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Review article

Relationship between congenital anomalies and contraception

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Most contraceptive methods are associated with defined maternal risks. These risks can be balanced against the benefits, with individual users arriving at informed decisions concerning propriety of using a given method. Physicians and patients alike have grown accustomed to benefits/risks analysis of this type.

More difficult to assess, however, would be fetal risks associated with contraceptive use. In the past decade, increasing attention has been given to allegations that various forms of contraception pose fetal risks. Sometimes risks are claimed as a result of exposure during pregnancy (teratogenesis). At other times long-term risks are claimed after discontinuation of the contraceptive agent. That is, there are risks due to exposure prior to pregnancy (mutagenesis).

In this communication we shall review the various allegations, attempt to define the more reasonable possibilities in order to suggest future studies assuring contraceptive safety, and provide information suitable for counseling of patients who inadvertently become pregnant while using contraception.

Mechanisms response for human malformations: genetic and teratogenic

Congenital abnormalities may result from several genetic mechanisms: (1) changes in chromosome number or structure or both; (2) changes at a single genetic locus (Mendelian inheritance); (3) cumulative effects of genes at several different loci (polygenic inheritance); and (4) interactions between genes and environmental (multifactorial inheritance) [1]. Sometimes abnormalities occur as a result of a mutant gene or chromosomal abnormality that is transmitted from parent to child. At other times parents have no genetic abnormalities, but an aberration arises in the sperm or oocyte responsible for fertilization. In the latter event a fresh *mutation* is said to occur; the agent causing the abnormality is said to be *mutagenic*. In addition, abnormalities may occur as a result of exogenous agents (*teratogens*) that deleteriously affect an embryo that was otherwise destined to develop normally. Agents that are teratogenic may or may not also be mutagenic.

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Genetic principles

Chromosomal abnormalities may be numerical or structural. The pertinent terms are standard, but perhaps worth defining again. The most common numerical abnormality in liveborn infants is *aneuploidy* – deviations in chromosomal number as a result of missing or additional whole chromosomes. *Monosomy* refers to the absence of one chromosome; *trisomy* refers to the presence of an additional chromosome. In humans, autosomal trisomies (Nos. 8, 9, 13, 14, 21, 22) are compatible with life, although all except one (No. 1) have been detected among spontaneous abortuses [1]. Autosomal monosomy is probably never observed, even among abortuses. Aneuploidy usually results from a failure of chromosomes to disjoin properly during cell division (*nondisjunction*). This phenomenon is related to advanced maternal age, but the biologic explanation is unknown.

Another type of numerical abnormality that will prove relevant is a change in the number of haploid sets – *polyploidy*. Instead of possessing the normal diploid number of chromosomes (in humans 2n=46), an organism may be *triploid* (69 chromosomes in humans) or *tetraploid* (92 chromosomes). Polyploidy may result from double fertilization (dispermy), failure of extrusion of a polar body, or incorporation of a polar body into the zygote.

Chromosomes may be structurally abnormal, usually as a result of chromosomal breakage followed by loss or rearrangement of chromosomal material. Examples include deletions and translocations.

The other genetic mechanisms, Mendelian or polygenic/multifactorial, are probably more familiar to readers. *Mendelian* disorders can be inherited in autosomal dominant, autosomal recessive, X-linked recessive, or X-linked dominant fashion. The mode of transmission depends upon (1) whether the mutant gene can be expressed in single (*heterozygous*) form (dominant inheritance) or only in double (homozygous) form (recessive inheritance) and (2) whether the gene is located on an autosome or on an X chromosome. Other complexities are less important to the present discussion.

Polygenic inheritance cannot ordinarily be distinguished from *multifactorial* inheritance in humans, although the two can theoretically be distinguished. It is appropriate to invoke *polygenic/multifactorial* inheritance for disorders in which recurrence risks for first-degree relatives (sibs, parents, offspring) are 1–5%. This is less than the 25–50% expected for dominant or recessive inheritance but much more than the incidence in the general population. Disorders fulfilling such criteria are typically anatomic defects limited to a single organ system, e.g. cleft lip, cardiac defect, hypospadias, spina bifida. Most allegations that contraceptive agents are mutagenic or teratogenic involve anomalies inherited in this fashion.

Teratogenic principles

Whether an agent is *teratogenic* in humans proves surprisingly difficult to determine. Presence or absence of a teratogenic effect depends upon several factors: (1) the specific agent, (2) the dose, (3) the time during gestation at which the fetus is exposed, and (4) the genotype (genetic susceptibility) of mother and fetus. Some of these factors are obvious. It is clear that certain agents are more teratogenic than

others. It is equally obvious that a fetus exposed to a high dose of a potential teratogen is more likely to be affected than a fetus exposed to a low dose.

Perhaps less obvious is the importance of the time during gestation when a fetus is exposed. During the first few weeks of life (perhaps 2 weeks in man), the embryo is relatively resistant to teratogenic insults. A major insult may be embryotoxic; however, if the embryo survives, no organ-specific anomalies occur. Presumably the fate of early embryonic cells is not irrevocably determined. Thus, at this early stage the function of one or more dead cells can be taken over by other cells.

By contrast, organ differentiation is usually occurring between embryonic weeks 3–8 (gestational weeks 8–10). During this period susceptibility is maximum for exerting a teratogenic effect in an organ-specific fashion. Following organogenesis, embryonic and fetal differentiation is characterized more by an increase in organ size than by continued organogenesis. During this (third) stage, a teratogen can affect the overall growth of the fetus, or size of a specific organ, but it is unlikely to produce a visible malformation (brain and gonadal tissues are exceptions).

Finally, not all fetuses exposed to a given teratogen will manifest the same anomalies, or even the presence of any anomaly. Administration of cortisol to pregnant mice causes cleft palate in 100% of A/Jax offspring, but in only 12% of CBA offspring [2]. In humans, one illustrative example involved a white epileptic woman who was delivered of dizygotic twins sired by different men – one white, one black. The infant sired by the white male manifested the hydantoin embryopathy, whereas its cotwin was normal [3]. The susceptibilities of the two fetuses must have differed because both were exposed to the same teratogen – diphenylhydantoin.

Several types of epidemiologic studies have been conducted in order to investigate the potential teratogenicity of drugs and other agents. Unfortunately, they differ widely in validity, a fact not readily appreciated by the unwary. Ideally, one prefers prospective studies, either of entire populations or of a cohort. However, such studies are laborious, expensive, and sometimes uncover only a few informative subjects. Case control retrospective studies have thus proved popular. In such a study one matches women with a specified abnormal outcome (e.g. infant with a cardiac anomaly) with controls who, perhaps after multivariate corrections, differ only with respect to the variable under investigation. Is drug exposure cited more frequently by the mothers of cases than by mothers of controls? The major pitfall in such a design is that women whose pregnancies resulted in an abnormal outcome search much harder for factors potentially responsible. Thus, case control studies inevitably yield spurious positive associations, a phenomenon called *recall biases*. Other biases exist as well [4]. Studies conducted prospectively with respect to fetal outcome should mitigate this bias. However, in some so-called prospective studies patients were interviewed before pregnancy outcome was known, yet months after drug exposure. Thus, recollections about drug exposure might still not be accurate. Finally, in neither retrospective nor prospective studies has infant morphology ordinarily been systematically assessed.

Oral contraceptives: claims of teratogenicity

Progestational and estrogenic components in oral contraceptives have been implicated as both teratogens and mutagens. Similar considerations must, by extension, be entertained for injectable or implantable progestins (medroxyprogesterone, norethisterone). In reality this author has found no substantive evidence for either oral contraceptives or implantable/injectable progestins being mutagenic or teratogenic. Of course, it is true that certain relevant progestins, namely the 19-nor-testosterone norethisterone and probably norgestrel (but not norethynodrel), can virilize female fetuses if administered in high doses at susceptible periods of pregnancy. (Details are provided elsewhere by the author) [5]. However, the doses required for virilization (e.g. 20–40 mg/day of norethisterone) are considerably in excess of those present in oral contraceptives unwittingly ingested by women already pregnant. Thus, virilization is truly not a practical consideration in the present context. Rather, we shall focus upon allegations that oral contraceptives cause hypospadias, cardiac defects, limb reduction defects, neural tube defects, pyloric stenosis, and indeed a generalized increase in anomalies.

In doing so, it would be desirable to consider separately the results of exposures to progestins only, estrogens alone, and oral contraceptives. Only a few of the available studies permit such analysis. The more typical experimental design has been to pool outcomes following exposure to a variety of sex hormones. Actually, the necessity of pooling exposures is not likely to pose serious problems for our present purpose – assessing risk of oral contraceptives or implantable/injectable agents. The only estrogen implicated as a teratogen is diethylstilbesterol, which is not a component in contraceptive agents, and the specific progestin seems unimportant with respect to somatic (non-genital) defects. Thus, extant data are less than ideal, but still serviceable.

Samples that have been used to assess the teratogenicity of progestins include pregnancies characterized by (1) administration of progestins for pregnancy maintenance (usually 20–40 mg/day); (2) administration of progestins for pregnancy diagnosis, based upon presence or absence of withdrawal uterine bleeding following 5 days of moderately high doses (10–20 mg/day); and (3) inadvertent progestin exposure (1 mg/day) during unrecognized gestation, associated either with oral contraceptives or progestogen-intrauterine devices. At present the former two indications are no longer appropriate. Nonetheless, one can retrospectively identify populations of fetuses exposed when those indications were valid. Of the prospective studies, Table 1 summarizes the larger samples.

Before systematically beginning our review of anomalies claimed to be associated with exposure to progestins, it should be added that almost no data specifically relate to injectable/implantable progestins. The one relevant study was conducted in Thailand [22]. Between 1975 and 1978, 190 of 8816 infants born in Chiang Mai, Thailand, were anomalous. The mothers of the 190 infants used medroxyprogesterone in the same proportion as mothers of the 8626 normal infants, but whether any fetuses were exposed during gestation is not known. Even fewer direct data exist concerning injectables or implantable norethisterone or norgestrel, but similarly no direct claims of teratogenicity exist to my knowledge.

Table 1 Major prospective studies evaluating effects of progestin exposure during pregnancy. The clear consensus is that progestins in the doses received were not teratogenic. Modified from Simpson [5]

Investigator	*Sample*	*Control*	*Anomalies*	*Comment*
Spira *et al.* (1972) [9]	9566 women, interviewed in the 3rd month, who received hormones (mostly for pregnancy support or diagnosis) (France)		171/9566 (1.8%)	Anomalies equally frequent in exposed and unexposed pregnancies
		8387 not receiving hormones	168/8387 (2.0%)	
Harlap *et al.* (1975) [10]	11 468 women, 432 receiving 'hormones' (Israel)		47/432 (10.9%) all anomalies, 21/432 (4.9%) major anomalies only	Small increase (25%) ($p<0.02$) observed, but recall bias possible because interviews were months after exposure
		11 036 unexposed	925/11 036 (8.4%); 426/11 036 (3.9%), major only	
Kullander and Källen (1976) [11]	6379 pregnancies from which 194 mothers had abnormal infants (Sweden)		5/194 exposed to progestogen (2.6%)	Exposure rates similar in both groups
		5002 women delivered normal infants	98/5002 exposed to progestogen (2.0%)	
Royal College of General Practitioners (1976) [12]	136 pregnancies conceived during oral contraceptive therapy		2/136 (1.5%)	No differences among groups
		11 009 pregnancies in nonusers	177/11 009 (1.6%)	
		5530 pregnancies in previous contraceptive users	86/5530 (1.6%)	
Goujard and Rumeau-Rouquette (1977) [13]	12 895 mothers interviewed in the first trimester, of whom 1165 were exposed (France) (same population as Spira *et al.*)		5/335 (1.5%) 'testosterone derivatives'; 15/830 (1.8%), 'progesterone derivatives'	Chromosomal anomalies excluded from analysis; no differences observed either overall or after separate analysis for cardiac and skeletal defects
		9822 nonexposed	160/9822 (1.6%) nonexposed	

continued

Table 1 (continued)

Investigator	*Sample*	*Control*	*Anomalies*	*Comment*
Heinonen *et al.* (1977) [14,15]	Collaborative Perinatal Project (50 282 women) 1958–66, of whom 1042 were exposed to 'sex hormones' and 866 to progestogens only (United States)		19/1042 (1.8%) cardiac after any sex hormone exposure; 75/866 (8.7%) all anomalies after progestin exposure alone	No significant differences for total anomalies but significantly increased for cardiac anomalies alone (relative risk 2.3, $p<0.05$). Relative cardiac risk 1.8 for progestogens alone ($p<0.05$). However, some infants exposed only during 1st lunar month; others exposed during 4th lunar month
		49 240 not exposed to any sex hormones; 49 416 not exposed to progestogens	385/49 240 (0.8%) cardiac; 3172/49 416 (6.5%) all anomalies	
Nora *et al.* (1978) [16]	118 women who received hormones in 'first trimester' (United States)		16/118 (13.6%)	Probably not truly prospective for controls, with bias toward unrecognized exposure in controls. Exposure interval not well defined
		At time of delivery of exposed women, 'control infant without . . . exposure . . . selected'	4/118 (3.4%)	
Torfs *et al.* (1978) [17]	Over 18 000 women, of whom 203 had 'hormonal pregnancy tests' (United States)		9/203 (4.4%)	No significant differences among groups
		689 with serum pregnancy tests;	30/617 (4.4%)	
		332 with urine pregnancy tests;	9/332 (2.7%)	
		17 057 with no pregnancy tests	650/17 047 (3.8%)	

continued

Table 1 (continued)

Investigator	*Sample*	*Control*	*Anomalies*	*Comment*
Goujard *et al.* (1979) [18]	3451 women, of whom 133 used progestins (France)		5/133 (3.8%)	Four of 5 anomalies occurring in subset of 35 women who used testosterone derivatives
		3318 nonexposed	3318 (2.3%) overall	
Vessey (1979) [19]	66 pregnancies conceived while on oral contraceptives (United Kingdom)	None	1/66 (1.5%)	
Savolainen *et al.* (1981) [20]	3002 mothers of malformed infants, of whom 38 conceived while receiving 'pills' (Finland)			Anomaly rates similar in sample and control, both for previous or concurrent contraceptive use
		3002 matched controls		
Michaelis *et al.* (1983) [21]	13643 pregnancies; about 10% of whom received hormones for diagnosis or support		4/320 (1.3%), progesterone alone; 11/610 (1.8%), progesterone and estradiol	No significant difference between exposed cases and their unexposed matched controls
		Matched controls within same population who were not exposed		

Hypospadias

Aarskog [23] claimed that progestins and medroxyprogesterone in particular cause penile or perineoscrotal hypospadias. Although Aarskog's data [23] are uncontrolled, two other studies later supported his hypothesis. A Latin American case control study [24] claimed a relative risk of 2.4 for hypospadias being associated with progestin exposure. Of 314 cases, 24 (7.6%) were exposed to one of the various progestins; 12 of 319 controls (3.8%) ($p<0.05$) were exposed. However, few details concerning the time of exposures were provided, and incidences varied widely among the reporting countries. A second study from Hungary reported that 28 of 294 mothers delivered of males with hypospadias received sex hormones, compared to 12 of an unspecified number of controls [25]. This difference was said to be significant; however, there is little confidence that controls were well matched, and a high prevalence of hypospadias in the proband's male relatives suggests selection bias.

In contrast to the two case control studies, a larger number of similar or better-designed studies failed to show an association between MPA or progestins and hypospadias [26–28]. Moreover, not a single prospective study (Table 1) has shown a relationship between progestins and hypospadias.

In conclusion, only a few studies have claimed a relationship between progestins and hypospadias, and more extensive case control studies have failed to confirm such an association. No prospective study found an effect. Progestins seem unlikely to adversely affect male genital development.

Cardiac anomalies

That progestins could be cardiac teratogens was initially claimed by Levy [29] in 1973. His case control study revealed that 7 of 76 mothers delivered of infants with transposition of the great vessels received 'hormones' in the first trimester. Significantly fewer (0/76) controls were exposed ($p<0.007$). Nora and Nora claim similar findings in a series of overlapping studies that were initially retrospective but later more prospective in design. In one case control study [30], 20 of 224 mothers delivered of infants with cardiac defects recalled receiving an estrogen/progestin compound, compared to only 4 of 262 controls ($p<0.001$). Nora then began a prospective study [16]. No significant differences were observed between the first 60 mothers and their controls. Despite these negative findings a second study was conducted with two controls per subject. In the second study, 31 of 176 mothers with affected offspring received hormones, compared to only 21 of 352 control mothers ($p<0.001$).

A few other case control studies have revealed positive correlations. In one, Janerich *et al.* [31] identified infants with cardiac defects through birth certificates. Of 104 mothers of affected infants, 18 received hormones; 16 were prescribed for pregnancy diagnosis and 2 were the result of inadvertent contraceptive use. Significantly fewer controls reported exposure. Cardiac anomalies were also among the anomalies said to be responsible for the finding by Greenberg *et al.* [32] of a relationship between progestins and generalized anomaly rates.

The US Collaborative Perinatal Project reported a positive association [14,15], and is the study that has generated the most attention in the United States. Of 1042 offspring said to have been exposed to 'sex hormones' during their gestation, 19 had cardiac defects (1.82%); 385 of 49240 (0.78%) unexposed offspring were affected (relative risk 2.3, $p<0.05$). Too few cases existed to allow analysis by specific hormones; however, exposure to progestins only had a lower but still significantly increased relative risk (1.8, $p<0.05$). Strangely, continued contraceptive use during the second and third lunar months was associated with a relative risk of 2.4, yet exposure to progestogens only in the same interval was associated with a risk of 1.5 and exposure to estrogens only with a risk of 1.4.

In contrast to these claims are several contrary case control reports, and the prospective studies summarized in Table 1. To take an example, Spira *et al.* [9] followed 20000 French women throughout pregnancy. Almost half (9566) received hormones, usually for pregnancy diagnosis or pregnancy maintenance. The anomaly rate in the exposed subjects did not differ from those in the unexposed group. In a later tabulation of the same population (12764 women) by Goujard and Rumeau-Rouquette [13], cardiac anomalies were no more frequent in exposed (43%) than unexposed (41%) mothers. Also impressive is the failure of Nishimura *et al.* [38] to detect cardiac anomalies in 108 microdissected embryos exposed to

hormones. By contrast, several controls had cardiac defects. Studies in Finland [20], Germany [21], Sweden [11], Great Britain [12,19], and the US [17] similarly reveal no increase in cardiac defects.

If the consensus fails to implicate progestins as cardiac teratogens, why did a minority of studies arrive at ostensible contradictory conclusions, generating fervent opposing opinions? Unavoidable statistical vicissitudes and differing genetic susceptibilities notwithstanding, methodological shortcomings are likely to furnish the explanation for those studies purporting to show positive associations between progestins and cardiac defects.

First, we have already suggested that recall biases are inherent in all retrospective (case control) studies. Investigations of Janerich *et al.* [31], Levy *et al.* [29], and the Noras [30] all potentially suffer from this bias.

Second, reasons for the attempt to maintain pregnancies, often the reason for administering progestins, were not sought.

Third, prior pregnancy outcome was rarely if ever taken into account. In fact, the birth of one child with a cardiac defect confers an increased risk (1–4%) in subsequent pregnancies. The increased risk might even pass unrecognized if a cardiac defect had unknowingly been responsible for a stillborn infant. Even more relevant is that occurrence of a stillborn infant in a previous pregnancy could tempt some obstetricians to administer hormones empirically.

Fourth, hormones could have been administered in pregnancies already manifesting problems (e.g. bleeding) indicative of underlying defects. Indeed, Matsunaga and Shioto [36] provide embryologic data indicating that not progestins, but rather the bleeding for which progestins were administered, was responsible for cardiac defects.

Fifth, few studies restrict analysis to the interval in embryogenesis during which exposure could produce cardiac defects. In the analysis of the US Collaborative Perinatal Project, the first 4 lunar months were considered that interval during which exposure could have produced anomalies. However, the first lunar month includes the 2 weeks before conception and the 2 weeks after. During this interval (all-or-none period), anomalies cannot ordinarily be produced. By the fourth lunar month, heart formation is complete. Among the 19 infants with cardiac defects identified in the Collaborative Perinatal Project, 4 were exposed only during the first lunar month and 3 were exposed only during the fourth month. Excluding these 7 cases would probably abolish any significant association with hormone exposure. In the only other prospective study claiming a positive association between progestins and abnormal outcome, namely that of Harlap *et al.* [10], the first lunar month exposure was also considered to be a susceptible interval.

We can thus conclude that not only do most retrospective and all but one prospective study fail to support the hypothesis that progestins are cardiac teratogens, but serious methodological flaws exist in the few reports claiming positive associations. The magnitude of the observed differences in the few positive case control studies seems consistent with recall biases, and the few prospective studies claiming effects are marred by potential selection biases and by inattention to exposure intervals. A prudent conclusion is that progestins are not cardiac teratogens.

Limb reduction deformities

Shortening or absence of a limb, finger, or a toe (limb reduction defects) has been alleged to be associated with progestin exposure. This possibility was first raised by Janerich *et al.* [37] who reported that 15 of 108 women with an affected infant received hormones (inadvertent oral contraceptive exposure, hormone pregnancy test, or hormones for pregnancy maintenance). Only 4 of 108 controls were exposed ($p<0.05$). Greenberg *et al.* [32] claimed an overall increase in anomalies following progestin exposure, apparently contributed in part by limb reduction defects. However, these studies invite invalidation because of pronounced recall bias. Interviews were sometimes conducted as long as 10 years after birth! In the study of Janerich *et al.* [37] some exposures may also have occurred during the all-or-none period.

By contrast, other case control investigations have failed to show an association betwen limb reductions and maternal hormonal exposure. Bracken *et al.* [26] showed no statistically significant association, and Oakley *et al.* [41] failed to observe a relationship in an especially well-constructed case control study. Controls in the latter study included women delivered of offspring with chromosomal abnormalities, a design presumably obviating recall biases.

Prospective studies have similarly failed to confirm an association (Table 1). Strengthening the value of prospective studies is that missing digits or severe limb shortening should be obvious to even the casual observer, unlike cardiac defects. Finally, Nishimura *et al.* [38] failed to observe limb reduction deformities in their microdissection of 108 embryos recovered from progestin-exposed mothers.

To recapitulate, the well-publicized report of Janerich *et al.* [37] was followed by a variety of studies failing to confirm a relationship between progestin exposure and limb reduction defects. This reassurance is enhanced by its being derived in part from studies in which exposure levels were much higher than those expected with injectable/implantable progestins or oral contraceptives.

Neural tube defects (NTD)

Neural tube defects – anencephaly and spina bifida – were claimed almost a decade ago to be associated with hormone exposure. The claim is now almost completely discounted, but worth a brief mention.

Using a case control design, Gal *et al.* [39] reported that 19 of 100 women delivered of infants with myelomenigocele or hydrocephalus received hormones (estradiol plus ethisterone or norethisterone) for pregnancy diagnosis; only 4 of 100 controls recalled hormone exposure ($p<0.01$). Unfortunately ignored in this study was prior pregnancy history, especially relevant because in the United Kingdom NTD recurrence risk is 5%. Also not considered was the stage of embryogenesis at which exposure occurred. The neural tube closes at 28 embryogenic days, earlier than attempts to diagnose pregnancy are usually made and, hence, earlier than hormones would ordinarily have been administered for such purpose. The one later study showing a possible relationship to NTD was that of Greenberg *et al.* [32]. The anomaly rate in the hormone-exposed group was increased, 25 of 93 malformed infants showing NTD.

On the other hand, other case control studies showed no association between NTD and progestins. A United Kingdom study was that of Laurence *et al.* [40], who showed no significant increase in a sample much larger than that of Gal *et al.* [39]. Another large negative case study is that of Bracken *et al.* [26]. Not a single prospective study showed an association between progestins and NTD. Also significant in view of the tendency of NTD fetuses to abort and, hence, possibly fail to be detected, is the absence of NTD in 108 progestin-exposed embryos microdissected by Nishimura *et al.* [38].

The hypothesis that progestins cause neural tube defects thus currently receives no support.

Other anomalies

A final consideration is whether a generalized (nonspecific) increase in anomalies occurs after progestin or estrogen exposure. Evaluating overall anomaly rates as an end point is hazardous because of etiologic heterogeneity. Moreover, it is unlikely that a generalized increase would exist without one or more organ systems subsequently being identified as primarily responsible.

Excess anomaly rates of a nonspecific nature have been claimed following oral contraceptive exposures in some case control studies. One study claiming an association is that of Greenberg *et al.* [32]. We have alluded before to this work, actually of uncertain validity because only a small and, hence, possibly unrepresentative proportion of eligible women participated. By contrast, no significant associations were found in case control studies of Bracken *et al.* [26] and Oakley *et al.* [41]. The frequency of anomalies was not increased in 541 'pill-failure' pregnancies pooled by Harlap and Eldor [42].

Only 2 of 15 prospective studies [6,12] showed an association. One is the previously cited US Collaborative Perinatal Project, as analyzed by Heinonen *et al.* [14,15]. There existed a significant association with 'hormones, hormone antagonists, and contraceptives', but not with 'progestational agents' alone. The Israeli prospective study of Harlap *et al.* [10] also showed a small increased relative risk. Thirteen other prospective studies failed to show any generalized increase (Table 1).

A refinement of the claim that an overall nonspecific increase in anomalies exists is the belief that the complex of vertebral, anal, cardiac, tracheoesophageal, renal and limb (VACTERL) anomalies is associated with maternal progestin exposure [43]. Defects involving any 3 of the 7 organ systems are said to justify the diagnosis. A further variant is the claim by Lorber *et al.* [44] for an 'EFESSES syndrome' (embryo-fetal exogenous sex steroid exposure syndrome), characterized by various dysmorphic features. Geneticists do not appear to subscribe seriously to the EFESSES concept, but the VACTERL complex requires comment.

After some prior anecdotal reports, Nora *et al.* [16] reported significantly increased frequency of progestin exposure in 30 VACTERL probands (11 exposures), compared to 60 controls (5 exposures) [16]. Not only is the sample size small, but recall bias would surely be amplified in mothers whose infants had multiple malformations. Knowledge of the purported relationship to progestin exposure

may also have tempted referring physicians to diagnose the VACTERL association. Even more importantly, we have already refuted individually claims of cardiac and limb reduction components of VACTERL. Other case control studies have also failed to reveal a significant relationship between progestin exposure and other VACTERL components, such as esophageal atresia [45]. In prospective studies, investigators have specifically sought and failed to observe the VACTERL complex [20,21].

Little evidence thus exists in support of the hypothesis that progestin exposure causes a generalized increase in malformations. This is the conclusion of not only this author but others [5,46–48]. The validity of the VACTERL association and its association with progestins seems doubtful.

Oral contraceptives: claims of mutagenicity

In addition to direct effects on the developing embryo (teratogenesis), a deleterious agent can cause abnormalities by inducing changes (mutations) in individual germ cells. A child subsequently conceived with that germ cell could be anomalous. Induction of both gene mutations and chromosomal abnormalities have been claimed.

Gene mutations

Whether progestins and estrogens result in mutations responsible for Mendelian or polygenic disorders among progeny conceived months or years later should ideally be assessed locus by locus, for the mutability almost certainly varies. However, this proves mathematically impossible, given even a large increase over baseline mutation rates of 10^{-5} to 10^{-6}/gamete/generation [1]. As a result, one must be content to compare overall anomaly rates in women exposed and not exposed to hormones. Additionally, most studies pool anomalies of Mendelian, polygenic, chromosomal, and environmental etiology. This approach is obviously less than ideal, for which reason it is fortunate that available data are sufficiently reassuring to minimize fears that more refined analyses will yield surprises.

There are several studies of offspring of women who used oral contraceptives prior to conception. All have failed to detect evidence for mutagenicity, the total samples surveyed being over 20 000 [47]. Further verifying the safety is the failure of national surveillance reports to record an increase in any Mendelian disorder after the introduction of oral contraceptives.

Sometimes overlooked as a source of data concerning gene mutations is the simple yet powerful analysis of sex ratio. Induction of lethal X-linked recessive mutations decreases the proportion of liveborn males. Mutations at any X-linked loci would contribute to the decrease. Moreover, observations would have general applicability because agents inducing X-linked mutations would also induce autosomal mutations. In fact, several large cohort studies provide no evidence of an altered sex ratio, contradicting some earlier studies of much smaller sample size [47].

Finally, neither progestins nor estrogens are representative of those classes of compounds known to be mutagens. Progestins do not yield mutations in the Ames test [49]. Incidentally, failure of progestins to induce mutations causing anomalies offers reassurance against unexpected mutagenic effects (e.g. cancer) later in life.

Numerical chromosomal abnormalities

That prior hormonal exposure could induce chromosomal abnormalities has been the subject of surprisingly intense investigation. Actually the hypothesis is not unreasonable. Oocytes remaining in dictyotene of meiosis I until ovulation might complete meiosis sluggishly as a result of hormone exposure resulting in abnormalities that would be chromosomal.

Initial concerns were generated by Carr [50], who reported an increase in chromosomally abnormal abortuses among women previously using oral contraceptives. In such women, 48% of abortuses were chromosomally abnormal, compared to only 22% in controls. Most of the excess was due to polyploidy. However, these observations were not confirmed in several later studies. Boué *et al.* [51] reported 16% abnormal complements (16% polyploidy) among 243 previous contraceptive users, compared to 63% (18% polyploidy) among 604 controls. Lauritsen [52] found a slight excess in those women previously using oral contraceptives (61% v. 49%). However, even this insignificant increase was due to monosomy X and structural abnormalities, and not polyploidy as Carr [50] had reported. Alberman *et al.* [53] observed 32% abnormalities in 524 prior users, compared to 26% in 428 controls. Dhardial *et al.* [54] found differences of a similar, but again insignificant, magnitude. In induced abortuses, Klinger *et al.* [55] similarly noted an insignificantly higher frequency in prior contraceptive users (1%) than in controls (0.5%).

Failure to confirm Carr's initial findings [50] is especially noteworthy because any of several biases would spuriously yield increased abnormality rates in exposed groups. First, unrecognized induced abortuses are surely more likely to be included inadvertently among controls than are women who ceased contraception in order to achieve pregnancy. Indeed, surreptitious illicit abortions furnish a highly likely explanation for the unusually low (22%) frequency of chromosomally abnormal abortuses in Carr's controls. Second, ovulation may be delayed immediately after contraception is continued. This will unwittingly produce pregnancies less advanced than in controls, who are more likely to have normal cycles. Because the frequency of chromosomal abnormalities is in general inversely related to gestational age, unrecognized earlier gestational ages would lead to ostensibly higher abnormality rates among prior contraceptive users.

Still other data suggest that oral contraceptives do not induce numerical chromosomal abnormalities. First, if prior contraceptive use leads to chromosomal abnormalities, the absolute frequency of spontaneous abortions should increase after hormonal discontinuation because 50–60% of first-trimester abortuses show cytogenetic abnormalities. To the contrary, several large prospective studies show no increase in abortion rates. Second, increased frequency of liveborns with Down's syndrome should be evident because trisomy in both abortuses and liveborns is

presumably caused by the same cytologic mechanism (nondisjunction). Neither case control nor prospective studies have shown any increase.

Thus, initial concern generated by Carr's observing an apparent excess of polyploid abortuses following cessation of oral contraceptives has abated. The consensus is that progestins do not predispose to chromosomally abnormal abortuses.

Structural chromosomal abnormalities

Several *in vitro* studies have claimed increased chromosomal breakage in lymphocytes of contraceptive users, raising the possibility that progestins or estrogens could be clastogenic [5]. Recall, however, that breakage studies are hazardous without rigorous 'blind' analysis. Confounding experimental variables are also legend. Reassuring is the lack of an increase in structural chromosomal abnormalities in the abortus studies cited above. Similarly, there is no evidence for an increase in anomalous liveborns. Finally, structural, chromosomal abnormalities in liveborns would be expected to contribute to an increased overall anomaly rate, a phenomenon we have already concluded does not exist.

In conclusion, the possibility of increased structural chromosomal abnormalities occurring in increased frequency among offspring of women previously exposed to oral contraceptives seems extremely remote.

Intrauterine devices (IUD): claims of teratogenicity

Although various maternal hazards have been claimed for intrauterine devices, a relationship to congenital malformations appears not to have been raised seriously until 1976. Prior to that time investigators seemed confident that there was no reason to suspect either teratogenic risks (i.e. anomalies associated with IUD remaining *in situ* during gestation) or mutagenic risks (i.e. anomalies induced in progeny conceived after IUD removal).

In 1976, Barrie [56] reported two infants whose mothers were using intrauterine devices (one Gräfenberg ring, one probable Dalkon Shield) at conception. Both devices had remained *in situ* throughout pregnancy, and both infants showed limb reduction defects (upper and lower extremities). Leighton *et al.* [57] later reported a further case associated with a copper-containing IUD.

Claiming that limb reduction defects result from a coexisting IUD is indeed superficially an attractive hypothesis. The author is aware of at least one animal study [58] consistent with a teratogenic relationship. Fetuses born of rats wearing a Silastic device showed increased anomaly rates compared to animals without devices. Further reflection reveals, however, that the IUD would need to penetrate both chorion and amnion to exert a direct mechanical action on the early embryo. Of course, infections could arise; however, the likely outcome of infection in early pregnancy is abortion. Even if the developing pregnancy envelops the IUD later in gestation, limb reduction defects and most other anomalies seem unlikely to arise. Indeed, amniotic bands and their sequelae would make a more plausible hypothesis.

Limb reduction defects probably invoked a special emotional response with respect to teratogens. Recalling the well-known association of phocomelia and thalidomide, the casual observer might think that limb reduction defects in general are likely to be caused by teratogens. In reality, limb reduction defects are very heterogeneous, and, like most defects limited to a single organ system, show heritable tendencies consistent with polygenic/multifactorial inheritance. Thus, postulating IUD-associated teratogenesis is initially reasonable, but upon further reflection is less reasonable.

Formal studies also reveal that IUDs are not teratogenic, at least not to any serious extent. Longitudinal studies of pregnancies conceived despite a coexisting IUD can be assessed. Tatum *et al.* [59] evaluated the outcome of 918 women conceiving while wearing a Copper T device. After excluding the 465 who chose elective abortion, abortion rates and anomaly rates were assessed. The spontaneous abortion rate was 20.3% (24 of 118) with the device removed or expelled and 54.1% (85 of 157) with the device *in situ*. The stillborn rate was 0.9% with removal and 1.9% without removal. However, only 1 anomaly (vocal cord fibroma) was observed among 166 embryos that developed to the stage at which anomalies could be detected. Albert [60] also failed to observe increased anomalies in women conceiving with copper devices. Snowden [61] reported no anomalies in 317 pregnancies conceived in the United Kingdom with IUDs (20 centers; 25 000 insertions). Guillebaud [62] reported 5 disparate anomalies among 167 full-term infants whose mothers had an IUD *in situ* throughout pregnancy. The initial sample consisted of 20 684 insertions and 714 pregnancies, prior to spontaneous and induced abortions. All 5 anomalies were distinct (bald spot, eyelid ptosis, lipoid tumor, congenital hip dislocation, spina bifida). At least four other authors have also failed to detect an association between IUDs and anomalies [63–66].

Because numbers of exposed subjects are inevitably small in longitudinal studies, case control designs have been utilized. As noted already (section on oral contraceptives), case control studies are sensitive in detecting existing associations but less specific in excluding other explanations. Layde *et al.* [67] conducted a case control study of 96 mothers delivered of infants with limb reduction defects. There existed no significant increase in IUD usage at time of conception. The exposure frequency was 2.1% (2 of 96) in the limb reduction group, 1.6% (15 of 915) in mothers of infants with all other major anomalies, and 1.2% (2 of 169) in mothers of infants with chromosomal abnormalities. The latter group served as an 'anomaly group' to obviate recall bias. A second case control study is that of Bracken and Vita [68], who conducted a large case control study of malformed and control infants in Connecticut. Of 2191 anomalous infants in the original sample, 1427 mothers were interviewed; 3001 controls were identified. Contraceptive usage of all types was sought, with comparisons made of IUD and other nonhormonal types. The only significant association among 154 categories was that of multiple malformation, but only 2 mothers wearing IUDs at conception were in the category.

In conclusion, pregnancies in women wearing an IUD are known to be associated with relatively increased frequencies of ectopic pregnancy and spontaneous abortions. However, infectious pathogenesis rather than teratogenic factors seems the likely explanation. Nothing other than anecdotal reports suggest an increased frequency of anomalies.

Vaginal spermicides: claims of mutagenicity

A teratogenic effect for spermicides has been claimed but is not particularly reasonable because women conceiving on spermicides should soon appreciate their pregnancy and stop using spermicides. Even continued use until the 'missed' menses would generally result only in exposures during the 'all-or-none' period, an event not likely to result in anomalies. Of course, a few women might fail to recognize pregnancies until several months later. Maternal storage of alcohol, mercury, nonoxynol and other spermicidal agents is also a formal consideration.

On the other hand, a *mutagenic* effect on sperm responsible for fertilization deserves more serious consideration, if spermicidal action is the rationale for using vaginal foams and similar agents.

The 1981 report of Jick *et al.* [69] seems to have been the first claiming an association between spermicides and anomalies. This well-publicized report involved a study of prescriptions filled by participants in a health maintenance organization in Seattle. Of 4772 pregnant women, 790 (17%) filled prescriptions for spermicidal contraceptives. Among 4665 liveborns were 84 anomalous infants. Of the 84, 18 infants were excluded because their defects were 'familial, minor, or positional'. Of the remaining 66, the frequency of spermicide prescription was 2.2%, compared to only 1.0% in the remaining women. An excess of cases existed in four groups: limb reduction defects, neoplasms, chromosomal abnormalities, and hypospadias.

A study of this type is never intended to do other than generate hypotheses, which the publicity associated with the Jick *et al.* study assured [69]. Most glaring among the deficiencies of experimental design is lack of verification that women even used the spermicides, especially during the cycle of conception or early pregnancy.

Two other reports claimed a relationship between spermicidal usage and anomalous liveborns. Smith *et al.* [70] also observed an association between limb reduction defects and spermicides. Rothman [71] found increased spermicidal exposure in Down's syndrome infants having cardiac defects, compared to normal controls. However, spermicide use was not increased in mothers of infants with cardiac defects not due to Down's syndrome. Thus, the increased association in the Down's syndrome group is probably the result of recall bias.

In contrast to the above reports implicating spermicides in birth defects are at least seven studies failing to show an effect [68, 72–77]. Several are well designed and deserve comment. First, the study of Bracken and Vita [68] has already been described. No association with spermicide use was observed. A second noteworthy study is the review by Shapiro *et al.* of the US Collaborative Perinatal Project data [75], a sample also described earlier. In that sample, 462 women had used spermicides other than phenylmercuric acetate, usually nonoxynol-9 (74%) or octoxynol (18%). The 462 reported use of spermicides during the first 4 lunar months, with 438 also reporting use during the month preceding the last menstrual period. Thus, both mutagenic and teratogenic effects were possible. The 462 exposed women were delivered of 23 anomalous offspring (5%), compared to 2254 anomalies among 49825 nonexposed controls (4.5%). The difference is not

statistically significant, nor was analysis when anomalies were grouped according to those claimed significant in the study by Jick *et al.* [69]: Down's syndrome, hypospadias, and limb reduction defect. Similar (negative) findings were also observed when analysis was restricted to women exposed to phenylmercuric acetate.

Polednak *et al.* [72] also used the case control approach to address specifically the anomalies implicated by Jick *et al.*, again finding no association. The relative risks were 0.43 for limb reduction defects (n=108), 1.10 for hypospadias (n=99), and 1.17 for Down's syndrome (n=103). No significant differences were observed either for neural tube defects (n=201), cardiovascular defects, and 'multiple defects'.

The most extensive search specific for a spermicidal effect is the latest study of Mills *et al.* [73], a case control study involving 34 660 women. Of these, 3146 had used spermicides before but not after their last menstrual period; 2282 used spermicides only after their last menstrual period. Confounding variables and other contraceptive usages were documented, and analysis by time of exposure (before or after last menstrual period) was conducted. No significant differences between controls and spermicidal users were noted for 157 types of malformations, nor for infants with 3 or more anomalies or a pattern of anomalies. Mills *et al.* also noted no change in the sex ratio following spermicidal exposure, offering reassurance concerning lethal X-linked recessive mutations and thus lethal mutations in general [77].

A few other fetal effects of spermicides have been claimed but not substantiated. Two studies [78,79] found an increase in fetal losses, but this was likewise not confirmed by Mills *et al.* [77]. A significant reduction in female (but not male) birth weight was found by Polednak *et al.* [72] but again not by others in larger samples [77].

Despite immense publicity and legal claims to the contrary, a clear scientific consensus exists. Vaginal spermicides are not associated with increased malformations in offspring of women who become pregnant either while using these agents or having just immediately discontinued usage prior to pregnancy.

Rhythm and delayed fertilization

Reproduction requires sperm, ovum, and synchronous union between the two. If coitus occurs relatively frequently, spermatozoa are ejaculated shortly after maturation, and neither sperm nor ovum is likely to languish in the female tract prior to fertilization. If coitus occurs infrequently, however, spermatozoa will inevitably age within the male tract, and either sperm or ovum could persist for prolonged periods in the female tract prior to fertilization. These relationships are illustrated in Figure 1.

Opportunities for delayed fertilization arise not only among those who have intercourse infrequently of their own volition, but among those who deliberately abstain for certain intervals. The latter circumstance occurs among couples who utilize rhythm for contraception. If ova and spermatozoa undergo aging as described, the effects may be (1) incompatible with fertilization, (2) compatible with

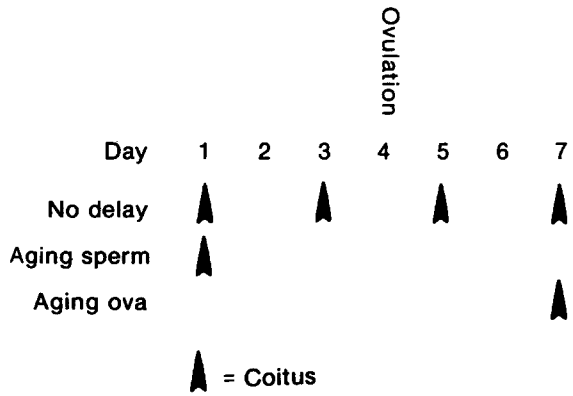

Figure 1 Diagrammatic representation of the consequences of infrequent coitus. If coitus occurs every other day it is unlikely that an ovum or sperm involved in fertilization would be retained in the female tract more than 48 hours. By contrast, coitus on day 1 only could lead to fertilization after retention of sperm in the female tract for 3 days. Likewise, coitus on day 7 might lead to fertilization 3 days after ovulation (delayed fertilization). From Simpson [80]

fertilization but incompatible with subsequent embryonic survival, or (3) compatible with survival, but not with normal development. The last possibility would obviously be cause for concern.

The above scenario is not idle speculation. A large body of animal data supports the occurrence of embryonic abnormalities secondary to fertilization involving aging sperm or aging ova. Moreover, these phenomena have been seriously proposed as explanations for congenital anomalies in humans. Although rhythm is a method that poses no maternal risk *per se* (discounting high failure rate and pregnancy health risks secondary to this) – the method may paradoxically be that contraceptive method posing the greatest fetal risk.

In deference to its extensiveness, we shall first review relevant animal data, considered more extensively elsewhere [80]. Thereafter, we consider human studies, addressing the possibility that practitioners of rhythm may be at increased risk for anomalous offspring.

Animal studies involving aging sperm

Sperm usually remain viable in mammalian females for a relatively short time. For example, fertilizing capacity is retained for about 6 hours in the mouse, 14–20 hours in the rat, 22 hours in the guinea pig, and 30 hours in the rabbit [80]. The length of the fertilizing capacity of human sperm is usually stated to be 24–48 hours, but in exceptional cases one could reason that sperm survived 9–10 days [80]. In rats [81] and guinea pigs [82] fertilization by sperm retained in the female for prolonged periods is said to result in progressively declining litter sizes, but apparently not in developmental abnormalities. On the other hand, Martin and Shaver [83] recovered rabbit blastocysts from rabbits inseminated by sperm aged *in utero*. Artificial insemination was performed 0–21 hours prior to injection of chorionic gonadotropin (hCG). Fewer blastocysts were recovered from rabbits

inseminated with sperm aged 18–32 hours *in utero*, compared to insemination with sperm aged 0–18 hours. Moreover, 13 of 134 blastocysts in the group fertilized by aged sperm were cytogenetically abnormal.

There are also data showing deleterious effects of fertilization involving sperm retained for prolonged periods in the male tract prior to ejaculation. This phenomenon is again potentially relevant to practitioners of rhythm. Young [84] studied the effects of fertilization by sperm aged within the male tract of guinea pigs. Two ligatures were applied to each epididymis. Proximal and distal epididymal sperm could thus be separated into two compartments, enabling their relative fertilizing capacity to be assessed over the subsequent 25-day period. Not surprisingly, the fertilizing capacity of proximal sperm increased, presumably reflecting maturation of younger sperm. By contrast, the fertilizing capacity of distal sperm decreased, presumably reflecting aging. In addition to this predictable decrease in fertilizability, however, the percentage of resorbed or aborted fetuses increased as the age of the distal sperm increased. Moreover, many fetuses were structurally abnormal. Tesh and Glover [85] later studied the phenomenon of sperm aging in rabbits. After 4 weeks of aging sperm in the epididymis, fertilizing capacity was greatly reduced; by 7 weeks fertilizing capacity was totally absent. Many offspring sired with aging sperm showed structural abnormalities, principally involving the skeletal system or gallbladder.

Martin-De Leon *et al.* [86] considered the cause for the embryonic abnormalities, studying blastocysts obtained from rabbits inseminated with aging sperm. Sperm were collected 7–35 days after epididymal ligation. Eight of 72 blastocysts (11%) resulting from insemination with 7–27-day-old sperm were chromosomally abnormal, compared to only 1 of 125 (0.8%) in the control group. Later studies also suggest that aging within the male tract causes cytogenetic abnormalities [87].

Additional investigations are obviously necessary to determine whether fertilization by aged sperm leads to abnormal embryos. Nonetheless, available data suggest that fertilization by sperm aged in either the male or female tract is a consideration in explaining abnormalities.

Animal data: postovulatory aging of ova (delayed fertilization)

That a time between normalcy and death of unfertilized ova might exist was first recognized by Pflüger [88] in 1882. Witschi [89] seemed to be the first to establish firmly that delayed fertilization not only leads to decreased fertilization, but also to structurally abnormal embryos.

Witschi initially studied *Rana temporaria* [89]. This frog releases ova directly into its abdominal cavity, to be collected by the oviducts and deposited in the uteri prior to fertilization. Retention of ova *in utero*, particularly under low temperatures, is compatible with normal embryonic development. However, prolonged retention under higher temperatures leads to embryonic anomalies following fertilization.

Witschi and Laguens [90] later showed that most of these malformed embryos have demonstrable chromosomal abnormalities. Of 25 *Rana pipien* embryos that were structurally abnormal as result of delayed fertilization, 20 (80%) showed an abnormal chromosomal complement; all 20 controls showed the normal diploid

complement. In the experimental group, 5 frogs were monosomic, 3 trisomic, 2 haploid, 1 tetraploid, 4 either triploid or hypotriploid (2n/3n), and 6 diploid mosaics. Three of the 6 mosaic embryos had both monosomic and trisomic cells (2n+1/2n−1 mosaicism), a form of mosaicism most readily explained by an abnormality at the first cleavage division; the 3 other mosaic embryos had multiple cell lines, suggesting an abnormality later in cleavage. In amphibians, one can conclude that delayed fertilization leads both to aneuploidy and polyploidy.

What happens in mammals? Approximately 15–20% of rabbit zygotes induced by delayed matings are trinuclear (polyploid), apparently because they contain two female haploid complements (polygyny) [91,92]. Such abnormalities are the result of cytogenetic aberrations. Shaver and Carr studied 6-day embryos, recovered after matings delayed 0–10 hours following intravenous injection of chorionic gonadotropin [93]. (Assuming 6 hours for capacitation, fertilization was delayed 6–16 hours.) The incidence of chromosomal abnormalities in matings occurring immediately after injection was 7% (5/73 blastocysts), compared to 2% (1/58) in control animals. Abnormalities included two mosaic, one trisomic, one pentaploid, and one structural rearrangement. The frequencies of chromosomal aberrations among animals mated 2, 4, and 6–9 hours after gonadotropin injection were 2/33 (6%), 1/24 (4%), and 10/80 (8%), respectively. The 10 chromosomally abnormal blastocysts in the latter group were all triploid, 3 XXX and 7 XXY. The lack of XYY blastocysts suggests that triploidy usually results from digyny. Austin [94] also observed that five of nine 10-day-old rabbit embryos conceived after delayed fertilization were triploid, compared to only two of 23 controls.

In rats, Blandau and Jordan [95] and Soderwall and Blandau [96] were the first to show that increasing interval between ovulation and fertilization decreased litter size, and decreased the proportion of resorbed pregnancies. Later, polyploidy was shown to be the most consistent genetic abnormality detected.

In mice, Vickers [97] showed that delayed fertilization slightly but significantly increased the incidence of polyploidy and possibly aneuploidy. Donahue [98] confirmed the increase in polyploidy, but failed to observe aneuploidy. Among Vickers' control group [97], 8 of 309 (2.6%) blastocysts recovered from animals mated 5–11 hours after ovulation were abnormal: 1 monosomic, 1 tetraploid, and 6 triploid. The differences in abnormalities in the two groups were statistically significant. In a second experimental group, 5 of 95 (5.3%) $9\frac{1}{2}$–$11\frac{1}{2}$-day fetuses were abnormal: 1 triploid, 1 monosomic, and 3 mosaic. Using the 'oldest fertilizable' embryos, Donahue observed suppression of the second polar body, large second polar body, and lack of female chromatin (i.e. expulsion of both female haploid groups from the spindle [98]). These abnormalities were verified by cytogenetic studies. On the other hand, none of 152 mouse zygotes developing from oldest fertilizable oocytes were aneuploid in other studies by Donahue. In mice, delayed fertilization thus results in polyploidy but not necessarily in aneuploidy.

In guinea pigs, Young and Blandau [99] observed that the proportion of pregnancies resulting in abortions or embryonic defects increased with increasing ovulation–insemination interval. Both growth retardation and structural anomalies occurred; however, all liveborn embryos were normal. Hancock [100] later showed that blastocysts recovered from gilts inseminated 0, 23, 30, and 40–43 hours after estrus showed 0%, 3%, 13%, and 41% trinuclear eggs (triploidy), respectively.

Deleterious effects of delayed fertilization have also been observed in hamsters. Yamamoto and Ingalls [101] mated females 5 hours before ovulation and 3, 5, and 9 hours after ovulation. Only 1 of 258 controls was abnormal, but 8% of all experimental offspring were abnormal; 9 triploid, 2 tetraploid, 5 monosomic, 1 trisomic, and 7 mosaic; that is, both aneuploidy and polyploidy were observed.

In summary, extensive animal literature reveals that delayed fertilization may lead to embryonic anomalies in amphibians and mammals. Infrahuman primates and humans have apparently not been subjected to direct experimentation. Chromosomal abnormalities are the only etiologic mechanisms known to be responsible for anomalies resulting from delayed fertilization. Aneuploidy, mosaicism, and polyploidy have been detected, but polyploidy and especially triploidy is most common. There seems to be no direct evidence that delayed fertilization increases mutation rates for single genes, although this is difficult to test.

Human studies

Given the animal studies reviewed above, it is certainly reasonable to hypothesize that fertilization involving aging ova or aging sperm could lead to chromosomal abnormalities in humans. It is less reasonable to postulate that Mendelian or polygenic/multifactorial disorders may result, although formally they should not be excluded.

In humans the relevant hypotheses have usually been tested only indirectly. Several studies have compared offspring conceived under circumstances in which one would postulate fertilization with an aging sperm or ovum. German [102] publicized the issue in 1968 when he suggested that trisomy 21 was caused by delayed fertilization secondary to decreased frequency of coitus at advanced parental ages. He tested this idea by comparing duration of marriage in various groups, taking advantage of the observation that coital frequency is said to be inversely related to duration of marriage [102]. After corrections for age, the length of marriage prior to the pertinent birth was significantly greater in mothers delivered of offspring with trisomy 21 than in mothers delivered of normal offspring. Thus, not age *per se*, but rather the decreased coital frequency said to accompany increased duration of marriage, was believed to be responsible for increased aneuploidy. German [102] also reasoned that if chromosomal abnormalities result from delayed fertilization, one might predict increased abnormalities in conceptions occurring under circumstances in which coitus occurred infrequently or irregularly, namely rhythm, or unexpected coitus following abstinence.

Studies by other investigators later furnished additional support that delayed fertilization could be deleterious in humans [103].

Jongbloet strongly accepts the concept of delayed fertilization having made a series of circumstantial observations [104–108]. In one study [107], he ascertained 127 couples who had mentally retarded children, and inquired about the circumstances in which the children were conceived. After excluding couples in which the child had a known 'genetic' or 'metabolic' defect, there remained 49 couples who experienced unintended pregnancies while practicing rhythm (calendar method in all except 2 cases). Percentages of abnormal progeny were highest

among couples who practiced rhythm 'carelessly' and among conceptions occurring after midcycle. These are the categories expected to correlate with fertilization involving aging sperm or aging postovulatory ova (delayed fertilization). (Preovulatory overripeness, as considered elsewhere [80], is an alternate possibility.) In other studies Jongbloet observed that anencephaly occurs with highest frequency in Roman Catholic regions [108], whose inhabitants are likely to practice rhythm. In a recent study, Milstein-Moscati and Becak [109] observed significantly longer intervals between intercourse in parents delivered of Down's syndrome offspring (n=33), compared to controls (n=80). However, in this retrospective study recall bias must be suspected.

Consistent also with deleterious effects of delayed fertilization are studies by Guerrero and Rojas [110], who used basal body temperature charts to determine likely time of conception in a series of couples undergoing artificial insemination. In an initial sample of 965 pregnancies there were 75 in which spontaneous abortion occurred. The fetal loss rate was 24% when insemination occurred 3 or more days after ovulation, but only 3.2% when occurring within 2 days of the thermal shift.

Noteworthy is the recent case control study of Bracken and Vita [68], whose design has already been cited. Of all contraceptive methods, the strongest associations with anomalies occurred in rhythm users; however, differences were not significant when corrected for multiple comparisons. Only 3.9% of controls (n=2859) practiced rhythm. However, the prevalences of rhythm usage was 10.5% in mothers having a child with cleft lip and palate (n=38), 15.8% in the hydrocephaly group (n=19) and 7.7% in the chromosomal abnormality group (n=52). Almost half of infants experiencing chromosomal abnormalities were born to mothers using rhythm.

On the other hand, dissenting voices question whether aging sperm or aging ova are associated with human malformations in general, and trisomy 21 in particular. Not all studies show an effect [111], as already noted. Other criticisms follow as well. First, most studies showing an effect have been only circumstantial in design, unavoidably but nevertheless not optimal. Second, retrospectively gathering data inevitably raises the risks of faulty memory and recall bias, as discussed earlier. Third, plausible mechanisms do not always exist to explain just why delayed fertilization would cause a particular anomaly. We have noted that chromosomal abnormalities are plausible, but Mendelian or polygenic/multifactorial traits are less so. Fourth, coital rates decrease roughly linearly with age, whereas rates for trisomy 21 rise exponentially. Thus, delayed fertilization would not seem likely to explain all trisomy cases on mathematical grounds alone. Finally, and most importantly, the error in trisomy 21 usually involves Meiosis I, especially in the female [112]. By contrast, an error arising as result of delayed fertilization would result in an error in Meiosis II. Thus, only a few cases of trisomy are candidates to be explained on the basis of delayed fertilization.

Conclusion concerning rhythm

Whether fertilization involving aging sperm or aging ova results in fetal abnormalities in humans is unresolved. Definitive proof of an association in humans is

lacking, but extensive animal data raise the possibility of a deleterious effect of delayed fertilization. One explanation for these ostensibly contradictory findings is that abnormalities are indeed induced in humans, but they are lethal. This is actually quite a reasonable postulate because polyploidy is the most likely aberration, and it would result not in anomalous liveborns but abortions. The study of Guerrero and Rojas [110] is consistent with this postulate.

Nonetheless, practitioners of rhythm are surely among those at highest likelihood for delayed fertilization, and it would be intemperate to assume that rhythm contraception carries no fetal risk. Rhythm should therefore not be chosen as a contraceptive method in the belief that fetal risks are lower than other methods, e.g. oral contraception.

Barrier methods

For barrier and other methods, there have been few if any serious claims of a relationship to anomalies. Indeed, postulating a relationship between anomalies and the diaphragm, condom, or withdrawal seem almost frivolous. Nonetheless, we can cite a study failing to observe an association. The case control study of Bracken and Vita [68] addressed these issues, finding no associations between anomalies and any of the contraceptive methods. In addition, the same study failed to observe an association between anomalies and either foams or creams.

Conclusion

From this critical assessment of published reports on various contraceptive methods and their possible relationship to teratogenic and mutagenic disorders, this reviewer concludes that no contraceptive method substantively increases fetal risks over the 2–3% likelihood that any given pregnancy results in anomalous offspring.

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