

Reproductive functions of the ageing male*

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Delayed childbearing is a common phenomenon in industrialized countries. This review focuses on age-associated alterations of male fertility and genetic risks. Semen volume, sperm motility and sperm morphology decrease with age, whereas the data concerning sperm concentration are conflicting. The age-related changes of semen parameters reflect the histological modifications which are found to varying degrees in individual testes. Men aged >40 years contribute to reduced fertility and fecundity of a couple, especially when the female partner is also of advanced age. Because relatively few children are born to older fathers and genetic diseases are rare, there is little statistical power supporting an association of genetic diseases in the offspring with advancing paternal age. Nevertheless, autosomal dominant diseases and some diseases of complex aetiology, such as schizophrenia, are associated with advancing paternal age. The single point mutations in sperm which are responsible for achondroplasia and Apert's syndrome, two autosomal dominant diseases, increase with the man's age. In case of Apert's syndrome this increase is believed to be due to a pre-meiotic selection of mutant spermatogonia. Although structural chromosome anomalies and disomies of certain chromosomes increase in sperm with the man's age, paternal age is, with the exception of trisomy 21, not associated with numerical or *de novo* structural chromosomal aberrations in newborns. However, even if the genetic risk for progeny from older fathers is slightly increased, the risk to the individual is low.

Key words: ageing male/fertility/genetic risk/mutation/semen parameters

Introduction

When reproductive functions of older men are discussed, several celebrities who became fathers at advanced age such as Pablo Picasso, Charlie Chaplin and Anthony Quinn are often cited as examples. While the public regards these cases with a mixture of admiration and skepticism, birth statistics show that there are quite a number of children born to fathers aged >50 years in the general population and this is true of Eastern and Western cultures alike (Figure 1 and Martin *et al.*, 2003). However, it is well known that practically no children are born to mothers aged >50 years and it is common to all older fathers that they have younger partners. The discrepancy in the reproductive span between males and females is astonishing and reduced fertility and higher reproductive risks associated with advancing maternal age prompt the question whether advanced paternal age is also associated with compromised fertility and increasing risks. This question can be extended to younger age groups since increasing numbers of couples postpone parenthood into their fourth or fifth decade of life. In Germany, the

mean age of men at first marriage has risen from 26.6 years in 1985 to 31.8 years in 2002 (Statistisches Bundesamt Deutschland) and the median age of married fathers has increased from 31.3 in 1991 to 33.1 years in 1999 (Statistisches Bundesamt, 2004b). Reasons for this continuing trend are the use of female contraception, rising female educational levels and the almost universal entry of women into the labour market. In addition, life expectancy is increasing and is associated with changing patterns of marriage and divorce so that remarriage and the wish to father a child in a new partnership are becoming increasingly common.

This delay in childbearing unmasks the phenomenon of reproductive ageing which is well documented for women. Besides a progressive decrease of fertility due to both quantitative and qualitative loss of oocytes, eventually ending in menopause, women experience an age-dependent increase of miscarriages, obstetric morbidities and chromosomal anomalies of the fetus (Lubna and Santoro, 2003). In contrast to the female, male reproductive functions do not cease abruptly, but androgen production and spermatogenesis continue lifelong.

Notwithstanding, age-dependent alterations of male reproductive functions have become increasingly obvious. Alterations of

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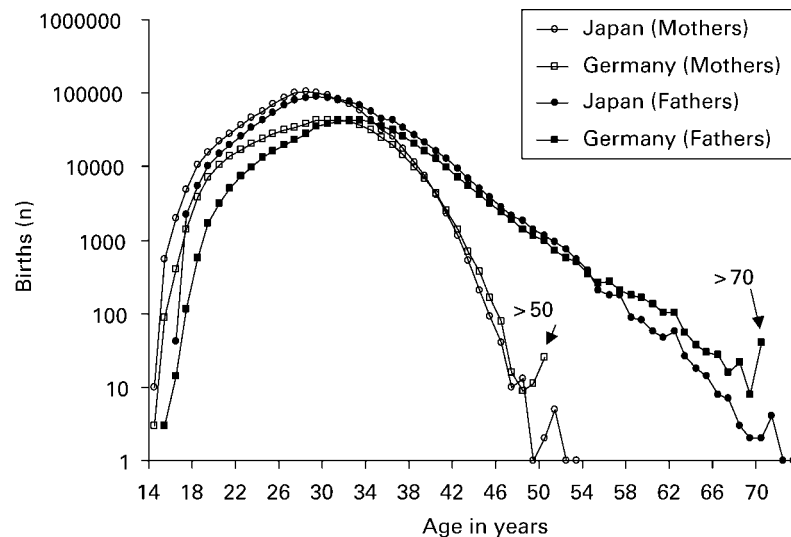


Figure 1. Parental age at the time of birth of offspring in Germany (2001; $n = 550\,659$) and in Japan (2002; $n = 1\,135\,222$). Birth data of German mothers aged >50 years and German fathers >70 years are represented as one dot respectively. (Updated from Rolf and Nieschlag, 2000.)

the endocrine testicular function have been reviewed by Juul and Skakkebaek (2002) in this journal; here we focus on the following questions: (i) do semen parameters show age-dependent alterations? (ii) is there a male contribution to reduced fertility and fecundity of older couples? (iii) is there a genetic risk for offspring attributable to increased paternal age?

Materials and methods

We reviewed the literature by a PubMed (<http://www.pubmed.de/data/nlm.link.html>) search using the key words 'ageing male AND fertility*'; or fecundity* or sperm*; 'paternal age AND fertility*'; or fecundity*; 'ageing male AND chromosomal anomalies*'; or aneuploidies*. Search criteria were limited to studies in humans published in English or German. We searched cited references in retrieved articles and reviewed relevant publications we have collected over 30 years. Book chapters and systematic reviews were also included because they provide a more extensive overview.

Age-dependent alterations in testes and semen

Age-dependent alterations of semen parameters may have several causes. In addition to age *per se*, factors such as urogenital infections, vascular diseases or an accumulation of toxic substances may be responsible for a deterioration in semen parameters. Indeed, a retrospective cross-sectional study in 3698 infertile men showed an infection rate of the accessory glands in 6.1% in patients aged <25 years but in 13.6% of patients >40 years, and total sperm counts were significantly lower ($P < 0.0001$) in patients with an infection of the accessory glands (Rolf *et al.*, 2002). In addition, an age-dependent increase of polychlorinated biphenyls (PBC) in men has been described and in men with normal semen parameters the PBC concentration is inversely correlated with sperm count and progressive motility (Dallinga *et al.*, 2002). The concentration of cadmium also increases with age in the human testis, epididymis and prostate, although lead and selenium remain constant over the whole age range in reproductive

organs (Oldereid *et al.*, 1993, 1998). However, no correlation between cadmium levels in seminal plasma and fertility could be established in humans (Keck *et al.*, 1995).

Testis volume, a rough indicator of spermatogenesis, remains constant over a long period of life. After exclusion of men with diseases shown to be associated with reduced testicular size, the specific effects of age demonstrated a reduction of testicular volume only in the 8th decade of life (Handelsman and Staraj, 1985). In healthy men of this age group, the testis volume is 31% lower than in 18–40 year old men (Mahmoud *et al.*, 2003). Although some histological studies of testes from autopsies of older men or from patients with prostate cancer reveal completely normal conditions (Doepfmer, 1960; Schlüter, 1978), degenerative changes to a highly variable extent are also described, in addition to the physiological germ cell loss observed in the germinal epithelium (Holstein, 1989). Consequently, spermatogenic efficiency, i.e. the number of sperm arising from one cell division of a spermatogonium, is reduced (Johnson *et al.*, 1990). Morphological characteristics of ageing testes include a reduced number of type-A dark spermatogonia, increased occurrence of multinucleated spermatogonia, megalospermatocytes as well as giant spermatids and, as a characteristic feature of senescence, multilayered spermatogonia and diverticula of the seminiferous tubules (Holstein *et al.*, 1988; Holstein, 1989). Sertoli cells accumulate cytoplasmic lipid droplets (Holstein, 1989) and are reduced in number (Harbitz, 1973), as are the Leydig cells (Johnson, 1986), which may also be multinucleated (Paniagua *et al.*, 1986). Tubule involution is associated with an enlargement of the tunica propria, leading to progressive sclerosis parallel to a reduction of the seminiferous epithelium with complete tubular sclerosis as an endpoint (Paniagua *et al.*, 1987). Testicular sclerosis is associated with defective vascularization of the testicular parenchyma and with systemic arteriosclerosis of affected men (Regadera *et al.*, 1985). Arteriographic patterns of the epididymis and the testes support these findings and are correlated with the degree of systemic arteriosclerosis (Regadera *et al.*, 1985). In addition, age-dependent alterations of the prostate are well known (Hermann *et al.*, 2000) and are detectable

histologically in 50% of 50 year old men, but in 90% of men aged >90 years (Coffey *et al.*, 1987).

Considering these age-dependent changes in reproductive organs of men, variations in semen parameters over time are not surprising. About 20 studies have analysed age-dependent variations of sperm parameters, but only few were controlled for abstinence time and covariates such as hypertension or smoking habits. Most studies are retrospective and rarely include males >60 years (Nieschlag *et al.*, 1982). An overview of the studies is presented in Table I. They clearly indicate a decrease of semen volume and sperm motility with age, when only those studies controlling for length of abstinence are included. Concerning semen volume, five studies of infertile (Rolf *et al.*, 1996; Andolz *et al.*, 1999; Rolf *et al.*, 2002) or healthy men (Fisch *et al.*, 1996; Eskenazi *et al.*, 2003) found a decrease with age. This decrease ranges between 0.5% per year (Andolz *et al.*, 1999) and a relative decrease in semen volume of 20% when comparing 50 year old with 30 year old men (Eskenazi *et al.*, 2003). The only study which controlled for length of abstinence but failed to detect a significant change of semen volume with age included only men up to an age of 50 years (Schwartz *et al.*, 1983). The bulk of semen volume is derived from the seminal vesicles, and one of their secretions, fructose, also decreases with age whereas zinc and α -glucosidase, secreted by the prostate and epididymis respectively, remain constant over the age range (Rolf *et al.*, 1996).

Six of seven studies which adjusted for time of abstinence found a significant decrease in sperm motility associated with age and a yearly decrease ranging between 0.17% (Fisch *et al.*, 1996) and 0.7% (Eskenazi *et al.*, 2003). These studies were performed in sperm donors (Schwartz *et al.*, 1983; Auger *et al.*, 1995; Fisch *et al.*, 1996; Eskenazi *et al.*, 2003) as well as in infertile patients (Rolf *et al.*, 1996; Andolz *et al.*, 1999) and three of these studies adjusted for confounding factors (Auger *et al.*, 1995; Andolz *et al.*, 1999; Eskenazi *et al.*, 2003).

When focusing on sperm concentration, abstinence-adjusted studies do not provide a uniform picture. A significant age-dependent decrease (Auger *et al.*, 1995; Eskenazi *et al.*, 2003) as well as constant values over the age range (Schwartz *et al.*, 1983) or even a non-significant age-dependent increase with age (Fisch *et al.*, 1996) have been detected in healthy men. Sperm concentration in infertile patients increases (Rolf *et al.*, 1996; Andolz *et al.*, 1999) or remains unaltered (Rolf *et al.*, 2002), as indicated in abstinence-adjusted studies. Total sperm count was calculated only in two of these studies and this decreases with age in fertile men (Eskenazi *et al.*, 2003) but remains constant in infertile men (Rolf *et al.*, 1996).

Concerning sperm morphology, four studies found a significant and five studies found a non-significant deterioration with age in both fertile and infertile patients; five studies found no relation between age and morphology. As methodologically stronger studies with high numbers and adjustment for confounding factors reflect a decrease of normal morphology with age, this finding may be accepted as evidence-based (Kidd *et al.*, 2001). The decrease per annum ranges between 0.2% (Andolz *et al.*, 1999) and 0.9% (Auger *et al.*, 1985).

All reported changes of histological and seminal parameters develop gradually without a sudden age threshold. The alterations in semen parameters fall within normal ranges.

Nevertheless, the age-dependent alterations of testicular histology and semen parameters are accompanied by a significant increase in FSH (Nieschlag *et al.*, 1982) and a slight but significant decrease in inhibin B (Baccarelli *et al.*, 2001; Mahmoud *et al.*, 2003), which are also found in men with apparently normal semen parameters.

Fertility of ageing men

Undoubtedly, male fertility is basically maintained until very late in life, and, in addition to anecdotal reports, it has been documented scientifically up to an age of 94 years (Seymour *et al.*, 1935). Age-dependent decreases of fertility in couples are usually attributed to female ageing, and indeed the strong female age effect, and the fact that male and female age are correlated, make studies on male age effect on fertility difficult. Besides female age, further confounders, such as reduced coital frequency, an increasing incidence of erectile dysfunction and smoking habits have to be considered in studies which analyse male fertility. In a large survey of Italian men, the frequency of erectile dysfunction rose from 4.6% in men <25 years to 37.6% in men >74 years (Mirone *et al.*, 2004). Cigarette smoking is not only associated with infertility but is a strong predictor of erectile dysfunction. Smoking doubled the likelihood of moderate or complete erectile dysfunction after a median follow-up interval of 8.9 years in the Massachusetts Male Aging Study (Feldman *et al.*, 2000).

All studies which focused on a non-clinical population found a significant negative relationship between male age and fertility of the couple.

According to descriptive birth rate data for the Irish population prior to 1911, male fertility declines almost linearly after the age of 42.5 years (Anderson, 1975). In a Mormon birth cohort with a pattern of young marriage, a moderately negative effect of the father's ageing was also detected (Mineau and Trussell, 1982) and the same is true for developing countries, such as Kenya and Syria (Goldman and Montgomery, 1989). All these studies were adjusted for female age and marital duration, which is negatively associated with coital frequency (Weinstein and Stark, 1994).

In a current study from Britain (Ford *et al.*, 2000) the odds ratio for conception within 12 months decreases by 3% per year of the man's age ($P < 0.001$). The authors compared the chance of conception within 12 months for women whose partner was ≥ 5 years older with women whose partner was the same age or younger and calculated an odds ratio of 0.73 ($P = 0.001$). The study was criticized as it evaluated the men's age at conception and not at onset of attempted pregnancy, so that paternal age may be overestimated (Sallmen and Lukkonen, 2001). Another current study found that men >45 years old are 4.6-fold more likely to have a time to pregnancy (TTP) of >1 year relative to men aged <25 years (Hassan and Killick, 2003). The results were comparable in estimating age at conception or at onset of pregnancy attempts and they remained similar when they restricted the results to young women.

The aforementioned studies cannot rule out any influence from subclinical or manifest abortions. Maternal age >35 years is a well-known risk factor for miscarriages but paternal age ≥ 40 years is also a risk factor for spontaneous abortion

Table I. Male age and semen parameters: overview of published data

Male category and reference	No. of subjects (n)	Male age (years)	Age effect on			
			Semen volume	Sperm motility	Sperm morphology	Sperm concentration
Men without fertility problems including semen donors (not adjusted for abstinence time)						
Nieschlag <i>et al.</i> , 1982	43	24–88	↓ (ns)	↓ ($P < 0.0005$)	↔	↑ ($P < 0.05$)
Homonnai <i>et al.</i> , 1982 ^a	555	20–68	30% ↓ ($P < 0.0005$)	↓	↔	↔
Dondero <i>et al.</i> , 1985	445	18–81	↓ (after age 40) (ns)	↓ (after age 40) (ns)	↓ (ns)	↓ (after age 40 ns) (after age 60 sig.)
Wang <i>et al.</i> , 1985	1239	19–53	↔	$r = -0.14$ ($P < 0.05$)	↔	↔
Bujan <i>et al.</i> , 1996	302	21–44	nd	nd	nd	3.3% ↑/year (ns)
Haidl <i>et al.</i> , 1996	64	26–69	↔	↓ ($P = 0.04$)	↓ (ns)	↓ ($P = 0.01$)
Irvine <i>et al.</i> , 1996	577	18–53	0.01% ↑/year (ns)	0.06% ↑/year (ns)	nd	2.1% ↑/year ($P < 0.001$)
Men without fertility problems including semen donors (adjusted for abstinence time)						
Schwartz <i>et al.</i> , 1983	809	26–50	↔	↓ ($P < 0.02$)	↓ ($P < 0.001$)	↔ ($n = 681$)
Auger <i>et al.</i> , 1995	1351	19–59	nd	0.6% ↓/year ($P < 0.001$)	0.9% ↓/year ($P < 0.001$)	3.3% ↓/year ($P < 0.001$)
Fisch <i>et al.</i> , 1996	1283	34.3	$r = -0.15$ ($P < 0.001$)	$r = -0.17$ ($P < 0.001$)	nd	$r = 0.03$ (ns)
Eskenazi <i>et al.</i> , 2003	97	22–80	0.03 ml ↓/year ($P < 0.01$)	0.7% ↓/year ($P < 0.01$)	nd	$r = -2.5\%/year$ ($P = 0.005$)
Infertile men (not adjusted for abstinence time)						
Mladenovic <i>et al.</i> , 1994	77	20–50	nd	↓ ($P < 0.001$)	↓ ($P < 0.01$)	nd
Gallardo <i>et al.</i> , 1996	345	≤30–64	↓ (ns)	↓ (ns)	↓ (ns)	↑ (ns)
Berling and Wolner-Hanssen (1997)	718	21–54	$r = 0.06$ (ns)	↔	↔	↔
Spandorfer <i>et al.</i> , 1998	821	≤39, 40–49, ≥50	↓ ($P < 0.05$)	↓ (ns)	↔	↔
Infertile men (adjusted for abstinence time)						
Andolz <i>et al.</i> , 1999	20 411	15–74	0.5% ↓/year ($P < 0.001$)	0.3% ↓/year ($P < 0.01$)	0.2% ↓/year ($P < 0.001$)	0.7% ↑/year ($P < 0.004$)
Rolf <i>et al.</i> , 1996	117	22–61	↓ ($P < 0.01$)	↓ ($P < 0.05$)	↓ (ns)	↑ (ns)
Rolf <i>et al.</i> , 2002	3437	19–63	↓ ($P < 0.0001$)	↔	nd	↔
Fertile and infertile men (not adjusted for abstinence time)						
Centola and Eberly, 1999	2065	19–67	$r = -0.04$ (ns)	$r = -0.1$ (% motility) ($P = 0.0001$)	$r = -0.5$ (ns)	$r = -0.06$ ($P = 0.02$)
Henkel <i>et al.</i> , 1999	90	22–57	nd	$r = -0.367$ ($P = 0.001$)	nd	nd

^aMiddle aged men referred to an outpatient clinic for treatment of partial impotence.

ns = not statistically significant; sig. = statistically significant; r = correlation coefficient; ↓ = decrease; ↑ = increase; ↔ = no age-related changes.

Paternal age	Maternal age		
	20-29 years	30-34 years	35-44 years
20-29 years			high risk zone
30-34 years	standard risk zone 1.00 (reference)		2.87 (1.86-4.45)
35-39 years			
40-64 years		high risk zone	highest risk zone 5.65 (3.20-9.98)

Figure 2. Miscarriage risk for couples in different age groups. The adjusted odds ratio and 95% confidence intervals of three couple-age zones are presented. (With permission from de la Rochebrochard and Thonneau, 2002.)

(de la Rochebrochard and Thonneau, 2002). A retrospective study of a large sample of European couples analysed the risk of difficulties (due to adverse pregnancy outcome, i.e. ectopic pregnancy, miscarriage or stillbirth or due to delayed conception) and the risk of delay in pregnancy onset (de la Rochebrochard and Thonneau, 2003). Both indicators of infertility are increased in couples with women aged 35–39 years and paternal age ≥ 40 years compared to paternal age < 40 years, but the former is a greater risk. Thus this reflects the increased risk of miscarriage with paternal age. Paternal age ≥ 40 years becomes relevant concerning miscarriages when the woman is > 30 years old and concerning pregnancy onset when pregnancy is delayed beyond female age > 35 years. Risk of delay in pregnancy onset and especially of miscarriage is highest when both partners are advanced in age (de la Rochebrochard and Thonneau, 2002, 2003). The risks for miscarriage for three different couple-age classes, i.e. standard risk, significantly increased risk ('high zone') and a major risk increase ('highest zone') are summarized in Figure 2. Age-related changes were also found in a prospective study which estimated day-specific probabilities for pregnancy relative to ovulation (Dunson *et al.*, 2002). Frequency of sexual intercourse was monitored by sexual diaries and ovulation was based on basal body temperature measurements (Figure 3). According to this study, fertility for men aged > 35 years is

significantly reduced and the age effect of men aged 35–40 years is about the same as when intercourse frequency drops from twice per week to once per week (Dunson *et al.*, 2004).

In studies dealing with subfertile couples, a significant decrease in pregnancy rates (Rolf *et al.*, 1996) or increase in TTP (Olson, 1990) were observed with female but not with male age, possibly indicating that male age-dependent alterations are masked by the infertility as such.

With methods of assisted reproduction, prerequisites for natural conception such as motility or fertilizing capacity are circumvented. The more invasive the treatment, the less important male age appears: success rates of ISCI (Spandorfer *et al.*, 1998) or IVF (Piette *et al.*, 1990; Gallardo *et al.*, 1996; Paulson *et al.*, 2001) are not associated with male age. On the other hand the success rate of intrauterine insemination (IUI), a method which requires much higher quality and capability of sperm, is related to male age (Mathieu *et al.*, 1995; Brzechffa and Buyalos, 1997).

However, when we analysed data from the German IVF register from 1998 to 2002, we found a significantly reduced pregnancy rate per embryo transfer in couples with male age ≥ 50 years and female age between 31 and 40 years, compared to couples with a male age < 50 years. This effect may have escaped the notice of the above-mentioned studies because of a lower number of couples in this male age category (Figure 4).

Taken together, the studies indicate that men start to contribute to reduced fertility of a couple in their late thirties and to a reduced fecundity at the beginning of their forties. The male age effect is less prominent than that of the female, but it becomes particularly relevant if the woman is also of advanced age.

Genetic risks of the ageing male

Down's syndrome (trisomy 21) is among the most common genetic diseases in humans and was already found to be strongly associated with maternal age in the 1930s (Penrose, 1933). A maternal age effect has been found for all trisomy conditions but varies among chromosomes, with an exponential increase of chromosome 21 and a linear increase, e.g. for chromosome 16 (Wyrobek *et al.*, 1996). The resulting trisomy frequency in

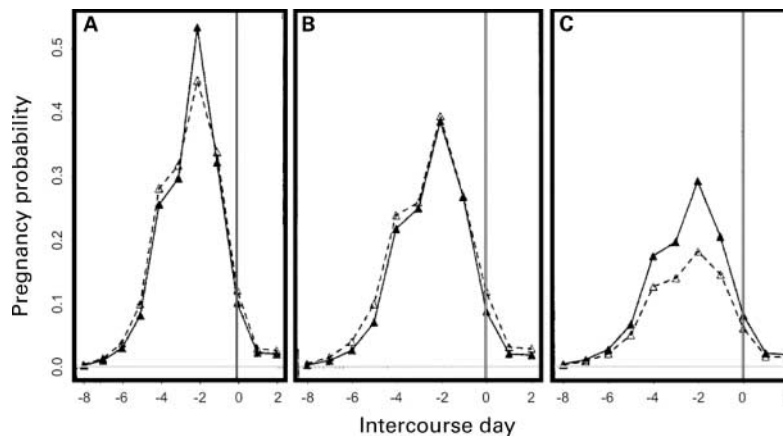
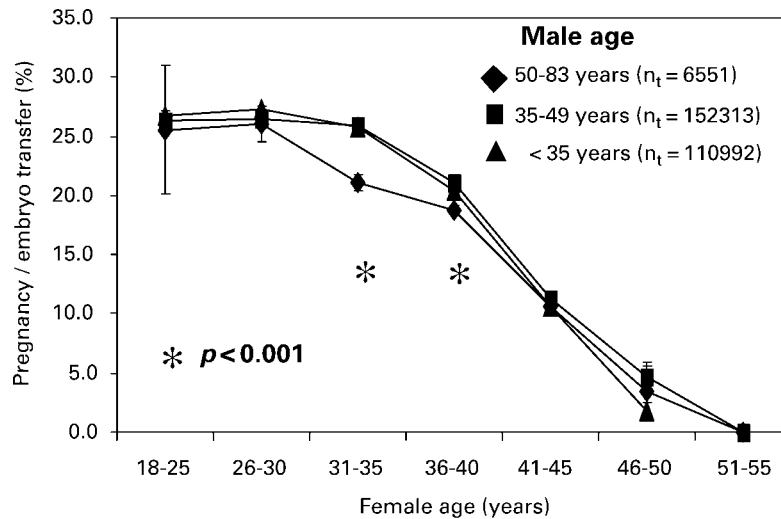


Figure 3. Probability of clinical pregnancy following intercourse on a given day relative to ovulation for women of three different age groups (A: 19–26 years; B: 27–34 years; C: 35–39 years) with average fertility having partners of the same age (—) or 5 years older (- - -). (With permission from Dunson *et al.*, 2002.)



$n_t(\blacklozenge) = 43$	380	149	2858	1626	143	11
$n_t(\blacksquare) = 1168$	13289	63413	62067	12019	349	8
$n_t(\blacktriangle) = 9871$	42766	46586	10203	1508	58	0

Figure 4. Pregnancy/embryo transfer (%) in relation to the couples' age undergoing IVF or ICSI. Nt = numbers of transfer. [Data compiled from the German IVF Register (DIR) 1998–2002.]

clinically recognized pregnancies among women aged 20–25 years is 2–3% but >30% for women in their forties (Hassold and Chiu, 1985), so that advanced maternal age represents an indication for prenatal chromosome analysis via amniocentesis or chorionic villus sampling (e.g. Bundesärztekammer, 1998).

On the other hand, early observations also associate paternal age with certain syndromes (Penrose, 1955). Meanwhile it has become evident that some mutations, consisting of single base substitutions in three different genes: RET, FGFR2 (fibroblast growth factor receptor 2) and FGFR3 (fibroblast growth factor receptor 3), are exclusively of paternal origin and increase non-linearly but sharply with male age (for review, see Crow, 2000). A possible explanation for this male-specific age effect is the much higher number of germ cell divisions in males than in females: in the fetal ovary, germ cells undergo 22 mitotic divisions before they enter the meiotic prophase (Drost and Lee, 1995). They remain in meiotic arrest and continue meiosis in adulthood when ovulation has taken place. Thus, while it was formerly believed that in women germ cell divisions are completed before birth, a recent publication suggests that adult mouse ovaries still possess mitotically active germ cells (Johnson *et al.*, 2004).

In males, germ cells divide continuously. It has been estimated that 30 spermatogonial stem cell divisions take place before puberty, when they begin to undergo meiotic divisions. From then on, 23 mitotic divisions per year occur, resulting in 150 replications by the age of 20 years and 840 replications by the age of 50 years (Crow, 2000). Because of these numerous divisions of stem cells, older men may have an increased risk of errors in DNA transcription.

The association between elevated paternal age and serious birth defects is the reason why the age of semen donors is limited to 40 years in certain countries (American Society for Reproductive Medicine, 1998; British Andrology Society, 1999).

Conversely, male age is not an indicator for prenatal diagnosis. The relevance of this will be discussed in the following sections.

Numerical chromosome disorders

Aneuploidy, the presence of an extra or missing chromosome, is the leading genetic cause of pregnancy loss. Aneuploidies are detected in 35% of spontaneous abortions, in 4% of stillbirths and in 0.3% of live births (Hassold and Hunt, 2001). Among spontaneous abortions, Turner's syndrome (45,X) and trisomy 16, 21 and 22 are the most prevalent aneuploidies. In live births, an additional sex chromosome (XXX; XXY; XYY) or an additional chromosome 21, followed by trisomy 13 or 18, are most common. In general, aneuploidies arise by the process of non-disjunction, i.e. the failure of paired chromosomes to separate in the first meiotic division of maternal meiosis, whereas some chromosomes show a significant proportion of paternal and/or maternal II errors (Eichenlaub-Ritter, 1996; Griffin, 1996). The aneuploidy rate of oocytes has been reported to be in the range of 13.2% (Jacobs, 1992). However, these data have to be considered with caution as the oocytes were generated from hyperstimulated ovaries and women of higher than average reproductive age.

Sperm reveal an aneuploidy incidence of 2% with a high variability of disomy frequency of individual sperm from different fluorescence in-situ hybridization (FISH) studies (Griffin, 1996). The disomy frequency was calculated to be 0.26% for the sex chromosomes and 0.15% for the autosomes with an exception for chromosomes 14, 21 and 22 which display higher disomy frequencies (for review, see Shi and Martin, 2000). Studies which analysed the age-dependent alteration of aneuploidy frequency in chromosomes are severely limited due to low case numbers. So far, no age effect has been found for aneuploidies in chromosomes 6, 8, 12, 13, 14 or 18 (Luetjens *et al.*, 2002)

and varying results for chromosomes 1, 9 and 21 (Martin *et al.*, 1995; McInnes *et al.*, 1998; Rousseaux *et al.*, 1998; Bosch *et al.*, 2001, 2003; Luetjens *et al.*, 2002).

With two exceptions (Bosch *et al.*, 2001; Luetjens *et al.*, 2002), all studies found an effect of age on the production of disomic sex chromosomes with varying results for XX, XY or YY (Griffin *et al.*, 1995; Robbins *et al.*, 1995; Kinakin *et al.*, 1997; Asada *et al.*, 2000; Guttenbach *et al.*, 2000; Lowe *et al.*, 2001). However, one of these studies found an increase in XY frequency in sperm from older infertile (but normozoospermic) men, compared to a group of younger men without known fertility problems (Asada *et al.*, 2000), so that their results are possibly not only related to age effects. Interestingly, the age-dependent increase of XY disomy was also detected in sperm from fathers of boys with Klinefelter's syndrome (Lowe *et al.*, 2001), irrespective of paternal or maternal inheritance of the extra X chromosome (Eskenazi *et al.*, 2002). Focusing on fathers of boys with paternally derived Klinefelter's syndrome, only one study demonstrated increased paternal age compared to maternally inherited Klinefelter's syndrome (Lorda-Sanchez *et al.*, 1992), whereas the other studies did not confirm a paternal age effect (Carothers and Filippi, 1988; Jacobs *et al.*, 1988; MacDonald *et al.*, 1994; Thomas *et al.*, 2000). Fifty per cent of Klinefelter's syndrome cases are of paternal origin and other gonosomal aneuploidies are even more often paternally inherited in live births, as are 80% of Turner's syndrome cases (45,X) and 100% of XYY karyotypes (Lorda-Sanchez *et al.*, 1992). None of these syndromes is related to paternal age (Bordson and Leonardo, 1991). Similarly the incidence of autosomal aneuploidies, such as trisomy 13, 16 and 18, is independent of paternal age (Hatch *et al.*, 1990; Bordson and Leonardo, 1991).

The paternal age effect for trisomy 21 remains to be elucidated. Early studies with small sample sizes reflect different results in the same study population depending on the method of statistical analysis (Hook and Cross, 1982; Stene *et al.*, 1987). With one exception (Stene *et al.*, 1981), most studies analysing prenatal diagnosis data find no paternal age effect (Roth *et al.*, 1983; Ferguson-Smith and Yates, 1984; Cross and Hook, 1987). In spontaneous abortions a non-significant paternal age effect was detected (Hatch *et al.*, 1990) and in live births, no age effect (Regal *et al.*, 1980; Roecker and Huether, 1983; de Michelena *et al.*, 1993; Stoll *et al.*, 1998) or a significant paternal age effect (McIntosh *et al.*, 1995; Fisch *et al.*, 2003) were evident. In a further study which included stillbirths, a weak but non-significant paternal age effect was observed (Kazaura and Lie, 2002). A further study indicated an effect of donor age on the incidence of trisomy 21 after artificial insemination with frozen donor sperm (Thepot *et al.*, 1996). It should be kept in mind that only 10% of Down's syndrome patients receive the excess chromosome from their father (Hassold and Sherman, 2000), so that an age effect could be confined to this small category of cases and subtle age effects might go undetected unless those derived paternally are considered separately. However, with respect to paternally inherited Down's syndrome cases, no paternal age effect became evident (Hook and Regal, 1984).

Moreover, it is difficult to identify whether there is an additional paternal age effect, due to the steep maternal age effect. Nevertheless, all studies undertaken within the last 10 years involving large sample sizes (McIntosh *et al.*, 1995;

Table II. Advanced paternal age and genetic abnormalities

Numerical chromosomal anomalies
In sperm:
No paternal age effect for chromosomes 6, 8, 12, 13, 14 or 18
Age effect equivocal for chromosomes 1, 9 and 21
Age effect for XY
In newborns:
Age effect for trisomy 21 highly probable
Structural chromosomal anomalies
In sperm: increase with age (cytogenetic studies)
FISH studies in individual chromosomes:
Duplications and deletions for the centromeric and subtelomeric regions of chromosome 9
Deletions of the centromere of chromosome 1
In newborns: no increase of <i>de novo</i> anomalies
Autosomal dominant diseases
In sperm:
Age-dependent increase of mutations for Apert's syndrome and achondroplasia
In newborns:
Exponential increase with paternal age in case of achondroplasia, Apert's syndrome, multiple endocrine neoplasia type 2B, Crouzon's syndrome, Pfeiffer's syndrome, thanatophoric dysplasia
Weak paternal age effect for: osteogenesis imperfecta, neurofibromatosis and bilateral retinoblastoma

Thepot *et al.*, 1996; Jyothy *et al.*, 2001; Kazaura and Lie, 2002; Fisch *et al.*, 2003) reflect an influence of paternal age to a varying degree, and the most recent study clearly shows that advanced paternal age combined with maternal age significantly influences the incidence of Down's syndrome (Fisch *et al.*, 2003). Paternal age effect was seen in association with a maternal age of ≥ 35 years, so that a paternal age effect in aged couples can no longer be neglected concerning trisomy 21, whereas other autosomal or sex chromosomal aneuploidies are not associated with increased paternal age (Table II).

Structural chromosomal anomalies

Structural chromosomal anomalies result from chromosomal breakage and the following abnormal rearrangement within the same or within different chromosomes. In 84% of cases *de novo* structural aberrations are paternal in origin (Olsen and Magenis, 1988) and they are found in 2% of spontaneous abortions and in 0.6% of live births (Jacobs, 1992). Cytogenetic studies on structural chromosomal anomalies in sperm are rare but consistently describe an increase of mutations with age (Martin and Rademaker, 1987; Estop *et al.*, 1995; Sartorelli *et al.*, 2001). FISH was used for the structural analysis of individual chromosomes: duplications and deletions for the centromeric and subtelomeric regions of chromosome 9 increase significantly with age (Bosch *et al.*, 2003) as do deletions of the centromere of chromosome 1 (McInnes *et al.*, 1998). Despite these age-dependent structural alterations in sperm, no increase of *de novo* structural chromosomal anomalies has been detected in newborns from older fathers (Hook *et al.*, 1984). However, it is assumed that older fathers more often bequeath balanced structural chromosomal anomalies to their offspring than younger fathers (Hook *et al.*, 1984).

Autosomal dominant diseases

Achondroplasia, the most common form of dwarfism, is the first genetic disorder that was hypothesized to have a paternal age component (Penrose, 1955). Meanwhile further autosomal dominantly inherited diseases have been shown to be the result of paternally inherited mutations and to be associated with increasing paternal age, such as multiple endocrine neoplasia type 2B and craniosynostotic diseases such as Apert's syndrome, Crouzon's syndrome and Pfeiffer's syndrome, which are characterized by a premature fusion of sutures of the skull (Risch *et al.*, 1987; Carlson *et al.*, 1994; Moloney *et al.*, 1996; Wilkin *et al.*, 1998; Glaser *et al.*, 2000). Achondroplasia occurs between 1 in 15 000 and 40 000 live births (Vajo *et al.*, 2000). It is caused by two mutations at the same nucleotide 1138 of the FGFR 3 gene, both resulting in the substitution of arginine for the glycine residue at position 380 in the protein. Apert's syndrome has a birth prevalence of ~ 1 in 70 000 (Cohen *et al.*, 1992; Tolarova *et al.*, 1997) and is due to two mutations in the FRGF 2 gene with a C-to-G transversion at position 755 and 758 (Glaser *et al.*, 2003). Apert's syndrome and achondroplasia have been amenable to direct sperm DNA mutation analysis (Tiemann-Boege *et al.*, 2002; Glaser *et al.*, 2003; Goriely *et al.*, 2003) and both are characterized by an age-dependent increase of mutations in sperm, but there are some peculiarities.

The age-related increase of the mutation for achondroplasia in sperm is lower than expected (Figure 5) and does not explain the exponential increase of the disease with paternal age (Tiemann-Boege *et al.*, 2002). This discrepancy might be explained by a selective process so that the mutant spermatozoon is more likely to fertilize an oocyte. Another possible reason is that, with the exception of four cases, mutation analysis was not performed in sperm of fathers of children with achondroplasia. They might constitute a group with distinct mutation properties. In fact, in fathers of children with Apert's syndrome, the increased frequency of mutations in sperm mirrors the paternal age effect of the disease, whereas fathers of unaffected children reveal a lower mutation frequency in sperm (Glaser *et al.*, 2003). Goriely *et al.* (2003) concluded that the apparently high mutation frequency for Apert's syndrome in older men is caused by a

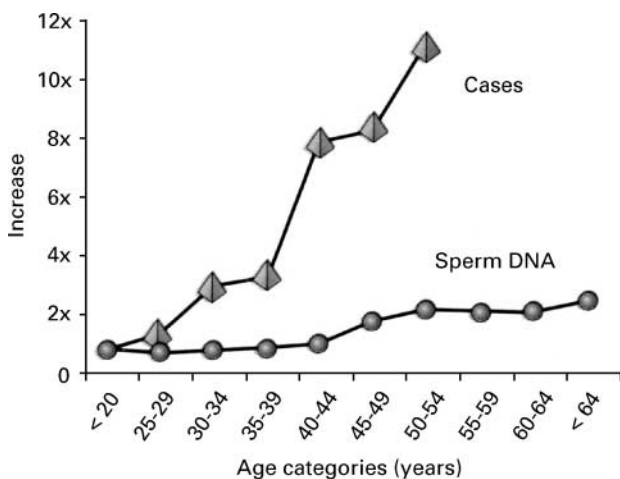


Figure 5. Increase of spontaneous cases of achondroplasia with paternal age and frequency of FGFR 3 mutations in sperm. (With permission from Tiemann-Boege *et al.*, 2002.)

selection of mutant spermatogonia before the start of meiosis. They detected a mutation frequency in sperm which corresponds to the age-dependent increase of the disease. The results were independent of whether the fathers had children with Apert's syndrome or not. The mutation frequency of Apert's syndrome is constant with age in leukocytes (Glaser *et al.*, 2003; Goriely *et al.*, 2003), suggesting that the increase of mutations or the selection of mutations is a tissue-specific effect of the testis.

For sporadic cases of Crouzon's or Pfeiffer's syndrome, 11 different mutations of the FGFR 2 gene are responsible, indicating that, unlike Apert's syndrome or achondroplasia, these are genetically heterogeneous conditions (Glaser *et al.*, 2000). These mutations also arise in the male germ line and advanced paternal age was noted for fathers of those patients.

Other mutations in the FGFR 3 gene have been identified as a cause of thanatophoric dysplasia, the most common type of neonatal lethal osteochondrodysplasias and characterized by extremely short ribs, tubular bones and macrocephaly (Sahinoglu *et al.*, 2003). The increase with paternal age is exponential (Orioli *et al.*, 1995).

The relationship between mutation frequency and paternal age is heterogeneous among autosomal dominantly inherited diseases (Risch *et al.*, 1987). In contrast to the above-mentioned diseases, osteogenesis imperfecta, neurofibromatosis or bilateral retinoblastoma show a weak paternal age effect (Riccardi *et al.*, 1984; Risch *et al.*, 1987; Jadayel *et al.*, 1990; Orioli *et al.*, 1995; Sivakumaran *et al.*, 2000). This may be due to the fact that a significant fraction of new mutations is not base substitutions (for review, see Crow, 2000). Many of the mutations of the neurofibromatosis gene are intragenic deletions. These deletions are not age-dependent because they occur by mechanisms other than the base substitutions and are maternally derived in 16 of 21 cases (Lazaro *et al.*, 1996).

Due to this heterogeneity of the paternal age effect in autosomal dominant diseases, the risk estimates proposed by Friedman for paternal age and autosomal dominant mutations may be overestimated (Friedman, 1981; Hook, 1986). Friedman calculated a risk for autosomal dominant diseases of 0.3–0.5% among offspring of fathers aged ≥ 40 years. This risk is comparable with the risk of Down's syndrome for 35–40 year old women. However, the calculation was based on the assumption that the paternal age effect found in achondroplasia is typical of all autosomal dominant diseases.

Currently, prenatal screening is not possible for all autosomal dominant diseases (American College of Obstetricians and Gynecologists, Committee Opinion, 1997), as there are plenty of mutations to be considered. At least 154 paternally derived mutations have been analysed in FGFR3, FGFR2 and RET (Crow, 2000). Achondroplasia is almost never detected on prenatal ultrasound before the third trimester and a reliable sonographic diagnosis of fetal *de novo* skeletal dysplasias is rarely possible (Mesoraca *et al.*, 1996; Modaff *et al.*, 1996). Taken together, prenatal screening for paternally transmitted autosomal dominant diseases is not yet performed routinely.

Genetic diseases of complex aetiology

This section focuses on diseases which are caused by genetic as well as environmental components. Specifically, congenital heart

defects belong to this category. In a retrospective analysis of 4110 cases, an increasing risk with paternal age was found for ventricular septal defects, atrial septal defects and patent ductus arteriosus (Olshan *et al.*, 1994). It was estimated that among men aged >35 years, ~5% of cases may be due to advanced paternal age, possibly through dominant mutations. A further analysis of paternal age in relation to birth defects indicated an odds risk for fathers aged ≥ 40 years relative to those <40 years of 1.69 for ventricular septal defects. For atrial septal defects, the risk for fathers aged ≥ 35 years was an odds ratio of 1.95 (Lian *et al.*, 1986). Conversely, three further studies did not support the hypothesis that paternal age *per se* is a risk factor for congenital heart disease (Ewing *et al.*, 1997; Loffredo *et al.*, 2001; Cedergren *et al.*, 2002). However, one of them found associations for paternal marijuana smoking and for cocaine use among fathers aged >34 years, thereby supporting the multifactorial aetiology of birth defects (Ewing *et al.*, 1997).

Similarly conflicting are the data for Alzheimer's disease. Four studies conclude that paternal age is a risk factor (Powell and Folstein, 1984; Urakami *et al.*, 1989; Whalley *et al.*, 1995; Bertram *et al.*, 1998). One of them found an increased paternal age associated with the disease only in men (Whalley *et al.*, 1995) and another showed a positive association only in patients without a major gene for Alzheimer's disease (Bertram *et al.*, 1998). Four studies found no association between paternal age and Alzheimer's disease (Corkin *et al.*, 1983; Hofman *et al.*, 1990; Fratiglioni *et al.*, 1993; Ptok *et al.*, 2000). These inconsistent results may be due to small sample sizes of the studies or due to the genetic heterogeneity of the disease.

There are more conclusive data with regard to schizophrenia: all studies identified an increased risk of schizophrenia with paternal age (Bertranpetit and Fananas, 1993; Malaspina *et al.*, 2001; Dalman and Allebeck, 2002). Patients without a family history of schizophrenia had significantly older fathers than familial patients, so that *de novo* mutations were considered responsible (Malaspina *et al.*, 2002). Pre-eclampsia, which is considered to be a risk factor for schizophrenia, is also associated with paternal age (Harlap *et al.*, 2002).

Besides the above-mentioned risk for retinoblastoma, advanced paternal age increases the risk of other cancers in offspring. According to the Swedish Family-Cancer Database, there is a marginal effect of paternal age on the incidence of sporadic breast and sporadic nervous system cancer in offspring (Hemminki and Kyyronen, 1999). A weak association between paternal age and death from breast cancer was confirmed by another Swedish study (Holmberg *et al.*, 1995). The association between advanced paternal age and acute lymphoblastic leukaemia is equivocal: it was deduced from two studies (Dockerty *et al.*, 2001; Murray *et al.*, 2002) but rejected by another (Hemminki and Kyyronen, 1999). Using data from the Framingham study, an association between paternal age and the son's risk of prostate cancer was found (Zhang *et al.*, 1999). The association of paternal age with early onset prostate cancer (<65 years) was greater than that with late onset.

Paternal age may influence life expectancy of the female offspring. A retrospective analysis in >8500 persons from aristocratic families found that daughters of fathers aged >50 years died 4.4 years earlier compared to daughters of younger fathers (20–29 years) (Gavrillov *et al.*, 1997). The authors speculate that

genes responsible for longevity are located on the X chromosome and may acquire more mutations during longer paternal life.

A syndrome of unknown cause which is associated with significantly higher paternal age at conception is the CHARGE syndrome (Tellier *et al.*, 1996). The syndrome refers to a non-random clustering of malformations, such as ocular coloboma, heart malformation, choanal atresia, retarded growth, malformations of the central nervous system, genital hypoplasia, ear abnormalities or deafness (Pagon *et al.*, 1981). Environmental factors could be excluded whereas genetic factors are suspected but could not yet be identified (Tellier *et al.*, 1998).

Conclusion

Although based on a small number of cases, the data presented for testicular morphology, semen parameters and fertility in ageing males are conclusive and reflect a gradual deterioration with age within a broad individual spectrum. Most studies suggest that reduced fertility begins to become evident in the late thirties in men. Increased male age is associated with an increased risk of miscarriages and both the risk of infertility and the risk of miscarriage strongly depend on female age. Among couples composed of a woman aged 35–39 years, the adjusted odds ratio for delay in pregnancy onset (failure to conceive within 12 months) for men ≥ 40 years compared to men <40 years is 2.21 and the odds ratio for difficulties in having a child is 3.02. The effect of paternal age on miscarriage becomes relevant in women aged >30 years; the effect on pregnancy onset becomes relevant in women aged >35 years (de la Rochebrochard and Thonneau, 2003).

Advancing paternal age is associated with diseases of complex aetiology such as schizophrenia and with autosomal dominant inherited diseases such as achondroplasia and Apert's syndrome. The literature also indicates an increased risk for trisomy 21.

Couples should be aware of these age-dependent alterations in fertility and predisposition to genetic risks. They should be informed, however, that the male contribution to reduced fertility is less pronounced than the female's and that the absolute risk for the aforementioned genetic disturbances is low. In addition, biological disadvantage may to a degree be balanced by social advantage, as it has been shown that children born to older parents do better at school than do those of very young parents (Zybert *et al.*, 1978; Cohen *et al.*, 1980).

Although at the moment increased paternal age is not an indication for prenatal diagnosis, there may be further developments in the future. Technical progress will resolve the conflicting data related to the effect of paternal age in achondroplasia and Apert's syndrome and the corresponding mutation in sperm. Mutations and relevant environmental factors in diseases with complex traits still have to be identified and the mechanisms and relevance of DNA damage in sperm with age have to be clarified. Other open questions, e.g. concerning sperm parameters, influence of paternal age on assisted reproduction and on Alzheimer's disease will be answered by studies with more cases. The increasing number of children born to older fathers will allow more highly powered studies on paternal age effects on the offspring, but will also increase the need to identify those men who carry an increased risk for affected children.

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