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The role of chromosome segregation and nuclear organisation in human subfertility

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Abstract

Spermatogenesis is central to successful sexual reproduction, producing large numbers of haploid motile male gametes. Throughout this process, a series of equational and reductional chromosome segregation precedes radical repackaging of the haploid genome. Faithful chromosome segregation is thus crucial, as is an ordered spatio-temporal “dance” of packing a large amount of chromatin into a very small space. Ergo, when the process goes wrong, this is associated with improper chromosome number, nuclear position and/or chromatin damage in the sperm head. Generally, screening for overall DNA damage is relatively commonplace in clinics, but aneuploidy assessment is less so and nuclear organization studies form the basis of academic research. Several studies have focussed on the role of chromosome segregation, nuclear organisation and analysis of sperm morphometry in human subfertility observing significant alterations in some cases, especially of the sex chromosomes. Importantly, sperm DNA damage has been associated with infertility and both extrinsic (e.g. lifestyle) and intrinsic (e.g. reactive oxygen species levels) factors, and whilst some DNA strand breaks are repaired, unexpected breaks can cause differential chromatin packaging and further breakage. A “healthy” sperm nucleus (with the right number of chromosomes, nuclear organization and minimal DNA damage) is thus an essential part of reproduction. The purpose of this review is to summarise the state of the art in the fields of sperm aneuploidy assessment, nuclear organization and DNA damage studies.

Introduction

Gametogenesis, the process of producing haploid gametes is central to successful sexual reproduction, and in male mammals, spermatogenesis describes the transformation of germ cells into spermatozoa. Taking place during three distinct phases, the mitotic proliferative phase, the meiotic phase and the cytodifferentiation (spermiogenesis) phase, a series of events that includes both equational and reductional chromosome segregation as well as radical repackaging of the haploid genome occurs. Faithful chromosome segregation is thus crucial for the process to continue normally, as is an ordered spatio-temporal "dance" of packing a large amount of chromatin into a very small space. Given this, it is hardly surprising that, when the process goes wrong it is associated with improper chromosome number, nuclear position or chromatin damage in the sperm head.

Given that infertility affects approximately one in six couples globally[1], and that male factor subfertility contributes to around 30% of these cases[2], there is an indisputable need for more research into the male gamete to be undertaken to understand the role of chromosome segregation and chromatin packaging in male infertility. To date however, studies have focused mostly on "spot counting" i.e. interphase cytogenetics to establish the proportion of aneuploid cells in an ejaculate[3], studies to assess the overall levels of DNA damage in sperm heads[4] and nuclear position of chromosome territories[5,6]. Whilst screening for overall DNA damage is relatively commonplace in some IVF clinics, aneuploidy assessment is less so (although such techniques are nonetheless offered by some companies). This review covers our current understanding of the importance of sperm nuclear organisation and the mechanisms of chromosome segregation in human sperm, with a focus on the differences between fertile and subfertile individuals. Given that the clinical definition of infertility refers to one year of unwanted non-conception following unprotected intercourse in the fertile phase of the menstrual cycle (WHO definition) and is sometimes used interchangeably with sterility, here we use the term "subfertility" throughout to refer to any form of reduced fertility that occurs over a prolonged period of time.

Screening of sperm and a possible role for aneuploidy assessment

When screening human semen for fertility evaluation, various different physical characteristics are routinely assessed, including the volume, appearance, viscosity and pH of the ejaculate, as well as the morphology of the sperm heads[7]. Given that some studies have suggested that these routinely assessed parameters are not entirely indicative of fertility[8], it is clear that other screening methods are necessary. Given that numerous studies have identified that there is a correlation between sperm aneuploidy and male infertility[9–13], irrespective of constitutional chromosome abnormalities (i.e. men that have normal karyotypes, but compromised semen parameters), aneuploidy assessment has been proposed as a potential alternative screening method that is currently not routinely implemented clinically.

Infertile men who were previously unable to procreate are now able to, due to the development of various methods of assisted reproductive technologies (ART) such as intracytoplasmic sperm injection (ICSI). Potentially therefore, those men who fit the referral category for ICSI, in theory run the risk of perpetuating aneuploidy to their offspring. Although the majority of autosomal aneuploidies are maternal in origin, 7% are paternally derived (this equates to around 1 in 10,000 children with paternally derived Down syndrome for instance)[14,15] and 50% of sex chromosome aneuploidies also arise in the male gamete. That is, it has been shown that almost half of XXY, three quarters of XO, 5% of XXX, and all XYY cases are a result of an aneuploid sperm[16]. Aneuploid events in sperm can be identified by fluorescence *in situ* hybridisation (FISH)[9,17], which permits thousands of sperm heads to be screened. The first reports which used FISH as a screening tool for human fertility[18,19] demonstrated that aneuploidy was far more common in men afflicted by severely comprised semen parameters such as concentration (oligozoospermia), morphology (teratozoospermia) and motility, (asthenozoospermia), together known as oligoasthenoteratozoospermia (OAT). The presence, or not, of sperm aneuploidy in both fertile (normozoospermic) and infertile men has been widely studied and the received wisdom is that all men produce a proportion of aneuploid sperm[3]. As described above,

the incidence of aneuploidy however has been positively correlated with reduced semen parameters[20], and these occurrences increase with the severity of the infertility. Initial studies on sperm aneuploidy involved the analysis of karyotypes of those human sperm cells that were capable of fertilising a hamster oocyte. Whilst this method permitted the detection of both structural and numerical chromosome aberrations, this approach is challenging and time consuming. Such studies revealed that structural chromosome abnormalities were more prevalent than numerical incidences, and that non-disjunction events were most common in chromosomes 21, 22, X and Y compared to the rest of the chromosome complement. Given that meiotic recombination assists homologous chromosomes to stay together and that these chromosomes often only cross over once during recombination, it is not unexpected that these would be the most affected pairs[21].

The genetic quality of sperm cells used in ART must therefore be considered, and ultimately the selection of a euploid sperm prior to ICSI is the ultimate goal[22]. In the meantime, being aware of the overall level of sperm aneuploidy (and hence the risks involved) is the primary option for patients. Even though IVF clinics have the ability to screen sperm for aneuploidy (by outsourcing to a company such as iGenomix), this is rarely performed. Given that aneuploid sperm are still capable of fertilisation, and that aneuploidy has been estimated to be more likely in samples from subfertile male[9,16], it has been argued that such screening would be worthwhile. Of course another solution is to screen the embryos of all ICSI patients by preimplantation genetic tests (PGT-A) and a recent study[23] has demonstrated that embryos from ICSI males have elevated levels of sex chromosome aneuploidies. Aneuploid embryos can result from a non-disjunction event in the oocyte or sperm cell, or via mitotic loss, mitotic gain or a non-disjunction event in the embryo itself. Thus, although PGT-A is in itself controversial[24] it is argued that severe male factor subfertility should be a referral category for it.

Sperm nuclear organisation

Genome condensation is necessary prior to the transmission of the male genome to the offspring; this involves the replacement of most histones with a family of small, arginine-rich proteins, protamines to ensure that the complexes occupy a minimal cell volume[25]. In fertile men, a small proportion of the haploid genome is retained in nucleosomes (between 4% and 15% depending on the source of information), that are enriched at developmental gene promoters and imprinted gene loci[26], including histone variant proteins such as testes-specific histone H2B[26,27]. Interestingly, this level of retention is far higher than in other mammalian gametes, but the distribution of histones and protamines in sperm has not yet been fully characterised. It has been suggested that histones in the sperm nuclei may influence which genes are transcribed following fertilisation, or that the unique packaging seen in these cells may be vital for unpacking of the male genome during the process of fertilisation[27]. Importantly, changes in the level of histone retention have been associated with infertility; for example, histone retention in subfertile men has been shown to be randomly distributed, which is unlike the distribution seen in fertile men[26,28].

This unique structure is important for two reasons; protection from DNA damage and a fast and full unpacking of the male genome to the oocyte[29]. Faithful sperm chromatin packaging has been implicated as essential for the establishment and continuation of a normal pregnancy[6,11]. Some studies have suggested however that the impact of abnormal sperm chromatin on embryo development is subject to not only how severe the damage is, but also how efficient the oocyte is at repairing any abnormalities[30].

The term nuclear organisation describes the spatiotemporal arrangement of the DNA and associated proteins in the interphase nucleus. It is often assayed by establishing the specific positions occupied by each chromosome territory (CT) and/or specific loci[31,32]. In humans, investigations into the organisation and spatial arrangement of CTs at interphase have provided valuable insights into genome function, particularly when considering higher levels of control that transcend the impact of

142 the DNA sequence alone. Studies of nuclear organisation in somatic cells have also revealed a
143 correlation between the gene density of the chromosome and the radial positioning of CTs[33]. In many
144 somatic cell types, it has long been established that gene-rich CTs are located towards the interior of
145 the nucleus, whereas gene-poor chromosomes are positioned in the peripheral regions[34]. This
146 arrangement has been shown to be cell-type and tissue-type specific, and is evolutionarily
147 conserved[35]. The structure of CTs has been shown to be dynamic, and less physically constrained than
148 once thought[36], thereby enabling genes to reposition from the periphery of the nucleus towards the
149 interior following a change in cell status caused by quiescence or senescence[37]. It is also evident that
150 the organisation is imperative for cellular functions (such as transcription) to proceed normally and it
151 has been hypothesised that chromatin organisation may be associated with epigenetic
152 modifications[38] (discussed later), genomic imprinting[39] and X chromosome inactivation[40]. In human
153 sperm, chromosomes are organised non-randomly[6] and centromeres form a chromo-centre (i.e. they
154 cluster) in the nucleus interior, with telomeres positioned nearer the periphery[6]. This pattern is
155 similar in many other mammalian species with the sex chromosomes also clustering nearer the centre
156 of the nucleus[41]. Further to this, it has been well documented that there is a functional significance
157 for the ordered pattern of chromosomes in human sperm cells[42], and that, in turn, aberrant
158 organisation is common in samples from subfertile men. Evidence suggests that irregular chromosome
159 organisation is correlated with delayed decondensation, impacting the zygote's first mitotic division,
160 and playing a role in sex chromosome aneuploidy events. Such studies have been performed in both
161 2D and 3D[5].
162
163 As described above, in recent years numerous studies have focussed on the role of chromosome
164 segregation, nuclear organisation and analysis of sperm morphometry in human subfertility[6,33,43–45].
165 To date however, such studies are still few and far between in other mammalian species, particularly
166 those in agriculturally important species such as cattle (*Bos taurus taurus*), pigs (*Sus scrofa*
167 *domesticus*), goats (*Capra aegagrus hircus*) and sheep (*Ovis orientalis aries*)[46–48]. A key goal in animal

production is to identification of subfertile animals (so that they can be removed from breeding programmes in a timely manner). That is, animals with fertility problems have the potential to produce reduced litter sizes throughout the breeding population, thereby impeding the production of foodstuffs[8]. Some of our current work aims to address this by comparing nuclear topology and chromosome positioning in fertile and subfertile pig samples.

DNA damage and the impact of epigenetic change

Sperm DNA damage has been related to numerous different factors that can be both extrinsic (e.g.: lifestyle factors)[49,50] and intrinsic (e.g.: levels of reactive oxygen species (ROS)[51,52]). Given the unique structure and function of spermatozoa, it has been shown that they are very susceptible to damage caused by oxidative stress. Free radicals, or ROS, have been show to cause protein, DNA and membrane damage, as well as lipid peroxidation, that results in base modification and chromatin cross-linking[53]. Naturally, affected sperm cells would not participate in fertilisation, hence making the impact of oxidative stress in ART of particular interest; given that seminal plasma contains many antioxidants that protect spermatozoa against oxidative stress, it is therefore important to provide *in vitro* protection against ROS throughout ART[54].

Whilst some DNA strand breaks are expected and subsequently repaired (for example those occur as part of chromatin remodelling[55]), unexpected breaks have the potential to cause chromatin to be packaged differently, and may lead to further DNA breakage. Interestingly, it has been shown that men with abnormal semen parameters present with high levels of an apoptotic protein, Fas[56]. The presence of this protein on spermatozoa is indicative of cells that had been set aside for programmed cell death, but have evaded this due to the high numbers of cells present in the ejaculate. This mechanism is known as ‘abortive apoptosis’ and can lead to oligozoospermia, azoospermia, or a high number of abnormal sperm, which in turn may have an impact on successful fertilisation. It has been well documented that men of reproductive age that are being, or that have been, treated with

chemotherapy present with impaired spermatogenesis, increased levels of sperm aneuploidy, and a higher rate of DNA fragmentation[57]. Whilst aneuploidy levels recover, levels of DNA damage may not. Lifestyle factors such as smoking and obesity have also been correlated with reduced semen quality and higher levels of DNA damage[49,58]. Further to this, it has been shown that an increase in the rate of DNA fragmentation is associated with lower natural, intrauterine insemination (IUI) and IVF conception rates[59,60].

Whilst the effect of maternal age on reproductive success has been extensively documented, it is important to also consider the relationship between sperm quality and paternal age. Whilst advanced maternal age is universally accepted to be above the age of 35, there is currently no consensus as to the age at which men should be considered to be in the category of "advanced paternal age". Some studies have however shown a correlation with increased risk of genetic disease, semen quality and reproductive function[38]. Conversely however, a study by Winkle and colleagues (2009) investigated the effect of male aging by analysing the ejaculates of 320 men who were attending a fertility centre; these findings suggested that routinely assessed semen parameters and DNA fragementation rates were not affected by male age[61]. Similarly, Dain and collagues (2011) concluded that there was insufficient evidence to establish an unfavourable effect on paternal age on ART outcomes following a systematic review of the literature[62]. Given the limited number of studies in this area, and the inconsistency between the results, it is clear that more research needs to be undertaken.

There are several different ways in which the levels of sperm chromatin damage can be assessed, as described in Figure 1. These include sperm nuclear matrix assays such as the chromatin dispersion test[63], the use of sperm chromatin structural probes such as the sperm chromatin structure assay (SCSA)[64], and DNA fragmentation assays such as the Terminal deoxynucleotidyl transferase dUTP Nick-End Labelling (TUNEL)[11] and comet assays[63]. It has been shown that if an SCSA test detects DNA fragmentation of over 30%, there is a far smaller chance for fertilisation to be a success via natural

pregnancy or IUI[65]. This does not however seem to be the case for ICSI cases, and can almost certainly be attributed to the fact that both the sperm and the subsequent embryo are carefully selected prior to implantation. Nevertheless, pregnancy loss following IVF or ICSI has been linked to abnormal levels of sperm DNA damage. It has been suggested that this is because embryonic genome expression does not happen until the 4-8 cell stage[66], and therefore DNA damage in sperm does not affect fertilisation *per se*, however there are only a limited number of studies that have focussed on the effect of DNA fragmentation on IVF or ICSI outcomes[59,60].

The role of epigenetics in human reproduction is an active and interesting field of research, particularly due to the transgenerational effects attributed to epigenetic modifications in both male and female gametes. Epigenetic patterns are metastable heritable changes in gene expression that can change due to endogenous and environmental factors[67,68]. For example, the epigenetics of ageing sperm has been linked to increased frequency of neurocognitive disorders such as autism, schizophrenia and other bipolar disorders, as well as metabolic dyshomeostasis and obesity in offspring[67]. At present, epigenetic modifications in sperm have been found to have an impact on four key areas of reproduction: 1) spermatogenesis failure, 2) embryogenesis, 3) success rates and overall outcomes associated with ART procedures and 4) long-term progeny effects[68]. Whilst several epigenetic modifications relating to DNA methylation and histone modifications have been found to occur during various stages of spermatogenesis (mitosis, meiosis and spermiogenesis), histone-protamine replacement has been found to be the main change in sperm cells[68]. The literature suggests that various features of male infertility, including oligozoospermia and OAT, chromosomal aneuploidies, DNA fragmentation and chromatin packaging could all be linked to epigenetic modifications occurring at various junctures of spermatogenesis. Paternal epigenetic changes have also been associated with childhood cancers and imprinting diseases, and that such changes are increased in offspring conceived via ART[67], and further to this, it has been shown that control of the paternal lifestyle (for example the use of dietary antioxidant supplements) before conception may have a downstream impact[68].

Perspectives

- **Importance of the field:** A “healthy” nucleus is an essential part of any cell or tissue. In chromatin terms this can mean the correct number of chromosomes, the appropriate organization of CTs and the absence of significant DNA damage. This is particularly apparent in the sperm cell, in part because (being haploid and the end of a very specialized developmental pathway) it does not have the opportunity to repair its DNA, eject offending chromosomes, nor reorganize its chromatin. In this respect, analysis of sperm chromatin can also be used to monitor the effects of toxic agents or environmental pollutants. Sperm are our legacy to the next generation and thus, in this regard, with eggs, perhaps the most important cells in our body.
- **Summary of current thinking:** Increased sperm aneuploidy, aberrant nuclear organization and DNA damage have all been associated with male infertility and this is particularly important for couples seeking fertility treatment such as ICSI. In this regard, the genetic quality of the sperm is important as an indicator of the likely success of the procedure and possibly could impact on the future health of the resultant child.
- **Comment on future directions**: One of the confounding factors in sperm head analysis is the necessity to score a large number of cells. In sperm aneuploidy studies, this can mean up to 20,000 cells in order to reach statistically significant result[69,70] which can be prohibitively laboriousAutomated scoring is thus a priority and adaptations of flow cytometry such as flow FISH are essential in this regard. Moreover, still relatively little is known about the basic mechanisms that lead to chromatin damage in sperm and this is a fascinating area that needs much deeper investigation, ultimately for future patient benefit.

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