

# The Role of Sperm Chromatin Anomalies on the Outcome of Assisted Reproductive Techniques

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## Abstract

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Sperm DNA is known to contribute one half of the genomic material to the offspring. The integrity of sperm DNA is important in fertilization, embryonic and fetal development, and postnatal child well being. The nature has created multiple barriers that allow only the fittest sperm to reach and fertilize an oocyte. However, assisted reproductive techniques (ART), like IVF and ICSI, may allow sperms with abnormal genomic material to enter the oocyte with minimal effort. This article describes structure of sperm DNA and different mechanism involved in sperm chromatin anomalies and DNA damage. Furthermore, this study elaborates possible sperm selection methods that may improve the outcome of ART.

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## Introduction

Assisted conception techniques are now responsible for 2-4% of new births in developed countries. While this technology has revolutionized the treatment of infertile couples, concerns have been raised about the incidence of birth defects in such children (1). The main disadvantage of ART is that it bypasses the natural selection barrier which is present throughout female reproductive tract until sperm enters the oocyte (2).

The nature has created multiple barriers that allow only the fittest sperm to reach and fertilize an oocyte. However, ART procedures, like IVF and ICSI, may allow sperm with abnormal genomic material to enter the oocyte with minimal effort (3, 4). One of the factors thought to be responsible for morbidity, as well as the high rate of early pregnancy loss associated with assisted conception cycles, is the presence of DNA damaged-sperm in fertilization (5, 6).

The quality of sperm DNA is very important in maintaining the reproductive potential of men. Sperm DNA is known to contribute one half of the genomic material to the offspring. Sperm normal genetic material is essential for fertilization, embryonic and fetal development, and postnatal child well being. Abnormal DNA can lead to disruption of these processes. Sperm DNA is resistant to many types of

insults that occur during its journey from the testis until it reaches the oocyte for fertilization. Normally, sperm DNA is very stable in the nucleus because of its structural organization (7).

### *Structural organization of sperm DNA*

The formation of mature spermatozoa is a unique process involving a series of mitotic and meiotic changes in cytoplasmic architecture, replacement of somatic cell-like histones with transition proteins, and the final addition of protamines leading to a highly packaged chromatin (8). Sperm DNA is organized in a specific manner that keeps the chromatin in the nucleus compact and stable. Therefore, this structure forms a nearly crystalline status that is at least six times more condensed than mitotic chromosomes (9). It occupies almost the entire nucleus volume, whereas somatic cell DNA only partly fills the nucleus.

Protamines are highly basic proteins about half the size of a typical histone (5-8 kDa) (9). Arginine forms 55 to 79% of the amino acid residues of protamines, permitting a strong DNA binding.

The mechanism by which protamines interact with DNA is still under debate. P1 and P2 may bind to the major groove of the DNA (10),

attach to both minor and major grooves (11), or electrostatically bind to the surface of the DNA by interacting with phosphate residues (12). Sperm epididymal maturation involves a final stage of chromatin organization. During this procedure, protamines contain a significant number of cysteine residues and

participate in sperm chromatin compaction by formation of multiple inter- and intra-protamine disulfide bridges. These interactions make mammalian DNA the most condensed eukaryotic DNA (13). The intermolecular and intramolecular disulfide cross-links between the cysteine-rich protamines are responsible for the compaction and stabilization of the sperm nucleus, and it is thought that this nuclear compaction protects the sperm genome from external stresses such as oxidation or temperature elevation in the female reproductive tract (14).

Recently, sperm DNA has been recognized as an independent measure of sperm quality that may have better diagnostic and prognostic capabilities than standard sperm parameters for both *in vivo* and *in vitro* fertility assessments. The cause of infertility in infertile men with normal semen parameters can be related to abnormal sperm DNA (15). Therefore, the evaluation of sperm DNA integrity, in addition to routine sperm parameters, can provide more information about the quality of spermatozoa. The damage to sperm DNA is critical in the context of assisted reproductive techniques (ART), which are increasingly used to treat infertile couples (16). Recent reports have raised concern about decreasing male fertility caused by genomic abnormalities. There are some reports of increased congenital anomalies and testicular cancer in children (17). The defects in the genomic material may take the form of condensation or nuclear maturity defects, DNA breaks or DNA integrity defects, and sperm chromosomal aneuploidy (18).

#### ***Mechanisms of sperm DNA damage***

The etiology of sperm DNA damage, much like male infertility, is multifactorial and may be due to intratesticular, post-testicular, or external factors. It is not known whether a single factor or multiple factors (possibly acting in a cascade) are responsible for sperm DNA damage. Potential etiologic factors reported to cause sperm DNA damage include: protamine deficiency, apoptosis, drugs, chemotherapy, radiotherapy, ROS, cigarette smoking, post-testicular factors, and varicoceles. The most important mechanisms of sperm DNA damage are abnormal

chromatin packaging (protamine deficiency), reactive oxygen species (ROS), and apoptosis (19, 20).

#### ***Defective sperm chromatin packaging (protamine deficiency)***

A frequent abnormality of sperm chromatin is partial or complete deficiency of sperm protamines (21). Because of critical role of the correct histone-to-protamine exchange for spermatid differentiation, one might expect aberrations in protamine expression or structure leading to male infertility.

Many studies have shown that protamine deficiency and aberrant P1/P2 ratio are adversely related to fertilization rates both in IVF and ICSI cases (21, 22, 23, 24). Moreover, abnormal P1/P2 ratios have been reported in spermatozoa of infertile men (25-29). In addition, Steger *et al.* reported an aberrant P1/P2 mRNA ratio in round spermatids of testicular biopsies (30). *In vitro* studies by Carrell and Liu (2001) suggest that an increased P1/P2 ratio is associated with decreased sperm penetration and fertilization rates (21).

Chromatin analysis of failed fertilized human oocytes has revealed that sperm premature chromosome condensation (PCC) is the next prevalent cause of fertilization failure in both IVF and ICSI, after aneuploidy (31-33). Our previous studies have shown that the proportion of sperm premature chromosomal condensation (PCC) in unfertilized human oocytes was higher in protamine deficient patients, and sperm protamine deficiency could be one of the factors that made sperm prone to PCC (34). Complementary studies in mice suggest that haplo-insufficiency of P2 leads to sperm DNA damage and early embryo death (35), while premature translation of P1 causes premature nuclear condensation, which resulted in an arrest of spermatid differentiation (36).

Unlike fertile men, a subset of infertile men (5% to 15%) possesses a complete protamine deficiency, and genetic analysis has shown that it may be due to a mutation in the protamine gene cluster (21). Although studies on transgenic animal models with targeted protamine deficiency suggest a relationship between protamine deficiency, sperm DNA damage, and poor fertilizing capacity during *in vitro* fertilization (IVF), no such association has been studied in humans (35). A single case report indicated that a febrile illness can cause a transient increase in the nuclear histone/protamine ratio and associated abnormalities of sperm chromatin structure (37).

McPherson and Longo (38) demonstrated the presence of endogenous DNA strand breaks in elongating rat spermatids, when chromatin structure and nucleoproteins are modified. They proposed (39) that the presence of endogenous DNA nicks in ejaculated spermatozoa might be indicative of incomplete maturation during spermiogenesis. They also postulated (38-40) that chromatin packaging might involve endogenous nuclease activity in order to create and ligate nicks during the replacement of histones by protamines, and that an endogenous nuclease, topoisomerase II, may play a role. Topoisomerase II functions by transiently introducing DNA double strand breaks, allowing the passage of one double helix through another, and resealing the double strand break (41). While the role of topoisomerase II in spermatogenesis is yet to be clarified, it is expressed in human testis (42) and the transient presence of DNA breaks has been reported in both mouse and human (43, 44).

#### *Reactive oxygen species*

Although low levels of ROS in semen may be important for normal sperm maturation, studies have shown that high levels of ROS can adversely affect multiple sperm functions and sperm DNA integrity (45, 46). High levels of ROS are detected in the semen of 25% of infertile men but not in the semen of fertile men (47). Some studies show that retention of cytoplasmic droplets (a morphologic feature associated with ROS production and a sign of sperm immaturity) is positively correlated with sperm DNA damage (48). Many studies have reported that ROS is a major cause of sperm DNA damage (49, 50).

Leukocytospermia is also associated with high levels of sperm DNA damage, likely secondary to elaboration of ROS by these cells. ROS may also cause hypercondensation of the sperm nucleus as a result of excessive oxidation of protein sulfhydryl groups.

#### *Apoptosis*

Apoptosis or programmed cell death during normal mammalian spermatogenesis results in up to 75% of potential spermatozoa death (51). Germ cell death during mammalian spermatogenesis occurs mainly via apoptosis (52-55). Evidence from animal models shows that the active proliferation of early germ cells is balanced by selective apoptosis of their progeny (56). Therefore, testicular germ cell apoptosis seems to occur physiologically and continuously throughout life. Apoptosis may play two putative roles during normal spermatogenesis: limitation of the germ cell

population to numbers that can be supported by the Sertoli cells and selective depletion of abnormal spermatozoa (57-59).

One of the factors postulated to be implicated in sperm apoptosis is the cell surface protein, Fas (54). Fas is a type I membrane protein that belongs to the tumor necrosis factor-nerve growth factor receptor family, and mediates apoptosis. Binding of Fas ligand (FasL) or agonistic anti-Fas antibody to Fas kills cells by apoptosis. In mice and rats, it has been shown that Sertoli cells normally express FasL and signal the killing of Fas positive germ cells, limiting the size of the germ cell population to numbers they can support. In addition, after injury, FasL expression of Sertoli cells increases to a new equilibrium that matches the reduced capacity of the dysfunctional Sertoli cells with fewer germ cells. Thus, upregulation of Fas in germ cells is seen as a self-elimination process for the cells that are destined to die because of inadequate support (52, 54).

#### *Evaluation of DNA status*

There is extensive ongoing research on quantifying the amount of abnormal DNA present in human spermatozoa. Multiple techniques are reported to measure sperm DNA defects in human spermatozoa (60, 18). Some methods like terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate nick end labeling (TUNEL), COMET, in situ nick translation, and sperm chromatin structure assay (SCSA) evaluate the integrity of sperm DNA.

Other techniques, like staining with chromomycin A3, aniline blue, sodium dodecyl sulfate (SDS), SDS + EDTA, and acridine orange, help identify the packaging defects of sperm chromatin. However, none of these tests are in routine clinical use.

#### *Sperm chromatin structure assay (SCSA)*

The SCSA is a flow cytometric assay that relies on the fact that abnormal sperm chromatin is highly susceptible to physical induction of partial DNA denaturation in situ (61, 62). The extent of DNA denaturation following heat or acid treatment is determined by measuring the metachromatic shift from green fluorescence (acridine orange intercalated into double-stranded nucleic acid) to red fluorescence (acridine orange associated with single stranded DNA) (63). The most important parameter of the SCSA is the DNA fragmentation index (%DFI), which represents the population of cells with DNA damage (60).



Developed over the course of more than 20 years, the SCSA is considered a highly robust statistical measure of male DNA-factor infertility.

The SCSA, now proposed clinically as a service, measures a number of parameters such as the DNA fragmentation index (DFI) and the highly DNA stainable cells (HDS), which show sperm fraction with detectable denaturable single stranded and double-stranded DNA, respectively (60).

As these parameters are not correlated to each other, they represent independent aberrations of the human mature male gamete in the ejaculate. DFI has been shown to influence normally-initiated pregnancy (64, 65). Increasing levels of DFI (>30%), independent of World Health Organization (WHO) standard semen parameters, were associated with a decreased probability to father a child.

#### **COMET Assay**

The COMET assay measures DNA damage by quantifying the single-stranded and double-stranded breaks associated with DNA damage (66). In this assay, spermatozoa are stained with a fluorescent DNA-binding dye.

*Sperm chromatin abnormalities and DNA damage in male infertility.* The resulting images, which resemble COMETs', are measured after staining to determine the extent of DNA damage (67). The characteristics that have been used for analysis include the diameter of the nucleus and the COMET length (68). One of the principles of the COMET assay is that nicked double-stranded DNA tends to remain in the COMET head, whereas short fragments of nicked double- and single-stranded DNA migrate into the tail area (69). Thus, spermatozoa with high levels of DNA strand breaks would show increased COMET tail fluorescent intensity (70) and COMET tail length (71). However, useful thresholds have not yet been established for the COMET assay.

#### **TUNEL Assay**

This assay is dependent on the terminal deoxynucleotidyl transferase-mediated d-UTP nick end labeling of fragmented DNA strands (72, 73). In brief, d-UTP is incorporated at single-stranded and double-stranded DNA breaks, and the reaction is catalyzed by the terminal deoxynucleotidyl transferase (72, 73). The incorporation of d-UTP is then amplified by a variety of secondary enzymatic reactions and measured by fluorescent or light microscopy. Spermatozoa are examined microscopically and labeled as positive or

negative on the basis of the presence or absence of head staining, respectively.

#### ***In-situ nick translation (NT) assay***

The NT assay quantifies the incorporation of biotinylated deoxyuridine triphosphate (d-UTP) at single-stranded DNA breaks in a reaction that is catalyzed by the template-dependent enzyme, DNA polymerase I. The NT assay identifies spermatozoa that contain appreciable and variable levels of endogenous DNA damage (74). The clinical value of the NT assay is severely limited, because no correlation has been proved with fertilization during in-vivo studies (75) and because of its lack of sensitivity compared with other assays (76).

#### ***Sperm chromatin dispersion (SCD) test***

This assay has been recently described as a simple and inexpensive method for the analysis of sperm DNA fragmentation. The SCD test is based on the principle that sperm with fragmented DNA fail to produce the characteristic halo when mixed with aqueous agarose following acid denaturation and removal of nuclear proteins (77).

#### ***Chromomycin A3 staining***

Protamines are required for normal DNA packaging, and assessment of protamines may be the most physiologic assessment of chromatin, as the role of protamines in chromatin condensation is well described. However, measuring the protamines in sperm is relatively difficult, and it remains primarily as a research tool at this time. Chromomycin A3 is a fluorochrome, which indirectly detects protamine deficiency in loosely packed chromatin. Some studies reported that Chromomycin A3 is a useful tool for evaluation of chromatin status and male fertility (78- 80). Specimens with more than 30% sperm CMA3 positivity (with bright yellow staining) have a lower fertilization rate during IVF and ICSI (22, 23, 78).

#### ***Aniline blue staining***

In abnormal sperm, histones are still present instead of protamines during spermiogenesis (81, 82). Some investigators have applied aniline blue staining, which binds to lysine-rich histones, to evaluate histone excess in chromatin structure (83). In this technique, normal sperm are colorless, and damaged sperm are blue (84, 85). The degree of sperm staining correlates with the amount of excessive histone. Although the results of aniline staining correlate well with chromomycin A3, sperm chromatin structure

assay, TUNEL, and COMET test, they measure different aspects of nuclear structure (21, 86).

#### *Acridine orange test*

The acridine orange test (AOT) was introduced as a simplified microscopic method of the SCSA that does not require expensive flow cytometry equipment and a SCSA-trained technician (87). It relies on visual interpretation of fluorescing spermatozoa and debris that fall into a broad range of colors under microscopic examination.

Indistinct colors, rapidly fading fluorescence and heterogeneous slide staining exacerbate problems with interpretation (88). Such conditions preclude using the AOT for critical clinical diagnosis and prognosis of a semen sample (89), since the AOT may introduce many sources of variation. Although some laboratories have used the AOT in an attempt to improve male fertility evaluations (90), the predictive value of the test for human fertility remains controversial.

#### *Sperm chromatin stability (SCS) and nuclear chromatin decondensation (SDS + EDTA) tests*

DNA stability of sperm depends on the amount and ratio of disulfide bonds (S-S), non-covalent bonds between Zn and sulfhydryl groups (SH...Zn...SH), and free thiol groups. It reflects the epididymal maturation and more or less the normal function of the male genital glands. The ability of nuclear chromatin decondensation (NCD) also depends on the amount of SH...Zn...SH bonds. Nuclear chromatin decondensation of spermatozoa and subsequent male pronucleus formation are essential for fertilization and normal embryonic development. Therefore upon removal of spermolemma with sodium dodecyl sulfate (SDS) as a detergent, nuclear decondensation can take place depending on chromatin stability (91, 92). The proportion of unstable sperms (partially or completely swollen) can reveal sperm chromatin stability. Moreover, removal of Zn by EDTA can result in nuclear chromatin decondensation (NCD). However we observed no correlation with fertilization rate when using SDS alone or with EDTA in IVF or ICSI cases (22, 23).

#### *Sperm DNA integrity and reproductive outcome*

Reproductive parameters including fertilization, blastocyst development, and pregnancy rates can be affected by increased chromatin anomalies in ejaculated spermatozoa. However, controversial results have been reported by different authors.

Investigation of the possible association between chromatin anomalies in spermatozoa and fertilization rates in patients undergoing ART did not show any relationship between DNA integrity of ejaculated spermatozoa and IVF and ICSI fertilization rates, using in situ nick translation (93), COMET assay (94, 24), TUNEL assay (95), or SCSA (96, 97). In contrast, a negative relationship between sperm DNA breaks and IVF (98) and ICSI (99) fertilization rates have been reported using the TUNEL assay. Different studies including report from our lab found a negative correlation between sperm chromatin anomalies and fertilization rates using chromomycin A3 and aniline blue staining post IVF (22, 79, 80). In contrast to IVF cases, no correlation was observed between excessive histones (aniline blue staining) and fertilization rate following ICSI (23, 100). Previous studies have revealed no significant correlation between chromatin assessed by SDS and SDS+EDTA with fertilization rate of both IVF and ICSI cases (22, 23, 101). Different authors found negative correlation between DNA damage evaluated by COMET or tunnel assay and embryo cleavage (24, 102-105). Activation of embryonic genome expression occurs at the 4–8-cell stage in human embryos (106), suggesting that the paternal genome may not be effective before genomic activation. Therefore, the lack of relationship between elevated DNA breaks in spermatozoa and fertilization rates is not surprising. The mentioned association seen between some of the sperm chromatin anomalies and fertilization rate may be due to direct effect of these anomalies or concurrent occurrence of these anomalies with other functional parameters, such as acrosomal status or acrosomal activity.

As expected, a negative relationship between the extent of nuclear DNA damage in ejaculated spermatozoa and blastocyst development after IVF and ICSI was reported using both the TUNEL assay for processed sperm for IVF (107) and the SCSA for unprocessed spermatozoa (108). In addition, pregnancy rates after IVF are reduced in couples who have higher percentages of spermatozoa with DNA strand breaks detected by in situ nick translation (93).

Some evidence shows that embryos derived from spermatozoa with damaged DNA have a lower potential to reach later or blastocyst stages. Janny and Menézo (1994) found a strong relationship between cleavage and blastocyst formation rate (109), and Shoukir *et al.* (1998) also demonstrated a lower blastocyst formation rate after comparing the

data between ICSI and IVF (110). This lower blastocyst rate is believed to be due to higher DNA damage or sperm chromatin anomalies in ICSI compared with IVF. Like other studies, no correlations were observed between COMET parameters or sperm protamine deficiency and embryo quality (111).

However, Morris *et al.* believed that the implantation and pregnancy outcome would not be so adversely affected by using sperm samples carrying high loads of DNA damage (102), since embryos derived from damaged sperm DNA have little chance of developing to blastocyst stage. However, it is still likely that low sublethal levels of sperm DNA damage are transmitted to embryos. Such levels of DNA damage may be insufficient to trigger a gross response such as cell cycle arrest or apoptosis prior to implantation, or early pregnancy failure, but may nonetheless be expressed in fetal or postnatal development.

On the other hand, there is strong evidence for a relationship between sperm nuclear DNA integrity, as assessed by SCSA and fertility rate after both normal intercourse (64, 65) and ART (96). Interestingly, Evenson *et al.* (1999) found cases with classical criteria (concentration, motility and morphology) within the normal ranges, but the SCSA values were poor and not compatible with good fertility after intercourse (64). Indeed, SCSA parameters are not strongly related with World Health Organization (WHO) parameters including concentration, motility and morphology (112). Based on these results, Evenson *et al.* speculated that SCSA parameters may be independent predictors of reproductive outcome beyond WHO parameters. On the other hand, in a recent study, Gardner *et al.* (2004) did not find a difference in the implantation and pregnancy rates between two groups that had 16 versus 40% fragmentation rates detected by SCSA (113). This result contradicts previous findings using the same technique. As SCSA is performed in the raw semen sample prior to processing, these results may reflect a selective elimination of spermatozoa with DNA fragmentation during sperm preparation for ART. If that is the case, revalidation of SCSA in spermatozoa processed for use in IVF may become necessary. Finally, Carrell *et al.* (2003) found that the proportion of spermatozoa with DNA fragmentation detected using the TUNEL assay is significantly increased in men whose wives suffer recurrent pregnancy loss (38 + 4.2) compared to donor sperm (11.9 + 1) or the general population (22 + 2). In the recurrent pregnancy loss group, no

relationship was observed between semen quality parameters and TUNEL positivity.

There is also accumulating evidence linking sperm nuclear DNA anomalies to poor reproductive outcome in relation to ART (114). Bartoov *et al.* demonstrated that the morphological state of the entire sperm cell, and especially of its nucleus, had predictive value for fertilization, pregnancy outcome, or both following ICSI (115). The tests currently available only provide an inkling of the impact of sperm nuclear DNA abnormalities on reproductive outcomes. More research is needed to improve our current knowledge in relation to DNA anomalies in spermatozoa, how to detect them more accurately, and how they may relate to failed reproductive outcomes. Additionally, more standardized, large-scale trials are needed to assess the predictive value of sperm DNA fragmentation techniques as useful pregnancy predictors in ART.

#### *Selection of appropriate sperm for ART*

By looking through the literature on sperm chromatin anomalies, it becomes apparent that sperm chromatin anomalies may directly or indirectly affect fertilization rate, embryo cleavage and quality, blastocyst formation, implantation, and finally pregnancy outcome. Therefore, selection of appropriate sperm for insemination, on the basis of factors other than sperm motility and morphology, is of importance in ART outcome. Selection of sperm at the level of semen processing has been considered as one of the steps required for isolation of appropriate sperm. The current techniques of sperm processing include density gradients centrifugation, glass wool filtration, swim up, and swim down. Some methods of density gradient centrifugation such as percoll, Pure Sperm, and Sil-Select isolate motile sperm with normal morphology directly from heterogeneous population of spermatozoa. Our previous study showed that Sil-Select was a suitable alternative to percoll to recover sperm with normal chromatin and morphology (116). However, some researchers have recently tried to select sperm on a different basis. Recently, Ainsworth *et al.* (2005) used electrophoresis-based microflow technology to separate spermatozoa by size and charge. The isolated sample was enriched of sperm with normal morphology and sperm with low levels of DNA damage, indicating that electrophoretic sperm isolation procedure has great potential as an extremely versatile, time- and cost-effective method for preparing spermatozoa for a wide



variety of assisted conception applications (117).

Said *et al.* (2005) also reported that magnetic-activated cell sorting (MACS) using annexin V-conjugated super-paramagnetic microbeads can effectively separate non-apoptotic spermatozoa from those with deteriorated plasma membranes based on the externalization of phosphatidylserine residues, and this novel technique provides spermatozoa of higher quality in terms of motility, viability and apoptosis indices compared with other conventional sperm preparation methods. MACS separation enhances the sperm-oocyte penetration

potential; therefore it may be of potential benefit during ART procedures such as intrauterine insemination (IUI) or IVF (118).

Jakab *et al.* (2005) reported a new method based on the hyaluronic acid binding ability of spermatozoa. Using this method, they were able to simulate ICSI techniques and select a spermatozoa population with a 4–5 fold decreased frequency of disomy or diploidy compared to unselected spermatozoa (119). While further validation is necessary, their findings are encouraging in providing a test that allows selection of spermatozoa that may be used in fertility treatment.



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## نقش اختلالات کروماتین اسپرم در نتایج حاصل از تکنیکهای کمک باروری

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### چکیده

دریافت مقاله: ۸۴/۱۱/۱، پذیرش مقاله: ۸۴/۱۱/۱۸

DNA اسپرم حاوی نیمی از مواد ژنتیکی بوده که به فرزند منتقل می‌شود و تمام محتویات ژنتیکی جهت لقاح، رشد و نمو جنین و حتی تکامل طبیعی پس از تولد الزامی است. در حالت طبیعی سدهای متعددی وجود دارد تا مناسب‌ترین اسپرم به تخمک وارد و سبب بارور نمودن آن شود. در این مقاله ساختار طبیعی کروماتین، سازماندهی DNA در هسته اسپرم و مکانیسم عواملی که در ایجاد آنومالی‌های ساختار کروماتین و آسیب DNA اسپرم موثر هستند، بیان شده است. همچنین روش‌های نوینی که جهت ارزیابی کیفیت کروماتین به کار می‌روند و از این طریق می‌توان اسپرم‌های سالم را انتخاب نمود تا کارآیی تکنیک‌های کمک باروری افزایش یابد، معرفی می‌شوند.

