



## Review

# Cross-talk between free and bound spermatozoa to modulate initial sperm:egg ratios at the site of fertilization in the mammalian oviduct

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## ABSTRACT

This essay proposes that highly localized communication between free and bound spermatozoa in the caudal portion of the oviduct acts to regulate the numbers detaching from the epithelium and progressing to the site of fertilization close to the time of ovulation. Low initial sperm:egg ratios are essential for monospermic fertilization. Liberation of surface macromolecules and metabolic prompting from activated spermatozoa, together with altered patterns of sperm movement and dynamic differences in intracellular  $Ca^{2+}$  ion status between neighboring sperm cells, would influence the progressive release of spermatozoa from the reservoir in the oviduct isthmus. Different intensities of preovulatory epithelial binding, reflecting a range of states in the sperm surface membranes and associated proteins, would provide a further explanation for a chronologically staggered periovulatory detachment of spermatozoa. Intimate sperm–sperm interactions within the confines of the oviduct isthmus offer a sensitive means of fine-tuning the vanguard of competent male gametes reaching the isthmo-ampullary junction.

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## 1. Introduction

In recent years, competition between spermatozoa in the female genital tract before fertilization has emerged as a prominent theme within the discipline of reproductive biology [1–7]. One inference has been that those spermatozoa directly involved in the events of fertilization are in some manner ‘superior’. In eutherian mammals, such putative superiority has generally been attributed to motility characteristics, primarily to the speed of swimming within the female tract [8–11]: the first spermatozoon to reach an egg’s investments is presumed to be the fertilizing spermatozoon. However, if there is indeed some form of

selection of a vanguard of superior spermatozoa, what does this mean genetically? Does the genome of a ‘superior’ fertilizing spermatozoon lead to the generation of a better quality zygote and thereby to a more viable embryo? Although the response to this question remains unknown, the incidence of prenatal mortality in spontaneously breeding mammals is some 30% to 40% [12–16], placing the notion of embryo viability in a realistic perspective.

An understanding of sperm competition that focuses on swimming speeds may overlook considerable subtlety in the nature of sperm interactions before and close to the onset of fertilization. In the respective fields of sperm competition and sperm selection within the female tract of mammals, new insights might be gained by considering the possibility of communication between individual sperm cells *en route* to the site of fertilization. This essay has such an objective and examines the following: (1) where and when sperm–sperm interactions might be found, (2) the

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potential nature of sperm–sperm interactions, and (3) why they could be beneficial.

Sperm cross-talk, as proposed in this article, would not include instances of sperm cooperation [17,18]. In the sense previously used, such cooperation acts to facilitate sperm progression in the lower genital tract, especially in the uterus, rather than acting quantitatively within the oviduct isthmus to regulate sperm release.

## 2. Where might sperm–sperm interactions occur?

In species in which ejaculation is into the vagina, such as rabbits, ruminants, and primates, nonrandom sperm contacts may have occurred during entry into the cervix and passage through the mucus-lined cervical canal. However, the initial focus of this essay will be on the uterus wherein gradients in the density of suspended spermatozoa are established toward the region of the utero-tubal junction [19]. After the much accentuated myometrial contractions induced at coitus have subsided and as subpopulations escape from immersion in seminal plasma in the anterior vagina and distal portion of the cervix, scope arises for specific sperm–sperm interactions. These may be initiated while in suspension in the lumen of the uterus or close to the endometrial surface and could occur during passage between and across the folds of the utero-tubal junction. Indeed, this specialized junctional region may itself impose a form of selection on those sperm entering the oviducts [19–21].

In species in which the ejaculate is introduced into the uterus at mating (e.g., dog, horse, pig, many rodents), the utero-tubal junction forms distended papilla-like processes that protrude into the uterus during estrus (see Fig. 1 in Ref. [22] and Figs 1 and 2 in Refs [23] and [24]). These processes act primarily as a valve to prevent bulk entry of seminal fluids into the lumen of the oviduct. In such species, vigorous sperm motility is essential for traversing this formidable barrier in the hours before ovulation. During passage across and through the utero-tubal junction, the concentration of suspended spermatozoa is reduced by  $X10^2 - X10^3$  (100-fold to 1000-fold) compared with that in the uterine lumen (see Figs in [25,26]). Transient interactions between motile spermatozoa could commence in this region of the tract, as cells proceed intimately across the epithelial surfaces. The nature of such putative cross-talk remains unknown. It might include physical cues concerned with movements of the sperm head and flagellum or molecular interactions concerned with the sperm surface or a combination of both. Each could be mediated by contact with specialized epithelial cells. Sperm-specific proteins may assist passage through the utero-tubal junction [27].

Interactions of an immunological nature are beyond the scope of this essay (see [19,28,29]), but the vast population of polymorphonuclear leukocytes that infiltrates the uterus in response to seminal products needs consideration. Sperm interactions with polymorphonuclear leukocytes take many forms and, as originally proposed Soupart [30], could contribute to modification of the sperm surface and the process of capacitation. Viable spermatozoa are resuspended in female genital fluids during ad-ovarian progression, so the potential for specific sperm–cell interactions should

increase, becoming most effective within the confines of the oviduct isthmus. The potential nature and significance of sperm–sperm interactions in this portion of the tract are considered in subsequent sections.

## 3. When might sperm–sperm interactions occur?

At this point of the essay, it is appropriate to recall extensive observations on sperm distribution and motility in the oviduct in the hours before and soon after ovulation. Following mating early in estrus and until close to the time of ovulation, highly motile spermatozoa entering the oviducts in laboratory rodents and domestic farm animals are arrested and bind to the epithelium of the caudal isthmus (reviews: [11,19,27,31–36]). Having gained this region of constriction just beyond the utero-tubal junction, spermatozoa undergo suppression of motility in a viscous glycoprotein milieu and make specific adhesion contacts between the sperm head and endosalpingeal cilia or microvilli or both [22,23,37]. A functional sperm reservoir is thereby established. When ovulation is imminent, and with the prospect of one or more oocytes being shed into the oviduct, discrete numbers of competent spermatozoa are activated and released from epithelial binding [27,32,38–40]. This controlled release of subpopulations generates initial sperm:egg ratios at the isthmo-ampullary junction—the site of fertilization—close to unity, reducing the risk of multiple sperm penetration of individual eggs [32,38,40]. Such tight regulation around the time of ovulation should be contrasted with postovulatory release of progressively increasing numbers of motile spermatozoa, although this occurs too late to influence the normality of fertilization: a stable block to polyspermy would have already been established in activated eggs [33,40,42–44].

Overall control of these pre- and periovulatory events within the genital tract resides in endocrine activity of the ipsilateral gonad [32,33,41,45]. Such an influence is mediated through the systemic circulation and, 10 to 20 times more powerfully, *via* a local countercurrent transfer of hormones, notably of progesterone, between the ovarian vein and oviduct branch of the ovarian artery [32,33,40,45]. Periovulatory Graafian follicles close to shedding a female gamete thereby communicate with the region of the oviduct containing a reservoir of male gametes and signal the imminence of ovulation. Binding of spermatozoa to the endosalpinx facilitates transfer of informational cues from the gonad-programmed mucosa *via* intercellular bridges. This ipsilateral endocrine regulation is proposed to act in concert with highly localized sperm interactions as a means of fine-tuning the number of spermatozoa activated and released from binding shortly before and soon after ovulation. Remodeling of the sperm surface, shedding of sperm proteins, and the onset of hyperactive motility may all be critical at this stage [27].

## 4. Why cross-talk could be beneficial

In this system for the generation of low *initial* sperm:egg ratios at the site of fertilization, a negative feedback influence of detaching and detached hyperactive spermatozoa would act to regulate the reserves of spermatozoa still

bound to the caudal isthmus. One consequence of this proposed cross-talk would be a chronologically staggered periovulatory detachment of further spermatozoa from epithelial binding, thus limiting the number proceeding to the isthmo-ampullary region at any one time. Post-ovulatory attenuation of sperm storage is due to increasing ovarian progesterone secretion and has been examined in some detail [32,40]. In the postovulatory situation, *activation* of multiple sperm release would gradually take precedence over *inhibition*. The physiological significance of cross-talk between individual spermatozoa would be presumed to decrease with time after ovulation.

An integral part of the preceding hypothesis concerns the number and condition of spermatozoa established in the caudal isthmus. Different intensities of preovulatory epithelial binding in multiple subpopulations of spermatozoa would be anticipated among the 5000 or more viable spermatozoa in each oviduct reservoir [19,46–48]. A range of states in sperm surface membranes and molecular expression, especially in different domains on and around sperm heads, would reflect maturational status and influence the extent of endosalpingeal intimacy. Indeed, each and every sperm cell in the oviduct may be distinct when viewed at a molecular level [49]. At the time of periovulatory release from epithelial contact, those sperm cells least avidly bound and presumed to be more mature would be liberated first. Subtle shades of difference in the continuously remodeled architecture of the sperm surface could therefore underlie a chronologically staggered release. Increasing numbers of spermatozoa would be liberated as capacitative changes proceed, these being prompted by ovarian endocrine signals to the mucosa.

## 5. Feedback regulation of sperm release

As to the nature of interactions between viable detached and bound spermatozoa to influence a progressive release of spermatozoa in the caudal isthmus, feedback signals from sperm already activated are proposed to involve:

1. Metabolic cues from spermatozoa expressing hyperactive motility, such as respiratory byproducts; these would impose local inhibition until sufficiently attenuated.
2. Liberation of surface macromolecules from activated spermatozoa, such as seminal plasma proteins and female genital tract proteins during remodeling of the sperm plasma membrane, notably in the portion overlying the acrosome.
3. Modification in the intensity and distribution of sperm surface charge, this form of membranous change permitting cross-talk between adjacent sperm cells. Increasing net charge during sperm maturation could act to regulate the extent of repulsion.
4. Dynamic differences in intracellular  $\text{Ca}^{2+}$  ion status between neighboring sperm cells in surface contact, acting to influence the capacitation status of individual cells. The CatSper calcium channel provides a link between progesterone signaling and changing calcium influx and content [50,51], the latter being monitored by cells on the threshold of release from binding.

5. Specific patterns of movement or vibration characteristics in the head and flagellum of activated spermatozoa, such altered beat generating and transmitting a highly localized resonance in the oviduct fluid micromilieu.
6. Modification of temperature microgradients in the fluid compartment of the oviduct isthmus [52,53].

The overall sensitivity to such forms of prompting would be presumed to increase close to the time of ovulation.

A changing combination of some or all of the preceding factors, and doubtless diverse others, could underlie a numerically controlled release of spermatozoa from endosalpingeal binding. In other words, sperm cells themselves would be acting to influence their binding reactions although, close to the time of ovulation, sperm–sperm signaling may also be mediated *via* the endosalpinx. Whatever the precise nature of chemical and physical signals, they would be presumed to demonstrate a concentration gradient, diminishing with distance from their principal source (i.e. liberated, hyperactive spermatozoa). Cells in closest proximity would have the potential to communicate most powerfully. In the light of these proposals, sperm interactions could be viewed as part of a selection process, but this would be a physiological selection based on sperm cell integrity and maturity.

### 5.1. Further signals influencing sperm release

Progressively overriding the local influences of sperm cross-talk, both negative and positive, could be attractant cues for subpopulations of spermatozoa derived from the peri- or postovulatory ovary. More specifically, the possibility of chemotactic attraction between the products of ovulation (i.e., oocyte, cumulus cells, follicular fluid) and a potentially fertilizing spermatozoon has resurfaced in the mammalian literature: attraction of discrete populations of spermatozoa toward sources of progesterone is a prominent theme [54–56], although the statistical interpretation of data from *in vitro* studies is open to improvement [57]. In any case, dissociating prefertilization events under physiological conditions will be complex, for progesterone is freely available in oviduct fluid (reviewed in [26]) and ovarian follicular sources of progesterone will be influencing both the cellular contents of the oviduct lumen and activities of the oviduct wall. Furthermore, local treatment of oviduct tissues with microinjections of a solution of progesterone in oil prompted a dramatically increased adovarian passage of spermatozoa, a 38% incidence of polyspermic fertilization, and large numbers of spermatozoa (100–250 per egg) on and in the zona pellucida [58]. Overall, therefore, demonstrating the phenomenon of progesterone-guided chemotaxis within a highly contractile periovulatory oviduct in the presence of ciliary beat, gradients in temperature and viscosity, and relative movements of luminal fluid will require sophisticated technology.

In parallel with a possible influence of chemotaxis [59], the presence of odorant receptors in the midpiece or flagellum of spermatozoa could suggest a supplementary

system for modulating ad-ovarian sperm progression in the oviduct. Recent reports center around G-protein-coupled odorant receptors in sperm [60,61], but details of their specific involvement relative to the time of ovulation and completion of capacitation remain to be clarified, as does a source of such molecules in the gonad or genital duct. Of potential relevance, however, the CatSper channel discussed above can be directly activated by odorants [51].

## 6. Sperm competition: outstanding questions

Returning to a point touched on in the Introduction section, if a faster swimming speed in the female tract is suggested to bestow a competitive advantage on a potentially fertilizing spermatozoon, then how can this be reconciled with preovulatory arrest and binding in the caudal isthmus followed by a tightly controlled peri-ovulatory sperm release? And, as a more specific but still related question, are the first spermatozoa entering the oviduct and binding to the epithelium those that will be released first when the time of ovulation approaches? How could this be demonstrated, and do such ‘vanguard spermatozoa’ bind to a region of the isthmus closer to the site of fertilization than subsequent spermatozoa? Whatever the answer to these questions, proponents of the competitive advantage of sperm swimming speed need to take account of events in the mammalian oviduct before and close to the time of ovulation. Moreover, because multiple preovulatory mating occurs in many populations of breeding mammals [62,63], the competitive relationship between those spermatozoa binding early in the period of receptivity (estrus) and those binding nearer to the time of ovulation also needs clarification. And, in particular, are there circumstances in which the fertilizing spermatozoon avoids a phase of endosalpingeal binding altogether? Such binding is not mandatory before fertilization [64].

## 7. Significance of sperm swimming speed

There is an alternative interpretation of the importance of sperm swimming speed in the female tract before arrival at the cumulus cell investment and contact with the zona pellucida. The notion of a *race* may very well be correct, but not in the sense of a competitive one between individual spermatozoa and based on quality of the genome. Rather, the race might be one to escape from the lower reaches of the tract and enter the privileged preovulatory environment of the oviduct. This would be for multiple reasons including those of avoiding the following:

1. Undue exposure to fluids present in the uterine lumen.
2. Progressive leaching of membrane-stabilizing macromolecules from the sperm surface.
3. Dissipation of limited energy reserves while swimming actively in uterine fluids.
4. Engulfment by infiltrating populations of polymorphonuclear leukocytes within the uterine lumen.

5. Untimely progression toward full capacitation by arrest in the viscous milieu of the cooler caudal isthmus with specific sperm head binding to the endosalpinx.

This last step would place cells of suitable integrity under the locally transmitted influence of ovarian endocrine events as a follicle(s) approaches ovulation, thereby enabling coordination in the final maturation of male and female gametes.

Despite the thrust of the above remarks, there may, nevertheless, be a sperm race—a preovulatory competition—to reach and occupy the most favorable binding sites in the caudal portion of the oviduct isthmus. Features of such binding sites also require clarification.

## 8. Conclusion

Putting the above views in an overall perspective, it may be helpful to return to some words written 20 years ago:

“It has long been accepted that, with the evolution of internal fertilisation in the higher vertebrates and the shedding of quite limited numbers of oocytes, there would be complex physiological processes to promote a successful union of the gametes. However, the notion of diverse local messenger molecules functioning within such systems is only now beginning to receive close attention.” [39]

The valedictory essay of [65] offers useful speculations on this theme.

The principal message of this article is that consideration should be given to a highly localized molecular strategy to monitor and coordinate the ad-ovarian progression of subpopulations of competent spermatozoa within the mammalian oviduct.

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## Competing interests

There is no conflict of interest of any kind whatsoever in the above work. The essay was conceived and discussed by both authors, written by RHFH, and approved in its final version by both authors.

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