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# The Sperm Journey to Fertilization: a Predetermined Encounter

Die Reise des Samens zur Befruchtung: ein vordefiniertes Aufeinandertreffen

**Abstract**

Sperm interact with various microenvironments, generating conditions for sperm capacitation and acrosome reaction in the right place and time. Such events are highly synchronized to ensure fertilization. This review describes and analyzes the various changes sperm experience during transport through the female genital tract: the influence of cervical, endometrial and oviductal secretions, interaction with the epithelium, and hormonal effects on sperm. Molecules in the microenvironments with which sperm interact include hormones, neurotransmitters, and other metabolites. Physiological events of gamete membrane fusion should be considered by basic and applied researchers working in reproductive biology.

**Keywords: acrosome reaction, cervix, fertilization, hormones, sperm migration**

**Zusammenfassung**

Spermien interagieren mit verschiedenen Faktoren der Mikroumgebung, wodurch die Voraussetzungen für die Endreifung der Spermien und die Akrosomreaktion am richtigen Ort und zur richtigen Zeit geschaffen werden. Die exakte Synchronisierung dieser Faktoren sichert die Befruchtung. Dieser Aufsatz beschreibt die Einflüsse, denen die Spermien bei ihrer Passage durch den weiblichen Genitaltrakt ausgesetzt sind, wie Sekrete der Cervix, des Endometriums und des Eileiters, sowie Interaktionen mit dem Epithel. Ferner interagieren die Spermien mit Molekülen in der Mikroumgebung, bestehend aus Hormonen, Neurotransmittern und verschiedenen Metaboliten.

**Schlüsselwörter: Akrosomreaktion, Zervix, Befruchtung, Hormone, Migration der Spermien**

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## Introduction

Sperm transport through the female genital tract starts with deposition in the upper third of the vagina. From here sperm migrate through the cervix and then ascend to the endometrial cavity towards the oviduct, in order to reach the site of fertilization. Fertilization will occur in the distal third of the oviduct, after a lengthy journey that can take from 5-10 minutes to about 6 days.[1] During transport sperm will interact with a number of structures and secretions of the female genital tract, which are heavily influenced by sex steroid hormones. Contact with these and other components will allow the sperm to undergo an ordered series of changes that will capacitate them for successful fertilization.

Hormone concentrations in the female reproductive tract vary considerably depending on anatomical location and phase of a woman's menstrual cycle. Interestingly, estrogens (e. g. estradiol), progesterone and testosterone will have different effects – they can even be antagonistic – on sperm changes.[2] Successful fertilization requires that these changes occur both in an ordered sequence and in appropriate temporal and spatial conditions.

The various processes that sperm go through from ejaculation are called sperm capacitation; they comprise all changes that prepare sperm for the acrosome reaction (AR).[3]

Sperm capacitation includes modification of flagellar activity, characterized by changes in the head's angle of movement and a rise in tail motility. Such movements go from low amplitude and high frequency, to facilitate rapid ascent through the female tract, to high amplitude and low frequency (known as hyperactivation), to facilitate passage among the granulosa cells surrounding the oocyte.[4] If, for instance, hyperactivation takes place long before sperm reach the oocyte, flagellar movement would not allow sperm to progress towards the site of fertilization.

Once capacitation is completed, sperm undergo the AR, *i. e.* sperm plasma membrane fusion with the outer acrosomal membrane. In the AR the contents of the acrosomal vesicle, comprised of enzymes such as proacrosin, acrosin, hyaluronidase and trypsin, are released, helping sperm to penetrate the oocyte coats.[5]

Gamete fusion usually occurs between the equatorial segment of the sperm plasma membrane and the oocyte plasma membrane.[6] The AR is essential for changes to take place in the equatorial and post-equatorial segments of the sperm plasma membrane, in order to enable binding and fusion.[7] Consequently, if a sperm does not undergo the AR, it will not acquire the conditions necessary for gamete membrane fusion.

The above phenomena are affected by sperm environment. Sex steroid hormones, substances secreted by the multiple cell types and cells forming the female reproductive tract play a crucial role in activating or inhibiting changes during capacitation, AR and fertilization. The purpose of this review is to describe and analyze sperm changes during transport through the female genital tract, and how these changes are influenced by sperm environment. Special attention will be given to ART (artificial reproduction techniques) and their implication for future generations.

## 1. Sperm passage through the cervix and interaction with cervical mucus

Cervical mucus is produced only in the cervix of some species such as rabbits, bovines and humans.[88] The existence of two forms of transport through human cervical mucus has been described: a fast one, in which the sperm deposited in the superior third of the vagina, would reach the oviduct in just 5-10 minutes, and a slow one, in which the sperm would be stored in the cervical crypts.[9] Sperm lifespan in the cervix is variable: from a few hours up to 6 days[10] in the presence of estrogenic mucus.

In humans it is essential that sperm pass through or remain in the cervix, because cervical mucus performs a series of biological functions,

namely: 1) selection of morphologically normal sperm, 2) preservation of the acrosome to maintain fertilizing ability, 3) antimicrobial action of certain metabolites present in cervical mucus, and 4) delivery of an adequate nutritive environment for the sperm. Importantly, not all mammalian species produce cervical mucus. Presence of this hydrogel in humans could derive from the evolutionary need for a selective barrier filtering out abnormal sperm, which can amount to 95% of the total sperm in one ejaculation.[11] In contrast, abnormal forms in rodents do not exceed 5 to 10%.[12]

The ability to select normal sperm entails blocking passage of those with morphological abnormalities or motility disorders.[13] It has been confirmed that sperm with morphological abnormalities of the head exhibit inadequate mucus penetration.[14] Cervical mucus not only selects sperm based on morphology and motility, it also acts on sperm with genetic and chromosomal abnormalities.[15] This is relevant because chromatin abnormalities are known to have a negative impact on male fertility, altering fertilization, embryonic development and pregnancy.[16]

It is well known that variations in sex steroid hormone levels during the menstrual cycle generate changes in the physical and biochemical characteristics of the mucus.[17] It has also been de-

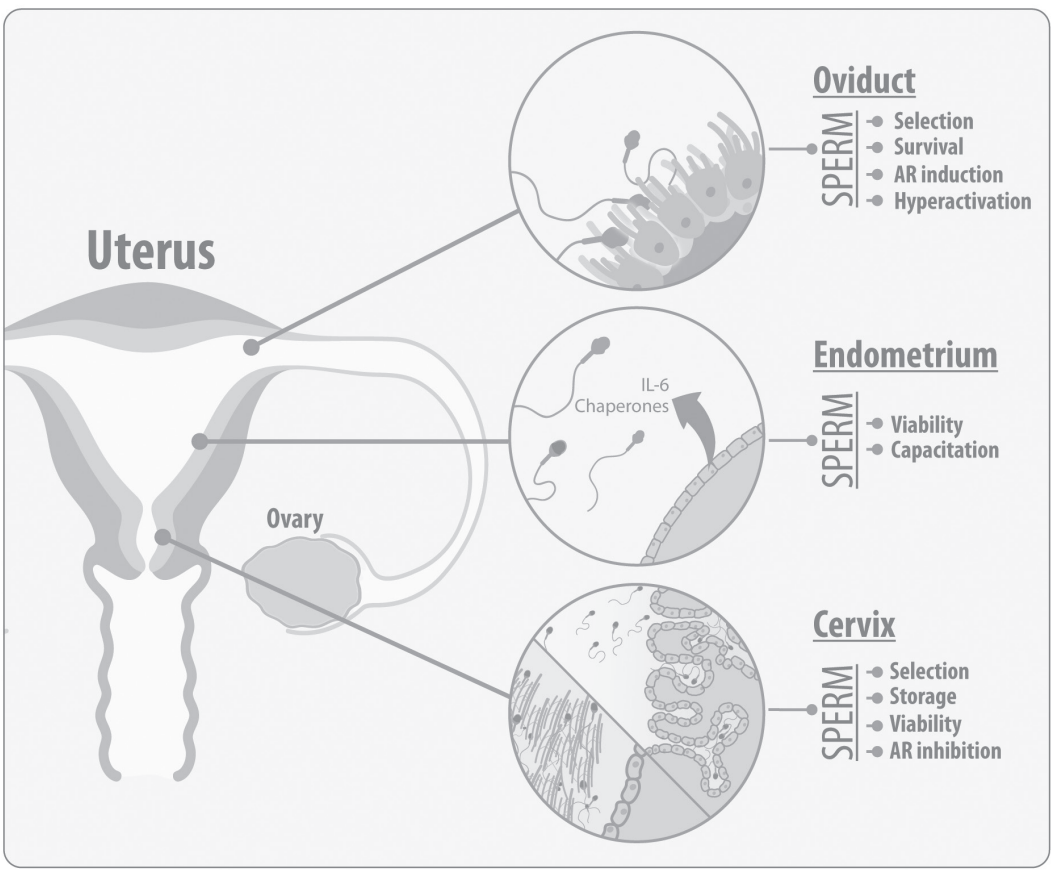


*Figure 1. Scheme of female reproductive tract where the main areas of interaction with the sperm are indicated. The cervix, the endometrium and the oviduct are illustrated in detail. The molecules with which sperm interact in the different areas and the processes occurring are also indicated. The filaments shown in the cervix represent the cervical mucus. GABA: gamma-aminobutyric acid; ANP: atrial natriuretic peptide; IL-6: interleukin-6.*

monstrated that steroid hormones have a direct effect on sperm physiology. An example is the inhibition of the sperm AR that occurs with the presence of estradiol in a dose dependent way *in vitro* and *in vivo*.[18] It is interesting to note that high estradiol concentrations are present in the aquous phase of cervical mucus during the woman's fertile period.[19] This could be an adaptive mechanism to facilitate fertilization, since sperm can remain in the cervical crypts for several days. High estrogen contents in this period could ensure preservation of acrosomal integrity, assuring that the AR occurs during the encounter with the oocyte. Regarding sperm, estradiol would act through a non-genomic pathway characterized by binding to membrane receptors, which generates calcium influx and tyrosine phosphorylations in certain proteins that would mediate non-genomic effects.[20]

Presence of testosterone has also been documented in cervical mucus.[21] *In vitro* studies have verified that testosterone inhibits the AR with mechanisms involving a change in sperm plasma membrane fluidity, and an increase in intracellular calcium concentrations.[22] Within this context, testosterone has been reported to generate minor disturbances in the fluidity of the plasma membrane and outer acrosomal membrane, which would prevent gamete membrane fusion. Recent studies have demonstrated that physiological testosterone concentrations inhibit acrosomal exocytosis in human sperm.[23] In this way, adequate testosterone levels in both cervical mucus and seminal plasma would enhance the arrival of a large percentage of acrosome-intact sperm to the uterine cavity. This suggests that hormonal variations in a woman's menstrual cycle would not only facilitate successful ovulation, but also would contribute to the ordered and essential sequence of events required by sperm to achieve fertilization.

## 2. Sperm transport through the endometrial cavity

After leaving the cervical canal sperm enter the uterine cavity, where they can remain for 2 to 2.5 days, and make contact with endometrial cells and their secretions. Some studies have suggested that endometrial cells could participate in sperm capacitation.[24]

Endometrial cells secrete a variety of cytokines, of which the most widely studied is interleukin-6 (IL-6), which induces the AR, but in high concentrations it has a deleterious effect on sperm, lowering motility and viability.[25] In the proliferative phase of the menstrual cycle, epithelial endometrial cells facilitate sperm capacitation by secreting IL-6. But in the luteal phase, production of endometrial IL-6 increases, potentially leading to a negative impact on sperm fertilizing ability.[26]

Two chaperone proteins have been described in the endometrium: heat shock protein (HSP60) and glucose-regulated protein 78 (GRP78), which would also take part in sperm capacitation.[27]

## 3. Sperm transport through the oviduct

In humans, after swimming up the endometrial cavity, a few thousand spermatozoa will ascend towards the oviduct in their journey to meet the oocyte. It is in the oviduct that sperm complete capacitation and the AR is triggered. In addition to allowing gamete passage, the oviduct plays an active role in modulating sperm binding to the oocyte. While moving up the oviduct, sperm develop a close relationship with oviductal epithelial cells.[28] This relationship between sperm and oviductal epithelial cells has been widely described in different mammalian species.[29] It has been proposed that sperm binding to oviductal epithelial cells, extends for variable periods of time and boosts sperm fertile lifespan.[30] Sperm-oviduct interaction has been proposed to depend on sperm membrane integrity and described as a sperm selection mechanism, which ends with the gradual release from the epithelium of hyperactivated sperm with high fertilizing ability.[31]

Sperm interaction with the oviduct goes beyond physical binding. Several studies suggest that sperm may modulate the oviduct environment. This modulation seems to be mediated through a differential genetic expression of the oviduct that is induced by sperm, and the magnitude of this modulation depends on whether it carries an X or Y chromosome.[32] These studies suggest that sperm modulate their own environment in a sexual dependent way which would have repercussions on the immune response of the oviduct generated by the future presence of the embryo.

In addition to interacting with oviduct cells, sperm can remain in contact with oviductal secretions for hours and even days.[33] Several molecules have been described in the oviductal fluid that interact directly with the sperm, including atrial natriuretic peptide (ANP), catecholamines and gamma-aminobutyric acid (GABA). There is evidence that ANP,[34] some catecholamines (adrenaline and noradrenaline),[35] and GABA[36] participate in the AR induction.[37] Dopamine, another catecholamine (commonly known for its role as a neurotransmitter) is found in substantial concentrations throughout sperm transport up the human oviduct.[38] Interestingly, high dopaminergic concentrations affect sperm motility and alter capacitation, possibly acting through the dopamine receptor.[39] Dopamine has been proposed to have an immobilizing effect on spermatozoa in the oviductal reservoirs.

Consequently, the oviduct accomplishes three significant roles in sperm transport: 1) sperm reservoir, 2) selection of the best sperm, and 3) modulation of the AR.

## 4. Sperm interaction with the follicular fluid

Later on its way, sperm interact with the follicular fluid. After ovulation, follicular fluid and the cumulus-oocyte complex are captured by the oviduct. This fluid is composed of sex steroid hormones such as progesterone, estrogen and testosterone; peptides such as ANP, angiotensin II and GnRH; and polysaccharides such as glycosaminoglycans. Although a large proportion of its content is contributed by granulosa cells, oocyte molecular products and theca interna secretions are also observed in follicular fluid.[40] After ovulation, the released follicular fluid generates chemoattraction on sperm. Apparently, sperm with the highest fertilizing ability respond better to this chemotactic activity.[41]

As described above, progesterone and ANP induce the AR, while estradiol and testosterone inhibit or delay it. Progesterone, specifically, which is produced in the follicle and accumulates in the antral fluid, is one of the most potent AR inducers. Interestingly, progesterone levels in humans rise just before ovulation.[42] High progesterone concentrations in the follicular fluid accompanying the oocyte at ovulation would encourage adequate oocyte-sperm interaction, encouraging a temporally and spatially suitable AR within the fertilization process.

In women with polycystic ovary syndrome and elevated androgen levels, the effect of testosterone could be relevant. Testosterone levels in the follicular fluid of these women have been demonstrated to be higher than those observed in control women with normal testosterone levels.[43] In this scenario, ovulation would generate the release of a cumulus-oocyte complex accompanied by testosterone-rich follicular fluid, which, when meeting the sperm, could delay the AR and affect oocyte fertilization.

## 5. Sperm passage through the cumulus cells

When sperm approach the oocyte, they must go through two surrounding layers: the cumulus cells and the zona pellucida.

The cumulus is not only a barrier to be overcome by sperm but it also secretes factors that impact sperm. The soluble components released by cumulus cells alter sperm motility, generating a forward movement pattern.[44] Some of the possible factors that are secreted by the oviduct and could modulate sperm movement, are prostaglandins. It has

been shown that blocking prostaglandin biosynthesis results in lower fertilization rates.[45] Progesterone has been identified as an additional candidate; since, as stated above, it is an inducer of flagellar hyperactivation and a promoter of the AR.[46] Also, proteins from the chemokine ligands family (CCL), secreted from the cumulus cells, facilitate sperm migration towards the oocyte.[47]

Additionally, it has been shown that the spermatozoa that are able to penetrate the zona pellucida and fuse with the oocyte membrane, begin the AR before reaching the zona pellucida, while passing through the cumulus.[48]

Cumulus cells, therefore, not only support and protect the oocyte but also actively participate in gamete interaction (Figure 2A).

## 6. Sperm passage through the zona pellucida

Continuing its journey, after penetrating the cumulus cells, sperm reach the zona pellucida (ZP). The presence of the acrosomal vesicle, with its enzymatic contents, is vital to sperm passage through the ZP. In humans it has been shown that round headed sperm, which do not have an acrosomal vesicle, are unable to adhere to or penetrate the ZP.[49]

The ZP of both rat and human oocytes is made up of a network of thin interconnected filaments forming a porous mesh.[50] The pores are vital for sperm to penetrate the ZP and reach the perivitelline space, where they make contact with the oocyte plasma membrane. In species such as rabbits, hamsters and humans, sperm follow an oblique path through the ZP. This is particularly relevant when considering that once the sperm is present in the perivitelline space, it allows the plasma membrane of the equatorial or post-equatorial segment to make contact and fuse with the oocyte plasma membrane[51] (Figure 2B).

The mammalian ZP consists of three or four glycoproteins designed as ZP1 (zona pellucida sperm-binding protein 1), ZP2 (zona pellucida sperm-binding protein 2), ZP3 (zona pellucida sperm-binding protein 3) and ZP4 (zona pellucida sperm-binding protein 4). Only humans, some primates and rats exhibit all four glycoproteins.[52]

The involvement and function of the multiple glycoproteins making up the ZP matrix during fertilization has generated great interest. In mice, ZP2 and ZP3 are the major zona pellucida-sperm binding mediators; and they participate in the AR as well.[53] In humans, though, several studies have shown that, in addition to ZP2 and ZP3, ZP4 also takes part in sperm binding and AR.[54]

It has been described in humans that a variety of oligosaccharide residues, such as N-acetyl glucosamine, fucose and mannose, are involved in sperm-ZP binding.[55] Certain glycodelin isoforms are potent inhibitors of sperm-ZP binding in humans,[56] but other isoforms promote binding even though the protein nucleus of these glycodelins is the same.[57] There is evidence that shows that a considerable portion of the specificity of these glycoproteins is given by their glycosylation patterns, thus strongly influencing the fine regulation of sperm-ZP binding.

## 7. Gamete plasma membrane fusion

Sperm-ZP binding leads to the AR, which enables sperm to penetrate the ZP and gain access to the perivitelline space, an extracellular region adjacent to the oocyte plasma membrane. This is where the last adhesion process in the path to fertilization occurs: binding of the sperm plasma membrane to that of the oocyte[58] (Figure 2C). Adequate binding (adhesion) precedes and is required for gamete plasma membrane fusion.

At the molecular level, at least two adhesion protein families involved in sperm-oocyte membrane fusion have been described. In mammals, CD9 and CD81, proteins of the tetraspanin family present in the oocyte membrane, are crucial to oocyte fusion. Studies in mutant mice for CD9[59] and double knockout mice for CD9 and CD81, confirmed partial and total infertility, respectively.[60]

Another group of surface proteins in the oocyte involved in the fusion process are those anchored

by glycosylphosphatidylinositol (GPI-Aps). They are quite diverse in functional terms and have been described as cellular adhesion, receptor, enzyme and cellular signaling components. It has been shown that enzymatic removal of two of these GPI-APs alters sperm-oocyte binding and blocks plasma membrane fusion.[61]

It has been described that ADAM family proteins (proteins with A Disintegrin And Metalloproteinase domains), present in the sperm plasma membrane, are required for fertilization. Studies in knockout mice for two proteins of this family, fertilin β and cyritestin, documented that sperm binding to ZP-free oocytes fell by 90%.[62]

Gamete plasma membrane fusion marks the end of the sperm journey: the male and female pronucleus are formed and the zygote is thus originated. The oocyte extrudes the second polar body and the zygote acquires the corresponding genetic load to the species to which it belongs.

This sequence of coordinated events ends with fertilization, giving origin to offspring whose gametes have gone through rigorous selection processes. When using certain assisted reproductive techniques, gamete selection and initial embryonic development occur differently and effects on the resulting offspring have not been thoroughly studied yet.

## 8. Risks for Offspring after using ART

At present, about 10% of couples present a fertility problem.[63] Since the first human born using in vitro fertilization (IVF), an assisted reproductive technique (ART),[64] was born in 1978, these methods have been employed all over the world as an infertility treatment. More than 4 million people are estimated to have been born as a result of this technology and the number continues to grow.[65]

For decades clinical reports concluded that ARTs did not increase risks for offspring, but the long-term consequences of these techniques were ignored. Only in the 2000s did studies show for the first time that individuals conceived with ARTs (mainly IVF and intracytoplasmic sperm injection (ICSI)) had a bigger risk of developing certain pathologies such as insulin resistance,[66] type 2 diabetes,[67] obesity,[68] hypertension,[69] coronary alterations[70] and thyroid dysregulation.[71] Some of these metabolic disorders, e. g. insulin resistance and hypothyroidism, will affect the reproductive function of such individuals in the future. Females will experience ovulatory dysfunction,[72] while males sperm production will be affected.[73] These alterations have been partially associated with differences in epigenetic imprinting when using ART.

The epigenetics revolution hit in the early 2000s, when scientists began reporting that

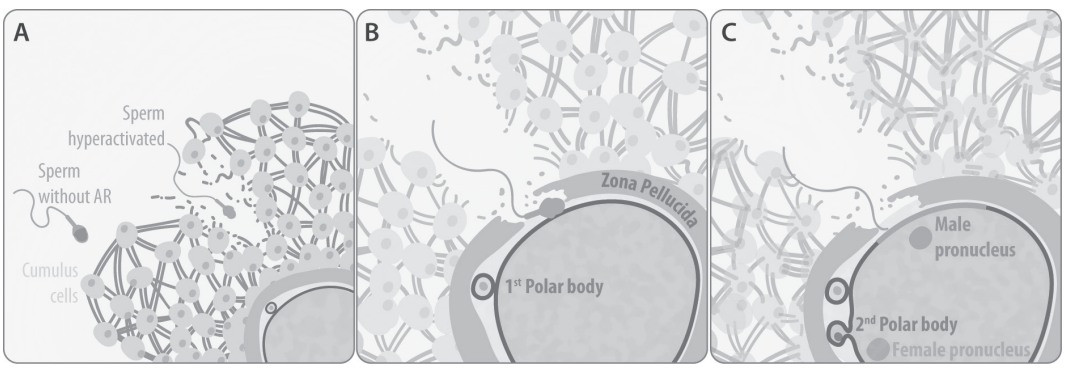


*Figure 2. Passage of sperm through the cumulus cells, the zona pellucida and fusion with the oocyte plasma membrane. Figure 2A shows the soluble molecules (progesterone, PGE1, and PGE2) with which sperm interact when contacting the cumulus cells. Figure 2B shows the degradation of the zona pellucida resulting from the interaction with reacted sperm. Figure 2C shows the fusion of the sperm membrane with the oocyte membrane. PGE1: Prostaglandin E1; PGE2: Prostaglandin E2.*

environmental factors can influence the addition or removal of chemical tags on DNA that turn genes on and off.[74] It was observed that, for some mammalian species, including humans, the environment in which the first stages of embryonic development occurred had a substantial impact on the metabolic conditions of adult individuals.[75] Researchers also demonstrated a relationship between malnutrition during pregnancy and risk of certain adult-life diseases, such as coronary disease, hypertension, type 2 diabetes and metabolic syndrome. Similarities in effects seen in women with nutritional deficiencies during pregnancy and individuals conceived with ART suggest, first, that the metabolic environment in the early stages of development plays a major role in homeostatic regulation and, second, that the environment surrounding the embryo conceived with ART would not be optimal for the species.

To prove this hypothesis in rats, animals were malnourished or fed with a low-protein diet in the preimplantation period, which resulted in low weight and abnormal blood pressure in offspring.[76] These preconception influences on development are believed to occur through environment-induced modification of the embryonic epigenome.[77]

DNA methylation, histone modification, miRNAs and high-order DNA packaging in nucleosomes[78] are the major mechanisms of epigenetics. DNA methylation – the addition of a methyl group to a cytosine located in a cytosine-phosphate-guanine dinucleotide – is by far the most studied epigenetic mechanism. DNA methylation usually turns genes off, because it causes the binding of transcriptional repressors to the modified dinucleotide. In humans, more than 50 genes have been described with one allele repressed through DNA methylations,[79] a phenomenon known as imprinting that ensures correct gene dosage. These epigenetic events take place during both the development of germinal cells (which originate the gametes) and the preimplantation stage of embryonic development.[80]

The metabolic disorders listed above could be explained by imprinting alterations, more prevalent in gametes and embryos from ART procedures than in their *in vivo* counterparts.[81] In animal models, these imprinting-specific alterations have also been associated with pathologies such as metabolic syndrome and glucose metabolism dysregulation.[82]

Additionally, some alterations found in ART-conceived individuals could derive from the gamete selection process. In both IVF and ICSI various selection techniques are used with sperm, such as swim-up and centrifugation in Percoll gradients with multiple washings in culture medium. After completing the selection process, the recovered spermatozoa are capacitated and highly motile, and possess high fertilizing ability. We have shown that the ratio of normal spermatozoa selected with these procedures exceeds the ratio found in the original seminal sample, however it is lower than when selection involves sperm passage through a column of estrogenic cervical mucus.[83] The recovered oocytes, in turn, are incubated in multiple culture media that are rich in nutrients, but incapable of accurately simulating the complex environment found in the oviduct at fertilization. Before collection, the oocytes are exposed to high hormone concentrations during the ovarian stimulation process. Upon extraction from the ovarian follicle the oocytes undergo thermal and mechanical stress, which prepares them for the different ARTs.

*In vitro* culture, selection and hormonal stimulation expose gametes to factors that could alter the correct establishment of imprinting, as well as other epigenetic and functional components. Possible epigenetic alterations generated by ARTs, particularly their long-term consequences, are hard to assess. The notion that epigenetic marks are transmitted across generations is even more provocative.[84]

Nevertheless, since ARTs are mostly applied to individuals with infertility problems, comparisons with the general population are difficult because

infertile individuals can differ genetically from fertile ones. This cannot, however, invalidate the available evidence of environment-induced modifications to the human genome.

## Conclusions

An analysis of the available evidence proves that cell recognition, adhesion and fusion depend on a complex network of molecular interactions. Variations in some of these components, vital to specificity in inter- and intraspecific gamete recognition, would lead to fertilization failure.

Although the evidence of sperm physiology in the female reproductive tract is abundant, it remains insufficient to fully understand this long and complex process of capacitation and fertilization. The evidence shown in this review suggests that many of the events and interactions that occur in vivo during this process appear to be important for gamete selection process, embryo development and future generations.

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## AUTHORS' CONTRIBUTIONS

PV wrote the paper together with IV-U and made the literature search. JPDR participated in the writing of the manuscript, provided critical feedback and editorial input. FGS drew the illustrations.

## CONFLICT OF INTERESTS

None of the authors declare competing financial interests.

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