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REVIEW

THE DEATH OF SPERM CELLS

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Abstract. *A major factor affecting male fertility is excessive death of germ cells, both immature germ cells and mature spermatozoa. It can be due to various factors causing testicular and/or post-testicular damage, such as infections, obstructive conditions, toxins, oxidative stress, hormonal imbalance, hyperthermia, and anti-sperm antibodies. Massive death of spermatozoa leads to a high proportion of dead sperm cells in the ejaculate (necrozoospermia or necrospermia) while death of immature germ cells can lead to low sperm count (oligozoospermia or oligospermia). Cell death can occur both by necrosis and by apoptosis; in recent decades, it has been found that apoptosis of mature spermatozoa is not only possible but quite common, and can contribute to infertility. Treatment approaches are primarily directed to the underlying condition, i.e. removing the cause(s) of sperm cell death whenever possible, but include also attempts to bypass the cell death event by intracytoplasmic sperm injection with testicular spermatozoa.*

Key words: *spermatozoa, male germ cells, necrozoospermia, male infertility, sperm apoptosis*

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Male infertility is a condition having a severe impact on individuals and families, and affecting more and more people: its age-standardized prevalence rate has increased by 0.291% since 1990 [1]. Studying its mechanisms is important because it can help identify the underlying causative factors and eventually find the best course of treatment. The first prerequisite for sperm cells to be functional is, of course, being alive. If there is excessive death of germ cells, either immature germ cells or mature spermatozoa, fertility will be reduced or abolished altogether. The aim of this review is to summarize the current state of knowledge about male germ cell death.

FACTORS CAUSING DEATH OF SPERM CELLS AND THEIR PRECURSORS

The presence of a high proportion of dead spermatozoa in the ejaculate, sometimes referred to as necrospermia or necrozoospermia, greatly reduces fertility. It can be due to various factors causing testicular and/or post-testicular damage, and their accurate assessment is needed to develop an adequate approach to every case [2, 3]. In addition, excessive death of immature germ cells in the testis can lead to too few of them completing their differentiation and ever reaching the ejaculate, leading to low sperm count – oligozoospermia (oligospermia) [4].

Examples of factors able to cause testicular damage leading to male germ cell death are varicocele, local hyperthermia, and hormonal imbalance. Examples of factors causing post-testicular damage include inflammatory conditions of the male genital tract such as epididymitis and prostatitis, and obstructive conditions, such as structural changes in autosomal dominant polycystic kidney disease, or a history of vasectomy even if it has been reversed. Systemic factors such as generalized infection or intoxication, spinal cord injury, aging, and anti-sperm antibodies can cause both testicular and post-testicular damage [5].

Infections of the male genital tract, while having only a minor impact on sperm viability in most cases, are thought to cause about 40% of cases of necrozoospermia. The mechanisms involved seem to differ between individual pathogens. They may involve substances directly produced by the pathogen, such as bacterial lipopolysaccharide, or mediators of inflammation caused by the infection, such as cytokines secreted in chronic bacterial prostatitis [3]. In this respect, sperm cells are similar to their partners, the oocytes, in which prostaglandin exposure can induce accelerated degeneration [6]. Viruses can also have an impact. In addition to the well-known correlation between mumps orchitis and future infertility risk, new data have shown acute decrease in sperm count and chronic reduction in testicular size and weight after SARS-CoV-2 infection of an experimental model. These effects seem to occur through Sertoli cell damage [7].

Toxic damage can be due to environmental, occupational or iatrogenic exposure to toxic substances, as well as substance abuse. Its negative effects on male germ cells, though difficult to evaluate especially with common pollutants, are pervasive and long-term [4]. When prescribing drugs to male patients, the potential impact on semen quality should be taken into account. For example, anti-parasitic drugs, being toxic to eukaryotic cells, can have detrimental effects on male germ cells. In particular, niridazole which is used to treat schistosomiasis has been reported to cause reversible spermatogenic arrest [8]. Oxidative stress is so important that it deserves a category of its own: some studies have found increased concentrations of reactive oxygen species in the seminal plasma of about a third of infertile men [9]. Oxidative stress can affect male germ cells by multiple mechanisms: it can directly damage mature spermatozoa, which are very susceptible to it due to the high content of polyunsaturated fatty acid in their membranes, and at the same time unable to repair the damage because of the loss of most of the cytoplasm; it can make late spermatids retain excess residual cytoplasm; and it might also disturb earlier stages of spermatogenesis. Chlorine disinfectants, which act by causing oxidative damage, have been shown to interfere with chromosome segregation in hematopoietic cells of a primate model [10], and data from patients with Robertsonian translocations suggest that problems with chromosome mis-segregation can lead to apoptosis [11]. The impact of varicocele on male fertility is thought to be partly based on generating oxidative stress in addition to heat stress [9].

Hormonal imbalance is another factor associated with male germ cell death. Hyperthyroidism has been shown by experiments in rats to cause prophase I arrest of spermatocytes, and in humans, it is associated with a decrease in the vitality, number and motility of spermatozoa [12]. Gonadotropins and testosterone regulate the survival of immature germ cells in the testis, with their effect being at least partly mediated by Sertoli cells creating the microenvironment for differentiating spermatogenic cells. Deprivation or excessive levels of these hormones can cause germ cell death. It is noteworthy that, while estradiol can support germ cell survival when appropriately balanced with follicle-stimulating hormone and testosterone, on its own it has a pro-apoptotic effect [13]. In recent decades, there is a widespread concern that environmental pollution with chemical mimicking the action of estrogens can be a contributing factor to the deteriorating sperm cell counts and male fertility worldwide [14].

Anti-sperm antibodies, both autoantibodies produced by the male and isoantibodies produced by his female partner, are especially important when directed against surface antigens. In addition to hindering sperm motility by agglutination, they could mediate their destruction by turning them into a target for defense mechanisms such as phagocytosis and complement. In fact, the sperm immobilization test, which is routinely used for detection of anti-sperm antibodies [15], detects immobilization resulting from lysis by complement. It is hypothesized that formation of anti-sperm antibodies is the basis of lingering effects after vasectomy reversal [3], and that such antibodies against epitopes cross-reacting with antigens of pathogens contributes to the impact of infections on male fertility [15].

NECROSIS VERSUS APOPTOSIS

In addition to the above described situations of spermatogenic cell death caused directly by an overwhelming external factor, i.e. necrosis, their death by apoptosis is the subject of a growing number of studies. Immature male germ cells, similarly to other dividing and differentiating cells, are well known to undergo apoptosis. During the first spermatogenic wave, apoptosis of large numbers of spermatogonia is used to optimize their ratio to Sertoli cells; in later life, testicular germ cells respond by apoptosis to moderately damaging factors [16]. Hormonal imbalance, especially

testosterone deficiency or excess, increases the level of male germ cell apoptosis [13, 17]. Certain toxins, such as estrogen-mimicking chemicals and plasticizers which are widespread environmental pollutants, have also been shown to exercise testicular damage by inducing germ cell apoptosis [4, 14]. This pro-apoptotic action can be based both on the extrinsic pathway using signaling though death receptors (Fas), and the intrinsic pathway using mitochondria-associated proteins of the Bcl-Bax family [13]. The well-known detrimental effect of hyperthermia on spermatogenesis is also due to inducing apoptosis, though it is still unclear why spermatogenic cells of humans and most other mammals are uniquely susceptible to heat-induced apoptosis and require a lower temperature than is maintained in the rest of the body [18]. Experiments on rats have shown that only prolonged and intensive heat can change the mode of cell death from apoptosis to necrosis, which is supposed to be caused by severe oxidative stress resulting from the treatment [19].

Apoptosis of mature spermatozoa was regarded as more controversial. It was initially thought that they cannot undergo this type of cell death due to their terminal differentiation including cessation of transcription and protein synthesis. This assumption was supported by the methodological difficulties in assessing apoptosis in sperm cells with their already highly condensed nuclei, leading to difficult detection of DNA fragmentation by TUNEL protocols standardized for somatic cells, and making it impossible to use as a criterion the morphological changes of the nucleus which are a hallmark of somatic cell apoptosis [20]. In recent years, however, evidence accumulated that spermatozoa possess the molecular apparatus of apoptosis, particularly caspases, and are able to perform it. TUNEL test has been optimized for sperm cells, and other tests to detect DNA damage have been introduced, such as the Comet assay quantifying the DNA fragmentation level. Both double- and single-strand breaks can be detected [20]. Moreover, the proportion of sperm cells displaying apoptotic markers has been shown to be higher in infertile patients than in healthy donors, and increases after subjecting the cells to potentially damaging treatment such as freezing [22, 23]. These data show that sperm cell apoptosis not only exists but also is of practical importance with regard to male infertility and assisted reproduction. In fact, spermatozoa may be better “suited” for apoptosis than mature oocytes which are arrested in metaphase II and need to exit meiosis in order to develop the full apoptotic sequence [24].

Numerous teams have studied the influence of factors able to trigger apoptosis in spermatozoa, as well as the possible mechanisms of the process. Mitochondria known to be important for apoptosis of somatic cells can have the same role in sperm cells, as shown by experiments using the betulinic acid, a plant terpenoid inducing release of cytochrome C from the mitochondria into the cytosol [25, 26]. Bacterial lipopolysaccharide, which is present in the seminal plasma in male genital tract infections, causes apoptosis of spermatozoa by binding their surface Toll-like receptor 4 (TLR4) [27]. Once inside the female genital tract, sperm cells undergo capacitation which, while a necessary prerequisite for their fertilizing ability, increases their susceptibility to apoptosis through oxidative stress [28]. There are yet no data whether anti-sperm antibodies against surface antigens can cause apoptosis but this possibility should be considered in future research, because such pathways have been described in other cell types [e.g. 29].

ASSESSMENT AND TREATMENT OF NECROZOOSPERMIA

During semen analysis, sperm motility is a proof of vitality. Dead spermatozoa are of course immotile, but live spermatozoa can also be immotile for various reasons, i.e. a molecular defect in their motility apparatus [30]. To distinguish between the two situations, if the observed motility of ejaculated spermatozoa is poor (< 40%), a vitality test is performed by assessing their membrane integrity. The recommended methods are staining by eosin (alone or in combination with nigrosin) or hypo-osmotic swelling [31]. If the result proves an excessive proportion of dead spermatozoa, more examinations and tests are performed to reveal the cause(s). An important parameter in this respect is sperm DNA fragmentation, which may be involved in the causation of cell death and invariably follows it [3]. Electron microscopic observation, though not obligatory, can also be very helpful and show interesting sequences of structural degeneration [32, 33]. The role of epididymal factors in some cases of necrozoospermia was first revealed by this method [34].

Treatment of necrozoospermia depends on its presumed cause, and there is yet no standardized protocol [35]. Depending on the revealed causative factors, there are two approaches to help the patient, which are not mutually exclusive. The first one is to address the supposed cause(s) of germ cell death: treating the infection, minimizing the exposure to heat, removing the toxins, surgically correcting the varicocele, correcting the hormonal profile etc. The second approach is to try to bypass the stage and place where cell death is induced. When epididymal factors are suspected, the exposure of sperm cells to them can be shortened by repeated ejaculations. In severe necrozoospermia, expecially when it is thought to be caused by post-testicular damage, intracytoplasmic sperm injection (ICSI) with testicular spermatozoa is recommended [3]. When perform-

ing ICSI with immotile spermatozoa, application of a modified hypo-osmotic swelling test is recommended in order to select a viable sperm cell [35]. In all cases, treatment should be personalized based on extensive examinations and tests to reveal the etiology of sperm damage, and the aim should be not only giving the patient an opportunity to reproduce but also restoration of his general health and well-being.

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