STRUCTURAL AND FUNCTIONAL ASPECTS OF SPERM AFTER TRANSIT THROUGH THE FALLOPIAN TUBE OF THE SHEEP

by

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CONTENTS

ABSTRACT

Ejaculated mammalian spermatozoa have the potential for fertilization. The acquisition of the ability to fertilize a fully invested oocyte occurs during the passage through the female reproductive tract. These post-ejaculatory maturational changes include the processes of capacitation and the acrosome reaction. The Fallopian tube has been accepted as the site of completion of these events while their initiation may occur either in the uterus or in the Fallopian tube. The role and/or interaction of the Fallopian tube with sperm to undergo these molecular events, are poorly understood. This study attempts to demonstrate the influence on sperm of the environment of the Fallopian tube which could initiate certain structural and functional alterations with respect to capacitation and the acrosome reaction; this study does not attempt to define the exact nature of the interaction between spermatozoa and the Fallopian tube.

In this study the *in vivo* situation is mimicked, exposing sperm to the oviduct under laboratory conditions. Pre- and postmigratory sperm were compared. The specific structural and functional aspects examined in this study include motility, morphology, localization of N-acetyl-D-glucosamine receptors and the acrosomal status.

Motility is important to ensure successful migration to the site of fertilization. Special attention was focussed on the incidence of hyperactivation after transit through the Fallopian tube. The term hyperactivation has been used to describe

the frantic movement of sperm before they undergo the acrosome reaction and often serves as a biological marker for capacitation. Motility analyses were performed using the CASMA system, the Sperm Motility Quantifier (SMQ). The biological state of hyperactivation occurred in a small population of spermatozoa after transit through the Fallopian tube.

A morphologically normal sperm is a direct product of spermatogenesis and epididymal maturation. No gross morphological alterations have been reported to occur to sperm within the female tract. Instead, elimination of sperm with gross morphological aberrations (whether as a direct function of the female tract or intrinsic sperm factors) has been shown. Measurement of the sperm head dimensions were performed in this study using the Flexible Image Processing System (FIPS). No obvious morphological disparities were present in the samples of ram sperm used. No obvious morphological alterations/selections occurred during transit through the Fallopian tube. Ram sperm head dimensions are defined.

Fusion between spermatozoa and the egg vestments is a crucial step in fertilization. Exposure of fusiogenic structures on sperm is a component of capacitation. The presence of these receptors is important in species-specific interaction and its absence play a significant role in infertility. FITC-conjugated wheatgerm agglutinin was used to identify and localize N-acetyl-D-glucosamine-like receptors on the sperm membrane surface. This surface component is believed to play an important role in sperm-egg interaction. Membrane alterations associated with receptor activity (allowing for sperm-zona binding)

appear to have occurred after transit through the Fallopian tube.

The acrosome reaction has, to date, been recognised as the most reliable indicator of the completion of capacitation. It is generally accepted that the acrosome reaction of the fertilizing sperm occurs at the zona pellucida surface, that previously acrosome-reacted sperm may also bind to the ZP. FITC-conjugated peanut agglutinin was selected as a probe for acrosomal status determination. An increase in the incidence of acrosome-reacted sperm was observed after transit through the Fallopian tube.

A small population of spermatozoa, therefore, appear to have acquired (wholly or partially) fertilizing potential after transit through the Fallopian tube.

ABSTRAK

Uitgestorte soogdier spermatosoa besit die potensiaal om te bevrug. Die verwerwing van die vermoë om 'n ten volle omklede oösiet te bevrug geskied ten tye van die deurvaart van spermatosoa deur die vroulike geslagsisteem. Hierdie post-ejakulatoriese rypwordings veranderinge sluit in die prosesse van kapasitasie en die akrosoomreaksie. Die fallopiese buis word aanvaar as dié plek vir die voltooïng van hierdie gebeurtenisse, terwyl die inisiasie geskied in óf die uterus óf die fallopiese buis. Die rol en/of interaksie van die fallopiese buis met spermatosoa om die molekulêre gebeure te ondergaan, word swak begryp. Hierdie studie poog om die invloed van die fallopiese buisomgewing op spermatosoa te demonstreer in so verre dat die spermatosoa sekere strukturele en funksionele veranderinge ondergaan met betrekking tot kapasitasie en die akrosoomreaksie; die studie poog nie om die presiese karakter van die interaksie tussen spermatosoa en die fallopiese buis te definïeer nie.

Dus probeer hierdie studie die *in vivo* situasie naboots; om spermatosoa aan die ovidukt onder labratoriumkondisies bloot te stel. Pre- en postmigrasie spermatosoa word vergelyk. Die spesifieke strukturele en funksionele aspekte wat in hierdie studie onder die loep kom, sluit in motiliteit, morfologie, lokalisasie van N-asetiel-D-glukosamien reseptore en akrosomale status.

Motiliteit is belangrik om die suksesvolle migrasie na die plek van bevrugting te verseker. Spesiale aandag was gevestig op die voorkoms van hiperaktivering na

migrasie deur die fallopiese buis. Die term hiperaktivering is gebruik om die hoë aktiwiteit beweging van spermatosoa te beskryf voor hulle die akrosoomreaksie ondergaan en dien dikwels as 'n biologiese indikator vir kapasitasie. Motiliteitsanalises was verkry deur die gebruik van die "CASMA" sisteem, die "Sperm Motility Quantifier (SMQ)". Die biologiese toestand van hiperaktivering het slegs in 'n klein populasie van spermatosoa na migrasie deur die fallopiese buis voorgekom.

'n Morfologies normale sperm is 'n direkte produk van spermatogenese en epididimale rypwording. Geen aanduiding van morfologiese veranderinge van spermatosoa binne die vroulike geslagsisteem, kon vasgestel word nie. Die eliminasie van spermatosoa met groot morfologiese afwykings (óf vanweë 'n direkte funksie van die vroulike geslagsisteem óf intrinsieke spermatosoön faktore) word egter aangetoon. Opmetings van die spermkop se dimensies was verkry deur gebruik te maak van die "Flexible Image Processing System (FIPS)". Geen ooglopende morfologiese verskille van die betrokke ram spermatosoa is waargeneem nie. Geen ooglopende morfologiese veranderinge/seleksies het geskied tydens die deurtog deur die fallopiese buis nie. Ram spermkop dimensies is gedefinïeer.

Binding tussen spermatosoa en die eier bedekkings is 'n belangrike stap in bevrugting. Blootstelling aan bindingsstrukture op spermatosoa is 'n komponent van kapasitasie. Die voorkoms van hierdie reseptore is belangrik in spesie-spesifieke interaksie en hul afwesigheid speel 'n onmisbare rol in infertiliteit. FITC-gekonjugeerde koringkiem agglutinien was gebruik om N-asetiel-D-

glukosamien-tipe reseptore op die spermmembraan oppervlakte te identifiseer en te lokaliseer. Dié oppervlakte komponent speel na bewering 'n belangrike rol in die sperm-eier interaksie. Membraan veranderinge wat geassosieer is met reseptor aktiwiteite (wat toelaat vir sperm-zona binding) blyk voor te kom na migrasie deur die fallopiese buis.

Die akrosoomreaksie is, tot op datum, erken as die mees betroubare indikator vir die voltooïng van kapasitasie. Dit word algemeen aanvaar dat die akrosoomreaksie van die bevrugtende sperm geskied op die zona pellucida oppervlakte, maar dat die akrosoom-gereageerde sperm ook mag bind aan die ZP. FITC-gekonjugeerde grondboontjie agglutinien was geselekteer as om akrosomale status aan te dui. 'n Verhoging in die voorkoms van akrosoom-gereageerde spermatosoa was waargeneem na migrasie deur die fallopiese buis.

'n Klein populasie van spermatosoa het blykbaar (geheel of gedeeltelik) bevrugtings potensiaal verwerf na migrasie deur die fallopiese buis.

CHAPTER ONE

General Introduction

1.1 Introduction

Fertilization may be described as the sequence of events that starts with the interaction of the male and female gametes and culminates with the formation of the zygote. The crucial event of fertilization is the fusion of one spermatozoon with one oocyte [Monroy & Rosati, 1983]. The mechanisms leading to fertilization point to the existence of a well-defined sequence of events which includes maturation of both the oocyte and the spermatozoon. Sperm maturation starts within the male reproductive tract and is completed within the female reproductive tract. This maturational process includes the development of the fertilizing capacity of the spermatozoa and involves the processes of capacitation and the acrosome reaction. Subsequent events include sperm passage through the zona pellucida and fusion of the fertilizing spermatozoon with the oocyte [Hafez, 1980].

The sperm cell is unique in that it has evolved to survive many different physiological microenvironments from the epididymis, the vas deferens and

seminal plasma of the male post-testicular reproductive tract, to the vagina, cervix, uterus, Fallopian tube and peritoneal cavity of the female reproductive tract. Although the autonomous migration of sperm possibly plays a large role in the transport of sperm through the different microenvironments of the female reproductive tract, the fusion of sperm and oocyte also depends on the actions of the female tract in which the sperm cell may be a passive participant. These actions are biophysical phenomena and include the muscular contractions of the viscera and the activity of the cilia lining the lumina within the tract. The epithelial surfaces and secretions of the female reproductive tract, as well as the egg vestments, appear to modulate sperm motility and may coordinate the transport and fusion of the gametes [Katz *et al.*, 1989]. The female hormones, secreted during the different phases of the oestrus cycle, may also have an indirect effect on the transport of spermatozoa through the female tract by altering the properties of the secretions found within the female reproductive tract.

The prerequisites for successful fertilization *in vivo* on the part of the sperm cell appear to include:

1) a morphologically normal sperm which is a product of spermatogenesis and/or epididymal maturational processes,
2) vigorous sperm motility to ensure successful migration to the site of fertilization and transit through the egg envelopes (the forces due to flagellar motion assist the sperm in penetrating the egg vestments to reach

the oolemma) [Katz *et al.*, 1989] and

3) exposure of fusiogenic structures (receptors) coupled with species-specific interaction between the spermatozoa and the egg envelopes [Epel, 1980].

Many studies have been undertaken to elucidate the complexity of sperm transport and the prefertilization changes that occur within the female reproductive tract. Some of these studies have focused on microenvironment-induced capacitational changes by studying the effect of various possible contributing factors. However, these factors were studied in isolation and, therefore, it is difficult to appreciate the influence of the female reproductive tract, in its entirety, on these capacitational changes. Possible contributing factors, from the oviduct to the transport and prefertilization changes of sperm, include mechanical activity of the oviducts, oviductal fluid, follicular fluid, oviductal mucosa and ovarian hormones. For obvious reasons, it is very difficult to study these events as they occur *in vivo*. In this study, where the *in vivo* situation was mimicked, sperm were exposed to the oviduct environment under laboratory conditions. Although every possible precaution was taken to accommodate and maintain a constant physiological environment, an obvious drawback to the study is the possible failure of the maintenance of tissue survival outside the body.

Fertilizing spermatozoa are sequestered in the lower part of the oviductal isthmus until ovulation begins so that sperm ascent to the ampulla occurs

synchronously [Yanagimachi, 1994]. The termination of the arrest of spermatozoa in the caudal isthmus results in the release of capacitated sperm [Hunter *et al.*, 1983; Smith & Yanagimachi, 1991]. Although the site of capacitation is uncertain and may vary from species to species, increasing evidence suggests that, in some species at least, capacitation must be completed in the oviduct [Barros, 1968; Bedford, 1972]. Also, Fallopian tube fluid [Barros & Austin, 1967; Barros, 1968] and follicular fluid [Yanagimachi, 1969] have been found to promote capacitation. These results have led me to target the oviduct for my study of structural and functional aspects of sperm after transit through the Fallopian tube.

This study, therefore, examines structural and functional aspects of sperm transit through the oviduct. Although many studies have focused on a single fertilizing requirement of sperm, such as motility or morphology, after exposure to the oviduct, no study thus far has examined structural and functional aspects relating to motility, morphology, receptors and acrosomal status within the same sperm sample.

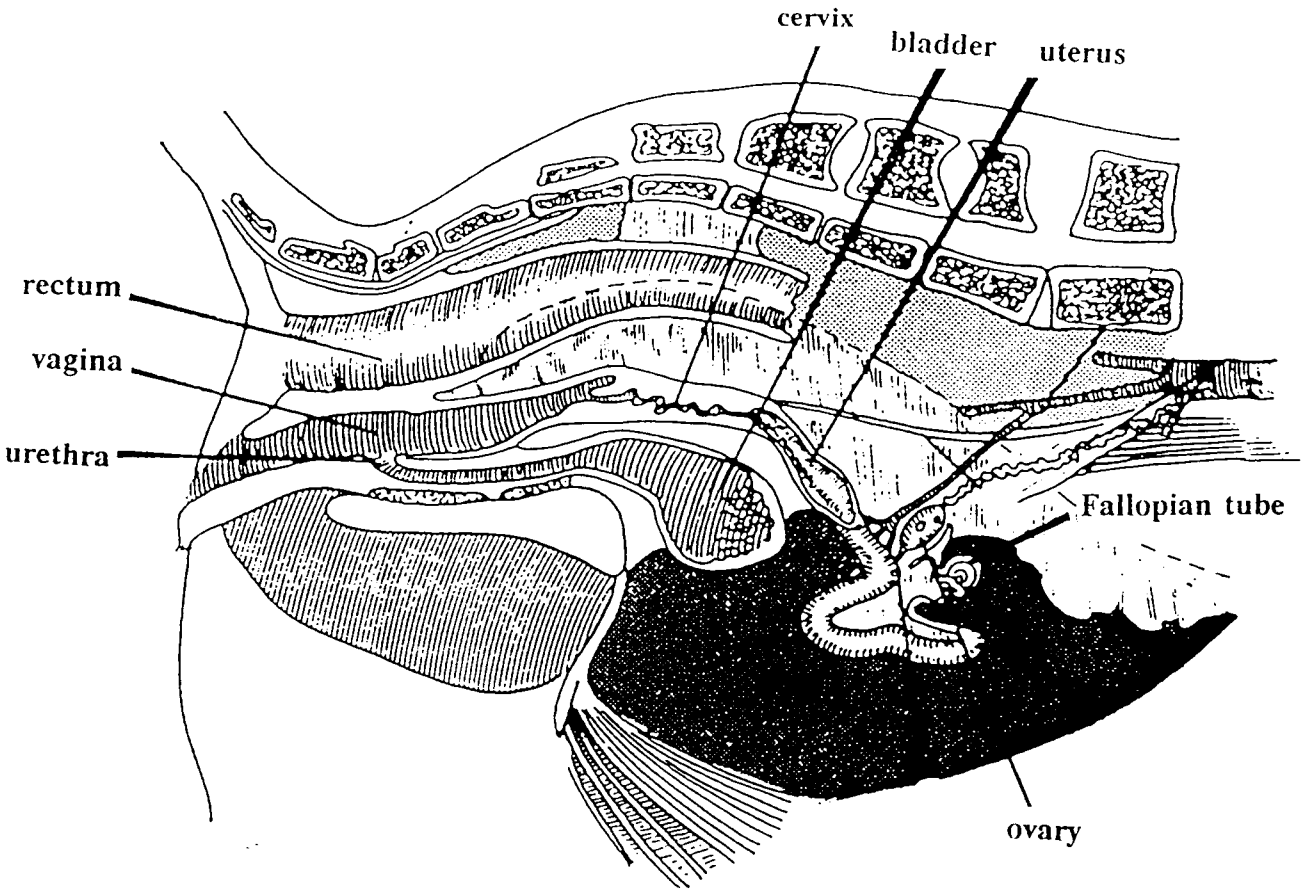

Fig.1.1 Saggital section through pelvic region (view of left side) showing urogenital tract of the ewe [Hafez, 1980]

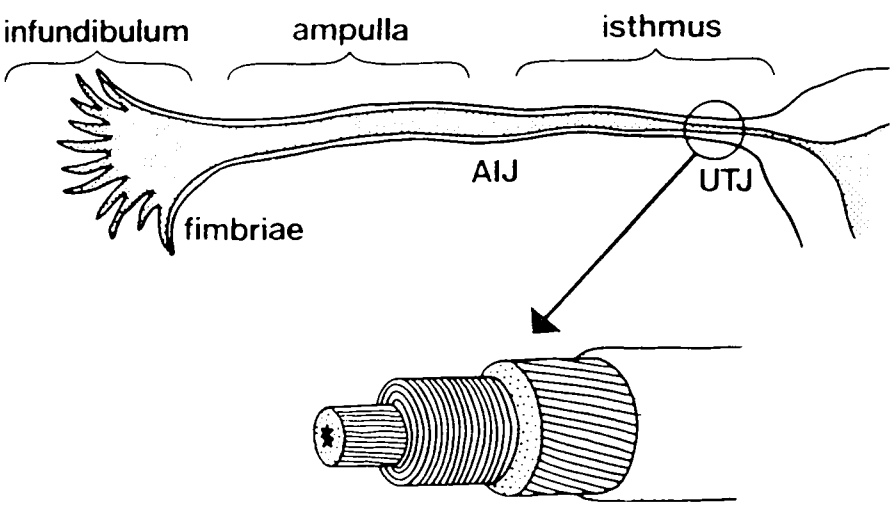

Fig.1.2 Major segment of oviduct [Hafez, 1980]

AIJ - ampullary-isthmic junction; UTJ - uterotubal junction

1.2 Migration through the female reproductive tract

Sperm forward progression is characterized as an interactive process between the cell and its environment. Flagellar movement is a fundamental expression of the vitality of the sperm cell where flagellar bending results in a distribution of local forces against the surrounding fluid. The viscosity of the suspending fluid can influence sperm flagellar motion. If viscosity increases, the flagellum must push harder to achieve the same local velocity as in a simple fluid. The cervical mucus is commonly seen as the first polymeric secretion with complex rheological behaviour which the sperm must traverse [Katz *et al.*, 1989].

Prior to exposure to cervical mucus, spermatozoa are exposed to seminal plasma. Although the properties conferred to sperm by seminal plasma are not fully known, Peitz and Olds-Clarke [1986] have shown that seminal vesicle removal results in decreased fertility in the house mouse. Stegmayr and Ronquist [1982] concluded that particulate elements in human seminal plasma were involved in stimulation of forward motility in spermatozoa, whereas Davis and Hungund [1976] observed that the particles in rabbit seminal fluid originating from the epididymis caused sperm decapacitation and inhibited fertility. The functional importance of the particles in seminal fluid remain contradictory. One of the important physical properties of cervical mucus is the exclusion of the seminal plasma from semen [Katz *et al.*,1989].

Sperm migration through the cervix involves distinct but interrelated factors. The

ability of spermatozoa to penetrate the mucus, the properties of mucus that enable it to participate actively in the process of sperm transport and, also, the morphologic configuration of cervical crypts and clefts that contribute to the storage and preservation of spermatozoa in the cervical canal and their suspended and prolonged release to the upper tract [Blandau & Moghissi, 1973]. Other factors which contribute to sperm penetration at the semen-mucus interface are seminal enzymes and external forces due to visceral contractility. Mechanical properties of the microstructure, the viscosity of the mucus plasma, and the size and shape of the sperm head and flagellum afford mechanical resistance to sperm penetration through the cervical mucus. There is variation among species in these biological factors [Katz *et al.*, 1989].

Subsequent movement of sperm into and through the uterus is probably the result of myometrial contractions rather than significant independent sperm motility [Mortimer, 1978]. The increased uterine activity found during orgasm in women may assist in sperm transport into the cervix and uterus [Edwards, 1980].

The spaces through which sperm migrate *in vivo* may have transverse dimensions of the same magnitude, or they may be smaller than the length of the body of the sperm. In some locations, such as the uterotubal junction and the lower oviductal isthmus, the spaces are small enough to restrict sperm passage physically. The presence of a nearby surface will have a profound effect on

sperm movement. When a sperm is swimming near a solid surface at a distance less than its own body length, the fluid motions induced by the flagellum are constrained by the surface; this is known as the wall effect. This phenomenon causes an increase in the dissipation of hydrodynamic energy and, consequently, the flagellum must push harder to achieve a local velocity equal to that attained in the absence of a nearby boundary. As in the case of increased viscosity, it is reasonable to expect that the flagellar bend propagation mechanism will respond to this wall effect [Katz *et al.*, 1989]. Experimental evidence demonstrating such a response was reported by Suarez *et al.* [1983] that, when sperm become constrained to beat two-dimensionally, their flagellar waves become less symmetrical resulting in circular trajectories. It is likely that these wall effects, so conspicuous *in vitro,* play a role in sperm migration *in vivo*. During much of their journey along the female reproductive tract, spermatozoa move in close proximity to epithelial surfaces. The two-dimensional flagellar wave propagation, with planar orientation of the sperm body, is well suited to migration along such surfaces. These sperm would progress along surfaces but could be trapped and eliminated from transport if led into cul-de-sacs of the epithelium. The presence of fluid currents due to ciliary activity would act to reorientate such sperm; this mechanism, along with visceral contractility, may contribute to sperm transport through the oviduct [Katz *et al.*, 1989; Mortimer & Swan, 1995].

The term hyperactivation has been used to describe the frantic movement of sperm before they undergo the acrosome reaction [Yanagimachi, 1994]. Most

spermatozoa in the ampulla are hyperactivated [Suarez *et al.*, 1983]. Hyperactivation is a useful biological marker as related to capacitation and it may mechanically promote a number of sperm functions, including transport through the oviduct and penetration of the cumulus and zona pellucida. However, despite the increasing attention to this visually striking phenomenon, it still lacks objective kinematic definition: the site, mechanisms and kinetics of its onset are unclear and its role in sperm function is not fully understood. Subjective visual assessments have led to a remarkable set of metaphors to characterize swimming trajectories of hyperactivated sperm. Such motion has been referred to as "bobbing" [Gwatkin & Anderson, 1969], "serpentine", "high amplitude" [Yanagimachi, 1970], "whiplash" [Cooper *et al.*, 1979], "figure-of eight" [Fraser, 1977] and "darting" [Corselli & Talbot, 1986]. Clearly these terms do not permit standardisation in the identification of hyperactivated sperm. Definition and interpretation of hyperactivation should derive from analysis of the sperm flagellar beat, which is the biophysical cause of the motion [Katz *et al.*, 1989].

1.3 Selection of a morphologically normal sperm for fertilization

A normal sperm has a head, neck, midpiece and tail. Two-thirds of the anterior surface of the head is covered by the acrosome. Both the midpiece and the tail are capable of independent motility, even in the absence of the head. The size and shape of the sperm head is species-specific [Van der Horst *et al.*, 1991].

Various morphological abnormalities can occur in semen [Salisbury & VanDemark, 1961].

In humans and certain other mammals, including sheep, sperm is deposited in the upper part of the vagina. The cervical mucus acts as a barrier to spermatozoa during most of the menstrual and oestrus cycle. Only in the periovulatory period does the structure of the cervical mucus alter to permit penetration of the spermatozoa [Blandau & Moghissi, 1973]. The ability of the cervix and its mucus to exclude many of the morphologically abnormal spermatozoa has long been recognised [Bergman, 1955; Botella-Lluisa, 1956]. Information on the morphology of the spermatozoa at the site of fertilization is limited but available literature indicates that there is indeed a morphological selection along the female reproductive tract.

Algren *et al.* [1974] reported that between 79 and 98% of human spermatozoa recovered from the ampullae were morphologically normal, while Asch [1976] reported that no abnormal forms were encountered. However, Mortimer and co-workers' [1982] results show that this may be an over-simplification, and that a few abnormal forms may reach the site of fertilization. Mortimer *et al.* [1982] conclude that the apparent selection of morphologically normal spermatozoa is not a direct function of the female tract, but that spermatozoa can effect their own selection because of differential motility. Detailed evaluation of spermatozoa in their study shows that the selection of sperm is largely achieved

by reductions in sperm with midpiece, tail and other defects which might be expected to impair their motility. A study undertaken by Ragni *et al.* [1985] supports Mortimer's work that the mucus acts as a "passive filter" with selection depending on spermatozoa themselves in relation to motility.

Katz *et al.* [1989] agree that morphologically abnormal sperm, as a group, have inferior motility compared with normal sperm in the same ejaculate, but that this differential swimming ability is not large enough to account for the exclusion of such a large population of abnormal sperm from mucus penetration. The properties of the human sperm surface possibly influence penetration. When antisperm antibodies are present on the sperm surface, sperm with vigorous motility may be unable to swim for more than a few sperm body-lengths into cervical mucus [Fjallbrandt, 1968 & 1969;Jager *et al.*, 1981; Bronson & Cooper, 1987]. Either by physical entanglement or by chemical linkage, antibodies interact with sperm in such a way as to resist forward progression. Similarly, antibodies secreted into the mucus can link to penetrating sperm, causing analogous impediment to motion. Basic hydrodynamic reasoning dictates that the variations in the dimensions of the sperm heads alone will not generate sufficient drag to prevent mucus penetration by these sperm. The origin of the increased resistance to these abnormal sperm may lie in their surface interactions with the mucus macromolecules [Katz *et al.*, 1989]. Studies with capacitated sperm have indicated that a more generalized cellular dysfunction may be associated with morphological abnormalities [Morales *et al.*, 1988]. Surface

changes in the sperm cell are closely related to the functional alterations of capacitation. It is possible, therefore, that the dysfunctional state of the sperm cell is revealed in its earliest interaction with the cervical mucus [Katz & Phillips, 1986].

Fredericcson and Björk [1977] additionally reported that, a barrier which is particularly active against spermatozoa with abnormal heads, exists at the level of the external os. Their study indicates the presence of a female factor in the selection of spermatozoa but it does not attempt to explain a mechanism for this selection. Foldesy *et al.* [1984] reported that there was no selection along the female tract in the rabbit of a special subpopulation of spermatozoa from the ejaculate for fertilization. They indicate that the progressive reduction in numbers during the ascension of spermatozoa through the female reproductive tract does not appear to be an important factor for fertilization but may be important in reducing the incidence of polyspermy. No morphological assessments were done; their study was based on the survival of rabbit embryos after fertilization.

1.4 Molecular events leading to fertilization

1.4.1 Capacitation

Capacitation is primarily an endogenous physiological change of the spermatozoon necessary for mammalian fertilization [Bedford, 1973; Austin,

1975]. It is a membrane event at the molecular level, achieved after spermatozoa have resided in the female genital tract. Sperm capacitation is believed to be a continuation of the maturational process initiated in the epididymis [Hinrichson-Kohane *et al.*, 1984]. Initial alterations in capacitation include modification, redistribution or loss of the epididymal and seminal plasma proteins coating the sperm surface [Brackett & Oliphant,1975; Oliphant, 1976; Kinsey & Koehler, 1978; Koehler,1976 & 1981]. Not only is the sperm plasma membrane surface altered during capacitation but changes intrinsic to the membrane have also been described and these are evidenced by the restriction in the ability of intramembraneous proteins to move laterally within the plasma membrane [O'Rand, 1977]. These intramembraneous modifications, which occur during capacitation, lead to the formation of glycoprotein-rich and glycoprotein-poor areas [Friend *et al.*, 1997]. These areas co-exist in a patchwork-like topography and are consistent with the pattern of membrane fusion seen during the acrosome reaction [Franklin, 1970]. Capacitation is now generally accepted to include all steps required to permit sperm to undergo the acrosome reaction without actually doing so [Bedford, 1970a]. For this reason, in the past, the acrosome reaction had been recognised as the principal event that signalled that capacitation had been completed [Burkman, 1991].

As stated earlier, hyperactivation is a useful biological marker for capacitation. Membrane changes during capacitation may be directly related to changes in flagellar motion, for example, in altering membrane ion conductance [Katz *et al.*,

1989]. Mohri and Yanagimachi [1980] showed that, upon exposure to ATP, demembranated cauda epididymal and ejaculated sperm displayed hyperactivated motility. They suggest that the characteristics of the sperm motor apparatus does not change during capacitation but that the motor apparatus, in fresh epididymal and ejaculated sperm, must merely be prevented by some (intrasperm?) mechanism from effecting the hyperactivated motility.

The site of physiological capacitation within the female reproductive tract is uncertain. In species in which spermatozoa are deposited in the vagina at coitus, sperm capacitation may begin while spermatozoa pass through cervical mucus. "Rubbing off" sperm-surface-adsorbed materials (including seminal plasma proteins) against the mucus network may facilitate capacitation [Gould *et al.*, 1985; Katz *et al.*, 1989]. Barros *et al.* [1988] reports that cervical mucus modifies human spermatozoa, as measured by their interaction with zona-free hamster eggs. They suggest that spermatozoa might achieve a state of partial capacitation while in the cervical mucus. Chang [1951, 1955] established that capacitation of rabbit spermatozoa can take place within the uterus. However, there is increasing evidence that, in some species at least, capacitation must be completed in the oviduct [Barros, 1971; Bedford, 1972]. This idea is further supported by the fact that the cumulus oophorus has been found to be a potent inducer of hamster capacitation *in vitro* [Gwatkin *et al.*, 1972]. In the presence of cumulus oophorus, spermatozoa, attach to cumulus cells , remain associated with them for two to three hours and are then released in a capacitated state. According to

Gwatkin [1977], cellular microfilaments and microtubules appear to be involved in the capacitation process. During this association, the spermatozoa are enveloped by the cumulus cell microvilli and become deeply embedded. Glycosidases released by the cumulus cells, at this stage, appear to alter the sperm plasma membrane [Carter, 1974; Gwatkin & Carter, 1974].

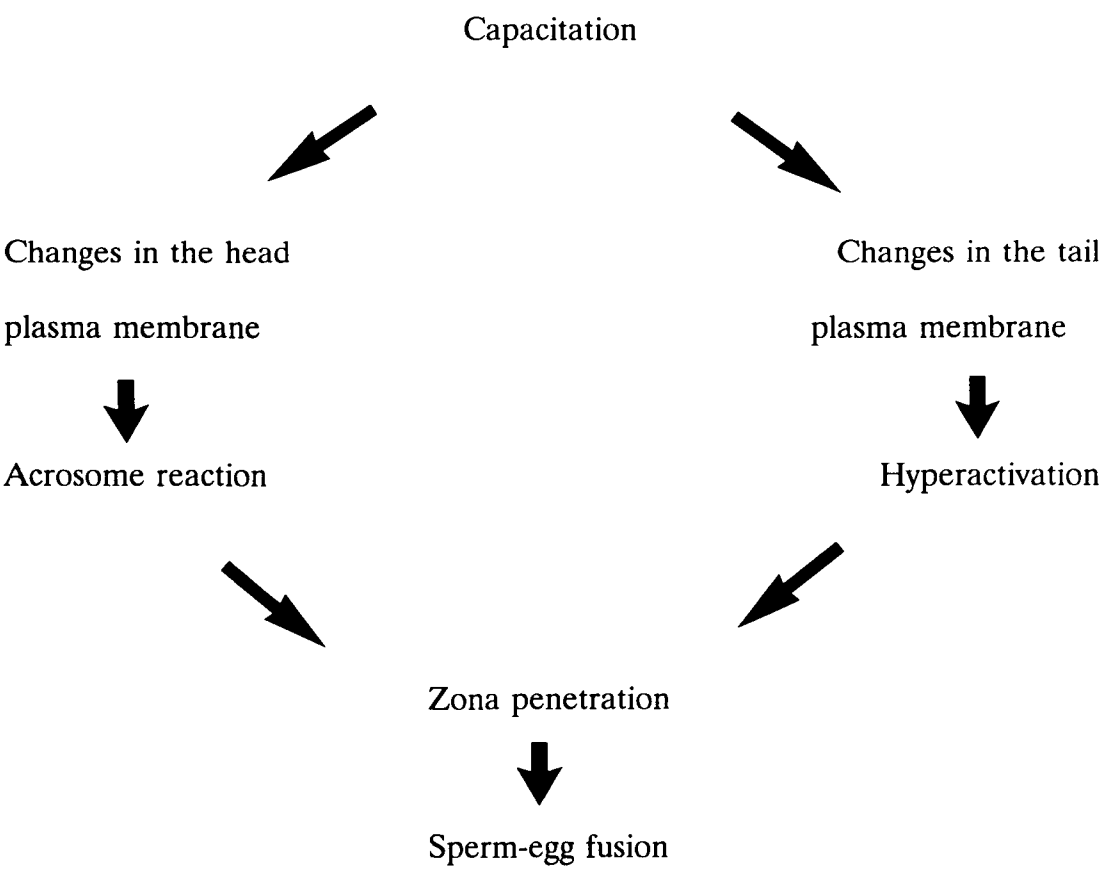

Fig.1.3 Possible relationships among sperm capacitation, acrosome reaction and hyperactivation [Yanagimachi, 1994].