

Consequences of sperm DNA fragmentation for assisted reproductive technology

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Summary

The possession of offspring is the most important human biological goal, which conditions the survival of the human species. The problem of the lack of offspring is a phenomenon concerning approximately 15% of married couples in Poland. In a half of the cases, the causative agent is the male factor infertility problem. It is considered that in approximately 20% of patients with idiopathic infertility, and elevated level of sperm DNA fragmentation may be the cause of failure in reproduction. High levels of sperm DNA fragmentation have also been associated with decreased oocyte fecundation, embryo quality and pregnancy rate. The quality of the genetic material carried in male sperm becomes a prognostic factor in the area of the effectiveness of treatment of an infertile couple, bearing a healthy child, as well as the risk of contracting cancer in the future generations. Present data on the consequences of sperm DNA fragmentation (SDF) for reproduction are scarce and, in many ways, inconsistent. The differences in the study conclusions might result from the different methods used to detect SDF, the study design and the inclusion criteria. Consequently, it is difficult to decide whether SDF testing should be carried out in fertility assessment and assisted reproductive technology (ART). It is clear that there is an urgent need for the standardization of the methods and the need for additional clinical studies on the impact of SDF on ART outcomes.

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The possession of offspring is the most important human biological goal, which conditions the survival of the human species. The problem of the lack of offspring is a phenomenon concerning approximately 15% of married couples in Poland. In a half of the cases, the causative agent is the male factor infertility problem. There is evidence that certain states of male fertility disorders are related with the disorders of the process of spermatogenesis. From the aspect of endocrinology, the course of normal spermatogenesis depends on proper secretion by the pituitary gland of follicle-stimulating hormone – FSH), luteinizing hormone (LH), as well as secretion of testosterone.

Hormonal testicular function is controlled by pituitary gonadotropins: LH - luteinizing hormone, via Leydig cells, stimulates the production of sex steroids, and FSH – folliclestimulin, together with testosterone, act on seminiferous tubules via Sertoli cells in order to support and maintain spermatogenesis.

An intensification of sperm DNA fragmentation is accompanied both extremely low and extremely high levels of FSH and LH. Sperm DNA fragmentation increases together with a decrease in the level of testosterone.

The secretion of these two gonadotropins by the pituitary gland is controlled by hypothalamic decapeptide GnRH (Gonadotropin Releasing Hormone). This peptide is released in an episodic pattern of pulses, every 15-30 minutes, synthesized in neurons located in the hypothalamus. Subsequently, it is secreted into the hypophyseal pituitary portal system and fixed on membrane receptors typical of gonadotropic cells of the anterior lobe of the pituitary gland. The secretory activity of the GnRh is modulated by sex hormones. FSH plays a major role in the hormonal control of spermatogenesis; however, an activity of androgens independently of FSH is also assumed. FSH stimulates cell division and differentiation, inhibits apoptosis during spermatogenesis, and stimulates meiosis, while testosterone controls the course of meiosis, the transformation of spermatids, especially their elongation during maturation phase, and adhesion of spermatids to Sertoli cells. Several types of Sertoli cells not correlated with individual stages of the cycle are distinguished in humans, which is probably associated with the patch-like distribution of the stages in the epithelium. In the vicinity of Sertoli cells sur-

face, in the region of the zonula occludentes, the presence of pinocytotic vesicles was confirmed, the number of which increases in conditions of stimulation by LH-like gonadotropin called human chorionic gonadotropin. Hormones control spermatogenesis indirectly by maintaining the activity of somatic cells, especially Sertoli cells. Directly, spermatogenesis is controlled due to the function of the local regulatory system. Gametogenic cells lack the FSH receptors and androgen. Sertoli cells, as the only receptor cells of these hormones in the epithelium, produce the mechanism of transmission of signals required at individual stages of spermatogenesis. This mechanism functions on the principle of cross-talk cellular interaction. This is a complex mechanism, in which intercellular junctions participate. It has been found that the process of spermatogenesis can neither be hormonally accelerated nor slowed down, but only inhibited [10]. Under the effect of hormones it is possible to increase the number of genatogenic cells, which are converted in the course of spermatogenesis, and end this process due to, among others, reduction in the number of cells undergoing apoptosis. FSH and LH deficiency occurs in males with hypogonatropic hypogonadism, as well as in those who receive treatment with antidepressants, whereas the level of these hormones is elevated in the case of primary (including genetic causes) or secondary testicular failure.

It is considered that in approximately 20% of patients with idiopathic infertility, and elevated level of sperm DNA fragmentation may be the cause of failure in reproduction [1,2]. High levels of sperm DNA fragmentation have also been associated with decreased oocyte fecundation, embryo quality and pregnancy rate [1,2]. Intriguingly, an intracytoplasmic sperm injection (ICSI) study of mouse oocytes, using spermatozoa with a high percentage of DFI, revealed that a significant proportion of adult offspring produced by this procedure, showed a significant increase in the incidence of abnormal behavioral tests, malformations, tumors and signs of premature aging [5]. DFI is also an informative biomarker in studies of reproductive toxicology, as exposure of the testis to reproductive toxicants, such as thermal stress, ionizing radiation, chemotherapy, pesticides, air pollution and smoking have all resulted in a higher incidence of sperm DNA damage [4,6,11]. The quality of the genet-

ic material carried in male sperm becomes a prognostic factor in the area of the effectiveness of treatment of an infertile couple, bearing a healthy child, as well as the risk of contracting cancer in the future generations [3].

Among the different DNA abnormalities that can be present in the male gamete, DNA fragmentation is the most common, especially in the infertile subjects. There is now a consistent proof that a sperm containing fragmented DNA can be alive, able to move, morphologically normal and able to fertilize an oocyte. There is also evidence that the oocyte is able to restore DNA damage; however, the extent of this repair is strongly associated with the type of DNA damage present in the sperm, as well as with the quality of the oocyte. That is why, it is important to understand the probable consequences of sperm DNA fragmentation (SDF) for embryo growth, implantation, pregnancy outcome and the health of progeny conceived naturally and by accompanied reproductive technology (ART) [16].

There is evidence that certain states of male fertility problems are associated with genetic disorders, as well as with an excessive production of reactive oxygen species. An appropriate concentration of reactive oxygen intermediates is regulated by means of antioxidant enzymes, to which belongs, among others, superoxide dismutase (SOD). The disorders of the balance in the oxidoreduction system in the human body may cause an excessive peroxidation of lipids, destruction of cellular membranes and organelles, damage to enzymes and nucleic acids [1]. Tobacco smoking is also an agent weakening the system of the body's defence against oxidants. As a result of nicotinism there occur modifications of nitrogenous bases and formation of DNA adducts. The level of natural antioxidants in blood plasma decreases (vit. C and vit. E), and there is an increase in lipid peroxidation (F₂-isoprostanes) in the cardiovascular system. The process of ageing is inseparably associated with the weakening of human antioxidant defence. With age, an increase occurs in the number of the DNA lesions, cholesterol deposits, lipid peroxidation, and atherosclerosis intensifies. An elevated cell membrane lipid peroxidation with age deteriorates the fluidity and functions of the cell and mitochondrial membranes. This leads to electron leakage, and disorders in energy production [2].

Sperm DNA fragmentation has always been referred

to as a constant parameter of sperm quality, without any specification of the period of time that had passed between sperm quality assessment and use for ART. However, this could be untrue because the sperm DNA fragmentation index may change rapidly when samples for artificial insemination are handled. In fact, in mammalian species it has been reported that temperature excursion episodes dramatically increase the rate of sperm DNA degradation, and after some hours of sperm incubation at 37°C, the basal values for the sperm DNA fragmentation index may be doubled [7].

The SDF dynamics evaluated by *in vitro* incubation provides important information about the evolution of DNA integrity in the mature spermatozoon that is typically not revealed on basal assessment of SDF.

A study conducted by Pregl et al. [12] determined the diagnostic value of the following sperm function tests in predicting the fertilizing ability of spermatozoa in conventional *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI): hyaluronan-binding assay (HBA), DNA fragmentation (Halosperm), and hyperactivity. Both FR and EQ in IVF cycles negatively correlated with sperm DNA fragmentation. Furthermore, a positive correlation was observed between FR and hyaluronan-binding ability or induced hyperactivity. The semen samples from the IVF cycles with low FR (group 1) were characterized by statistically significantly higher sperm DNA fragmentation and lower hyaluronan-binding ability in comparison with semen samples from the group with high levels of fertilization (group 2). In ICSI cycles, no relationship was found between sperm function tests and FR or EQ.

The study of Kumar et al. [9] assessed DNA integrity in cases experiencing recurrent pregnancy loss (rPL) following spontaneous conception. Their data indicate that sperm from men with a history of rPL have a higher percentage of DNA damage as compared to control group, and this can explain pregnancy loss in these patients.

Studies performed by Jiang et al. [8] explored the predictive value of sperm chromatin integrity test (SCIT) in assisted reproductive technology (ART) by analyzing the relationship of sperm chromatin integrity (SCI) with the outcomes of IVF-ET and ICSI. The clinical pregnancy rate of ICSI was significantly higher than

that of IVF in the high DFI group, while the clinical outcomes showed no significant differences between the high and low DFI groups in either the IVF or the ICSI subgroup. These studies are consistent with the results obtained by us. Simon et al. [15] investigated relationships between DNA fragmentation, sperm protamines and assisted reproduction treatment. Fertilization rates and embryo quality decreased as sperm DNA fragmentation increased. Sperm DNA fragmentation was lower in couples achieving pregnancies after IVF, but not after ICSI. Increased sperm DNA fragmentation was associated with abnormal protamination and resulted in lower fertilization rates, poorer embryo quality and reduced pregnancy rates.

Sadeghi et al. [14] evaluated the effect of sperm source on chromatin integrity and ICSI outcomes. They claimed that abnormal sperm chromatin condensation and DNA fragmentation were not correlated with fertilization rate and embryo quality.

Rougier et al. [13] considered that DFI depends on the preparation of semen before ICSI. Sperm DNA fragmentation significantly decreased after centrifugation gradient, regardless of the initial levels of the sample. Samples with TUNEL $\geq 20\%$ were more susceptible to a significant increase in DNA fragmentation over time, with similar increases being observed over time for samples that were incubated in HA or PVP. These data may be relevant for sperm preparation for intracytoplasmic sperm injection.

Present data on the consequences of SDF for reproduction are scarce and, in many ways, inconsistent. The differences in the study conclusions might result from the different methods used to detect SDF, the study design and the inclusion criteria. Consequently, it is difficult to decide whether SDF testing should be carried out in fertility assessment and ART. It is clear that there is an urgent need for the standardization of the methods and the need for additional clinical studies on the impact of SDF on ART outcomes.

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