

The Meaning of Sperm Capacitation

A Historical Perspective

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C. R. Austin (1951) reported in an Australian journal that "when sperms were introduced into the fallopian tube of rabbit before ovulation, most of the eggs subsequently recovered were fertilized. However, if the sperms were introduced after ovulation the eggs rarely showed signs of penetration." He concluded that "there seems to be a need for sperms to spend some time, apparently a few hours, in the female tract before they can penetrate the zona." The same year, Chang (1951) published a paper entitled "Fertilizing Capacity of Spermatozoa Deposited into the Fallopian Tubes" in which he stated that "It is quite clear that fertilization occurs when the spermatozoa have been in the tube for six hours before ovulation, which is perhaps the time required for a physiological change in the spermatozoa enabling them to attain fertilizing capacity." Based upon these two reports and his observations on the penetration of rat eggs examined at various times after mating, Austin (1952) introduced the term "Capacitation" to the literature of reproductive biology and concluded that "the sperm must undergo some form of physiological change or capacitation before it is capable of penetrating the egg."

Our knowledge of fertilization before 1951 was mainly from the study of sea urchins. One would expect that mammalian spermatozoa from the male genital tract are capable of penetrating the egg. When these experiments demonstrated that

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mammalian spermatozoa do need to spend some time in the female genital tract to achieve their final fertilizing capacity, it was a stimulus to many scientists to seek the changes that occur in the spermatozoa during their sojourn in the female genital tract. But capacitation is a general term and may include many physiological and morphological changes about which we had no ideas at that time.

Due to the progress in the study of mammalian fertilization in recent years, capacitation of spermatozoa became a commonly used term, and the meaning of capacitation was held differently among scientists and even by the same author. This article, based mainly on review articles and some work done in the author's laboratory, attempts to clarify some of the confusion.

Capacitation and Decapacitation

There are various ways to study the capacitation of spermatozoa. By depositing ejaculated, epididymal spermatozoa, or spermatozoa recovered from the uterus at various times after mating, into the oviducts of rabbits soon after ovulation, Chang (1955) found that fertilization occurred only following deposition of spermatozoa recovered from the uterus; this showed that capacitation can be achieved in the uterus. Further experiments (Chang, 1957) revealed that when the capacitated spermatozoa recovered from rabbit uteri were treated with 5 to 20% rabbit, bull, or human seminal plasma, and deposited into rabbit oviducts soon after ovulation, fertilization did not occur. When treated uterine spermatozoa were deposited 6 hours before ovulation, fertilization was pos-

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sible. These findings show that capacitated spermatozoa can be "decapacitated" by treatment with seminal plasma, while the decapacitated spermatozoa can be "recapacitated" in the oviducts. The so-called "decapacitation factor" was further studied by Williams et al (1967), and discussed by Austin (1969) and McRorie and Williams (1974) in polypeptide, molecular, and antifertility terms.

The Acrosome Reaction

The acrosome reaction of sea urchin spermatozoa upon contact with the jelly coat of the egg was first described by Dan (1952, 1956). The role of the mammalian sperm acrosome during fertilization was first studied by Austin and Bishop (1958) with a phase contrast microscope. They concluded that "the acrosome becomes modified in spermatozoa passing through the female genital tract and is detached before the spermatozoa penetrates the zona pellucida. These changes in the acrosome are considered to constitute 'capacitation'." The acrosome reaction is an obvious morphological change of spermatozoa during their sojourn in the female genital tract. By means of electron microscopy it was observed that the acrosome reaction involves membrane vesiculation or multiple fusion between the plasma and the underlying outer acrosomal membrane for the rat (Piko any Tyler, 1964) and for the hamster and rabbit (Barros et al, 1967). Ultrastructural changes in the sperm head during fertilization in the rabbit were also described by Bedford (1968). The acrosome of guinea pig spermatozoa, a very conspicuous structure, disappears after 14 to 18 hours in culture, and these capacitated spermatozoa are capable of penetrating eggs immediately (Yanagimachi, 1972). Various aspects of acrosome reaction *in vivo* and *in vitro* were discussed recently by Yanagimachi (1981).

Capacitation *In Vivo* and Fertilization *In Vitro*

The inhibition of capacitation in the uterus of pseudopregnant or progesterone-treated rabbits was reported by Chang (1958). The capacitation of rabbit spermatozoa in the isolated bladder, isolated colon, anterior chamber of the eye, and glandula vesicularis was described by Noyes and associates (1958). All these experiments, however, were conducted by depositing spermatozoa recovered from the uterus, or from these other organs, into the oviducts of rabbits soon after ovulation and examining them for possible fertilization.

Up to 1951, the possibility of fertilizing mammalian eggs *in vitro* using ejaculated or epididymal spermatozoa without capacitation was never authentically demonstrated. Since the recognition of capacitation of mammalian spermatozoa in the female tract, cytological evidence of fertilization of rabbit eggs *in vitro* by capacitated spermatozoa was reported by Thibault and associates (1954). The production of young, genetically true to their parents, following the transfer of rabbit eggs fertilized *in vitro* by spermatozoa recovered from the uterus was reported by Chang (1959). Later, *in vitro* fertilization of denuded rabbit eggs by spermatozoa recovered from the vagina was described (Chang et al, 1971). Thus, the successful *in vitro* fertilization of rabbit eggs by capacitated spermatozoa further reinforced the validity and importance of capacitation of spermatozoa in the female genital tract.

Capacitation of Spermatozoa *In Vitro*

The first successful fertilization of hamster eggs *in vitro* was described by Yanagimachi and Chang (1963). They reported that in Tyrode's solution containing glycine, 30 to 66% of the eggs were fertilized by spermatozoa recovered from the uterus 0.5 to 5 hours after mating but only 17% of the eggs were fertilized by epididymal spermatozoa. This shows that the capacitated spermatozoa recovered from the uterus are better able to fertilize eggs. If the final ability to penetrate the egg requires capacitation, then the epididymal spermatozoa must have capacitated *in vitro*. This finding changed the original notion that capacitation is achieved only in the female genital tract, and also opened the door to study capacitation *in vitro*. The possibility of fertilization of hamster eggs *in vitro* by epididymal spermatozoa was further confirmed by Barros and Austin (1967), who showed that a period of 4 hours is needed for sperm capacitation *in vitro* and described the close correlation between the acquisition of fertilizing capacity by spermatozoa and the occurrence of the sperm acrosome reaction. *In vitro* capacitation, including the acrosome reaction of hamster spermatozoa in the presence of tubal fluid of mouse and rat, was described by Barros (1968), and *in vitro* fertilization of hamster eggs in the presence of bovine follicular fluid was reported by Yanagimachi (1969).

Although the fertilization of mouse eggs *in vitro* by spermatozoa recovered from the uterus was described by Whittingham (1968), *in vitro* fertilization of mouse eggs by epididymal spermatozoa in the

presence of bovine follicular or rabbit tubal fluid was reported by Iwamatsu and Chang (1969). Finally, capacitation, including the acrosome reaction of hamster spermatozoa in the presence of blood sera, was described by Barros and Garavagno (1970) and Yanagimachi (1970a).

Successful *in vitro* fertilization of Chinese hamster eggs in media containing 1% bovine serum albumin was reported by Pickworth and Chang (1969), who also pointed out the advantage of preincubation of spermatozoa. By introducing 4 g/l of bovine albumin into the culture medium, Toyoda and associates (1971) were able to fertilize mouse eggs *in vitro* and concluded that "mouse epididymal spermatozoa can be capacitated *in vitro* in a chemically defined medium without the presence of female reproductive tissue fluid." These two studies revealed clearly that capacitation and fertilization *in vitro* can be achieved without the participation of specific substances from the female reproductive tissue and biological fluids.

The importance of serum albumin and metabolic intermediates for capacitation of spermatozoa and fertilization of mouse eggs *in vitro* was further described by Miyamoto and Chang (1973). *In vitro* fertilization of rat eggs in a chemically defined medium was achieved, and the development of such eggs following transfer was described (Toyoda and Chang, 1974). The capacitation of rabbit epididymal spermatozoa *in vitro* appeared to be difficult, but finally succeeded according to a procedure of washing twice and employing a longer preincubation time of 12 hours (Hosoi et al 1981). Based upon the study of *in vitro* fertilization of hamster eggs by epididymal spermatozoa (Yanagimachi and Chang, 1963) and the induction of sperm capacitation in the presence of tubal and follicular fluid (Barros and Austin, 1967), Edwards, Bavister, and Steptoe (1969) reported the first authentic evidence of *in vitro* fertilization of human eggs. They stated that "Our impression is that this preincubation (of human spermatozoa) led to the attachment of more spermatozoa to the zona pellucida, and to a higher incidence of penetrated and pronucleate eggs." Austin and associates (1973) estimated that the time required for capacitation of human spermatozoa *in vitro* was about 7 hours, which was much longer than the present estimation. It should be pointed out here that most investigators working on fertilization *in vitro* consider capacitation to include all the changes before

penetration because they consider capacitated spermatozoa as those able to fertilize eggs, not those only having some molecular changes in their membrane.

Capacitation and Hyperactivation

It was reported by Hamner and Williams (1963) that the uptake of oxygen by rabbit spermatozoa increased four-fold after they had been incubated for 6 hours in the uterus of estrous rabbit. Mounib and Chang (1964) found that "both uptake of oxygen and glycolytic activity of sperm were increased after incubation in the uterus of rabbit and the rise of consumption of oxygen was utilized to oxidize endogenous and exogenous substrates with a promotion of the hexose monophosphate shunt." The different motility pattern of golden hamster spermatozoa before and after capacitation was first noticed (1969) and described by Yanagimachi (1970b). It was observed that when hamster spermatozoa were incubated in media containing biological fluid, the spermatozoa agglutinate head to head within 30 minutes. About 2 to 3 hours later, agglutinated spermatozoa dispersed spontaneously, and free spermatozoa showed an extraordinary active movement, with vigorous whip-lash-like beating of the flagellum. Later on, Yanagimachi (1981), named this sperm movement "hyperactivation of spermatozoa" rather than "activation of spermatozoa", and stated that "the spermatozoa began to move extremely vigorously shortly before the acrosome reaction was initiated." A similar type of sperm motility was also described in the guinea pig (Yanagimachi, 1972; Barros et al, 1973) dog (Mahi and Yanagimachi, 1976) mouse (Fraser, 1977), rabbit (Cooper et al, 1979) and sheep (Cummins, 1982). It is difficult to say from these reports whether the hyperactivation of spermatozoa occurred before or after the acrosome reaction. According to Yanagimachi (1981), the acrosome reaction and hyperactivation can occur independently and should be considered as separate phenomena. If capacitation is defined to include all the changes in spermatozoa before they are capable of penetrating eggs, then the hyperactivation of spermatozoa is also one stage of capacitation. Whether hyperactivation starts before, during, or after the acrosome reaction was not exactly determined.

Separation of Capacitation and Other Changes

In a study of morphological aspects of sperm capacitation in mammals, Bedford (1970a) stated that "observations in the phase contrast and electron microscope fail to reveal any structural changes in rabbit sperm which can be interpreted as morphological concomitant of capacitation," because he found that capacitated rabbit uterine spermatozoa had intact acrosomes. In a review paper on sperm capacitation and fertilization in mammals, Bedford (1970b) remarked that "no morphological changes occur before the onset of acrosome reaction, which is not considered as a facet of capacitation itself. After capacitation, sperm become competent to undergo the acrosome reaction in response to stimuli which seem to exist in the vicinity of the egg, and in follicular fluid." Such statements rather deviate from the original meaning of capacitation, which includes all the changes that enable spermatozoa to penetrate eggs. On the other hand, the absence of acrosome in capacitated hamster (Yanagimachi, 1966) mouse (Iwamatsu and Chang, 1969) and guinea pig spermatozoa (Yanagimachi, 1972) has been reported. If we accept Bedford's notion (1970a) that the acrosome reaction of capacitated rabbit spermatozoa *in vivo* only occurs in contact with eggs, it does not necessarily contradict the original meaning of capacitation (changes undergone by mammalian spermatozoa in the female genital tract) because the acrosome reaction of rabbit spermatozoa occurs when the sperm and eggs are in the oviducts, which are part of the female genital tract. Moreover, we cannot say that all capacitated rabbit uterine spermatozoa have intact acrosomes because the acrosome reaction of rabbit spermatozoa is difficult to examine, and the acrosome reaction of rabbit uterine spermatozoa has not been thoroughly examined as far as the author is aware.

In an article entitled "Capacitation of Golden Hamster Spermatozoa During Incubation in Culture" Bavister (1973) remarked that "since there is some controversy over the meaning of the term 'capacitation,' it is used below to denote only those changes undergone by spermatozoa after leaving the male reproductive tract and before the occurrence of acrosome reaction." But the results he presented in his article were based on the penetration of the eggs, which requires all the changes in spermatozoa before fertilization.

In an article entitled "Components of Capacitation" Austin and associates (1973) reported their study of *in vitro* sperm penetration in the golden hamster, mouse, and human. It was concluded that "capacitation is an essential physiological change in all three species studied, and in each, the spermatozoa were clearly capable of undergoing this process *in vitro*." Because preincubation of spermatozoa in medium without eggs for 3 to 4 hours presumably did not induce acrosome reaction, but produced a large reduction in the ultimate time between insemination and penetration, they further concluded that "the separate nature of capacitation and acrosome reaction is indicated by observation on preincubation of hamster spermatozoa." It should be pointed out here that "these experiments gave variable results," as the same authors stated, and that they did not determine the occurrence of acrosome reaction after preincubation. Moreover, even after preincubation for 3 to 4 or 6 to 7 hours, it still requires 1 to 2 hours after semination for the spermatozoa to penetrate the eggs. This shows that what has happened during preincubation is only a part of, or a preparation for capacitation, rather than the whole process of capacitation. The definition of capacitation applied originally did not fragment the process; it denoted all the changes in the spermatozoa that enable them to penetrate and fertilize the eggs.

Dealing with membrane fusion and fertilization, Austin (1975) further stressed the separation of capacitation and the acrosome reaction. He considered capacitation to involve the removal of the glycoprotein coat from spermatozoa. The removal of the extraneous coat unlocks the acrosome reaction, while the acrosome reaction allows the escape of hydrolytic enzymes for the penetration of the zona pellucida. Johnson (1975) discussed capacitation and acrosome reaction separately from membrane reaction and immunological reaction, based upon extant knowledge at the macromolecular level. In a recent review article by Yanagimachi (1981), capacitation and acrosome reaction were discussed separately. He listed nineteen detected or suspected phenomena in the sperm membrane associated with sperm capacitation from 56 references, but he was not certain whether to define capacitation strictly as a preparation for the acrosome reaction. The acrosome reaction, however, can be induced rapidly in guinea pig spermatozoa without preliminary incubation by causing calcium

uptake through the influence of ionophore A23187 (Singh et al, 1978). In a recent review article entitled "Significance of the Need for Capacitation Before Fertilization in Eutherian Mammals," Bedford (1983) stressed the influx of calcium during capacitation for acrosome reaction. He postulated that due to the loss of the oocytes' stimulation of the acrosome reaction, as in the sea urchin, and the unusually formidable egg investments in the vertebrate, mammalian spermatozoa must undergo capacitation in the female genital tract.

As sea urchin spermatozoa undergo the acrosome reaction without obvious capacitation, it is inconsistent to consider the acrosome reaction as an isolated event in one animal group and as an integral part of a more complex sequence of events in mammals. Whether or not the acrosome reaction of sea urchin spermatozoa may have some fast reactions similar to capacitation remains to be investigated.

The confusion created in recent years is mainly due to the fact that in the title of their articles, Austin et al (1973), Bavister (1973), and Bedford (1970a,b; 1983) imply that capacitation includes all the changes in the spermatozoa before they have the capacity to fertilize. But in the text of their articles, they have treated capacitation as a preparation for hyperactivation and acrosome reaction. The confusion is often caused by the change in the original meaning of the word.

Summary and Conclusions

It should be recalled that sperm capacitation was originally defined in 1952 as some physiological changes of the spermatozoa in the female genital tract before they are capable of penetrating and fertilizing the eggs. It was found further that capacitation can be achieved outside the female tract, first in the presence of biological fluids, and then in the absence of biological fluids. Later on it was found that capacitated rabbit uterine spermatozoa still have acrosome and that the acrosome reaction of rabbit spermatozoa occurred in contact with eggs in the oviduct. Thus, several authors separated acrosome reaction from capacitation and considered capacitation as a preparation for the acrosome reaction, even though the titles of their articles still implied that capacitation included acrosome reaction. During the past 30 years we have found many membrane changes on the molecular and immunological level in spermatozoa

that prepare them for physiological changes such as "hyperactivation," and morphological changes such as "the acrosome reaction." These events lead to more vigorous motility and to the release of various enzymes for the penetration of the egg. Undoubtedly, further study will reveal more molecular, physiological, and morphological changes in the mammalian spermatozoa before they are capable of fertilization. There are definite changes before hyperactivation and acrosome reaction, but these changes are parts of capacitation, if we prefer to keep its original meaning. It is proposed here that in order to save further confusion, capacitation of spermatozoa should be defined as originally proposed, that is, to include all the events that lead to the development of the capacity of mammalian spermatozoa to penetrate eggs. All the changes in the spermatozoa before hyperactivation and acrosome reaction should be defined as the first part of capacitation. Certainly the writers should clearly state whether or not acrosome reaction is included in their work on capacitation.

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