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Sperm DNA Damage Causes, Assessment and Relationship with Fertility: A Review

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ABSTRACT

Evaluation of sperm quality has been mainly based on subjective parameters included in the spermiogram. Results of these parameters have been correlated with fertility but this relationship is not always true. Recently, for bull fertility assessment, sperm DNA integrity assessment has been proposed as an important index. Sperm DNA integrity has got an important role in success of fertilization process and fetal and offspring development. DNA integrity assessment has got a pivotal role in assisted reproductive techniques such as *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), besides sperm quality assessment and putative fertility predictor. Various techniques for assessment of sperm DNA integrity have been proposed. Using various sperm DNA integrity assays for detection and characterization of DNA fragmentation will aid in improving semen storage procedures by identification of various protocols which are less likely to be associated with DNA damage. Moreover, sperm DNA assays may help in screening bulls that produce good freezable semen with reasonable fertility.

Keywords: Sperm, DNA, damage, causes, fertility

Various subjective parameters included in the spermiogram have been traditionally used for evaluation of semen quality (Colenbrander *et al.,* 2003; Hidalgo *et al.,* 2009). These routine semen parameters have been correlated with fertility but this relationship is not always true. For predicting fertilizing potential of semen, developing more sophisticated and advanced technologies continues to be a priority (Alkmin *et al.,* 2013). An important aspect of sperm quality is sperm DNA integrity (Serafini *et al.,* 2016). The integrity of sperm DNA is very important for the success of fertilization and the development of fetus and offspring (Lopes *et al.,* 1998). Semen samples that had normal results in spermiogram were found infertile when assayed by DNA fragmentation assay (Giwercman *et al.,* 2003). The assessment of DNA integrity has got a significant importance in case of assisted reproductive techniques such as *in vitro* fertilization or intracytoplasmic sperm injection (Carretero *et al.,* 2012). DNA damage of 1.2–3% is normal in bulls with high fertility (Bochenek *et al.,* 2001). Sperm DNA damages have been associated with the poor embryonic development and genetic abnormalities in the offspring (Kasimanickam *et al.,* 2007; Loft *et al.,* 2003). Sperm DNA assays may have an

important role in improving the selection of cattle and buffalo bulls having better quality frozen-thawed spermatozoa.

Causes of sperm DNA damage

Factors responsible for DNA damage in ejaculated spermatozoa are still not clear but two possibilities may be there (Sakkas *et al.,* 1999): programmed cell death (apoptosis) during spermatogenesis and errors in maturation during spermiogenesis. Apoptosis during spermatogenesis facilitates elimination of defective germ cells and contributes to efficiency of spermatogenesis. At the time of ejaculation, the DNA breakdown is only partially completed in some spermatozoa which cause a higher percentage of DNA fragmentation index (%DFI)in otherwise intact spermatozoa (Sakkas *et al.,* 1999). Incomplete maturation during spermatogenesis also leads to increased %DFI due to the fact that positive association exists between sperm DNA damage and poor packaging of chromatin due to under protamination in mature sperm (Gorczyca *et al.,* 1993). The freezing and thawing process performed on ram (Peris *et al.,* 2004) and bovine (Celeghini *et al.,* 2008; Januskauskas *et al.,* 2003) sperm were shown to cause permanent structural alterations to DNA that, in turn, reduces fertility. The organization of sperm chromatin into stable and compact structures called toroids, which are attached to the nuclear matrix by toroid linker regions. These linker regions are highly prone to DNA damage (Ward and Coffey, 1991; Sotolongo *et al.,* 2003). Enzymatic or oxidative damage can result in single (ssDNA) or double strand breaks (dsDNA) (Aitken *et al.,* 2013). Breaks in ssDNA may result in impairment of fertilizing capacity (Ribas-Maynou *et al.* 2012b; Simon and Lewis, 2011), whereas dsDNA breaks may be responsible for interference in embryonic development and implantation (Lewis and Aitken, 2005). Three major hypotheses have been proposed to explain cellular mechanisms that result in the altered sperm DNA molecule. The first is related to torsional stress in unconstrained DNA supercoils and is a direct consequence of histone-protamine replacement during mid-spermiogenesis (McPherson and Longo, 1992; Marcon and Boissonneault, 2004). The second hypothesis regards DNA fragmentation as a consequence of oxidative stress in the male reproductive tract (Aitken *et al.* 1998; Agarwal *et al.,* 2003). The third hypothesis concerns apoptotic-related DNA strand breakage, similar to that which occurs in abortive apoptosis in somatic cells; the presence of caspase 9 in the midpiece and the occurrence of activated caspases 8, 1 and 3 in the post acrosomal region appeared to support this view (Paasch *et al.,* 2004a). However, the etiologies of DNA damage are many and varied, ranging from bacterial infections (Gallegos *et al.,* 2008), chemical toxicity (Rubes *et al.,* 2005), elevated temperature (Evenson *et al.,* 2000), diabetes (Agbaje *et al.,* 2007), age (Singh *et al.,* 2003; Wyrobek *et al.,* 2006), body mass (Kort *et al.,* 2006), and genetic background (Rubes *et al.,* 2007). Whereas many of the factors resulting in sperm DNA fragmentation are typically unavoidable, certain types of induced iatrogenic sperm DNA damage can become exacerbated when sperm are inappropriately manipulated in the laboratory. Reports have previously shown how changes in temperature excursions can affect the rate of sperm DNA fragmentation during *in vitro* incubation (Rubes *et al.,* 2007). Oxidative stress has been implicated as a primary mechanism of DNA fragmentation in sperm (Aitken *et al.,* 2006) and given that cryopreservation has also been shown to increase the level of reactive oxygen species in sperm (Mazzilli *et al.,* 1995), it is likely also a primary cause of DNA fragmentation in cryopreserved sperm (Paasch *et al.,* 2004a). Due care in the assessment of sperm DNA is particularly important in species with sperm predisposed to high rates of sperm DNA damage, including sheep (López-Fernández *et al.,* 2008), humans (Gosálvez *et al.,* 2009), and fish (López-Fernández *et al.,* 2009). This effect seems to be less critical in other species, e.g. pigs and

cattle, where sperm have much lower rates of DNA fragmentation following cryopreservation (López-Fernández *et al.,* 2009). Spermatozoa from rams with superior field fertility displayed increased sperm motility, viability, and sperm nuclear size. Exposure of sperms to UV light was also effective in producing fragmented DNA (Carretero *et al.,* 2012). Bull spermatozoa that were irradiated with UV light, used in IVF, failed to produce embryos beyond 2-cell stage, suggesting functional destruction of sperm genomic component by UV irradiation (Bordignon and Smith, 1999). DNA integrity of post-thaw sperm is affected by freezing distance and cooling velocity in both manual and automated freezing processes. Higher DNA fragmentation has been reported in fresh sperm of inbred mice compared to outbred and hybrid mice. Hypo-osmotic solutions result in higher DNA damage as compared to hyper-osmotic solutions in mouse spermatozoa (Yildiz *et al.,* 2010). Chill storage of semen for 48 hours leads to significantly increased DNA fragmentation in canine (Hidalgo *et al.,* 2009) and equine (López-Fernández *et al.,* 2007) semen. It is suggested that DNA damage in case of chilled spermatozoa occurs before than decline sperm quality (Hidalgo *et al.,* 2009).

Assessment of sperm DNA damage

For evaluation of different aspects of the sperm DNA structure, many techniques including single cell gel electrophoresis method (Comet assay), Sperm Chromatin Structure Assay (SCSA) and Sperm Bos Halomax (SBH) assay have been developed. SCSA identifies the ratio of ssDNA (abnormal) to dsDNA (native) in the exposed toroid linker regions, but not in the more compact toroids (Shaman and Ward, 2006). SCSA has been widely used to assess sperm DNA quality in men (Evenson *et al.,* 1980), bulls (Fortes *et al.,* 2012; D'Occhio *et al.,* 2013), stallions (Love and Kenney, 1998) and boars (Evenson *et al.,* 1994). Contrarily to SCSA, for identification of ssDNA and dsDNA breaks. Comet assays allow accessibility to both toroid and toroid linker regions (Shaman *et al.,* 2007). Sperm DNA breaks move away from the head region to form comets following electrophoresis, while as intact DNA remains in the actual head position (Shaman and Ward, 2006). Breaks in ssDNA and dsDNA are identified by alkaline Comet assay, whereas neutral Comet assay identifies mainly dsDNA breaks. In contrast Baumgartner *et al.* (2009) proposed that alkaline Comet assay identifies only ssDNA breaks and neutral Comet assay identifies dsDNA breaks and closely related ssDNA breaks. Based on Sperm Chromatin Dispersion Test (SCDT) for humans, the sperm DNA integrity in bulls has been assessed by Sperm Bos Halomax (SBH) assay (Fernandez *et al.,* 2003). SBH assay is similar to Comet assay except that treated spermatozoa remain unexposed to an electrophoretic field in the former. Larger halos are produced due to greater DNA fragmentation and less fragmentation of DNA yields smaller halos (García-Macías *et al.,* 2007). Which Comet assay (neutral or alkaline) may be more accurate for sperm DNA quality evaluation remained unclear (Zee *et al.,* 2009; Enciso *et al.,* 2011; Ribas-Maynou *et al.,* 2012a; Serafini *et al.,* 2015). Cortes-Gutierrez *et al.,* (2007) classified DNA fragmentation evaluation techniques into two groups. The first group includes various methods that are used to mark double and simple strand breaks. These include incorporation of marked nucleotides in situ such as terminal dUTP nick-end labeling (TUNEL) or in situ nick translation (ISNT) by application of enzymatic processes. The second group comprises the techniques that assess the denaturalizing ability of chromatin after treatment. This group includes techniques such as sperm chromatin structure assay (SCSA), single-cell-gel-electrophoresis (SCGE), or comet assay, the DNA breakage detection-fluorescence in situ hybridization (DBD-FISH) and the sperm chromatin dispersion (SCD) test. Using neutral and alkaline Comet assays, lesser ssDNA and dsDNA breaks had been reported in fertile men as compared to subfertile men (Ribas-Maynou *et al.,* 2012b).

Sperm DNA damage in relation to fertility

An important index of fertility potential in vitro is sperm DNA integrity (Nijs *et al.,* 2011). Using neutral and alkaline Comet assays, lesser ssDNA and dsDNA breaks had been reported in fertile men as compared to subfertile men (Ribas-Maynou *et al.,* 2012b). In case of non sex-sorted sperm as compared to sex-sorted sperm of bull, neutral Comet assay revealed more DNA breaks in non-sorted sperm as compared to sex-sorted sperm (Boe-Hansen *et al.,* 2005).

Fertile males have high sperm motility, morphology and SCSA measures and consistent with sperm quality (Ballachey *et al.,* 1988; Love and Kenney, 1998; Serafini *et al.,* 2016). In equines (Serafini *et al.,* 2014) and bovines (Serafini *et al.,* 2016), the sensitivity values of these DNA assays were high (80–86%) and specificity values were low (15–26%), which provides an indication that these tests are better predictor of likelihood of pregnancy (i.e., sensitivity) compared to non-pregnancy (i.e., specificity). Sperm DNA assays may have an important role in improving the selection of buffalo bulls having better quality frozen-thawed spermatozoa. Regarding DNA quality assessment, complementary information to the SCSA may be provided by neutral Comet assay (Serafini *et al.,* 2016). Using the SCSA technique, in sexually mature rams, under nutrition leads to higher sperm DNA damage than in sperm from well-fed rams (Guan *et al.,* 2014). Variation in DNA fragmentation index (DFI %) among different breeds of sheep has already been reported (Malama *et al.,* 2013). Semen collection frequency affects DNA damage in boars (Strzeiek *et al.,* 1995) and may also be responsible for alteration in DFI (%) (Guan *et al.,* 2014). In human spermatozoa, distinct cutoff values for % DFI associated with low fertility (15%) and sterility (30%) have been established (Evenson *et al.,* 1999). Due to well establish differences between chromatin packaging density between humans and rams, the %DFI in rams is much lower (Evenson *et al.,* 2002). Presences of genetic material defects viz., impairments in chromatin condensation, DNA integrity or chromosomal abnormalities are associated with infertility (Aravindan *et al.,* 1997; Tsarev *et al.,* 2009). Heat stress is one of the potent causes responsible for compromised sperm DNA integrity (Banks *et al.,* 2005; Perez-Crespo *et al.,* 2008; Carretero *et al.,* 2012). Exposure of sperms to UV light was also effective in producing fragmented DNA (Carretero *et al.,* 2012). DNA damages in spermatozoa have been associated with poor embryonic development and offspring genetic abnormalities (Kasimanickam *et al.,* 2007; Loft *et al.,* 2003). Functionally intact sperm membranes are essential to achieve fertilization and integrity of DNA is necessary for development of embryo. Although, Reactive oxygen species (ROS) in semen play roles in normal fertilization processes (Agarwal *et al.,* 2006) but high levels of ROS cause damage to spermatozoa and result in infertility (Yeni *et al.,* 2010). Sperm DNA damage is related to semen quality and DNA damage is higher in poor quality semen samples as compared to good quality semen samples (Aoki *et al.,* 2005; Sailer *et al.,* 1995). Abnormal spermatozoa have capability to fertilize but lack ability to maintain pregnancy and before morulla stage, embryos are lost (Paul *et al.,* 2009; Fatehi *et al.,* 2006; Saleh *et al.,* 2003).

In conclusion, sperm DNA quality is vital for conveyance of genetic material to the next generation. In assisted reproductive techniques such as *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), assessment of sperm DNA integrity has now a day's got a pivotal role. Alterations in sperm DNA integrity may be responsible for impaired development of embryo and hence offspring abnormalities. Apoptosis and erroneous maturation are some potent factors leading to DNA damage in ejaculated spermatozoa. Three hypotheses proposed to explain alterations in DNA integrity include torsional stress due to histone-protamine replacement during mid-spermiogenesis, fragmentation of DNA

in the male tract due to oxidative stress and apoptotic-related DNA strand breakage. DNA fragmentation detection and characterization using various assays will not only help in selection of good freezable bulls but also play role in identification of various protocols that are less likely to be associated with DNA damage and thus aid in improving semen storage procedures.

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