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Sperm Cell in ART

Dejan Ljiljak¹, Tamara Tramišak Milaković², Neda Smiljan Severinski²,
Krunoslav Kuna¹ and Anđelka Radojčić Badovinac²

¹University Hospital Center "Sisters of Mercy", Zagreb

²University Hospital Center Rijeka, Rijeka
Croatia

1. Introduction

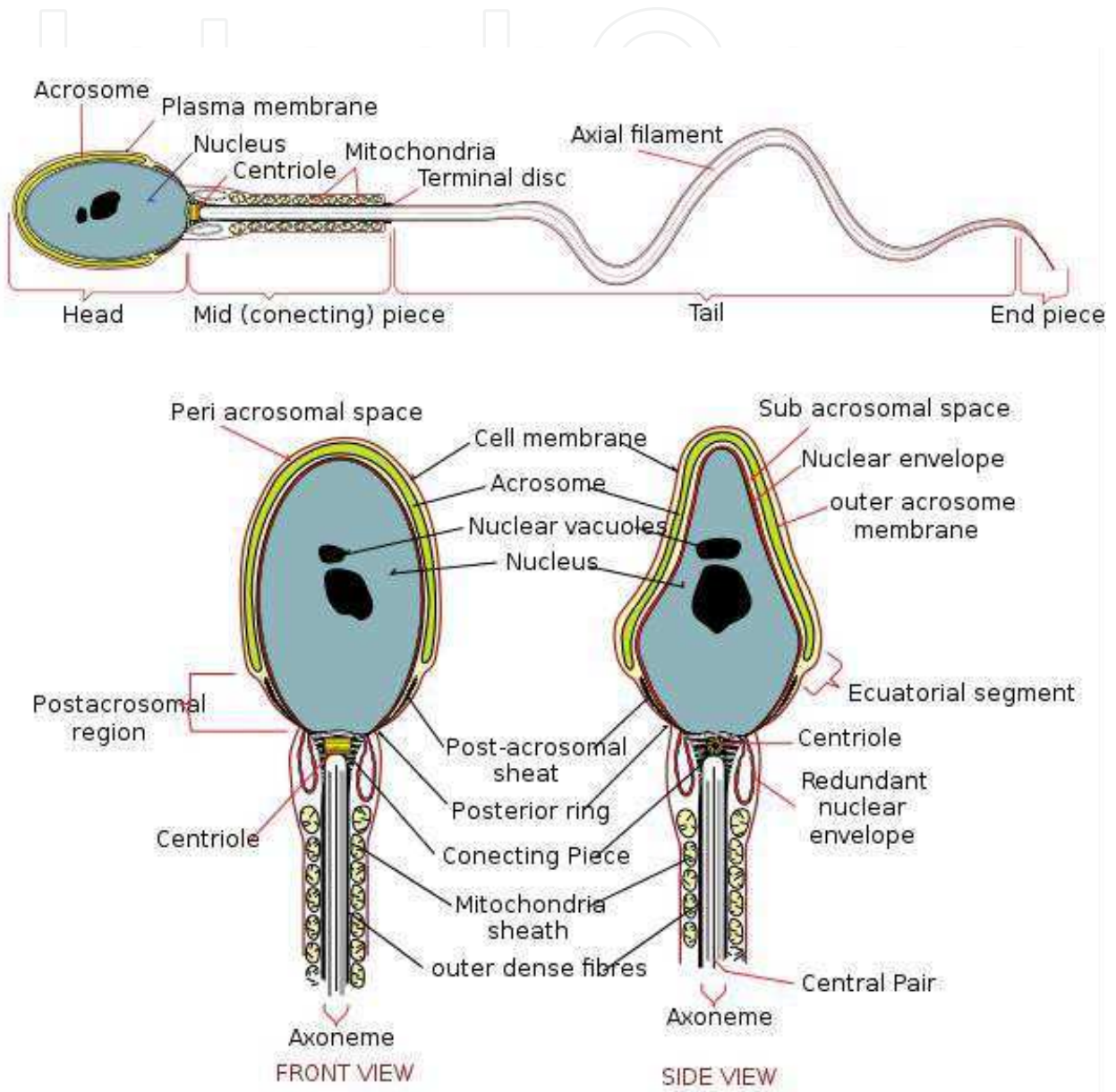
Infertility today represents a global problem. Male factor contributes in approximately 50% of infertile couples. In the last decade we are witnesses of the decreased quality of semen and the increased frequency of testicular cancer and cryptorchidism. Currently, the assessment of semen quality is based on the routine semen analysis including sperm count, morphology and motility. Although variation and combination among these three main factors articulate few diagnosis, nowadays developed assisted reproduction techniques (ART), especially intracytoplasmatic sperm injection (ICSI) may be used to treat most of the male infertility problems. In general we can say that traditional semen parameters provide a limited degree of diagnostic information, thus we are aware that these indexes of diagnosis should be revisited, which includes more specific test of sperm assessments, such as DNA tests and sperm proteome.

Spermatogenesis is a process that includes physiological, morphological and biochemical changes. After a complex process from the round diploid spermatogonia to haploid spermatozoa, a mature sperm just has an ability to fertilize a mature oocyte. If any errors occur during spermatogenesis process, appropriate sperm will not be produced. Thus, in our ART practice, it is important to understand normal physiology of spermatogenesis and find the reason of abnormal situation. In this chapter we will review today knowledge about:

- - Spermatogenesis
- - Sperm chromatin structure (sperm nucleus proteins),
- - DNA damage through spermatogenesis,
- - Apoptosis,
- - Oxidative stress (testicular and posttesticular factors),
- - Y chromosome microdeletions,
- - Centrosome (epigenetic factors)
- - New techniques of sperm selection in ART

2. Spermatogenesis

The sperm cell is composed of a sperm head, a sperm neck and a sperm tail. Whole sperm is covered by the sperm plasma membrane called plasmalemma (Picture 1.). The sperm head



Picture 1. Diagram of human spermatozoon

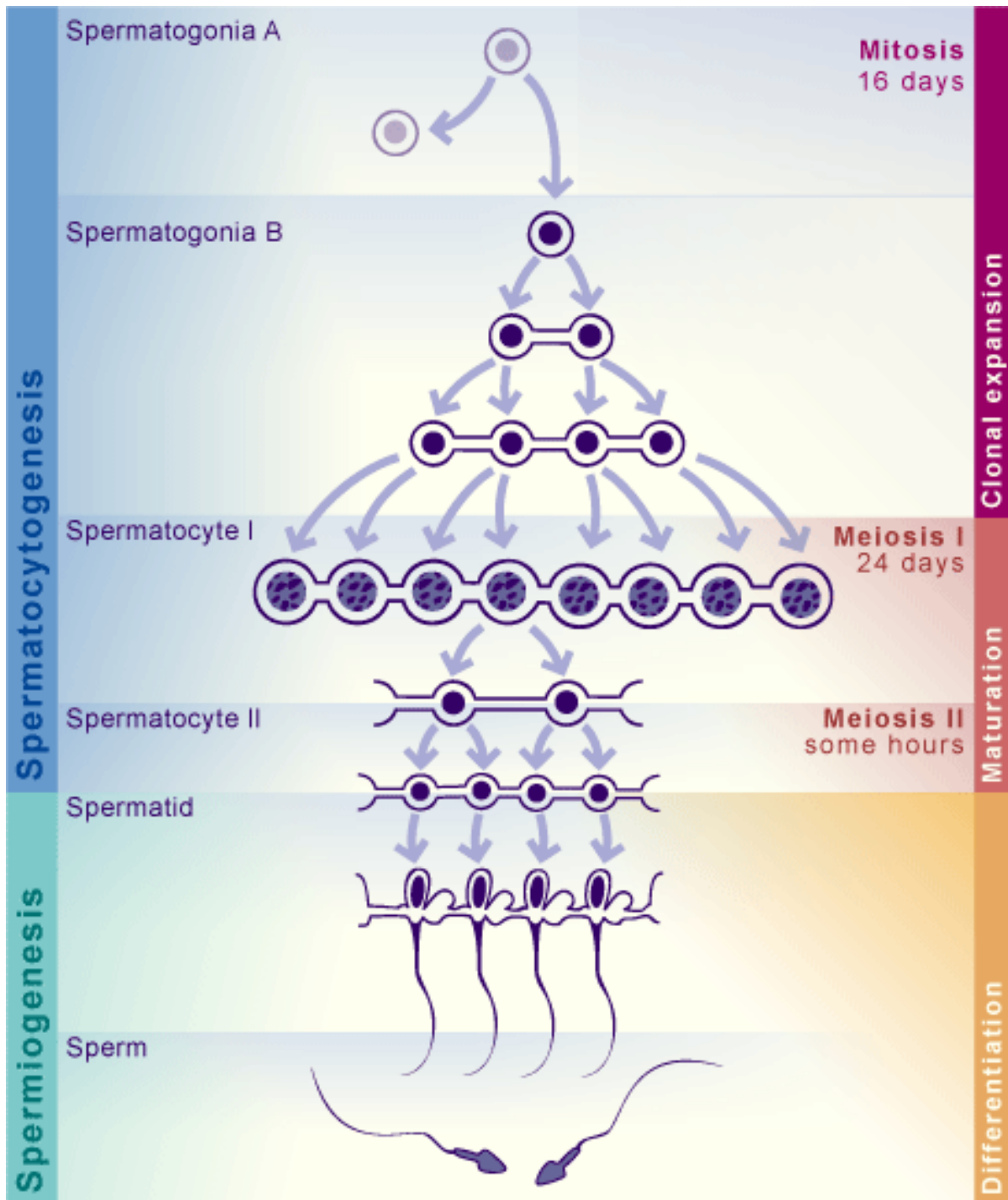
is composed of a nucleus and an acrosome. The nucleus contains sperm DNA (half number of chromosomes) and the acrosome has important enzymes for fertilization. The sperm neck or midpiece has 100 sperm mitochondria which generate energy for the sperm tail. The sperm tail is based on 9 + 2 microtubules. The microtubule doublets are connected doublet-to-doublet by dynein arms.

A spermatogenesis basically includes the mitotic expansion of stem cells, the meiotic recombination of genetic information and the haploid spermatid production (Picture 2.). The aim of the process is to produce a highly specialized mature sperm cell which can bind to the oocyte. The paternal inherited centrosome is essential for normal fertilization, chromatin packaging and early embryogenesis. The complete matured spermatozoon must undergo acrosome development, nuclear elongation and condensation, the formation of middle piece and tail, and the reduction of cytoplasm.

The primordial germ cells in fetal testis are enclosed in tubules which form very proliferative active cells called the gonocytes. The meiotic prophase is inhibited. Spermatogonia are positioned on seminiferous tubules and have a connection with the Sertoli cells. The basement membrane and Sertoli cells form the blood-testis barrier. After birth, gonocytes rise to a population of spermatogonia which constitute the stem cell pool. The spermatogonia are characterized by mitotic division and they are inactive until the puberty. The diploid spermatogonia differentiate into primary spermatocytes. The primary spermatocytes undergo first meiotic division and create secondary spermatocytes. After the second meiotic division four haploid spermatids are created. After the morphological differentiation a mature sperm is formed. The Leydig cells are responsible for the production of testosterone which is necessary for spermatogenesis. Some other hormones are also responsible for spermatogenesis. The Luteinising hormone and the follicle stimulating hormone have important influence on right spermatogenesis as well. The first one (FSH) stimulates the Leydig cells to make testosterone and maintain mitotic division. The second one (FSH) is obligated for the influence on Sertoli cells. Sertoli cells produce inhibin B. Spermatogenesis process takes about 3 weeks.

| Cell | Ploidy / number of chromosomes | Process |
|------------------------|--------------------------------|--------------------------------|
| Spermatogonium | Diploid / 46 | Spermatocytogenesis |
| Primary spermatocyte | Diploid / 46 | Spermatidogenesis (Meiosis I) |
| Secondary spermatocyte | Haploid / 23 | Spermatidogenesis (Meiosis II) |
| Spermatid | Haploid / 23 | Spermiogenesis |
| Mature sperm cell | Haploid / 23 | Spermiogenesis |

Table 1.



Picture 2. Spermatogenesis

3. Sperm chromatin structure

The sperm chromatin is very tightly compacted and the result is a paternal genome inactivation. Nuclear proteins and DNA are connected in a unique way. Nuclear remodelling and condensation in the spermatid combine with histone modification and displacement with transition proteins and then by protamines. However, around 15% of sperm DNA remains packaged by histones. The reason lies in the specific manner of oocyte activation after sperm entry. Disulfide cross-links between the cysteine-rich protamines are responsible for the compaction of chromatin and the stabilization of paternal genome. The genome is protected from oxidation or temperature elevation in the female reproductive tract. Human testis express two protamines: protamine 1 (P1) and protamine 2 (P2). Equal amounts of P1 and P2 are considered normal for human spermatozoa. Any unbalance of P1 and P2 ratio is associated with male infertility. Mouse knockout models demonstrate that the sperm protamine haploinsufficiency directly impairs spermatogenesis and embryo development. Through evolution protamines have increased the number of positively charged residues. These positively charged residues create highly condensed complex with DNA.

The protamine 1 is synthesized as a mature protein and protamine 2 as a precursor and protamine 1 and 2 differ from each other only by the N-terminal extension of 1-4 residues.

Protamine 2 is zinc-finger protein with one Cys2-His motif and they are expressed only in some mammals. Both, P1 and P2 undergo post-transcriptional modifications before binding to the DNA. After binding, protamines and DNA make highly compact nucleoprotamine complex. Khara et al., (1997) showed first comparison P1/P2 ratio related to IVF and found a P1/P2 ratio between 0.55 and 0.29 in the group with fertilization index (FI) $\geq 50\%$ and three of the infertile patients who had a FI below 50% had a ratio outside this range (Khara et al., 1997). Carrel and Liu (2001) describe the undetectable protamine 2 in infertile males.

4. DNA damage during spermatogenesis

The sperm DNA damage is clearly associated with male infertility. Small part of spermatozoa from fertile men also has detectable levels of DNA damage. Factors that cause the DNA damage include protamine deficiency, apoptosis, chemotherapy, ROS, cigarette smoking and varicoceles. The DNA fragmentation is characterized by single and double strand breaks. Oxidative stress is a result of the production of reactive oxidative species (ROS). Spermatozoa are vulnerable to ROS because they have a small amount of cytoplasm which does not contain antioxidant molecules and repair system. The DNA fragmentation is associated with diminished motility, morphology and sperm count. Also the DNA fragmentation has predictive value for unsuccessful IVF cycle. The most interesting group of patients which go to IVF is idiopathic infertility. Significant paternal contribution to sex chromosome trisomy has been described. Spermatozoa with numerical chromosome abnormalities are able to fertilize oocyte. The increase of sperm aneuploidy rate is associated with lower implantation and pregnancy rate. The sperm mitochondria represent the biggest source of reactive oxygen species. Oxidative stress induces activation of free radicals by the mitochondria which later induce oxidative DNA damage and DNA fragmentation. The majority of DNA damage is caused by oxidative stress.

5. Apoptosis

The apoptosis represents normal physiological process in spermatogenesis. About 75% potential spermatozoa are destructed by the programmed cell death. The apoptosis acts like selective factor of the early germ cells and prevents overproliferation of germ cells. Also the abnormal sperm formation is excluded from spermatogenesis. Sertoli cells can support a specified number of germ cells. Some spermatozoa with DNA damage or fragmentation escaped apoptosis and exist in the semen. Men with abnormal sperm parameters have higher levels of apoptotic protein Fas. Apoptotic protein Fas is strongly correlated with poor sperm concentration and abnormal sperm morphology. Also some other apoptotic markers can be found in human sperm such Bcl-x, p53, caspase and annexin V.

6. Oxidative stress

Reactive oxygen species (ROS) are the product of the normal metabolism in a cell. Free radicals are highly chemically reactive because of the unpaired electrons. Also ROS are produced by leukocytes in phagocytic process. ROS can effect on sperm DNA integrity. High levels of reactive oxygen species are detected in the semen of infertile men. Reactive oxygen species cause hypercondensation of DNA as a result of the oxidation of sperm protein sulfhydryl groups. The post-testicular genital infection results in the leukocytospermia and the increased levels of DNA damage. ROS can damage the DNA by causing deletions and mutations. Antioxidant therapy has shown a decreasing sperm DNA fragmentation.

7. Y chromosome microdeletions

Microdeletions in the Y chromosome genes are associated with impaired or absent spermatogenesis. Three regions of the Y chromosome azoospermic factor AZFa, AZFb and AZFc are crucial for an adequate process of spermatogenesis. Deletions in AZFa region are associated with Sertoli cells only, deletions in AZFb region with spermatogenic arrest and deletions in AZFc region are associated with the spermatogenic arrest at the spermatid stage. The frequency of Y chromosome microdeletions affects approximately 5-15% infertile men. Previous studies have shown that boys born from oligozoospermic men treated using ICSI have an increased risk of carrying Y chromosome deletions.

8. Centrosome

The centrosome consists of two centrioles in a perpendicular arrangement and pericentriolar material. After the fusion of sperm and oocyte, sperm tail is incorporated into ooplasm and centriolar region forms the sperm aster which acts to guide the female pronucleus towards the male pronucleus. The maternal centrosome is fully degraded, thus the centrosome of zygote is mainly inherited from the sperm. After fertilization normally formed centriole is an essential proper cell division. The centrosome disfunction may lead to the numerical chromosomal abnormalities. The human sperm centrosome is responsible for normal syngamy and an early embryonic development.

9. New techniques of sperm selection in ART

The routine sperm preparation techniques are density gradient centrifugation and swim-up. They depend on the sedimentation or migration ways to separate spermatozoa. The sperm

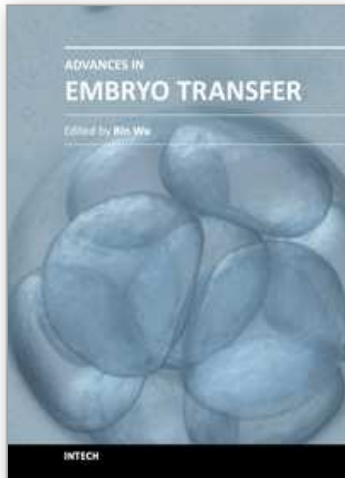
characteristics such as apoptosis, DNA integrity and membrane maturation are not directly targeted by routine sperm preparation techniques. At this moment magnet activated cell sorting (MACS) becomes novel technique for sperm separation based on presence of annexin V as apoptotic marker. Also modified ICSI called PICSi is commonly used for single sperm selection on the level of membrane maturity for sperm binding on hyaluron acid binding sites. Generally, sperm selection can be based on the sperm surface charge (electrophoresis-based technology), non-apoptotic sperm selection, selection based on the sperm membrane maturity and selection based on the sperm ultramorphology. Electrophoresis-based technology separates spermatozoa based on the size and electronegative charge. The externalization of phosphatidylserine (apoptotic marker) allows binding with Annexin-V-conjugated paramagnetic microbeads which separates apoptotic spermatozoa using a magnetic-activated cell sorting system (MACS, Miltenyi Biotec GmbH, Germany).

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Embryo transfer has become one of the prominent high businesses worldwide. This book updates and reviews some new developed theories and technologies in the human embryo transfer and mainly focus on discussing some encountered problems during embryo transfer, which gives some examples how to improve pregnancy rate by innovated techniques so that readers, especially embryologists and physicians for human IVF programs, may acquire some new and usable information as well as some key practice techniques. Major contents include the optimal stimulation scheme for ovaries, advance in insemination technology, improved embryo transfer technology and endometrial receptivity and embryo implantation mechanism. Thus, this book will greatly add new information for readers to improve human embryo transfer pregnancy rate.

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Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
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InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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