

INTERRELATIONSHIPS BETWEEN SPERMATOZOA, THE FEMALE REPRODUCTIVE TRACT, AND THE EGG INVESTMENTS

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This chapter describes some principal physiological events during transport of spermatozoa in the female reproductive tract from the time of ejaculation until penetration of the egg investments at the start of fertilization. The objective is to review our current understanding of the relevant processes and to highlight areas requiring further research; it is also hoped to contrast certain aspects of sperm transport in pigs with those in ruminants. Although much of the material discusses processes not obviously susceptible to manipulation, topics related to the successful practice of artificial insemination are emphasized.

Previous reviews of sperm transport in the female reproductive tract of pigs include those of Hunter (1973a; 1975a,b; 1980), Polge (1978) and Einarsson (1980), and a comprehensive coverage of the early literature is found in these works. The volume edited by Hafez and Thibault (1975) compares mechanisms of sperm transport and storage in a wide range of vertebrates, whilst fertilization itself is described in pigs by Thibault (1959), Hancock (1961), Baker, Dziuk and Norton (1967), Hunter (1972a, 1974), Szollosi and Hunter (1973; 1978), Baker and Polge (1976) and Polge (1978).

Deposition of semen and uterine transit

The specific stimulus provoking ejaculation in the boar is the interlocking of the glans penis in the cervical ridges of an oestrous gilt or sow. Due to the influence of ovarian steroid hormones, these muscular ridges become taut and oedematous at oestrus (Burger, 1952; Smith and Nalbandov, 1958), and their arrangement is such that the lumen of the cervix tapers towards the internal os, enabling the glans penis to be gripped by the female tissues (*Figure 3.1*). During thrusting of the penis through the anterior vagina and into the cervical canal, there is also a twisting component to the penile shaft, causing the spiral folds of the glans to interdigitate tightly with the cervical wall. The pressure derived from this 'lock' leads to ejaculation, a situation which is mimicked during manual collection of boar semen.

As a result of these anatomical specializations and because of the large volume of the boar ejaculate (150–500 ml), semen makes only passing

contact with the innermost portion of the cervical canal before entering the body of the uterus (*Figure 3.1*). Deposition is therefore effectively *intrauterine* and, in the context of sperm transport to the site of fertilization, little further consideration needs to be given to the structure of the cervix. An initial distribution of semen in the uterus may be achieved due to the force of ejaculation and the volume of fluid involved, assuming that little or no leakage occurs into the vagina. However, contractions of the myometrium are vigorous during oestrus (Corner, 1923; Keye, 1923), and

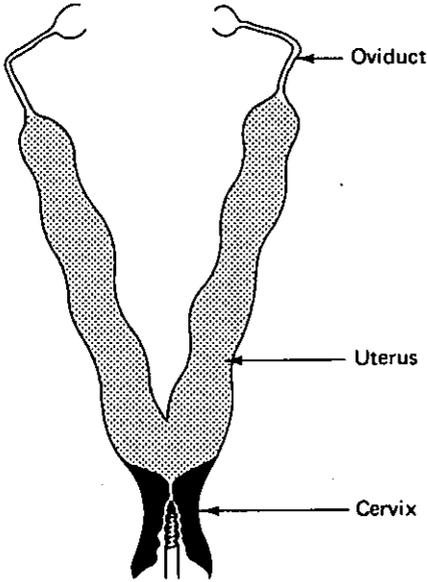
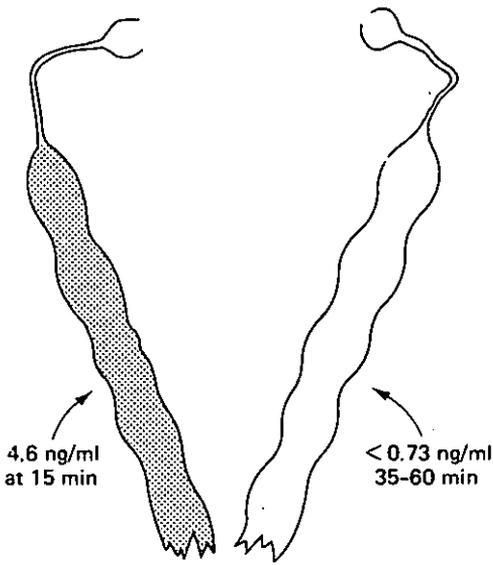


Figure 3.1 Diagram to illustrate the manner in which the spirally-arranged folds of the glans penis interlock with the prominent muscular ridges of the cervix in oestrous pigs. As a result of these specializations, ejaculation is effectively into the uterus and the large volume of semen distends the two cornua by the end of the protracted period of mating.

these should assist transport of the male secretions and, in the pre-ovulatory interval, redistribution of fluid between the two horns. Reflex release of oxytocin would enhance uterine contractions (see Knifton, 1962) and a stimulatory rôle of the semen itself should be considered. Although very low concentrations of prostaglandins E_2 and $F_{2\alpha}$ were reported in boar semen (Hunter, 1973a), an increased concentration of prostaglandin $F_{2\alpha}$ was found in the uterine venous blood of a gilt sampled 15 minutes after mating when the uterus was still full of semen (*Figure 3.2*). This result was not endorsed in six other animals sampled 35–60 minutes after mating, but the notion that semen may activate a local release of smooth muscle stimulants is worth examining further.

One other aspect concerning myometrial contractions should be mentioned at this stage. Unilateral fertilization, that is the fertilization of eggs in only one Fallopian tube with failure of spermatozoa to reach eggs in the contralateral tube, is a frequent sequel to the delayed insemination of pigs (Kvasnitsky, 1959; Hancock, 1962; Hunter, 1967). Since uterine contractions are programmed by the balance of ovarian steroid hormones, and

because ovarian steroid secretion will have changed in favour of progesterone under conditions of delayed insemination, an altered pattern of uterine contractions may explain this failure of sperm transport and fertilization. If contractile waves of low frequency occlude one horn of the uterus during insemination, then the semen would be delivered unilaterally. Also the more relaxed condition of the cervix leads to leakage of semen with a belated mating or insemination, and with insufficient fluid remaining in the uterus to enable a redistribution between the two horns, this may also be a cause of diminished fertility.



Prostaglandins in boar semen (ng/ml)

	E ₂	F _{2α}
Collection	< 0.27	< 0.34
Uterus	< 1.1	< 2.8

Figure 3.2 Diagrammatic representation of the uterine horns in a group of gilts to indicate the concentration of prostaglandin F_{2α} in uterine venous blood at various intervals after mating. The concentration for the left horn (4.6 ng/ml) represents the situation 15 minutes after the end of mating when the lumen is still distended by the ejaculate. The concentration shown against the right horn (<0.73 ng/ml) represents the highest concentration in a series of six gilts sampled 35–60 minutes after mating when the bulk of the ejaculate was no longer grossly detectable. From Hunter and Poyser (unpublished). These figures are compared in the lower part of the diagram with the concentration of prostaglandins in freshly-ejaculated semen and in semen aspirated from the uterus shortly after mating (see Hunter, 1973a).

During the protracted period of coitus (e.g. 5–15 minutes), semen is not emitted as a homogeneous fluid. If the ejaculate is collected as a series of fractions, these will consist first of watery and gel pre-sperm secretions, then a sperm-rich fraction, followed by a post-sperm and gelatinous fraction (Rodolfo, 1934a; McKenzie, Miller and Bauguess, 1938). Such a sequence may take 3–5 minutes and can be considered as one full wave of

ejaculation. After renewed thrusting and re-establishment of the cervical lock, a second and indeed third wave of ejaculation may follow. Whether the fractions have a special significance in the transport of spermatozoa is uncertain, but they may favour displacement of the sperm-rich fraction to the region of the utero-tubal junctions (McKenzie, Miller and Bauguess, 1938; Du Mesnil du Buisson and Dautzier, 1955a). In any event, under conditions of natural mating performed in the pre-ovulatory interval, the uterine horns should be fully distended with semen by the time mating is finished, a situation that has been verified by laparotomy and at autopsy. The gelatinous material of the ejaculate acts as a cervical plug for a variable period after mating, thereby preventing immediate leakage of the uterine contents.

The total number of spermatozoa deposited may vary from $4-8 \times 10^{10}$ cells, with the concentration of spermatozoa in the whole ejaculate being $1-3 \times 10^8$ cells/ml (Rodolfo, 1934b; McKenzie, Miller and Bauguess, 1938; Polge, 1956). A concentration of this order, or even higher if there is preferential transport of the sperm-rich fraction, will therefore bathe the utero-tubal junction by the completion of mating with a mature boar; uterine transport of semen should not therefore be a physiological problem. Accordingly, discussion of the process of sperm transport after mating now needs to focus on the rôle of the tract between the utero-tubal junction and the site of fertilization. Diminished fertility may arise, however, after insemination of a reduced volume of diluted semen due to ineffective transport of spermatozoa to the utero-tubal junction. The use of frozen-thawed boar semen may further exacerbate the problem, as will insemination too early in oestrus.

Sperm transport through the utero-tubal junction

As far as the transport of spermatozoa is concerned, the utero-tubal junction of pigs functions, in several respects, in a manner similar to the cervix of ruminants. Before developing this analogy, the morphology of the utero-tubal junction must be briefly described. As shown in *Figure 3.3* the longitudinal folds of the isthmus extend caudally into the tip of the uterine horn, where they are arranged as a series of polypoid processes. These have a prominent mucosa which is sensitive to the balance of ovarian steroids and during the period of oestrus the polypoid processes swell due to oedema in the mucosa. This swelling is particularly pronounced in the pre-ovulatory phase of oestrus (*Figure 3.3*), but begins to subside within 8-12 hours of the time of ovulation. The processes therefore form a powerful barrier in the tract during the first 40-48 hours of oestrus, and are the principal physical obstacle to sperm transport. Although a series of quite recent studies has examined the rôle of the utero-tubal junction in relation to sperm transport in pigs (Hunter, 1972b; 1973a; 1973b; 1975b; 1981; Hunter and Hall, 1974a,b), the anatomy of this region first received attention in the classical studies of Andersen (1928) and Lee (1928).

The utero-tubal junction of oestrous pigs is not patent to the passage of fluids from the uterus when tested by injection into the uterine lumen. Indeed, as observed by Lee as long ago as 1928, the wall of the uterus will

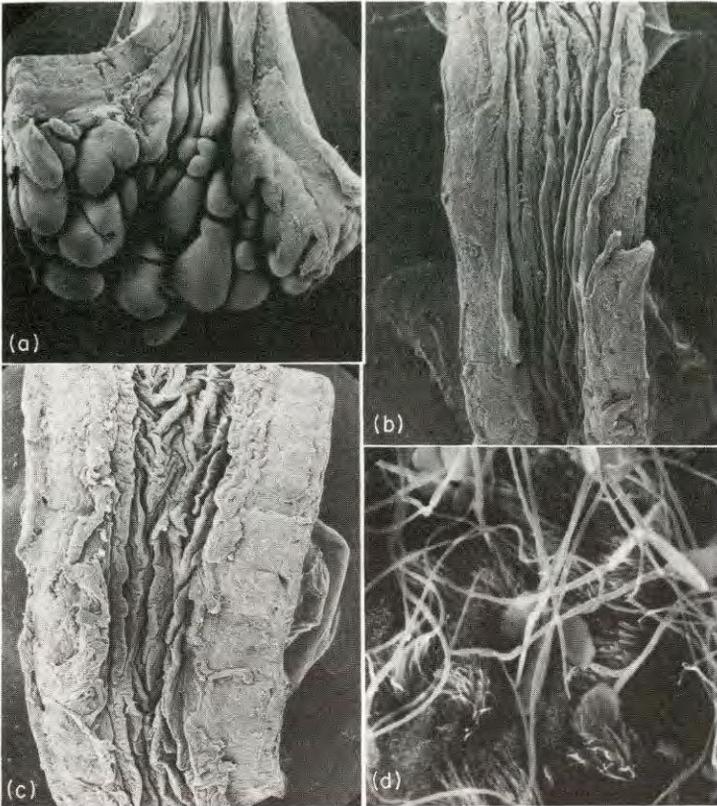


Figure 3.3 Scanning electron micrographs of the utero-tubal junction and isthmus of pigs during the period of oestrus. (a) The utero-tubal junction showing the highly swollen polypoid processes that extend rostrally into the isthmus as longitudinal folds. The oedematous polypoid processes act as a major barrier to seminal plasma and immotile spermatozoa in the pre-ovulatory phase of oestrus. (b) and (c) To show the thick muscular wall in the lower portion of the isthmus which, together with the swollen longitudinal ridges and folds, functions to regulate the ascent of spermatozoa. (d) Spermatozoa on the surface of a furrow between the terminal folds of the isthmus. Many of the sperm heads appear to be engaged by their tips in the epithelial surface and occasionally in contact with cilia. From Fléchon and Hunter (1981)

rupture before fluid can be forced through the junction into the Fallopian tube. Although these remarks concern the potential for bulk passage of fluids, there is no evidence from insemination of radio-opaque material that even minor quantities of whole semen would cross the utero-tubal junction (Polge, 1978). This report endorses an earlier failure to trace constituents of seminal plasma in the Fallopian tubes at oestrus by biochemical methods (Mann, Polge and Rowson, 1956). Taken together, these findings strongly suggest that whole semen does *not* enter the tubes of pre-ovulatory animals in detectable amounts after mating, and infers that spermatozoa gain the lumen of the isthmus by virtue of their own motility. Both these comments bring out the analogy between the rôle of the utero-tubal junction in pigs and that of the cervix in ruminants.

Further evidence that the utero-tubal junction divests the ascending spermatozoa of any gross association with seminal plasma was reported by Hunter and Hall (1974a). Using measurement of capacitation time as a sensitive physiological indicator, they noted that spermatozoa passing through the utero-tubal junction had a consistent advantage of approximately two hours compared with those instilled directly into the tubes. This observation was strengthened by the two hours delay in capacitation time after introduction of 0.02–0.1 ml aliquots of cell-free seminal plasma into the tubal isthmus (Hunter and Hall, 1974a). Once more, sperm motility was considered essential for negotiation of the utero-tubal junction prior to ovulation, and those studies claiming passage of dead cells have been criticized either as being unphysiological (e.g. Baker and Degen, 1972) or possibly performed late in oestrus when the utero-tubal junction is no longer fully oedematous and fertilization should have occurred (e.g. First *et al.*, 1968a). Moreover, if dead spermatozoa can freely negotiate the utero-tubal junction prior to ovulation, then perhaps the same facility should be extended to polymorphonuclear leucocytes and bacteria present in the uterine lumen after mating. There is no evidence that this is the case, nor did a study of the tubal isthmus by scanning electron microscopy reveal such cells (Fléchon and Hunter, 1981).

Despite all this evidence in favour of the utero-tubal junction acting to prevent passage of whole semen, polymorphs and bacteria in the pre-ovulatory phase of oestrus, one recent report has claimed that boar seminal plasma does enter the tubes after insemination on the second day of oestrus (Einarsson *et al.*, 1980). However, the method of homogenizing Fallopian tube tissues in order to detect radiolabelled compounds suspended in the inseminate was unable to distinguish between radioactivity in the lumen and that in the wall, as might have been brought about by local vascular or lymphatic transport. Even so, muscular movements associated with the region of the utero-tubal junction might explain the observations of Einarsson *et al.*, but the failure of sperm capacitation and fertilization in the presence of seminal plasma would then need to be considered.

Sperm ascent up to and through the utero-tubal junction is relatively rapid when compared with the situation in ruminants. Whilst spermatozoa have been observed in the ampulla within 15–30 minutes of mating or artificial insemination (Burger, 1952; Ito, Kudo and Niwa, 1959; First *et al.*, 1968b), the interval required for spermatozoa to traverse the utero-tubal junction in numbers sufficient to fertilize the eggs has been calculated from post-coital separation of the isthmus from the utero-tubal junction (Hunter and Hall, 1974b; Hunter, 1981). The conclusion here was that a population of spermatozoa sufficient to give *maximum* fertilization is established in the tubes within 1–2 hours of mating. Because this rate of formation of adequate sperm reservoirs in the tubes corresponds closely with the time course of seminal plasma elimination from the uterus, one explanation for the exceptional volume of the boar ejaculate can be offered. The intrauterine deposition of 150–500 ml of fluid may have evolved to ensure successful passage of whole semen to the utero-tubal junction of all oestrous females in a breeding herd (i.e. gilts and parous sows) so that spermatozoa are present at the junctions for a period of time

sufficient to enable formation of reservoirs in the caudal isthmus of each tube. This argument is supported by the fact that surgical insemination of as little as 0.05 ml semen directly into the caudal isthmus leads to fertilization (Polge, Salamon and Wilmut, 1970; Hunter, 1973b). Therefore, from the point of view of the future development of artificial insemination, a technique that permits deposition of a few millilitres of whole semen at the top of each uterine horn against the utero-tubal junction should be fruitful, and should also enable a much wider propagation of each boar ejaculate.

Whilst the utero-tubal junction has been suggested as a reservoir for spermatozoa (Du Mesnil du Buisson and Dauzier, 1955a; Rigby, 1966), it has been argued from several points of view that the physiological reservoir is more likely to be established in the lowermost portions of the isthmus (Hunter and Léglise, 1971; Hunter, 1972b, 1973a); the evidence from experiments involving post-coital transection of the tract would support this argument (Hunter and Hall, 1974b; Hunter, 1981). Nonetheless, there are glandular openings within the processes of the utero-tubal junction (Fléchon and Hunter, 1981), so short-term storage of spermatozoa might be expected in this region.

Sperm regulation and release by the isthmus

The tactical problem after completion of mating and distension of the uterus with semen is to secure transport of an appropriately-sized population of viable spermatozoa from the utero-tubal junction to the site of

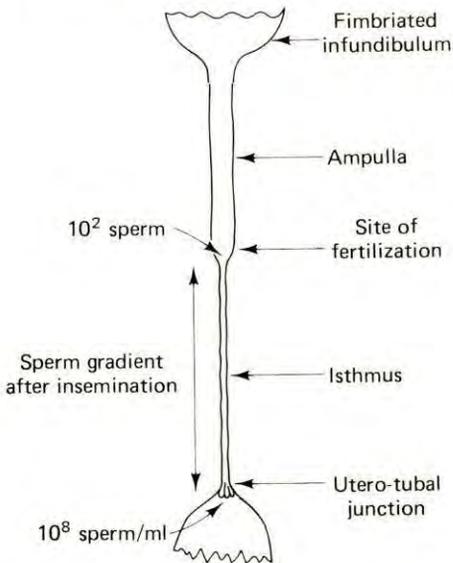


Figure 3.4 A semi-diagrammatic portrayal of the Fallopian tube to illustrate the extremely steep sperm gradient that must be obtained between the utero-tubal junction and the site of fertilization at the ampullary-isthmic junction if polyspermy is to be avoided. The figures indicate the concentrations of spermatozoa that would bathe the utero-tubal junction at the completion of mating and the total number of spermatozoa that might achieve the site of fertilization at any one time.

fertilization at the ampullary-isthmic junction. However, in the light of the concentration of spermatozoa bathing the utero-tubal junction at the end of mating, the rôle of the isthmus must be seen more as a *regulator* of sperm transport than as a section of the tract *facilitating* transport of cells. This is illustrated in *Figure 3.4*: a gradient in sperm concentration decreasing from 10^8 cells/ml semen to an approximate total of 10^2 cells *at any one time* must be achieved between the utero-tubal junction and the site of fertilization, a distance of less than 10–12 cm in most gilts and sows. If the number of capacitated spermatozoa in the vicinity of the freshly ovulated eggs were to increase above such a figure, then the risk of polyspermic fertilization would be high. Polyspermy entails penetration of more than one spermatozoon into the egg cytoplasm, a lethal condition in mammals (*Figure 3.5*).

The precise manner in which the isthmus forms a gradient in sperm concentration is unknown. Even so, the lumen of this portion of the tube is extremely constricted in the pre-ovulatory period, for the longitudinal

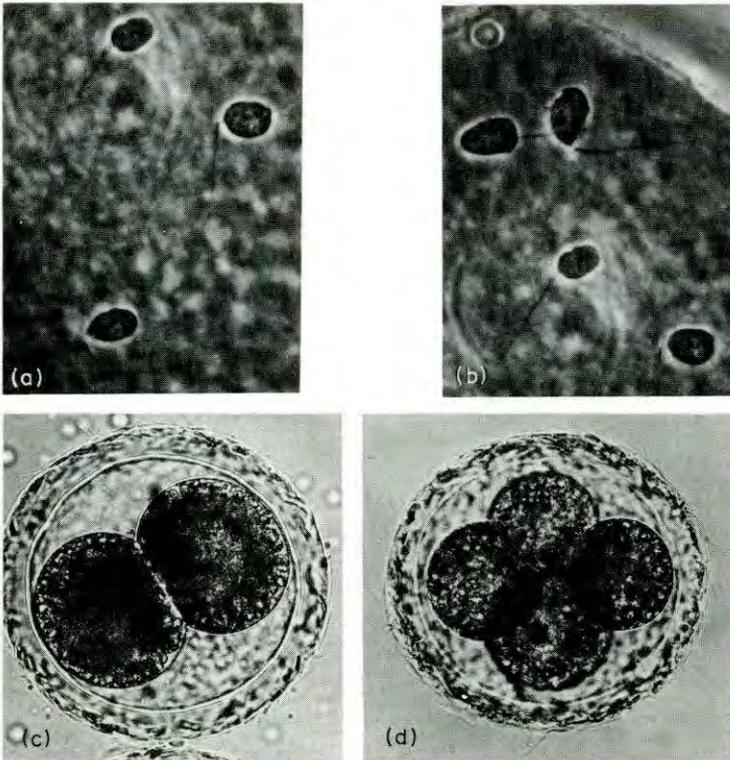


Figure 3.5(a) and (b) Whole-mount preparations of pig eggs fixed in 25% acetic-alcohol and stained with 0.5% aceto-orcein to show polyspermic penetration of the vitellus. The similar stages of transformation of the sperm heads suggest closely synchronous (and recent) penetration of the egg membranes. (c) and (d) Whole-mount preparations of living pig embryos at the 2- and 4-celled stages of development to show the very large numbers of sperm heads trapped in the zona pellucida as a result of the block to polyspermy in the innermost part of this membrane. More than 200 spermatozoa are within the zona of the 4-celled embryo.

folds protrude inwards and are oedematous. Physical reduction in the patency of the tube therefore serves to regulate the number of spermatozoa passing to the site of fertilization. Although these comments concentrate on swelling in the mucosa, isthmic patency is also controlled through its powerful layers of circular and longitudinal muscles. Two aspects of myosalpingeal function are of interest (reviewed by Hunter, 1977; 1980). First, the α -adrenergic receptors in the isthmic muscle are known to be activated under oestrogen dominance, leading to contraction, whereas β -adrenergic receptors are potentiated by progesterone secretion, leading to relaxation. Second, prostaglandins of the F series in the myosalpinx may act to cause contraction with oestrogen dominance, whereas those of the E series may promote relaxation under progesterone dominance. Thus, the influence of pre-ovulatory oestrogen secretion ensures regulation of sperm ascent by enhancing contraction of the myosalpinx, a situation that changes close to the time of ovulation.

Instead of a smoothly decreasing sperm gradient along the length of the isthmus, there is evidence that spermatozoa are largely restricted to the caudal tip (1–2 cm) of the isthmus during much of the pre-ovulatory interval (Hunter, 1981). Release of spermatozoa from this reservoir commences shortly before the time of ovulation, probably under the influence of a local transfer of ovarian follicular hormones to the wall of the isthmus. Pre-ovulatory secretion of progesterone may be involved, but a local vascular or lymphatic transfer of follicular relaxin and prostaglandins should also be considered. Whatever the nature of the mechanisms, such ovarian regulation of the patency of the isthmus would explain why peri- or post-ovulatory mating can lead to a more rapid transport of spermatozoa to the site of fertilization than mating early in oestrus (Du Mesnil du Buisson and Dauzier, 1955b; Hunter, 1981).

Discussion of sperm gradients in the isthmus would not be complete without stressing the deleterious influence of removing or overcoming such gradients. Three experimental approaches have all led to the condition of polyspermy, inferring that the gradient is no longer functional and that the egg membranes have been confronted simultaneously by more than one competent spermatozoon. First was the approach of surgical resection of most of the isthmus followed by end-to-end anastomosis of the remaining portions of the tube (Hunter and Léglise, 1971). Second was the reduction in oedema and myosalpingeal contraction obtained by microinjections of a solution of progesterone under the serosal layer of the isthmus and utero-tubal junction (Hunter, 1972b). Third was the instillation of excessive numbers of spermatozoa into the tube by introducing a 'rounded' insemination needle through the utero-tubal junction and into the isthmus (Polge, Salamon and Wilmot, 1970; Hunter, 1973b).

Apart from their intrinsic motility, sperm transport in the isthmus is assisted by waves of muscular activity and by the influence of cilia. Both have been examined in post-mortem preparations of pig material (Gaddum-Rosse and Blandau, 1973; 1976; Blandau and Gaddum-Rosse, 1974) and in tissues obtained at laparotomy (Fléchon and Hunter, 1981); ciliary activity and waves of contraction appear to progress from the utero-tubal junction to the site of fertilization. Again, these activities are under endocrine control and although of an ad-ovarian direction at the time of

ovulation, they are reversed within 48 hours when the embryos or unfertilized eggs are descending to the uterus. In fact, the phase of accelerated sperm transport close to the time of ovulation is probably associated more with myosalpingeal activity than with the beat of tubal cilia or sperm flagella. On the other hand, contractions and ciliary beat in the ampulla are programmed in an ab-ovarian direction towards the site of fertilization, which explains why an appropriately timed intraperitoneal insemination may lead to fertilization (Hunter, 1978).

Capacitation and sperm interactions with the egg investments

Ovulation occurs approximately 40 hours after the onset of oestrus, and eggs released on to the densely ciliated surface of the fimbriated infundibulum are propelled to the site of fertilization within 45 minutes, still invested in cumulus cells (Hunter, 1974). These cells surrounding individual eggs aggregate very soon after ovulation so that the eggs shed by each ovary reach the ampullary-isthmic junction assembled within a plug of cumulus cells (Hancock, 1961). Such a plug may persist for several hours after ovulation in unmated animals, but spermatozoa promote its rapid dissolution, presumably by means of acrosomal enzymes. Whilst the endocrine events associated with ovulation may stimulate a release of spermatozoa from the isthmus (see above, p.57), initial contact of the follicular contents with the tubal epithelium could also provoke a final phase of sperm transport to the ampullary-isthmic junction. This is not to infer that the follicular fluid passes down the tube, but because it is rich in hormones such as prostaglandins of the E and F series and relaxin, transient contact with the epithelium could influence the function of the isthmus.

Capacitation of boar spermatozoa requires some 2–3 hours after mating or intracervical insemination (Hunter and Dziuk, 1968). By contrast, if aliquots of whole semen are introduced directly into the Fallopian tubes, the process of capacitation is delayed by approximately two hours. The uterus and tubes therefore act synergistically to accelerate achievement of the capacitated state, primarily by removing elements of the seminal plasma during sperm passage through the utero-tubal junction (Hunter and Hall, 1974a). Contact of spermatozoa with the egg investments or, alternatively, with the microenvironment around denuded eggs may be necessary for the completion of capacitation and the concomitant increase in sperm motility.

Swelling of the acrosome is thought to be an immediate sequel to capacitation of boar spermatozoa, and precedes the vesiculation reaction between the plasma and outer acrosomal membranes (Szollosi and Hunter, 1978). Despite the intense progressive motility of capacitated spermatozoa, hyaluronidase is considered necessary to depolymerize a passage between the follicular cells (see Austin, 1961). Again, dogma holds that the acrosome reaction is necessary before hyaluronidase can be released from viable spermatozoa, but Szollosi and Hunter (1978) inferred escape of this enzyme from the swollen acrosome *before* actual commencement of vesiculation. This would enable the fertilizing sperm to reach the surface of the zona before exhibiting the acrosome reaction. Constituents of the zona



Figure 3.6 Electron micrographs of boar spermatozoa, pig eggs and cells of the cumulus oophorus. (a) Spermatozoon on the surface of the zona pellucida showing the extensive vesiculation between the outer acrosomal and plasma membranes that constitutes the acrosome reaction. (b) Spermatozoon in the substance of the zona pellucida showing the distinct penetration pathway that is inferred to be digested by lytic enzymes of the inner acrosomal membrane. Note the spongy outer and more compact inner regions of the zona pellucida. (c) Section through the peripheral region of an egg showing the microtubules and mid-body of the spindle, and completion of the second meiotic division shortly after activation by the fertilizing sperm. Note the absence of organelles in the cytoplasm of the second polar body, and that the chromatin in this vestigial body has not attracted elements of a nuclear envelope. (d) and (e) Follicle cells of the corona radiata still intimately associated with the zona pellucida and containing engulfed spermatozoa. Note the transverse section through the sperm tail in (e). From Szollosi and Hunter (1973; 1978)

pellucida or indeed factors emanating from the egg cytoplasm might therefore trigger the acrosome reaction, which would be a means of conserving acrosomal enzymes until the egg itself was confronted. Boar spermatozoa penetrate the zona under the dual influence of incisive progressive motility and that of lytic enzymes. Whilst ultrastructural studies have failed to reveal the sperm penetration filament reported by Dickmann and Dziuk (1964), a pathway can be detected in the substance of the zona after passage of the fertilizing sperm (Szollosi and Hunter, 1973). Spermatozoa not penetrating the zona pellucida may be incorporated by cells of the corona radiata (*Figure 3.6*).

One of the components of egg activation initiated by membrane fusion of the fertilizing sperm with the egg plasma membrane is the cortical reaction whereby the contents of the cortical granules are released into the perivitelline space (Austin, 1956; Szollosi, 1967; Fléchon, 1970). The manner in which this reaction causes a block to polyspermy in the zona pellucida still requires clarification, but there is no obvious structural change in this accessory membrane. The block is located in the innermost portion of the zona pellucida, for the heads of accessory spermatozoa continue to penetrate into the zona of newly fertilized eggs (Thibault, 1959; Hancock, 1961). Despite the stability of the block to polyspermy, the outermost portion of the zona remains permeable to spermatozoa for up to two days after initial penetration, a situation which explains the massive increase in zona sperm numbers as the egg descends through the isthmus. In fact, 2- and 4-cell eggs may each contain in excess of 200 spermatozoa (*Figure 3.5*), and yet only one sperm has crossed the perivitelline space to enter the cytoplasm. Spermatozoa in the isthmus not entering the zona pellucida or being incorporated by follicular cells may be transported back into the uterus at the time of embryo passage (Hunter, 1978), but whether this accounts for the whole of the residual tubal population remains uncertain, as does its ultimate fate.

Acknowledgements

Published and unpublished studies of the author referred to in the text were supported by grants from the Agricultural Research Council, the Wellcome Trust and the Ford Foundation. Drs J.E. Fléchon, N.L. Poyser and D. Szollosi kindly gave permission to publish photographs and data arising from collaborative projects.

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