non-ART group. The risk of low birth weight (LBW; <2,500 g) decreased significantly in Cryo versus fresh group in the adjusted analyses, but for very low BW (VLBW) risks, there were no statistically significant differences between singletons from frozen and fresh cycles. An analysis of 25,777 children in the national assisted reproduction registry of Japan [102] found that the mean birth weight after FET was significantly higher compared with fresh ET and all Japanese births (3,100.7 ± 387.2 g, 3,009.8 ± 376.8 g, and 3,059.6 ± 369.6 g, respectively). For sex, the risk of LBW was higher in female neonates compared with male neonates. Another study by Shih W [103] found that the mean birth weight in first births was significantly lower, and LBW were more frequent for IVF (3,166 ± 676 g, LBW = 11.7 %) and ICSI (3,206 ± 697 g, LBW = 11.5 %) than for FET (3,352 ± 615 g, LBW = 6.5 %) and non-ART conceptions (3,341 ± 634, LBW = 7.1 %).

Although a higher rate of very low birth weight was observed among IVF twins from frozen cycles than children from fresh cycles, Cryo ICSI twins had a significantly higher mean birth weight than their fresh peers [99]. In addition, there was no difference in very low birth weight rate between cryo IVF and cryo ICSI.

In Australian and Swedish studies the low birth weight rate in singletons was significantly lower for children born from cryopreserved embryos than children born after fresh cycles [103, 104]. In the former study, singleton first babies born

after fresh embryo transfer had a lower mean birthweight than those born after cryopreservation [103]. And for twins, low birthweight rates in frozen cycles were lower than fresh cycles [103].

Many factors may result in low birthweight, including fetal sex, birth defects, parity and maternal age. Culture media may also affect perinatal outcome including low birthweight [105]. Culture in Cook's medium after fresh transfer resulted in singletons with lower mean birthweight, and, more singletons with low birthweight, when compared to singletons born after culture in Vitrolife AB medium. Inter-related health, lifestyle and socio-economic factors may also be important but the reason why the risk for low birthweight in FET was significantly lower than fresh ET continues to be studied. In FET cycles, supplements of oestrogen and progesterone may improve the uterine environment leading to better development of the placenta, subsequent fetal growth, and heavier birth weight. Ovarian stimulation is one of the causes of differences in mean birthweight following fresh ET and FET cycles. Birthweight was also affected by the duration of embryonic culture, the medium for culture and the procedure of freezing and thawing.

10.5.4 Preterm Birth Rate

A systematic review of children born after cryopreservation of embryos [106] shows that the preterm birth rate (PBR; <37 weeks) for cryopreserved singletons varies between 9.2 and 12.0 %. And the corresponding PBR for fresh IVF/ICSI singletons varies between 7.4 and 14 %. In the Swedish and the Australian studies [103, 104], the PBR in singletons was significantly lower for children born after cryopreservation than children born after fresh cycles. Although in other studies, there were no significant differences in preterm birth rates.

The Danish cohort study showed that, in singletons after frozen cycles, gestational age was 2.4 days longer when compared with the fresh group. The risk of preterm birth decreased significantly in cryo versus fresh groups, but for very preterm birth (<34 weeks), there were no significant differences. A systematic review and meta-analysis of perinatal outcomes in singleton pregnancies resulting from FET versus fresh ETs [107] shows that relative risks (RR) of having a delivery at <37 weeks was 0.84 in singleton pregnancies after FETs, when compared to those after fresh ETs with a risk of 2 %. However, when compared to singletons from the general population, significantly higher rates of extreme preterm birth (<28 weeks) were found in singletons from cryopreserved cycles [100].

For twins, the PBR for infants born after cryopreservation, varies between 33 and 62 %, while for "fresh" twins, it was between 47.6 and 61.3 %. Although there were no significant differences between "frozen" and "fresh" ICSI, a study found a significantly higher PBR for "frozen" versus "fresh" IVF twins [99]. But in other studies no significant differences were found between these groups of twins.

To explore the association between infertility and preterm birth, Basso et al. [108] examined the relationship in various subsets of the study population, after combining data for primiparas and multiparas. When excluding women aged

>30 years or restricting the samples to women with a BMI of 20±24.99, or with regular menstrual cycles of 27±31 days, or nonsmokers, the results were similar to those before. It is proposed that the fetus determines the length of gestation, whereas mother's size determines the size of the baby at birth.

10.5.5 The Sex Ratio

The sex ratio of neonates may be associated with genetics, differential survival of male fetuses in utero, and, other intrapartum factors. In the Danish cohort study, the male sex ratio of Cryo-ICSI was 57.0 %, and it was 48.9 % in the fresh-ICSI group [101]. In previous reports, the use of ICSI was associated with a decrease in the sex ratio of male infants [109] though the reasons for the sex-ratio imbalance remain unclear. One report suggests over-use of ICSI as there is a general disruption in genomic imprinting during the process of ICSI, while others suggested differential death of male and female embryos in the early stages of embryogenesis. Sperm separation procedures or culture media may also affect the sex ratio of the children from cryo and fresh ETs [110]. A study of the effect of energy sources during culture on the development of in vitro bovine embryos, demonstrates that the energy substrate during in vitro culture affects both the production and viability of blastocysts. Furthermore, manipulating the metabolic profile of embryos during in vitro culture may have an impact on sex ratio [111].

If some component of the fresh-ICSI procedure are likely to cause reductions in the male sex ratio, other components drawing in the opposite direction may cause an increased male sex ratio in Cryo-ICSI children. A comparison of the offspring sex ratio between fresh and thawed blastocyst transfer (BT) find a significant sex-ratio imbalance towards female offspring from thawed BT, compared with those from fresh BT. In the subgroups of either singleton or multiple deliveries, the female-to-male ratio increased significantly in the thawed BT group [112]. Such findings may be associated with iatrogenic factors including the embryo morphology selection strategy. Because top-quality embryos are selected by experienced embryologists and then given priority for fresh cycle transfer, most of the thawed embryos are also fresh cycle transfers.

10.5.6 Birth Defects and Mortality

A summary of 17 studies show that the rate of congenital malformations in all frozen cycles varies between 0.7 and 8.6 %, and, the corresponding figures for fresh cycles varied between 0.7 and 8.7 % [102]. Although adjusted risk differences in malformation rates between cryopreserved and fresh or non-ART were not found in the Danish cohort study, there was a tendency for highest rates in fresh and lowest rates in non-ART, with cryopreserved somewhere in between [101]. The rates of total malformations, particularly cardiac and musculoskeletal, increased in fresh and cryopreserved versus non-ART though there were no significant

differences of weeded malformations or any specific organ system problems between cryo-ICSI and cryo-IVF. There were no significant differences in rates of mental retardation and cerebral palsy in cryopreserved compared to either fresh or non-ART groups. The Swedish study showed similar results, and, no significant difference was found between frozen and fresh cycles when adjusting for year of birth, maternal age and number of infants born [104]. But in the Belgian study, malformations were more frequent in cryo-ICSI liveborns (6.4 %) compared to cryo-IVF liveborns (3.1 %) and fresh-ICSI liveborns (3.4 %) [99]. And there was a significantly higher rate of malformations in the total cryopreserved group, compared to the total fresh group. Compared to fresh-ICSI, pre- and post-natal rates of chromosome aberrations in cryo-ICSI were similar. In the study by Olson [113], compared with fresh IVF/ICSI twins, only in cryo IVF/ICSI twins higher malformation rates were found.

With regard to perinatal mortality, there were no differences between singletons and twins from fresh and frozen cycles in a UK study [103], while a significantly higher perinatal mortality rate for singletons from fresh versus frozen cycles in an Australian study. Although the crude figures for most obstetric measures were better for cryo-ICSI compared with cryo-IVF, no statistically significant differences were found between the two groups.

An increased risk of congenital malformations after IVF is largely independent of preterm births, which is associated with the upper respiratory tract infections, convulsions, and accidents. It seems unlikely that IVF itself increases the risk of these problems. Compared with parents of spontaneously conceived children, parents of IVF children are more likely to ask for medical help for less severe conditions, and that may affect hospitalization for these conditions and contribute to elevated risks for children born preterm.

10.5.7 Growth and Childhood Morbidity

Data are limited for growth, childhood morbidity and mental development, and, few differences were found between children born after frozen and fresh embryo transfer. In a Swedish study, 255 children from cryopreserved embryos were followed up to 18 months, and growth was normal. No differences were found between children from fresh and frozen embryos or spontaneous pregnancies. In the questionnaire study by Nakajo et al. including 78 born after IVF, 343 children born after ICSI and 81 after cryopreservation, the children were followed up to 2 years old [114]. For singletons, growth was similar to non-ART children, however, compared with naturally-conceived singletons, the growth of IVF, ICSI and cryo multiples was significantly delayed but had caught up by 6 months of age.

In a study of 16,280 children born after IVF with a median follow-up time of 5.5 years, which included 1,474 children from frozen embryos, three children in this group had a cancer diagnosis. In another study, there was no difference in the prevalence of chronic disease at 18 months of age among the cryopreserved, fresh IVF and naturally-conceived groups.

10.5.8 Cryopreservation of Zygotes

Clinical applications for oocyte cryopreservation include fertility preservation in cancer patients, oocyte accumulation in low-responder patients, ovum donation programs, fertility preservation for social reasons, minimization of ovarian hyperstimulation syndrome risk, and surplus oocyte storage after controlled ovarian stimulation when embryo cryopreservation is not feasible [115]. There is no significant difference in the potential of fertilization, embryogenesis, and pregnancy between from oocytes derived from vitrification warming cycles and from that of fresh oocytes. But there are rare reports of offspring's ill-health following oocyte cryopreservation.

Children born after cryopreservation have a better outcome as compared to those born after fresh transfer. The risks of low birthweight and preterm birth are significantly lower in infants from FET than those from fresh ETs. The caesarean section rate is significantly higher in the cryopreserved than the fresh groups, and is almost doubled compared with the non-ART group. The difference of sex ratio between frozen and fresh transfer is mostly in ICSI and blastocyst. For the birth defects and mortality, there were no significant differences for children from frozen and fresh transfer, but all have a higher risk than non-ART children. The long term follow-up investigations of FET children have found no significantly adverse outcomes; however, the data are limited. Prospective studies are necessary to fully evaluate the outcomes of FET.

10.6 Conclusions

Superovulation, in vitro culture, IVM, ICSI, embryo biopsy and cryopreservation, may all contribute to increased risks of adult diseases. Large, prospective, epidemiological studies to systematically assess the potential risk factors associated with adult diseases will be necessary to establish precise risks.

References

1. Motrenko T. Embryo-fetal origin of diseases – new approach on epigenetic reprogramming. Arch Perinat Med. 2010;6:11–5.
2. Savage T, Peek J, Hofman PL, et al. Childhood outcomes of assisted reproductive technology. Hum Reprod. 2011;26:2392–400.
3. Ludwig AK, Sutcliffe AG, Diedrich K, et al. Post-neonatal health and development of children born after assisted reproduction: a systematic review of controlled studies. Eur J Obstet Gynecol Reprod Biol. 2006;127:3–25.
4. Cetin I, Cozzi V, Antonazzo P. Fetal development after assisted reproduction – a review. Placenta. 2003;24:S104–13.
5. Ceelen M, van Weissenbruch MM, Vermeiden JP, et al. Growth and development of children born after in vitro fertilization. Fertil Steril. 2008;90:1662–73.
6. Rosenwaks Z, Bendikson K. Further evidence of the safety of assisted reproductive technologies. Proc Natl Acad Sci U S A. 2007;104:5709–10.

7. Sutcliffe AG, Ludwig M. Outcome of assisted reproduction. Lancet. 2007;370:351–9.
8. Sunderam S, Kissin DM, Flowers L, et al. Assisted reproductive technology surveillance – United States, 2009. MMWR Surveill Summ. 2012;61:1–23.
9. Hazekamp J, Bergh C, Wennerholm UB, Hovatta O, et al. Avoiding multiple pregnancies in ART: consideration of new strategies. Hum Reprod. 2000;15:1217–19.
10. Zhu JL, Basso O, Obel C, et al. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. BMJ. 2006;333:679.
11. de Mouzon J, Lancaster P, Nygren KG, et al. World collaborative report on Assisted Reproductive Technology, 2002. Hum Reprod. 2009;24:2310–20.
12. Kalra SK, Molinaro TA. The association of in vitro fertilization and perinatal morbidity. Semin Reprod Med. 2008;26:423–35.
13. Welmerink DB, Voigt LF, Daling JR, et al. Infertility treatment use in relation to selected adverse birth outcomes. Fertil Steril. 2010;94:2580–6.
14. Steel AJ, Sutcliffe A. Long-term health implications for children conceived by IVF/ICSI. Hum Fertil (Camb). 2009;12(1):21–7.
15. Diaz-Garcia C, Estella C, Perales-Puchalt A, et al. Reproductive medicine and inheritance of infertility by offspring: the role of fetal programming. Fertil Steril. 2011;96:536–45.
16. Romundstad LB, Romundstad PR, Sunde A, et al. Effects of technology or maternal factors on perinatal outcome after assisted fertilisation: a population-based cohort study. Lancet. 2008;372:737–43.
17. Basatemur E, Sutcliffe A. Follow-up of children born after ART. Placenta. 2008;29: 135–40.
18. Lidegaard O, Pinborg A, Andersen AN. Imprinting diseases and IVF: Danish National IVF cohort study. Hum Reprod. 2005;20:950–4.
19. Stromberg B, Dahlquist G, Ericson A, et al. Neurological sequelae in children born after in-vitro fertilisation: a population-based study. Lancet. 2002;359:461–5.
20. Middelburg KJ, Haadsma ML, Heineman MJ, et al. Ovarian hyperstimulation and the in vitro fertilization procedure do not influence early neuromotor development; a history of subfertility does. Fertil Steril. 2010;93:544–53.
21. Kramer S, Ward E, Meadows AT, et al. Medical and drug risk factors associated with neuroblastoma: a case–control study. J Natl Cancer Inst. 1987;78:797–804.
22. Skora D, Frankfurter D. Adverse perinatal events associated with ART. Semin Reprod Med. 2012;30:84–91.
23. Moll AC, Imhof SM, Cruysberg JR, et al. Incidence of retinoblastoma in children born after in-vitro fertilisation. Lancet. 2003;361:309–10.
24. Bruinsma F, Venn A, Lancaster P, et al. Incidence of cancer in children born after in-vitro fertilization. Hum Reprod. 2000;15:604–7.
25. Vulliemoz NR, McVeigh E, Kurinczuk J. In vitro fertilisation: perinatal risks and early childhood outcomes. Hum Fertil (Camb). 2012;15:62–8.
26. Wen J, Jiang J, Ding C, et al. Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. Fertil Steril. 2012;97:1331–7. e1–4.
27. Bukulmez O. Does assisted reproductive technology cause birth defects. Curr Opin Obstet Gynecol. 2009;21:260–4.
28. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012;366:1803–13.
29. Odom LN, Segars J. Imprinting disorders and assisted reproductive technology. Curr Opin Endocrinol Diabetes Obes. 2010;17:517–22.
30. Eroglu A, Layman LC. Role of ART in imprinting disorders. Semin Reprod Med. 2012;30: 92–104.
31. Kuentz P, Bailly A, Faure AC, et al. Child with Beckwith-Wiedemann syndrome born after assisted reproductive techniques to an human immunodeficiency virus serodiscordant couple. Fertil Steril. 2011;96:e35–8.
32. Choufani S, Shuman C, Weksberg R. Beckwith-Wiedemann syndrome. Am J Med Genet C Semin Med Genet. 2010;154C:343–54.

33. Manipalviratn S, DeCherney A, Segars J. Imprinting disorders and assisted reproductive technology. Fertil Steril. 2009;91:305–15.
34. Owen CM, Segars Jr JH. Imprinting disorders and assisted reproductive technology. Semin Reprod Med. 2009;27:417–28.
35. Fortier AL, Lopes FL, Darricarrere N, et al. Superovulation alters the expression of imprinted genes in the midgestation mouse placenta. Hum Mol Genet. 2008;17:1653–65.
36. Stouder C, Deutsch S, Paoloni-Giacobino A. Superovulation in mice alters the methylation pattern of imprinted genes in the sperm of the offspring. Reprod Toxicol. 2009;28:536–41.
37. Market-Velker BA, Zhang L, Magri LS, et al. Dual effects of superovulation: loss of maternal and paternal imprinted methylation in a dose-dependent manner. Hum Mol Genet. 2010;19:36–51.
38. Wilkins-Haug L. Assisted reproductive technology, congenital malformations, and epigenetic disease. Clin Obstet Gynecol. 2008;51:96–105.
39. Marchesi DE, Qiao J, Feng HL. Embryo manipulation and imprinting. Semin Reprod Med. 2012;30:323–34.
40. Gilchrist RB. Recent insights into oocyte-follicle cell interactions provide opportunities for the development of new approaches to in vitro maturation. Reprod Fertil Dev. 2011;23:23–31.
41. Son WY, Tan SL. Laboratory and embryological aspects of hCG-primed in vitro maturation cycles for patients with polycystic ovaries. Hum Reprod Update. 2010;16:675–89.
42. Suikkari AM. In-vitro maturation: its role in fertility treatment. Curr Opin Obstet Gynecol. 2008;20:242–8.
43. Sutton ML, Gilchrist RB, Thompson JG. Effects of in-vivo and in-vitro environments on the metabolism of the cumulus-oocyte complex and its influence on oocyte developmental capacity. Hum Reprod Update. 2003;9:35–48.
44. Merriman JA, Whittingham DG, Carroll J. The effect of follicle stimulating hormone and epidermal growth factor on the developmental capacity of in-vitro matured mouse oocytes. Hum Reprod. 1998;13:690–5.
45. Vanderhyden BC, Armstrong DT. Role of cumulus cells and serum on the in vitro maturation, fertilization, and subsequent development of rat oocytes. Biol Reprod. 1989;40:720–8.
46. Singh J, Adams GP, Pierson RA. Promise of new imaging technologies for assessing ovarian function. Anim Reprod Sci. 2003;78:371–99.
47. Ye J, Campbell KH, Craigon J, et al. Dynamic changes in meiotic progression and improvement of developmental competence of pig oocytes in vitro by follicle-stimulating hormone and cycloheximide. Biol Reprod. 2005;72:399–406.
48. Lin YH, Hwang JL. In vitro maturation of human oocytes. Taiwan J Obstet Gynecol. 2006;45:95–9.
49. Cha KY, Chung HM, Lee DR, et al. Obstetric outcome of patients with polycystic ovary syndrome treated by in vitro maturation and in vitro fertilization-embryo transfer. Fertil Steril. 2005;83:1461–5.
50. Holzer H, Scharf E, Chian RC, et al. In vitro maturation of oocytes collected from unstimulated ovaries for oocyte donation. Fertil Steril. 2007;88:62–7.
51. Li Y, Feng HL, Cao YJ, et al. Confocal microscopic analysis of the spindle and chromosome configurations of human oocytes matured in vitro. Fertil Steril. 2006;85:827–32.
52. Shu-Chi M, Jiann-Loung H, Yu-Hung L, et al. Growth and development of children conceived by in-vitro maturation of human oocytes. Early Hum Dev. 2006;82:677–82.
53. Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril. 1994;62:353–62.
54. Cao YX, Chian RC. Fertility preservation with immature and in vitro matured oocytes. Semin Reprod Med. 2009;27:456–64.
55. Lucifero D, Chaillet JR, Trasler JM. Potential significance of genomic imprinting defects for reproduction and assisted reproductive technology. Hum Reprod Update. 2004;10:3–18.
56. Palermo GD, Neri QV, Takeuchi T, et al. Genetic and epigenetic characteristics of ICSI children. Reprod Biomed Online. 2008;17:820–33.

57. Price TM, Murphy SK, Younglai EV. Perspectives: the possible influence of assisted reproductive technologies on transgenerational reproductive effects of environmental endocrine disruptors. Toxicol Sci. 2007;96:218–26.
58. Palermo GD, Neri QV, Takeuchi T, et al. ICSI: where we have been and where we are going. Semin Reprod Med. 2009;27:191–201.
59. Fulka H, Fulka Jr J. No differences in the DNA methylation pattern in mouse zygotes produced in vivo, in vitro, or by intracytoplasmic sperm injection. Fertil Steril. 2006;86:1534–6.
60. Santos F, Hyslop L, Stojkovic P, et al. Evaluation of epigenetic marks in human embryos derived from IVF and ICSI. Hum Reprod. 2010;25:2387–95.
61. Tierling S, Souren NY, Gries J, et al. Assisted reproductive technologies do not enhance the variability of DNA methylation imprints in human. J Med Genet. 2010;47:371–6.
62. Feng C, Tian S, Zhang Y, et al. General imprinting status is stable in assisted reproduction-conceived offspring. Fertil Steril. 2011;96:1417–1423.e9.
63. de Waal E, Yamazaki Y, Ingale P, et al. Gonadotropin stimulation contributes to an increased incidence of epimutations in ICSI-derived mice. Hum Mol Genet. 2012;21:4460–72.
64. Dommering CJ, van der Hout AH, Meijers-Heijboer H, Marees T, Moll AC. IVF and retinoblastoma revisited. Fertil Steril. 2012;97(1):79–81.
65. Alukal JP, Lamb DJ. Intracytoplasmic sperm injection (ICSI) – what are the risks. Urol Clin North Am. 2008;35:277–88. Ix–x.
66. Bowen JR, Gibson FL, Leslie GI, et al. Medical and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection. Lancet. 1998;351:1529–34.
67. Sutcliffe AG, Taylor B, Saunders K, et al. Outcome in the second year of life after in-vitro fertilisation by intracytoplasmic sperm injection: a UK case-control study. Lancet. 2001;357:2080–4.
68. Bonduelle M, Wennerholm UB, Loft A, et al. A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. Hum Reprod. 2005;20:413–19.
69. Rajender S, Avery K, Agarwal A. Epigenetics, spermatogenesis and male infertility. Mutat Res. 2011;727:62–71.
70. Aitken RJ, De Iuliis GN. Origins and consequences of DNA damage in male germ cells. Reprod Biomed Online. 2007;14:727–33.
71. Avendano C, Franchi A, Taylor S, et al. Fragmentation of DNA in morphologically normal human spermatozoa. Fertil Steril. 2009;91:1077–84.
72. Avendano C, Franchi A, Duran H, et al. DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. Fertil Steril. 2010;94:549–57.
73. Fernandez-Gonzalez R, Moreira PN, Perez-Crespo M, et al. Long-term effects of mouse intracytoplasmic sperm injection with DNA-fragmented sperm on health and behavior of adult offspring. Biol Reprod. 2008;78:761–72.
74. Fatehi AN, Bevers MM, Schoevers E, et al. DNA damage in bovine sperm does not block fertilization and early embryonic development but induces apoptosis after the first cleavages. J Androl. 2006;27:176–88.
75. Woldringh GH, Besselink DE, Tillema AH, et al. Karyotyping, congenital anomalies and follow-up of children after intracytoplasmic sperm injection with non-ejaculated sperm: a systematic review. Hum Reprod Update. 2010;16:12–9.
76. Paoloni-Giacobino A. Implications of reproductive technologies for birth and developmental outcomes: imprinting defects and beyond. Expert Rev Mol Med. 2006;8:1–14.
77. Sutcliffe AG, Manning JT, Katalanic A, et al. Perturbations in finger length and digit ratio (2D:4D) in ICSI children. Reprod Biomed Online. 2010;20:138–43.
78. van der Heijden GW, van den Berg IM, Baart EB, et al. Parental origin of chromatin in human monopronuclear zygotes revealed by asymmetric histone methylation patterns, differs between IVF and ICSI. Mol Reprod Dev. 2009;76:101–8.
79. Qiao J, Chen Y, Yan LY, et al. Changes in histone methylation during human oocyte maturation and IVF- or ICSI-derived embryo development. Fertil Steril. 2010;93:1628–36.

80. Yoshizawa Y, Kato M, Hirabayashi M, et al. Impaired active demethylation of the paternal genome in pronuclear-stage rat zygotes produced by in vitro fertilization or intracytoplasmic sperm injection. Mol Reprod Dev. 2010;77:69–75.
81. Ajduk A, Yamauchi Y, Ward MA. Sperm chromatin remodeling after intracytoplasmic sperm injection differs from that of in vitro fertilization. Biol Reprod. 2006;75:442–51.
82. Zhang YL, Chen T, Jiang Y, et al. Active demethylation of individual genes in intracytoplasmic sperm injection rabbit embryos. Mol Reprod Dev. 2005;72:530–3.
83. Perry AC, Wakayama T, Kishikawa H, et al. Mammalian transgenesis by intracytoplasmic sperm injection. Science. 1999;284:1180–3.
84. Chan AW, Luetjens CM, Dominko T, et al. Foreign DNA transmission by ICSI: injection of spermatozoa bound with exogenous DNA results in embryonic GFP expression and live rhesus monkey births. Mol Hum Reprod. 2000;6:26–33.
85. Moreira PN, Fernandez-Gonzalez R, Rizos D, et al. Inadvertent transgenesis by conventional ICSI in mice. Hum Reprod. 2005;20:3313–17.
86. Ronquist GK, Larsson A, Ronquist G, et al. Prostasomal DNA characterization and transfer into human sperm. Mol Reprod Dev. 2011;78:467–76.
87. de Waal E, Yamazaki Y, Ingale P, et al. Primary epimutations introduced during intracytoplasmic sperm injection (ICSI) are corrected by germline-specific epigenetic reprogramming. Proc Natl Acad Sci U S A. 2012;109:4163–8.
88. Ciapa B, Arnoult C. Could modifications of signalling pathways activated after ICSI induce a potential risk of epigenetic defects. Int J Dev Biol. 2011;55:143–52.
89. Zechner U, Pliushch G, Schneider E, et al. Quantitative methylation analysis of developmentally important genes in human pregnancy losses after ART and spontaneous conception. Mol Hum Reprod. 2010;16:704–13.
90. Okamoto I, Otte AP, Allis CD, et al. Epigenetic dynamics of imprinted X inactivation during early mouse development. Science. 2004;303:644–9.
91. Staessen C, Verpoest W, Donoso P, et al. Preimplantation genetic screening does not improve delivery rate in women under the age of 36 following single-embryo transfer. Hum Reprod. 2008;23:2818–25.
92. Mastenbroek S, Twisk M, van Echten-Arends J, et al. In vitro fertilization with preimplantation genetic screening. N Engl J Med. 2007;357:9–17.
93. Duncan FE, Stein P, Williams CJ, et al. The effect of blastomere biopsy on preimplantation mouse embryo development and global gene expression. Fertil Steril. 2009;91:1462–5.
94. Yu Y, Wu J, Fan Y, et al. Evaluation of blastomere biopsy using a mouse model indicates the potential high risk of neurodegenerative disorders in the offspring. Mol Cell Proteomics. 2009;8:1490–500.
95. Kuleshova LL, Lopata A. Vitrification can be more favorable than slow cooling. Fertil Steril. 2002;78:449–54.
96. Aflatoonian A, Oskouian H, Ahmadi S, et al. Can fresh embryo transfers be replaced by cryopreserved-thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial. J Assist Reprod Genet. 2010;27:357–63.
97. Vutyavanich T, Sreshthaputra O, Mongkolchaipak S, et al. Slow programmable and ultra-rapid freezing of human embryos. J Obstet Gynaecol Res. 2008;34:457–63.
98. Henningsen AK, Pinborg A, Lidegaard O, et al. Perinatal outcome of singleton siblings born after assisted reproductive technology and spontaneous conception: Danish national sibling-cohort study. Fertil Steril. 2011;95:959–63.
99. Belva F, Henriet S, Van den Abbeel E, et al. Neonatal outcome of 937 children born after transfer of cryopreserved embryos obtained by ICSI and IVF and comparison with outcome data of fresh ICSI and IVF cycles. Hum Reprod. 2008;23:2227–38.
100. Sazonova A, Kallen K, Thurin-Kjellberg A, et al. Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos. Hum Reprod. 2012;27:1343–50.
101. Pinborg A, Loft A, Aaris HAK, et al. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995–2006. Fertil Steril. 2010;94:1320–7.

102. Nakashima A, Araki R, Tani H, et al. Implications of assisted reproductive technologies on term singleton birth weight: an analysis of 25,777 children in the national assisted reproduction registry of Japan. Fertil Steril. 2013;99(2):450–5.
103. Shih W, Rushford DD, Bourne H, et al. Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. Hum Reprod. 2008;23:1644–53.
104. Kallen B, Finnstrom O, Nygren KG, et al. In vitro fertilization (IVF) in Sweden: infant outcome after different IVF fertilization methods. Fertil Steril. 2005;84:611–17.
105. Nelissen EC, Van Montfoort AP, Coonen E, et al. Further evidence that culture media affect perinatal outcome: findings after transfer of fresh and cryopreserved embryos. Hum Reprod. 2012;27:1966–76.
106. Wennerholm UB, Soderstrom-Anttila V, Bergh C, et al. Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. Hum Reprod. 2009;24:2158–72.
107. Maheshwari A, Pandey S, Shetty A, et al. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril. 2012;98:368–77. e1-9.
108. Basso O, Baird DD. Infertility and preterm delivery, birthweight, and Caesarean section: a study within the Danish National Birth Cohort. Hum Reprod. 2003;18:2478–84.
109. Luke B, Brown MB, Grainger DA, et al. The sex ratio of singleton offspring in assisted-conception pregnancies. Fertil Steril. 2009;92:1579–85.
110. Fedder J, Gabrielsen A, Humaidan P, et al. Malformation rate and sex ratio in 412 children conceived with epididymal or testicular sperm. Hum Reprod. 2007;22:1080–5.
111. Rubessa M, Boccia L, Campanile G, et al. Effect of energy source during culture on in vitro embryo development, resistance to cryopreservation and sex ratio. Theriogenology. 2011;76:1347–55.
112. Lin PY, Huang FJ, Kung FT, et al. Comparison of the offspring sex ratio between fresh and vitrification-thawed blastocyst transfer. Fertil Steril. 2009;92:1764–6.
113. Nakajo Y, Fukunaga N, Fuchinoue K, et al. Physical and mental development of children after in vitro fertilization and embryo transfer. Reprod Med Biol. 2004;3:63–7.
114. Olson CK, Keppler-Noreuil KM, Romitti PA, et al. In vitro fertilization is associated with an increase in major birth defects. Fertil Steril. 2005;84:1308–15.
115. Cobo A, Diaz C. Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril. 2011;96:277–85.

ERRATUM

Gamete/Embryo-Fetal Origins of Diabetes

4

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The spelling of author 'Guo-Dian Ding' was wrong. The correct spelling is 'Guo-Lian Ding'.

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