Arch. Biol. Med. Exp. *24:* 339-348 (1991)
Printed in Chile

Behaviour of spermatozoa in the human oviduct

Biología del espermatozoide en el oviducto humano

DAVID MORTIMER

Gamete Biology Unit of the Reproductive Medicine Research Group,
University of Calgary Health Sciences Centre,
3330 Hospital Drive NW, Calgary, Alberta T2N 4N1, Canada.

This review considers the possible mechanisms that may regulate human sperm fertilizing ability *in vivo*, including evidence for the modern hypothesis of sperm transport whereby spermatozoa migrate to the oviductal isthmus during the pre-ovulatory period and there complete (or reach a late stage of) capacitation. Spermatozoa are then released from this isthmic sperm reservoir in the immediate peri-ovulatory period and transported to the site of fertilization in the oviductal ampulla. Possible roles for oviductal micro-environments (*e.g.* extra-cellular potassium concentrations, taurine, progesterone) in the regulation of capacitation, hyperactivation and the acrosome reaction are examined. In addition, consideration that the spontaneous human sperm acrosome reaction is of no physiological relevance is discussed, along with evidence for the non-specific induction of acrosome reactions by such products of ovulation as follicular fluid and the cumulus mass. Evidence for the specific induction of the human sperm acrosome reaction by the zona pellucida is reviewed, concurring with the modern theory that the fertilizing human spermatozoon undergoes its acrosome reaction on the zona pellucida, after sperm-zona binding. Consequent to these concepts the quality and efficiency of *in vitro* culture systems, especially their physiological appropriateness for experimental studies on human sperm physiology, are reviewed. Several considerations for the design of future experiments in this area are presented: 1) the need to use physiologically appropriate levels of albumin (but avoiding serum supplementation which cannot be standardized); 2) the inclusion of important components such as bicarbonate and steroid hormones, particularly progesterone; 3) the need for an extra non-bicarbonate buffer to obviate pH-induced artifacts during laboratory manipulations; and (4) possible variation of the culture conditions to duplicate the sequence of micro-environments spermatozoa probably experience *in vivo*.

INTRODUCTION

Unlike other mammalian species in which reliable studies on spermatozoa within, or removed from, the female reproductive tract have been performed, such observations area not available for the human. Many studies on human sperm function (as distinct to physiology) *in vitro* have used simple culture media, cumulus-free zonae pellucidae or zona-free oocytes. Many physiologists are concerned that the extrapolation from such studies has limited value in terms of what happens under physiological conditions *in vivo*. This paper will consider how unifying hypotheses of sperm transport and the regulation of sperm fertilizing ability may be applied to humans and, in addition, also review some of the recent experimental studies on human spermatozoa *in vitro* with particular reference to helping us understand what is happening *in vivo*.

In short, the aim is to show how little we really know of human sperm physiology and to try and suggest how future studies could be designed and/or improved so as to provide information more likely to be of relevance to what could be occurring in the natural situation of the female reproductive tract. An evident benefit of such knowledge will be an improved understanding of human gamete manipulation *in vitro* and, hopefully, improved success in clinical *in vitro* fertilization (IVF).

SPERM TRANSPORT

Sperm transport is a process whereby spermatozoa pass from the site of insemina-

tion in the upper vagina (primarily around the external cervical os) to the site of fertilization in the oviductal ampulla.

It is well-established that the process of sperm selection occurs at the level of sperm penetration into the cervical mucus. This selection is based upon the differential motility of the spermatozoa, the positive relationship between sperm morphology and motility also causing a simultaneous selection for spermatozoa of normal morphology.

Probably the commonest approach to understanding mammalian, including human, sperm transport is that of a series of barriers and reservoirs as propounded by Hafez (1). While it is true that the cervical mucus at the external os (in species with vaginal insemination), and also the utero-tubal junction, do function as selective barriers, there is no evidence that the internal cervical os or the ampullary-isthmic junction have any such function. In addition, since spermatozoa are rapidly immobilized in the vagina after ejaculation, the vaginal seminal pool cannot be considered as a functional sperm reservoir.

The concept of a cervical sperm reservoir originates primarily from work in the sheep but, although it may be an attractive hypothesis and has been widely accepted for all species with vaginal insemination, there is really only minimal evidence for any such mechanism in the human female tract. Spermatozoa certainly populate the crypts of the cervical epithelium, but a shift from the lower to the upper levels of the cervix has not been demonstrated (2). Since a continuous migration from the vaginal pool into the lower crypts, which could mask such an upward transfer of spermatozoa, is known not to occur, we are forced to accept that spermatozoa "stored" in any particular crypt remain there until being either transferred directly to the uterus or disposed of (probably by phagocytosis since there is a pronounced leukocytosis from the cervical epithelium after insemination (3)). In a continued absence of any definite evidence that any spermatozoa actually leave a crypt after entering it, there is no sound basis for considering the crypts of the cervical mucosa to be organs of sperm storage in the human.

Indeed, a more plausible hypothesis is that because spermatozoa swimming in cervical mucus are constrained to follow lines of the mucin structure, it may be that those spermatozoa that follow this orientation back to its secretory origin in the crypts are those that are later found there; only those spermatozoa which continue to swim within the mucus column to the internal os eventually reach the uterine cavity. This population of spermatozoa, each swimming at their own speed, may well be the real "cervical reservoir" —a concept that is supported by various observations in the dynamics of sperm distributions within the cervical mucus (review: 4).

The occurrence of a phase of "rapid" sperm transport, whereby spermatozoa are transported by the actions of the female tract from the site of insemination to the oviducts within a very few minutes after insemination, has been based upon studies in other species (which are probably of minimal relevance to the human situation) and upon poorly controlled studies in women. This literature has been extensively and critically reviewed elsewhere (4-6) and the existence of such a physiological process cannot be substantiated in the human. Indeed, even in the rabbit where rapid sperm transport has been unequivocally demonstrated, the rapidly transported spermatozoa are non-functional and do not participate in fertilization.

Spermatozoa probably enter the uterine cavity from the internal cervical os by virtue of their own motility. This is in accordance with the reported 90 minute delay between insemination and the appearance of spermatozoa in the uterine cavity (7).

In the adult nulliparous state the uterine cavity is merely a slit, more of a potential rather than an actual lumen, although it does remain somewhat larger in parous women. Spermatozoa entering the uterine cavity are probably suspended in a small pool of uterine fluid bathing the endometrium. Their intrinsic motility may help maintain them in suspension while

the fluid is mixed throughout the uterus by segmental pattern myometrial contractions. More or less uniform distribution of spermatozoa throughout the uterine cavity would therefore be established. There is no evidence to support nor disprove this hypothesis.

Likewise, only theories exist as to how spermatozoa traverse the uterotubal junction to reach the tubal isthmus. The utero-tubal junction is clearly at least a physical barrier since pressure has to be exerted to force fluid through it. Sperm motility is probably also important, and there is some evidence that the utero-tubal junction constitutes a barrier to spermatozoa with impaired motility (8).

Critical review of all careful studies to date on the dynamics of sperm transport in the eutherian female tract have supported the common existence of a sperm reservoir in the lower isthmus (5). These isthmic reservoir spermatozoa apparently show reduced motility and switch to the hyperactivated pattern upon transfer into ampullary fluid or culture medium (9, 10). This quiescence seems to be regulated by a high extracellular potassium ion concentration (11).

Spermatozoa from this isthmic reservoir are redistributed in the immediate periovulatory phase, so that they appear in the ampulla of the oviduct synchronous with the egg. This system is regulated in some way by local messengers produced by the ovary (12).

To date, only anecdotal evidence is availables as to the existence of an isthmic sperm reservoir in the human female tract. Laparoscopic sperm recovery 8 to 12 hours after insemination in couples with idiopathic infertility was positive in 26/47 cases (55.3%) (13). However, in 15 couples in whom a bilateral salpingectomy was performed at comparable times after insemination, only 5/15 cases (33.3%) had sperm in the isthmic and/or ampullary regions (A.A. Templeton and D. Mortimer, unpublished observations), contrary to the expectation of a higher rate of sperm recovery in normal fertile couples compared to cases of idiopathic infertility. It may be considered significant, however, that in cases of salpingectomy the distal part of the oviductal isthmus, being located within the wall of the uterus, would not be removed. Furthermore, in two normal fertile couples the intact uterus and oviducts were removed at hysterectomy, again some 8 to 12 hours after insemination, thereby allowing the oviducts to be flushed from the utero-tubal juction. Large numbers of spermatozoa were recovered in both cases (actual numbers unavailable: A.A. Templeton and D. Mortimer, unpublished observations).

While such limited observations clearly cannot constitute anything more than circumstantial evidence for the existence of an isthmic reservoir in the "intra-mural" portion of the human oviductal isthmus, they are the only data available.

SYNCHRONIZATION OF SPERM FERTILIZING ABILITY

Human spermatozoa, like those of all other eutheria, are incapable of fertilizing oocytes immediately after ejaculation. A final stage of maturation, which has been termed capacitation (defined as the process by which spermatozoa acquire the capacity to undergo the acrosome reaction to fertilize eggs) is required (14). Capacitation is essential for fertilization both *in vivo* and *in vitro*, and commences upon release of the spermatozoa from the inhibitory environment of the seminal plasma. Capacitation is more or less completed before sperm reach the site of fertilization in the oviductal ampulla, and it may be hypothesized that spermatozoa reach a late, or even terminal, stage of capacitation during their stay in the putative isthmic reservoir.

The characteristic highly active, but non-progressive, pattern of motility that is associated with capacitation ("hyperactivated motility": see below) has been described in most species not to occur *in vivo* until the spermatozoa enter the oviductal ampulla. *In vitro*, human spermatozoa seem to show hyperactivated motility quite early in capacitation, perhaps suggesting that hyperactivation may be an early concomitant of the capacitation

process as opposed to an expression of the acquisition of the capacitated state. It might also be a reflection of heterogeneity of human sperm populations in that the time required for the capacitation of some spermatozoa could be far shorter than previously thought (7, 15).

By definition, capacited spermatozoa are in a state ready to undergo the acrosome reaction. While it has generally been accepted that the acrosome reaction occurs prior to a fertilizing spermatozoon's penetration of a cumulus (14) there is increasing support for a "modern" hypothesis that the acrosome reaction is induced by some component(s) of the zona pellucida (16). Consequently the occurrence of spontaneous acrosome reactions are most probably related to sperm senescence and are not associated with fertilization. Totally capacitated spermatozoa are highly labile and it may be that some spermatozoa respond to non-specific stimuli such as follicular fluid (17).

HYPERACTIVATED MOTILITY

Mammalian capacitation is characterized in most, if not all, species by the development of a highly active but non-progressive pattern of motility known as "hyperactivation" (14). Although the expression of hyperactivated motility by human spermatozoa was doubted for many years, it is now accepted that changes in movement analogous to those described for other eutheria do occur (18-20).

In rodent species there is a rather synchronous shift from progressive motility into a "whiplash" or "figure-of-eight" pattern of movement associated with the completion of capacitation (21, 22). Capacitated rabbit spermatozoa show a biphasic motility pattern switching between a whiplash phase and a progressive phase (10). The whiplash pattern described for free-swimming capacitated spermatozoa *in vitro* has been ascribed to the development of large amplitude proximal waves by the flagellum. Because (a) the sperm head is free to move in three dimensions and (b) the propagation of these waves seems to be slightly delayed, this results in marked lateral movements of the sperm head (i.e. a high amplitude of lateral head displacement or *ALH*). Consequently, this pattern of flagellar beating causes the head to "thrash about" with no net space gain, especially in rodent spermatozoa with their long tails. Such whiplash motility has now been described for numerous species of eutheria (14) and is generally considered to be true hyperactivation. The more progressive phase, in which the spermatozoa still show relatively large amplitude flagellar waves but do gain space in a more or less defined direction, is also characterized by marked lateral displacements of the sperm head about the average axis of progression.

Many hyperactivated spermatozoa have been recovered from the oviductal ampullae of several species around the time of ovulation (14), and oviductal hamster spermatozoa *in situ* show the biphasic pattern of hyperactivation (23) produced by flagellar waves of large amplitude and curvature similar to those described for capacitated hamster spermatozoa *in vitro* (21). Upon attachment to the zona pellucida similar, although more symmetrical, waves are propagated (24), demonstrating that this pattern of motility is specifically related to the fertilization of zona pellucida-intact oocytes both *in vitro* and *in vivo* (25).

Studies on human sperm capacitation *in vitro* have reported the existence of four general patterns of movement: (i) typical progressive motility with only slightly larger *ALH* than seen in seminal spermatozoa = "forward progressive" phase cells; (ii) progressive motility but with a more irregular track and large *ALH* = "transition phase" cells; (iii) a more characteristic whiplash pattern of motility with no progression = "hyperactivated" or "star-spin" cells; and (iv) a period of axonemal rigor where the spermatozoa are "frozen" in a state of high flagellar curvature with no propagation of the wave="freeze-flex" cells (18, 19, 26, 27).

It is also apparent from microscopic observations that human spermatozoa switch repeatedly between these various patterns of movement (S.T. Mortimer & D.

Mortimer, unpublished observations), indicating that the development of hyperactivated motility is not an irreversible change in the cells. Technically this phenomenon is also problematic in that an observer will miss a substantial proportion of hyperactivated spermatozoa if the observation window is short.

Hyperactivated motility can now be described in objective terms and analyzed reliably using the digital image analysis technology now available in automated sperm motility analyzers (19, 20, 27, 28). However, few studies have investigated possible roles for the products of ovulation or the micro-environment(s) of the oviduct in regulating the expression of hyperactivated motility in eutherian spermatozoa and no information is available at all for human spermatozoa. Nevertheless, sperm populations from fertile donors show substantial levels of hyperactivated motility *in vitro* in the first few hours after separation from seminal plasma. Furthermore, the expression of hyperactivated motility is dependent not only upon the availability of an adequate extracellular concentration of calcium ions, but also a minimum requirement of 20 mM bicarbonate ions (29).

Consequently, the establishment of an *in vitro* assay for human sperm hyperactivation is now both biologically and technically feasible using modern motility analyzers, which will facilitate the validation of hyperactivated motility as a clinically relevant marker of capacitation and sperm fertilizing ability.

THE COMPOSITION OF HUMAN OVIDUCT FLUID AND THE ELABORATION OF A SYNTHETIC MEDIUM FOR IN VITRO STUDIES

Although the precise nature of the capacitation process remains uncertain (14), conditions suitable for its occurrence *in vitro* have been reported for a number of eutherian species including man (30). Many of the culture media used for the *in vitro* fertilization of eutherian gametes have been based loosely upon blood plasma, often with subsequent empirical modifications (31). Most studies on the fertilization of human gametes *in vitro* have employed either pre-existing media or modifications of other media such as Biggers, Whitten and Whittingham's "BWW" medium, Earle's salts, Ham's F10 medium, Krebs-Ringer-bicarbonate or Tyrode's medium. Especially in the case of media used for human gametes in the early days of clinical IVF, crude attempts to improve a basic formulation have involved the addition of serum.

Probably the most physiological approach has been that of Menezo's B_2 medium (32), originally developed for bovine embryos based upon the compositions of bovine uterine, tubal and follicular fluids and serum. This medium is also now widely used for human clinical IVF, and can even be used for this purpose without serum supplementation (33).

The formulation of culture media based upon the composition of human tubal fluid has also been reported. A relatively simple medium based upon the ionic composition of human tubal fluid, and usually supplemented with 10% serum, improved the pregnancy rate in clinical IVF - for which it is now commonly used ("human tubal fluid" or "HTF medium" (34)). We have also described a complex medium, intended for use without serum supplementation, whose formulation is based upon the known or assumed composition of fluid from the human oviductal ampulla, including typical periovulatory concentrations of estradiol and progesterone ("synthetic tubal fluid" or "STF medium" (35)). This medium has been used in several series of *in vitro* experiments on human sperm physiology (see below).

When human spermatozoa were tested in the zona-free hamster egg penetration test (HEPT) after capacitation in STF medium for 3 or 5 hours either without taurine or with a typical serum concentration of this β-amino acid (8.3 mg/l: 66.3 μM), there were significantly higher levels of penetration after preincubation in the presence of taurine after 3 h of preincubation (35). While the average penetration rates were still higher after 5 hours of preincubation in STF with taurine, the difference was no longer significant. These results were in-

terpreted as evidence of a possible physiological role for taurine as a capacitation mediator; an effect perhaps only revealed *in vitro* at such low concentrations because of the complex formulation of the STF medium and its ability to support possibly multi-factorial, even synergistic effects.

THE HUMAN SPERM ACROSOME REACTION

The population of spermatozoa obtained from an ejaculate seems to be heterogeneous with respect to the period of time taken to achieve the capacitated state and hence be capable of undergoing the acrosome reaction (36). Although capacitation *in vitro* may be followed by a spontaneous acrosome reaction, whether such an acrosome reaction is physiological or not remains unknown. While it is generally accepted that the occurrence of spontaneous acrosome reactions *in vitro* is very low, use of a medium such as STF has demonstrated that higher levels may be achieved, especially with prolonged incubation (37). Elevated extracellular potassium ion concentrations have also been reported to promote increased acrosome loss in human spermatozoa (38).

The classical consideration of the site of occurrence and function of the acrosome reaction is a subject of debate. One opinion is that some component of the products of ovulation (i.e. follicular fluid and/or the oocyte-cumulus complex) provides a stimulus which induces the natural acrosome reaction (39). Another is that neither the oviduct, the vestments of the egg, other products of ovulation, nor the egg itself, are in any way essential under quasi-physiological conditions for the onset of the normal acrosome reaction in eutherian spermatozoa (40, 41). While it was classically accepted that the acrosome reaction occurred prior to the fertilizing spermatozoon's penetration of the freshly-ovulated oocyte-cumulus complex (14, 42, 43), there is increasing support for the "modern" hypothesis that the acrosome reaction actually occurs on the zona pellucida, induced by some component(s) of the zona itself, not prior to or during penetration of the cumulus (14, 16, 43, 44).

Certainly both acrosome-intact and acrosome-reacted human spermatozoa can be seen within the cumulus as well as bound to, or partially embedded in with the zona pellucida of oocytes fertilized *in vitro*. However, only acrosome-reacted spermatozoa are found deep in the zona pellucida and all those in the perivitelline space show completed acrosome reactions (45). Completion of the acrosome reaction is also necessary for fertilization following sperm microinjection into the perivitelline space (46, 47). These findings, in conjunction with the ability of acid-solubilized human zonae pellucidae to induce an acrosome reaction in capacitated spermatozoa (48), support the concept that the fertilizing human spermatozoon undergoes its acrosome reaction in contact with the zona pellucida.

Follicular fluid has been shown to induce the acrosome reaction in a number of eutherian species, perhaps due to its high glycosaminoglycan content (14). The situation for human spermatozoa seems to be that while follicular fluid may promote the acrosome reaction, it is not a specific inducer of it (17, 49). Some other workers, however, believe there is a specific factor in human follicular fluid which triggers "capacitated" spermatozoa (*e.g.* 50; but see below). One suggestion has been that this active component may be serum albumin, but other studies have implicated more specific substances of follicular origin, possibly produced by the cumulus cells (39, 51-54). Most recently, this factor has been suggested to be progesterone (55), an effect that has been reproduced *in vitro* using 1 μg/ml of this steroid. At the cellular level, this amount of progesterone causes a rapid, transitory increase in intracellular calcium ion concentration which then returns to a new, slightly elevated resting level (56).

Studies on the possible induction of the human sperm acrosome reaction by homologous cumulus are constrained by the limited availability of human cumulus masses but there is evidence for increased levels of acrosome loss when spermatozoa

are cultured in the presence of cumulus (51) and also when incubated for 16 hours with oocyte-comulus complexes in an IVF situation (57). Comparative co-culture experiments using cumulus obtained from superovulated hamsters, as well as from clinical IVF, with preincubated motile sperm populations in STF medium, have also been performed (D. Mortimer and A.R. Camenzind, unpublished results). Comparison of spermatozoa free in suspension around the cumulus, loosely adherent on the cumulus surface, and within the cumulus mass with spermatozoa either incubated in isolation (background spontaneous acrosome loss) or treated with calcium ionophore to induce maximum acrosome loss, demonstrated the existence of some factor(s) in cumulus which induce(s) the acrosome reaction in capacitated human spermatozoa. Although hamster cumulus seemed to be a more powerful stimulator of acrosome loss than did human cumulus masses in these experiments, another study using HTF medium did not see an induction of acrosome loss in human spermatozoon by hamster cumulus (58).

Therefore, while substantial evidence for a stimulation of *in vitro* sperm fertilizing ability by cumulus cells, follicular fluid, and/or oviduct fluid exists for a number of eutherian species, specific roles for the oocyte-cumulus complex or oviduct fluid in normal (i.e. in vivo) fertilization remain to be demonstrated conclusively.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

In conclusion, the following general statements can be made:

1. Anecdotal evidence exists for an isthmic sperm reservoir in the human female reproductive tract, most probably within the intra-mural part of the oviduct.
2. Human sperm function can be modulated by elevated extracellular potassium ion concentrations, such as may be present within the oviductal isthmus.
3. Follicular fluid and cumulus promote capacitation and the acrosome reaction *in vitro*, but they are *not specific inducers* of it.
4. While both acrosome-intact and –reacted spermatozoa may bind to the human zona pellucida, some component of the zona pellucida itself appears to be the specific inducer of the acrosome reaction in *fertilizing* spermatozoa.
5. The state of the fertilizing spermatozoon *in vivo*, i.e. where the oocyte is surrounded not only by the zona pellucida but also by the corona radiata and cumulus oophorus cells, remains *unknown*.

An integrated physiological hypothesis

From these observations, a working hypothesis can be constructed whereby during physiological sperm transport spermatozoa do not proceed past the tubal isthmus until close to the time of ovulation. During their sojourn in the isthmus the activity of the spermatozoa is suppressed by high extracellular potassium ion concentration and capacitation is either completed or reaches a penultimate stage. Upon movement into the non-inhibitory environment of the ampulla the spermatozoa become hyperactivated and are in a state ready to undergo the acrosome reaction after binding to the sperm receptor on the zona pellucida. However, some spermatozoa undergo premature acrosome loss while swimming free in ampullary fluid, perhaps in response to follicular fluid (elevated progesterone or glycosaminoglycan concentrations) or during migration through the cumulus mass. Some early aspect(s) of the acrosome reaction, including possible swelling of the acrosomal matrix, may occur during cumulus penetration, but the vesiculation phenomenon of the acrosome reaction (the "morphological" acrosome reaction) would not occur in a fertilizing spermatozoon until after zona binding.

Recommendations for future studies

It is hoped that this paper has adequately explained the foundation of, and justification for, the principle that studies on the physiological regulation of human fertilization, which must perforce be carried out *in vitro*, must duplicate as closely as possible the *in vivo* situation. To this end, the

following recommendations would seem pertinent:

1. Serum supplementation of culture medium should be avoided in order to achieve the most consistent and reproducible experimental conditions.
2. For macromolecular supplementation of culture media an appropriate concentration of human serum albumin should be used. Since the periovulatory oviductal fluid protein concentration is about 3.0% (59), concentrations of 30 mg/ml should probably be used.
3. Steroid hormones, especially progesterone, should be included in culture media at concentrations reflecting the *in vivo* situation. This should at least be typical circulating serum levels of these hormones, although further observations on the concentration of such hormones in oviductal fluid, especially in view of its possible "contamination" with follicular fluid, are urgently required.
4. Bicarbonate, being essential for sperm hyperactivation, must be used at a concentration of at least 20 mM, although some other buffer (*e.g.* HEPES) should also be included to avoid pH artifacts that might occur during *in vitro* manipulation of sperm populations when a CO_2 atmosphere (essential for maintaining pH with a bicarbonate buffer) cannot be maintained.
5. Since spermatozoa experience a sequence of changing micro-environments during their passage through the cervical mucus, uterine lumen, oviductal isthmus and ampulla, it would seem appropriate that the influence of sequential changes in potential regulatory factors (*e.g.* extracellular potassium ion concentrations) should be studied.

Lastly, there is the question as to whether or not we should incorporate biological components in our *in vitro* culture systems. For example, should we add a small proportion of normal follicular fluid to culture medium in an attempt to duplicate the presence of some follicular fluid in the oviductal ampulla? Should we consider components (in addition to progesterone) that may be produced by the cumulus cells that would contribute to the environment of the ampullary fluid? Finally, it would seem important to pursue studies on sperm interaction with the oocyte-cumulus complex to establish the nature of sperm-zona binding when surrounded by cumulus.

ACKNOWLEDGEMENTS

Financial support from the Alberta Heritage Foundation for Medical Research, the Medical Research Council of Canada and the Nat Christie Foundation is gratefully acknowledged with respect to the author's studies on human sperm function. Earlier (unpublished) studies on human sperm transport and selection were supported by grants from the Wellcome Trust to the author and Dr. A. Allan Templeton of the Department of Obstetrics and Gynaecology, University of Edinburgh.

REFERENCES

1. HAFEZ, ESE. (1980) The cervix and sperm transport. In: Hafez ESE (ed.), *Human Reproduction. Conception and Contraception*, 2nd ed. Hagerstown: Harper & Row; pp. 221-252.
2. INSLER, V.; GLEZERMAN, M.; ZEIDEL, L.; BERNSTEIN, D.; MISGAV, N. (1980) Sperm storage in the human cervix: a quantitative study. *Fertil Steril 33:* 288-293.
3. PANDYA, I.J.; COHEN, J. (1985) The leukocytic reaction of the human uterine cervix to spermatozoa. *Fertil Steril 43:* 417-421.
4. MORTIMER, D. (1983) Sperm transport in the human female reproductive tract. In: Finn, C.A. (ed.), *Oxford Reviews of Reproductive Biology*, Vol. 5. Oxford: Oxford University Press; pp. 30-61.
5. HUNTER, R.H.F. (1987) The timing of capacitation in mammalian spermatozoa-a reinterpretation. *Res Reprod 19:* 3-4.
6. HUNTER, R.H.F. (1987) Human fertilization *in vivo*, with special reference to progression, storage and release of competent spermatozoa. *Hum Reprod 2:* 329-332.
7. SETTLAGE, D.S.F.; MOTOSHIMA, M.; TREDWAY, D.R. (1974) Sperm transport from the external cervical os to the fallopian tubes in women: a time and quantitative study. In: Hafez, E.S.E.; Thibault, C.G. (eds.), *Sperm Transport, Survival and Fertilizing Ability in Vertebrates, Vol. 26:* Paris: INSERM, pp. 201-217.
8. MORTIMER, D. (1978) Selectivity of sperm transport in the female genital tract. In: Cohen, J.; Hendry, W.F. (eds.) *Spermatozoa, Antibodies and Infertility.* Oxford & London: Blackwell Scientific Publications; pp. 37-53.
9. OVERSTREET, J.W.; KATZ, D.F.; JOHNSON, L.L. (1980) Motility of rabbit spermatozoa in the secretions of the oviduct. *Biol. Reprod. 22:* 1083-1088.
10. JOHNSON, L.L.; KATZ, D.F.; OVERSTREET, J.W. (1981) The movement characteristics of rabbit spermatozoa before and after activation. *Gamete Res. 4:* 275-282.
11. BURKMAN, L.J.; OVERSTREET, J.W.; KATZ, D.F. (1984) A possible role for potassium and pyruvate in

the modulation of sperm motility in the rabbit oviducal isthmus. *J. Reprod. Fertil. 71:* 367-376.
12. HUNTER, R.H.F. (1989) Ovarian programming of gamete progression and maturation in the female genital tract. *Zool. J. Linn. Soc. 95:* 117-124.
13. TEMPLETON, A.A.; MORTIMER, D. (1982) The development of a clinical test of sperm migration to the site of fertilization. *Fertil. Steril. 37:* 410-415.
14. YANAGIMACHI, R. (1988) Mammalian fertilization. In: Knobl, E.; Neill, J.D.; Ewing, L.L, Markert, C.L.; Greenwald, G.S.; Pfaff, D.W. (eds.), *The Physiology of Reproduction.* Vol. 1. New York: Raven Press, Ltda.; pp. 135-185.
15. McMASTER, R.; YANAGIMACHI, R.; LOPATA, A. (1978) Penetration of human eggs by human spermatozoa *in vitro. Biol. Reprod. 19:* 212-216.
16. WASSARMAN, P.M. (1987) Early events in mammalian fertilization. *Annu. Rev. Cell. Biol. 3:* 109-142.
17. MORTIMER, D.; CAMENZIND, A.R. (1985) The role of follicular fluid in inducing the acrosome reaction of human spermatozoa incubated *in vitro. Hum. Reprod. 4:* 169-174.
18. MORTIMER, S.T.; MORTIMER, D. (1990) Kinematics of human spermatozoa incubated under capacitating conditions. *J. Androl 11:* 195-203.
19. BURKMAN, L.J. (1990) Hyperactivated motility of human spermatozoa during *in vitro* capacitation and implications for fertility. In: Gagnon, C. (ed.) *Controls of Sperm Motility: Biological and Clinical Aspects.* Boca Raton: CRD Press, pp. 303-329.
20. MORTIMER, D.; MORTIMER, S.T.; ANDERSON, S.J.; ROBERTSON, L. (1991) Hyperactivated motility of human spermatozoa. In: Baccetti, B. (ed.), *Comparative Spermatology 20 Years After.* New York: Raven Press; in press.
21. YANAGIMACHI, R. (1970) The movement of golden hamster spermatozoa before and after capacitation. *J. Reprod. Fertil. 23:* 193-196.
22. FRASER, L.R. (1977) Motility patterns in mouse spermatozoa before and after capacitation. *J. Exp. Zool. 202:* 439-444.
23. KATZ, D.F.; YANAGIMACHI, R. (1980) Movement characteristics of hamster spermatozoa within the oviduct. *Biol. Reprod. 22:* 759-764.
24. KATZ, D.F.; YANAGIMACHI, R. (1981) Movement characteristics of hamster and guinea pig spermatozoa upon attachment to the zona pellucida. *Biol. Reprod. 25:* 785-791.
25. KATZ, D.F.; DROBNIS, E.Z.; OVERSTREET, J.W. (1989) Factors regulating mammalian sperm migration through the female reproductive tract and oocyte vestments. *Gamete Res. 22:* 443-469.
26. BURKMAN, L.J. (1984) Characterization of hyperactivated motility by human spermatozoa during capacitation: comparison of fertile and oligozoospermic sperm populations. *Arch. Androl. 13:* 153-165.
27. ROBERTSON, L.; WOLF, D.P.; TASH, J.S. (1988) Temporal changes in motility parameters related to acrosomal status: identification and characterization of populations of hyperactivated human sperm. *Biol. Reprod. 39:* 797-805.
28. MORTIMER, D. (1990) Objective analysis of sperm motility and kinematics. In: Keel, B.A.; Webster, B.W. (eds.), *Handbook of the Laboratory Diagnosis and Treatment of Infertility.* Boca Raton: CRC Press, pp. 97-133.
29. ANDERSON, S.J.; ROBERTSON, L.; MORTIMER, D. (1985) Investigation of the regulation of hyperactivated motility in human spermatozoa. In: Serio, M. (ed.) *Serono Symposia Review* Supplement I. *IV International Congress of Andrology Miniposters.* Rome: Ares-Serono Symposia; p. 117.
30. ROGERS, B.J. (1978) Mammalian sperm capacitation and fertilization *in vitro*: A critique of methodology. *Gamete Res. 1:* 165-223.
31. BAVISTER, B.D. (1981) Analysis of culture media for *in vitro* fertilization and criteria for success. In: Mastroianni, L. Jr.; Biggers, J.D.; Sadler, W.A. (eds.) *Fertilization and Embryonic Development In Vitro.* New York: Plenum Press; pp. 41-60.
32. MENEZO, Y. (1976) Milieu synthétique pour la survie et la maturation des gamètes et pour la culture de l'oeuf fécondé. *C.R. Acad. Sci. Paris, Sér D. 272:* 1967-1970.
33. MENEZO, Y.; TESTART, J.; PERRONE, D. (1984) Serum is not necessary in human *in vitro* fertilization, early embryo culture, and transfer. *Fertil Steril 42:* 750-755.
34. QUINN, P.J.; KERIN, J.F.; WARNES, G.M. (1985) Improved pregnancy rate in human *in vitro* fertilization with the use of a medium based on the composition of human tubal fluid. *Fertil Steril 44:* 493-498.
35. MORTIMER, D. (1986) Elaboration of a new culture medium for physiological studies on human sperm motility and capacitation. *Hum. Reprod. 1:* 247-250.
36. PERREAULT, S.D.; ROGERS, B.J. (1982) Capacitation pattern of human spermatozoa. *Fertil. Steril. 38:* 258-260.
37. MORTIMER, D.; CURTIS, E.F.; CAMENZIND, A.R.; TANAKA, S. (1989) The spontaneous acrosome reaction of human spermatozoa incubated *in vitro. Hum Reprod 4:* 57-62.
38. ROBLERO, L.; GUADARRAMA, A.; ORTIZ, M.E.; FERNANDEZ, E.; ZEGERS-HOCHSCHILD, F. (1988) High potassium concentration improves the rate of acrosome reaction in human spermatozoa. *Fertil Steril 49:* 676-679.
39. BAVISTER, B.D. (1982) Evidence for a role of post-ovulatory cumulus components in supporting fertilizing ability of hamster spermatozoa. *J. Androl. 3:* 365-372.
40. BEDFORD, J.M. (1983) Significance of the need for sperm capacitation before fertilization in eutherian mammals. *Biol. Reprod. 28:* 108-120.
41. BEDFORD, J.M. (1983) Form and function of eutherian spermatozoa in relation to the nature of egg vestments. In: Beier, H.M.; Lindner, H.R. (eds.) *Fertilization of the Human Egg In Vitro. Biological Basis and Clinical Application.* Berlin: Springer-Verlag; pp. 133-146.
42. BEDFORD, J.M. (1972) Sperm transport, capacitation and fertilization. In: Balin, H.; Glasser, S. (eds.) *Reproductive Biology.* Amsterdam: Excerpta Medica, pp. 338-392.
43. FRASER, L.R. (1984) Mechanisms controlling mammalian fertilization. In: Clarke, J.R. (ed.) *Oxford Reviews of Reproductive Biology*, Vol. 6. Oxford: Oxford University Press; pp. 174-225.
44. TALBOT, P. (1985) Sperm penetration through oocyte investments in mammals. *Am. J. Anat 174:* 331-346.
45. SATHANANTHAN, A.H.; TROUNSON, A.O.; WOOD, C.; LEETON, J.F. (1982) Ultrastructural observations on the penetration of human sperm into the zona pellucida of the human egg *in vitro. J. Androl. 3:* 356-364.
46. LAWS-KING, A.; TROUNSON, A.; SATHANANTHAN, H.; KOLA, I. (1987) Fertilization of human

oocytes by microinjection of a single spermatozoon under the zona pellucida. *Fertil Steril 48:* 637-642.

47. YAMADA, K.; STEVENSON, A.F.G.; METTLER, L. (1988) Fertilization through spermatozoal microinjection: significance of acrosome reaction. *Hum. Reprod. 3:* 657-661.
48. CROSS, N.L.; MORALES, P.; OVERSTREET, J.W.; HANSON, F.W. (1988) Induction of acrosome reactions by the human zona pellucida. *Biol. Reprod. 38:* 235-244.
49. STOCK, C.E.; BATES, R.; LINDSAY, K.S.; EDMONDS, D.K.; FRASER, L.R. (1989) Extended exposure to follicular fluid is required for significant stimulation of the acrosome reaction in human spermatozoa. *J. Reprod. Fertil. 86:* 401-411.
50. SIITERI, J.E.; GOTTLIEB, W.; MEIZEL, S. (1988) Partial characterization of a fraction from human follicular fluid that initiates the human sperm acrosome reaction *in vitro. Gamete Res. 20:* 25-42.
51. TESARIK, J. (1985) Comparison of acrosome reaction-inducing activities of human cumulus oophorus, follicular fluid and ionophore A23187 in human sperm populations of proven fertilizing ability *in vitro. J. Reprod. Fertil. 74:* 383-388.
52. TESARIK, J.; KOPECNY, V.; DVORAK, M. (1984) Selective binding of human cumulus cell-secreted glycoproteins to human spermatozoa during capacitation *in vitro. Fertil. Steril. 41:* 919-925.
53. SIITERI, J.E.; DANDEKAR, P.; MEIZEL, S. (1988) Human sperm acrosome reaction-initiating activity associated with the human cumulus oophorus and mural granulosa cells. *J. Exp. Zool. 246:* 71-80.
54. TESARIK, J.; PILKA, L.; DRAHORAD, J.; CECHOVA, D.; VESELSKY, L. (1588) The role of cumulus cell-secreted proteins in the development of human sperm fertilizing ability: implication in IVF. *Hum. Reprod. 3:* 129-132.
55. OSMAN, R.A.; ANDRIA, M.L.; JONES, A.D.; MEIZEL, S. (1989) Steroid induced exocytosis: the human sperm acrosome reaction. *Biochem. Biophys. Res. Comm. 160:* 828-833.
56. McLAUGHLIN, E.A.; FORD, W.C.L.; HULL, M.G.R. (1990) Effect of progesterone on the intracellular calcium concentration of human spermatozoa. *J. Reprod. Fert. Abstr. Ser. 5:* 23 (Abstract Nº 35).
57. DE JONGE, C.J.; RAWLINS, R.G.; ZANEVELD, L.J.D. (1988) Induction of the human sperm acrosome reaction by human oocytes. *Fertil. Steril. 50:* 949-953.
58. ROBLERO, L.S.; GUADARRAMA, A.; ORTIZ, M.E.; FERNANDEZ, E.; ZEGERS-HOCHSCHILD, F. (1990) High potassium concentration and the cumulus corona oocyte complex stimulate the fertilizing capacity of human spermatozoa. *Fertil. Steril. 54:* 328-332.
59. LIPPES, J.; KRASNER, J.; ALFONSO, L.A.; DACALOS, E.D.; LUCERO, R. (1981) Human oviductal fluid proteins. *Fertil. Steril. 36:* 623-629.