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Behaviour of spermatozoa in the oviduct of farm animals

Comportamiento de los espermatozoides en el oviducto de los animales de hacienda

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Extensive studies in the large farm animals (sheep, cow, pig) have established that the functional sperm reservoir –that is, site in which spermatozoa that will subsequently fertilise the eggs are stored during the pre-ovulatory interval– is located in the caudal portion of the oviduct isthmus. In this region of the tract, sperm cells are: 1) no longer exposed to constituents of the seminal plasma, 2) protected from engulfment by polymorphonuclear leucocytes, and 3) suppressed in terms of motility during the pre-ovulatory interval. Episodes of multiple mating early in oestrus do not displace spermatozoa from this reservoir. Viable spermatozoa progress from the caudal isthmus to the site of fertilisation in a tightly-regulated manner close to the time of ovulation. On the basis of careful analysis in farm animals with their relatively long pre-ovulatory interval (26-42 hours), it is important to stress that sperm activation and release from the ishtmus reservoir commence shortly before ovulation rather than as a consequence of ovulation itself. Regulation of ad-ovarian sperm progression seemingly involves a local counter-current transfer of follicular hormones from the ovarian vein to the tubal branch of the ovarian artery, an endocrine influence that is transduced via the oviduct epithelium and noted in a changing composition of the luminal fluids. As judged by both the acrosome reaction and activated patterns of motility, completion of capacitation is a peri-ovulatory event, but it remains uncertain whether these changes in the membranes and flagellar activity of the sperm cell are expressed in the ishtmus or await arrival at the ampullary-isthmic junction. This initial progression of spermatozoa to the site of fertilisation involves specific interactions with swollen microvilli or cilia of the endosalpinx, especially those engaging the anterior portion of the head. However, once eggs are fertilised and the block to polyspermy is irreversibly established, control of sperm ascent to the ampulla is conspicuously relaxed. Indeed, 2-4 cell pig embryos may each contain 400 or more sperm heads embedded in the zona pellucida.

INTRODUCTION

Timely apposition of the male and female gametes is an essential prerequisite for successful membrane fusion and completion of the process of fertilisation. Under conditions of free access of competent males to cyclic females, a sperm suspension would be introduced into the vagina or uterus at the onset of oestrus, whereas the oocyte(s) would be liberated from the gonad at the time of ovulation. Those spermatozoa directly involved in the events of fertilisation are now widely accepted to be stored before ovulation in the caudal region of the oviduct isthmus –the so-called functional sperm reservoir (Yanagimachi, 1981; Harper, 1982; Overstreet, 1983). The question therefore arises as to whether some form of synchronisation underlies meeting of the gametes and, more specifically, whether there is a programming of sperm activation and release from the isthmus reservoir by a Graafian follicle(s) on the verge of ovulation. This question will be addressed in the pages that follow, but first a paragraph concerning progression along the oviduct of the female gamete, the oocyte, to the site of fertilisation at the ampullary-isthmic junction.

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Initial transport of eggs

The oocyte enveloped in its much expanded and loosened cumulus mass is stripped from the surface of the collapsing Graafian follicle by a concerted action of dense arrays of cilia lining the inner surface of the fimbriated infundibulum (Blandau, 1969, 1973). In mammals in which an ovarian bursa is absent, these fimbriated extremities become engorged and embrace the ovaries intimately at the time of ovulation. The beat of the cilia is orientated towards the ostium of the oviduct, and the rate of beat is maximal close to ovulation (Blandau & Verdugo, 1976). In polytocous species such as hamsters and pigs, granulosa cell masses surrounding individual oocytes aggregate during the initial phase of egg transport to form a cumulus plug (Hancock, 1961). In these species, transport of eggs along the ampulla to the site of fertilisation occurs within the cumulus plug, but in monotocous species such as cows and sheep, granulosa cells remain only briefly around recently-ovulated eggs (Lorton & First, 1979).

Whether progression of the plug or indeed of single oocytes is primarily due to the action of cilia or of a contracting myosalpinx has been the subject of extensive experimentation and debate (Pauerstein & Eddy, 1979). However, the consensus would appear to be that smooth muscle activity (peristalsis) and an abovarian beat of the cilia lining the ampulla are together needed to assure a timely passage of eggs to the ampullary-isthmic junction. In laboratory species such as rabbits, this phase of transport requires 9-13.5 minutes (Harper, 1961) whereas in the larger farm species such as pigs, some 30-45 minutes may represent the usual time course (Hunter, 1974). Initial egg transport in primates appears to be significantly slower (Croxatto & Ortiz, 1975), but the precise site of fertilisation in women remains unknown. If psychosomatic influences do indeed play a rôle in gamete transport within the human oviduct, a variable site of fertilisation might be anticipated. With the possible exception of the situation in equids, eggs released at spontaneous ovulation are invariably secondary oocytes, even though a final phase of maturation of the egg membranes and ooplasm may still occur during transport to the site of fertilisation. This would be in response to (1) proteoglycan secretory activity of the corona cells and (2) specific macromolecular secretions of the endosalpinx.

Rate of formation of functional sperm reservoirs

As noted in the Introduction, extensive studies in the large farm animals (sheep, cow, pig) have demonstrated that the functional sperm reservoir –that is the site in which spermatozoa that will subsequently achieve fertilisation are stored during the protracted preovulatory interval– is located in the caudal portion of the oviduct isthmus (sheep: Hunter *et al.*, 1980, Hunter & Nichol, 1983; cows: Hunter & Wilmut, 1984, Wilmut & Hunter, 1984; pigs: Hunter, 1981, 1984). These studies therefore endorse earlier observations made in rabbits by Harper (1973a, b), based on the technique of egg transplantation combined with recovery at different intervals after mating. Harper's conclusions in rabbits received support from the study of Overstreet & Cooper (1975), showing a much reduced flagellar activity of rabbit spermatozoa during the pre-ovulatory interval. It should be noted that spermatozoa stored in the isthmus reservoir are(a) no longer exposed to elements of the male secretions –the seminal plasma– and (b) are protected from engulfment by the massive post-coital invasion of polymorphonuclear leucocytes into the uterus. Except in pathological situations, polymorphs seem not to enter the lumen of the oviduct.

Concerning the rate of establishment of these functional sperm reservoirs, there was no evidence in the farm species of an extremely rapid phase of transport of viable spermatozoa to the oviducts in a period of seconds or even in a small number of minutes (*cf.* Van Demark & Moeller, 1951, in cows; Overstreet & Cooper, 1978, Overstreet *et al.*, 1978, in rabbits). By

contrast, formation of sperm reservoirs in the isthmus of sheep and cows mated at the onset of oestrus was a slow progressive process (see Thibault, 1973), requiring a period of 6-8 hours or more under conditions of spontaneous oestrous cycles (Hunter *et al.*, 1980; Hunter & Wilmut, 1983). It is important to emphasise that these judgements concern viable spermatozoa able to bind to and penetrate the zona pellucida. The timing of sperm transport is significantly faster in pigs than in ruminants, due to the intra-uterine accumulation of a voluminous ejaculate. This latter situation enables the utero-tubal junctions to be bathed with a dense sperm suspension (1-2 x 10^8 cells per ml) by the completion of mating, which in turn facilitates entry of viable boar spermatozoa into the oviducts within as short a period as 15 minutes (Hunter & Hall, 1974; Hunter, 1981). In a majority of animals studied, sufficient boar spermatozoa had entered the oviducts one hour after mating at the onset of oestrus to ensure subsequent fertilisation of > 90% of the eggs ovulated (Hunter, 1981, 1984). Moreover, these studies indicated that viable boar spermatozoa were sequestered in the caudal 1-2 cm of the oviduct isthmus for the majority of the pre-ovulatory interval. Only in the last hour or two before ovulation did viable spermatozoa beging to progress onwards towards the site of fertilisation from reservoirs in the caudal isthmus (Table 1), a rather precise regulation that was also noted in sheep (Table 2; Hunter & Nichol, 1983). Episodes of multiple mating early in oestrus did not displace viable spermatozoa from this reservoir in sheep or pigs –an observation which raises major questions if contractile activity ensued.

Storage conditions in the isthmus sperm reservoir

Based on observations in the scanning electron microscope (Fléchon & Hunter, 1981; Hunter *et al.*, 1987), boar spermatozoa stored in the caudal portion of the oviduct isthmus *before* ovulation are associated with a viscous mucus that acts to occlude the narrow lumen. In scanning electron micrographs, this mucus appears as large spheres or globules together with a 'flocculent' material dispersed in an intermittent but seemingly organised manner on the surface of the sperm head. Attempts to obtain samples of isthmus mucus at surgery by inserting fine glass pipettes through the utero-tubal junction indicate that it is whitish-cream in colour and extremely viscous *before* ovulation, invariably being pulled back into the isthmus by its elastic qualities. Whether spermatozoa are physically arrested and their flagellar activity suppressed by the viscous nature of such pre-ovulatory mucus cannot yet be stated with certainty (see Suarez, 1987), but our own observations in anaesthetised pigs suggest that this mucus becomes reduced in viscosity *after* ovulation. If this is correct and represents the physiological situation, then the physical condition of secretions in the caudal portion of the oviduct lumen must contribute to the phase of sperm storage, even though a majority of the viable spermatozoa may be located deep in folds of the epithelium rather than distributed more centrally in the lumen. The observations of both Suarez (1987) and Suarez & Osman (1987) in mice and Smith & Yanagimachi (1990) in hamsters bear critically on such a sperm distribution.

Although the physical condition of mucus in the isthmus would be regulated by ovarian endocrine events, the precise influence of increasing numbers of capacitated spermatozoa and/or their acrosomal enzymes on the changing condition of the mucus remains unclear.

Changes in chemical composition of fluid in the oviduct isthmus may also contribute to the means of pre-ovulatory storage and then peri-ovulatory activation, and forms one of our current themes of research (unplublished with H. Leese & R. Nichol). Earlier studies in rabbits had suggested that K^+ ions inhibited sperm motility in the isthmus whereas pyruvate stimulated such motility, there being some evidence for differences in the composition of isthmic and ampullary fluids before ovulation (Burkman *et al.*, 1984).

TABLE 1

The influence of transecting the oviduct isthmus of pigs 1.5-2.0 cm above the utero-tubal junction at increasing intervals after mating at the onset of oestrus on the proportion of eggs subsequently fertilised (6 animals/group). The data demonstrate restriction of viable spermatozoa to the caudal portion of the isthmus until shortly before ovulation. (Modified from Hunter, 1984)

Interval from mating to transection (hours)	Condition of ovaries at transection	Transected isthmus			Control isthmus*		
		eggs recovered	N° of eggs fertilised	%	eggs recovered	N° of eggs fertilised	%
3	Pre-ovulatory	34	0	0	32	32	100
6	Pre-ovulatory	40	0	0	35	33	94
12	Pre-ovulatory	42	0	0	41	41	100
24	Pre-ovulatory	50	0	0	41	39	95
30	Pre-ovulatory	53	0	0	33	32	97
36	Pre-ovulatory	51	1	2	41	41	100
38	Pre-ovulatory	39	2	5.1	49	49	100
40	Pre-ovulatory	48	19	39.6	35	35	100
42-44	Post-ovulatory	46	46	100	34	34	100
Total		403	68	16.9	341	336	98.5

* Double ligatures were placed around the control oviduct and then removed.

TABLE 2

The proportion of sheep yielding fertilised eggs and the incidence of fertilisation when the interval from mating to transection of the isthmus 1.5-2.0 cm above the utero-tubal junction increased from 10 to 26 hours*. (Taken from Hunter & Nichol, 1983)

Interval from mating to transection (hours)	Condition of ovaries at transection	N° of ewes:		N° of eggs:		N° of accessory sperm per egg:	
		examined	with some fertilised eggs	recovered	fertilised	mean	range
10	Pre-ovulatory	8	0	9	0	0	–
12	Pre-ovulatory	8	0	8	0	0	–
14	Pre-ovulatory	8	0	8	0	0	–
18	Pre-ovulatory	8	0	8	0	0	–
20	Pre-ovulatory	8	0	11	0	0	–
21	Pre-ovulatory	8	0	8	0	0	–
22	Pre-ovulatory	8	1	8	1	0	–
23	Pre-ovulatory	10	1	11	1	0	–
24	Pre-ovulatory	11	0	14	0	0	–
25	Peri-ovulatory	12	3	14	3	0.7	0-2
26	Post-ovulatory	13	11	16	13	7.9	0-26
Total		102	16	115	18	7.4	0-26

* All ewes operated on 26 hours after the onset of oestrus had recent ovulations.

A further factor which may be implicated in sperm storage concerns a temperature gradient within the oviduct lumen. The caudal isthmus has been noted to be approximately 0.75°C cooler than the rostral ampulla *before* ovulation in mated pigs, a temperature differential that is effectively eliminated after ovulation (Hunter & Nichol, 1986a). Such small changes in temperature would nonetheless have an ability to influence markedly the potential configuration of the sperm flagellum. Regional

differences in pre-ovulatory temperatures had previously been recorded in rabbit oviducts (David *et al.*, 1972), although a physiological interpretation in the context of gametes and/or embryos was not offered.

Peri-ovulatory activation of spermatozoa

As already noted, viable spermatozoa progress from the caudal isthmus to the site of fertilisation in a strictly-regulated manner close to the time of ovulation. On the basis of a careful anatomical analysis in farm animals with their relatively long pre-ovulatory interval (26-42 hours), it is important to stress that sperm activation and release from the isthmus reservoir commence shortly *before ovulation* rather than as a consequence of the process of ovulation itself. Thus, mechanisms must be involved which precede and are quite distinct from the "products of ovulation" entering into the oviduct ampulla. Evidence in support of these statements is presented in Tables 1 and 2 for pigs and sheep, respectively. A working model of the pre- and peri-ovulatory events is presented in Figure 1, which also takes account of observations in cows by Herz *et al.* (1985).

The left-hand site of the model (Figure 1a) is intended to portray the situation in mated animals soon after the onset of oestrus. The pre-ovulatory Graafian follicles are not yet of terminal diameter, the wall of the isthmus is oedematous and, as a consequence, the lumen is extremely narrow. A majority of viable sperm cells at this early stage of oestrus have intact acrosomes, and the flagellum as seen in scanning electron micrographs is relatively straight or only slightly undulating. The cell is judged to be stabilised and motility suppressed. Follicles on the verge of ovulation are depicted in Figure 1b. Associated with increasing pre-ovulatory secretion of progesterone, the extent of oedema in the wall of the isthmus is already diminishing and as a consequence the patency of the oviduct lumen is thereby increasing. Acrosome-reacted spermatozoa are conspicuous, and the form of the flagellum in scanning electron micrographs indicates

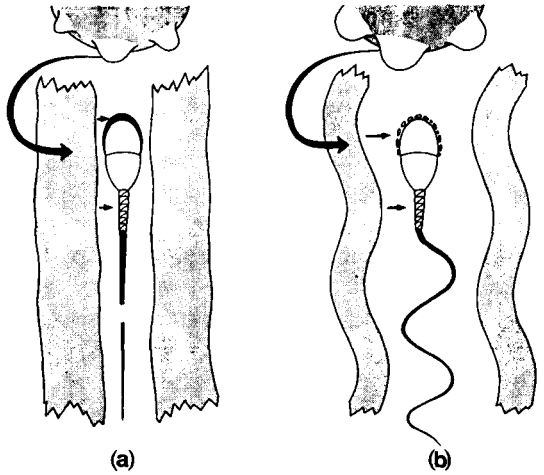

Fig. 1: Model to illustrate the manner whereby the endocrine activity of pre- or peri-ovulatory Graafian follicles acts locally to programme the membrane configuration and motility status of spermatozoa in the lumen of the oviduct isthmus. Gonadal hormones (from the follicles) act on the tubal epithelium whose transudates and secretions in turn influence the nature of the luminal fluids. Expression of capacitation is reasoned to be a peri-ovulatory event, at least in the large farm species with a protracted interval between the gonadotrophin surge and ovulation.

a) Intact, relatively quiescent spermatozoon under the overall influence of *pre-ovulatory* follicles. Membrane vesiculation on the anterior part of the sperm head is suppressed, as is the development of whiplash activity in the flagellum, presumably due to local molecular control mechanisms. The lumen of the oviduct isthmus is extremely narrow and contains viscous secretions, and myosalpingeal contractions are reduced.

b) An acrosome-reacted, hyperactive spermatozoon under the influence of Graafian follicles on the point of ovulation. The patency of the isthmus has commenced to increase, enabling expression of a more powerful pattern of flagellar beat.
Progression of such spermatozoa to the site of fertilisation is also aided by enhanced contractile activity of the myosalpinx, and yet involves a strict numerical regulation.

This model accords with the observation that high proportions of spermatozoa undergoing the acrosome reaction in ruminants are found predominantly in the ampulla adjoining the ovulatory ovary and only at or following ovulation.

activation. Whether true hyperactivation of spermatozoa (Katz *et al.*, 1978; Yanagimachi, 1981; Fraser, 1984) can and does actually occur in the caudal isthmus of farm animals remains to be clarified: the size of the lumen shortly before ovulation may preclude full expression of this pattern of flagellar beat.

None of the preceding argument is intended to deny a potent influence of the

ovulated cumulus mass on the subsequent phases of sperm transport.

Capacitation of spermatozoa

On the basis of the above analysis, and in the light of straightforward biological reasoning, classical views on the time-course of mammalian capacitation have been modified in a significant way since its discovery forty years ago (Austin, 1951; Chang, 1951). The summary that follows offers an important new perspective, presented in detail elsewhere (Hunter, 1987a, 1987b).

Since first being reported, the process of capacitation in mammalian spermatozoa has been viewed as a final maturation of gametes in the female genital tract, conferring upon a proportion of the cells an ability to penetrate the egg investments (Bedford, 1970). Ejaculated or cauda epididymal spermatozoa deposited at the site of fertilisation in the oviducts cannot penetrate and activate the egg(s) for a period of time, usually measured in hours. Capacitation has been thought primarily to involve escape from the seminal plasma, resuspension of spermatozoa in uterine or tubal fluids, and an altered metabolic activity. More subtle cellular changes were always suspected, but remained elusive until the 1970's. Overall, capacitation has been considered to require a time interval characteristic of each species (Table 3), a timing which may be closely mimicked in systems for *in vitro* fertilisation.

More recent studies have focused on events that are now interpreted as a consequence of the capacitation process: these include (1) membrane vesiculation on the anterior portion of the sperm head, termed the acrosome reaction, that facilitates release of proteolytic enzymes (Barros *et al.*, 1967), and (2) the dramatically modified flagellar beat –the so-called whiplash or hyperactivated motility that gives an incisive force of penetration (Yanagimachi, 1981). These changes are thought to be associated with a Ca^{2+} influx into the sperm cell and increased levels of cAMP.

Because of these changes in membrane configuration and flagellar beat, and bearing in mind the negligible reserves of cytoplasm, capacitated spermatozoa are accepted to be fragile, unstable and short-lived. This situation would seem to pose problems in females undergoing a single mating at the onset of receptivity, especially in species in which the period of oestrus extends for 1-2 days or more. Explanation for the prolonged availability of capacitated spermatozoa have therefore invoked the heterogeneous condition of spermatozoa in the ejaculate, with flexibility arising from a progressive ripening of cell in different states of maturity at deposition: in other words, a series of curves of ripening and decay within the millions of cells in an ejaculate. However, studies in farm animals indicate that this interpretation and the rather specific chronology presented in Table 3 may need to be modified, at least in species with a protracted interval between the gonadotropin surge and ovulation. Two examples of the dilemma will suffice. Ram spermatozoa have been reported to require 1.0-1.5 hours for capacitation (Mattner, 1963), and yet sheep ovulate approximately 26 hours after the onset of oestrus. Boar spermatozoa require 2-3 hours for capacitation (Hunter & Dziuk, 1968), whereas pigs ovulate 40 hours after the onset of oestrus. Even invoking the heterogeneous condition of the ejaculate and the subsequent curves of ripening, the time relationships suggest that these do not furnish a full explanation for fertility.

The new understanding of oviduct physiology in domestic animals indicates

TABLE 3

Approximate values for the capacitation time of mammalian spermatozoa *in vivo* available in a number of established textbooks. As argued in the text, these figures are only meaningful if interpreted in a post-ovulatory situation

Species	Interval (hours)
Sheep	1-1.5
Pig	2-3
Rat	2-3
Hamster	3-4
Cow	3-5
Rabbit	5-6

that traditional views on the process of capacitation do require modification. Rather than this terminal maturation needing a given period of time in the female tract (e.g. 1-5 hours according to species; Table 3), capacitation appears to be related to peri-ovulatory events. Clearly, there would be little value in achieving capacitation within a few hours of ejaculation if such capacitated cells were to be non-functional by the time of ovulation. In fact, specific oviduct proteins may act to suppress the completion of capacitation, the acrosome reaction and whiplash motility until shortly before ovulation; the rôle of the Wolffian duct protein, immobilin, may be matched by comparable Mullerian duct secretions. Moreover, ram spermatozoa display hyperactivated motility in the oviduct at the time of ovulation (Cummins, 1982). Finally, it is important to note that classical studies of capacitation, both *in vivo* and *in vitro*, have invariably been performed in a post-ovulatory environment, i.e. in the presence of eggs and/or their investments, and this may have led to a misinterpretation.

Ovarian regulation of sperm suppression and activation

If the preceding observations on pre-ovulatory storage of spermatozoa in the caudal portion of the isthmus are accepted, together with the suppressed motility and stabilised cell membranes, then some explanation is required for the means of activation of intensive motility and ad-ovarian sperm progression that occurs close to the time of ovulation. Although precise mechanisms are not yet fully understood, there is (1) some appreciation of the manner of gonadal control and (2) already information on a changing composition of oviduct luminal fluids with stage of the cycle (Restall, 1966; Mastroianni *et al.*, 1970; Borland *et al.*, 1980). Overall control of sperm activity appears to reside in the endocrine function of the Graafian follicle(s) close to ovulation (Figure 1), that is, in the structure that will release the female gamete. However, the mechanism seems to operate primarily through a local transfer of such endocrine information rather than via a systemic route, so that both oviduct and sperm function are reprogrammed in an incisive and sensitive manner at a time just preceding release of the egg(s). Evidence for a counter-current transfer of relatively high concentrations of follicular hormones such as steroids and prostaglandins has been presented elsewhere (Hunter *et al.*, 1983), the counter-current stemming from the ovarian vein into the oviduct branch of the ovarian artery (reviewed by Einer-Jensen, 1988). The experimental evidence did not preclude a contribution from lymphatic transfer of hormones, for the caudal portion of the oviduct is particularly rich in lymphatic sinuses (Andersen, 1927). Nor should it be overlooked that follicular hormones would also reach the oviduct isthmus through the systemic circulation, although at a much-reduced concentration. An influence of the changing endocrine information would be transduced through the tissues of the oviduct wall and be expressed in modified chemical constituents and perhaps altered gas tensions in the lumen.

Regulation of sperm ascent

Concerning progression of spermatozoa to the site of fertilisation at the ampullary-isthmic junction (Figure 2), a number of perplexing questions remain to be answered. Since the first reports of Stefanini *et al.* (1969) in mice, there has been a growing appreciation that sperm: egg ratios in the ampulla may be close to unity at the time of initial penetration and activation. In fact, at this particular moment, there are seldom any other spermatozoa in the immediate vicinity of the egg(s). Certainly, this seems to be the case in rats (Shalgi & Kraicer, 1978), hamsters (Bavister, 1979), sheep (Cummins & Yanagimachi, 1982), pigs (Hunter & Dziuk, 1968) and probably cows (Hunter & Wilmut, 1984). However, if there is a local programming of completion of capacitation and sperm activation, then large numbers of spermatozoa in the isthmus reservoir might be expected to respond more

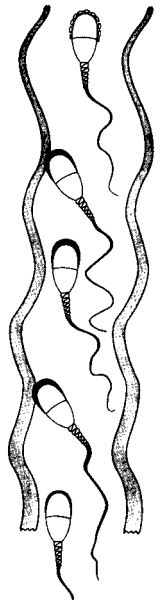

Fig. 2: Diagram to depict the manner of peri-ovulatory progression of spermatozoa in the isthmus of the mammalian oviduct based on *in situ* observations in mice and hamsters. Spermatozoa arrested in the caudal isthmus during a relatively prolonged pre-ovulatory interval become activated at the time of ovulation and proceed towards the site of fertilisation at the ampullary-isthmic junction by phases of highly-active free swimming alternating with phases of contact adhesion to the epithelium by the rostral tip of the head. Such phases of adhesion may offer one means of reducing the risk of polyspermic fertilisation by regulating the number of competent spermatozoa confronting the newly-ovulated eggs.

Three critical facts remain to be clarified in this model:

1) Whether vesiculation of membranes on the anterior portion of the sperm head –the acrosome reaction– commences in the isthmus or is induced physiologically in the vicinity of the eggs at the site of fertilisation. Scanning electron micrographs of both boar and bull spermatozoa in peri-ovulatory animals have recorded either a full acrosome reaction or an incipient reaction in the isthmus of the oviduct.
2) Whether the dramatic increase in beat of the flagellum that assumes a whiplash form –the so-called hyperactivation response– can be fully expressed in the isthmus or if the spermatozoon needs first to progress to the ampullary-isthmic junction. In rodents in particular, the size of the duct lumen may be a factor limiting full expression of hyperactivation.
3) Precisely how sperm:egg ratios of close to unity at the time of initial penetration of the egg(s) are obtained. Interactions between the sperm head and microvilli and/or cilia on the endosalpinx are thought to be vital.

or less simultaneously to the physiological cues, thereby overriding the sperm: egg ratios emphasised above. Perhaps the answer to this conundrum is that full hyperactivation of the flagellum can indeed only be expressed in the ampulla and that, preceding this condition, there is of necessity a tight regulation of sperm progression within the viscous secretions of the isthmus. An alternative argument might be that completion of capacitation among individual sperm cells in the isthmus is not achieved precisely synchronously, but rather is staggered in terms of significant numbers of seconds. Even so, any explanation for the regulation of sperm ascent must also take account of the dramatic peri-ovulatory waves of myosalpingeal contraction that are sufficient in the golden hamster, for example, to displace droplets of oil into the ampulla (Battalia & Yanagimachi, 1979).

A second difficulty concerns interpretation of sperm-epithelial interactions. Recent observations in mice (Suarez, 1987) and hamsters (Smith & Yanagimachi, 1990) suggest that spermatozoa ascend from the isthmus reservoir to the site of fertilisation by intermittent phases of adhesion to the endosalpinx followed by a period of active free swimming in the lumen (Fig. 2). Although it is not yet certain that such spermatozoa represent the fertilising ones, it seems reasonable to presume that this is so. If comparable alternating phases of free swimming and epithelial immobilisation occur with the spermatozoa of farm animals, then this may act to prevent bulk transport although some explanation for the underlying mechanism is needed. Distribution and exposure of glycoproteins on the sperm head are thought to be in a dynamic state close to the time of capacitation, rendering the sperm cell susceptible to phases of interaction and adhesion with the swollen tips of the epithelial microvilli in cows and/or cilia in pigs (Hunter *et al.*, 1987). But the precise nature of changes leading to release (i.e. end of an adhesive phase) is, as yet, undetermined. Migration of protein molecules on and within the anterior portion of the sperm head membranes may be a prime cause, associated with renewed energetic inputs expressed through the sperm flagellum. In such a manner, for the moment at least, one must try to interpret the putative progression of

a fertilising spermatozoon from the caudal isthmus to the site of fertilisation. Most importantly, differential timing of the release from epithelial contact may be a means of achieving the initial sperm: egg ratios highlighted above.

Fate of non-fertilising oviduct spermatozoa

Although substantial numbers of spermatozoa reach the ampulla in the golden hamster (Yanagimachi & Chang, 1963; Battalia & Yanagimachi, 1979), massive numbers of those arrested in the isthmus are reported to be moribund or dead by the time of ovulation (Smith & Yanagimachi, 1990). A high incidence of sperm death has also been noted in rabbit oviduct, although this may be principally associated with the initial very rapid phase of sperm transport (Overstreet & Cooper, 1978; Overstreet *et al.*, 1978). In some of the farm species, by contrast, large numbers of spermatozoa may remain alive and competent in the oviducts, even after mating very early in oestrus. This can be demonstrated by examination of the fertilised eggs. In those of the domestic pig, for example, although there are initially very few spermatozoa present in the zona pellucida of a single-celled zygote, the number of sperm heads within the zona increases significantly with time after fertilisation. In fact, by the time the 2-4 celled stage of development is reached, each individual zona pellucida may contain 400 or more spermatozoa embedded in its substance (Hunter, 1974). The strategy appears to be a strict regulation of sperm ascent within the oviduct isthmus until fertilisation is complete and the block to polyspermy irreversibly established. Thereafter, large numbers of spermatozoa can pass into the ampulla, as also monitored in sheep (Hunter & Nichol, 1983, 1986b), not least because the myosalpinx is relaxing and oedema of the mucosa diminishing. Surgical introduction of fresh suspensions of spermatozoa into the isthmus after fertilisation is complete does not lead to a breakdown in the stability of the block to polyspermy.

As to the ultimate fate of oviduct spermatozoa that do not associate with the zona pellucida, large numbers may be engulfed by granulosa (cumulus) cells (see Szollosi & Hunter, 1973). In species without an ovarian bursa, further spermatozoa may pass into the peritoneal cavity, this being especially well documented in women and indeed used as a test for tubal patency (Settlage *et al.*, 1975; Croxatto *et al.*, 1975; Templeton & Mortimer, 1980; Mortimer, 1983). Incorporation of sperm heads by the oviduct epithelium, as originally described by Austin (1959), seems not to have been documented in the large domestic animals. Finally, at the time of egg or embryo entry into the uterus, when the direction of bulk flow of a reduced volumen of oviduct fluid is reversed to become ab-ovarian, a suspension of dead or moribund spermatozoa has been found to pass back to the uterus (Hunter, 1978). The ultimate fate of these sperm cells may be incorporation by those of the trophoblast.

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