Fertility and ageing

ESHRE Capri Workshop Group*

The late 20th century trend to delay birth of the first child until the age at which female fecundity or reproductive capacity is lower has increased the incidence of age-related infertility. The trend and its consequences have also stimulated interest in the possible factors in the female and the male that may contribute to the decline in fecundity with age; in the means that exist to predict fecundity; and in the consequences for pregnancy and childbirth. In the female, the number of oocytes decreases with age until the menopause. Oocyte quality also diminishes, due in part to increased aneuploidy because of factors such as changes in spindle integrity. Although older male age affects the likelihood of conception, abnormalities in sperm chromosomes and in some components of the semen analysis are less important than the frequency of intercourse. Age is as accurate as any other predictor of conception with assisted reproductive technology. The decline in fecundity becomes clinically relevant when women reach their mid-30s, when even assisted reproduction treatment cannot compensate for the decline in fecundity associated with delaying attempts at conceiving. Pregnancies among women aged >40 years are associated with more non-severe complications, more premature births, more congenital malformations and more interventions at birth.

Key words: ageing/demographics/fecundity/fertility/infertility

Introduction

Two fertility trends of the 21st century are already evident in the Western countries; women are having fewer children and they are delaying births to a later age than in previous centuries (Daguet, 2002). Populations are ageing at a rate that is without precedent because not only are there fewer children, but the decline in mortality rates at age >60 years has also increased the number of older people. A further consequence is that women who choose to delay their attempts at conception may encounter delays and/or disappointment due to decreased fecundity. Decreased fecundity with increasing female age has long been recognized from demographic and epidemiological studies, which consistently found that fertility declined beginning as early as the middle of the third decade (Figure 1) (Menken *et al.*, 1986; Leridon, 2004).

The biological basis of this decline in fecundity with increasing female age appears to involve several factors; germ cells in the female are not replenished during life, attrition and utilization of follicles leads to a decline in the number of oocytes from birth to menopause, the quality of existing oocytes diminishes with age and on an average, intercourse frequency declines with age.

The demographic fertility trend and the individual woman's fecundity are usually studied by different methods. Demographic studies of fertility involve indicators such as the fertility rate, average age at birth and time to pregnancy. However, in the management of infertile couples, it is hoped that the chronological age of the individual does not completely determine the quality of the oocytes. Therefore, laboratory and dynamic tests have been introduced to evaluate whether chronological ageing or a possibly independent course of ovarian ageing is the dominant factor in a given woman. Whether declining fecundity might be advanced or retarded in some women can be addressed by prediction studies on the association between assumed markers of ovarian ageing and conception or a related outcome. The putative predictors may be biochemical or imaging variables, or dynamic tests of ovarian function (Table I).

Demographic aspects

During most of the 20th century, the decline of fertility in Western societies went together with a trend to lower the mean age of maternity. Both trends essentially stemmed from the marked reduction in births of parity ≥ 3. In France, for example,
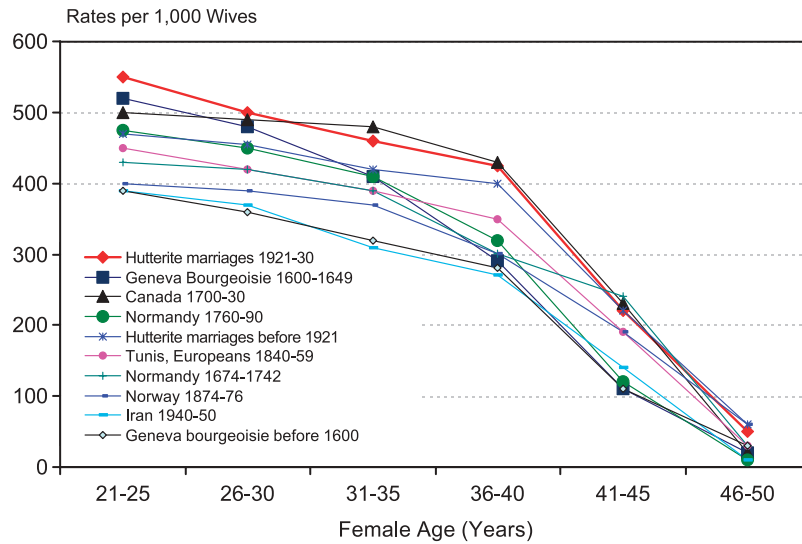
Figure 1. Marital fertility rates. [Drawn from Menken *et al.* (1986), Reprinted (abstracted/excepted) with permission from Science. Copyright (1986) APAS.]

the mean age of women at birth was 29.5 years in 1900 and 26.5 years in 1977 (Daguet, 2002). The trend in the mean age at birth began to change, however, in the late 1970s, and the mean age at maternity was again 29.5 years by 2000 (Figure 2). The impressive recent rise in the mean age at maternity is the result of postponing the first (and subsequent) births rather than a rise in fertility (the number of births per woman) at later ages. An upward trend did appear at the end of the 1970s in the rates for the 35–39 and 40–49 years age groups (Figure 3), but these rates in 2000 are still far below those observed in 1900. Similar changes can be observed in most developed countries.

In the same time-period, another major change has taken place, in that births are now more strictly planned, whatever their rank. A birth to a woman aged 35 years is often her first or second birth, or the first birth in a new union; a few decades ago a birth at 35 years of age was usually a birth to a woman of higher parity, and it was not always wanted. It is thus very likely that many couples who are also trying to have a child at around this age do not succeed because of the decline in fecundity with age.

An argument has been advanced that the postponement of births is not a problem when assisted reproduction techniques are available. Can assisted reproduction treatment (ART) really compensate for the natural decline of fertility with age? The answer is no. This can be shown by means of a simulation model of reproduction, combining the monthly probability of conceiving, the risk of miscarriage and the probability of becoming permanently sterile, all depending on age (Leridon, 2004).

Under natural conditions, 75% of women starting to try to conceive at age 30 years will have a conception ending in a live birth within 1 year, 66% at age 35 years and 44% at age 40 years. Within 4 years the success rates will be 91, 84 and 64%, respectively. If women turn to ART after 4, 3 or 2 years, respectively, without conception, and if the rate of success is as observed after two cycles of IVF (Templeton *et al.*, 1996), ART makes up for only half of the births lost by postponing a first attempt to conceive from age 30 to 35 years, and <30% after postponing from 35 to 40 years (Table II). Even if we relax some of the assumptions, ART in its present form cannot make-up for all births lost by the natural decline of fertility after age

Table I. Potential predictors of ovarian function

Biochemical
  FSH
  Inhibin A
  Anti-Müllerian hormone
  E_2
  Inhibin B
Imaging
  Antral follicle count
  Ovarian volume
  Uterine artery flow dynamics
Dynamic tests
  CCCT: clomiphene citrate challenge test
  EFORT: inhibin and E_2 response to FSH
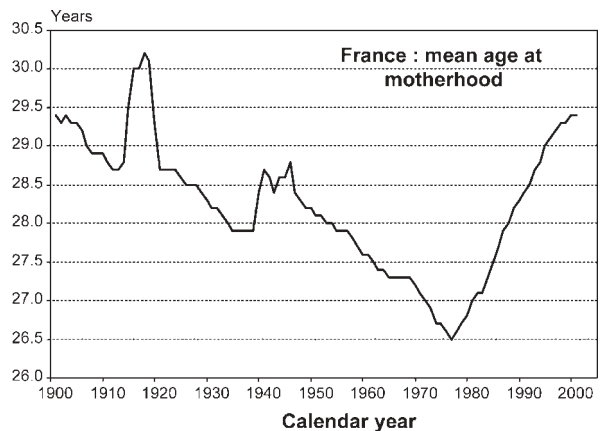 GAST: inhibin and E_2 response to GnRH agonist

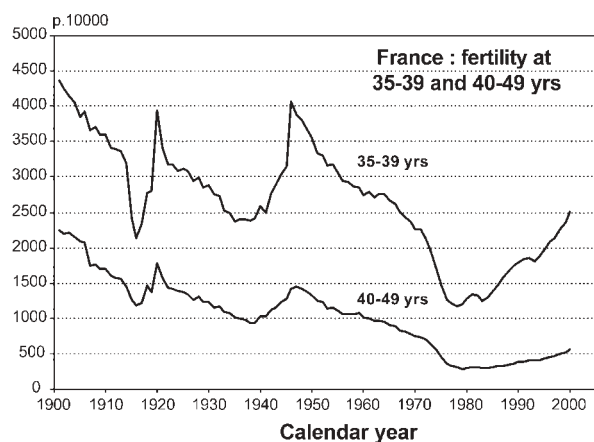**Figure 2.** Mean age of women at birth (Daguet, 2002).

Figure 3. Variation in the fertility rate (births per 10 000 women at each age range) between 1900 and 2000.

35 years. The data indicate that women should not defer their pursuit of motherhood to later ages, but if they have waited, there is a reasonable likelihood of success even if treatment is deferred.

The ageing ovary

The ovary is no exception to the ravages of ageing, which occur in every organ of the body. In contrast to somatic cells, the germ-line oogonia are potentially immortal with a minority being passed on to the next generation (Kirkwood, 1998). Although recently it has been suggested that germ-line stem cells can be found in the adult mouse ovary from which new oogonia can be formed by mitosis and meiosis (Johnson *et al.*, 2004), it is generally accepted that a finite number of oogonia are laid down in fetal and/or early neonatal life, after which no new germ cells are formed (Block, 1952; Baker, 1963; Peters, 1970). In our own species mitosis of oogonia is completed by birth. Between the fourth and sixth months of intrauterine life, the mesenchyme is

Table II. Spontaneous and induced live births according to the woman's age

	Woman's age when starting to try to become pregnant		
	30 years	35 years	40 years
Years before starting ART	4	3	2
Age when starting ART	34	38	42
Number of women per 1000 women of each age			
(a) No conception before ART	93	178	430
(b) Total conceptions (LB) with ART in 2 years	28	42	71
(c) Conceptions with no treatment in 2 years	14	25	67
Percentage success			
Apparent rate of success for ART = $100 \times$ (b/a)	30.1	23.6	16.5
Net rate of success for ART = $100 \times$ (b − c)/a	15.1	9.6	0.9

Data adapted from Leridon (2004).
LB = live birth.

organized round the oocyte as pre-granulosa cells to form primordial follicles. The oocyte is arrested at the dictyate stage of the first meiotic division until very late in the final stages of folliculogenesis just prior to ovulation (Peters, 1970).

Every day a given number of primordial follicles are recruited from this pool of resting follicles for further growth. The process of folliculogenesis takes ∼4–6 months, during which time the oocyte and surrounding somatic cells undergo a series of changes that eventually result in a large antral follicle capable of ovulating a mature oocyte (Gougeon, 1996). The proportion of the pool of primordial follicles that is recruited for the development increases with age and is related inversely to the density of follicles in the ovary (Krarup *et al.*, 1969). It is postulated that inhibiting factors such as anti-Müllerian hormone (AMH) produced by developing follicles inhibit the recruitment of primordial follicles (Gruijters *et al.*, 2003).

Folliculogenesis continues throughout life from before birth until the menopause (Figure 4). At puberty the levels of gonadotrophins rise to a concentration that is sufficient to permit the final stages of folliculogenesis, which results in the ovulation and formation of a corpus luteum. Unless pregnancy occurs, this cyclical ovarian activity is manifest by a series of monthly menses as the endometrium is shed in response to the fall in the concentration of progesterone during luteal regression. After the establishment of menses at puberty, the length of the menstrual cycle for most women remains remarkably constant until a few years before the menopause (Treloar *et al.*, 1994). There is a significant shortening of the cycle with age due to a decrease in the length of the follicular phase. Most women maintain regular ovulatory cycles until a few months before the menopause. The increased variation in length at this time probably reflects the reduced availability of antral follicles at the appropriate stage of development suitable for selection as the ovulatory follicle.

As the ovary ages there is a decline in the total number of oocytes and in their quality. Only four small studies have attempted to measure the total number of follicles in human ovaries at different ages (Block, 1952; Baker, 1963; Richardson *et al.*, 1987; Gougeon *et al.*, 1994). At any one age there is a considerable variation between individuals and within the same woman between different parts of the ovary. From these limited data mathematical models have been constructed to describe the dynamics of the decay. The original Faddy/Gosden model that describes two phases of linear decline with a sudden acceleration at 37 years ('broken stick') has been modified to one which assumes a single exponential decline (Gosden, 1985; Faddy *et al.*, 1992; Faddy and Gosden, 1995). This model is more biologically plausible and the age of onset of the ovarian failure predicted by this model fits the observed variation in the age of menopause.

As mentioned above, there is a gradual shortening of the length of the menstrual cycle as women age (Treloar *et al.*, 1994). This shortening of the follicular phase probably reflects a slight increase in the secretion of FSH, particularly during the early follicular phase when selection of the dominant follicle occurs (Ahmed Ebbiary *et al.*, 1994; Klein *et al.*, 1996). The significant increase in the incidence of dizygotic twinning in older women is probably due to the increased chance of selecting more than one ovulatory follicle by extending the 'window' of recruitment (Lambalk *et al.*, 1998).
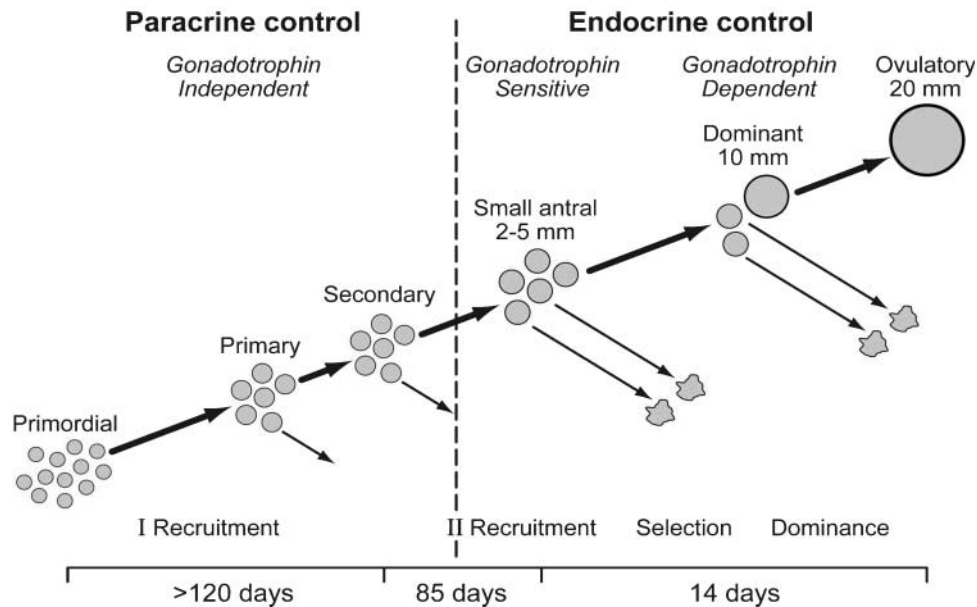
Figure 4. Folliculogenesis in primate and ruminant [adapted from Baird and Mitchell (2002) and McGee and Hsueh (2000)]. In B = inhibin B; In A = inhinbin A; AMH = anti-Müllerian hormone.

Over the last few years there have been extensive efforts to develop hormone and/or biophysical tests of ovarian ageing (te Velde and Pearson, 2002). By estimating the total number of oocytes left in the ovary (ovarian reserve), a prediction of the number of remaining years of reproductive life could be made as well as the likely success of assisted reproductive techniques such as IVF. None of the tests measures the total number of oocytes directly. Rather, they assume that the number of developing follicles (pre-antral and antral) is directly related to the total oocyte pool. However, as we have seen above, a relatively greater proportion of the total pool is recruited for development with age. Moreover, a smaller proportion of the developing pool is lost due to atresia during folliculogenesis with age because of the supportive influence of increased levels of FSH and other trophic factors. Hence, there is a tendency for these tests to over-estimate the total pool of follicles.

Probably the simplest and most accurate test of ovarian reserve is the measurement of total ovarian volume as measured by high resolution ultrasound (Wallace and Kelsey, 2004). As the bulk of the ovary is made up of antral follicles in the absence of a corpus luteum, total volume relates closely with total antral follicles (Yong et al., 2003). Not surprisingly, therefore, the levels of inhibin B and AMH secreted by developing follicles also associate well with total follicle count (Danforth et al., 1998; De Vet et al., 2002). In contrast, because >90% of estradiol (E_2) and inhibin A is secreted by the dominant follicle and/or the corpus luteum, the levels remain normal as long as ovulatory cycles persist. The increased level of FSH, which occurs in the early follicular phase of older women is probably related to the low levels of inhibin B at a time in the cycle when the inhibitory effects of E_2 are minimal (Klein et al., 1996).

It has been shown that the level of AMH is an excellent marker for ovarian ageing and response to gonadotrophins (van Rooij et al., 2000; Fanchin et al., 2003). AMH, such as inhibin B, is secreted by the granulosa cells of pre-antral and small antral follicles (Gruijters et al., 2003). However, the secretion of AMH in contrast to that of inhibin B declines strikingly when the follicle reaches the pre-ovulatory phase (Danforth et al., 1998; Gruijters et al., 2003; Weenen et al., 2004). Thus, its concentration reflects the small follicles only, not confounded by a significant contribution from the dominant follicle.

To sum up, the main component of ovarian ageing is the decline in oocytes. There are several indicators of the number of residual oocytes, including ovarian volume, antral follicle count (AFC), AMH and FSH. The difficulty is that they all test the number of developing follicles that may not reflect the number of primordial follicles. Nevertheless, they can be useful to predict ovarian responses and the remaining time to ovarian failure. Unfortunately, none of these measurements directly reflect the quality of the oocyte, to which the genetic composition contributes a significant amount.

Fecundity and the age of the woman

Fecundity, the capacity to bear a child, declines markedly with female age. The fundamental aspect of reproductive senescence in women is a decrease in the population of ovarian follicles. This appears to be inevitable and irreversible, with no evidence in individual women that the process can be slowed. Furthermore, there is clearly a difference in the threshold number of follicles required to maintain cyclicity, with an average period of 10 years between the loss of fecundity and the complete cessation of ovarian function. Other factors contributing to the loss of fecundity appear to be much less important, for example ageing of the reproductive tract and particularly the uterus. However, the frequency of intercourse may be contributory, and independently the male age is relevant when >40 years. In clinical terms the difficulty is that there is no external sign or marker of reduced or absent fecundity, although response to ovarian stimulation has some prognostic significance.

Until ∼20 years ago it was generally accepted that a woman's fecundity declined only slightly until the age of 35 years, when there was a sharp fall. However, the Centre d' Etude et de Conservation des Oeufs et du Sperme (CECOS) study of women having donor insemination, whose husbands had azoospermia, indicated that fertility fell significantly with age >30 years (Federation CECOS, 1982) (Figure 5). Fecundity has been studied traditionally in populations, although this is increasingly difficult in contemporary communities because of the widespread use of fertility control, among other factors. Generally speaking, historical data point to a slow decline in fecundity until after the age of 35 years. For example, compared to women aged 20–24 years, fertility is reduced by 31% in women aged 35–39 years (Menken *et al.*, 1986). It is recognized that the historical approach is of limited value in predicting fecundity in individual patients or clinical situations. Using both historical and contemporary data relating to fertility, it has been possible to estimate the proportion of women who remain childless according to their age at marriage (Menken *et al.*, 1986) (Table III).

Many people came to believe that controlling fertility was the real problem and that in contrast having children was easy. However, delay in childbearing to a much less fecund age has brought home the reality, and has also engendered unrealistic expectations of what modern fertility treatment and particularly assisted conception can achieve. Even with multiple embryo replacement there is a marked age-related decline in live birth rates among women in their late 30s having IVF treatment (Templeton *et al.*, 1996). On the other hand, a further study of donor insemination for azoospermia (van Noord-Zaadstra *et al.*, 1991) has indicated that the effect of age on fertility can be partially compensated by inseminating more cycles, at least among women in their 30s (Table IV). Even so, a woman of 35 years
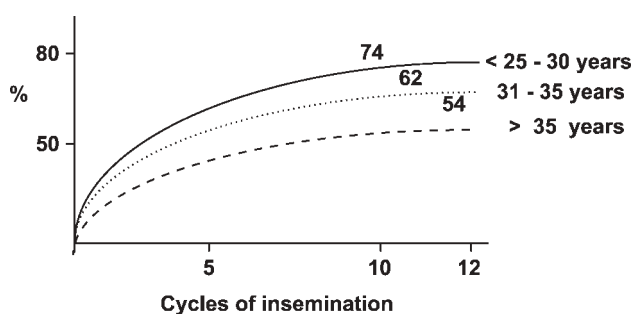

Figure 5. Cumulative success rates according to age (Federation CECOS, *New England Medical Journal*, 1982; ©2005 Massachusetts Medical Society. All rights reserved).

Table III. Age of marriage and risk of childlessness

Age (years)	Risk of childlessness (%)
20–24	5.7
25–29	9.3
30–34	15.5
35–39	29.6
40–44	63.5

From Menken *et al.* (1986); by permission from *Science*.

Table IV. Effect of age and number of cycles of insemination on pregnancy rates (%)

Age (years)	Number of cycles	
	12	24
20–31	74	85
>31	54	75

From van Noord-Zaadstra *et al.* (1991) (*British Medical Journal*, 1991, Vol. 302, pp. 1361–1365, amended and reproduced with permission from the BMJ Publishing Group).

was only half as likely to have a healthy baby as a woman of 25 years.

A questionnaire-based study of 782 couples using natural family planning methods showed that almost all pregnancies are conceived in a six day fertile window, which apparently did not decrease with age (Dunson *et al.*, 2002). However, fecundity declined from the late 20s onwards with women of 35–39 years being half as fecund as women of 19–26 years. A further study using the same data-set seemed to indicate that a reduced frequency of intercourse, at least up to the age of 39 years, could have a significant effect on the time to conceive. This was independent of the age of the male partner, which only became important from the late 30s onwards (Dunson *et al.*, 2004). The impact of sterility did not appear to make a significant contribution to the observed decline in fecundity with age. However, the authors conceded that things may be different in couples aged >40 years.

It is well established that the considerable increase in aneuploidy seen in embryos from older women contributes to their inability to bear a child by increasing both implantation loss and pregnancy failure (Munné *et al.*, 1995). Poor response to ovarian stimulation during IVF treatment, particularly in women aged <40 years, is a strong predictor of reduced ovarian reserve, resulting in reduced fecundity and early menopause (de Boer *et al.*, 2002; Lawson *et al.*, 2003). Poor response was a stronger predictor of declining ovarian function than raised FSH. In a prospective observational study it was shown that age remains the critical factor associated with poor response and embryo quality. Women aged ≥41 years and with normal FSH fared much worse than women aged <41 years who had elevated FSH levels (van Rooij *et al.*, 2003). A retrospective study also emphasized the importance of age, more than FSH levels, in the prediction of assisted reproduction success (Abdalla and Thum, 2004). The results support the concept that age is associated with increasing aneuploidy and that lower fertility is more a function of oocyte quality than oocytes numbers.

The genesis of human aneuploidy

Aneuploidy is defined as having an abnormal number of chromosomes which is not an exact multiple of the haploid number, in contrast to having multiple complete haploid sets of chromosomes, as in diploid or triploid states. The lack of aneuploidy is a key determinant of oocyte quality. Although the addition or loss of a single chromosome usually occurs at

meiosis, it can occur before or after that point in oocyte development. In the human species, aneuploid fetuses may survive to term and aneuploidy is the most common cause of mental retardation. In spite of these devastating clinical consequences little is known of how trisomy and monosomy originate in humans.

Imbalance in even one pair of chromosomes can result in non-viability. It is a rare phenomenon in most lower species such as yeast (1 in 10 000) and *Drosophila* (1 in 6000–7000); it occurs more frequently in mammals: in mice the trisomy or monosomy rate of fertilized oocytes is 1–2%; in the human, however, 10–30% of fertilized oocytes have the 'wrong' number of chromosomes (Koehler *et al.*, 1996).

Meiosis and meiotic abnormalities

The meiotic pathway is extraordinarily well-conserved in different species including the human. Meiosis generates haploid gametes through a round of DNA replication followed by 2-cell divisions, meiosis I (MI) and MII. In the unique meiotic prophase, homologous chromosomes synapse and undergo recombination. The first division, or MI, involves segregation of homologous chromosomes from each other and MII involves segregation of sister chromatids that is analogous to mitotic division.

These basic features hold for both human males and females but there are important sex-specific differences in the time of onset, duration and outcome of the meiotic processes (Figure 6).

Successful segregation of homologues in MI requires: (i) maintenance of physical connections in the chiasmata and (ii) centromeres of sister chromatids must attach to the same spindle poles. MII follows MI immediately without an intervening S phase (Smith and Nicolas, 1998).

There are several possible patterns of abnormal MI segregation: (i) 'true' non-disjunction, in which homologues travel together to the same pole; (ii) 'achiasmate' non-disjunction, in which homologues that failed to pair and/or recombine travel independently to the same pole and (iii) premature separation of
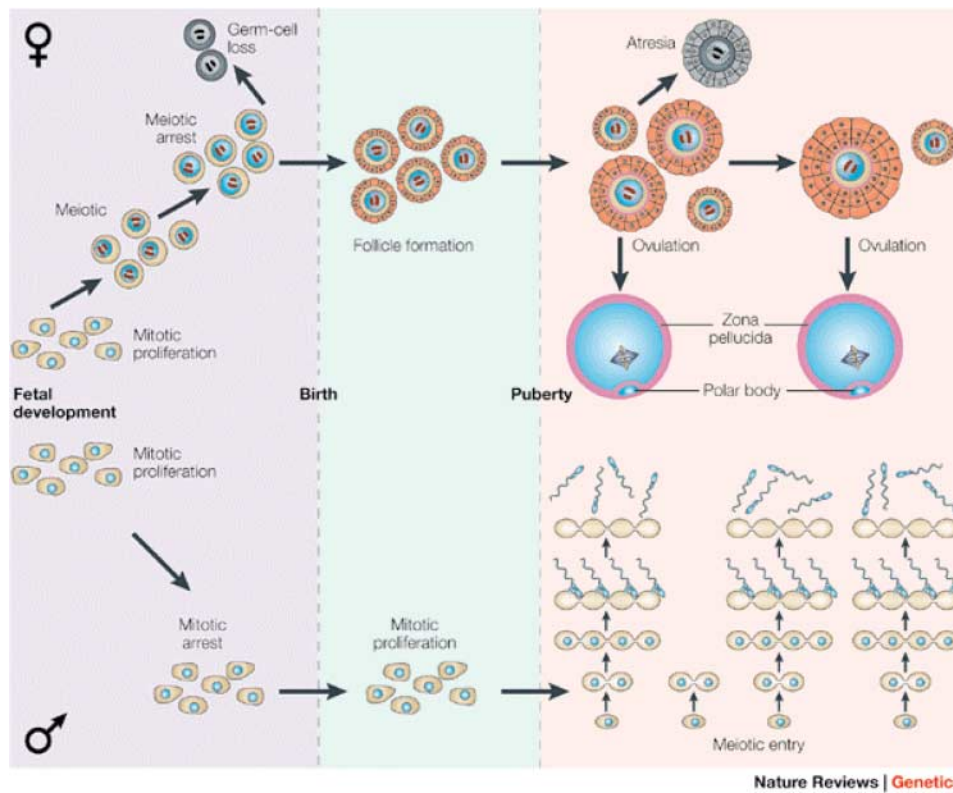

Figure 6. Sex-specific differences in the time of onset and duration of meiotic processes (Hassold and Hunt, 2001). Meiotic 'timelines' for humans. The fate of germ cells is dictated by the somatic environment. In both the developing ovary and the testis, germ cells undergo mitotic proliferation prenatally, but the time of entry into meiosis and the duration of meiosis is strikingly different between the sexes. Females: in the fetal ovary, a brief period of mitotic proliferation is followed by the entry of all cells into meiotic prophase. Several germ cells undergo apoptosis during this time, substantially reducing the pool of developing oocytes. Before birth, all surviving oocytes enter a period of extended meiotic arrest and, by the time of birth, all quiescent oocytes have become surrounded by somatic cells, forming primordial follicles. In a sexually mature woman, individual primordial follicles are stimulated to initiate growth throughout the reproductive lifespan. Typically, one fully grown oocyte is ovulated each month and several growing oocytes become atretic. This process continues until the cohort of oocytes is depleted and the woman enters menopause. Males: in the fetal testis, a brief period of mitotic proliferation is followed by an extended period of mitotic arrest. After birth, the male germ cells, or spermatogonia, resume mitotic proliferation and, with sexual maturity, cells are stimulated to undergo meiotic cell divisions. Because spermatogonia continue to proliferate mitotically and to send daughter cells into meiosis, sperm production is maintained throughout the lifetime of the male. Throughout the meiotic divisions, individual spermatocytes remain connected by cytoplasmic bridges. These connections are lost during the post-meiotic process of spermiogenesis, which involves tight packing of the chromatin, growth of the sperm tail and the sloughing of virtually all the cytoplasm into the residual bodies (depicted as empty cells). [Figure and legend reproduced with permission from *Nature Reviews Genetics* (Vol. 2, No. 4, pp. 280–291, 2001), ©2005 Macmillan Magazines Ltd.]
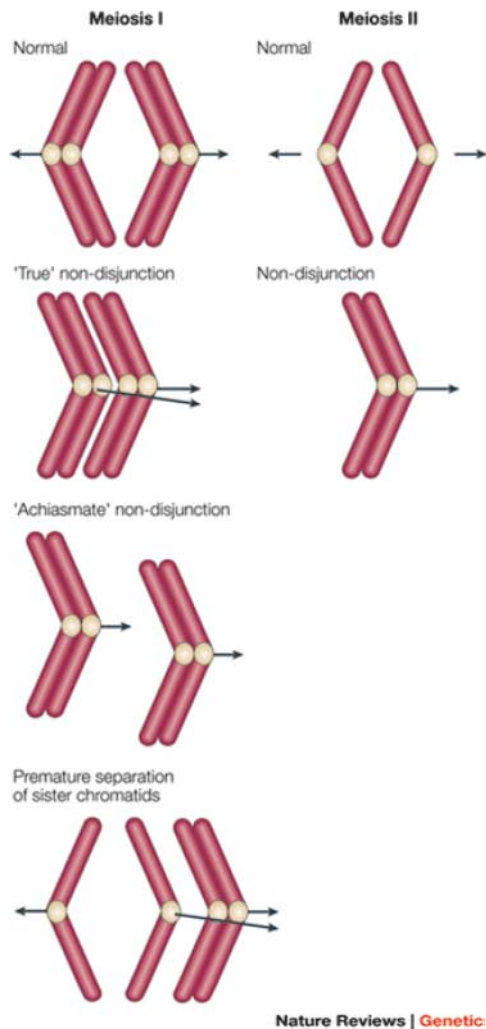
Figure 7. Mechansims of non-disjunction (Hassold and Hunt, 2001). [Reproduced with permission from *Nature Reviews Genetics* (Vol. 2, No. 4, pp. 280–291, 2001), ©2005 Macmillan Magazines Ltd.]

sister chromatids (PSSC), in which chromatids—rather than homologues—segregate from one another (Figure 7).

Non-disjunction at MII is assumed to result from the failure of sister chromatids to separate. More complicated errors have also been proposed.

Incidence of aneuploidy

The observed level of aneuploidy in humans varies enormously, depending on the developmental point being examined: (i) in newborns it is 0.3% (trisomy 21 and sex-chromosome trisomies being most prevalent); (ii) in stillbirths it is 4% (prevalent abnormalities similar to newborns) and (iii) in clinically recognized abortions (between 6–8 and 20 weeks gestation) 35% of such conceptions are trisomic or monosomic for various chormosomes (Hassold *et al.*, 1996), whereas in early missed abortion the rate of chromosomally abnormal embryos is higher (67–75%) (Ferro *et al.*, 2003; Philipp *et al.*, 2003).

Assuming that 15% of recognized pregnancies abort spontaneously, that 1–2% are stillborn and the rest are liveborn, we can estimate that ≥5% of all human conceptions are aneuploid.

However, this is a gross underestimation of the real incidence in humans, as it does not include information on 'occult' pregnancies or data from preimplantation embryos and gametes (Macklon *et al.*, 2002).

Mechanisms of origin of aneuploidy

The origin of different aneuploid conditions has been elucidated by examining DNA polymorphism. For monosomies this information is only available on the 45,X condition. Most 45,X (70–80%) monosomies have a single maternally derived X chromosome, that is the paternal X or Y is lost in meiosis or in early embryogenesis (Jacobs *et al.*, 1997). Many trisomic conditions are also compatible with some development; therefore, information on the origin is available for several different trisomies. There is a remarkable variation among trisomies with regard to the parent and meiotic stage of origin of the additional chromosome. Similarly, the importance of MI versus MII errors varies among chromosomes. It is likely that there are chromosome-specific effects that influence the patterns of non-disjunction.

In spite of this there is at least one general theme: maternal MI errors predominate among almost all trisomies. This may be due to the fact that this first division is initiated prenatally and is not completed until the time of ovulation (Hassold and Hunt, 2001).

Little is known so far about the mechanism that underlies non-disjunction. In yeast and *Drosophila*, it could be demonstrated that some mutations reduce or abolish recombination and that there is an influence of the location of recombination; exchanges too near or too far from the centromere increase the risk for non-disjunction.

Genetic mapping techniques can be used to study the inheritance of DNA polymorphism in trisomic human concepti by comparing the frequency and distribution of meiotic exchanges in trisomy-generating meioses with those from chromosomally normal meioses. There is a significant reduction in recombination in all MI-derived trisomies that have so far been studied. Two processes might be responsible: (i) chromosomes fail to recombine, that is the non-disjoining bivalent is 'achiasmate' and (ii) there are more subtle reductions in recombination (Wolstenholme and Angell, 2000). Recombination errors in MII-derived oocytes have also been identified, but generally cases of human female non-disjunction have their origin at the first meiotic division.

Cytogenetic methodology alone or in combination with immunofluorescence and fluorescence in-situ hybridization (FISH) technology revealed that non-disjunction and PSSC may be involved in oocytes; the former accounts for two-thirds of cases.

Putative aneuploidy inducing factors

The frequency of aneuploidy is high, but surprisingly we know very little about factors that modulate the risk of meiotic non-disjunction. Increasing maternal age is the one factor which is known to be incontrovertibly linked to human aneuploidy.

As early as 1933, the association between maternal age and Down's syndrome was recognized. This association was also recognized for other human trisomies. The magnitude of this effect is extraordinary: the incidence of trisomy among clinically

recognized pregnancies is 2% among women aged <25 years and nearly 35% among women aged >40 years. The effect is hard-wired into our species and there is no known influence of race, geography or socioeconomic status.

Despite the important impact of maternal age we know very little about the basis of this age effect. The uterus cannot be involved because these aneuploidies are all of maternal origin. Also, while the age effect is chronological, there is a small degree of biological variability among women. Women with a known trisomic pregnancy entered menopause 1 year earlier than those in a control group. This can be consistent with the 'limited oocyte pool' hypothesis that the age effect might be due to the relative scarcity of oocytes at optimal stages of maturation (Warburton, 1989).

Besides these observations, little else is certain about the maternal-age effect. Several models have been proposed, but they require further validation and necessitate appropriate animal models.

Other environmental or genetic risk factors have been suggested as predisposing factors, but none of them have been proven. Lack of proof may reflect either that such mechanisms do not exist or that the study designs used were inappropriate.

In 1999, Down's syndrome was linked to a maternal polymorphism for an enzyme involved in folic acid metabolism. Point mutations in methylenetetrahydrofolate reductase were studied in Down's syndrome individuals and age-matched controls. Mutations lead to reduced enzyme activity, which is a known risk factor for neural tube defects. The enzyme affects folate metabolism and cellular methylation reactions (James *et al.*, 1999).

The hypothesis that the aberrant methylation might increase the likelihood of meiotic non-disjunction was appropriate since there is a high significant increase in the proportion of heterozygotes and mutant homozygotes among mothers of Down's syndrome individuals. The same association was later also found for a second gene in the folate pathway, methionine synthase reductase. These observations are provocative because the magnitude of the effect is remarkable and the results indicate the possibility of a relatively straightforward preventative measure (dietary folate supplementation). However, recent studies indicate that the link between folate metabolism variants and Down's syndrome might be less important than originally thought or missing altogether (Petersen *et al.*, 2000).

There has further been persistent conjecture that maternal smoking might be a risk factor for Down's syndrome, but the results have been contradictory. In a recent study, a significant association emerged with the effect being confined to MII cases that involve younger women (Yang *et al.*, 1999).

Although a link between maternal age and Down's syndrome was established >60 years ago and the last 10 years have seen many important advances in understanding human aneuploidy, including the characterization of parental and meiotic origins, an understanding of molecular mechanisms and the maternal effect on trisomy remains elusive.

Genetics of the oocyte

Successful reproduction depends on oocyte quality which is determined largely by the genetics of the oocyte. The key contributing elements are oocyte maturation, spindle formation, energy supply, syngamy and early embryonic development and epigenetics; as will be seen, errors in some of these factors are more likely with advancing age. During oogenesis, oocytes acquire molecular and cellular properties in a sequential manner that confers meiotic and developmental competence to the female gamete. Following the LH surge, a competent oocyte can complete meiosis, sustain fertilization and oocyte activation, and organize the transition from maternal transcripts to gene products of embryonic origin. Several processes are involved in these complex mechanisms, including remodelling of the chromatin and the cytoskeleton, with activation of pathways involving genetic and epigenetic processes.

Disturbances during oocyte maturation and development may perturb the oocyte's energy supply by interfering with the function and distribution of mitochondria, or alter the motor proteins of the spindle. Such disturbances may affect the expression of spindle components, leading to abnormal chromosome segregation, which has negative consequences for oocyte viability.

Oocyte maturation

Oocyte maturation is controlled by maturation-promoting factor (MPF), which is present in an inactive phosphorylated form in immature oocytes as a complex of Cdk1 and cyclin B (Anderiesz *et al.*, 2000). This phase corresponds to the first point of arrest in oocyte growth, when oocytes at the germinal vesicle stage are quiescent. Following dephosphorylation, the activity of MPF reaches a peak during metaphase I, decreases during the anaphase to telophase transition and rises again during metaphase II. This phase is the second point of arrest in oocyte growth where the activity of MPF remains stable under the regulation of a cytostatic factor. Degradation of MPF occurs after fertilization.

Oocyte viability depends on the accumulation of specific molecules during oogenesis, including mRNA, proteins and imprinted genes (Eichenlaub-Ritter and Pschke, 2002). Active cell growth involves high rates of gene expression during oogenesis, alternating with phases of low transcriptional activity during cell growth arrest. The genes responsible for replication, pairing and recombination are expressed in the embryonal ovary and lead to primordial follicle formation. This probably occurs via the expression of transcription factors, which in turn depend on the transcription of genes whose products are essential in oocyte–somatic cell interactions.

Spindle formation

Meiotic and mitotic spindles in eukaryotic cells are essential for the normal chromosome segregation of chromosomes and to maintain the genomic stability after cell division (Eichenlaub-Ritter *et al.*, 2003). Spindles are dynamic structures, composed of bundles of microtubules which are polar polymers of α- and β-tubulin heterodimers. These tubulin polymers form in the presence of γ-tubulin at microtubule organizing centres and require energy derived from an exchange of GDP/GTP. GTP hydrolysis takes place at the extremities of the microtubules; the fast extremity is attached to the centromere of the individual chromosomes and the slow extremity to the spindle pole. The kinetics of the process are modulated by motor proteins, exchange factors, capping proteins and the concentration of cations.

In humans, spindles undergo a rapid turnover of shrinkage and repolymerization that is highly sensitive to temperature conditions; fast depolymerization occurs, for example, under cooling influence.

Disturbances in spindle function in the oocyte would cause errors in chromosome segregation, resulting in aneuploidy. Chromosomal imbalance is a significant cause of infertility and most of the errors appear to occur during oogenesis, as suggested by data from trisomic fetuses and analyses from polar bodies and preimplantation embryos. A specific spindle regulator mechanism or checkpoint governs the correct reductional chromosome segregation at meiosis. The mechanism may be supported by a back-up mechanism similar to that demonstrated in somatic cells, by which progression to meiosis only occurs when all chromosomes have properly aligned at the equator. Oocytes from aged women or those matured under suboptimal conditions are probably tolerant for a non-efficient spindle checkpoint, permitting cell cycle progression despite the lack of congression when chromosomes fail to align correctly. An additional factor contributing to aneuploidy is the loss of cohesion between homologues or sister chromatids. Cohesion loss is especially frequent in oocytes in which the spindle or the cell cycle progression was perturbed during oogenesis. The most common defects in ageing oocytes (both in women and mouse) involve the spindle (shortening or disorganization) and congression failure of chromosomes. Both conditions predispose the oocyte to aneuploidy or maturation arrest with increasing age. The implication for programmes involving oocyte maturation and handling is the vital need to preserve cytoskeletal integrity and to avoid perturbing spindle formation and function.

Energy supply

Oocytes are the largest cells in the body and require sufficient energy to support transcription and translation during maturation and the minor insufficiencies in ATP availability could irreversibly compromise oocyte quality (Eichenlaub-Ritter and Pschke, 2002). Mitochondria are the major source of the oocyte's energy supply and most mitochondrial proteins derive from nuclear transcripts. Their translocation to the mitochondrial compartment depends on the redox potential and intracellular pH. Any deficiency in these proteins could compromise spindle formation, checkpoint control and chromosome alignment and have consequent effects on chromosome segregation and the likelihood of aneuploidy.

Oocytes require pyruvate as their energy source during maturation, but once the embryo has begun to divide there is a switch to glycolysis. Until the resumption of meiosis, granulosa cells supply some ATP and nutrients to the oocyte via gap junctions, implying that defective mitochondria in granulosa cells could impair oocyte quality. Interestingly, granulosa cells have fewer normal mitochondria in women aged >38 years than in younger women. In addition, low mitochondrial membrane potential has an effect on spindle formation in the oocyte that is associated with chromosome non-disjunction and chaotic mosaicism in human embryos.

Following the resumption of meiosis, oocytes become transcriptionally inactive. As an exception, certain mRNA acquire polyadenylation and expression in a manner which is highly coordinated and specific to the stage of development. In this transcriptionally inactive stage, ATP is needed only to sustain basal metabolism and spindle formation. Mitochondria play a pivotal role in controlling the local intracellular pH by clustering around the spindle, conditioning protein function and cytoskeletal organization, and regulating intracellular calcium homeostasis (Van Blerkom et al., 2002). In conjunction with the endoplasmic reticulum, mitochondria contribute to transmit calcium oscillations which are of primary importance in oocyte activation, and modulate calcium/calmodulin-dependent protein kinase II that is implicated in the transition from metaphase I to anaphase I.

A close association exists between the translocation of mitochondria during oocyte maturation and the integrity of the microtubule network. Therefore, damage to the cytoskeleton during maturation not only affects spindle formation directly, but also indirectly by compromising mitochondrial clustering and distribution. The result is an increased risk of errors at chromosome segregation and a higher risk of aneuploidy.

Syngamy and early embryonic development

Beside providing its genome to the zygote, the mammalian oocyte accumulates the molecules and information needed to sustain initial divisions in the embryo and supplies the factors that will be implicated in regulating the expression from the paternal genome (Gianaroli et al., 1994). At this stage, the epigenetic processes occurring during oocyte growth and maturation impose marking factors acting at fertilization and, in combination with maternal modifiers expressed post-zygotically, at embryogenesis by controlling gene methylation and expression.

In humans, the sperm centrosome represents the division centre regulating the apposition of the two pronuclei and the plane of the first mitotic division (Sathananthan et al., 1996). The sperm centrosome consists of two centrioles in a perpendicular arrangement with pericentriolar material that coordinate nucleation of microtubules and formation of the mitotic spindle. At fertilization, while the sperm head is decondensing, the centriolar region from the sperm tail forms the sperm aster, a radial microtubule-containing structure. By using proteins derived from the oocyte to assemble microtubule structures, the aster brings the female and male pronuclei into close proximity and finally to apposition in the centre of the cell. ICSI of a sperm tail gives rise to an aster, confirming that the centrosome is a paternal inheritance. Conversely, human oocytes have been demonstrated not to contain any centrosomal elements, and the metaphase II spindle is peripherally located and anastral. The occasional traces of electron-dense material found in the oocyte could represent a non-functional maternal centrosome. During the pronuclear stage, the sperm centriole duplicates and locates at syngamy at opposite poles of the first mitotic spindle. The sperm tail generally remains in close proximity to one of the spindle poles. Centrioles are detectable even in the hatching blastocyst.

One of the most relevant morphological modifications occurring after fertilization is the appearance of nuclear precursor bodies (NPB) that migrate and merge into nucleoli according to an orderly sequence. The sites for synthesis of pre-rRNA are in the nucleoli, and the location of these sites in the chromosomes corresponds to the loci where the genes coding for rRNA map.

The synthesis of NPB is initiated by an early transcriptional activity in each pronucleus that is regulated by cytoplasmic factors accumulated during oocyte maturation. A strictly coordinated sequence of these events is extremely important since the newly synthesized rRNA is necessary to translate embryonic transcripts. Polarization of both chromatin and NPB occurs within the pronuclei, which rotate to position their axes toward the second polar body and achieve a proper orientation for subsequent cleavage. These are related phenomena that regulate the design of the embryonic axis, a fundamental step for cell determination in the developing embryo. Alterations in any of these strictly related events may cause abnormalities in embryo division and lead to uneven cleavage, aneuploidy or fragmentation (Gianaroli *et al.*, 2003).

Epigenetics

Epigenetics refers to a process that regulates gene activity without affecting DNA code and is heritable through cell division. Gene activation in the zygote and early embryonic development are regulated by both genetic and epigenetic mechanisms. While the genetic mechanisms depend on the DNA code, epigenetic mechanisms are not DNA sequence-based, even though they determine inheritable modifications that play a key role in the regulation of gene expression (Lucifero *et al.*, 2004). The major epigenetic modifications involve DNA methylation, modification of histones and chromatin remodelling; they are closely linked and act at the transcriptional level. Epigenetic reprogramming occurs at gametogenesis and the correct establishment of epigenetic modifications are essential for normal embryo growth and viability.

Genetic abnormalities in sperm of aged men

Numerical and structural abnormalities in sperm increase somewhat with ageing in men, but since the initial incidence is very low, the increase in observed abnormalities is not clinically relevant. The types of genetic abnormalities that occur in sperm are more likely to cause early abortion than result in the birth of abnormal children (Table V). Although many chromosomal anomalies, such as *de novo* structural rearrangements (84%), the XYY karyotype (100%) and 45,X Turner's syndrome (80%) are

Table V. Advanced paternal age and chromosome anomalies

Numerical anomalies in sperm:
  No paternal age affect for chromosomes 6, 8, 12, 13, 14, 18
  Possible age affect for chromosomes 1, 9, 21
  Probable age effect for XY and linear effect for diploidy
  In newborns:
    Probable effect for trisomy 21
Structural anomalies in sperm:
  Increase in centromere/telomere deletions/duplications of chromosomes 1, 9, 3
  In newborns:
    No increase of *de novo* anomalies (as expected from mechanisms of origin)

Modified from Kühnert and Nieschlag (2004).

of paternal origin, a relationship between paternal ageing and an increase in the proportion of chromosome abnormalities in the offspring has not been clearly established, probably because most of them do not produce a recognizable phenotype, and also because the increase is not as marked as in some anomalies of maternal origin.

Initial attempts to relate paternal age and chromosome abnormalities were only carried out when the interspecies human/hamster fertilization system became available. The results were controversial, in part because there were few analysable metaphases, and also because the oldest men studied were just 44 years of age.

With the advent of FISH, it became possible to analyse a much larger number of sperm. Several studies were designed to determine whether the age affected either sperm disomy or structural rearrangements. Studies on numerical anomalies did not identify an age-related increase for chromosomes 8, 9, 12, 13, 14, 17 and 18.

The incidence of structural anomalies of chromosome 1 was evaluated in control men and in aged men. In these studies, reviewed by Sloter *et al.* (2000), the only structural anomaly that was significantly increased with age (up to the age of 58 years) was the deletion of the centromere region of chromosome 1. These studies also revealed that duplications and deletions were observed in approximately a 1:1 proportion, an indication of the underlying mechanisms of these anomalies.

A related result (an increase in the centromere deletions of chromosome 1) was also found in the only study designed to analyse the frequency of chromosome anomalies in aged men (McInnes *et al.*, 1998).

More recently, Bosch *et al.* (2001, 2003 and unpublished data) have carried out a more systematic study on the relationship between the age and the sperm chromosome anomalies. These studies involved 18 donors (three from each decade of life between the 20s and the 70s) and their ages ranged from 24 to 74 years. In different studies, the same individuals were analysed for numerical anomalies of chromosomes 3, 6, 9, 21, X and Y, as well as for structural anomalies of chromosomes 9 and 3. Chromosome 9 is prone to structural rearrangements, especially paracentric inversions involving the heterochromatic region. Chromosome 3 also shows a tendency to rearrangements of its short arms.

No age-related increase in disomy frequency was found for chromosomes 6, 21 or the sex chromosomes (XX, XY or YY). On the other hand, a statistically significant tendency to a linear increase of diploidy with age was observed.

Regarding chromosome 9, there was a significant tendency to a linear increase of disomy with age. Structural rearrangements increased with age in a linear fashion, and included centromere duplications and the duplication or (even more frequently) the deletion of the long arm telomeric region.

The risk of transmitting a chromosome 9 anomaly to the offspring was 29% higher for every 10 year period for disomy, 19% higher for diploidy, 21% for centromere duplications, and 28% for 9qtel deletions. Although these relative values seem high, they apply to the baseline anomaly values, which are only 0.14% for disomy 9 and ~0.2% for diploidy. Although disomy and diploidy risks would rise by ~100% over 40 years, the final incidences would be only 0.28 and 0.4%, respectively.

A statistically significant tendency to a linear increase with age was also found, in the same donors, for disomy 3, duplications of 3cen and duplications of 3ptel. The risk of transmission per decade increased by 21% for disomy 3, 12% for diploidy, 9% for 3cen duplications and 16% for 3ptel duplications. These studies have not identified an increase in chromosome 6 or Y chromosome disomy.

The mechanisms underlying the events observed probably include numerical and structural anomalies. Since centromere and telomere probes are used in the same round of FISH, in structural anomalies the mechanism involved must separate the centromere from the telomere to obtain the observed 1:1 proportion between duplications and deletions.

In the case of disomy, any non-disjunctional event (including predivison) would fulfil the model. For instance, Luetjens et al. (2002) had found an increased disomy rate in chromosome 9 among older donors (aged >60 years) compared to younger ones (<30 years), and Guttenbach et al. (1997) observed that chromosome 9 had a tendency to non-disjunction. Diploidy could result from failure of either the pachytene or the spindle checkpoints.

As for the structural anomalies, in chromosome 9 the mechanism described above should keep the centromere and telomere separate. This could result from a deletion and non-disjunction of the telomere region, or from a paracentric inversion or an intracentromeric insertion, provided that the resulting dicentric did not produce an anaphase bridge to enter the bridge–breakage–duplication fusion cycle, and the acentric fragment was often lost. In the case of chromosome 3, on the other hand, the acentric fragment should usually have been retained.

Finally, a non-polarized, X-type chromatid exchange in meiosis II could also explain the increase in centromere duplications and telomere duplications of chromosome 3, but chromatid exchange is probably too unusual to be the responsible mechanism. While some age-related increases in the frequency of chromosomal abnormalities observed in sperm are statistically significant, these are small increases and together with the low baseline rates the impact is not clinically relevant.

Among the numerical and structural chromosomal defects, trisomy 21 incidence may be affected by paternal age, although there is too little power to evaluate these associations with certainty. In two autosomal dominant diseases, achondroplasia and Apert's syndrome, there is an association with paternal age. The frequency of the age-related increase in the mutation for achondroplasia is insufficient to explain the age-related rise in the incidence, prompting speculation that the mutant sperm are more capable of fertilizing an oocyte (Kühnert and Nieschlag, 2004). Apert's syndrome is one of the more common craniostosis syndromes, having in addition to the skull abnormality a variety of hand abnormalities. The frequency of the mutation parallels the rising frequency of the disease when observed in sperm but not in lymphocyte chromosomes (Kühnert and Nieschlag, 2004).

Although frequency of chromosomal anomalies and disomies increases with male age, paternal age is not associated with numerical or de novo structural abnormalities in newborns, possibly excepting trisomy 21 (Martin and Rademaker, 1987).

Age and sperm quality

In most Western countries, the mean age of fathers has increased and a significantly larger proportion of men now father a child in their 50s (Plas et al., 2000; Kidd et al., 2001). The trend is mainly attributable to women's decisions to delay the first child until later in life when there is a well-documented age-related decline in female fertility. Whether an age-related decline in male fertility is contributing to the downward trend in fecundity is still an open question. It is also unclear whether the deterioration in some components of the semen analysis or spermiogram with age is clinically meaningful.

Age-related changes are known to occur in the testis. The Leydig cells decrease in number and accumulate the 'ageing' pigment lipofuscin. Age-related changes also occur in the basal membranes, the seminiferous tubules and the tunica albuginea (Johnson et al., 1984; Neaves et al., 1984; Meacham and Murray, 1994).

Age causes localized changes in spermatogenesis, which include a reduction in dark type and intratubular clustering of pale type spermatogonia A. Spermatogenesis is arrested at the spermatocyte I stage and there are numerous malformations of spermatids. These signs of degeneration are usually found in relatively small areas which are diffusely distributed in the testis, and frequency varies among individuals (Holstein, 1986).

In keeping with the decline in Leydig cell numbers, serum testosterone and free testosterone levels decrease by 0.4 and 1.2% per year, respectively, after the age of 50 years (Gray et al., 1991). Reduced mitochondrial steroid supply and reduced perfusion secondary to arteriosclerosis may contribute to this decline (Suoranta, 1971; Takahashi et al., 1983).

Changes in the seminal characteristics involve reductions in seminal volume and sperm cell motility that may be secondary to age-related changes in the function of the epididymis, the prostate and the seminal vesicles. Many studies have evaluated changes in the spermiogram with respect to age, but the selection of subjects, the age groups and the methods of analysis are heterogeneous and the results are conflicting. The methodologically superior studies suggest that the semen volume, the percentage of motile sperm cells and the percentage of the sperm cells with normal morphology decline with age. However, no consistent data confirm that sperm concentration also declines with advancing years (Schwartz et al., 1983; Auger et al., 1995; Rolf et al., 1996; Andolz et al., 1999; Plas et al., 2000; Kidd et al., 2001).

There are too few studies of sperm cell function, of which one study found no age-related change in the ability of human sperm to bind to the hamster zona pellucida (Nieschlag et al., 1984).

The observed endocrine and sperm cell changes with increasing age do not necessarily imply a decline in fertility, although the incidence of subfertilty and time to pregnancy increase significantly when the male partner is aged >50 years (Goldman and Montgomery, 1989; Olsen, 1990; Mathieu et al., 1995; Rolf et al., 1996). The data on whether paternal age affects the outcome of ART are conflicting. Many of the relevant studies do not control for maternal age, which is a natural confounder, affecting both the variable of interest (paternal age) and the outcome (conception). In one study, there were no differences between couples where the males were aged >40 or <40 years

(Spandorfer *et al.*, 1988). In other studies, when male partners were >50 years of age, the pregnancy rates were significantly lower even when the female partner was relatively young (Rolf *et al.*, 1996; Brzechffa *et al.*, 1998).

Prediction of conception among ageing women

Declining fertility might be advanced or retarded in some women, an issue that can be addressed by prediction studies of the association between the assumed markers of ovarian ageing and the conception or a related outcome. In prediction studies, accuracy is a crucial benchmark because forecasting is a hazardous statistical undertaking and the process of prediction is far from perfect.

Among the assumed predictors of ovarian ageing, biochemical markers include FSH, E_2, inhibin A, inhibin B and AMH. Imaging predictors include the number of antral follicles, total ovarian volume and uterine artery flow dynamics. Dynamic tests of ovarian responses include the clomiphene challenge test, the inhibin and E_2 response to FSH and the inhibin and E_2 response to GnRH agonist. The preferred or gold standard outcome of prediction studies should be live birth, but other outcomes are common, such as the relation with chronological age, remaining reproductive lifespan and time of last pregnancy. Among infertile couples, outcomes include the likelihood of conception with or without treatment, poor ovarian response or conception during IVF treatment and various intermediate outcomes of IVF, such as the number of mature follicles or implantation rate.

An unalterable feature of diagnosis and prediction is that accuracy is diminished if there are intervening factors between the test and the outcome of interest. Outcomes that are distant in time involve intermediate influences that may confound earlier predictions. For example, basal FSH predictions of ovarian response to stimulation are more accurate than the basal FSH predictions of IVF outcomes, because factors such as the male contribution, the fertilization environment and the endometrial receptivity are superimposed on the test that characterizes ovarian function (Bancsi *et al.*, 2003). Although IVF is the more uncertain outcome, it is of greatest interest to patients and this discussion will focus on commonly tested predictors of the IVF outcome.

The most widely cited predictor is basal FSH concentration estimated from day 2 to day 4 of the menstrual cycle. An elevated basal FSH level predicts that the ovarian response to gonadotrophin will be reduced and conception will be less likely in IVF cycles (Kwee *et al.*, 2003). Thresholds for FSH level range from <10 to 25 IU/l. A meta-analysis involving 18 studies up to the end of 1999 and 8082 cycles concluded that basal FSH had moderate predictive value for ovarian response and low predictive value for conception in IVF cycles (Bancsi *et al.*, 2003). The likelihood ratio [LR (+)] for conception was >5 in only five of 23 estimates at various thresholds and for those estimates the FSH threshold was usually ≥20 IU/l. When the chosen threshold is far into the severe end of the spectrum of disease, the number of patients in the abnormal range is small (5–11%). In a later meta-analysis that included some studies involving non-ARTs, average sensitivity was 6.6% [95% confidence interval (CI) 5.7, 7.2] and average specificity was 99.6% (95% CI 99.0, 99.9) (Jain *et al.*, 2004). This balance of sensitivity and specificity means that conception is less likely but far from impossible after an abnormal test result, but a normal test result is not reassuring.

The clomiphene citrate challenge test (CCCT) was expected to improve on the predictive value of basal FSH because a second estimation of FSH concentration was included after clomiphene citrate was administered. A meta-analysis by Jain *et al.* (2004) included seven CCCT studies, of which five had IVF success as an outcome, and involved 1352 patients with mean age 34.5 years. Average sensitivity was 25.9% (95% CI 23.0, 29.0) and average specificity was 98.1% (95% CI 96.5, 99.1). Although sensitivity was better with the CCCT than with basal FSH alone, the authors concluded that there was too little difference between basal FSH and CCCT prediction to justify the additional cost and drug exposure.

AFC on cycle day 3 appear to relate with ovarian response in IVF cycles (Chang *et al.*, 1998; Fratarelli *et al.*, 2003; Bancsi *et al.*, 2004). When various expressions of AFC serve to predict ovarian response during IVF cycles, the area under the receiver operating characteristics curve was ≥0.85 compared with 0.61 for age alone (Bancsi *et al.*, 2004). However, only 6–11% of patients are identified as abnormal with the use of a threshold at four follicles; for predicting conception in IVF cycles, AFC is operationally similar to FSH and CCCT (Chang *et al.*, 1998; Fratarelli *et al.*, 2003).

It appears that the basal FSH estimation and other tests are not sufficiently accurate predictors of IVF success (Abdalla and Thum, 2004; van Rooij *et al.*, 2004). Chronological age is more reliable: among 118 young women (≤38 years of age) an elevated FSH (≥10 IU/l) did prevent success (21% live birth rate) while among 577 older women (>38 years) a normal FSH (<10 IU/l) did not improve success (12% live birth rate) (Abdalla and Thum, 2004). Although some women may experience ovarian ageing that is out of synchrony with chronological age, such women are infrequent and none of the putative markers of ovarian ageing currently available are sufficiently accurate to provide a sound basis for clinical policies on eligibility for ART (Table VI).

Pregnancy in women aged >40 years

The endometrium is not a limiting factor for successful pregnancy in women of advanced maternal age (WAMA). Although gene expression profiling by microarray technology has shown that the endometrium consists of ≥19 different

Table VI. Test properties for various predictors of conception with IVF and/or ICSI treatment

Predictor (threshold)	Abnormal test result (%)	LR (+)	LR (−)
FSH (15 IU/l)	11	1.3	1
FSH (20 IU/l)	2	3	1
CCCT	18	12	0.75
AFC (four follicles)	7	7	0.9
Age (38 years)	23	2.6	0.8

LR = likelihood ratio; (+) = abnormal test result; (−) = normal test result; CCCT = clomiphene challenge test; AFC = antral follicle count.

cell types, sophisticated new research techniques such as genomics, transcriptomics, proteomics and metabolomics so far have failed to elucidate the enigmatic process of embryo implantation in the inner uterine lining (Christian *et al.*, 2002). Nevertheless, simple sequential exogenous stimulation with one estrogen and one type of progestogen will allow the endometrium of a 63-year-old post-menopausal uterus to develop and become receptive for an embryo to implant. Ample evidence exists that, well beyond the age of natural menopause, the uterus (at least the endometrium) can preserve its competence and support implantation and early pregnancy (Paulson *et al.*, 1997).

But the uterus is an ageing organ, with its inherently compromised vascularization, which—in the case of pregnancy—makes increased demands on the maternal organism to support the developing fetus, especially beyond the first few gestational weeks.

In a study involving $>24\,000$ pregnant women aged >40 years, in nulliparous WAMA compared with nulliparous 20–29-year-old controls, birth asphyxia (6 versus 4%), fetal growth restriction (2.5 versus 1.4%), malpresentation (11 versus 6%) and gestational diabetes (7 versus 1.7%) were more common in the older women (Gilbert *et al.*, 1999). Similar but smaller differences were found for multiparous WAMA and young controls (birth asphyxia 3.4 versus 2.4%, fetal growth restriction 1.4 versus 1%, malpresentation 6.9 versus 3.7% and gestational diabetes 7.8 versus 1.6%). The mean birthweights were 3201 and 3317 g (nulliparous WAMA babies and controls) and 3381 and 3387 g (multiparous WAMA and young controls). Gestational age at delivery was 273 and 278 days (nulliparous WAMA and young controls) and 274 and 278 days (multiparous WAMA and young controls). For the newborns, there was a greater likelihood in WAMA compared with young controls of pre-term birth (18 and 8% before 37 weeks), low birthweight (11 and 4% <2500 g) and intensive care admission (7 and 4%) (Dulitzki *et al.*, 1998).

The effect of age on the cardiovascular system is complicated to study because BMI is related to blood pressure and increases with age. In a logistic regression of pregnancy data from 910 women, age alone was a risk factor for hypertension and severe hypertension in pregnancy, whereas BMI was an independent risk factor for proteinuria, hypertension and pre-eclampsia (Hrazdilova *et al.*, 2001).

Several reports have shown that WAMA are more likely than younger women to have operative vaginal instrumental deliveries and WAMA have higher Caesarean section rates (47% in WAMA and 23% in young controls) (Dulitzki *et al.*, 1998; Gilbert *et al.*, 1999). The excess rate of Caesarean sections is only partially accounted for by gestational complications (Dulitzki *et al.*, 1998). The majority of women aged >50 years who have oocyte donation pregnancies experience antenatal complications underscoring the importance of high risk obstetric surveillance and care in this group (Sauer *et al.*, 1995).

Although there are few comparative studies, pregnancies after age 40 years have more non-severe complications, more interventions and more premature births, notwithstanding the fact that many of the reported conceptions are in highly selected and well-screened oocyte donation recipients.

Conclusions

Demographic studies show that with women delaying childbirth the mean age at motherhood has been rising since 1980, after decreasing throughout the 20th century. The risk of childlessness increases at higher ages and ovarian ageing is the apparent factor. The most striking manifestation of ovarian ageing is not the decline in oocyte numbers but the decline in the quality of the oocyte, as reflected in the high incidence of early pregnancy loss. The endocrine function of the ovary declines more gradually than its reproductive function: ovulatory cycles are maintained until ovarian failure at the time of the menopause, usually 10 years after the average time of last birth. The decline in follicle number that occurs as women age causes no more than subtle changes in cycle length. Tests reflecting follicle number include ovarian volume, total number of antral follicles and estimates of AMH, inhibin B or FSH concentrations. Although these tests relate with follicle number, the variation between subjects is such that they have limited value in predicting when the ovary will fail in individual women (and they are even less accurate in predicting the likelihood of conception). The lower number and diminished quality of oocytes causes a marked decline in fecundity, which is clinically relevant in women from their mid-30s.

Unfortunately, even ART cannot compensate for $>30–50\%$ of the fecundity that is lost by delaying attempts at conceiving.

Aneuploidy is an important cause of the decline in oocyte quality. During the last decade, the parental and meiotic origins of the most important aneuploid conditions have been characterized and alterations in meiotic recombination have been identified as the first molecular relate of human non-disjunction.

An understanding of the molecular mechanisms responsible for meiotic non-disjunction remains elusive, however, and little is known about the effect of maternal age on trisomy.

A high proportion of chromosomal defects in oocytes arise during the initial phase of development, at meiotic maturation. During fertilization and early divisions of the zygote, mitotic errors are also quite frequent, leading in most cases to developmental arrest. Age induces relatively minor changes in sperm chromosomes, but a small decline in semen parameters: seminal volume, percentage of motile sperm cells and percentage of sperm cells with normal morphology are reduced, but not the concentration of sperm cells.

When the male partner is aged >50 years, the incidence of subfertilty and time to pregnancy seem to increase and the pregnancy rates in ART seem to drop. In women having pregnancies after 40 years of age, gestational diabetes, pregnancy-induced hypertensive disorders, instrumental deliveries and Caesarean sections occur more frequently than in younger women. The newborns of women aged >40 years were more likely to be premature, have low birthweight and be admitted to intensive care units.

There is a need for research to use new approaches to the study of meiotic chromosome segregation if we are to gain a better understanding of the genesis of the most common class of human genetic disorder. There is also a need for better understanding of how the genetics of human oocytes contribute to nuclear maturation and embryo viability. Clinical research is

needed, given that FSH and other predictors of conception are inadequate, to identify robust and accurate markers of ovarian ageing that could identify young patients with a poor prognosis for conventional infertility treatment and assisted reproduction. Finally, health services research is needed to identify treatment programmes for older women that have an optimal balance of cost and effectiveness.

Acknowledgements

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