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REVIEW

The impact of obesity on egg quality

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Abstract Obesity in women is a concern in many countries. This causes numerous health issues; however, this review focuses on the impact of obesity on women's reproduction, and in particular the oocyte. Data from infertility clinics and experimental animal models that address the effects of obesity are presented. Bidirectional communication and metabolic support from the surrounding cumulus cells are critical for oocyte development, and the impact of obesity on these cells is also addressed. Both oocyte maturation and metabolism are impaired due to obesity, negatively impacting further development. In addition to reproductive hormones, obesity induced elevations in insulin, glucose, or free fatty acids, and changes in adipokines appear to impact the developmental competence of the oocyte. The data indicate that any one of these hormones or metabolites can impair oocyte developmental competence in vivo, and the combination of all of these factors and their interactions are the subject of ongoing investigations.

Keywords Oocyte · Cumulus cell · Obesity · Metabolism · Infertility

Capsule Clinical and experimental data indicate that through various mechanisms obesity has a negative impact on oocyte maturation and metabolism, which affect subsequent development.

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Introduction

Maternal obesity is a growing problem in many parts of the world. It is generally defined using the body mass index (BMI) measurement in the units kg/m^2. Based on World Health Organization (WHO) standards, a BMI of 18.5–24.9 is considered normal, 25–29.9 overweight, and ≥30 as obese. In the US it is estimated that almost a quarter of reproductive-age women have a body mass index (BMI) ≥30 [1] and obesity is estimated to affect more than one-third of all US adults [2]. This has consequences for reproductive health. Obese women are almost three times more likely than non-obese women to have some degree of infertility and may take longer to conceive, even if cycling regularly [3, 4]. Studies have shown that obese women who do become pregnant have an increased risk of miscarriage, preeclampsia, gestational diabetes, and congenital defects in offspring [5]. In addition to obesity alone, polycystic ovarian syndrome (PCOS) is a common metabolic disorder in reproductive-aged women commonly associated with luteinizing hormone (LH) hypersecretion, hyperandrogenism, insulin resistance, and anovulation [5–7]. Compounding the effects of PCOS, 50% of patients with PCOS also have a BMI associated with obesity; more than the average population [8]. Obese women with PCOS who also have hyperinsulinemia and impaired glucose tolerance are reported to have decreased fertilization and implantation rates compared to women with PCOS alone [9]. Morbidly obese women (BMI ≥40 kg/m) with PCOS have significantly lower pregnancy rates than women with PCOS alone [10]. The impact of obesity on reproductive health is particularly important because it can have long lasting consequences on future generations. This concept that maternal health and nutrition during pregnancy can have long-term effects on offspring is commonly known as the

Barker Hypothesis, or the developmental origins of adult diseases [11].

When analyzing results from human clinical studies, it may be difficult to separate out the effect of obesity on the oocyte, sperm, embryo, placenta, or uterine environment; as any of these tissues can be affected by obesity. Indeed, some studies of third party reproduction indicate that obesity has a negative impact on development through its effects on the uterine environment [12–15], and recent studies suggest obesity may be affecting sperm function as well [16–19]. This review will focus solely on how obesity and insulin resistance affect the oocyte.

Because women are not born with an infinite number of oocytes, the impact of the maternal environment on this cell is particularly consequential. Negative environmental exposures may affect the developmental competence of the oocyte; defined as the ability of the oocyte to be fertilized and support embryo development. There have been many systematic reviews that assessed the impact of obesity on reproductive outcome, including effects on the oocyte, following assisted reproductive technology (ART) procedures. Many of these have produced conflicting results. A recent review illustrates this point [20]. In regards to the effect of obesity on (1) oocyte retrieval: eight studies reported lower numbers, nine found no difference; (2) oocyte quality or maturity: six studies found an adverse effect, one found no effect; (3) fertilization: three studies found decreased rates, eight found no difference; (4) embryo quality: two found decreased quality, two found no difference [20]. Other recent reviews have also shown conflicting reports on oocyte number, oocyte maturity, and fertilization rate [21, 22]. Recently, a very large study of 45,163 ART cycles in the US reported that increasing obesity (based on BMI) is associated with decreased clinical pregnancy rates from autologous oocytes, but not donor oocytes indicating that the oocyte quality alone can affect pregnancy [23]. Despite the variations in results from these clinical studies, obesity is clearly associated with subfertility [24, 25]. Here, we will summarize the data from both experimental animal models and clinical data from infertility clinics related to the impact of obesity on oocyte maturation and fertilization, and impacts on oocyte metabolism.

Oocyte maturation and fertilization

The mammalian oocyte remains arrested in prophase I (germinal vesicle stage) of meiosis from oogenesis through follicle formation and growth. It is not until the preovulatory GnRH and LH surge that maturation continues to metaphase II of meiosis and the first polar body is extruded. The ooctye does not fully complete maturation and extrude the second polar body until fertilization. Specialized granulosa cells called cumulus cells that have surrounded the oocyte since the antral follicle stage expand and secrete hyaluronic acid following the LH surge. Prior to the LH surge, the oocyte and cumulus cells are coupled by transzonal processes and communicate through gap junction and paracrine signaling [26]. The bidirectional communication that exists between the somatic cumulus cells and the oocyte [27, 28] and the metabolic support from the cumulus cells [29, 30] are both essential for oocyte viability and developmental competence. Suboptimal conditions during the oocyte maturation stage can negatively impact further embryo development [31].

Obesity may affect oocyte competence and maturation through alterations in various hormones, particularly those hormones that trigger oocyte maturation. Because adipose tissue is an important site for steroid hormone production and metabolism, its excess in obesity can alter concentrations of steroid hormones. Frequently, obese women require greater amounts of gonadotropins for IVF, a situation that can lead to alterations in oocyte maturation and competence [20, 32]. One of the major effects of obesity is increased serum insulin concentrations and resistance to insulin action in cells. Excess insulin can decrease steroid hormone binding globulin (SHBG), which elevates testosterone, dihydrotestosterone, and androstenediol [21]. In response to decreased peripheral insulin sensitivity, insulin production increases leading to further decreases in SHBG and increases in androgens [21]. In women, increasing BMI was associated with decreased SHBG and increased insulin, glucose, lactate, triglycerides, and C-reactive protein, an inflammatory marker in follicular fluid [33]. In the theca and granulosa cells of the ovary, insulin also stimulates steroidogenesis and upregulates LH receptor expression [34]. Due to LH hypersecretion and altered LH:FSH ratio, ovulation and the resumption of oocyte maturation is frequently impacted by obesity in women [21, 35].

Obesity and insulin resistance can also lead to type II, or "adult-onset" diabetes that is characterized by hyperglycemia, as well as hyperinsulinemia. Many studies using mouse models of type I diabetes have shown the negative impact of hyperglycemia alone on oocyte maturation. Oocytes from type I diabetic mice exhibit delays in maturation [36–38] accompanied by spindle defects and chromosome misalignment during oocyte maturation [37]. Maternal type I diabetes also causes increased granulosa cell apoptosis [37]. The above-mentioned gap junction communication that is important in mediating signals from the cumulus cells to the oocyte is also impaired due to maternal diabetes [39]. Mice fed a high fat diet for 16 weeks led to an increase in weight and elevated glucose, but not to the point of being considered diabetic. Even without severe

hyperglycemia, this diet resulted in delayed oocyte maturation, increased apoptosis in ovarian follicles, and smaller oocyte size [40]. Studies from human ART clinics have shown an impairment in oocyte maturation is associated with increasing BMI [41–43]; however, others found no difference in oocyte maturation between different BMI scores [44]. Another study found that number of cumulus-oocyte-complexes (COCs) collected was lower, and stage of maturation delayed, but fertilization rate and pregnancy rate were not impacted by obesity. However, the number of cycles with good quality cryopreservable embryos was lower in obese women [45].

The effects of obesity on the preovulatory LH surge can be particularly complicated in women with PCOS who frequently have LH hypersecretion and anovulation [5–7]. Treatment with insulin sensitizing drugs such as rosiglitizone, a peroxisome proliferator-activated receptor-γ (PPARγ) agonist, may improve ovulation. However, it has been reported that women with PCOS still have increased pregnancy loss [46, 47], possibly due to changes in the oocyte. The prolonged folliculogenesis and altered hormonal environment present in follicles of women with PCOS may alter oocyte gene expression patterns. Indeed, microarray analysis followed by real-time PCR indicated varied gene expression in oocytes of normal ovulatory women compared to women with PCOS; particularly in genes associated with meiotic cell cycle regulation [6]. Women with PCOS and obesity were also reported to have smaller oocyte diameter at the time of retrieval; and these effects are independent from each other [48]. Fertilization rates in women with PCOS are also reported to be lower [49, 50]. Despite the above studies; studies have found no difference in aneuploidy rates between PCOS and control oocytes [49] and pregnancy rates do not always differ [49, 50].

Besides alterations in the ovulatory and steroid hormones, obesity can also alter the concentration of certain adipokines. Leptin is a secreted protein produced in adipocytes; its secretion increases with food intake and decreases during starvation. Its primary site of action is the hypothalamus; however, the leptin receptor has also been indentified in rodent and human theca cells, granulosa cells, oocytes, and embryos [51, 52]. In rodent models, high concentrations of leptin can impair follicle development, ovulation, and oocyte maturation [53, 54]. The leptin deficient (ob/ob) mouse [55] is frequently used to study the effects of obesity. Female ob/ob mice have reduced numbers of antral follicules, impaired folliculogenesis, reduced ovulation rate, and elevated granulosa apoptosis [56]. Treatment of ob/ob mice with gonadotropins and leptin replacement can improve whole body metabolism, as well as ovulation and fertilization [56]. In bovine oocytes high concentrations of leptin decreased cleavage rates and development to blastocyst stage, but did not affect gene expression [57]. In women, elevated leptin is associated with higher BMI [58], and in vitro studies of human granulosa cells indicate leptin can impair steroidogenesis [59]. Lower intrafollicular leptin does not differ between PCOS and control women, but is associated with higher BMI. However, some have found women with PCOS experience elevated serum leptin [60]. Another protein secreted by adipocytes is adiponectin. Concentrations of adiponectin decrease with obesity and insulin resistance and increase with weight loss. Adiponectin has been found in the ovary and oocyte [61]. While its role in the ooctye is unknown, it can alter folliculogenesis in animal models [21]. Other adipokines such as interluekin-6 (IL6), plasminogen activator inhibitor (PAI) type-1, or tumor necrosis factor (TNF) family members may affect oocyte competence or maturation through alterations in steroidogenesis and interaction with other metabolic hormones [21]. While the mechanisms by which adipokines impact the oocyte quality have not been elucidated yet, their altered concentrations due to obesity represent another potential factor to consider when addressing the impact of obesity on oocyte competence.

Oocyte metabolism

Lipids

Abnormally high or low rates of metabolism may impair oocyte and embryo development [62, 63]. The idea that embryos and oocytes have a “quiet” metabolism was proposed in 2002 [64] and the nutritional excess provided by a high-fat or nutrient-rich diet may alter the metabolism and development. While excess fat in diet may cause deleterious effects, there is evidence that normal beta-oxidation of fatty acids occurs in oocytes and embryos and is necessary for development [65, 66]. Oocytes do contain lipid droplets and their localization and expression of related genes changes during oocyte maturation in the mouse, suggesting a role for lipid droplets in normal development. Lipid droplets become larger and more centrally located as the oocyte matures [67]. The lipid droplet protein, perlipin-2 was co-localized with these lipid droplets; and perlipin-2 mRNA increased after hCG administration in mice [67]. In some species such as cattle, sheep, and pigs lipid content is relatively high in the oocyte under normal conditions [68]. In follicular fluid of women the most abundant free fatty acids detected were oleic, palmitic, linoleic, and stearic acids; respectively [69]. Elevated follicular free fatty acids in women were associated with poor COC morphology. Interestingly, there is little correlation between follicular and serum free fatty

acids [69]. Thus far, no studies have investigated the role of lipids in human oocytes.

Various researchers have used mice fed a high fat diet to mimic the effects of diet-induced obesity and insulin resistance. This generally results in weight gain, elevated insulin and free fatty acids, but not severe hyperglycemia. After only 4 weeks on a high fat diet increased lipid content was observed in mouse COCs [70]. This excess lipid accumulation in non-adipose tissues can lead to lipotoxicity and cell death. Lipotoxicity occurs through accumulation of reactive oxygen species, which induce endoplasmic reticulum (ER) stress, the unfolded protein response (UPR), and cell death [71]. The ER is the site of synthesis, folding, and modification of proteins. When unfolded proteins accumulate in the lumen of the ER this induces the UPR. Insulin resistance and type 2 diabetes are also commonly associated with ER stress in other tissues. Four weeks on a high fat diet resulted in increased mRNA expression of ER stress markers in COCs and granulosa cells. One of these ER stress genes, ATF4, was also elevated in granulosa cells of obese women [70]. Besides these markers of ER stress, a 4 weeks high fat diet in mice also caused apoptosis in cumulus and granulosa cells, and decreased mitochondrial membrane potential by JC-1 staining [70]. Mitochondria are maternally derived; so maternal nutrition could have major effects on this organelle. Their primary function is production of ATP by oxidative phosphorylation; they also provide cellular guanosine-5′-triphosphate (GTP) and are a site of amino acid synthesis and calcium reservoir. Other studies have also reported altered mitochondrial function in oocytes in response to 6 weeks on a high fat diet, including increased mitochondrial DNA, mitochondrial membrane potential, mitochondrial biogenesis; as well as increased reactive oxygen species and reduced glutathione, an important antioxidant. These oocytes also had reduced development to the blastocyst stage [72].

Glucose

Glucose consumption in oocytes is low and pyruvate is the major energy source [30, 73]. Previous work has shown that oocytes have low glycolytic activity [74] as well as low phosphofructokinase (PFK) activity [75]. As described above, the oocyte and cumulus cells exist together until after fertilization as the cumulus-oocyte-complex (COC). The cumulus cells metabolize the majority of the glucose in the COC and provide metabolic intermediates such as pyruvate to the oocyte and also accumulate these intermediates in the follicular fluid and oviduct [31, 73, 76]. In contrast to the low PFK activity in oocytes [75], the cumulus cells also have high PFK activity [77]. Oocyte maturation cannot occur without either cumulus cells or pyruvate provided in the media [30, 78] and glucose alone will not support maturation of denuded oocytes without the addition of cumulus cells [29]. Therefore it is important to consider the cumulus cells when addressing oocyte metabolism. In women with high BMI there is elevated glucose and lactate in follicular fluid; however, the inverse correlation between glucose and lactate remained regardless of BMI indicating anaerobic glycolysis still occurred normally [33]. Even if the glycolytic pathway was not affected, other metabolic pathways in the COC such as the pentose phosphate pathway, hexosamine biosyntheis pathway, or polyol pathway could be altered [31]. Besides energy production, the COC uses glucose for nucleic acid and purine synthesis during maturation [79].

Glucose enters cells by through facilitative glucose transporters (GLUTs). There are 14 members of the GLUT family, GLUT1–12, the H^+ coupled myo-inositol-transporter and GLUT14 [80, 81]. The GLUTs exhibit a high degree of sequence homology but differ in their substrate specificity, kinetic characteristics and tissue and subcellular distribution[80]. GLUT4 is the primary insulin sensitive transporter and translocates to the cell surface to increase tissue glucose uptake. However, other glucose transporters besides GLUT4 may mediate insulin stimulated glucose uptake. The preimplantation embryo is insulin sensitive and expresses both the insulin and IGF-1 receptor [82]. In the blastocyst-stage embryo GLUT8 translocates to the cell surface in response to insulin and mediates insulin stimulated glucose uptake [83]. Additionally, recent data suggests that GLUT12 may also function as an insulin sensitive glucose transporter [84, 85]. It is unknown if GLUT8 or GLUT12 function in the oocyte or cumulus cells. The oocyte contains GLUTs 1, 3, and 8, but GLUT4 has not been reported in oocytes [31]. There are reports of GLUT4 in granulosa cells of the mouse by immunohistochemistry [86] in human granulosa cells by mRNA [33]; and in granulosa cells of sheep and cattle by mRNA [87, 88]. However, others have failed to detect GLUT4 in mouse or rat granulosa cells by western blotting and mRNA hybridization [89, 90]. Whether the cumulus cells or oocytes display insulin stimulated glucose uptake, and how insulin resistance may affect this process is unknown at this time.

While hyperglycemia is not always present with obesity and insulin resistance, results from some studies using the hyperglycemic type I diabetic mouse may be relevant to the discussion of glucose metabolism and obesity. Hyperglycemic conditions either in-vivo or in-vitro cause down-regulation of glucose transporters, decreased glucose transport into cells, abnormal metabolism, and increased apoptosis in cells of preimplantation embryos [91–93]. This hyperglycemia-induced apoptosis of progenitor cells in the embryo can affect differentiation of the remaining cells, manifesting later as malformations or miscarriages. Similar

events may occur in the oocyte as well as the surrounding cumulus cells of mice under hyperglycemic conditions. In mouse models of type I diabetes, oocytes exhibit altered mitochondria ultrastructure, increased mtDNA copy number, and reduced function of mitochondria; evidenced by reductions in TCA cycle metabolites [94]. Mitochondrial dysfunction has also been noted in the cumulus cells of type I diabetic mice, evidenced as decreased ATP and citrate, as well as increased apoptosis [95]. Using embryo transfer experiments, it is evident that maternal hyperglycemia present only up to the one-cell stage results in growth retardation and malformations in offspring [96]. It is clear that hyperglycemia alone has severe negative consequences for the oocyte. Thus far, few studies have investigated how the combination of both hyperglycemia and hyperinsulinemia in type II diabetes may affect oocyte competence.

Insulin

Two of the major functions of insulin that may affect oocytes and embryos are (1) its growth/anti-apoptotic effects on cells and (2) its metabolic ability to increase glucose uptake into cells. Both of these functions can be impaired during insulin resistance. The phosphatidylinsositol 3-kinase (PI3K) pathway mediates cell growth and survival, as well as insulin stimulated glucose uptake. It is present and functional in the preimplantation embryo [97] and studies conducted using embryos may shed light on what could be happening in the oocyte or cumulus cells where fewer studies have been conducted. Insulin signaling through the PI3K pathway mediates insulin stimulated glucose uptake in the blastocyst stage embryo, and inhibition of this pathway blocks insulin stimulated glucose uptake and increases apoptosis [98]. Elevated insulin or IGF-1 in-vitro can reduce insulin stimulated glucose uptake and lead to apoptosis in mouse blastocysts [99, 100]. In-vitro or in-vivo exposure of preimplantation mouse embryos to high IGF-1 also leads to decreased implantation rates on d 14.5 [101].

The ovary is a target organ for insulin [21] and both the oocyte and surrounding cumulus cells of mice contain the insulin receptor [102]. In humans the insulin and IGF-1 receptor have been found in the granulosa, theca, and ovarian stroma [21]. The PI3K pathway plays a role in oocyte maturation following the LH surge. During oocyte maturation Akt mRNA concentration in oocytes remains the same from germinal vesicle to MI, then drops following in-vivo maturation or EGF treatment. Phospho-Akt protein also decreased at the MII stage [103]. Other studies in COCs showed that inhibition of the PI3K pathway reduced glucose uptake in response to FSH, but did not investigate the role of insulin [86]. The terminal enzyme in insulin signaling, glycogen synthase kinase 3A/B (GSK3A/B) is present in denuded oocytes [102]. However, 19 h insulin treatment of denuded oocytes did not alter phosphorylation of GSK3A or B. The effect of insulin treatment on other enzymes in the insulin signaling pathway in oocytes or cumulus cells is unknown [102]. The IGF-1 knockout mice have decreased granulosa cell proliferation and decreased GLUT1 [89, 104], similar to results observed in blastocysts following PI3K inhibition [98]. Feeding mice a high fat diet for 16 weeks leads to weight gain and hyperinsulinemia, poor oocyte quality, and impaired ovulation [105]. Even after removal from the maternal environment and culture and fertilization in-vitro, oocytes from high fat fed mice had reduced development to blastocyst and abnormal embryonic cell differentiation (decreased inner cell mass to trophoblast ratio) [105]. This study also tested various insulin sensitizing agents and found that 4 days of treatment prior to mating with rosiglitizone improved embryo development to the blastocyst stage and normalized cell allocation to the inner cell mass [105]. Other insulin sensitizing agents such as AICAR; a glucose and lipid lowering AMP kinase activator, or sodium salicylate, an IKK inhibitor, did not have an effect on embryo development [105]. It is unclear if the effects on embryo development were due to improvements in systemic insulin sensitivity and glucose metabolism or due to a direct effect on the oocyte and embryo.

In women with high BMI (≥30) decreased embryo quality has been reported [43, 44]. Follicular fluid samples collected from women indicate that the insulin receptor substrate-2 (IRS-2) mRNA and GLUT4 mRNA were not affected by BMI, but were found in different components, with IRS-2 being more abundant in the cumulus and GLUT4 more abundant in the granulosa cells [33]. Other glucose and insulin regulated genes were similarly unaffected by BMI but did differ in their cell specificity [33]. The authors suggest that metabolic responses to insulin remain unaffected but lipogenic pathways are upregulated based on upregulation of two lipogenic genes (CD36 and SR-BI) in granulosa cells [33]. Cultured granulosa cells from women with insulin resistant PCOS had down regulation of insulin receptor protein, and insulin induced lactate production in media was reduced, indicating some insulin resistance [106].

Conclusions and perspectives

It is evident from both clinical data and animal studies that obesity negatively impacts the developmental competence of oocytes. This occurs through both disruption of systemic maternal endocrinology and metabolism, and direct effects on the oocyte. However, the complex interaction of how these various hormones and metabolites impact the oocyte

is still being actively researched. Fortunately, modest weight loss and exercise in obese women seems to ameliorate many of the negative impacts of obesity on reproduction [21, 107, 108]. Further research into the mechanisms of obesity-induced reproductive failure may provide avenues for pharmaceutical intervention when weight loss is not easily achieved. In particular, studies using models of both hyperglycemia and hyperinsulinemia and studies on lipotoxicity in oocyte and cumulus cells would be especially beneficial.

Conflict of Interest Disclosure The authors have no conflict of interest to report.

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