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# How Relevant are Male Factors for Fertilization and Early Embryo Development? Looking into the (Epi)genome, Proteome and Metabolome

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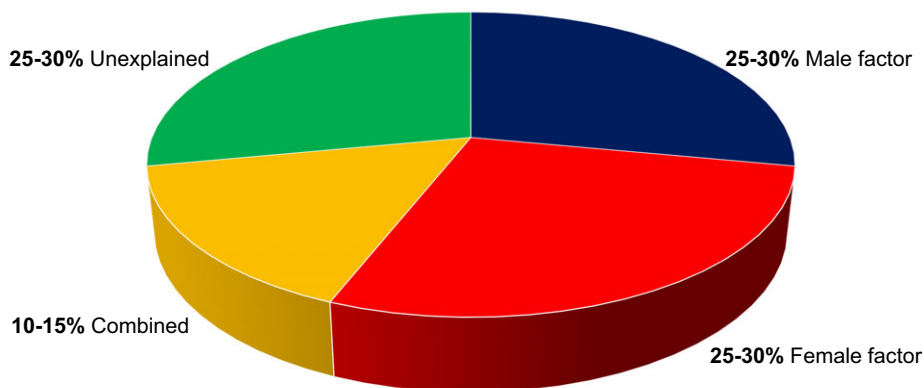
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Infertility affects 10–15% of couples at the age of conception. Mounting evidence supports that not only are paternal factors crucial during fertilization, but also for embryogenesis. This review aims to provide some clues about the contribution of male factors to reproductive success and live birth, as such contributions can be as important as that of the female. Semen is composed of two fractions: sperm and seminal plasma. Regarding the former, the integrity of sperm components (i.e., centrioles, DNA integrity and methylation, histone-to-protamine ration, specific proteins, etc.) has been proven to be essential for some of the events occurring upon engulfment of the spermatozoon into the oocyte cytoplasm. The metabolic status of sperm also seems to shape their potential fertilizing capacity. Furthermore, seminal plasma appears to modulate the female reproductive tract, and has been suggested to support embryo implantation. In spite of the aforementioned, it remains largely unaddressed how paternal factors interact with maternal ones, and whether the latter may mask the former. While assisted reproductive techniques (ART) are useful to rescue infertility, a better understanding about the contribution of semen to fertilization, embryo development and implantation can increase the efficiency of these techniques, and address further the causes of total fertilization failure, implantation deficiency and recurrent miscarriage.

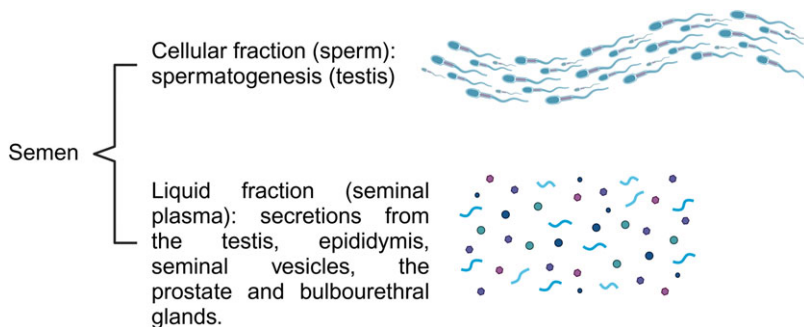


**Figure 1. Causes of human infertility.** About 25–30% of infertility cases are exclusively due to male factors, and a similar figure of cases is related to female factors (25–30%). In addition, about 10–15% of infertility cases are due to a combination of male and female factors, and between 25% and 30% of cases are unexplained. Percentages are given in ranges, as there are some differences between clinical studies.

### Introduction

The incidence of infertility, which is defined as the inability of a couple at reproductive age of conceiving after one year of unprotected intercourse, is around 10–15% worldwide (Simon *et al.* 2017a). Primary infertility can be distinguished from secondary infertility depending on whether the couple has never been able to conceive (primary), or was able to conceive but is no longer capable to do so (Zegers-Hochschild *et al.* 2008). The causes of each type of infertility differ. In the case of male infertility, the contribution of male factors can be as important as that of the female. Indeed, while single male factors account for 30–35% of cases, as too do single female factors, there are still about 30–40% of cases that could arise from both male and female sides, and about of 20% of unexplained/idiopathic infertility that could also involve the participation of male factors (Figure 1) (Yeste *et al.* 2016).

In order to understand the contribution of semen, one needs to dissect each of its fractions and components, as their integrity may have an influence. In most cases, diagnosis of male infertility is based on the evaluation of spermogram variables (ejaculate volume, pH, concentration, motility, morphology) which, despite being very useful, does not provide a complete picture of what can be wrong with male gamete components (Colaco and Sakkas 2018; Bashiri *et al.* 2021; Tarozzi *et al.* 2021). In effect, great attention to motility and concentration has been paid, as sperm have been regarded as a means of bringing the haploid genome of the father to the female's oocyte. The shortcoming of this approach is that it neglects that other paternal factors can also play a crucial role during fertilization and, even, at the start of embryogenesis, as emerging research supports (reviewed in Yeste *et al.* 2016; Vallet-Buisan *et al.* 2023).



**Figure 2. Semen composition.** The semen is composed of cellular (sperm) and liquid (seminal plasma, also known as seminal fluid) fractions. Sperm are produced in the testis through spermatogenesis and then enter the efferent duct and epididymis, where they mature. The seminal plasma (SP) comprises secretions from the testis, epididymis, seminal vesicles, the prostate and bulbourethral glands.

While great advances in rescuing male infertility through assisted reproductive techniques have been made over the last 40 years, allowing for the birth of more than eight million babies until 2018, according to figures released by the European Society of Human Reproduction and Embryology (Niederberger *et al.* 2018; ESHRE 2018), a full comprehension about which paternal factors can underlie fertilization failure, impaired embryo development and miscarriage has not been achieved. The aim of this review is to discuss the paternal factors that can potentially contribute to the events taking place during fertilization and beyond (including their influence on embryo development and implantation, and on offspring health), and how this may help improve diagnosis and treatment of male infertility. For this purpose, the following sections approach sperm and seminal plasma separately.

### Composition of Semen: Sperm and Seminal Plasma

The contribution of paternal factors, which, as aforementioned, is at least 30%, comes from the semen. This differs from the case of the female, where the respective contribution of 30–35% does not only involve ovarian dysfunction and advancing maternal age, which have repercussions on oocyte quality and competence, but also problems derived from uterine, pelvic and Fallopian tube alterations (including endometriosis), and other factors such as psychosexual ones (Child 2013).

The semen is composed of a cellular fraction (sperm) and a liquid one (seminal plasma, also known as seminal fluid) (Figure 2). Regarding the former, three parts can be distinguished in a spermatozoon: head (composed of nucleus and acrosome), neck (or connecting piece), and tail (where mitochondrial, principal and end pieces are distinguished). Remarkably, the spermatozoon is a very peculiar, complex and differentiated cell, as it is the only one that performs its mission (i.e., bring the father haploid genome to the oocyte) in another body (the female's). It is formed through

spermatogenesis, and then completes its maturation during the transit along the epididymis. This transit is featured by the remodelling of the plasma membrane, nuclear compaction, and incorporation of proteins, including cytosine-rich secretory protein 1 (CRISP1) (Cohen *et al.* 2011; Weigel Muñoz *et al.* 2018), acrosin binding protein (ACRBP) (Nagdas *et al.* 2010; Yin *et al.* 2018), and disintegrins/metalloproteinases (ADAM) (Inoue *et al.* 2005; Nishimura *et al.* 2007), through extracellular vesicles (James *et al.* 2020; Barrachina *et al.* 2022). In addition, motile ability is acquired in the epididymis cauda (Tourzani *et al.* 2021). As discussed later, epididymosomes also contain non-coding RNAs, and could be involved in passing the paternal epigenetic inheritance to the offspring (Zhang *et al.* 2018; Chan *et al.* 2020).

In humans, sperm are deposited in the vagina, and then enter the cervical canal, where they must pass through the cervical mucus. Active and passive (myometrium) contractions let sperm travel along the uterus. In the uterus, sperm trigger NETosis, which is a process through which polymorphonuclear granulocytes, a type of white blood cells, release their DNA into the extracellular environment to generate neutrophil extracellular traps (NETs), which can capture and kill bacteria and sperm (Zambrano *et al.* 2016). NETosis appears to have a selective mission, removing the excess of sperm, particularly those that have less motility and potentially less fertility, as studies in animal models indicate (Alghamdi and Foster 2005; Alghamdi *et al.* 2009; Zambrano *et al.* 2021). Those sperm that escape from leukocytes reach the utero-tubal junction (UTJ) (Ishimoto and Gaffney 2016). This junction has a selection role, as it presents different folds that allow for the retention of poor-quality sperm. Sperm cells capable of overcoming the UTJ then enter the fallopian tube, where, based on observations performed in animal models and in vitro studies in humans (Pacey *et al.* 1995; Ziskind *et al.* 2000; Massa *et al.* 2019), they appear to attach to the epithelial cells of the isthmus, one of the fallopian tube's sections, and form a 'reservoir' (Sharif *et al.* 2022). Only sperm in good shape can bind these epithelial cells; hence, this reservoir has been posited to exert a selective function (Rath *et al.* 2016), and would drive the transient storage of sperm, maintaining their survival until the oocyte is available (Teijeiro and Marini 2012). Moreover, an in vitro study conducted in humans reported that binding of sperm to OE-E6/E7, an immortalized human cell line of fallopian tube cells, triggers a transcriptomic response in these epithelial cells. This results in a downregulation of inflammatory molecules (cytokines and chemokines) that ultimately allow for a greater immunological tolerance with regard to sperm (Mousavi *et al.* 2021). When, in contrast, sperm incubated with the OE-E6/E7 cell line have high levels of DNA fragmentation (evaluated with the TUNEL assay), they rather activate a toll-like receptor signalling pathway in these cells, upregulating the expression of inflammatory cytokines and chemokines (Mohammadi *et al.* 2022).

Around ovulation, sperm detach from the epithelial cells of the isthmus and capacitate. Only capacitated sperm are able to trigger the acrosome reaction, which is required for the male gamete to pass through the cumulus cells and the zona pellucida (Molina *et al.* 2018; Gupta 2021). Some defective sperm cells are unable to

undergo capacitation and acrosome reaction, and/or penetrate the oocyte, whereas others – even when they fuse with the oocyte – are not able to activate the oocyte and drive embryo development (Colombero *et al.* 1999). For example, microinjection of parts of the sperm (such as the head or the tail) gives rise to an embryo that is not able to withstand several mitotic divisions (Moomjy *et al.* 1999).

The following sections scrutinize what we know about sperm components that are potentially involved in the interaction with the oocyte (i.e., gamete fusion), and what happens thereafter (oocyte activation and onset of embryogenesis). This includes: sperm proteome, centrioles, chromatin condensation and DNA integrity, epigenome and metabolome. The seminal plasma is approached in a separate section.

### **The Sperm Proteome**

The sperm proteome differs between fertile and infertile men (Légaré *et al.* 2014). Castillo *et al.* (2018) conducted a meta-analysis, identifying 103 sperm proteins that could be involved in fertilization and 93 that could play a role during early embryo development. This section is split into two different subsections, referring to sperm proteins that could play a role (i) during, and (ii) after fertilization.

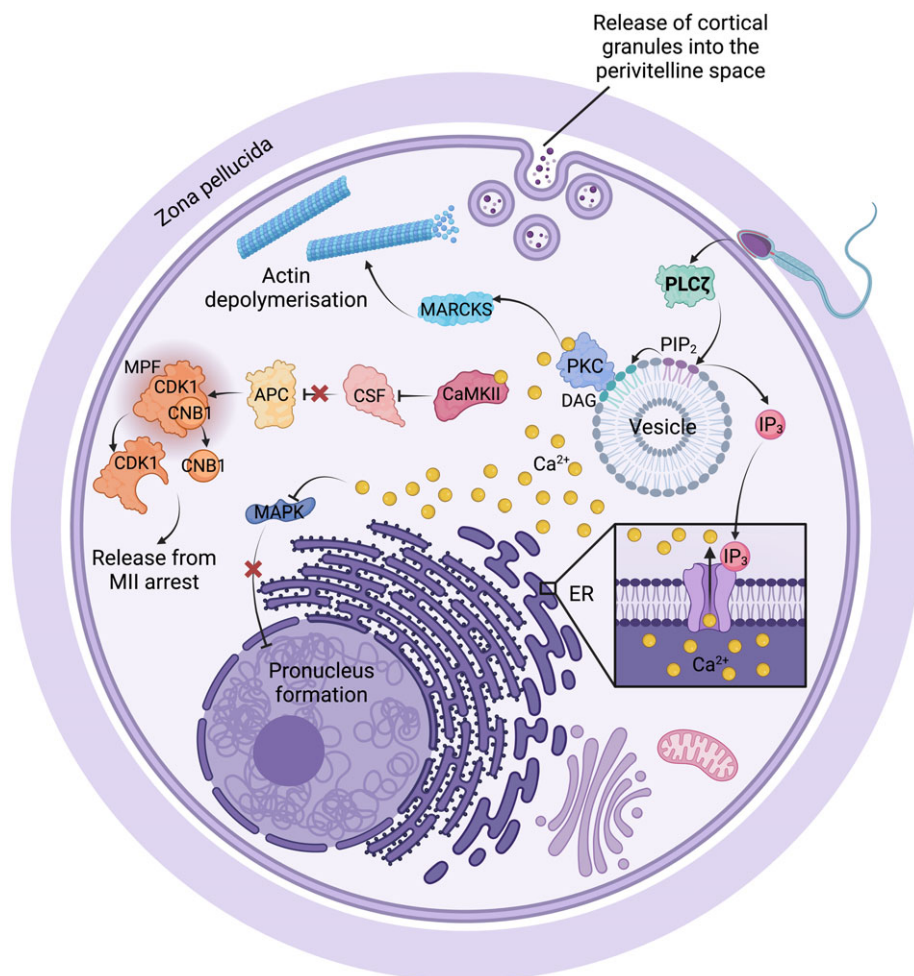
#### ***Sperm Proteins Involved in Fertilization***

The fusion of sperm and oocyte membranes is a complex process that appears to involve many proteins; some of the protein–protein interactions have been established in the last decade. From the sperm side, the proteins that interact with their oolemma counterparts are located in the equatorial region. One of these proteins is IZUMO1 (Inoue *et al.* 2005; Hirohashi and Yanagimachi 2018), which interacts with JUNO, an oocyte membrane protein; this interaction is needed for gamete fusion and the subsequent engulfment of the spermatozoon into the oocyte (Bianchi *et al.* 2014; Jean *et al.* 2019).

In addition to IZUMO1, other sperm proteins have been suggested to mediate gamete fusion. For example, tetraspanins CD9, CD81 and CD151 are located in the equatorial region of sperm, and have been found to be involved in the fusion of oocyte and sperm membranes (Jankovicova *et al.* 2020). Another protein found in the sperm plasmalemma, TMEM95, appears to play a similar role (Lamas-Toranzo *et al.* 2020).

#### ***Sperm Proteins Involved after Fertilization***

Whether the male proteome can contribute to early embryo development has been a subject of research, despite the technical difficulties to address the matter. From the 93 sperm proteins potentially involved in post-fertilization events, 11 have been suggested to have a mission during the first embryo divisions (up to eight-cell stage), 29 in the formation of morulae, and 19 in blastocyst development.



**Figure 3. Protein phospholipase C zeta (PLC $\zeta$ ).** Sperm-specific protein PLC $\zeta$  is involved in oocyte activation. Upon gamete fusion, it is released into the ooplasm, where it triggers the signalling pathway that alleviates the oocyte from the metaphase-II arrest. Abbreviations: APC, Anaphase-promoting complex/cyclo-some; CaM/CaMKII, Calmodulin/Calmodulin-dependent protein kinase II; CSF, Cytostatic factor; CNB1, Cyclin B1; CDK1, Cyclin-dependent kinase 1; DAG, Diacylglycerol; IP<sub>3</sub>, Inositol 1,4,5-trisphosphate; IP<sub>3</sub>R, IP<sub>3</sub> receptor; MAPK, Mitogen-activated protein kinase; PIP<sub>2</sub>, Phosphatidylinositol 4,5-bisphosphate; PKC, Protein kinase C. Reproduced from Yeste et al. (2023) with permission.

A sperm-borne protein that has been largely confirmed to be relevant for what occurs after gamete fusion is phospholipase C Zeta (PLC $\zeta$ ), a sperm-specific phospholipase C located in the equatorial and post-acrosomal regions (Figure 3) (Yeste *et al.* 2023). Mounting evidence supports that this protein is involved in oocyte activation, which is the process that releases the oocyte from its metaphase-II arrest, then allows for the onset of embryogenesis, and is featured by Ca<sup>2+</sup> oscillations in the



oocyte cytoplasm (Saunders *et al.* 2002; Horner and Wolfner 2008; Yeste *et al.* 2016, 2023). Sperm devoid of PLC $\zeta$  are unable to trigger Ca<sup>2+</sup> oscillations, which leads to oocyte activation deficiency and lower fertilization rates (Heytens *et al.* 2009; Heindryckx *et al.* 2013; Kashir *et al.* 2011, 2013; Yelumalai *et al.* 2015). Mutations in the coding sequence of this gene result in an impaired activity of this protein and total fertilization failure (Escoffier *et al.* 2016). A study conducted by Hachem *et al.* (2017) used a *Plcz1* knock-out male mouse model and confirmed the crucial role of this protein for oocyte activation, although the sperm from *Plcz1* knock-out males were still able to initiate embryogenesis after oocyte fertilization. In addition, total fertilization failure has been found to occur when sperm present the adequate levels of PLC $\zeta$ . All these data suggest that sperm proteins other than PLC $\zeta$  could be involved in oocyte activation. For this reason, other sperm proteins, such as post-acrosomal sheath WW domain-binding protein (WBP2NL), have been suggested to play a role during oocyte activation (Wu *et al.* 2007; Aarabi *et al.* 2010, 2014; Kennedy *et al.* 2014; Kaya *et al.* 2022). Yet, the exact function of WBP2NL has not been ascertained, as other studies did not observe the same results (Nomikos *et al.* 2014, 2015; Freour *et al.* 2017).

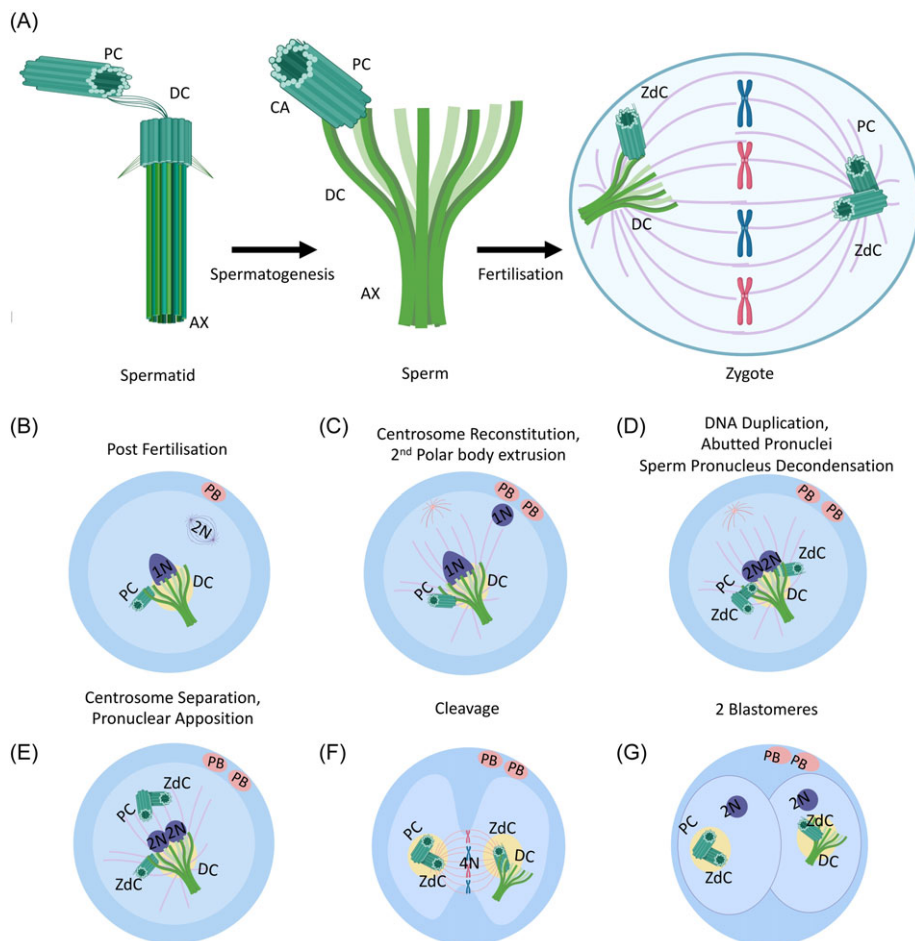
Other proteins that could play a role after fertilization are desmocollin 3 (DSC3), which is delivered via sperm to the zygote (Den *et al.* 2006) and has been proposed to regulate cell adhesion in blastomeres before embryo genome activation (EGA) (Castillo *et al.* 2018); lactosylceramide 1,3-N-acetyl-beta-D-glucosaminyltransferase (B3GNT5), which would be implied in morula formation (Biellmann *et al.* 2008); and choline-phosphate cytidyltransferase A (PCYT1A), which could be implicated in blastocyst development and embryo implantation (Wang *et al.* 2005).

### The Centrioles

Centrioles are crucial organelles in eukaryotic cells, and are involved in the organization of the cytoskeleton, cell division, and formation of flagellum. Mature sperm have two centrioles located in the neck: the proximal (PC) and the distal centriole (DC) (Figure 4) (Khanal *et al.* 2021; Leung *et al.* 2021). In most eutherian mammals except rodents, paternal centrioles are inherited by the fertilized oocyte, which does not have centrioles. For this reason, paternal centrioles organize the cytoskeleton of the zygote, mediate pronuclei migration (Scheffler *et al.* 2021) and drive cell cleavage (Rawe *et al.* 2002; Amargant *et al.* 2021; Avidor-Reiss *et al.* 2019; 2022). Sperm from infertile men have been identified to have abnormal centrioles (Turner *et al.* 2021), and the fact that paternal centrioles in humans have a crucial function after fertilization has been suggested to be one of the reasons for the greater proportion of aneuploid embryos in humans compared with rodents (Cannarella *et al.* 2020).

### The Relevance of Nuclear Integrity: Chromatin and DNA

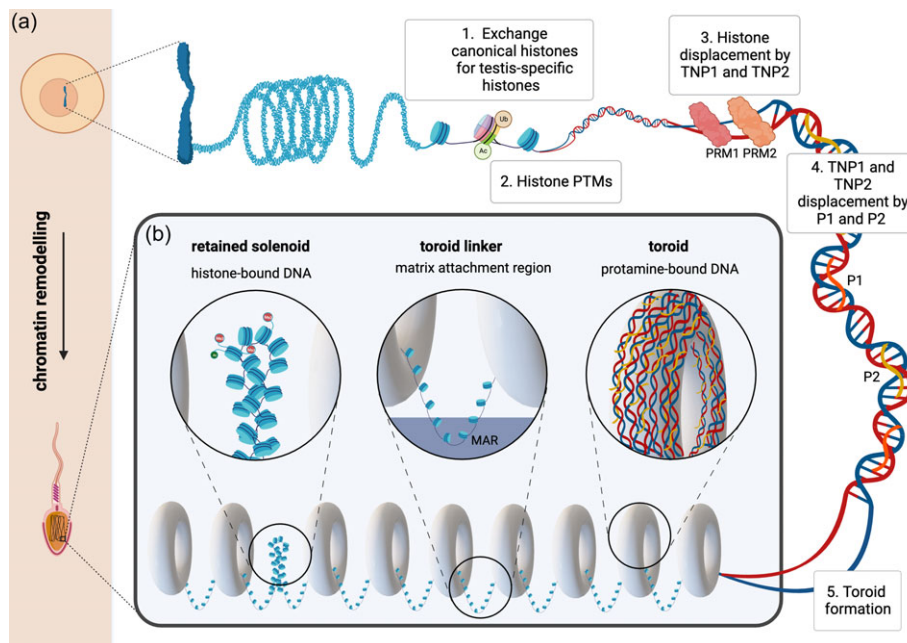
In contrast to prokaryotic cells, DNA is bound to proteins in the eukaryotic ones forming a structure called chromatin, which, during interphase, is isolated from the



**Figure 4. Sperm centrioles.** Centrioles are crucial organelles in eukaryotic cells, which are involved in the organization of the cytoskeleton, cell division, and flagellum formation. Mature sperm have two centrioles located in the connecting piece: the proximal (PC) and the distal centriole (DC) (A). After gamete fusion (B), the sperm centrosome forms an aster while the oocyte completes meiosis II and the second polar body is extruded (C). Thereafter, centrioles begin to duplicate to produce two daughter centrioles (D), which is followed by the separation of the zygote centrosomes (E). Subsequently, the zygote undergoes mitosis (F), leading to the formation of two blastomeres. Abbreviations: Ax, Axoneme; Ca, Centriole adjunct; DC, Distal centriole; PC, Proximal centriole; PCL, Proximal centriole-like structure; ZdC, Zygotic daughter centriole; PB, Polar body; N, Ploidy. Reproduced from Vallet-Buisan *et al.* (2023) with permission.

rest of the cytoplasm thanks to the nuclear envelope. While chromatin is formed by DNA and histones (which may bear epigenetic signatures) in somatic cells (Bartosovic *et al.* 2021), mature sperm get most of these histones (85–90%) replaced by protamines (protamine 1 and protamine 2, in the case of humans) (Figure 5)





**Figure 5. (a) Chromatin remodelling during spermiogenesis.** Some histones are replaced with testis-specific histone variants. Histones then undergo post-translational modifications. Following this, histones are replaced by transition nuclear proteins, and these by protamines, which ultimately increases chromatin packaging. **(b) Organization of chromatin in sperm.** In sperm, chromatin is organized into three components: the most abundant, which consists of DNA bound to protamines; histone-bound DNA; and nuclear matrix attachment regions (MAR). Protamine-bound DNA is coiled into toroids, and histone-bound DNA is present in retained solenoids and toroid linkers. Abbreviations: MAR, Matrix attachment region; PTM, post-translational modification; P1, protamine 1; P2, protamine 2; TNP, transition nuclear protein. Reproduced from Balder *et al.* (2024) with permission.

(Brykczynska *et al.* 2010; Jung *et al.* 2017). This occurs during spermiogenesis, when the canonical organization of chromatin with nucleosomes changes to a toroidal one around protamines (Barral *et al.* 2017). The different toroids, each containing about 200 kb of DNA, are linked with toroid-linker regions, which contain histones and mediate the attachment to the proteinaceous nuclear matrix (Narwade *et al.* 2019). Unlike histones, protamines contain arginine to allow for stronger binding to DNA in order to form a toroid-like ridged structure. As sperm do not have all the machinery required for repairing DNA damage, the greater degree of condensation provided by protamines has been suggested as a strategy to maintain DNA integrity better (Ni *et al.* 2016). Remarkably, the 10–15% of histones retained in the sperm chromatin may bear epigenetic marks, such as H3K23me, and are organized in nucleosomes (Champroux *et al.* 2018). The potential contribution of these epigenetic signatures brought into the zygote by sperm is discussed later (see the section about the sperm epigenome).

### ***Chromatin Protamination and Condensation***

An insufficient replacement of histones by protamines is associated with chromatin immaturity, and may lead to an impaired embryo development ( Ribas-Maynou *et al.* 2023); the relevance of this sperm protamination may be masked by oocyte factors. Specifically, when the histone-to-protamine ratio is lower than 6% or greater than 26%, embryo development is impaired (Fournier *et al.* 2018). On the other hand, and as aforementioned, chromatin in human sperm contains two protamines (P1 and P2), which should be in a 1:1 ratio (Sarasa *et al.* 2020). Alterations in this ratio are related to male infertility and poor embryo quality (Rogenhofer *et al.* 2017; Amor *et al.* 2019).

On the other hand, chromatin condensation can be evaluated through the integrity of disulphide bridges between protamines, and has been found to be a consequence of deficient protamination. A lower degree of chromatin condensation is linked to male infertility (Ribas-Maynou *et al.* 2023).

### ***DNA Integrity***

Damage in sperm DNA occurs in one (single) or two (double) DNA strands and underlies male infertility and impaired embryo, and may negatively affect offspring health (Fernández-González *et al.* 2008; Sedó *et al.* 2017; Borges *et al.* 2019; Ribas-Maynou *et al.* 2021b; 2022a). Sperm DNA damage can arise from different factors including lifestyle habits, such as nutrition (Jurewicz *et al.* 2018) and smoking (Cui *et al.* 2016; Muñoz *et al.* 2024); diseases, such as diabetes (Condorelli *et al.* 2018), obesity (Fullston *et al.* 2015), cancer (Meseguer *et al.* 2008), and male genital tract infections (Han *et al.* 2021); advancing male age (Evenson *et al.* 2020; Vaughan *et al.* 2020; Guo *et al.* 2023); altered histone-to-protamine ratio (Yoshida *et al.* 2018); insufficient chromatin condensation; abortive apoptotic-like changes (Shukla *et al.* 2012), and oxidative stress (Dorostghoal *et al.* 2017). The detrimental impact of sperm DNA damage on the embryo can be more apparent after embryo genome activation – which includes the expression of paternal-inherited genes – occurring at the 4/8-cell stage (Wong *et al.* 2010).

As aforementioned, sperm do not have all the machinery required for repairing DNA breaks. While the higher degree of chromatin condensation is intended to prevent it, DNA fragmentation may still occur (Smith *et al.* 2013). Although, in this scenario, the oocyte plays a crucial role in restoring the integrity of paternal DNA (Shimura *et al.* 2002; Lord and Aitken 2015), its repair capacity is limited, so that it may not be able to fix all paternal DNA damage. Thus, and as clinical data support, alterations in paternal DNA may be passed on to the embryo, which increases the mutational load, and may lead to embryo development arrest, failure to implant, miscarriage, and reduced clinical pregnancy and birth rate (Avendaño *et al.* 2010; Robinson *et al.* 2012; Lane *et al.* 2014; Ohno *et al.* 2014; Zhao *et al.* 2014; Simon *et al.* 2017b; Haddock *et al.* 2021; Ribas-Maynou *et al.* 2021b, 2022a). It is worth noting that the effects of sperm DNA fragmentation on embryo development and

other reproductive outcomes have been found to not completely agree across studies, as a meta-analysis compiling data from 25,000 IVF and ICSI cycles indicated (Ribas-Maynou *et al.* 2021b). These disparities could be explained by the different tests – the most used being sperm chromatin structure assay, sperm chromatin dispersion test, single-cell gel electrophoresis, also known as Comet, and TUNEL – employed to evaluate sperm DNA damage (Vallet-Buisan *et al.* 2023). The ART utilized also matters, as a negative correlation between sperm DNA fragmentation and reproductive outcomes (blastocyst development and pregnancy rates) was observed in the case of IVF but not in that of ICSI (Ruvolo *et al.* 2013; Cankut *et al.* 2019; Ribas-Maynou *et al.* 2021b). All this evidence supports the need for testing sperm DNA integrity when total fertilization failure and/or impaired embryo development occur, in order to discard male factor infertility.

### ***Other Influences from Altered Paternal Genome***

Alteration in paternal DNA can also lead to aneuploid embryos, although in most cases aneuploidy results from problems in the first meiotic division of the oocyte (Oldereid *et al.* 2018, Wang *et al.* 2020). Aneuploidies in sperm result from aberrant spermatogenesis, which manifests in poor morphology (e.g., macrocephalic sperm, teratozoospermia, oligoastheno-teratozoospermia) (Mehdi *et al.* 2012; Braham *et al.* 2019, Nayel *et al.* 2021; Saei *et al.* 2021).

### **The Sperm Transcriptome**

Although mammalian sperm are widely regarded as transcriptionally silent cells and they have about 600 times less mRNA than somatic cells (Zhao *et al.* 2006; Ren *et al.* 2017), their nuclei exhibit residual DNA and RNA polymerase activity (Bianchi *et al.* 2018). Whether sperm transcripts could play a function is, however, not clear. Many have suggested that sperm mRNAs could be delivered to the oocyte, where they could be involved in the onset of embryogenesis, perhaps before embryo genome is activated (Ko *et al.* 2000; Hayashi *et al.* 2003; Ostermeier *et al.* 2004; Jodar *et al.* 2015; Ntostis *et al.* 2017). Different sperm-borne transcripts, such as *PSGI* and *HLA-E*, have been hypothesized to be involved in embryo implantation (Avendaño *et al.* 2008), perhaps because they play an immunotolerance function, with the sperm levels of *PSGI* and *HLA-E* mRNA being higher in fertile than in infertile men (Avendaño *et al.* 2009). Other transcripts, such as *SSFA2* and *SESNI*, have been suggested to be involved in the development of pig embryos until the 4-cell stage, when EGA occurs (Guo *et al.* 2017). Embryo development is also related to the levels of protamine-1 (*PRM1*), protamine-2 (*PRM2*), POU domain class 5 transcription factor 1 (*POU5F1*, also known as *OCT4*), glutathione peroxidase 4 (*GPX4*), and heat shock protein 90 (*HSP90AA1*) transcripts in sperm (Cho *et al.* 2003; Meseguer *et al.* 2006; Hwang *et al.* 2013; Rogenhofer *et al.* 2017; Sadakierska-Chudy *et al.* 2020). As most of these studies assume that sperm transcripts play a role during embryogenesis

because they are present in the embryo, further research to ascertain their specific function is needed. It also remains unaddressed when and how they are degraded, as in the mouse this happens as early as the zygote stage (Hayashi *et al.* 2003).

### **The Sperm Epigenome**

Epigenetics involve a series of regulatory mechanisms that modify gene expression without altering the DNA. In sperm, the epigenome includes DNA methylation, histone modifications and non-coding RNAs (Figure 6); all these elements can also contribute to embryo development (Smith *et al.* 2014).

#### ***DNA Methylation***

Methylation of DNA is one of the epigenetic modifications that modulates gene expression. Methylation and demethylation are mediated through DNA-methyltransferases (DNMTs) and TET enzymes (Kaneda *et al.* 2004; Yagi *et al.* 2020). Sperm DNA methylation is established during spermatogenesis, and active demethylation of paternal DNA by dioxygenase ten-eleven translocation 3 (TET3) enzyme occurs in the early embryo before EGA (Lee *et al.* 2018; Cheng *et al.* 2019). Alterations in sperm DNA methylation profile are linked to male infertility, impaired embryo development and miscarriage (Aston *et al.* 2015; Cao *et al.* 2020; Richard Albert *et al.* 2020). Paternal DNA regions linked to H3K9me2 are not demethylated, and can be related to imprinted loci, as in the case of *H19* and *RASGRF1* genes (Nakamura *et al.* 2007). Alterations in the imprinting of these genes are associated with congenital syndromes, including Angelman, Prader-Willi and Beckwith-Wiedemann syndromes (Kobayashi *et al.* 2009; Hattori *et al.* 2019; Inoue *et al.* 2020). In addition, altered methylation patterns in the regions of sperm DNA that have retained histone CpG islands are linked to impaired embryo development (Denomme *et al.* 2017). Specifically, high levels of hypomethylated DNA are related to reduced fertilizing ability (Pacheco *et al.* 2011).

#### ***Modifications in Histones***

Acetylation, methylation, phosphorylation and chronotylation are post-translation modifications in histones that regulate gene expression. As aforementioned, retained paternal histones can bear epigenetic signatures and be inherited by the embryo (Ozturk *et al.* 2021). These retained histones have been found to be associated with developmental genes – such as HOX gene clusters – in human sperm (Hammoud *et al.* 2009). In addition, these retained histones could be located in the linker regions (Ribas-Maynou *et al.* 2021a; 2022b), and their alterations could be related to impaired embryo development (Hammoud *et al.* 2011; Vieweg *et al.* 2015; Glanzner *et al.* 2017; Huang *et al.* 2019). Specific lysine modifications of sperm-borne H3, such as H3K4me3 (Deng *et al.* 2020; Lambrot *et al.* 2021) and H3K27me3 (Sun *et al.*

2021), are involved in EGA. Also, before EGA, the male chromatin packaged into protamines is reorganized, thanks to the involvement of nucleoplasmins; and maternal histones, such as H3.3, TH2A and TH2B, are deposited onto paternal chromatin (Ishiuchi *et al.* 2021). Interestingly, sperm from infertile men have reduced levels of six histone variants, which has been proposed as one of the reasons for impaired embryogenesis (Azpiazu *et al.* 2014).

### ***Non-coding RNAs***

Non-coding mRNAs include miRNAs, siRNAs, piRNAs, circular (circ) RNAs and lnc-RNAs, and are found in human sperm, where they could be involved in fertilization and embryo development (Salas-Huetos *et al.* 2020). Most of the evidence comes from microRNAs, which are small single-strand RNAs that regulate gene expression, and are associated with male infertility (Lian *et al.* 2010; Marcet *et al.* 2011; Romero *et al.* 2011; Comazzetto *et al.* 2014; Gou *et al.* 2017). miR-34c, one of the most abundant miRNAs in human sperm (Salas-Huetos *et al.* 2014), plays an essential role during the first cleavage in mice (Liu *et al.* 2012b). Another important sperm miRNAs is miR-216b, which regulates the expression of a protein that participates in cell proliferation and differentiation in two-cell embryos, and is known as KRAS (Alves *et al.* 2019). Most of these miRNAs could be acquired during epididymal maturation, via extracellular vesicles, and could be one of the ways of passing epigenetic marks from the father to the offspring (Zhang *et al.* 2018; Chan *et al.* 2020). Other ncRNAs, such as tRNAs, can also be passed onto the offspring from the father (Sharma *et al.* 2018; Chen *et al.* 2016; Zhang *et al.* 2018). Furthermore, the miRNA profile differs between fertile and infertile patients (Liu *et al.* 2012a).

On the other hand, lncRNAs and circRNAs in sperm, which are circular single-strand molecules of RNA, can regulate the function of proteins, mRNAs and miRNAs, and even have a role in embryo development (Dang *et al.* 2016; Corral-Vazquez *et al.* 2021; Li *et al.* 2021). For instance, circCNOT6L is brought by sperm to the oocyte, where it participates in the transition from zygote to 2-cell stage (Chioccarelli *et al.* 2021). It is worth mentioning that lncRNA and circRNA cargo in sperm differs between men with good and poor sperm quality (Chioccarelli *et al.* 2019; Manfredola *et al.* 2020).

### **The Sperm Metabolome**

Metabolomics has been emerging over the last decade and has been proved to have many applications. Most of the studies focused on semen investigated the metabolites of seminal plasma. One of these studies revealed that men suffering from oligoasthenospermia had significantly lower values of free L-carnitine, polyunsaturated fatty acids, glutamate, aspartate, methionine, tryptophan, proline, and alanine; and four biogenic amines (spermine, spermidine, serotonin,

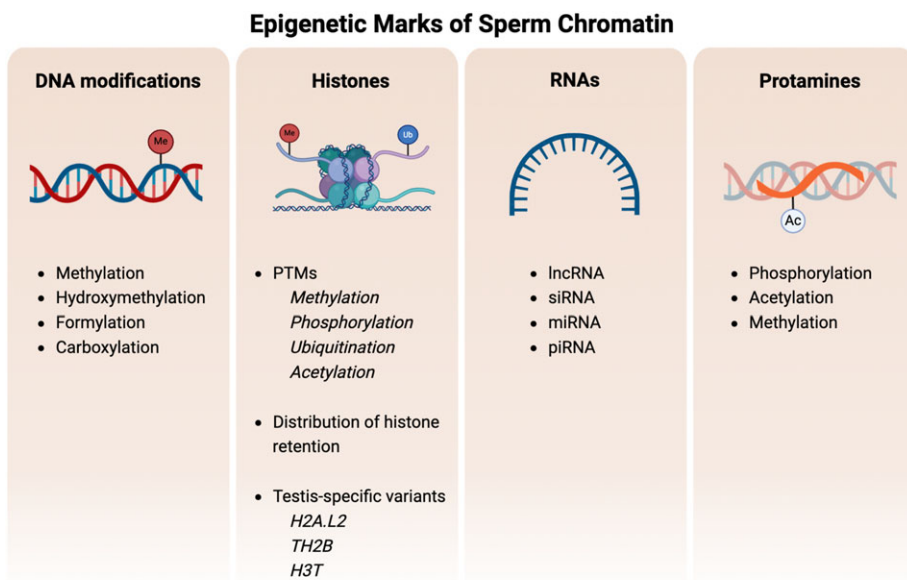
and alpha-aminoadipate). This deficiency in L-carnitine could be related to a decreased activity of  $\beta$ -oxidation of fatty acids, and oxidative phosphorylation, whereas reduced levels of amino acids and biogenic amines could be related to dysregulated signalling pathways (Boguenet *et al.* 2020).

Other research, which also targeted seminal plasma, compared fertile and infertile patients and identified up to 40 molecules (metabolomics/lipidomics approach) differing between fertile and infertile men. Acylcarnitines, phosphatidylserine (PS) (40:2) and lactate were lower, and PE (18:1; 18:1), phosphatidic acid (PA) (O-19:2; 18:1), lysophosphatidylethanolamine (LPE) (O-16:1) and phosphatidylcholine (PC) (O-16:2; 18:1)-CH<sub>3</sub> were higher in infertile patients (Correnti *et al.* 2023). In a systematic review, Llavanera *et al.* (2022) compiled a list of metabolites whose levels in seminal plasma were significantly different between fertile and infertile men, including lactate, alanine, choline, citrate, glycerophosphocholine, glutamine, tyrosine, histidine, phenylalanine, and uridine. Another study found that smoking alters the metabolism of sperm, which could potentially affect fertilizing ability, as it decreases the uptake of fatty acids by sperm mitochondria, which in turn decreases energy supply (Engel *et al.* 2021).

On the other hand, and given the events occurring during gamete fusion and thereafter, it seems quite unrealistic that sperm metabolites have a direct influence on fertilization and early embryo development. In spite of this, Guo *et al.* (2023) compared sperm from young and aged men, and identified 129 differentially expressed metabolites; from these, four, such as pipamperone, 2,2-bis(hydroxymethyl)-2,2',2''-nitrioltriethanol, Arg-Pro and triethyl phosphate, were more abundant in aged men. These findings are supported by investigations performed in animal models. In cattle, a previous study found that from 3704 metabolites identified in sperm, bulls with different fertility differed in the levels of 33 metabolites. These metabolites were related to taurine and hypotaurine, whose levels were reduced in bulls with low fertility, and also with different routes, such as glycolysis,  $\beta$ -oxidation of fatty acids, and synthesis of pyrimidines (Talluri *et al.* 2022).

Also in cattle, a study using liquid chromatography–mass spectrometry (LC-MS) identified up to 3704 metabolites in sperm, and identified five metabolites (hypotaurine, selenocysteine, l-malic acid, d-cysteine, and chondroitin 4-sulfate) that differed between bulls of high and low fertility (Saraf *et al.* 2020). In pigs, sperm with higher quality and fertilizing ability, and giving rise to more embryos at day 6 having greater levels of glycolysis-derived metabolites than those with poor quality, were found to have greater amounts of metabolites related to oxidative phosphorylation. This suggests the basal metabolism in mammalian sperm could be relevant for the ability of sperm to fertilize the oocyte and give rise to a viable embryo (Mateo-Otero *et al.* 2023). Finally, another study also conducted in pigs found that alteration of sperm mitochondrial activity, which impacts oxidative phosphorylation and metabolism, could increase ROS levels, which could in turn affect sperm DNA integrity and ultimately embryo development (Mateo-Otero *et al.* 2024).





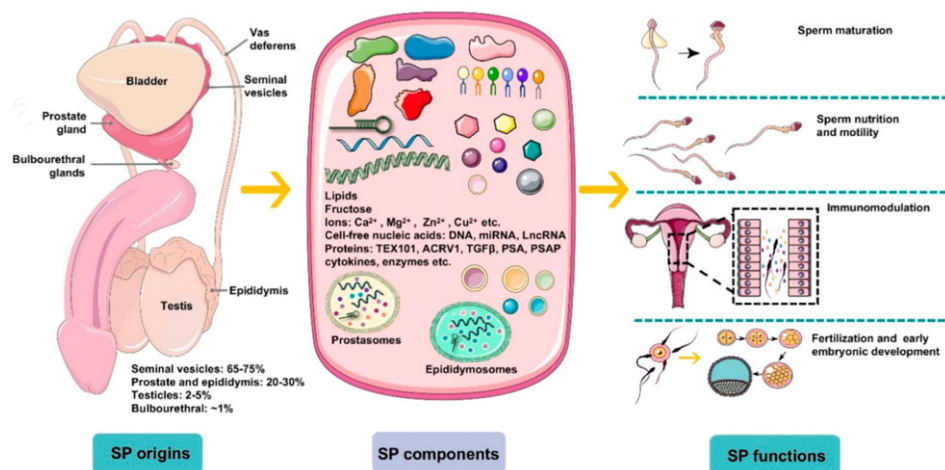
**Figure 6. Epigenetic signatures in sperm.** The contributors to the sperm epigenome include modifications to DNA and histones, RNAs and protamines. Abbreviations: lncRNA, long noncoding RNA; miRNA, microRNA; piRNA, piwi-interacting RNA; PTMs, post-translational modifications; siRNA, small interfering RNA. Reproduced from Balder *et al.* (2024) with permission.

All this compiled evidence, therefore, indicates that sperm metabolic fate influences fertilizing ability and could be even relevant while giving rise to a viable embryo, as the yet few studies in humans and animal models suggest.

### Does the Seminal Plasma have Any Role?

Seminal plasma is the liquid part of semen, and is produced by epididymis and male accessory glands (prostate, seminal vesicles) (Figure 7). While this fluid has largely been regarded as a mere vehicle for sperm upon ejaculation, growing evidence supports that it interacts with the female reproductive tract and can be involved in the modulation of the uterine environment, embryo development, and foetal growth (Bromfield *et al.* 2014; Watkins *et al.* 2018). In mice, seminal plasma modulates the expression of tumour necrosis factor (TNF), interleukin-1 $\beta$  (IL-1 $\beta$ ), interferon- $\gamma$  (IFN- $\gamma$ ), microphage inflammatory protein 1- $\alpha$  (MIP-1 $\alpha$ ) and colony stimulating factor 3 (CSF3), and the expression of regulatory T-cell genes (Watkins *et al.* 2018) in the endometrium, which supports its immunomodulation role. This would increase the maternal tolerance to paternal and foetal antigens, and facilitate embryo implantation in mice (Robertson *et al.* 1996, 2001; Bromfield *et al.* 2014).

Since humans do not ejaculate in the uterus, as mice do, but in the vagina, the role that seminal plasma could play in this species is less clear, as it remains unknown



**Figure 7. Origin, components, and functions of seminal plasma (SP).** Seminal plasma represents more than 95% of the semen volume, whereas testicular secretions containing sperm account for 2–5%. The seminal plasma is composed of a complex set of heterogeneous molecules, such as proteins (enzymes, cytokines, TEX101, ACRV1, TGFβ, prostate-specific antigen (PSA), prostatic-specific acid phosphatase (PSAP), etc.), lipids, sugars (fructose), cell-free nucleic acid (DNA, microRNA, and LncRNA), ions ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ , etc.), and small-molecule metabolites. Not only does SP modulate sperm function, but some of its components, such as cytokines, also recognize receptors on epithelial cells lining the cervix and uterus and induce the synthesis of pro-inflammatory cytokines and chemokines that recruit and activate inflammatory leukocytes. The SP also modulates the release of cytokines and growth factors, which appear to regulate embryo development in the oviduct and uterus before implantation. Reproduced from Wang *et al.* (2020), which was published under the terms and conditions of a Creative Commons Attribution license (CC BY 4.0).

whether it reaches the uterus. Studies in other animal models that, like cattle, also ejaculate in the vagina indicate that seminal plasma factors could adhere onto the sperm surface, thereby influencing the uterine environment (Recuero *et al.* 2020), as in this species they also induce the production of cytokines and chemokines in the female genital tract (Schjenken *et al.* 2015; Nongbua *et al.* 2020). These findings would match those in humans, where seminal plasma has been proposed to prime the maternal endometrium for implantation (Ibrahim *et al.* 2019; George *et al.* 2020; Ajdary *et al.* 2021), as it increases pregnancy rate after embryo transfer (Wolff *et al.* 2009; Chicea *et al.* 2013; Friedler *et al.* 2013; Crawford *et al.* 2015).

Seminal plasma contains extracellular vesicles, mainly epididymosomes and prostatosomes. These vesicles are membrane-bound particles released by cells (Yáñez-Mó *et al.* 2015). They have been found to be synthesized by the epididymis and prostate. In the case of the epididymis, these extracellular vesicles, called epididymosomes, fuse with epididymal sperm (Păunescu *et al.* 2014). Thanks to this fusion, epididymal cells can transfer proteins and miRNA to sperm (Twenter *et al.* 2020). Proteins identified in

these extracellular vesicles include enzymes, and others having a function for sperm motility (Barrachina *et al.* 2022), and even galactin-3, which is a lectin transferred from the seminal plasma to the sperm surface, that is involved in the binding of capacitated sperm to the zona pellucida (Mei *et al.* 2019).

Prostasomes, which are produced by prostate epithelial cells (Brody *et al.* 1983), also transfer proteins to sperm (Ronquist *et al.* 2011), and have been suggested to modify the composition of sperm plasmalemma (Dubois *et al.* 2015). Prostasomes cargo also seems to regulate sperm motility (Andrews *et al.* 2015; García-Rodríguez *et al.* 2018), capacitation (Pons-Rejraji *et al.* 2011; Aalberts *et al.* 2013), and even protect sperm from the female immune system (Milutinović *et al.* 2019; Paktinat *et al.* 2019). In humans, seminal plasma vesicles have been suggested to fuse with endometrial stromal cells, induce decidualization, and increase the secretion of prolactin (Rodríguez-Caro *et al.* 2019). All these data indicate that these seminal plasma vesicles play a role with regard to both sperm function and the female reproductive tract, and should be further investigated in the future.

### **Conclusions**

Mounting evidence supports that semen, which is composed of cellular (sperm) and liquid (seminal plasma) fractions, does not only play a role during oocyte fertilization, but may even be relevant to embryo development and implantation, and modulate the female reproductive tract. Sperm components such as centrioles, chromatin condensation/protamination, DNA integrity, transcriptome, and epigenome appear to be important for post-fertilization events, including implantation. In addition, the sperm metabolome tells about their fertilizing ability, and some specific metabolomics signatures may indicate that the male gamete is more capable of giving rise to a viable embryo. All these findings contribute to shedding light onto the paternal factors underlying infertility and suggest that further research is needed to address to what extent these factors are crucial for embryo development and implantation, and whether they may influence offspring health.

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### **Conflict of Interest**

The author declares no conflict of interest.

## References

- Aalberts M, Sostaric E, Wubbolts R, Wauben MWM, Nolte-T Hoen ENM, Gadella BM, Stout TAE and Stoorvogel W (2013) Spermatozoa recruit prostasomes in response to capacitation induction. *Biochimica et Biophysica Acta* **1834**, 2326–2335.
- Aarabi M, Balakier H, Bashar S, Moskovtsev SI, Sutovsky P, Librach CL and Oko R (2014) Sperm-derived WW domain-binding protein, PAWP, elicits calcium oscillations and oocyte activation in humans and mice. *FASEB Journal* **28**, 4434–4440.
- Aarabi M, Qin Z, Xu W, Mewburn J and Oko R (2010) Sperm-borne protein, PAWP, initiates zygotic development in *Xenopus laevis* by eliciting intracellular calcium release. *Molecular Reproduction & Development* **77**, 249–256.
- Ajdary M, Ashrafi M, Aflatoonian R and Mehdizadeh M (2021) The role of sperm in inducing genomic changes in the implantation: An experimental study. *Andrologia* **53**, 4077.
- Alghamdi AS and Foster DN (2005) Seminal DNase frees spermatozoa entangled in Neutrophil Extracellular Traps. *Biology of Reproduction* **73**, 1174–1181.
- Alghamdi AS, Lovaas BJ, Bird SL, Lamb GC, Rendahl AK, Taube PC and Foster DN (2009) Species-specific interaction of seminal plasma on sperm–neutrophil binding. *Animal Reproduction Science* **114**, 331–344.
- Alves MBR, Arruda RP de, Bem THC De, Florez-Rodriguez SA, Sá Filho MF de, Belleannée C, Meirelles FV, Silveira JC da, Percin F and Celeghini ECC (2019) Sperm-borne miR-216b modulates cell proliferation during early embryo development via K-RAS. *Scientific Reports* **9**, 10358.
- Amargant F, Pujol A, Ferrer-Vaquer A, Durban M, Martínez M, Vassena R and Vernos I (2021) The human sperm basal body is a complex centrosome important for embryo preimplantation development. *Molecular Human Reproduction* **27**, gaab062.
- Amor H, Shelko N, Hamad MF, Zeyad A and Hammadeh ME (2019) An additional marker for sperm DNA quality evaluation in spermatozoa of male partners of couples undergoing assisted reproduction technique (IVF/ICSI): Protamine ratio. *Andrologia* **51**, e13400.
- Andrews RE, Galileo DS and Martin-DeLeon PA (2015) Plasma membrane Ca<sup>2+</sup>-ATPase 4: interaction with constitutive nitric oxide synthases in human sperm and prostasomes which carry Ca<sup>2+</sup>/CaM-dependent serine kinase. *Molecular Human Reproduction* **21**, 832–843.
- Aston KI, Uren PJ, Jenkins TG, Horsager A, Cairns BR, Smith AD and Carrell DT (2015) Aberrant sperm DNA methylation predicts male fertility status and embryo quality. *Fertility and Sterility* **104**, 1397.e1385.
- Avendaño C, Franchi A, Duran H and Oehninger S. (2010) DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. *Fertility and Sterility* **94**, 549–557.
- Avendaño C, Franchi A, Jones E and Oehninger S (2009) Pregnancy-specific  $\beta$ -1-glycoprotein 1 and human leukocyte antigen-E mRNA in human sperm: differential expression in fertile and infertile men and evidence of a possible functional role during early development. *Human Reproduction* **24**, 270–277.
- Avendaño C, Franchi A and Oehninger S (2008) Human sperm mRNA: differential expression between fertile and infertile men and maintenance of transcripts after fertilization. *Fertility and Sterility* **90**(Suppl), 194–195.
- Avidor-Reiss T, Achinger L and Uzbekov R (2022) The centriole's role in miscarriages. *Frontiers in Cell and Developmental Biology* **10**, 864692.

- Avidor-Reiss T and Fishman EL** (2019) It takes two (centrioles) to tango. *Reproduction* **157**, R51.
- Azpiazu R, Amaral A, Castillo J, Estanyol JM, Guimerà M, Ballescà JL, Balasch J and Oliva R** (2014) High-throughput sperm differential proteomics suggests that epigenetic alterations contribute to failed assisted reproduction. *Human Reproduction* **29**, 1225–1237.
- Balder P, Jones C, Coward K and Yeste M** (2024) Sperm chromatin: Evaluation, epigenetic signatures and relevance for embryo development and assisted reproductive technology outcomes. *European Journal of Cell Biology* **103**, 151429. <https://doi.org/10.1016/j.ejcb.2024.151429>.
- Barrachina F, Battistone MA, Castillo J, Mallofré C, Jodar M, Breton S and Oliva R** (2022) Sperm acquire epididymis-derived proteins through epididymosomes. *Human Reproduction* **37**, 651–668.
- Barral S, Morozumi Y, Tanaka H, Montellier E, Govin J, Dieuleveult M de, Charbonnier G, Couté Y, Puthier D, Buchou T, et al.** (2017) Histone variant H2A.L.2 guides transition protein-dependent protamine assembly in male germ cells. *Molecular Cell* **66**, 89–101.e8.
- Bartosovic M, Kabbe M and Castelo-Branco G** (2021) Single-cell CUT&Tag profiles histone modifications and transcription factors in complex tissues. *Nature Biotechnology* **39**, 825–835.
- Bashiri Z, Amidi F, Amiri I, Zandieh Z, Maki CB, Mohammadi F, Amiri S and Koruji M** (2021) Male factors: the role of sperm in preimplantation embryo quality. *Reproductive Sciences* **28**, 1788–1811.
- Bianchi E, Doe B, Goulding D and Wright GJ** (2014) Juno is the egg Izumo receptor and is essential for mammalian fertilization. *Nature* **508**, 483–487.
- Bianchi E, Stermer A, Boekelheide K, Sigman M, Hall SJ, Reyes G, Dere E and Hwang K** (2018) High-quality human and rat spermatozoal RNA isolation for functional genomic studies. *Andrology* **6**, 374–383.
- Biellmann F, Hülsmeier AJ, Zhou D, Cinelli P and Hennet T** (2008) The Lc3-synthase gene B3gnt5 is essential to pre-implantation development of the murine embryo. *BMC Developmental Biology* **8**, 109.
- Boguenet M, Bocca C, Bouet PE, Serri O, Chupin S, Tessier L, Blanchet O, El Hachem H, Chao de la Barca JM, Reynier P and May-Panloup P** (2020) Metabolomic signature of the seminal plasma in men with severe oligoasthenospermia. *Andrology* **8**, 1859–1866.
- Borges E Jr, Zanetti BF, Setti AS, Braga DPAF, Provenza RR and Iaconelli A Jr** (2019) Sperm DNA fragmentation is correlated with poor embryo development, lower implantation rate, and higher miscarriage rate in reproductive cycles of non-male factor infertility. *Fertility and Sterility* **112**, 483–490.
- Braham A, Ghedir H, Zidi I, Sallem A, Hajlaoui A, Ajina M, Saad A and Ibal-Romdhane S** (2019) Nuclear sperm quality in total polymorphic teratozoospermia and its impact on intracytoplasmic sperm injection outcome. *Andrologia* **51**, e13252.
- Brody I, Ronquist G and Gottfries A** (1983) Ultrastructural localization of the prostasome - an organelle in human seminal plasma. *Uppsala Journal of Medical Sciences* **88**, 63–80.
- Bromfield JJ, Schjenken JE, Chin PY, Care AS, Jasper MJ and Robertson SA** (2014) Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 2200–2205.
- Brykczynska U, Hisano M, Erkek S, Ramos L, Oakeley EJ, Roloff TC, Beisel C, Schübeler D, Stadler MB and Peters AHFM** (2010) Repressive and active histone

- methylation mark distinct promoters in human and mouse spermatozoa. *Nature Structural & Molecular Biology* **17**, 679–687.
- Cankut S, Dinc T, Cincik M, Ozturk G and Selam B** (2019) Evaluation of sperm DNA fragmentation via Halosperm technique and TUNEL assay before and after cryopreservation. *Reproductive Sciences* **26**, 1575–1581.
- Cannarella R, Condorelli RA, Mongioi LM, La Vignera S and Calogero AE** (2020) Molecular biology of spermatogenesis: novel targets of apparently idiopathic male infertility. *International Journal of Molecular Sciences* **21**, 1728.
- Cao M, Shao X, Chan P, Cheung W, Kwan T, Pastinen T and Robaire B** (2020) High-resolution analyses of human sperm dynamic methylome reveal thousands of novel age-related epigenetic alterations. *Clinical Epigenetics* **12**, 192.
- Castillo J, Jodar M and Oliva R** (2018) The contribution of human sperm proteins to the development and epigenome of the preimplantation embryo. *Human Reproduction Update* **24**, 535–555.
- Champroux A, Cocquet J, Henry-Berger J, Drevet JR and Kocer A** (2018) A decade of exploring the mammalian sperm epigenome: paternal epigenetic and transgenerational inheritance. *Frontiers in Cell and Developmental Biology* **6**, 50.
- Chan JC, Morgan CP, Adrian Leu N, Shetty A, Cisse YM, Nugent BM, Morrison KE, Jašarević E, Huang W, Kanyuch N, Rodgers AB, Bhanu NV, Berger DS, Garcia BA, Ament S, Kane M, Neill Epperson C and Bale TL** (2020) Reproductive tract extracellular vesicles are sufficient to transmit intergenerational stress and program neurodevelopment. *Nature Communications* **11**, 1499.
- Chen Q, Yan M, Cao Z, Li X, Zhang Y, Shi J, Feng GH, Peng H, Zhang X, Zhang Y, Qian J, Duan E, Zhai Q and Zhou Q** (2016) Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science* **351**, 397–400.
- Cheng H, Zhang J, Zhang S, Zhai Y, Jiang Y, An X, Ma X, Zhang X, Li Z and Tang B** (2019) Tet3 is required for normal in vitro fertilization preimplantation embryos development of bovine. *Molecular Reproduction & Development* **86**, 298–307.
- Chicea R, Ispasoiu F and Focsa M** (2013) Seminal plasma insemination during ovum-pickup—a method to increase pregnancy rate in IVF/ICSI procedure. A pilot randomized trial. *Journal of Assisted Reproduction and Genetics* **30**, 569–574.
- Child T** (2013) Causes and investigations of male and female infertility. In Coward K, Wells D (eds). *Textbook of Clinical Embryology*. Cambridge, UK: Cambridge University Press, pp. 152–160.
- Chioccarelli T, Falco G, Cappetta D, De Angelis A, Roberto L, Addeo M, Ragusa M, Barbagallo D, Berrino L, Purrello M, Ambrosino C, Cobellis G, Pierantoni R, Chianese R and Manfredola F** (2021) FUS driven circCNOT6L biogenesis in mouse and human spermatozoa supports zygote development. *Cellular and Molecular Life Sciences* **79**, 50.
- Chioccarelli T, Manfredola F, Ferraro B, Sellitto C, Cobellis G, Migliaccio M, Fasano S, Pierantoni R and Chianese R** (2019) Expression patterns of circular RNAs in high quality and poor quality human spermatozoa. *Frontiers in Endocrinology* **10**, 435.
- Cho C, Jung-Ha H, Willis WD, Goulding EH, Stein P, Xu Z, Schultz RM, Hecht NB and Eddy EM** (2003) Protamine 2 Deficiency Leads to Sperm DNA Damage and Embryo Death in Mice. *Biology of Reproduction* **69**, 211–217.
- Cohen DJ, Maldera JA, Vasen G, Ernesto JI, Muñoz MW, Battistone MA and Cuasnicú PS** (2011) Epididymal protein CRISP1 plays different roles during the fertilization process. *Journal of Andrology* **32**, 672–678.



- Colaco S and Sakkas D** (2018) Paternal factors contributing to embryo quality. *Journal of Assisted Reproduction and Genetics* **35**, 1953–1968.
- Colombero L., Moomjy M, Sills E., Rosenwaks Z and Palermo G** (1999) The role of structural integrity of the fertilising spermatozoon in early human embryogenesis. *Zygote* **7**, 157–163.
- Comazzetto S, Giacomo M Di, Rasmussen KD, Much C, Azzi C, Perlas E, Morgan M and O’Carroll D** (2014) Oligoasthenoteratozoospermia and infertility in mice deficient for miR-34b/c and miR-449 Loci. *PLoS Genetics* **10**, e1004597.
- Condorelli RA, Vignera S La, Mongioi LM, Alamo A and Calogero AE** (2018) Diabetes mellitus and infertility: Different pathophysiological effects in type 1 and type 2 on sperm function. *Frontiers in Endocrinology* **9**, 268.
- Corral-Vazquez C, Blanco J, Aiese Cigliano R, Sarrate Z, Rivera-Egea R, Vidal F, Garrido N, Daub C and Anton E** (2021) The RNA content of human sperm reflects prior events in spermatogenesis and potential post-fertilization effects. *Molecular Human Reproduction* **27**, gaab035.
- Correnti S, Preianò M, Fregola A, Gamboni F, Stephenson D, Savino R, D’Alessandro A and Terracciano R** (2023) Seminal plasma untargeted metabolomic and lipidomic profiling for the identification of a novel panel of biomarkers and therapeutic targets related to male infertility. *Frontiers in Pharmacology* **14**, 1275832.
- Crawford G, Ray A, Gudi A, Shah A and Homburg R** (2015) The role of seminal plasma for improved outcomes during in vitro fertilization treatment: review of the literature and meta-analysis. *Human Reproduction Update* **21**, 275–284.
- Cui X, Jing X, Wu X, Wang Z and Li Q** (2016) Potential effect of smoking on semen quality through DNA damage and the downregulation of Chk1 in sperm. *Molecular Medicine Reports* **14**, 753–761.
- Dang Y, Yan L, Hu B, Fan X, Ren Y, Li R, Lian Y, Yan J, Li Q, Zhang Y, Li M, Ren X, Huang J, Wu Y, Liu P, Wen L, Zhang C, Huang Y, Tang F and Qiao J** (2016) Tracing the expression of circular RNAs in human pre-implantation embryos. *Genome Biology* **17**, 130.
- Den Z, Cheng X, Merched-Sauvage M and Koch PJ** (2006) Desmocollin 3 is required for pre-implantation development of the mouse embryo. *Journal of Cell Science* **119**, 482–489.
- Deng M, Liu Z, Chen B, Wan Y, Yang H, Zhang Y, Cai Y, Zhou J and Wang F** (2020) Aberrant DNA and histone methylation during zygotic genome activation in goat cloned embryos. *Theriogenology* **148**, 27–36.
- Denomme MM, McCallie BR, Parks JC, Schoolcraft WB and Katz-Jaffe MG** (2017) Alterations in the sperm histone-retained epigenome are associated with unexplained male factor infertility and poor blastocyst development in donor oocyte IVF cycles. *Human Reproduction* **32**, 2443–2455.
- Dorostghoal M, Kazeminejad SR, Shahbazian N, Pourmehdi M and Jabbari A** (2017) Oxidative stress status and sperm DNA fragmentation in fertile and infertile men. *Andrologia* **49**, e12762.
- Dubois L, Ronquist KG, Ek B, Ronquist G and Larsson A** (2015) Proteomic Profiling of Detergent Resistant Membranes (Lipid Rafts) of Prostrasomes. *Molecular & Cellular Proteomics* **14**, 3015–3022.
- Engel KM, Baumann S, Blaurock J, Rolle-Kampczyk U, Schiller J, von Bergen M and Grunewald S** (2021) Differences in the sperm metabolomes of smoking and nonsmoking men. *Biology of Reproduction* **105**, 1484–1493.
- Escoffier J, Lee HC, Yassine S, Zouari R, Martinez G, Karaouzène T, Coutton C, Kherraf ZE, Halouani L, Triki C, et al.** (2016) Homozygous mutation of PLCZ1

- leads to defective human oocyte activation and infertility that is not rescued by the WW-binding protein PAWP. *Human Molecular Genetics* **25**, 878–891.
- ESHRE** (2018) <https://www.focusonreproduction.eu/article/ESHRE-News-GlobaIIVF18> (accessed 8 January 2024).
- Evenson DP, Djira G, Kasperson K and Christianson J** (2020) Relationships between the age of 25,445 men attending infertility clinics and sperm chromatin structure assay (SCSA®) defined sperm DNA and chromatin integrity. *Fertility and Sterility* **114**, 311–320.
- Fernández-González R, Moreira PN, Pérez-Crespo M, Sánchez-Martín M, Ramirez MA, Pericuesta E, Bilbao A, Bermejo-Alvarez P, de Dios Hourcade J, de Fonseca FR and Gutiérrez-Adán A** (2008) Long-Term Effects of Mouse Intracytoplasmic Sperm Injection with DNA-Fragmented Sperm on Health and Behavior of Adult Offspring. *Biology of Reproduction* **78**, 761–772.
- Fournier C, Labrune E, Lornage J, Soignon G, d’Estaing SG, Guérin J-F and Benchaib M** (2018) The impact of histones linked to sperm chromatin on embryo development and ART outcome. *Andrology* **6**, 436–445.
- Freour T, Barragan M, Ferrer-Vaquer A, Rodríguez A and Vassena R** (2017) WBP2NL/PAWP mRNA and protein expression in sperm cells are not related to semen parameters, fertilization rate, or reproductive outcome. *Journal of Assisted Reproduction and Genetics* **34**, 803–810.
- Friedler S, Ben-Ami I, Gidoni Y, Strassburger D, Kasterstein E, Maslansky B, Komarovsy D, Bern O, Ron-El R and Raziel A** (2013) Effect of seminal plasma application to the vaginal vault in in vitro fertilization or intracytoplasmic sperm injection treatment cycles—a double-blind, placebo-controlled, randomized study. *Journal of Assisted Reproduction and Genetics* **30**, 907–911.
- Fullston T, McPherson NO, Owens JA, Kang WX, Sandeman LY and Lane M** (2015) Paternal obesity induces metabolic and sperm disturbances in male offspring that are exacerbated by their exposure to an “obesogenic” diet. *Physiological Reports* **3**, e12336.
- García-Rodríguez A, la Casa M de, Peinado H, Gosálvez J and Roy R** (2018) Human prostasomes from normozoospermic and non-normozoospermic men show a differential protein expression pattern. *Andrology* **6**, 585–596.
- George AF, Jang KS, Nyegaard M, Neidleman J, Spitzer TL, Xie G, Chen JC, Herzig E, Laustsen A, Menezes EGM de, et al** (2020) Seminal plasma promotes decidualization of endometrial stromal fibroblasts in vitro from women with and without inflammatory disorders in a manner dependent on interleukin-11 signaling. *Human Reproduction* **35**, 617–640.
- Glanzner W, Wachter A, Coutinho AR, Albornoz M, Duggavathi R, GonÇalves P and Bordignon V** (2017) Altered expression of BRG1 and histone demethylases, and aberrant H3K4 methylation in less developmentally competent embryos at the time of embryonic genome activation. *Molecular Reproduction & Development* **84**, 19–29.
- Gou LT, Kang JY, Dai P, Wang X, Li F, Zhao S, Zhang M, Hua MM, Lu Y, Zhu Y, et al.** (2017) Ubiquitination-deficient mutations in human piwi cause male infertility by impairing histone-to-protamine exchange during spermiogenesis. *Cell* **169**, 1090–1104.e13.
- Guo L, Chao S-B, Xiao L, Wang Z-B, Meng T-G, Li Y-Y, Han Z-M, Ouyang Y-C, Hou Y, Sun Q-Y, et al.** (2017) Sperm-carried RNAs play critical roles in mouse embryonic development. *Oncotarget* **8**, 67394–67405.

- Guo Y, Li J, Hao F, Yang Y, Yang H, Chang Q, Kong P, Liu W, Jiao X and Teng X** (2023) A new perspective on semen quality of aged male: the characteristics of metabolomics and proteomics. *Frontiers in Endocrinology* **13**, 1058250.
- Gupta SK** (2021) Human Zona Pellucida glycoproteins: binding characteristics with human spermatozoa and induction of acrosome reaction. *Frontiers in Cell and Developmental Biology* **9**, 619868.
- Hachem A, Godwin J, Ruas M, Lee HC, Buitrago MF, Ardestani G, Bassett A, Fox S, Navarrete F, Sutter P De, et al.** (2017) Plc $\zeta$  is the physiological trigger of the Ca $^{2+}$  oscillations that induce embryogenesis in mammals but conception can occur in its absence. *Development* **144**, 2914–2924.
- Haddock L, Gordon S, Lewis SEM, Larsen P, Shehata A and Shehata H** (2021) Sperm DNA fragmentation is a novel biomarker for early pregnancy loss. *Reproductive BioMedicine Online* **42**, 175–184.
- Hammoud SS, Nix DA, Hammoud AO, Gibson M, Cairns BR and Carrell DT** (2011) Genome-wide analysis identifies changes in histone retention and epigenetic modifications at developmental and imprinted gene loci in the sperm of infertile men. *Human Reproduction* **26**, 2558–2569.
- Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT and Cairns BR** (2009) Distinctive chromatin in human sperm packages genes for embryo development. *Nature* **460**, 473–478.
- Han T, Huang J, Gu J, Xie Q, Zhong Y and Huang T** (2021) Hepatitis B virus surface protein induces sperm dysfunction through the activation of a Bcl2/Bax signaling cascade triggering AIF/Endo G-mediated apoptosis. *Andrology* **9**, 944–955.
- Hattori H, Hiura H, Kitamura A, Miyauchi N, Kobayashi N, Takahashi S, Okae H, Kyono K, Kagami M, Ogata T, et al.** (2019) Association of four imprinting disorders and ART. *Clinical Epigenetics* **11**, 21.
- Hayashi S, Yang J, Christenson L, Yanagimachi R and Hecht NB** (2003) Mouse preimplantation embryos developed from oocytes injected with round spermatids or spermatozoa have similar but distinct patterns of early messenger RNA expression. *Biology of Reproduction* **69**, 1170–1176.
- Heindryckx B, Nikiforaki D, Caluwaerts L, vanden Meerschaut F, Deroo T and de Sutter P** (2013) Analysis of calcium oscillations pattern triggered by human sperm showing failed or low fertilization. *Fertility and Sterility* **100**(Suppl), S236–S237.
- Heytens E, Parrington J, Coward K, Young C, Lambrecht S, Yoon SY, Fissore RA, Hamer R, Deane CM, Ruas M, et al.** (2009) Reduced amounts and abnormal forms of phospholipase C zeta (PLC $\zeta$ ) in spermatozoa from infertile men. *Human Reproduction* **24**, 2417–2428.
- Hirohashi N and Yanagimachi R** (2018) Sperm acrosome reaction: Its site and role in fertilization. *Biology of Reproduction* **99**, 127–133.
- Horner VL and Wolfner MF** (2008) Transitioning from egg to embryo: Triggers and mechanisms of egg activation. *Developmental Dynamics* **237**, 527–544.
- Huang X, Gao X, Li W, Jiang S, Li R, Hong H, Zhao C, Zhou P, Chen H, Bo X, et al.** (2019) Stable H3K4me3 is associated with transcription initiation during early embryo development. *Bioinformatics* **35**, 3931–3936.
- Hwang JY, Mulligan BP, Kim H-M, Yang B-C, Lee C-K, Hwang JY, Mulligan BP, Kim H-M, Yang B-C and Lee C-K** (2013) Quantitative analysis of sperm mRNA in the pig: relationship with early embryo development and capacitation. *Reproduction, Fertility and Development* **25**, 807–817.
- Ibrahim LA, Rizo JA, Fontes PLP, Lamb GC and Bromfield JJ** (2019) Seminal plasma modulates expression of endometrial inflammatory mediators in the bovine. *Biology of Reproduction* **100**, 660–671.

- Inoue K, Ogonuki N, Kamimura S, Inoue H, Matoba S, Hirose M, Honda A, Miura K, Hada M, Hasegawa A, et al.** (2020) Loss of H3K27me3 imprinting in the Sfbmt2 miRNA cluster causes enlargement of cloned mouse placentas. *Nature Communications* **11**, 2150.
- Inoue N, Ikawa M, Isotani A and Okabe M** (2005) The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. *Nature* **434**, 234–238.
- Ishimoto K and Gaffney EA** (2016) Mechanical tuning of mammalian sperm behaviour by hyperactivation, rheology and substrate adhesion: a numerical exploration. *Journal of the Royal Society Interface* **13**, 20160633.
- Ishiuchi T, Abe S, Inoue K, Yeung WKA, Miki Y, Ogura A and Sasaki H** (2021) Reprogramming of the histone H3. 3 landscape in the early mouse embryo. *Nature Structural & Molecular Biology* **28**, 38–49.
- James ER, Carrell DT, Aston KI, Jenkins TG, Yeste M and Salas-Huetos A** (2020) The role of the epididymis and the contribution of epididymosomes to mammalian reproduction. *International Journal of Molecular Sciences* **21**, 5377.
- Jankovicova J, Frolikova M, Palenikova V, Valaskova E, Cerny J, Secova P, Bartokova M, Horovska L, Manaskova-Postlerova P, Antalikova J, et al.** (2020) Expression and distribution of CD151 as a partner of alpha6 integrin in male germ cells. *Scientific Reports* **10**, 4374.
- Jean C, Haghighirad F, Zhu Y, Chalbi M, Ziyat A, Rubinstein E, Gourier C, Yip P, Wolf JP, Lee JE, et al.** (2019) JUNO, the receptor of sperm IZUMO1, is expressed by the human oocyte and is essential for human fertilisation. *Human Reproduction* **34**, 118–126.
- Jodar M, Sendler E, Moskovtsev SI, Librach CL, Goodrich R, Swanson S, Hauser R, Diamond MP and Krawetz SA** (2015) Absence of sperm RNA elements correlates with idiopathic male infertility. *Science Translational Medicine* **7**, 295re6.
- Jung YH, Sauria MEG, Lyu X, Cheema MS, Ausio J, Taylor J and Corces VG** (2017) Chromatin states in mouse sperm correlate with embryonic and adult regulatory landscapes. *Cell Reports* **18**, 1366–1382.
- Jurewicz J, Radwan M, Sobala W, Radwan P, Bochenek M and Hanke W** (2018) Dietary patterns and their relationship with semen quality. *American Journal of Men's Health* **12**, 575–583.
- Kaneda M, Okano M, Hata K, Sado T, Tsujimoto H, Li E and Sasaki H** (2004) Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature* **429**, 900–903.
- Kashir J, Jones C, Lee HC, Rietdorf K, Nikiforaki D, Durrans C, Ruas M, Tee ST, Heindryckx B, Galione A, et al.** (2011) Loss of activity mutations in phospholipase C zeta (PLC $\zeta$ ) abolishes calcium oscillatory ability of human recombinant protein in mouse oocytes. *Human Reproduction* **26**, 3372–3387.
- Kashir J, Jones C, Mounce G, Ramadan WM, Lemmon B, Heindryckx B, Sutter P De, Parrington J, Turner K, Child T, et al.** (2013) Variance in total levels of phospholipase C zeta (PLC- $\zeta$ ) in human sperm may limit the applicability of quantitative immunofluorescent analysis as a diagnostic indicator of oocyte activation capability. *Fertility and Sterility* **99**, 107–117.
- Kaya A, Dogan S, Vargovic P, Kutchy NA, Ross P, Topper E, Oko R, van der Hoorn F, Sutovsky P and Memili E** (2022) Sperm proteins ODF2 and PAWP as markers of fertility in breeding bulls. *Cell and Tissue Research* **387**, 159–171.
- Kennedy CE, Krieger KB, Sutovsky M, Xu W, Vargovič P, Didion BA, Ellersieck MR, Hennessy ME, Verstegen J, Oko R, et al.** (2014) Protein expression pattern of PAWP in bull spermatozoa is associated with sperm quality and fertility following artificial insemination. *Molecular Reproduction & Development* **81**, 436–449.

- Khanal S, Leung MR, Royfman A, Fishman EL, Saltzman B, Bloomfield-Gadêlha H, Zeev-Ben-Mordehai T and Avidor-Reiss T** (2021) A dynamic basal complex modulates mammalian sperm movement. *Nature Communications* **12**, 3808.
- Ko MS, Kitchen JR, Wang X, Threat TA, Wang X, Hasegawa A, Sun T, Grahovac MJ, Kargul GJ, Lim MK, et al.** (2000) Large-scale cDNA analysis reveals phased gene expression patterns during preimplantation mouse development. *Development* **127**, 1737–1749.
- Kobayashi H, Hiura H, John RM, Sato A, Otsu E, Kobayashi N, Suzuki R, Suzuki F, Hayashi C, Utsunomiya T, et al.** (2009) DNA methylation errors at imprinted loci after assisted conception originate in the parental sperm. *European Journal of Human Genetics* **17**, 1582–1591.
- Lamas-Toranzo I, Hamze JG, Bianchi E, Fernández-Fuertes B, Pérez-Cerezales S, Laguna-Barraza R, Fernández-González R, Lonergan P, Gutiérrez-Adán A, Wright GJ, et al.** (2020) TMEM95 is a sperm membrane protein essential for mammalian fertilization. *eLife* **9**, e53913.
- Lambrot R, Chan D, Shao X, Aarabi M, Kwan T, Bourque G, Moskovtsev S, Librach C, Trasler J, Dumeaux V, et al.** (2021) Whole-genome sequencing of H3K4me3 and DNA methylation in human sperm reveals regions of overlap linked to fertility and development. *Cell Reports* **36**, 109418.
- Lane M, McPherson NO, Fullston T, Spillane M, Sandeman L, Kang WX and Zander-Fox DL** (2014) Oxidative stress in mouse sperm impairs embryo development, fetal growth and alters adiposity and glucose regulation in female offspring. *PLoS One* **9**, e100832.
- Lee J, Matsuzawa A, Shiura H, Sutani A and Ishino F** (2018) Preferable in vitro condition for maintaining faithful DNA methylation imprinting in mouse embryonic stem cells. *Genes to Cells* **23**, 146–160.
- Légaré C, Droit A, Fournier F, Bourassa S, Force A, Cloutier F, Tremblay R and Sullivan R** (2014) Investigation of male infertility using quantitative comparative proteomics. *Journal of Proteome Research* **13**, 5403–5414.
- Leung MR, Roelofs MC, Ravi RT, Maitan P, Henning H, Zhang M, Bromfield EG, Howes SC, Gadella BM and Bloomfield-Gadêlha H** (2021) The multi-scale architecture of mammalian sperm flagella and implications for ciliary motility. *EMBO Journal* **40**, e107410.
- Li S, Li X, Xue W, Zhang L, Yang L-Z, Cao S-M, Lei Y-N, Liu C-X, Guo S-K and Shan L** (2021) Screening for functional circular RNAs using the CRISPR–Cas13 system. *Nature Methods* **18**, 51–59.
- Lian J, Tian H, Liu L, Zhang X-S, Li W-Q, Deng Y-M, Yao G-D, Yin M-M and Sun F** (2010) Downregulation of microRNA-383 is associated with male infertility and promotes testicular embryonal carcinoma cell proliferation by targeting IRF1. *Cell Death & Disease* **1**, e94.
- Liu T, Cheng W, Gao Y, Wang H and Liu Z** (2012a) Microarray analysis of microRNA expression patterns in the semen of infertile men with semen abnormalities. *Molecular Medicine Reports* **6**, 535–542.
- Liu WM, Pang RTK, Chiu PCN, Wong BPC, Lao K, Lee KF and Yeung WSB** (2012b) Sperm-borne microRNA-34c is required for the first cleavage division in mouse. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 490–494.
- Llavanera M, Delgado-Bermúdez A, Ribas-Maynou J, Salas-Huetos A and Yeste M** (2022) A systematic review identifying fertility biomarkers in semen: a clinical approach through Omics to diagnose male infertility. *Fertility and Sterility* **118**, 291–313.

- Lord T and Aitken RJ** (2015) Fertilization stimulates 8-hydroxy-2'-deoxyguanosine repair and antioxidant activity to prevent mutagenesis in the embryo. *Developmental Biology* **406**, 1–13.
- Manfrevola F, Chioccarelli T, Cobellis G, Fasano S, Ferraro B, Sellitto C, Marella G, Pierantoni R and Chianese R** (2020) CircRNA role and circRNA-dependent network (ceRNET) in asthenozoospermia. *Frontiers in Endocrinology* **11**, 395.
- Marcet B, Chevalier B, Luxardi G, Coraux C, Zaragosi L-E, Cibois M, Robbe-Sermesant K, Jolly T, Cardinaud B, Moreilhon C, et al.** (2011) Control of vertebrate multiciliogenesis by miR-449 through direct repression of the Delta/Notch pathway. *Nature Cell Biology* **13**, 693–699.
- Massa E, Prez G, Zumoffen C, Morente C and Ghersevich S** (2019) S100 A9 is expressed and secreted by the oviduct epithelium, interacts with gametes and affects parameters of human sperm capacitation in vitro. *Journal of Cellular Biochemistry* **120**, 17662–17676.
- Mateo-Otero Y, Llavanera M, Torres-Garrido M and Yeste M** (2024) Embryo development is impaired by sperm mitochondrial-derived ROS. *Biological Research* **57**, 5.
- Mateo-Otero Y, Madrid-Gambin F, Llavanera M, Gomez-Gomez A, Haro N, Pozo OJ and Yeste M** (2023) Sperm physiology and in vitro fertilising ability rely on basal metabolic activity: insights from the pig model. *Communications Biology* **6**, 344.
- Mehdi M, Gmidene A, Brahem S, Guerin JF, Elghezal H and Saad A** (2012) Aneuploidy rate in spermatozoa of selected men with severe teratozoospermia. *Andrologia* **44**(Suppl 1), 139–143.
- Mei S, Chen P, Lee C-L, Zhao W, Wang Y, Lam KKW, Ho P-C, Yeung WSB, Fang C and Chiu PCN** (2019) The role of galectin-3 in spermatozoa-zona pellucida binding and its association with fertilization in vitro. *Molecular Human Reproduction* **25**, 458–470.
- Mesguero M, Santiso R, Garrido N and Fernandez JL** (2008) The effect of cancer on sperm DNA fragmentation as measured by the sperm chromatin dispersion test. *Fertility and Sterility* **90**, 225–227.
- Mesguero M, de los Santos MJ, Simón C, Pellicer A, Remohí J and Garrido N** (2006) Effect of sperm glutathione peroxidases 1 and 4 on embryo asymmetry and blastocyst quality in oocyte donation cycles. *Fertility and Sterility* **86**, 1376–1385.
- Milutinović B, Goč S, Mitić N, Kosanović M and Janković M** (2019) Surface glycans contribute to differences between seminal prostasomes from normozoospermic and oligozoospermic men. *Upsala Journal of Medical Sciences* **124**, 111–118.
- Mohammadi R, Mousavi SO, Sheibak N, Amjadi F, Zandieh Z, Aghajanzpour S, Aflatoonian K, Sabbaghian M, Eslami M and Aflatoonian R** (2022) Sperm-oviduct interaction: Differential gene expression of growth factors induced by sperm DNA fragmentation. *Andrologia* **54**, e14378.
- Molina LCP, Luque GM, Balestrini PA, Marin-Briggiler CI, Romarowski A and Buffone MG** (2018) Molecular basis of human sperm capacitation. *Frontiers in Cell and Developmental Biology* **6**, 72.
- Moomjy M, Colombero LT, Veeck LL, Rosenwaks Z and Palermo GD** (1999) Sperm integrity is critical for normal mitotic division and early embryonic development. *Molecular Human Reproduction* **5**, 836–844.
- Mousavi SO, Mohammadi R, Amjadi F, Zandieh Z, Aghajanzpour S, Aflatoonian K, Sabbaghian M, Eslami M, Madani T and Aflatoonian R** (2021) Immunological response of fallopian tube epithelial cells to spermatozoa through modulating cytokines and chemokines. *Journal of Reproductive Immunology* **146**, 103327.



- Muñoz E, Fuentes F, Felmer R, Arias ME and Yeste M (2024) Effects of reactive oxygen and nitrogen species on male fertility. *Antioxidants & Redox Signaling* **40**, 802–836.
- Nagdas SK, Hamilton SL and Samirsubas R (2010) Identification of acrosomal matrix-specific hydrolases binding proteins of bovine cauda epididymal spermatozoa. *Journal of Andrology* **31**, 177–187.
- Nakamura T, Arai Y, Umehara H, Masuhara M, Kimura T, Taniguchi H, Sekimoto T, Ikawa M, Yoneda Y, Okabe M, *et al.* (2007) PGC7/Stella protects against DNA demethylation in early embryogenesis. *Nature Cell Biology* **9**, 64–71.
- Narwade N, Patel S, Alam A, Chattopadhyay S, Mittal S and Kulkarni A (2019) Mapping of scaffold/matrix attachment regions in human genome: a data mining exercise. *Nucleic Acids Research* **47**, 7247–7261.
- Nayel DM, Mahrous HSED, Khalifa EED, Kholeif S and Elhady GM (2021) The effect of teratozoospermia on sex chromosomes in human embryos. *Application of Clinical Genetics* **14**, 125–144.
- Ni K, Spiess AN, Schuppe HC and Steger K (2016) The impact of sperm protamine deficiency and sperm DNA damage on human male fertility: a systematic review and meta-analysis. *Andrology* **4**, 789–799.
- Niederberger C, Pellicer A, Cohen J, Gardner DK, Palermo GD, O'Neill CL, Chow S, Rosenwaks Z, Cobo A, Swain JE, *et al.* (2018) Forty years of IVF. *Fertility and Sterility* **110**, 185–324.e5.
- Nishimura H, Myles DG and Primakoff P (2007) Identification of an ADAM2-ADAM3 complex on the surface of mouse testicular germ cells and cauda epididymal sperm. *Journal of Biological Chemistry* **282**, 11, 17900–17907.
- Nomikos M, Sanders JR, Kashir J, Sanusi R, Buntwal L, Love D, Ashley P, Sanders D, Knaggs P, Bunkheila A, *et al.* (2015) Functional disparity between human PAWP and PLC $\zeta$  in the generation of Ca $^{2+}$  oscillations for oocyte activation. *Molecular Human Reproduction* **21**, 702–710.
- Nomikos M, Sanders JR, Theodoridou M, Kashir J, Matthews E, Nounesis G, Lai FA and Swann K (2014) Sperm-specific post-acrosomal WW-domain binding protein (PAWP) does not cause Ca $^{2+}$  release in mouse oocytes. *Molecular Human Reproduction* **20**, 938–947.
- Nongbua T, Guo Y, Ntallaris T, Rubér M, Rodriguez-Martinez H, Humblot P and Morrell JM (2020) Bull seminal plasma stimulates in vitro production of TGF- $\beta$ , IL-6 and IL-8 from bovine endometrial epithelial cells, depending on dose and bull fertility. *Journal of Reproductive Immunology* **142**, 103179.
- Ntostis P, Carter D, Iles D, Huntriss J, Tzetis M and Miller D (2017) Potential sperm contributions to the murine zygote predicted by in silico analysis. *Reproduction* **154**, 777–788.
- Ohno M, Sakumi K, Fukumura R, Furuichi M, Iwasaki Y, Hokama M, Ikemura T, Tsuzuki T, Gondo Y and Nakabeppu Y (2014) 8-Oxoguanine causes spontaneous de novo germline mutations in mice. *Scientific Reports* **4**, 4689.
- Olderid NB, Wennerholm U-B, Pinborg A, Loft A, Laivuori H, Petzold M, Romundstad LB, Söderström-Anttila V and Bergh C (2018) The effect of paternal factors on perinatal and paediatric outcomes: a systematic review and meta-analysis. *Human Reproduction Update* **24**, 320–389.
- Ostermeier GC, Miller D, Huntriss JD, Diamond MP and Krawetz SA (2004) Delivering spermatozoan RNA to the oocyte. *Nature* **429**, 154.
- Ozturk N, Dansranjav T, Gies S, Calay D, Shiplu S, Creppe C, Hendrickx J and Schagdarsurengin U (2021) H4K20me3 marks distal intergenic and repetitive regions in human mature spermatozoa. *Development* **148**, dev196477.

- Pacey AA, Hill CJ, Scudamore IW, Warren MA, Barratt CLR and Cooke ID (1995) Andrology: The interaction in vitro of human spermatozoa with epithelial cells from the human uterine (Fallopian) tube. *Human Reproduction* **10**, 360–366.
- Pacheco SE, Houseman EA, Christensen BC, Marsit CJ, Kelsey KT, Sigman M and Boekelheide K (2011) Integrative DNA methylation and gene expression analyses identify DNA packaging and epigenetic regulatory genes associated with low motility sperm. *PLoS One* **6**, e20280.
- Paktinat S, Hashemi SM, Ghaffari Novin M, Mohammadi-Yeganeh S, Salehpour S, Karamian A and Nazarian H (2019) Seminal exosomes induce interleukin-6 and interleukin-8 secretion by human endometrial stromal cells. *European Journal of Obstetrics & Gynecology and Reproductive Biology* **235**, 71–76.
- Păunescu TG, Shum WWC, Huynh C, Lechner L, Goetze B, Brown D and Breton S (2014) High-resolution helium ion microscopy of epididymal epithelial cells and their interaction with spermatozoa. *Molecular Human Reproduction* **20**, 929–937.
- Pons-Rejraji H, Artonne C, Sion B, Brugnion F, Canis M, Janny L and Grizard G (2011) Prostatomes: inhibitors of capacitation and modulators of cellular signalling in human sperm. *International Journal of Andrology* **34**, 568–580.
- Rath D, Knorr C and Taylor U (2016) Communication requested: Boar semen transport through the uterus and possible consequences for insemination. *Theriogenology* **85**, 94–104.
- Rawe VY, Terada Y, Nakamura S, Chillik CF, Olmedo SB and Chemes HE (2002) A pathology of the sperm centriole responsible for defective sperm aster formation, syngamy and cleavage. *Human Reproduction* **17**, 2344–2349.
- Recuero S, Sánchez JM, Mateo-Otero Y, Bagés-Arnal S, McDonald M, Behura SK, Spencer TE, Kenny DA, Yeste M, Lonergan P, *et al.* (2020) Mating to intact, but not vasectomized, males elicits changes in the endometrial transcriptome: insights from the bovine model. *Frontiers in Cell and Developmental Biology* **8**, 547.
- Ren X, Chen X, Wang Z and Wang D (2017) Is transcription in sperm stationary or dynamic? *Journal of Reproduction and Development* **63**, 439–443.
- Ribas-Maynou J, Garcia-Bonavilla E, Hidalgo CO, Catalán J, Miró J and Yeste M (2021a) Species-specific differences in sperm chromatin decondensation between eutherian mammals underlie distinct lysis requirements. *Frontiers in Cell and Developmental Biology* **9**, 1143.
- Ribas-Maynou J, Nguyen H, Valle R, Wu H, Yeste M and Ward WS (2022b) Sperm degradation after vasectomy follows a sperm chromatin fragmentation-dependent mechanism causing DNA breaks in the toroid linker regions. *Molecular Human Reproduction* **29**, gaac029.
- Ribas-Maynou J, Novo S, Salas-Huetos A, Rovira S, Antich M and Yeste M (2023) Condensation and protamination of sperm chromatin affect ICSI outcomes when gametes from healthy individuals are used. *Human Reproduction* **38**(3), 371–386.
- Ribas-Maynou J, Novo S, Torres M, Salas-Huetos A, Rovira S, Antich M and Yeste M (2022a) Sperm DNA integrity does play a crucial role for embryo development after ICSI, notably when good-quality oocytes from young donors are used. *Biological Research* **55**, 41.
- Ribas-Maynou J, Yeste M, Becerra-Tomás N, Aston KI, James ER and Salas-Huetos A (2021b) Clinical implications of sperm DNA damage in IVF and ICSI: updated systematic review and meta-analysis. *Biological Reviews of the Cambridge Philosophical Society* **96**, 1284–1300.
- Richard Albert J, Au Yeung WK, Toriyama K, Kobayashi H, Hirasawa R, Brind'Amour J, Bogutz A, Sasaki H and Lorincz M (2020) Maternal DNMT3A-

dependent de novo methylation of the paternal genome inhibits gene expression in the early embryo. *Nature Communications* **11**, 5417.

- Robertson SA, Mau VJ, Tremellen KP and Seamark RF** (1996) Role of high molecular weight seminal vesicle proteins in eliciting the uterine inflammatory response to semen in mice. *Journal of Reproduction and Fertility* **107**, 265–277.
- Robertson SA, Sjöblom C, Jasper MJ, Norman RJ and Seamark RF** (2001) Granulocyte-macrophage colony-stimulating factor promotes glucose transport and blastomere viability in murine preimplantation embryos. *Biology of Reproduction* **64**, 1206–1215.
- Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, Kirkman-Brown J and Coomarasamy A** (2012) The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. *Human Reproduction* **27**, 2908–2917.
- Rodriguez-Caro H, Dragovic R, Shen M, Dombi E, Mounce G, Field K, Meadows J, Turner K, Lunn D, Child T, et al.** (2019) In vitro decidualisation of human endometrial stromal cells is enhanced by seminal fluid extracellular vesicles. *Journal of Extracellular Vesicles* **8**, 1565262.
- Rogenhofer N, Ott J, Pilatz A, Wolf J, Thaler CJ, Windischbauer L, Schagdarsurengin U, Steger K and von Schönfeldt V** (2017) Unexplained recurrent miscarriages are associated with an aberrant sperm protamine mRNA content. *Human Reproduction* **32**, 1574–1582.
- Romero Y, Meikar O, Papaioannou MD, Conne B, Grey C, Weier M, Pralong F, Massy B De, Kaessmann H, Vassalli J-D, et al.** (2011) Dicer1 Depletion in Male Germ Cells Leads to Infertility Due to Cumulative Meiotic and Spermiogenic Defects. *PLoS One* **6**, e25241.
- Ronquist GK, Larsson A, Ronquist G, Isaksson A, Hreinsson J, Carlsson L and Stavreus-Evers A** (2011) Prostatic DNA characterization and transfer into human sperm. *Molecular Reproduction & Development* **78**, 467–476.
- Ruvolo G, Roccheri MC, Brucculeri AM, Longobardi S, Cittadini E and Bosco L** (2013) Lower sperm DNA fragmentation after r-FSH administration in functional hypogonadotropic hypogonadism. *Journal of Assisted Reproduction and Genetics* **30**, 497–503.
- Sadakierska-Chudy A, Patrylak J, Janeczko J and Chudy J** (2020) Downregulation of gene expression and the outcome of ICSI in severe oligozoospermic patients: a preliminary study. *Molecular Reproduction & Development* **87**, 1219–1230.
- Saei P, Bazrgar M, Gourabi H, Kariminejad R, Eftekhari-Yazdi P and Fakhri M** (2021) Frequency of sperm aneuploidy in oligoasthenoteratozoospermic (OAT) patients by comprehensive chromosome screening: a proof of concept. *Journal of Reproduction and Fertility* **22**, 57–64.
- Salas-Huetos A, Blanco J, Vidal F, Mercader JM, Garrido N and Anton E** (2014) New insights into the expression profile and function of micro-ribonucleic acid in human spermatozoa. *Fertility and Sterility* **102**, 213–222.e4.
- Salas-Huetos A, James ER, Aston KI, Carrell DT, Jenkins TG and Yeste M** (2020) The role of miRNAs in male human reproduction: a systematic review. *Andrology* **8**, 7–26.
- Saraf KK, Kumaresan A, Dasgupta M, Karthikkeyan G, Prasad TSK, Modi PK, Ramesha K, Jeyakumar S and Manimaran A** (2020) Metabolomic fingerprinting of bull spermatozoa for identification of fertility signature metabolites. *Molecular Reproduction & Development* **87**, 692–703.
- Sarasa J, Enciso M, García L, Leza A, Steger K and Aizpurua J** (2020) Comparison of ART outcomes in men with altered mRNA protamine 1/protamine 2 ratio

- undergoing intracytoplasmic sperm injection with ejaculated and testicular spermatozoa. *Asian Journal of Andrology* **22**, 623–628.
- Saunders CM, Larman MG, Parrington J, Cox LJ, Royse J, Blayney LM, Swann K and Lai FA** (2002) PLC $\zeta$ : A sperm-specific trigger of Ca $^{2+}$  oscillations in eggs and embryo development. *Development* **129**, 3533–3544.
- Scheffler K, Uraji J, Jentoft I, Cavazza T, Mönnich E, Mogessie B and Schuh M** (2021) Two mechanisms drive pronuclear migration in mouse zygotes. *Nature Communications* **12**, 841.
- Schjenken JE, Glynn DJ, Sharkey DJ and Robertson SA** (2015) TLR4 signaling is a major mediator of the female tract response to seminal fluid in mice. *Biology of Reproduction* **93**, 68.
- Sedó CA, Bilinski M, Lorenzi D, Uriondo H, Noblíá F, Longobucco V, Lagar EV and Nodar F** (2017) Effect of sperm DNA fragmentation on embryo development: Clinical and biological aspects. *JBRA Assisted Reproduction* **21**, 343–350.
- Sharif M, Hickl V, Juarez G, Di X, Kerns K, Sutovsky P, Bovin N and Miller DJ** (2022) Hyperactivation is sufficient to release porcine sperm from immobilized oviduct glycans. *Scientific Reports* **12**, 6446.
- Sharma U, Sun F, Conine CC, Reichholf B, Kukreja S, Herzog VA, Ameres SL and Rando OJ** (2018) Small RNAs are trafficked from the epididymis to developing mammalian sperm. *Developmental Cell* **46**, 481–494.e6.
- Shimura T, Inoue M, Taga M, Shiraishi K, Uematsu N, Takei N, Yuan Z-M, Shinohara T and Niwa O** (2002) p53-dependent S-phase damage checkpoint and pronuclear cross talk in mouse zygotes with X-irradiated sperm. *Molecular and Cellular Biology* **22**, 2220–2228.
- Shukla K, Mahdí A and Rajender S** (2012) Apoptosis, spermatogenesis and male infertility. *Frontiers in Bioscience-Elite* **4**, 746–754.
- Simon L, Emery BR and Carrell DT** (2017a) Review: Diagnosis and impact of sperm DNA alterations in assisted reproduction. *Best Practice & Research Clinical Obstetrics & Gynaecology* **44**, 38–56.
- Simon L, Zini A, Dyachenko A, Ciampi A and Carrell D** (2017b) A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. *Asian Journal of Andrology* **19**, 80–90.
- Smith TB, Dun MD, Smith ND, Curry BJ, Connaughton HS and Aitken RJ** (2013) The presence of a truncated base excision repair pathway in human spermatozoa that is mediated by OGG1. *Journal of Cell Science* **126**, 1488–1497.
- Smith ZD, Chan MM, Humm KC, Karnik R, Mekhoubad S, Regev A, Eggan K, Meissner A** (2014) DNA methylation dynamics of the human preimplantation embryo. *Nature* **511**, 611–615.
- Sun Y, Gao F, Xu D, Lu L, Chen Q, Yang Z, Wang X and Pan X** (2021) Wenshen Shengjing Decoction Improves Early Embryo Development by Maintaining Low H3K27me3 Levels in Sperm and Pronuclear Embryos of Spermatogenesis Impaired Mice. *Evidence-Based Complementary and Alternative Medicine* **2021**, 8035997.
- Talluri TR, Kumaresan A, Sinha MK, Paul N, Ebenezer Samuel King JP and Datta TK** (2022) Integrated multi-omics analyses reveals molecules governing sperm metabolism potentially influence bull fertility. *Scientific Reports* **12**, 10692.
- Tarozzi N, Nadalini M, Coticchio G, Zacà C, Lagalla C and Borini A** (2021) The paternal toolbox for embryo development and health. *Molecular Human Reproduction* **27**, gaab042.

- Teijeiro JM and Marini PE** (2012) The effect of oviductal deleted in malignant brain tumor 1 over porcine sperm is mediated by a signal transduction pathway that involves pro-AKAP4 phosphorylation. *Reproduction* **143**, 773–785.
- Tourzani DA, Battistone MA, Salicioni AM, Breton S, Visconti PE and Gervasi MG** (2021) Caput ligation renders immature mouse sperm motile and capable to undergo cAMP-dependent phosphorylation. *International Journal of Molecular Sciences* **22**, 10241.
- Turner KA, Fishman EL, Asadullah M, Ott B, Dusza P, Shah TA, Sindhwani P, Nadiminty N, Molinari E and Patrizio P** (2021) Fluorescence-Based Ratiometric Analysis of Sperm Centrioles (FRAC) Finds Patient Age and Sperm Morphology Are Associated With Centriole Quality. *Frontiers in Cell and Developmental Biology* **9**, 658891.
- Twenter H, Klohonatz K, Davis K, Bass L, Coleman SJ, Bouma GJ and Bruemmer JE** (2020) Transfer of microRNAs from epididymal epithelium to equine spermatozoa. *Journal of Equine Veterinary Science* **87**, 102841.
- Vallet-Buisan M, Mecca R, Jones C, Coward K and Yeste M** (2023) Contribution of semen to early embryo development: fertilization and beyond. *Human Reproduction Update* **29**, 395–433.
- Vaughan DA, Tirado E, Garcia D, Datta V and Sakkas D** (2020) DNA fragmentation of sperm: a radical examination of the contribution of oxidative stress and age in 16945 semen samples. *Human Reproduction* **35**, 2188–2196.
- Vieweg M, Dvorakova-Hortova K, Dudkova B, Waliszewski P, Otte M, Oels B, Hajimohammad A, Turley H, Schorsch M, Schuppe HC, et al.** (2015) Methylation analysis of histone H4K12ac-associated promoters in sperm of healthy donors and subfertile patients. *Clinical Epigenetics* **7**, 31.
- Wang D, Cheng L, Xia W, Liu X, Guo Y, Yang X, Guo X and Xu EY** (2020) LYPD4, mouse homolog of a human acrosome protein, is essential for sperm fertilizing ability and male fertility. *Biology of Reproduction* **102**, 1033–1044.
- Wang L, Magdaleno S, Tabas and Jackowski S** (2005) Early Embryonic Lethality in Mice with Targeted Deletion of the CTP:Phosphocholine Cytidylyltransferase  $\alpha$  Gene (*Pcyt1a*). *Molecular and Cellular Biology* **25**, 3357–3363.
- Watkins AJ, Dias I, Tsuru H, Allen D, Emes RD, Moreton J, Wilson R, Ingram RJM and Sinclair KD** (2018) Paternal diet programs offspring health through sperm- and seminal plasma-specific pathways in mice. *Proceedings of the National Academy of Sciences of the United States of America* **115**, 10064–10069.
- Weigel Muñoz M, Battistone MA, Carvajal G, Maldera JA, Curci L, Torres P, Lombardo D, Pignataro OP, Da Ros VG and Cuasnicú PS** (2018) Influence of the genetic background on the reproductive phenotype of mice lacking Cysteine-Rich Secretory Protein 1 (CRISP1). *Biology of Reproduction* **99**, 373–383.
- Wolff M von, Rösner S, Thöne C, Pinheiro RM, Jauckus J, Bruckner T, Biolchi V, Alia A and Strowitzki T** (2009) Intravaginal and intracervical application of seminal plasma in in vitro fertilization or intracytoplasmic sperm injection treatment cycles—a double-blind, placebo-controlled, randomized pilot study. *Fertility and Sterility* **91**, 167–172.
- Wong CC, Loewke KE, Bossert NL, Behr B, Jonge CJ De, Baer TM and Pera RAR** (2010) Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. *Nature Biotechnology* **28**, 1115–1121.

- Wu ATH, Sutovsky P, Manandhar G, Xu W, Katayama M, Day BN, Park KW, Yi YJ, Yan WX, Prather RS, *et al.* (2007) PAWP, a sperm-specific WW domain-binding protein, promotes meiotic resumption and pronuclear development during fertilization. *Journal of Biological Chemistry* **282**, 12164–12175.
- Yagi M, Kabata M, Tanaka A, Ukai T, Ohta S, Nakabayashi K, Shimizu M, Hata K, Meissner A and Yamamoto T (2020) Identification of distinct loci for de novo DNA methylation by DNMT3A and DNMT3B during mammalian development. *Nature Communications* **11**, 3199.
- Yáñez-Mó M, Siljander PRM, Andreu Z, Zavec AB, Borràs FE, Buzas EI, Buzas K, Casal E, Cappello F, Carvalho J, *et al.* (2015) Biological properties of extracellular vesicles and their physiological functions. *Journal of Extracellular Vesicles* **4**, 27066.
- Yelumalai S, Yeste M, Jones C, Amdani SN, Kashir J, Mounce G, da Silva SJM, Barratt CL, McVeigh E and Coward K (2015) Total levels, localization patterns, and proportions of sperm exhibiting phospholipase C zeta are significantly correlated with fertilization rates after intracytoplasmic sperm injection. *Fertility and Sterility* **104**, 561–568.e4.
- Yeste M, Delgado-Bermúdez A, Jones C and Coward K (2023) Functions and gene expression alterations of phospholipase C in gametes. In Chakraborti S (ed), *Phospholipases in Physiology and Pathology (Volume 4): Role of Phospholipases in Inflammation, Gene Expression, and Apoptosis*. London: Elsevier, pp. 355–389.
- Yeste M, Jones C, Amdani SN, Patel S and Coward K (2016) Oocyte activation deficiency: a role for an oocyte contribution? *Human Reproduction Update* **22**, 23–47.
- Yin Q, Shen J, Wan X, Liu Q, Zhou Y and Zhang Y (2018) Impaired sperm maturation in conditional Lcn6 knockout mice. *Biology of Reproduction* **98**, 28–41.
- Yoshida K, Muratani M, Araki H, Miura F, Suzuki T, Dohmae N, Katou Y, Shirahige K, Ito T and Ishii S (2018) Mapping of histone-binding sites in histone replacement-completed spermatozoa. *Nature Communications* **9**, 3885.
- Zambrano F, Carrau T, Gärtner U, Seipp A, Taubert A, Felmer R, Sanchez R and Hermosilla C (2016) Leukocytes coincubated with human sperm trigger classic neutrophil extracellular traps formation, reducing sperm motility. *Fertility and Sterility* **106**, 1053–1060.e1.
- Zambrano F, Namuncura C, Uribe P, Schulz M, Pezo F, Burgos R, Taubert A, Hermosilla C and Sanchez R (2021) Swine spermatozoa trigger aggregated neutrophil extracellular traps leading to adverse effects on sperm function. *Journal of Reproductive Immunology* **146**, 103339.
- Zegers-Hochschild F, Schwarze JE and Alam V (2008) Infertility. In Heggenhougen, HK (ed), *International Encyclopedia of Public Health*. New York: Academic Press, pp. 576–587.
- Zhang Y, Zhang X, Shi J, Tuorto F, Li X, Liu Y, Liebers R, Zhang L, Qu Y, Qian J, *et al.* (2018) Dnmt2 mediates intergenerational transmission of paternally acquired metabolic disorders through sperm small non-coding RNAs. *Nature Cell Biology* **20**, 535–540.
- Zhao J, Zhang Q, Wang Y and Li Y (2014) Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Fertility and Sterility* **102**, 1005.e8.



- Zhao Y, Li Q, Yao C, Wang Z, Zhou Y, Wang Y, Liu L, Wang Y, Wang L and Qiao Z** (2006) Characterization and quantification of mRNA transcripts in ejaculated spermatozoa of fertile men by serial analysis of gene expression. *Human Reproduction* **21**, 1583–1590.
- Ziskind G, Paltieli Y, Eibschitz I, Ohel G and Weichselbaum A** (2000) The effect of human fallopian tube epithelium on human sperm velocity motility and binding. *Journal of Assisted Reproduction and Genetics* **17**, 147–150.

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**Marc Yeste** graduated with a Bachelor of Science (Biology), and earned a European PhD in Cell Biology. He also graduated with a Bachelor of Political and Social Sciences, and a Bachelor of Laws, and read additional BA (Phil) courses. He was previously Visiting Researcher at the Institute of Zoology, Zoological Society of London; Postdoctoral Researcher (Juan de la Cierva) at the Autonomous University of Barcelona; Senior Postdoc (Marie Curie) at the University of Oxford; and Senior Research Fellow (Ramón y Cajal) at the University of Girona. Currently, he is an ICREA Academia Professor at the Department of Biology, University of Girona. His research is focused on Reproductive Biology and Fertility in humans and other mammals. He is a Fellow of the Higher Education Academy (HEA), Fellow of the Royal Society of Biology (RSB), Fellow of the Young Academy of Europe (YAE), and a Member of the Royal European Academy of Doctors. Since 2023, he has been the Secretary of the Young Academy of Europe.