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Getting to and away from the egg, an interplay between several sperm transport mechanisms and a complex oviduct physiology

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Abstract

In mammals, the architecture and physiology of the oviduct are very complex, and one long-lasting intriguing question is how spermatozoa are transported from the sperm reservoir in the isthmus to the oocyte surface. In recent decades, several studies have improved knowledge of the factors affecting oviduct fluid movement and sperm transport. They report sperm-guiding mechanisms that move the spermatozoa towards (rheotaxis, thermotaxis, and chemotaxis) or away from the egg surface (chemorepulsion), but only a few provide evidence of their occurrence *in vivo*. This gives rise to several questions: how and when do the sperm transport mechanisms operate inside such an active oviduct? why are there so many sperm guidance processes? is one dominant over the others, or do they cooperate to optimise the success of fertilisation? Assuming that sperm guidance evolved alongside oviduct physiology, in this review we propose a theoretical model that integrates oviduct complexity in space and time with the sperm-orienting mechanisms. In addition, since all of the sperm-guidance processes recruit spermatozoa in a better physiological condition than those not selected, they could

potentially be incorporated into assisted reproductive technology (ART) to improve fertility treatment and/or to develop innovative contraceptive methods. All these issues are discussed in this review.

Keywords: sperm transport – oviduct peristalsis – rheotaxis – thermotaxis – chemotaxis

1. Is there a need for sperm transportation to the egg?

The mammalian spermatozoon is a small (around 60 µm long in humans), highly differentiated cell with an optimised hydrodynamic shape, which carries only the organelles it will need to reach and fertilise the egg. For instance, it has an acrosome Golgi-derived vesicle to facilitate penetrating the egg vestments, a highly compacted DNA, and a long flagellum with a mitochondria network that generates theATP necessary to propel it (Eddy, 2006). It might seem that, with the minuscule cargo it carries, its auto-propulsion would be enough to find the egg. But, is it? Apparently not. Even though the spermatozoon can swim some tens of micrometres per second, the distance it has to cover through the complex and very active female reproductive tract is several times its size (Boyd et al., 2018). Its own movement is evidently not enough to reach the egg. For instance, in the case of humans (Suarez and Pacey, 2006), semen is deposited at the bottom of the vagina, and from there the spermatozoa must first pass through the uterine cervix (around 4 cm long). This is a very complex structure with crypts and folds leading to the uterine cavity. The mucus filling the cervix is less dense at the bottom of the channels and this means that sperm is transported more easily along these than

through the central lumen, where strong fluid currents towards the vagina hinder the entrance of microorganisms. Once in the uterus, spermatozoa must cross a long distance (around 7 cm in humans) to get to the oviduct entrance, but this is facilitated by the peristalsis of the uterine muscle. Hence spermatozoa pass through the uterus very fast without major complications. Then, they must go through the uterus-tubal junction, which is a simple anatomical structure in humans and of variable complexity in other species (Harper et al., 1982; Suarez and Pacey, 2006).

Once inside the oviduct, spermatozoa attach to the isthmus epithelium where they are physiologically prepared. It should be noted that spermatozoa coexist in different physiological states, and it is preferably the capacitated sperm subpopulation that reach and fertilise the egg (Cohen-Dayag et al., 1995; Oren-Benaroya et al., 2007; Uñates et al., 2014). Once spermatozoa complete capacitation, they detach from the epithelium, but there is still a long labyrinthine road (around 7 cm in humans and 25 cm in cows) to the fertilisation site, which is full of crypts and folds masking the egg's position (Burkitt et al., 2011; Yániz et al., 2014, 2006, 2000).

Up to the isthmus reservoir, the sperm's trip seems to be in a one-way direction. However, after detaching from the isthmus epithelium, the spermatozoon does not seem to follow a predetermined route. Finding the precise location of an egg at the fertilisation site is a major challenge for a cell without a "GPS". Fortunately, the egg environment expresses widgets that help the spermatozoon to reach the egg. Its transportation inside the oviduct is influenced by the movement and viscosity of the oviduct fluid, peristalsis, its confinement in small oviduct spaces, its physiological

state, and several aligning and guiding navigation mechanisms. Together, these factors seem to orchestrate the regulation of fertilisation, not only facilitating gamete encounter but also preventing the entry of supernumerary spermatozoa to the egg.

2. Conditions affecting sperm movement

The spermatozoon is propelled by the flagellum (Lindemann and Lesich, 2016; Ounjai et al., 2012), which is a complex structure connected to the sperm head by the neck. It can be divided into three regions: midpiece, principal piece and end piece. It contains a central axoneme which extends up to the end of the principal piece. The axoneme has 9 microtubule doublets, connected to a central pair of microtubules by radial spokes. There are 9 outer dense fibres (ODF), which externally surround each microtubule doublet. The ODF are encircled by the mitochondria sheath along the midpiece and by the fibrous sheath along the principal piece. The propelling force of the flagellum is generated by the hydrolysis of ATP, mediated by dynein motor proteins that are anchored to each microtubule doublet, producing a sliding movement between microtubules. However, as the microtubules are fixed to the neck, a bending movement is generated and a wave is propagated along the flagellum, the shape of which is determined by the force provided by the dynein, the ODF, the fibrous sheath structure, and the hydrodynamic drag force of the fluid in which the spermatozoon is moving (Lindemann and Lesich, 2016).

Sperm trajectories in quasi two-dimensional confinement enabled three different patterns of movement to be identified, which may reflect different physiological

states (Mortimer and Mortimer, 1990): progressive, where the flagellum adopts a helical movement which propels sperm forward (Mortimer and Mortimer, 1990); transitional (Fabro et al., 2002; Suarez, 1988), where the flagellum moves with a wider amplitude but the spermatozoon still swims progressively; and hyperactivated, characterised by large amplitude bending of the flagellum, which in turn generates a non-progressive tumbling motion (Mortimer and Mortimer, 1990; Yanagimachi, 1970). However, the confinement conditions should be taken into consideration because interaction with walls affects sperm movement. For instance, sperm swimming up to 1 µm from a solid surface showed a slithering movement, with a planar flagellar bending with no head rotation, resulting in a circular sperm trajectory (Guidobaldi et al., 2015; Nosrati et al., 2015). But when the spermatozoon swims up to 4 µm from the surface, it shows a helical flagellar beating with a rotating head, and the typical forward progressive trajectory (Guidobaldi et al., 2015; Nosrati et al., 2015). When spermatozoa are constrained by top, bottom and lateral walls under ultra-confinement conditions (e.g., at the edge of microchannel), they swim following the shape of the border delimited by the lateral walls (Guidobaldi et al., 2014; Lord Rothschild, 1963), with a partial decrease in progressive velocity (Guidobaldi et al., 2015; Bettera Marcat et al., 2020). However, spermatozoa swimming far from surfaces without flagellar movement restriction show a three-dimensional helical or twisted ribbon pattern (Su et al., 2012, 2013).

Another factor directly influencing sperm motility is the viscosity of the medium, as this can affect the features of flagellum movement (i.e. planarity, torsion, waveform) without altering the progressive velocity (Kirkman-Brown and Smith,

2011; Smith et al., 2009). It is interesting to note that, under *in vivo* conditions, sperm velocity decreases when it is near to the oviduct wall (Wang and Larina, 2018a). The pattern of sperm movement is thus a consequence of the flagellar architecture, the medium viscosity, the conditions of confinement and the interaction with surfaces, which influence the directionality of the spermatozoon.

3. Oviduct complexity

The oviduct is an intricate three-dimensional organ with several functional and morphological regions: the uterus-tubal junction, the isthmus, the isthmus-ampullary junction, the ampulla, and the infundibulum, which are described below (Fig. 1; Burkitt et al., 2011; Yániz et al., 2014, 2006, 2000). The oviduct presents a variable number of longitudinal folds, delimiting the oviduct lumen, which is filled with oviductal fluid (Koyama et al., 2016; Shi et al., 2014). Thus, in the uterus-tubal junction, the primary folds are flat and wide, and are linked by prominent oblique secondary folds, which form cul-de-sac-like structures open towards the uterus. The areas between the folds have shallow pockets with narrow crypts. In the isthmus, there are four to six primary folds that rarely converge or diverge. An oblique secondary branching can be observed from the apex to the base of the primary folds, and becomes more transverse towards the ampullary-isthmic junction. At the base of the folds, there are flat shallow pockets towards the uterus that occasionally contain tight crypts. In the ampullary-isthmic junctionthe principal folds are interconnected by lateral bifurcated branches that are organised in rows perpendicular to the principal folds, with irregular grooves between them. In the ampulla, the longitudinal folds become undulated and increase in number and size

145 towards the infundibulum. Six to ten principal folds can be observed (up to 1300
146 μm high) alternating with lower folds (up to 500 μm high). The intricate branching in
147 the ampulla leads to a thin lumen between folds of about tens of micrometres, as
148 observed in histology slides (Yániz et al., 2014, 2006, 2000), but can be thicker
149 under *in vivo* conditions (Burton et al., 2015). At the upper end of the ampulla,
150 there is the Infundibulum, which has a funnel shape. The proximal end of the
151 infundibulum presents numerous tortuous folds in continuity with those from the
152 ampulla. Then, they diverge and become less sharp and lower in height towards
153 the wider opening of the infundibulum. Between the primary folds, there are oblique
154 secondary folds forming cul-de-sac structures, open in the ovarian direction. Thus,
155 the lumen spaces along the oviduct become very narrow, generating micro-
156 confinement spaces for gamete transport and storage. The particularities of its
157 anatomy and histology make the oviduct a complicated organ for transporting
158 gametes and embryos.
159

160 **4. Oviduct fluid movement**

161 The epithelium of the oviduct consists of ciliated and secretory cells. The
162 proportion of these cells varies among regions, with a larger number of secretory
163 cells in the isthmus, and ciliated cells predominant in the ampulla and infundibulum
164 (Abe, 1996; Stewart and Behringer, 2012), while the numbers are also influenced
165 by the cyclic hormonal changes (Abe, 1996). Even though the oviduct physiology
166 experiences particular changes during the hormonal cycle, to describe the
167 movement of the oviduct fluid we refer only to the periovulatory period. The fluid
168 moves inside the oviduct because of the activity of secretory cells, the beating of

cilia from ciliated cells, and the oviduct muscle contractility or peristalsis, all of which are cyclically regulated by sex hormones (Fig. 2; Hunter, 2012).The oviductal fluid is produced by the contribution of the serum exudate coming from the blood-oviductal barrier and the exocytic activity of the oviduct secretory cells (Hunter, 2012). The amount of oviduct fluid during oestrous varies according to the species secretion rate (Gott et al., 1988; Hino and Yanagimachi, 2019; Iritani et al., 1974).The movement of the oviduct fluid also depends on cilia beating in the multi-ciliated cells, which are organised in patches of different sizes, beating synchronically at a rate below 25 Hz along the longitudinal axis of the oviduct towards the uterus (Koyama et al., 2019; Shi et al., 2014; Wang et al., 2015; Wang and Larina, 2018b). The orientation of the ciliary beating current is maintained towards the uterus in the ampulla in most of the species. However, in the isthmus, the cilia beating current may be towards the uterus (human, cow, and sheep), pro-ovarian (pig and rabbit) or not detected (guinea pig and rat) (Gaddum-Rosse et al., 1973; Gaddum-Rosse and Blandau, 1976; Gaddum-Rosse and Blandau, 1973). In the isthmus, the cilia movement generates turbulence that moves fluid from the bottom of the folds, while a current is formed in the middle of the lumen by the cilia located in the apical ridge of the folds. In contrast, in the ampulla, where the folds are larger, the main fluid current is observed along the lumen between folds (Kölle et al., 2009). In addition, the ovulated egg is transported from the infundibulum to the fertilisation site by cilia beating (Halbert et al., 1989, 1976). Peristalsis consists of alternate contraction and relaxation of the smooth muscle cells of the oviduct, an effect that is propagated like a wave along the oviduct. As a consequence, in the isthmus, the oviductal fluid is pushed in drops back and forwards, with a net

advance in the ovary direction, while the movement in the ampulla is unidirectional towards the peritoneal cavity, at least in the mouse (Battalia and Yanagimachi, 1980, 1979; Blandau and Gaddum Rosse, 1974; see supplementary video 1 in Guidobaldi et al., 2012; see videos in Hino and Yanagimachi, 2019). Hence, the net fluid flow depends on the combination of these three factors.

5. Sperm sweeping by oviduct fluid movement

The complex movement of the oviduct fluid described above apparently influences sperm transportation to the fertilisation site. However, under natural conditions, the distribution of spermatozoa along the oviduct is heterogeneous. Most of the spermatozoa are observed along the isthmus, while few are present at the fertilisation site (Guidobaldi et al., 2012; Hino and Yanagimachi, 2019; Ishikawa et al., 2016; La Spina et al., 2016). The back and forward fluid movement spreads spermatozoa along the isthmus, moving a few of them to the fertilisation site. Indeed, when peristalsis was inhibited, the few spermatozoa found at the fertilisation site were halved, suggesting that peristalsis contributes to some extent to transporting sperm to the fertilisation site (Guidobaldi et al., 2012). Other mechanisms may thus be cooperating to facilitate the gamete encounter.

6. Sperm taxis towards and away from the egg

Several somatic cells can migrate, for instance, during embryonic development, wound healing and immune responses, processes in which cell guidance is modulated by several transportation mechanisms, known as “taxes”,. Spermatozoa are also sensitive to taxes, i.e, rheotaxis, thermotaxis, and chemotaxis (which may

attract or repel the spermatozoon). Rheotaxis refers to sperm alignment according to the fluid flow, so that when the fluid flows against the sperm head it is called positive rheotaxis (Fig. 3A). Thermotaxis is sperm movement oriented by gradual differences in temperature towards the warmest place (Fig. 3B). In chemotaxis, cell orientation is mediated by the substance concentration gradient, and is positive when the sperm move towards the source of the substance (chemoattraction, hereinafter referred to as chemotaxis as it is commonly known), or negative, when they move away from it (chemorepulsion) (Fig. 3C). We next describe what is known about these sperm transportation mechanisms.

6.a. Sperm rheotaxis

Sperm rheotaxis was first reported by Bretherton and Rothschild (1961), and to date has been characterised in humans (Bretherton and Lord Rothschild, 1961; De Martin et al., 2017; Miki and Clapham, 2013; Zhang et al., 2016), bulls (Bretherton and Lord Rothschild, 1961; El-Sherry et al., 2017, 2014; Johnson et al., 2017), mice (Miki and Clapham, 2013), stallions and boars (Fair and Romero-Aguirregomezcorta, 2019; Romero-Aguirregomezcorta et al., 2018). It seems that rheotaxis selects motile spermatozoa regardless of the physiological state, since both non-capacitated and capacitated spermatozoa immersed in low- and high-viscosity media elicit rheotactic behaviour, aligning their movement against the fluid flow current (Miki and Clapham, 2013). The rheotactic sperm pattern is characterised by an increase in velocity (Rappa et al., 2018), rotation of the tail and hyperactivated behaviour (Miki and Clapham, 2013). A three-dimensional study also shows that the cell's turning direction is defined by the asymmetrical movement of the flagellum midpiece (Bukatin et al., 2015). Interestingly, studies

based on sperm movement simulation under flow predicted that rheotaxis is dependent on sperm confinement (Ishimoto and Gaffney, 2015) and surface interactions (Kantsler et al., 2014). The changes in motility associated with rheotaxis seem to be accompanied by extracellular calcium mobilisation by means of the CatSper channel (Miki and Clapham, 2013). In addition, bull spermatozoa best express rheotaxis at pH 6.4–6.6, the range at which constant values of nitric oxide, potassium and calcium are observed (El-Sherry et al., 2017). However, other authors report that there are no significant differences in sperm flagellar beating amplitude and asymmetry and that no calcium influx was observed during sperm rheotaxis turning (Zhang et al., 2016).

6.b. Sperm thermotaxis

This behaviour was first described almost 20 years ago in a small subpopulation of capacitated rabbit spermatozoa; consistently, the ampulla is about 2ºC warmer than the isthmus region of the oviduct (Bahat et al., 2003). This temperature difference is enhanced in the periovulatory phase in the rabbit (Bahat et al., 2005). Intriguingly, human spermatozoa can respond to temperature gradients as low as 0.014ºC/mm (Bahat et al., 2012). The sperm pattern of thermotactic movement involves changes in the frequency of turns and sperm velocity while searching for the temperature gradient; but when it is found, the values of those sperm parameters decrease, and the spermatozoon begins swimming straightforwardly aligned with the temperature gradient (Boryshpolets et al., 2015). The thermotaxis signalling mechanism seems to be initiated by the activation of the opsins (G-protein-coupled receptors), rhodopsin and melanopsin, which are co-localised mainly in the sperm head membrane in human and mouse spermatozoa (Pérez-

Cerezales et al., 2015; Roy et al., 2020). The rhodopsin receptor is involved in the cyclic-nucleotide pathway and the melanopsin activates the phospholipase C pathway (Bahat and Eisenbach, 2010; Pérez-Cerezales et al., 2015; Roy et al., 2020). Moreover, the transient receptor potential vanilloid (TRPV1 and TRPV4) has also been reported to mediate sperm thermotaxis in mice (Hamano et al., 2016) and humans (De Toni et al., 2016). The phospholipase C pathway may also reinforce the activity of TRPV1 (De Toni et al., 2016).

6.c. Sperm chemoattraction

In mammals, sperm chemotaxis was first reported for human spermatozoa towards peptides that attract bacteria (Gnessi et al., 1985). A few years later it was reported that human spermatozoa were attracted by follicular fluid from fertile oocytes (Ralt et al., 1991). This positioned sperm chemotaxis as an interesting mechanism that had the potential to improve the outcome of ART. It has so far been observed in small subpopulations of capacitated human, mouse, rabbit, bovine and equine spermatozoa (Dominguez, 2019; Dominguez et al., 2018; Eisenbach and Giojalas, 2006; Giojalas et al., 2015; Moreno-Irusta et al., 2020). The chemotactic response lasts up to two hours (Cohen-Dayag et al., 1995; Fabro et al., 2002), fluctuating with an ultradian cycle of two hours, and also with an infradian seasonal rhythm, at least in humans (Moreno-Irusta et al., 2019). Interestingly, the chemotactic state is associated with the time a fertilisable oocyte is available in the oviduct. Thus, in humans where ovulation does not necessarily coincide with ejaculation, the capacitated-chemotactic state is prolonged over time, but in the rabbit, where ovulation occurs upon the stimulus of coitus, it lasts a few hours (Giojalas et al., 2004). In addition, the acrosome must be intact for the occurrence of sperm

chemotaxis (Fabro et al., 2002; Guidobaldi et al., 2017b). Concerning the chemotactic pattern, capacitated spermatozoa swimming in a spatial concentration gradient orient their movement towards the source of the attractant with a transitional forward pattern (Blengini et al., 2011). But when the spermatozoon is aligned to the gradient, it swims straight forward, with turns and hyperactivation being suppressed (Armon and Eisenbach, 2011; Ernesto et al., 2015).

The first biological source of chemoattractants was follicular fluid in humans (Ralt et al., 1991) and mice (Oliveira et al., 1999). Other sources, like oviductal fluid in mice (Oliveira et al., 1999), the cumulus oophorus in humans (Oren-Benaroya et al., 2008; Sun et al., 2005) and rabbits (Guidobaldi et al., 2008), and the egg in humans (Sun et al., 2005), were also reported. But these sources are usually a mixture of molecules of different chemical nature, like hormones, peptides, reactive species, etc, some of which have been tested for chemotaxis.

Atrial natriuretic peptide is present in oviductal and follicular fluids and was reported to chemoattract human and mouse spermatozoa (Anderson et al., 1995; Bian et al., 2012; Zamir et al., 1993). Several chemokines and cysteine-rich secretory proteins, produced by granulosa, cumulus cells and/or the egg, have been reported to chemoattract human or mouse spermatozoa (Ernesto et al., 2015; Giojalas et al., 2015) and the corresponding receptor for some of these was identified in spermatozoa (Giojalas et al., 2015). Progesterone is a steroid secreted by the cumulus cells from the time of ovulation (Bar-Ami et al., 1989; Vanderhyden and Tonary, 1995) and was reported to induce chemotaxis upon gradients of low concentration (picomolar range) in human (Blengini et al., 2011; Gatica et al., 2013; Guidobaldi et al., 2017a; Teves et al., 2010, 2009, 2006), rabbit (Guidobaldi

et al., 2017a; Teves et al., 2006), mouse (Ernesto et al., 2015; Guidobaldi et al., 2017a, 2017b), bovine (Dominguez et al., 2018) and equine (Dominguez, 2019) spermatozoa, the chemotactic activity of which is modulated by the corticosteroid-binding globulin (Teves et al., 2010). The chemotactic response of sperm to progesterone seems to be mediated by a surface receptor located in the head (rabbit) or tail (human) (Guidobaldi et al., 2008). Even though several membrane proteins have been claimed as progesterone receptors (Giojalas et al., 2015; Miller et al., 2016), none of these studies provided experimental evidence of the participation of those proposed progesterone receptors in the chemotactic response of sperm. Nitric oxide, a free radical with a brief lifespan, secreted by the cumulus cells (Bu et al., 2003), chemoattracts human spermatozoa (Miraglia et al., 2007); interestingly, spermatozoa also release this molecule (Lewis et al., 1996), and thus a homologous attraction cannot be discarded. Odorant substances have been also reported to attract human and mouse spermatozoa (Fukuda et al., 2004; Spehr et al., 2003; Veitinger et al., 2011), and the presence of odorant receptors was described in spermatozoa (Fukuda et al., 2004; Spehr et al., 2003). However, the natural source of odorants in the female genital tract is not known. Cyclic nucleotides and reactive oxygen species are second messengers in chemotaxis signalling (Miraglia et al., 2007; Moreno-Irusta et al., 2020; Teves et al., 2009). Moreover, when spermatozoa are exposed to a concentration gradient of these small molecules, they emulate the action of a chemoattractant as reported for human (Teves et al., 2009) and equine (Moreno-Irusta et al., 2020) spermatozoa. Therefore, the use of concentration gradients of these molecules is an interesting tool to investigate the chemotactic molecular mechanism; however, they cannot be

considered putative attractants. There are also attractants of unknown chemical identity that have been isolated from follicular fluid or the oocyte, which can stimulate human sperm chemotaxis (Armon et al., 2014; Ralt et al., 1994). Studies of chemotactic signalling have examined the attractants, progesterone, nitric oxide, odorants, and atrial natriuretic peptide. Interestingly, the pathways studied in each case are elicited by more than one attractant. In general, signalling has been reported via adenylyl cyclase - cyclic adenosine monophosphate - protein kinase A and via guanylate cyclase - cyclic guanosine monophosphate - protein kinase C, reactive oxygen species, protein phosphorylation in tyrosine and calcium mobilisation from different origins (see Fig.3 in Giojalas et al., 2015; Moreno-Irusta et al., 2020)

6.d. Sperm chemorepulsion

Sperm repulsion has been observed in human, rabbit and mouse spermatozoa, sharing several characteristics with sperm chemotaxis. For instance, only the small subpopulation of capacitated spermatozoa can respond with repulsion, which is characterised by orientation of its movement away from the source of a substance, swimming with a transitional pattern (Guidobaldi et al., 2017a). The repellents tested so far are synthetic, like progesterone receptor ligands (sPRL) that are used as contraceptives (mifepristone, levonorgestrel, and ulipristal acetate), or physiological, like zinc released immediately and transiently after fertilisation (Kim et al., 2011; Que et al., 2017), suggesting an effect for blocking polyspermy. Indeed, rabbit spermatozoa treated with ulipristal are prevented from reaching the oocyte surface (Guidobaldi et al., 2017a). Interestingly, chemorepulsion was also observed when spermatozoa were exposed simultaneously to a concentration

gradient of progesterone (at the picomolar chemotactic range) and to a homogeneous distribution of sPRL or zinc. Thus, the chemotactic gradient of progesterone is converted by sPRL or zinc into a repellent gradient (Guidobaldi et al., 2017a). The molecular signalling of sperm chemorepulsion seems to be like that described for chemotaxis towards progesterone (unpublished data from our lab).

7. A theoretical model to explain sperm transportation to and away from the egg

The complexity of spatial-temporal oviduct architecture and functionality led to the notion that the sperm's own motility is not sufficiently capable of reaching the oocyte surface, and that extra guiding mechanisms may help to accomplish this goal. Based on the literature mentioned in this review, we propose a general theoretical model to explain how spermatozoa manage to complete this journey, particularly from the isthmus sperm reservoir to the oocyte membrane (Fig. 4), though there may be variations between species. The few spermatozoa that accomplish capacitation are released from the epithelium of the oviduct reservoir. Then, the cilia beating moves the spermatozoa from the bottom towards the ridge of the folds, where they are exposed to the current of oviduct fluid and peristalsis, which transport spermatozoa in the ad-ovarian direction. During oviduct contraction, spermatozoa may be transported inside drops of oviduct fluid along the isthmus; however, during oviduct relaxation, the oviductal fluid current is maintained by cilia beating towards the uterus, thus orienting the sperm by rheotaxis. It is interesting to note that the number of spermatozoa that are

transported along the isthmus is drastically decreased to tens in the ampulla. This may be due, in part, to the increase in the number of folds and the lumen surface, which may create resistance to the movement of the fluid by peristalsis coming from the isthmus. The complexity of the ampulla provides numerous alternative pathways between the folds, where interaction with the oviduct surface in micro-confined spaces guides the spermatozoon to swim next to the walls along the folds. Upon ovulation, the egg enters the infundibulum and is carried towards the fertilisation site by cilia beating, where it remains in one of the multiple intricate spaces between folds. In such a labyrinthine scenario, how can gametes encounter each other? Peristalsis, thermotaxis, and rheotaxis may move spermatozoa along the ampullary lumen. But since none of these mechanisms can guide the spermatozoon to the precise location of the egg, the gamete encounter would be by chance. Thus, spermatozoa may find the particular pathway where the egg is located by chemotaxis. During relaxation, concentration gradients of chemoattractants secreted by the egg may be spread by cilia beating along the small lumen between folds, increasing the probabilities of gamete encounter. But the spermatozoa must still penetrate the egg vestments to reach the oocyte surface. This step may be operated by chemotaxis due to the gradual secretion of one or more chemoattractants, either from the cumulus cells or the oocyte. Once fertilised, the oocyte secretes zinc which, in combination with the progesterone gradient, would rapidly repel arriving capacitated spermatozoa, preventing the entry of additional sperm cells. This complex interplay suggests to us that the intricate oviduct structure evolved in association with a multiplicity of sperm-guiding

mechanisms not only to ensure the gamete encounter but also to guarantee that one oocyte is fertilised by only one spermatozoon.

8. Can sperm taxis be applied as a technological tool to improve or to prevent fertilisation?

Even though the participation *in vivo* of these taxes has not yet been fully demonstrated, their ability to select spermatozoa in a better physiological state has encouraged the development of methods that may improve ART outcomes in humans and animals. For instance, microfluidic devices based on fluid flow select spermatozoa by rheotaxis, providing them with higher fertilisation ability, morphology, motility or low uncondensed chromatin (De Martin et al., 2017; Hwang et al., 2019; Zaferani et al., 2018). In any event, further studies are needed to test whether the spermatozoa selected by rheotaxis indeed improve the ART outcome. In thermotaxis, sperm quality is favoured by temperature selection since faster spermatozoa have been obtained with less damaged DNA (Pérez-Cerezales et al., 2018). Moreover, spermatozoa isolated by thermotaxis were used to fertilise mice oocytes, giving rise to a greater number of good quality embryos and pregnancies (Pérez-Cerezales et al., 2018). In the case of chemotaxis, spermatozoa selected by the chemoattractant progesterone elicited a higher level of capacitation, and lower DNA fragmentation and oxidative stress (Dominguez, 2019; Dominguez et al., 2018; Gatica et al., 2013). Moreover, when spermatozoa selected by chemotaxis towards progesterone were used to fertilise the oocyte by different ART procedures, an improvement was observed in the rate of cleavage or embryo quality (Dominguez, 2019; Dominguez et al., 2018). Thus, to improve the outcome

of ART, simple devices and procedures may be designed to select the best spermatozoa by means of one or more sperm taxes (perhaps combined with some oviductal features), without much perturbing the ART logistics. Some attempts have already been made in this direction (Bhagwat et al., 2018; Ko et al., 2018). And for contraception purposes, new protocols based on sperm repulsion may be designed to prevent gamete encounter without secondary effects.

9. Conclusions and future perspectives

Knowledge of the factors influencing sperm arrival at the egg has increased in recent decades, suggesting a much more complex scenario than that imagined in the past. Considering that sperm capacitation and sperm transport mediated by taxis are closely related, being able to obtain a subpopulation containing capacitated sperm provides a model to better comprehend the dynamics between the capacitated and post-capacitated state. The molecular level fine-tuning that regulates the orchestration of sperm transport towards and away from the egg still needs much further research. Another intriguing issue is the possibility that the female chooses the best spermatozoon, a hypothesis postulated that must be tested at molecular level. As a whole, such knowledge would not only illuminate the mechanisms underlying the regulation of fertilisation but also enable the manipulation of sperm transport for biotechnological purposes.

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827 **Legends for figures**

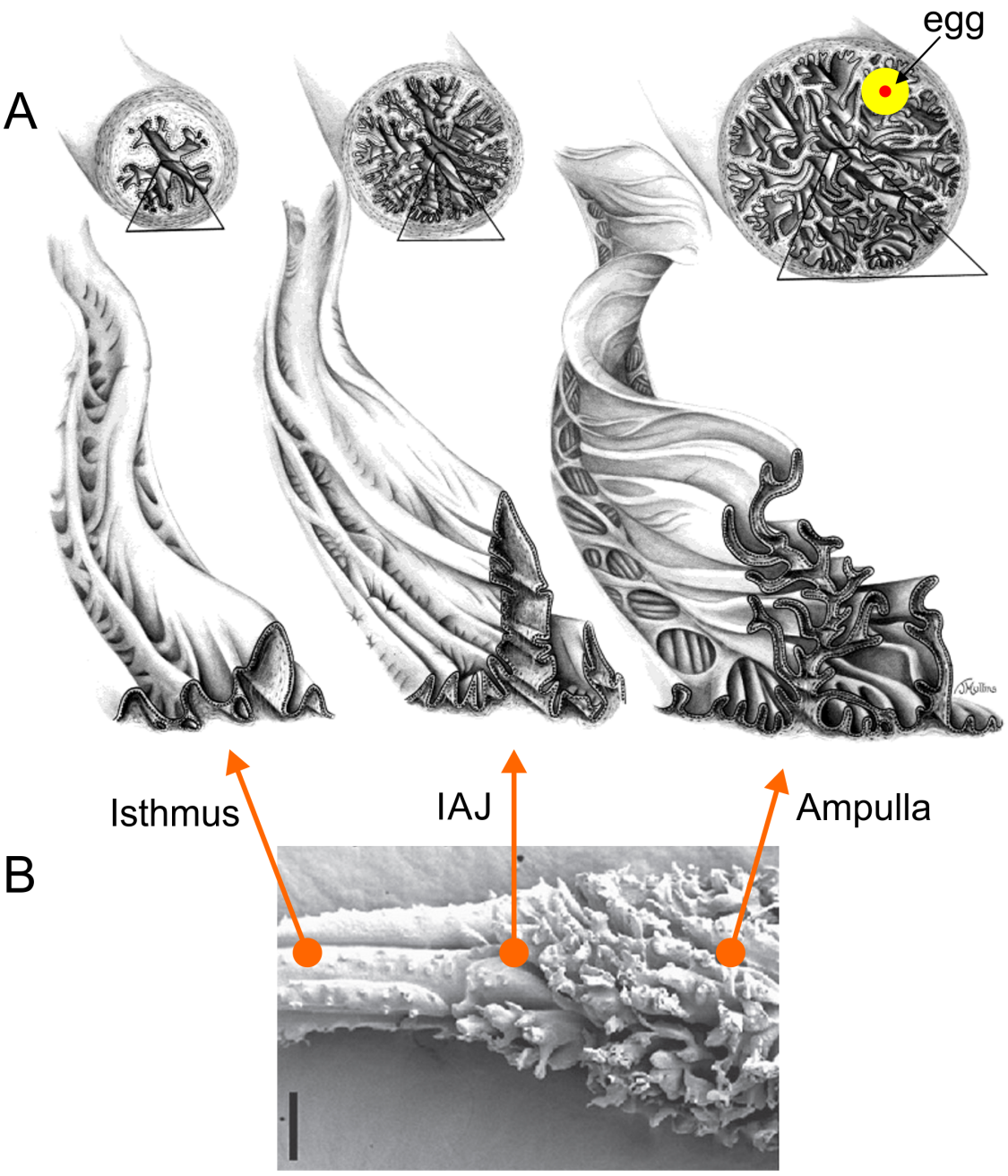
828 **Fig. 1. Anatomy of the oviduct regions. (A)** Transversal and the corresponding
829 longitudinal representations of the isthmus, isthmus-ampulla junction (IAJ), and
830 ampulla regions are shown. An oocyte is drawn to scale inside the ampulla cross-
831 section. **(B)** A three-dimensional representation of the lumen of the oviduct
832 corresponding to the three regions shown in A. IAJ, isthmus-ampulla junction.
833 (Figure adapted from Yániz et al., 2000; 2014).

834

835 **Fig. 2. Factors affecting oviduct fluid movement.** All images are schematic
836 representations of the isthmus, the isthmus-ampulla junction (IAJ), and the
837 ampulla. **(A)** Secretion of oviduct fluid by the secretory epithelial cells,
838 represented by vertical arrows, which are of different sizes to show that the
839 amount of fluid is high in the isthmus and gradually diminishes towards the
840 ampulla. The dotted line ending in an arrow at the right, represents the
841 direction of the fluid flow. **(B)** Cilia from ciliated cells, present in the oviduct
842 epithelium, beat in the direction of the uterus. Dotted lines and arrows
843 represent the direction of the fluid movement along the lumen between folds.
844 The red lines indicate the position of cilia responsible for forming the fluid flow.
845 **(C)** The wave of peristalsis is shown along the oviduct. The drops of fluid are
846 represented in the lumen of the tube. In the lower isthmus, the drops move
847 equally back and forward, in the middle they move mainly forward, and in the
848 ampulla they continue moving ahead (see the direction of the arrows in each
849 drop). The arrow to the right of the tube shows the direction of the oviduct fluid
850 flow.

Fig. 3. Representation of sperm taxis. The position of the spermatozoa show the direction they are swimming. In the case of rheotaxis **(A),** the spermatozoa are aligned against the fluid flow, which is represented with an arrow pointing its direction. In thermotaxis **(B),** spermatozoa orient themselves by following a temperature gradient, swimming from the cooler to the warmer place. In chemotaxis **(C),** spermatozoa are guided by following a concentration gradient of an attractant substance, swimming in the direction of its source. In chemorepulsion **(D),** spermatozoa swim against the source of the substance down its concentration gradient.

Fig. 4. A theoretical model to explain sperm getting to and away from the oocyte. See the explanation in the text.


A
egg
Isthmus
IAJ
Ampulla
B