The oviduct: a key to unlocking reproductive science

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ABSTRACT

Despite significant advances, assisted reproductive techniques (ARTs) remain suboptimal due to challenges linked to embryo development and quality, often attributed to the epigenetic reprogramming process and environmental factors. The aim of this review is to summarize the function of the oviduct, the organ where fertilization takes place. This organ plays a crucial but often overlooked role in natural fertilization, where it selectively allows only a few sperm to reach the egg, contrary to what happens in *in vitro* conditions, where oocytes are exposed many sperm, without this always resulting in fertilization. The oviduct environment is vital for fertilization, since it creates a reservoir for sperm, extending their viability and readiness for fertilization. In addition, the environment provided by this organ is optimal for the early stages of embryo development. Interactions between embryos and oviduct cells affect gene expression, improving embryo quality. Mimicking the conditions of the oviduct, by using oviductal fluid in culture media, for example, or developing advanced 3D cultures and microfluidic systems, can improve ART outcomes and embryo quality. In conclusion, the oviduct is essential for creating the optimal environment for fertilization and embryo development. Replicating its conditions can enhance ART success and the health of the offspring, highlighting its importance in reproductive processes.

KEYWORDS

Oviduct, ART, reproduction.

Introduction

As is well known, the problem of infertility has grown in recent years, leading to an increase in the use of assisted reproductive technologies (ARTs), resulting in more than two million children in Europe being born through use of these techniques [1].

Since their creation, ARTs have undergone continuous improvements and refinements. However, despite the advances achieved, ARTs are still suboptimal. Not all oocytes successfully develop into embryos, and the ones that do reach the blastocyst stage may not exhibit the same quality as their *in vivo* counterparts, showing reduced developmental capacity in bovine [2] and swine [3] species, for example. This suboptimal quality can primarily be attributed to the epigenetic reprogramming process that occurs during the early stages of mammalian embryo development when the embryo consists of just a few cells [4], making it susceptible to environmental factors. For instance, maternal malnutrition has been observed to affect imprints on the offspring's epigenome [5]. Furthermore, this susceptibility to the environment makes embryos exceptionally vulnerable to suboptimal *in vitro* conditions, which notably differ from the natural conditions of the female reproductive tract. These differences in environmental factors, such as oxygen levels or temperature, can influence the developmental trajectory of the embryo [6].

The purpose of this review is to compile and highlight the functions of the fallopian tube or oviduct, the organ where life begins and fertilization takes place. Often overlooked in discussions about reproductive health, the fallopian tube plays a crucial role in the early stages of conception.

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The forgotten organ

While millions of ejaculated sperm can be counted in the vagina or cervix after mating, depending on the species, only a few hundred manage to reach the oviduct, the organ where fertilization takes place [7]. However, even though the vast majority of sperm are lost during their journey, the few that reach the oviduct are capable of *in vivo* fertilization. In *in vitro* conditions, on the other hand, we have a different situation, and even though the oocytes are exposed to thousands/millions of spermatozoa, fertilization is not always successful. These discrepancies between natural and laboratory environments can be explained by the strong selection that spermatozoa undergo in the female genital tract. For instance, in some species, cervical mucus is hypothesized to serve as a selective barrier for sperm, as it is highly effective at impeding defective spermatozoa that

are unable to swim normally or possess a poor hydrodynamic profile due to morphological anomalies [8], eliminating around 70–85% of the sperm during this step [9]. After overcoming this barrier, spermatozoa enter the uterus, where another portion of the sperm population appears to be attacked by neutrophilic granulocytes, which are present in significant numbers following their post-insemination migration into the uterus [10]. Subsequently, the spermatozoa reach the utero-tubal junction, the part of the oviduct that connects with the uterus. While in humans this part does not play a significant role in sperm selection due to the strong anatomical continuity between the oviduct and the uterus, in certain species such as ruminants, pigs, rats, hamsters and mice, it does indeed play a role in sperm selection, being considered the second main selective barrier after the cervix. In those species, the utero tubal-junction only allows live [11], unreacted [12] and motile [13] spermatozoa to pass. Although the mechanisms of sperm selection at this point are still unknown, a single molecule-dependent interaction has been suggested, since the sperm of ADAM3 knockout mice are unable to get beyond this point [14].

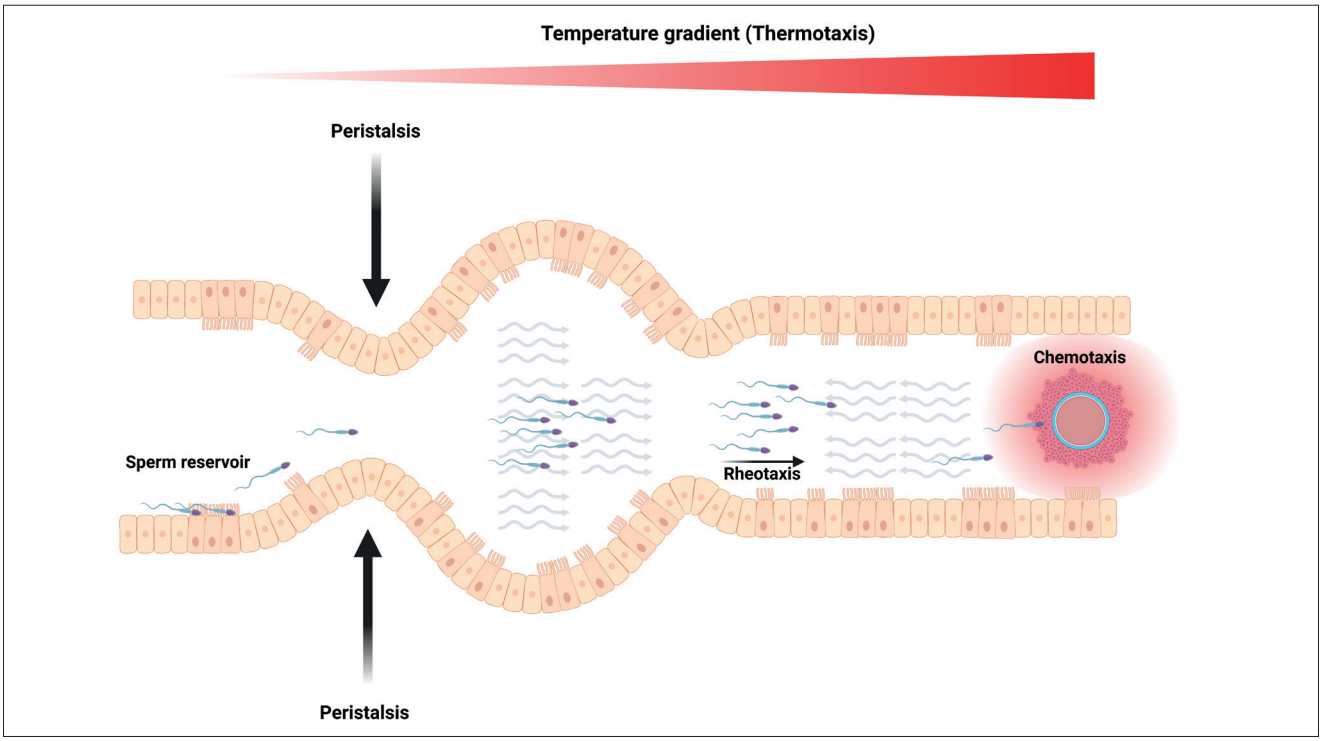
Finally, after this nightmarish journey, the few fortunate sperm that have overcome all the barriers enter the oviduct, where the environment is much more favorable. Here, where there seem to be no leukocytes waiting to attack them [15], they instead bind to the carbohydrates of the ciliated cells in the oviduct epithelium through lectin-like protein present in the head of the sperm (reviewed by Coy *et al*.[16]), forming the sperm reservoir. These ciliated cells "take care" of spermatozoa, delaying the process of sperm capacitation [17], and extending their viability from hours to months, depending on the species [18]. This process ensures that sperm are ready at the time of ovulation, when they are released, and acquire their fertilization capacity [19].

However, once released, they still need to navigate through a long and intricate passage, overcoming all the folds of the oviduct until they reach the oocyte. For this reason, it has been proposed that the meeting of the spermatozoa and the oocyte is not a coincidence [20]. *In vitro* studies have shown that spermatozoa can respond to temperature gradients. This phenomenon is not limited to a single species; it has been documented in rabbits [21] and humans [22], and is recognized as a common trait among mammalian sperm [23]. In conjunction with the finding in certain animals, such as rabbits [24] and pigs [25], that after ovulation, the temperature in the place of fertilization is higher than in the sperm reservoir, researchers propose that thermotaxis could be one of the mechanisms guiding sperm to the site of fertilization.

On the other hand, it has been shown that spermatozoa are able to align against the fluid flow, a phenomenon called rheotaxis. Again, this effect has been observed in several species, including humans [26,27]. In view of this ability of the sperm, together with the fact that there is a current flow that goes from the ovaries to the uterus [28], rheotaxis has been proposed as another mechanism serving to guide the spermatozoa on its journey to the oocyte. Lastly, sperm are capable of moving in response to chemical gradients of certain substances. One of the first chemoattractants reported was follicular fluid in humans [29], and since then the phenomenon has been observed in species such as mice [30] or cattle [31]. However, follicular fluid is not the only fluid that could have chemoattractants. The cumulus cells accompanying the oocyte produce progesterone [32], a steroid capable of inducing chemotaxis in humans and rabbits, among others [33].

Although these proposed mechanisms (summarized in Figure 1) have been heavily questioned, as peristaltic movements

Figure 1 Representation of the mechanisms of sperm guidance in the oviduct.



may play a more significant role in the *in vivo* oviduct [34], they have proven to be useful *in vitro*, improving sperm selection and *in vitro* production (IVP) outcome [35].

After fertilization, the first division of the embryo takes place. It has been observed that the presence of the embryo can induce distinct gene expression patterns in the oviductal epithelial cells (OECs) [36]. This diverse gene expression could play essential roles, such as facilitating embryo transport (e.g., ciliary motility) or creating an optimal environment for early embryo development. In a bidirectional way, it has been observed that co-culture of embryos with OECs promotes a different gene expression profile of genes involved in embryo quality [37]. In fact, when the embryos are produced in *ex vivo* oviducts, even in a heterologous manner, they show higher quality, [38]. The aforementioned effects have been observed in both *in vitro* and *in vivo* experiments.

Traditionally, the oviduct has been regarded simply as a tube, mainly because pregnancy can be achieved without exposing gametes or embryos to the oviduct environment. However, as we have discussed, its functions should not be underestimated; we are dealing with a highly complex system that is even capable of reacting differently depending on the sperm's sex, since the presence of X- or Y-sperm populations can change the oviductal transcriptome [39].

Mimicking the physiological environment

Numerous research groups are currently trying to improve the ARTs, mainly by mimicking the physiological environment of the oviduct. One of the most common strategies is the use of oviductal fluid (OF) as a supplement in the culture media, which has been used in different species, improving the yield of the IVP system [40]. Canovas *et al*., in the swine model, showed that the quality and methylation pattern of embryos produced *in vitro* with OF as an additive in the culture media were closer to their *in vivo* counterparts than to embryos produced *in vitro* without OF [41]. Even after birth, the growth of those piglets was more similar to that of piglets born through artificial insemination than that of piglets produced without OF [42].

Due to ethical concerns surrounding studies of this type in humans, determining the impact of these techniques remains challenging. However, the fact that the embryo culture medium can influence perinatal outcome [43] and children's growth [44] suggests that the potential consequences of a suboptimal non-physiological environment should not be underestimated. In fact, the use of OF has been already proposed, and other reproductive fluids, such as uterine fluid, have already been used as supplements in culture media, leading to the birth of healthy offspring [45].

It is important to highlight that the abovementioned *in vitro* studies focusing on the phenomena that occur in the oviduct have predominantly relied on monolayer cultures. Those standard 2D growth techniques have played an important role in advancing our understanding of how OECs respond to different stimuli. Nevertheless, 2D cell cultures may not always represent the optimal approach for conducting studies, since they fail to closely mimic the physiological conditions. As Watson *et al*. pointed out, *in vitro* studies of this kind give us results describing the 2D life, where the cells "talk only to cells of like mind, live in the dark and in their own excrement, and don't bury their dead". A solution to this problem could be to adopt a 3D approach, providing us with a more realistic perspective on how these responses might translate to an *in vivo* environment [46].

In recent years, several studies have been published in which the 3D culture approach was used to study different areas of the female reproductive tract [47]. Meanwhile, there is a growing interest in the development of microfluidics, a field that focuses on the control and manipulation of fluids at microscopic level to recreate the dynamic *in vivo* flow. Applying that technology to 3D cultures, it has even been possible to recreate the menstrual cycle *in vitro* [48]. By combining both 3D cultures and microfluidics technology, it has proved possible to create "oviducts on a chip", which represent a significant advance in improving IVP [49]. These innovative scaffold devices accurately replicate the physiological conditions of the oviduct, providing a more realistic environment for sperm-egg interaction. This technology has been shown to enhance bovine epigenetic reprogramming, making it more similar to the profiles of *in vivo*-derived embryos [49]. In humans, a similar device has been created and, although used with endometrial cells, was found to improve the blastocyst rate by mimicking the physiological environment [50].

Conclusion

In conclusion, the oviduct plays crucial roles beyond merely sperm selection and capacitation; it is not simply a tube for gamete encounter or embryo transport. Instead, this organ is responsible for creating the optimal environment for both gametes and the embryo, and its functions should not be underestimated. Mimicking the oviduct environment has proven to be a valuable strategy for enhancing ART yields and a potential tool for ensuring the health of the offspring.

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