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Review Article

Recent Advances in the Human Male Gamete: Focus on Testicular Varicocele

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ABSTRACT

From the XVII century, sperm has been studied for its function in fertilization and its unique cell characteristics. Successively, the new innovative techniques rendered possible to clarify, at last in part, the organization and structure of the male gamete, however, it remains to clarify the composition at molecular level and the mechanisms through which it regulates its functions. Previous studies have been mainly focused on morphological and biochemical changes related to the processes of capacitation and acrosome reaction. It is interesting to note that the human sperm during his life goes through two stages of development: the first in the male genital tract, where it acquires the morpho-anatomical maturation; the second in the female genital tract, where it acquires the functional maturation during the capacitation process which prepare the gamete to the acrosome reaction. Our studies have shown the presence of different hormone/hormone receptor system inside sperm e.g.: Aromatase/ERs, 5α-reductase/ARs, Insulin/IR-B, Leptin/OB-R allowing the sperm to perform its functions. Interestingly, the presence of different types of mRNAs was observed. From others and ours studies it emerges that the sperm can regulate its function trough an autocrine short loop and that it possesses mRNAs needed to produce new proteins during capacitation or to give him the possibility to be accepted by oocyte to proceed to the syngamy and embryo development.

Testicular varicocele impairs male fertility in a variety of ways: on spermatogenesis, semen quality and functionality of the gametes. Recently, it was showed that the disease causes a damage in the male gamete at the molecular level including a steroid receptors reduction and this open a new chapter in the already multifaceted physiopathology of varicocele. These studies constrain the need of further research on gamete composition and on the diseases related the male genital apparatus, considering the high couple infertility linked to the male. The mini review focalizes on new intriguing advances on sperm cell biology. Furthermore, we emphasize the impact of varicocele on the male gamete performance.

Keywords: Spermatozoa; Testicular varicocele; Hormones; Steroid receptors; Male infertility; Reproduction

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INTRODUCTION

To date it is known that the morphologically mature male gamete presents unique features: It is a highly differentiated cells that display extreme polarization of cellular composition and function. For example, the sperm head has evolved to interact with the egg's extracellular matrix and to transport paternal genetic material, whereas the sperm flagellum acts to provide motility. We also know that sperm possesses little cytoplasm, and therefore have reduced ability to translocate substrates from one region to another. It is considered transcriptionally inactive, due to the DNA hypercondensation by protamine, and therefore cannot make new proteins in response to the changing needs. The human sperm during its life passes through two states of development: the first in the male genital tract, where it acquires the morpho-anatomical maturation; the second in the female genital tract, where it acquires the functional maturation during the capacitation process which prepares the gamete to the acrosome reaction. During recent years, new and intriguingly aspects emerge from this cell some of which need to be mentioned: The autocrine regulation of its functions by sperm-derived factors, presence of different types of mRNAs, sperm ability to translate through mitochondrial ribosome-type, both by mRNA carried from the early phases of spermatogenesis or by fresh RNA. From others and our studies, it emerges the importance to study the anatomy of human sperm at molecular level, since we discovered new site of expression of molecules which have been always confined into specialized cells, such as insulin, leptin, estrogens, possibly through a short autocrine loop [1-3] Further, the different types of mRNAs have been associated to sperm translation. Altogether, these studies open new chapters in the andrology studies since sperm apparently normal from a morphological point of view are not always able to fertilize an oocyte.

From this it may be disclosed that sperm can be considered a mobile endocrine unit independent from the hormonal systemic regulation [1-3]. This is likely due to the peculiar sperm characteristics, since at any given time is free from the supervision of the organism producer: the spermatozoon leaves the testis going to meet the unknown. It may move through the female genital tract in the host body of the opposite gender, altogether sperm needs to be autonomous. We have studied the autocrine regulation of various sperm activities including metabolism. Our studies, therefore, define a new endocrine and metabolic sphere for human sperm [1-3].

Some years ago, in sperm was observed, the presence of a population of mRNAs some of which are delivered into the oocyte together the paternal DNA. Particularly, it has been shown that the presence of some of these mRNAs is essential in the early stages of embryonic development, since when they are silenced, this does not proceed [4-7]. The mRNAs present in the sperm include thousands of distinct species [5]. Knowing the messages that the male gamete carry as mRNAs may lead to a redefinition of the paternal and maternal roles both in fertilization and embryonic development [7,8]. The oocyte recognizes the sperm as a potential harmful intruder. In our recent study we have shown that sperm can defend itself in physiological and pathological conditions, through a sperm fight/flight response, since it produce cortisol which is aimed at assaulting the immune system [9].

The transmission of parasites genetic paternal derivation (retrotransposons and endogenous retroviruses) is a mechanism that uses both PIWI-interacting RNA (piRNAs) and microRNA (miRNAs) in oocyte's plasma [10]. However, these interactions at the level of mRNAs can be associated with chromatin modification of histones that scan the paternal genome input to assess the genetic compatibility. The sperm that passes the recognition for the cytoplasmic and genetic compatibility can proceed to syngamy [7]. May be formed pronuclei regardless of genetic compatibility, but after the failure to pass, the checks will lead to a failure of the activation of the embryonic genome [7]. Until recently, it was thought that the sperm acts as a cargo only to transport the paternal genome to the oocyte. However, other components of the spermatozoon that are essential for normal syngamy in all investigated mammalian orders (except for some rodents) have come to light in recent years. These include an oocyte activating factor (a member of the phospholipase group), an essential component of the zygote's microtubule-organizing center (the spermatozoa centrosome) and mitochondria [11]

Over the past years, an increasing number of reports have appeared, describing the presence of various mRNAs in the male gametes of many species including gymnosperms and angiosperms [12,13]. These studies have reawakened interest in RNA carriage by spermatozoa because it is an anomalous component of this apparently 'quiescent' specialized cell. Indeed, nuclear gene expression in mature spermatozoa is progressively shutdown during spermiogenesis (the haploid phase of spermatogenesis) to allow substitution of histones by the highly charged and physically smaller protamines, facilitating further compaction of the haploid genome [14]. Although, sperm mRNAs have been a subject of debate for over 40 years, it is now generally accepted that spermatozoa carry a large population of mRNAs, antisense and miRNAs into the oocyte together the paternal DNA [4]. However, its functional significance, is still a matter of debate. This reflects the accepted description that spermatozoa have a compacted nucleus and are transcriptionally inactive. Although, spermatozoa do transmit mRNAs to the oocyte at fertilization as part of the multilayered paternal contribution that also provides essential organelles (centriole and mitochondria in humans and primates) as well as male-specific proteomic components (PLCz, PT32, STAT4, ETS)[15].

The localization of mRNAs in the sperm nuclei has been reported and its compartmentalization within the euchromatic and heterochromatic regions of the genome described [10]. The retention of mRNAs and its association with the nuclear matrix is particularly intriguing given the differential packaging of spermatozoal DNA. During spermiogenesis, most histones are progressively replaced by the smaller, basic protamines, compacting the nucleus to 1/13th that of the oocyte [14].

During this period, transcription ceases and spermatids rely only on stored mRNAs to support protein synthesis [16]. However, protamine repackaging is not complete in many species as it in human spermatozoa, retaining approximately 15 % of their

DNA as histone bound and then likely available for transcription [16].

Different outcomes were proposed for RNA populations, mRNA, antisense and microRNA, retained in mature sperm:

1. Antisense and microRNAs may epigenetically modify and modulate early embryonic gene expression.
2. Sperm mRNAs may also have a structural role. For example, nuclear RNA may target chromosome regions remaining histone bound.
3. mRNA located in the midpiece may be translated under certain conditions, for example, during capacitation.
4. The vast majority of DNA within the head is packaged by highly charged protamines [14]. However, a small proportion of the genome is packaged by histone-containing nucleosomes. It is proposed that these segments are marked with paternally derived mRNAs [7,17].

LITARATURE REVIEW

Translation ability

It was shown that the male gametes are capable of *de novo* translation by ribosomes like those mitochondrial during capacitation [18-22]. The mechanism by which the nuclear-encoded mRNA transcripts are translated by ribosomes mitochondrial presently is unclear. Two possible mechanisms have been hypothesized: The first provides that mRNA transcripts can be translated by CP-sensitive ribosomes (Chloramphenicol) that are located outside of the mitochondria but anchored to its outer surface; alternatively, the mRNA nuclear power could be imported into the mitochondria to be translated by CP-sensitive ribosomes within the mitochondria [21,22]. The import of tRNA and rRNA in the mitochondria has been described in several species, import of mRNAs in mammalian mitochondria is unprecedented [7]. One way to ensure the transport of a nuclear protein to another cellular organelle, is to transport the mRNAs that encodes the protein to the ribosome which is located into the correct subcellular compartment. The mitochondrial precursors of the proteins carry the targeting sequence of amino terminal in such a way as to ensure their passage in the mitochondria [18,21,22]. The mRNA transcripts encoding mitochondrial proteins do not meet the mitochondria to mere chance, they use a special mechanism for fast track of mRNA, which is the first step of the sorting of mitochondrial proteins [18]. The second hypothesis suggests that protein translation takes place within the mitochondria. Studies demonstrated that the RNA translation in mature sperm happens in the mitochondria and seek to further localize the site of mRNA translation [21-22]. In mammalian sperm nuclear mRNA is translated by ribosomes like mitochondria in the cytoplasm and mitochondria, later translated proteins are activated [21-22].

Imprinting

Mounting evidence established that antisense RNAs play a critical role in unchanging silenced chromatin domains. There is the possibility, that the recently discovered antisense spermatozoa mRNAs could provide an epigenetic mark that is necessary for establishing and/or maintaining paternal imprints [6,17].

Under certain conditions, some cytoplasmic mRNAs are apparently translated *de novo*, possibly in mitochondrial polysomes. When this translation is prevented, sperm maturational events associated with capacitation are not observed [21-22]. This would suggest that the production of proteins from spermatozoal mRNAs is necessary for the maintenance of male fertility. In addition to structural roles, it has been suggested that in common with oocyte mRNA stores, spermatozoa transcripts may be functionally important to the zygote to promote post-fertilization development. Recent evidence would also suggest that paternal RNAs can provide epigenetic marks to the developing embryo that influence the phenotype of the offspring. Embryonic development, pattern formation, embryogenesis, and tissue organization [6,7,17]. These terms all refer to the processes by which an embryo develops its specific spatial arrangement of cells and tissues. Combining the identification of spermatozoa mRNAs within the midpiece and the dependence of mRNAs distribution by the paternally derived centrosome, it may be speculated that paternal transcripts play a key role in spatial patterning of the developing zygote [7].

The oocyte recognizes the sperm as a potential harmful intruder and then it requires a careful control before sperm-derived factors can be accepted for the syngamy. From the oocyte's perspective, the incoming sperm poses a significant challenge. The sperm is a "foreign" body that may carry with its undesirable factors into the egg [7].

The activation of the embryonic genome requires interactions of complementary mRNAs from oocyte and sperm which scan the paternal genome to assess genetic compatibility. The sperm that passes the recognition for the cytoplasmic and genetic compatibility can proceed to syngamy. A pronuclei regardless the check in for genetic compatibility may be formed but this will lead to a failure of the embryonic genome activation. Many failures of fertilization or developmental failure (through both natural and assisted conception) could be attributed to the sperm being unable to pass the checks necessary for successful syngamy and embryonic genome activation [7].

de-novo translation of spermatozoa mRNAs has been convincingly demonstrated in human and bovine spermatozoa as visualized by autoradiography and fluorescence microscopy, by the incorporation of labeled amino acids into polypeptides during sperm capacitation [21,22]. Surprisingly, mitochondrial (D-Chloramphenicol (CP), tetracycline and gentamycin) but not cytoplasmic translation inhibitors (cycloheximide) prevented translation. Inhibition of protein translation significantly reduced sperm motility, capacitation, and in vitro fertilization rate. Thus, contrary to the accepted dogma, nuclear genes are expressed as proteins in sperm during their residence in the

female reproductive tract until fertilization. It was shown that the male gametes are capable of *de novo* translation by 55S ribosomes like those mitochondrial during capacitation [21,22].

The spermatozoon's mitochondrial transcription-translational machinery, however, is active; hence the work demonstrating translation *de novo* essentially confirms the conclusions drawn 50 years ago in this regard [21,22]. In general, the prevailing consensus is that although mRNA and protein synthesis does occur in mature spermatozoa, they are confined to the cells' mitochondria. What is entirely novel about the most recent work is the evidence that polynomial complexes containing mitochondrial ribosomes may support the translation of nuclear-encoded cytoplasmic mRNAs. The mechanism by which the nuclear-encoded mRNA transcripts are translated by ribosomes mitochondrial presently is unclear. It is known that 16S and 12S ribosomal RNAs are present in the nucleus of mouse and human spermatozoa [21,22].

With respect to the role(s) of spermatozoa RNA, it has been discussed the evidence. This suggests that it is not residual from spermatogenesis. Possible functions include *de-novo* translational replacement of degraded proteins (now demonstrated), structural (repackaging of chromatin), post-fertilization and epigenetic. Perhaps the most exciting development to date is the inference of naturally occurring spermatozoa RNA-mediated epigenetic effects on the zygote, but detecting these effects remains a challenge for future research [6,7]. Certainly, the renewed interest in the male gamete is both welcoming and timely.

Active spermatozoa

Although it is now widely accepted that under normal conditions, spermatozoa are transcriptionally silent, recent evidence indicates that the spermatozoa nucleus is itself more dynamic than was originally considered. In addition to the new evidence for active translation of stored mRNAs, the transcription of fresh RNA, was hypothesized because (i) spermatozoa contain RNA polymerase (ii) and abundant transcription factors are present [23]. It is likely that spermatozoa synthesize new proteins needed for capacitation to replace proteins that have degraded. Protein translation is essential for sperm functions that contribute to fertilization, such as motility, actin polymerization, and the acrosome reaction. Thus, the ability of spermatozoa to synthesize proteins, including nuclear-encoded proteins, by the 55 S ribosomal machinery is critical for the final maturation step leading to successful fertilization [21-22].

Steroids

Spermatogenesis is one of the most important targets of steroids and it was recently demonstrated their ability to influence the activities of the male gamete [1-3, 24]. Nevertheless, in the literature controversial data are reported, the main problem for andrologists is to accept the presence of transcription factors, such as the steroid receptors, in a cell that appears silent from a transcriptional point of view [25,26]. The most of studies have supported the hypothesis of membrane receptors for steroids by which they can induce rapid effects. It is now clearly knowing that the classical steroid receptors act also by inducing rapid effects [27]. Estrogen Receptors (ERs): the effects of estrogens are mediated by two distinct nuclear receptors, ERα and ERβ. The receptors are encoded by two different genes and are expressed in germ cells. Besides the genomic effect mediated by the two nuclear receptors, estrogens also because non-genomic effects mediated through membrane receptors. Estrogen stimulates the phosphorylation of proteins involved in the cascade of Phosphatidylinositol-3-OH Kinase (PK3)/Akt and stimulates ERK1/2, which are involved in sperm function. The presence of ERα has been reported only in the midpiece of sperm, ERβ exhibit wider distribution patterns along the sperm tail, extending through the midpiece and the principal piece of flagellum. The sperm head appears to be totally devoid of both these proteins [28]. The Transmission Electron Microscopy (TEM) with immunogold analysis, confirmed the abovementioned location of these steroids' receptors [29].

Androgen receptor: androgens are steroid hormones important for the onset and maintenance of spermatogenesis as well as the development of secondary sexual characteristics. They act by genomic and non-genomic effects, mediated by nuclear receptors [30].

By immunofluorescence and confocal microscopy, have been localized ARs in the midpiece and in the mitochondria of human ejaculated sperm [30]. Furthermore, the classical AR-B and AR-A isoforms were shown to be present in sperm by western blot and TEM mostly within the head and in the midpiece [30].

Progesterone receptor: progesterone, a female hormone, is also involved in male reproduction. The physiological responses of progesterone are mediated by two types of nuclear receptors, the PR-A and PR-B receptor. These two classic nuclear receptors are encoded by a single gene. Similarly, to the estrogens and androgens, progesterone also binds to the surface of the sperm through specific receptors located on the membrane. Recently, the expression of the conventional intracellular PRs has been demonstrated, and their functional role has been related to capacitation, p-Akt and p60c-src activities, acrosome reaction, lipid, and glucose metabolism. TEM with immunogold examination localized the PRs not only to membranous compartments but also in the entire sperm body as component of the nucleus, the midpiece and the flagellum [31].

Testicular varicocele

Although varicocele is the most common cause of secondary male infertility, pathogenesis and its effects are still topics of investigation. The etiology and physiopathology of varicocele remain unclear and the mechanisms by which varicocele causes testicular dysfunction and infertility are not completely known. The effects of varicocele occur at different levels [32,33](Figure1).

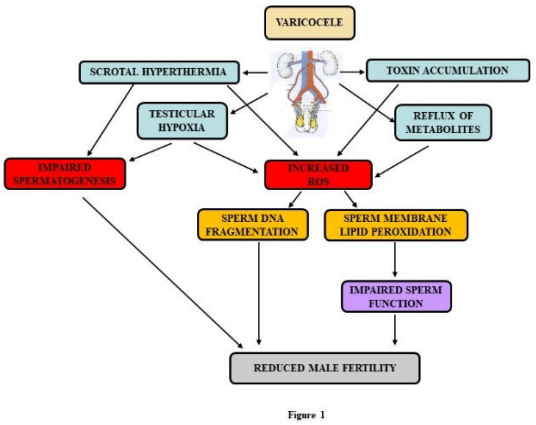

Figure1: Schematic representation of varicocele influence on male fertility.

Effect on spermatogenesis: Experimental data from clinical and animal models show that rising temperatures because of venous reflux, damage spermatogenesis [34]. In addition, the varicocele causes endocrine imbalance and hyperthermia in the testicles, oxidative stress, apoptosis and dysfunction of the epididymis [35].

Effect on seminal fluid: Many studies have tried to highlight the seminal indicators of the disease, but even here there are controversial data. In fact, the condition of the varicocele may be associated with several seminal paintings that veer from normozoospemia to moderate oligoastenoteratozoospermia or azoospermia [36].

Effect on gamete: The impact of the disease on the functionality of the gametes is still debated by scientists. Some studies have shown a significant association between varicocele and low quality of gametes; however, few studies have focused on the effect of the disease on the functions and structure of the human male gamete [32].

The varicocele induces an altered morphology with a prevalence of pointed heads, in fact, semen quality in varicocele patients is characterized by tapered sperm cells, furthermore, studied the effects of the disease by TEM showing a combination of generic characteristics as non-condensed nuclei, malformed acrosome, big waste cytoplasmic, not tightly assembled mitochondria, rolled axonemes [37]. Sperm ultra-morphology as a pathophysiological indicator suggested that varicocele may induce deleterious alterations in early spermatid head differentiation, causing sperm acrosome and nucleus malformations. In this context, our studies have shown that this pathology induces damage in the male gamete at the molecular level which includes a reduction of some steroid receptors, going beyond the morphological abnormalities described to date. Our research group has shown firstly the presence of classical receptors for steroids in human ejaculated male gamete at both the protein and mRNA [1-3]. Their function was related to motility, survival, capacitation, and acrosome reaction. To evaluate the possible involvement of ERs, PR-A / PR-B and ARA / ARB steroid receptors in the pathophysiology of varicocele, we investigated their expression and ultrastructural localization in human sperm from normozoospermic and oligoastenoteratozoospermic patients with or without varicocele. Interesting to note, in samples from patients with varicocele, the expression of steroid receptors is reduced, suggesting a role in the physiopathology of testicular varicocele. Importantly, as a consequence of the low steroid's receptors expression in varicocele, sperm exhibit a reduced responsiveness to the steroids [38]. Given the important role of steroids on sperm physiology, the missing receptors may cause impaired sperm activity, since in the male genital tract, sperm life is prevalently under the control of the androgens, while in the female genital tract the main hormones are the estrogens and progesterone.

The detrimental effects of varicocele and sperm metabolism: In the human male gamete, given its polarization, the proteins localization may be indicative of their own function. Given the common location of the steroid's receptors at mitochondrial level, we hypothesized their involvement in energy metabolism, aspect of the gamete biology that was less studied and therefore unclear. Therefore, we treated our samples with physiological activators for each steroid receptor. To highlight the involvement of each receptor in mediating the studied effects, we have made co-treatments with specific inhibitors, ICI for ERs, RU for PRs and Casodex, a specific AR antagonist [32,33,38]. As above mentioned, the sperm, during his lifetime, passes through two physiological stages: A steady state, uncapacitated, during which the gamete economize and / or stores energy, and a state of functional maturation, during which the gamete becomes precisely capacitated with considerable energy expenditure. Generally, it might be briefed that to the uncapacitated gamete is associable an anabolic metabolism, while in the capacitated state to catabolic one. Our previous studies have shown that steroids also induce capacitation, in new studies we observed that, in the gametes from normozoospermic patients, they activate both the carbohydrate and lipidic metabolism. In varicocele, where a reduced or absent response to steroids was observed in each metabolic assay considered, an accumulation of glycogen and triglycerides was obtained. This metabolic condition is like that observed in uncapacitated gametes. Therefore, it may be deduced that the gamete from patient with varicocele have difficulties to switch into capacitation [39, 40].

CONCLUSION AND PERSPECTIVE

Although, exciting progress has been made over the last years, sperm molecular set up and the regulation of its functions are at the beginning of the knowledge. It will be very hard to fully elucidate the almost unknown molecular sperm composition, however, we think that new studies are needed, since not all the apparently normal spermatozoa are able to fertilize. Furthermore, the functional roles of the great mRNA populations need to be deepened, since it was shown that the presence of some of these mRNAs is essential in the early stages of embryo development.

A recent report showing that an epigenetic alteration mediated by mRNAs to the phenotype, is sperm-derived, supporting the view that the RNA delivered at fertilization can act as an epigenetic modifier of early embryo development.

In conclusion, the paternal contribution to the fertilization process was reassessed since, till now, it was thought that the sperm acts as a cargo only to transport the paternal genome to the oocyte. However, other components of the sperm are essential for normal syngamy in all the investigated mammalian orders (with exception of some rodents). These include an oocyte activating factor (a member of the phospholipase group).

Far from being the quiescent cell we have also seen that spermatozoa are capable to regulate its own functions independently by the systemic regulation and of surprising intra-nuclear dynamics that includes novel functions for mRNAs. Knowing the messages that the male gamete carry as mRNAs could lead to a redefinition of the paternal and maternal roles both in fertilization and embryo development. Metabolomics and proteome of human spermatozoa were done, however currently the exact role of many proteins in male infertility is unknown. Altogether, we hypothesize that new intriguing discoveries await to be known about the biology of this cell.

DECLARATIONS

Data Availability

Not applicable because the study only presents a review of existing data. No new data were generated.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest.

AUTHOR'S ROLE

A.S. and V.A. wrote the original draft and edited the manuscript. A.S. and R.V. organized the design and supervised the referenced article. U.L helped to organize, edited and revised the review. P.G. and S. M. contributed to revised the methodology, conceptualization, and the manuscript: S.M. and A.H helped to revised the review. All Authors have read and agreed to the published version of the manuscript.

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