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Review Article

What is Wrong with the Human Reproductive System?

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Abstract

Human reproduction is highly inferior by mammalian standards. This is manifest as: (i) poor semen quality; (ii) a lower probability of conception; (iii) an increased risk of aneuploidy embryos (e.g. Down syndrome); (iv) a high risk of embryonic loss (early miscarriage); and (v) the recent rise of germ-cell testicular cancer, which occurs in few non-human mammalian species. Review and synthesis of the evidence from several distinct research areas reveals a shared pathogenesis underlying at least some cases of all of them. This involves an intergenerational process, starting with DNA damage to round spermatids. DNA repair in the oocyte is faulty, leading to abnormal chromosome structure in the surviving embryos. This is amplified during meiosis due to delayed synapsis, leading to cellular disorganization. This mechanism also explains the association of aneuploidy with maternal age: structural mismatch between maternal and paternal chromosomes reduces the number of crossovers, making segregation errors more likely.

Spermatid DNA damage can be initiated by numerous chemical agents, including ubiquitous ones. However, the leading candidate to explain these observations is heat, as it best fits the descriptive epidemiology. Intra-testicular temperature is higher among men in cool climates wearing trousers than among men in the tropics wearing loose garments; and the twentieth-century rise in sedentary occupations could explain the rise of testicular cancer.

Keywords

Semen quality; Infertility; Embryo loss; Aneuploidy; Testicular cancer; Sperm DNA damage; Meiosis; Aneuploidy relationship with maternal age

The Decline of Man

During the twentieth century, major adverse changes were observed in the reproductive systems of men in some populations, notably the rise of testicular cancer. In addition, there is reason to believe that even before these developments, reproduction in humans was inferior to that of other mammals, and included a high frequency of genetic abnormalities; this affects both sexes.

The male changes may be regarded as a sentinel event for the wider human impairments, as well as providing a clue that helps in understanding the mechanisms at work. The purpose of this paper is to explain how genetics and reproduction are specifically impaired in humans. This builds on a previous synthesis of the evidence on the recent/current problems affecting the male reproductive system, and an explanation of these phenomena, which is the starting point of this paper.

It is not suggested that all abnormalities of the human reproductive system have the same origin – for example there are clearly many causes both of male and female subfertility - but rather that there is a widespread pathogenetic process that can have many different manifestations. The present paper outlines this pathogenesis and the evidence for it, and then examines what etiology could have brought it into being.

These different manifestations are usually seen as separate issues, and are studied in different sub-disciplines that normally remain in unconnected silos. However, when the totality of the evidence across sub-disciplines is reviewed and synthesized, it becomes clear that these apparently distinct manifestations have a common origin. The relevant sub-disciplines include epidemiology, genetics (including genetic epidemiology and population genetics), cell cycle research, and clinical research. In a synthesis of this kind, it is clearly not feasible to approach this task with a systematic literature review. The possibility therefore exists that some degree of bias may be present.

In order to elucidate a phenomenon of this kind, it is necessary to consider the range of different types of observation that are relevant. By bringing these diverse items of evidence together, it is possible to build up a clear description of the main features of what needs to be explained – the *explanandum*. Once this has been made as clear as possible, one can consider the evidence on how these bodily changes have occurred – the *pathogenesis*; and also, what may have initiated these changes – the *etiology*. All three aspects clearly need to correspond with each other, and each can act as a clue for the others. It is important to be clear that to explain the phenomenon, it is not enough merely to find an association of one of the outcome variables with an agent; it needs to have a sufficient magnitude of effect, and to correspond with what is known about the spatial and temporal distribution as well as the magnitude of the exposure. These points may seem obvious, but they have not informed all of the literature that covers the relevant areas of research, a topic to which I will return.

The Explanandum

The best established change in the male reproductive system is the rise of germ-cell testicular cancer. This affects men usually between the ages of 20 and 45 years, and was virtually unknown until the late nineteenth century, when it began to increase in England and Wales [1]. It then rose in other European countries [2], starting in men born around 1905 (Figure 1) [3]- a birth cohort perspective is appropriate, because it is well established that the tendency to testicular cancer originates before birth [4], and there is some epidemiological evidence that reinforces this [3]. The magnitude eventually increased to the point where the lifetime risk in the most affected countries, Denmark and Norway, reached almost one percent, in men born around 1960 [5]. A similar but lesser increase has been seen in populations of European descent elsewhere in the world, and also among Polynesians (e.g. Maoris), but other populations have been far less affected if at all, including inhabitants of China and Japan, as well as African Americans [5]. The disease remains rare in the tropics.

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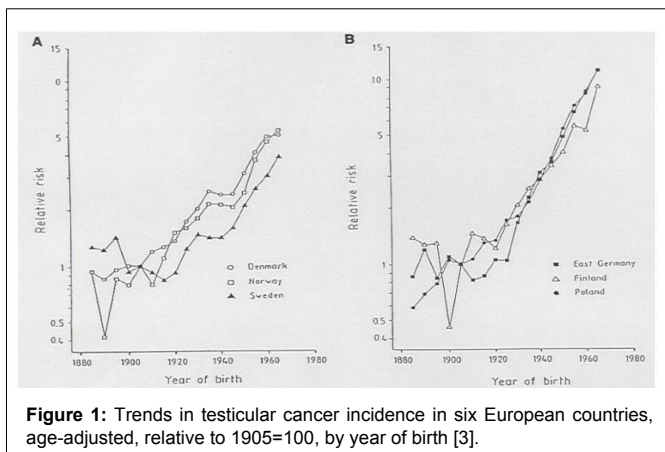
Figure 1: Trends in testicular cancer incidence in six European countries, age-adjusted, relative to 1905=100, by year of birth [3].

It only occurs in a few mammalian species other than humans, e.g. in dogs.

Germ-cell testicular cancer appears to be a severe form of a condition that is more widespread. Men with testicular cancer have lower fertility even before they develop the disease [6], and there are pathophysiological similarities [7].

Semen quality has also been subject to debate and research, particularly focused on the question of whether there was a worldwide decline sometime in the second half of the twentieth century [8,9], which remains controversial [10]. This literature includes studies of candidates for semen donation, which are comparatively reliable because this is a relatively unselected group – or at least, the selection factors are unlikely to change greatly over time – and laboratory methods of assessing semen quality within each clinic can be argued to be fairly stable over time. Such studies have shown a decline in sperm concentration in some places [11-13] but not others [14-16] during the 1970s and 1980s. Interestingly, in the places where sperm concentration fell, there was also deterioration in quality, affecting both motility and morphology. This timing could correspond to a birth cohort effect, relating to men born after about 1950. This is probably the appropriate viewpoint, because defective spermatogenesis is a lifelong tendency (apart from specific types of damage due to e.g. mumps or trauma) that is set early in life. In fact, each man with oligoasthenoteratospermia (OAT, a combination of reduced concentration, impaired mobility and abnormal morphology) tends to have a characteristic pattern of disturbance of spermatogenesis [17-19], which one can loosely describe as a "fingerprint" [20].

One of the intriguing features both of testicular cancer [21-24] and of impaired spermatogenesis [25-31] is that there is evidence of some degree of heritability. Most cases are not inherited, but once a case has occurred subsequent male relatives are at increased risk. It is intriguing because in evolutionary terms, a "gene for infertility" should quickly disappear [20], and also because the heritability coexists with strong evidence of an environmental component, from migrant studies [32] as well as because of the rapid time trends. This coexistence can be interpreted in various ways, but the one that best fits the observations is that an environmental factor has increased the frequency of damage to the genetic apparatus, and that this can then be passed on to the next generation [20]. In addition, the brother-brother link is stronger than the father-son association in testicular cancer, suggesting either an additional maternal factor (as discussed in the cited literature), or alternatively that the father passes on a more severe impairment to his son than he himself has experienced.

This links with another set of observations. Whilst testicular cancer may be relatively new, some other disorders are apparently part of the human condition. Reproduction is relatively impaired in humans, in several respects. Semen quality is greatly inferior to that of other mammals, in terms of quality as well as quantity [33,34]. Fecundability (the probability of conception) is also low [35,36]. Moreover, humans have an increased risk of embryonic loss [37-39], and the embryos are far more liable to aneuploidy, together with mosaicism [40-43]; these are connected, as genetically abnormal embryos are far less likely to continue to term.

Clearly these are not all features of male reproduction: reduced fecundability can be due to female as well as male factors, and most embryonic aneuploidy is female-mediated, except for that involving the sex chromosomes [44]. There are some additional specifically female observations: ovarian cells from apparently normal female fetuses exhibit mosaicism, with a varying proportion containing an extra chromosome 21 [45]. I will return to the question of women later.

A final observation is that it is unclear what "human" means in this context. Almost all the studies have been conducted on people who live in modern societies, implying that we do not really know whether the unique features listed here are biological species-specific characteristics, or whether they are socio-historical, perhaps cultural, features that are relatively recent in evolutionary terms, and may not in fact apply to all of humanity.

The evidence, taken together, suggests a longstanding impairment of the human reproductive system [46]. It also suggests the possibility that this became exacerbated in some populations a little more than a century ago, giving rise to germ-cell testicular cancer [46]. The fact that we are dealing with human specificity means that any findings from animal models need to be examined critically, as they may not be transferable to humans.

Pathogenesis

I will start by outlining some features of the pathogenesis of testicular cancer, and then explore to what extent similar processes, albeit less severe, could explain at least some of the other features of human reproductive-system impairment. This account has already been outlined elsewhere in relation to testicular cancer and OAT [20,46], but without exploring the wider implications.

The reason that the tendency to testicular cancer originates in early life is that the testicular germ cells of affected infants are characterized by aneuploidy and a block in differentiation – *carcinoma-in-situ* (*CIS*) [47-49] – which is already present at birth. The testes are not homogeneous: each affected tubule has its own characteristic pattern and severity of abnormality [49]. The contralateral testis also has an increased risk of *CIS*, indicating a more widespread disturbance. This heterogeneity suggests mosaicism, arising from an unstable genome.

When cancer arises twenty or more years later, it is surrounded by *CIS* tissue, indicating that the tumour arose in one of the *CIS* tubules [49]. The transformation to malignancy involves a deregulated cell cycle [50], and structural chromosome abnormalities and hyperploid [51].

The difficult question is, how does *CIS* arise in the embryo/fetus? In principle, it could be caused by a normal zygote becoming impaired in some way during pregnancy (a gestational issue), and/or from the progression of an already-existing zygotic abnormality (a genetic one)

[20]. I argue that it is more likely that a zygotic abnormality is already present at the time of conception, and that this in turn ultimately arises from fertilization by a sperm with chromosomal impairment in a previous generation, because this hypothesis unifies a large number of disparate observations. This evidence is outlined in the remainder of this section.

Zygotes that result from fertilization by a sperm with DNA damage undergo DNA repair, a process that is controlled by the oocyte [18]. Crucially, this is often faulty, so that the embryo has structurally abnormal chromosomes [18]. (The oocyte's repair capacity varies 10-fold between populations [18], which could be relevant to the ethnic variations in testicular cancer - research is needed on this.) The relevance of structural abnormality in chromosomes is indicated by the observation that embryos resulting from IVF when there is a severe male-factor problem are subject to cellular abnormalities. These include cell cycle delay, centrosome amplification, distorted cellular architecture and an abnormal spindle, chromosome mis-segregation and aneuploidy [52,53].

There is further evidence for the role of DNA structural abnormalities in at least some cases of male-factor infertility. Sperm DNA fragmentation is associated with infertility [54] and with early pregnancy loss [55]. Sperm chromosome aneuploidy is associated with recurrent miscarriage [56]. Nevertheless, fertilization can occur despite this impairment, and the fetus may well survive – and the consequences are driven by the damaged survivors. Synapsis, the coming together of the maternal and paternal homologous chromosomes in early meiosis, is abnormal in the spermatogenesis of infertile men [57-60]. In addition, the outcome of the ICSI procedure (Intra-Cytoplasmic Sperm Injection) is greatly improved by selection of spermatozoa having a smooth, symmetric and oval configuration, with no extrusion or invagination of the nuclear chromatin mass [61,62] - in other words, cellular architecture that is relatively undistorted.

The same is true of embryos. In the context of IVF, a population characterized by severe subfertility, relatively high implantation rates are seen with embryos that have an even, rounded shape, and also with those having more cells (i.e. less delay in cell division) [63,64]. There is now abundant evidence that phenomena related to the pathogenesis described here, such as abnormal division patterns, fragmentation and developmental arrest, adversely affect the formation of good-quality blastocysts and subsequent development [65-67].

The next stage of the pathogenic process occurs at meiosis, which is far more sensitive than mitosis. It starts with pairing of the maternal and paternal chromosomes. If the two chromosomes do not have an identical structure, irregularities such as unpaired loops occur [68,69]. The consequence is that the next stage, synapsis, is delayed by the process of synaptic adjustment (Figure 2) [68,69]. The cell cycle delay may then lead to centrosome amplification, with loss of the normal cellular architecture and possible aneuploidy [52].

Such cellular disruption can trigger apoptosis. This would cause loss of sperm cells, and therefore low sperm concentration. The disrupted cellular architecture of the surviving spermatozoa could be the source of abnormalities of morphology and motility. Thus, the three components of OAT could be due to DNA structural alterations stemming from synaptic delay and the ensuing abnormal spindles. Whilst the abnormal spermatogenesis of some men could result from damage to their own spermatozoa, the lifelong tendency to subfertility and low semen quality that is typically seen in infertile

men is plausibly a consequence of disordered meiosis due to a structural mismatch between their paternal and maternal DNA, from a faultily repaired zygote – an abnormality that was already present shortly after conception. It is trans-generational.

To summarize (Figure 3), my explanation is that DNA strand breaks in the F_0 generation give rise to DNA strand mismatch in the zygote (F_1); when spermatogenesis in the F_1 generation commences at puberty, meiosis characterized by delayed synapsis ensues, leading to cellular disorganization, which affects semen quality throughout life. The degree of chromosomal instability is insufficient to cause *CIS* in the F_1 generation, but in the F_2 generation a more severe degree occurs which can do so [20].

In each generation, I suggest that meiosis exacerbates the pre-existing abnormality because the structural mismatch increases. However there would be an upper limit to the severity of the cellular disorganization at the clone level, because beyond a certain point of chromosomal instability, the cell would not be able to continue beyond a checkpoint or other quality-control mechanism such as fertilizing capacity - it would disappear (Figure 4). The process is akin to natural selection, with the main pathological features being driven by the damaged survivors [20]. Once established in one individual, this process is transmitted to male offspring, explaining the observations outlined above on heritability but with most cases being newly arising.

This account is supported by the fact that the centrosome is paternally inherited in most mammals, including humans (but not in rodents), so that the disordered cell structure is passed down the male line [52]. Each clone would be differently affected by the chromosomal instability, a scattergun, giving rise to a particular persisting pattern of abnormality; this explains the observation of a characteristic "fingerprint" in relation to spermatogenesis, and a similar heterogeneity among the tubules of *CIS* [20]. This is the origin of the observed mosaicism. Germ cells are the target tissue of this process, so it is unsurprising that the testes of affected individuals contain Sertoli-cell only tubules that are entirely devoid of germ cells [7].

In addition, the account accords with the observation that breaks and fragments are relatively frequent in the sperm of healthy men (over 75% of all chromosomal aberrations); duplications and deletions are less common (5-13%); and aneuploidies less frequent still (1-3%) [17,70]. And it explains the ubiquity of duplications and deletions and copy number variations in the human genome [71-73], i.e. "old" structural changes that have persisted.
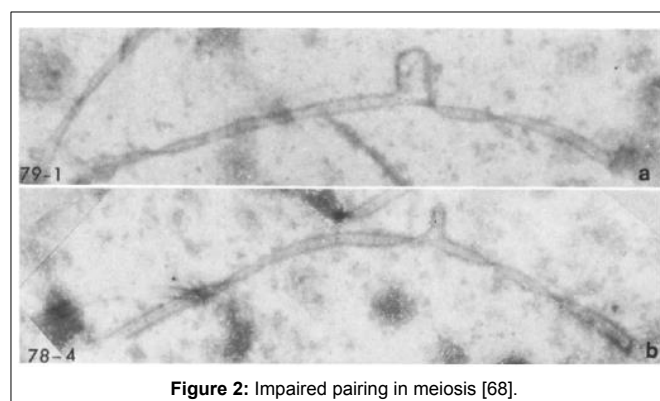

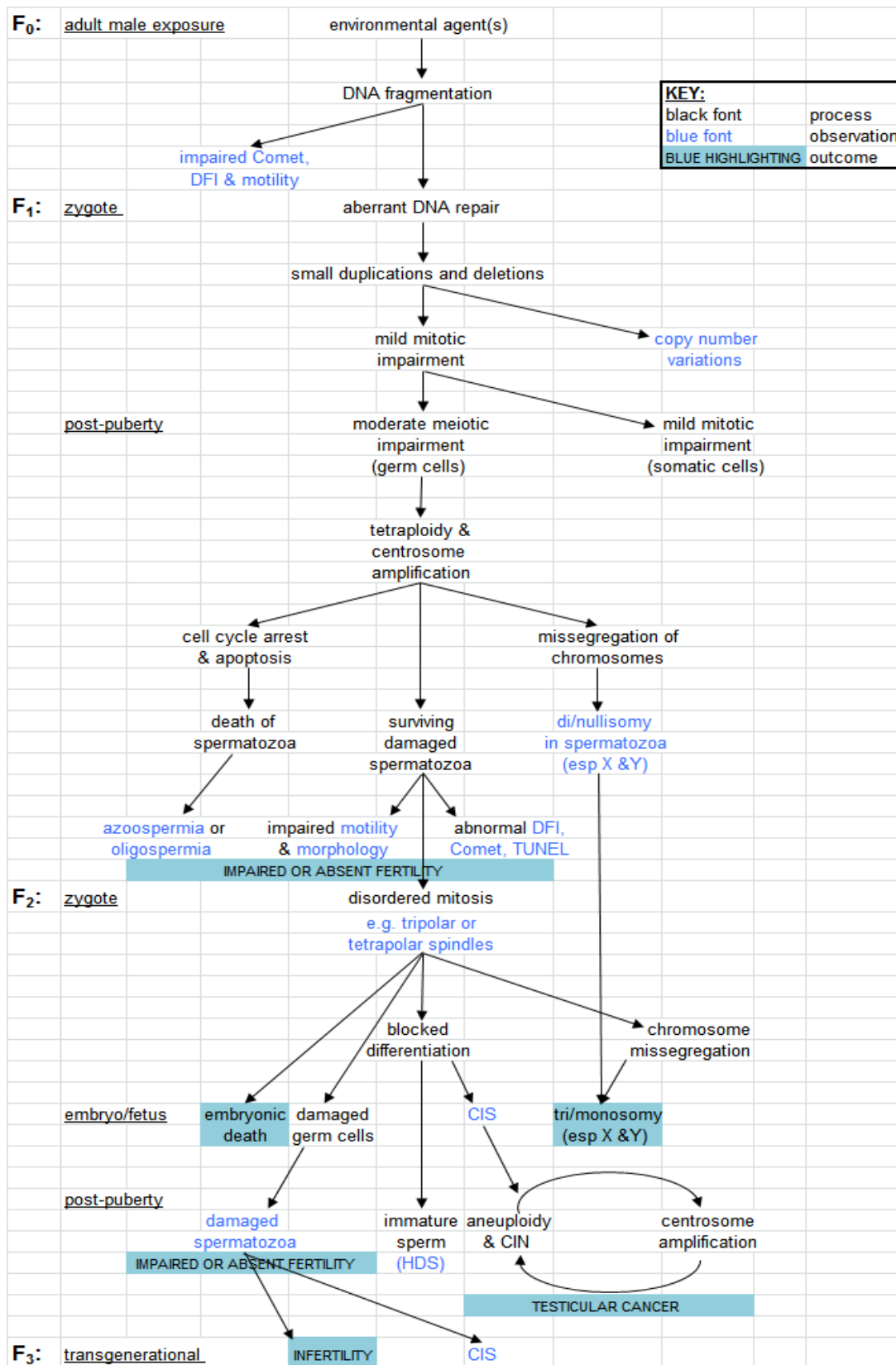**Figure 2:** Impaired pairing in meiosis [68].

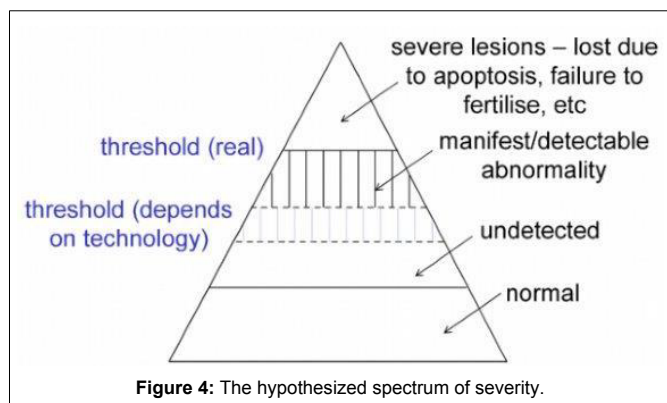**Figure 3:** Main causal pathways in the male line [20].

Figure 4: The hypothesized spectrum of severity.

One implication is that as duplications and deletions accumulate in the human genome, reproduction involving partners from very different populations would be less successful because of the mismatch of their DNA structure. There is some evidence for this: the greatest reproductive success, measured by the number of grandchildren, has been observed in couples who were third or fourth cousins [74,75]. This indicates that genetic similarity (inbreeding) and genetic distance (structural incompatibility) are both associated with reduced inclusive fitness, with an optimum somewhere between. A second implication is that the increased instability of the genetic process in humans could have led to faster biological evolution.

Possible etiology

According to this account, the whole process is started by DNA strand breaks in the F_0 generation. It has long been known that round spermatids are particularly susceptible to this type of damage [76]. Also, DNA repair is absent or greatly impaired at this stage of spermatogenesis [18].

There is a large literature on sperm DNA damage (reviewed in Lewis et al. [54]). Tests include the Comet assay, SCSA (sperm chromatin structure assay), the sperm chromatin dispersion ("Halo") test, the TUNEL assay, and DNA adduct analysis. There is strong evidence for an impact on impaired fertilization, with odds ratios higher than 50 in some cases, and for disrupted pre-implantation embryo development, miscarriage, and birth defects in the offspring. Sperm DNA damage is not predictive of the immediate outcome of ICSI, but early pregnancy loss is increased, which is compatible with the idea that oocyte DNA repair occurs but is faulty. In general, this literature does not routinely distinguish between damage to current spermatogenesis and that which is a pre-existing person-specific feature of a person's sperm production – corresponding respectively to a period effect and a birth cohort effect in epidemiological terms. However, a test such as DNA adducts analysis would be specific for the former.

Sperm DNA damage is thought to be generally a result of oxidative stress, which itself can be caused by a wide variety of factors. These include age, smoking, alcohol, phthalate esters, exposure to radio-frequency electromagnetic radiation, genital tract inflammation, paracetamol, acrylamide, and heat [54]. All of these are exposures that could affect a large proportion of the population. There is no specific evidence relating to any of these, and unfortunately it is difficult to study paternal exposures of these types, except perhaps age at fatherhood, and even more so to study such exposures occurring two or more generations before a case of testicular cancer. Spatio-temporal patterns may suggest that some are implausible, e.g. phthalate esters

which are of rather recent origin, whereas others might fit better with the epidemiological observations. For example, acrylamide is present in many foods, especially starchy food cooked at high temperatures such as potato chips and crisps [77,78], and their consumption has increased over the period relevant to the rise of testicular cancer; also, the metabolism of acrylamide may be ethnically highly variable [79].

Heat is a particularly interesting possibility: it is highly damaging to sperm DNA, even at temperatures that are commonly encountered [80,81]. Men living in temperate or cold climates tend to have a higher intra-testicular temperature than those living in the tropics who wear traditional clothing, due to the effect of trousers [82]. A more recent exacerbation has occurred due to the increase in sedentary living, which further raises the intra-testicular temperature [82,83]. A useful recent review of testicular heat stress can be found at [84].

In the past twenty years, the dominant hypothesis concerning impairment of the male reproductive system has been endocrine disruption. The idea was that exposure in early life to environmental chemicals, initially suggested to be estrogenic [85] and later anti-androgenic [86], was responsible. There is indeed some evidence that certain hormonally active substances can damage the male reproductive system, but this cannot explain these epidemiological observations [87]. First, the estrogenic version fails because even a huge dose of a potent estrogen (diethyl stilbestrol, DES) given during early pregnancy does not lead to the endpoints that concern us here, even according to one of the originators of the hypothesis [88].

Secondly, although the anti-androgenic account has more biological plausibility, it does not provide the answer either. It may be that DDE (the major stable breakdown product of the insecticide DDT) and certain phthalates (e.g. DBP, dibutyl phthalate), or mixtures of such anti-androgens, affect male reproductive development in humans by increasing the risk of cryptorchism and hypospadias, or by reducing the anogenital distance at birth [89-92]. But this hypothesis does not succeed in *explaining the epidemiological observations described above*, even if one accepts the view that these congenital conditions are associated with testicular cancer and male infertility (in addition to the long-established observation that cryptorchism is a risk factor for testicular cancer) in a "testicular dysgenesis syndrome", which is now discredited [93,94]. For one thing, these chemicals were only developed decades after the rise in testicular cancer started [46]. Also, DDE would have caused an epidemic of testicular cancer in malaria-affected areas by now, but this has not occurred; and, the "phthalate syndrome" seen in experimental animals has other manifestations that are not seen in human cases [87]. In any case, whilst endocrine disrupters may be able to cause quantitative damage to spermatozoa, it is very doubtful that they can cause qualitative damage such as impaired motility or morphology [87].

Is there also a Decline of Woman?

So far we have been concerned only with males. The endpoints considered are specific to men, and even the intergenerational pathways have only referred to father-son transmission. But if a process of this kind is operating, female zygotes and the resulting individuals would also be affected. This raises two questions: what pathogenic changes would ensue? And how would they be manifest?

Little directly relevant evidence is available. The focus of research has primarily been on males, and females are intrinsically more difficult to study because they do not produce any equivalent of semen that is available without invasive procedures.

The effects on reproduction would likely be affected by the difference in the meiotic process between the sexes. In men, this is continuous throughout post-pubertal life and affects all the germ cells, whereas in women meiosis starts in fetal life but is then quiescent until ovulation begins, and even then it only continues in a very few oocytes. The maternal centrosome is not passed on to the zygote, although the oocyte does contain transcripts that remain active after fertilization [53].

One observation is that ovarian germ-cell cancer (not ovarian cancer in general, which is mainly of an epithelial type) is more than 10 times less common than testicular cancer. This could be related to the sex difference in meiosis: whereas all the germ cells are continuously involved in meiotic division in post-pubertal males, in females meiosis continues only in a few selected ova. A second is that *germ-cell* cancer in women appears to have had a similar increase over time as has testicular cancer, and a comparable age distribution, suggesting a similar pathogenesis – although the evidence is unclear due to its rarity [95].

Certain additional features are similar to those operating in the male. As stated above, embryonic aneuploidy from male-mediated processes primarily affects the sex chromosomes, so it is likely that the autosomal equivalent could be explained by the same pathogenesis operating in females. In favor of this, mosaicism, cell cycle delay and chromosomal gain and loss are seen also in the female-mediated case [39,44,96-98], fitting well with the process described above. This is supported by experimental evidence: for example in female mice, age-related embryonic aneuploidy appears to be related to progressive shortening of meiotic prophase, leaving less time for accurate chromosome attachment [76]. Moreover, such a mechanism would readily explain female-factor infertility, and a tendency to embryonic loss.

The same pathogenic process could explain why human trisomies increase with maternal age. They result from segregation errors, when the sister centromeres fail to be held tightly together during the decades elapsing between embryonic life and the time of ovulation. Again, the impairment is due to a structural mismatch between the maternal and paternal chromosomes, and its effect on pairing. It has been observed that disturbance of meiotic pairing causes a failure of recombination in the affected chromosome [99]. Such lack of crossovers is a feature specific to a subgroup of women, and is more frequent in women than in other mammals, or in men [100]. This is important because crossovers are a major factor in keeping the sister chromatids together. This would not be evident in mouse models, or in women who do not have non-disjunction events. (However, in such situations a similar process may occur due to a decrease in cohesin protein rather than an absence of recombination [101-103]: cohesin protein and crossovers are both ways that the chromatids are kept together.) The idea that structural mismatch between maternal and paternal chromosomes is the source of trisomy, resulting from fewer crossovers, is supported by the observations that a high recombination count increases the chance of a live birth, especially with increasing maternal age, and that mothers with a high recombination rate tend to have more children; also, there is a large genetic component to the recombination rate [104].

Conclusion

In conclusion, I propose a pathogenic process that starts with sperm DNA damage and propagates through subsequent generations. In its earlier, ancient version it underlies the specifically human tendencies to poor semen quality, low fecundity, aneuploidy, mosaicism and embryonic loss. A more malignant recent version has given rise to an epidemic of testicular cancer in some populations during the twentieth century.

References

1. Davies JM (1981) Testicular cancer in England and Wales: some epidemiological aspects. Lancet 1: 928-932.

2. Adami HO, Bergström R, Möhner M, Zatoński W, Storm H, et al. (1994) Testicular cancer in nine northern European countries. Int J Cancer 59: 33-38.

3. Bergström R, Adami HO, Möhner M, Zatonski W, Storm H, et al. (1996) Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon. J Natl Cancer Inst 88: 727-733.

4. Skakkebaek NE, Berthelsen JG, Giwercman A, Muller J (1987) Carcinoma-in-situ of the testis: possible origin from gonocytes and precursors of all types of germ cell tumours except spermatocytoma. Int J Androl 10: 19-28.

5. Parkin DM (2005) Cancer Incidence in Five Continents. IARC Press, Lyon.

6. Møller H, Skakkebaek NE (1999) Risk of testicular cancer in subfertile men: case-control study. BMJ 318: 559-562.

7. Skakkebaek NE, Rajperts-de Meyts E, Main KM (2001) Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 16: 972-978.

8. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE (1992) Evidence for decreasing quality of semen during past 50 years. BMJ 305: 609-613.

9. Swan SH, Elkin EP, Fenster L (2000) The question of declining sperm density revisited: an analysis of 101 studies published 1934-1996. Environ Health Perspect 108: 961-966.

10. te Velde ER, Bonde JP (2013) Misconceptions about falling sperm counts and fertility in Europe. Asian J Androl 15: 195-198.

11. Auger J, Kunstmann JM, Czyglik F, Jouannet P (1995) Decline in semen quality among fertile men in Paris during the past 20 years. N Engl J Med 332: 281-285.

12. Irvine S, Cawood E, Richardson D, MacDonald E, Aitken J (1996) Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. BMJ 312: 467-471.

13. Van Waeleghem K, De Clercq N, Vermeulen L, Schoonjans F, Comhaire F (1996) Deterioration of sperm quality in young healthy Belgian men. Hum Reprod 11: 325-329.

14. Wittmaack FM, Shapiro SS (1992) Longitudinal study of semen quality in Wisconsin men over one decade. Wis Med J 91: 477-479.

15. Bujan L, Mansat A, Pontonnier F, Mieusset R (1996) Time series analysis of sperm concentration in fertile men in Toulouse, France between 1977 and 1992. BMJ 312: 471-472.

16. Fisch H, Goluboff ET, Olson JH, Feldshuh J, Broder SJ, et al. (1996) Semen analyses in 1,283 men from the United States over a 25-year period: no decline in quality. Fertil Steril 65: 1009-1014.

17. Sloter ED, Lowe X, Moore II DH, Nath J, Wyrobek AJ (2000) Multicolor FISH analysis of chromosomal breaks, duplications, deletions, and numerical abnormalities in the sperm of healthy men. Am J Hum Genet 67: 862-872.

18. Marchetti F, Wyrobek AJ (2005) Mechanisms and consequences of paternally-transmitted chromosomal abnormalities. Birth Defects Res C Embryo Today 75: 112-129.

19. Sergerie M, Laforest G, Boulanger K, Bissonnette F, Bleau G (2005) Longitudinal study of sperm DNA fragmentation as measured by terminal uridine nick end-labelling assay. Hum Reprod 20: 1921-1927.

20. Joffe M (2011) Genetic damage and male reproduction, In Reproduction and Adaptation [AS Parkes Memorial volume] Cambridge University Press, Cambridge, 17-49.

21. Forman D, Oliver RT, Brett AR, Marsh SG, Moses JH, et al. (1992) Familial testicular cancer: a report of the UK family register, estimation of risk and an HLA class 1 sib-pair analysis. Br J Cancer 65: 255-262.

22. Li FP (1993) Molecular epidemiology studies of cancer in families. Br J Cancer 68: 217-219.

23. Goldgar DE, Easton DF, Cannon-Albright LA, Skolnick MH (1994) Systematic population-based assessment of cancer risk in first-degree relatives of cancer patients. J Natl Cancer Inst 86: 1600-1608.

24. Swerdlow AJ, De Stavola BL, Swanwick MA, Maconochie NE (1997) Risks of breast and testicular cancers in young adult twins in England and Wales: evidence on prenatal and genetic aetiology. Lancet 350: 1723-1728.

25. Czyglik F, Mayaux MJ, Guihard-Moscato ML, David G, Schwartz D (1986) Lower sperm characteristics in 36 brothers of infertile men, compared with 545 controls. Fertil Steril 45: 255-258.

26. Lilford R, Jones AM, Bishop DT, Thornton J, Mueller R (1994) Case-control study of whether subfertility in men is familial. BMJ 309: 570-573.

27. Meschede D, Lemcke B, Behre HM, De Geyter C, Nieschlag E, et al. (2000) Clustering of male infertility in the families of couples treated with intracytoplasmic sperm injection. Hum Reprod 15: 1604-1608.

28. Christensen K, Kohler HP, Basso O, Olsen J, Vaupel JW, et al. (2003) The correlation of fecundability among twins: evidence of a genetic effect on fertility?. Epidemiology 14: 60-64.

29. Storgaard L, Bonde JP, Ernst E, Andersen CY, Spanô M, et al. (2006) Genetic and environmental correlates of semen quality: a twin study. Epidemiology 17: 674-681.

30. Gianotten J, Westerveld GH, Leschot NJ, Tanck MW, Lilford RJ, et al (2004) Familial clustering of impaired spermatogenesis: no evidence for a common genetic inheritance pattern. Hum Reprod 19: 71-76.

31. van Golde RJ, van der Avoort IA, Tuerlings JH, Kiemeney LA, Meuleman EJ, et al. (2004) Phenotypic characteristics of male subfertility and its familial occurrence. J Androl 25: 819-823.

32. Parkin DM, Iscovich J (1997) Risk of cancer in migrants and their descendants in Israel: II. Carcinomas and germ-cell tumours. Int J Cancer 70: 654-660.

33. Sharpe RM (1994) Regulation of spermatogenesis In the Physiology of Reproduction, Raven Press, Ltd, New York, USA.

34. França LR, Avelar GF, Almeida FF (2005) Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. Theriogenology 63: 300-318.

35. Wood JW (1994) Dynamics of Human Reproduction: Biology, Biometry, Demography (Foundations of Human Behavior). Aldine de Gruyter, New York, 285-289.

36. Zinaman MJ, Clegg ED, Brown CC, O'Connor J, Selevan SG (1996) Estimates of human fertility and pregnancy loss. Fertil Steril 65: 503-509.

37. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, et al. (1988) Incidence of early loss of pregnancy. N Engl J Med 319: 189-194.

38. Norwitz ER, Schust DJ, Fisher SJ (2001) Implantation and the survival of early pregnancy. N Engl J Med 345: 1400-1408.

39. Coonen E, Derhaag JG, Dumoulin JCM, et al (2004) Anaphase lagging mainly explains chromosomal mosaicism in human preimplantation embryos. Human Reprod 19: 316-324.

40. Sosnowski J, Waroczyk M, Switonski M (2003) Chromosome abnormalities in secondary pig oocytes matured in vitro. Theriogenology 60: 571-581.

41. Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, et al. (2009) Chromosome instability is common in human cleavage-stage embryos. Nat Med 15: 577-583.

42. Vanneste E, Voet T, Melotte C, et al (2009) What next for preimplantation genetic screening? High mitotic chromosome instability rate provides the biological basis for low success rate. Human Reprod 24: 2679-2682.

43. Fragouli E, Alfarawati S, Spath K, Jaroudi S, Sarasa J, et al. (2013) The origin and impact of embryonic aneuploidy. Hum Genet 132: 1001-1013.

44. Delhanty JD (2001) Preimplantation genetics: an explanation for poor human fertility? Ann Hum Genet 65: 331-338.

45. Hultén MA, Patel SD, Tankimanova M, Westgren M, Papadogiannakis N, et al. (2008) On the origin of trisomy 21 Down syndrome. Mol Cytogenet 1: 21.

46. Joffe M (2010) What has happened to human fertility? Hum Reprod 25: 295-307.

47. Mayer F, Stoop H, Sen S, Bokemeyer C, Oosterhuis JW, et al. (2003) Aneuploidy of human testicular germ cell tumors is associated with amplification of centrosomes. Oncogene 22: 3859-3866.

48. Frigyesi A, Gisselsson D, Hansen GB, Soller M, Mitelman F, et al. (2004) A model for karyotypic evolution in testicular germ cell tumors. Genes Chromosomes Cancer 40: 172-178.

49. Rajpert-De Meyts E (2006) Developmental model for the pathogenesis of testicular carcinoma in situ: genetic and environmental aspects. Hum Reprod Update 12: 303-323.

50. Bartkova J, Rajpert-De Meyts E, Skakkebaek NE, et al (2003) Deregulation of the G1/S-phase control in human testicular germ cell tumours. Acta Pathologica, Microbiologica, et Immunologica Scandinavica 111: 252-266.

51. Ottesen AM, Larsen J, Gerdes T, et al (2004) Cytogenetic investigation of testicular carcinoma in situ and early seminoma by high-resolution comparative genomic hybridization analysis of subpopulations flow sorted according to DNA content. Cancer Genet Cytogenet 149: 89-97.

52. Chatzimeletiou K, Morrison EE, Prapas N, et al (2005) Spindle abnormalities in normally developing and arrested human implantation embryos in vitro identified by confocal laser scanning microscopy. Human Reprod 20: 672-682.

53. Chatzimeletiou K, Rutherford AJ, Griffin DK, Handyside AH (2007) Is the sperm centrosome to blame for the complex polyploidy chromosome patterns observed in cleavage stage embryos from an OAT patient? Zygote 15: 81-90.

54. Lewis SE, John Aitken R, Conner SJ, Iuliis GD, Evenson DP, et al. (2013) The impact of sperm DNA damage in assisted conception and beyond: recent advances in diagnosis and treatment. Reprod Biomed Online 27: 325-337.

55. Zini A, Boman JM, Belzile E, Ciampi A (2008) Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Human Reprod 23: 2663-2668.

56. Carrell DT, Wilcox AL, Lowy L, Peterson CM, Jones KP, et al. (2003) Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. Obstet Gynecol 101: 1229-1235.

57. Vendrell JM, García F, Veiga A, Calderón G, Egozcue S, et al. (1999) Meiotic abnormalities and spermatogenic parameters in severe oligoasthenozoospermia. Hum Reprod 14: 375-378.

58. Guichaoua MR, Perrin J, Metzler-Guillemain C, Saias-Magnan J, Giorgi R, et al. (2005) Meiotic anomalies in infertile men with severe spermatogenic defects. Hum Reprod 20: 1897-1902.

59. Codina-Pascual M, Navarro J, Oliver-Bonet M, et al (2006) Behaviour of human heterochromatic regions during the synapsis of homologous chromosomes. Human Reprod 21: 1490-1497.

60. Sciurano R, Rahn M, Rey-Valzacchi G, Solari AJ (2007) The asynaptic chromatin in spermatocytes of translocation carriers contains the histone variant gamma-H2AX and associates with the XY body. Human Reprod 22: 142-150.

61. Berkovitz A, Eltes F, Lederman H, Peer S, Ellenbogen A, et al. (2006) How to improve IVF-ICSI outcome by sperm selection. Reprod Biomed Online 12: 634-638.

62. Delaroche L, Yazbeck C, Gout C, Kahn V, Oger P, et al. (2013) Intracytoplasmic morphologically selected sperm injection (IMSI) after repeated IVF or ICSI failures: a prospective comparative study. Eur J Obstet Gynecol Reprod Biol 167: 76-80.

63. Van Loendersloot L, van Wely M, van der Veen F, et al (2014) Selection of embryos for transfer in IVF: ranking embryos based on their implantation potential using morphological scoring. Reprod Biomed Online 29: 222-230.

64. Molina I (2014) A minimally invasive methodology based on morphometric parameters for day 2 embryo quality assessment. Reprod Biomed Online 29: 470-480.

65. Meseguer M, Herrero J, Tejera A, Hilligsøe KM, Ramsing NB, et al. (2011) The use of morphokinetics as a predictor of embryo implantation. Hum Reprod 26: 2658-2671.

66. Hlinka D, Kaľatová B, Uhrinová I, Dolinská S, Rutarová J, et al. (2012) Time-lapse cleavage rating predicts human embryo viability. Physiol Res 61: 513-525.

67. Yang ST, Shi JX, Gong F, Zhang SP, Lu CF, et al. (2015) Cleavage pattern predicts developmental potential of day 3 human embryos produced by IVF. Reprod Biomed Online 30: 625-634.

68. Moses MJ, Poorman PA (1981) Synaptosomal complex analysis of mouse chromosomal rearrangements. II. Synaptic adjustment in a tandem duplication. Chromosoma 81: 519-535.

69. Moses MJ, Poorman PA, Roderick TH, Davisson MT (1982) Synaptonemal complex analysis of mouse chromosomal rearrangements. IV. Synapsis and synaptic adjustment in two paracentric inversions. Chromosoma 84: 457-474.

70. Schmid TE, Brinkworth MH, Hill F, Sloter E, Kamischke A, et al. (2004) Detection of structural and numerical chromosomal abnormalities by ACM-FISH analysis in sperm of oligozoospermic infertility patients. Human Reprod 19: 1395-1400.

71. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, et al. (2006) Global variation in copy number in the human genome. Nature 444: 444-454.

72. De Stahl TD, Sandgren T, Piotrowski A, Nord H, Andersson R, et al. (2008) Profiling of copy number variations (CNVs) in healthy individuals from three ethnic groups using a human genome 32K BAC-clone-based array. Human Mutation 29: 398-408.

73. Levy S, Sutton G, Ng PC, Feuk L, Halpern AL, et al. (2007) The diploid genome sequence of an individual human. PLoS Biol 5: e254.

74. Helgason A, Pálsson S, Gudbjartsson DF, Kristjánsson T, Stefánsson K (2008) An association between the kinship and fertility of human couples. Science 319: 813-816.

75. Labouriau R, Amorim A (2008) Comment on "An association between the kinship and fertility of human couples". Science 322: 1634.

76. Chandley AC (1991) On the parental origin of de novo mutation in man. J Med Genet 28: 217-223.

77. Adler ID, Baumgartner A, Gonda H, Friedman MA, Skerhut M (2000) 1-Aminobenzotriazole inhibits acrylamide-induced dominant lethal effects in spermatids of male mice. Mutagenesis 15: 133-136.

78. Tyl RW, Marr MC, Myers CB, Ross WP, Friedman MA (2000) Relationship between acrylamide reproductive and neurotoxicity in male rats. Reprod Toxicol 14: 147-157.

79. Ghanayem BI, Witt KL, El-Hadri L, Hoffler U, Kissling GE, et al. (2005) Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: evidence supporting a glycidamide-mediated effect. Biology of Reproduction 72: 157-163.

80. Karabinus DS, Vogler CJ, Saacke RG, Evenson DP (1997) Chromatin structural changes in sperm after scrotal insulation of Holstein bulls. J Androl 18: 549-555.

81. Paul C, Murray AA, Spears N, Saunders PT (2008) A single, mild, transient scrotal heat stress causes DNA damage, subfertility and impairs formation of blastocysts in mice. Reproduction 136: 73-84.

82. Mieusset R, Bengoudifa B, Bujan L (2007) Effect of posture and clothing on scrotal temperature in fertile men. J Androl 28: 170-175.

83. Bujan L, Daudin M, Charlet JP, Thonneau P, Mieusset R (2000) Increase in scrotal temperature in car drivers. Hum Reprod 15: 1355-1357.

84. Durairajanayagam D, Agarwal A, Ong C (2015) Causes, effects and molecular mechanisms of testicular heat stress. Reprod Biomed Online 30: 14-27.

85. Sharpe RM, Skakkebaek NE (1993) Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 341: 1392-1395.

86. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, et al. (1995) Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. Nature 375: 581-585.

87. Joffe M (2003) Infertility and environmental pollutants. Br Med Bull 68: 47-70.

88. Sharpe RM (2003) The 'oestrogen hypothesis'- where do we stand now? Int J Androl 26: 2-15.

89. Christiansen S, Scholze M, Axelstad M, Boberg J, Kortenkamp A, et al. (2008) Combined exposure to anti-androgens causes markedly increased frequencies of hypospadias in the rat. Int J Androl 31: 241-248.

90. Rider CV, Furr J, Wilson VS, Gray LE Jr (2008) A mixture of seven antiandrogens induces reproductive malformations in rats. Int J Androl 31: 249-262.

91. Sharpe RM (2008) "Additional" Effects of Phthalate Mixtures on Fetal Testosterone Production. Toxicological Sciences 105: 01-04.

92. Swan SH, Sathyanarayana S, Barrett ES, Janssen S, Liu F, et al. (2015) First trimester phthalate exposure and anogenital distance in newborns. Human Reprod 30: 963-972.

93. Akre O, Richiardi L (2009) Does a testicular dysgenesis syndrome exist? Hum Reprod 24: 2053-2060.

94. Thorup J, McLachlan R, Cortes D, Nation TR, Balic A, et al. (2010) What is new in cryptorchidism and hypospadias--a critical review on the testicular dysgenesis hypothesis. J Pediatr Surg 45: 2074-2086.

95. Møller H, Evans H (2003) Epidemiology of gonadal germ cell cancer in males and females. APMIS 111: 43-46.

96. Hassold T, Hunt P (2001) To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2: 280-291.

97. Daphnis DD, Delhanty JD, Jerkovic S, Geyer J, Craft I, et al. (2005) Detailed FISH analysis of day 5 human embryos reveals the mechanisms leading to mosaic aneuploidy. Hum Reprod 20: 129-137.

98. Hassold T, Hunt P (2009) Maternal age and chromosomally abnormal pregnancies: what we know and what we wish we knew. Curr Opin Pediatr 21: 703-708.

99. Tease C, Hartshorne G, Hultén M (2006) Altered patterns of meiotic recombination in human fetal oocytes with asynapsis and/or synaptonemal complex fragmentation at pachytene. Reprod Biomed Online 13: 88-95.

100. Cheng EY, Hunt PA, Naluai-Cecchini TA, Fligner CL, Fujimoto VY, et al. (2009) Meiotic recombination in human oocytes. PLoS Genet 5: e1000661.

101. Lister LM, Kouznetsova A, Hyslop LA, Kalleas D, Pace SL, et al. (2010) Age-related meiotic segregation errors in mammalian oocytes are preceded by depletion of cohesin and Sgo2. Curr Biol 20: 1511-1521.

102. Chiang T, Duncan FE, Schindler K, Schultz RM, Lampson MA (2010) Evidence that weakened centromere cohesion is a leading cause of age-related aneuploidy in oocytes. Curr Biol 20: 1522-1528.

103. Tsutsumi M, Fujiwara R, Nishizawa H, Ito M, Kogo H, et al. (2014) Age-related decrease of meiotic cohesins in human oocytes. PLoS One 9: e96710.

104. Kong A, Barnard J, Gudbjartsson DF, Thorleifsson G, Jonsdottir G, et al. (2004) Recombination rate and reproductive success in humans. Nat Genet 36: 1203-1206.

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