

FERTILIZATION

(Invited Paper)

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I. INTRODUCTION

My first comment on fertilization is that an acceptable meaning for the word should be agreed upon among those working in reproductive physiology and suitable names found for the other meanings at present attributed to the term. Austin and Walton (1960) have defined fertilization as covering the period from the formation of the gametes up to the first zygotic cleavage. However, according to Rothschild (1956) it begins with sperm-egg collision and ends with syngamy, fusion or apposition of sperm and egg nuclei. Although usually accepted as being concerned with the initiation of development of the egg as well as the transfer of hereditary material to the ovum by the spermatozoon, the word may be used to signify only one of these, the fusion of the male and female nuclei, since in parthenogenesis activation is said to take place without fertilization. In this sense the word is used to describe only one small part of the complex chain of events that is commonly understood to be included in fertilization. It is clear there is no one generally accepted meaning for the word, which makes for difficulty in communication, and I regret the necessity to use it in more than one sense in this paper.

Recently Rothschild (1962) thought we had come to a stop for the time being in determining how the spermatozoon fertilizes the egg and that the subject should be left until some clever person thought of a new way of tackling it. This may be true of the phenomenon in lower animals, such as the sea urchin, in which it has received considerable attention. Because the mammalian egg is less readily available, and *in vitro* fertilization has been difficult to achieve due to the necessity for capacitation of the sperm and for avoiding experimentally induced parthenogenesis, knowledge of fertilization of mammalian ova, which must concern a Society of Animal Production, has some distance to go to equal that in lower animals. By concentrating on mammalian fertilization I hope to avoid masquerading as Rothschild's "clever person".

II. FERTILIZATION *IN VITRO*

There have been many claims to have achieved fertilization of mammalian eggs *in vitro*, the proof often being not completely satisfactory because it relied on histological examination of developing ova. Even when living young were born following transplantation of ova apparently fertilized *in vitro*, the possibilities had not been eliminated that parthenogenetic development had occurred or that fertilization had taken place *in vivo* of transferred ova, or of the recipient's ova, by spermatozoa accompanying the transferred eggs. Although other workers had probably

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been successful, Chang (1959) appears to have been the first to demonstrate unequivocally the fertilization *in vitro* of mammalian ova by recovering capacitated sperm from the female reproductive tract, incubating sperm and ova and storing the latter until he observed apparently normal cleavage, transferring these cleaved ova to recipients in which ovulation had been artificially induced and obtaining the birth of living young. With this advance, the way appears to lie open for the further study *in vitro* of such mechanisms of mammalian fertilization as the penetration of the zona pellucida and vitellus, the activation of the egg, the block to polyspermy and the fate of spermatozoan components.

III. SPERM TRANSPORT

In addition to advancing our knowledge of the events which take place following the meeting of the sperm and egg, we require to learn more of the physiological phenomena involved in the bringing together of the gametes both in space and time.

To achieve this meeting the sperm have to traverse the male and the female reproductive tracts. For the meeting to be fruitful the ovum must be recently ovulated, so that the timing of insemination relative to ovulation is significant.

These journeys are important not only in bringing the sperm to the ovum, but also because the sperm undergo maturation and capacitation in the male and female tracts respectively, so ensuring that the sperm arrive ready to perform their function.

(a) Sperm transport in the male

Although it is of both theoretical and practical interest, the mechanism of sperm transport through the male tract is obscure and has been the subject of little experimentation. Even the recent advances in electro-ejaculation of rams and bulls have given but slight impetus to the study of the physiology of sperm transport.

It appears that the immotile spermatozoa are moved from the seminiferous tubules through the efferent ducts into the epididymis partly by the pressure from the extrusion of spermatozoa and secretion of fluid into the tubules and partly by the movement of cilia lining the ducts. Cross (1959) observed rhythmic contractions of the tunica albuginea without any change in volume of the rabbit testes, and thought it likely that these contractions would have the effect of massaging the seminiferous tubules and facilitating the passage of sperm towards the efferent ducts. In several instances, injection of Pitocin seemed to accelerate these contractions but there also appeared to be spontaneous fluctuations in the contractions, so the evidence for the effect of oxytocin was not clear.

The epididymis undergoes both pendular and peristaltic contractions. Cross (1959) made a small opening into a rabbit epididymis and observed the ejection of tiny jets of fluid approximately six times per minute. It seems clear that contractions of the epididymis contribute to sperm transport. The epididymal contractions and flow of spermatozoa are apparently continual (Ewy, Bielanski and Zapletal 1963) and about 1000 million sperm pass daily into the urine of the ram (Bielanski and Wierzbowski 1961).

Ejaculation is the result of contractions of smooth muscle in the male reproductive tract, controlled by a spinal reflex transmitted via the hypogastric plexus. From the position of the electrodes of the probe in the rectum of the ram and

bull it appears that stimulation of the hypogastric plexus gives the optimal results at electro-ejaculation.

Cross (1959) described experiments in the rabbit showing that the hypothalamus also influenced sperm transport. Stimulation of the sympathetic zone of the hypothalamus caused contraction of the tail of the epididymis and vas deferens which forced sperm from the cannulated vas deferens. Adrenaline injection produced similar results. He was unable to demonstrate any influence of the neurohypophysis or of oxytocin injection. However, there is evidence suggesting the presence of oxytocin in the blood of males. This would presumably influence the transport of sperm, especially at ejaculation. Massage of the seminal vesicles and ampullae of the ram produced a rise in pressure in the mammary gland of a cross-circulated lactating ewe (Debackere, Peeters and Tuytens 1961) and injected oxytocin greatly increased the flow of sperm from the cannulated vas deferens of the ram (Ewy, Bielanski and Zapletal 1963). The presence and concentration of oxytocin in the blood of males particularly at mating can now be determined by the specific assay technique of Tindall and Yokoyama (1962) using the lactating guinea pig.

Our knowledge of the hormonal, nervous and psychological factors affecting sperm transport in the male remains very incomplete.

(b) Sperm transport in the female reproductive tract

There has been considerable interest in determining how quickly, following insemination, sperm can reach the Fallopian tubes. In the ewe, estimates of the minimal time required, reviewed by Edgar and Asdell (1960), have ranged from six minutes to eight hours. Mattner (1963a) recently provided a possible explanation for these variable findings by showing that the speed of initial transport depended on the individual reaction of ewes to the conditions at the time of insemination. In tractable ewes the initial rate of transport was higher than in ewes unaccustomed to handling, although there was little difference in subsequent sperm passage between the two.

It does appear that undue stress has been laid on the rapidity of the arrival of the sperm in the Fallopian tubes following insemination. Sperm require a period of some hours in the female reproductive tract while they undergo capacitation, but sperm survive for a shorter period in the Fallopian tube than in the uterus or cervix. Further, it is now thought (Mattner and Braden 1963) that sperm continue through the Fallopian tubes into the peritoneal cavity. These two factors probably increase the likelihood that sperm that have undergone capacitation in the uterus rather than in the Fallopian tubes will fertilize the eggs. Thus females in which sperm transport to the Fallopian tubes is delayed a few hours may be at no disadvantage compared with those having rapid initial transport.

As sperm travel in the female tract faster than they can swim *in vitro*, and as inert material passes from the vagina to the Fallopian tubes, it appears that movements of the female tract are largely responsible for sperm transport, particularly immediately following mating. Akester and Inkster (1961) have shown that radio-opaque material passes from the cervix in small "puffs" into the uterus of the rabbit and this mechanism may be responsible for the controlled release of sperm. Apparently, oestrogen in the blood at oestrus causes uterine movements which are augmented perhaps by the output of oxytocin under the stimulus of mating, al-

though this action can apparently be inhibited by stress at that time. Cross (1959) considered there were three possible mechanisms of uterine activation during mating, spinal reflex initiated by vaginal stimulation, hypothalamic influences mediated by sympathetic outflow, or neurohypophyseal secretion. As mentioned in connection with movements of the male tract, the new-found ability to assay oxytocin in blood should shed some light on this aspect. The degree of artificiality in experimentally recording uterine movements should be reduced by using a tiny remote passive transducer implanted in the uterine wall rather than balloons inserted in the lumen which may themselves stimulate the output of oxytocin or reflexly induce activity.

The passage of immotile sperm and inert material through the tract has emphasized the part played by movements of the female reproductive organs in sperm transport and led to the suggestion that sperm motility plays little or no part. As mentioned earlier, this is probably true during the short period of strong uterine contractions which follows mating, but studies of the distribution of sperm in the female reproductive tract have shown that sperm are not spread equally throughout, but that the cervix and uterotubal junction appear to form barriers to their progress (Austin and Braden 1952).

By injecting sperm into one horn of the uterus of ewes at laparotomy, Mattner (1963b) was able to show that, in the absence of stimulation by natural or artificial vaginal insemination, live and dead sperm were distributed equally through both horns of the uterus, but that motile sperm passed more readily than dead sperm through the cervix and uterotubal junction. One would like to see this experiment repeated using motile and immotile live sperm, but present knowledge does suggest that contractions of the female tract play a major part in sperm transport through the uterus and Fallopian tubes, while sperm motility is important in the passage through the cervix and uterotubal junctions. The combination of all these factors ensures over a period of some hours the continual arrival at the site of fertilization of an adequate but not superfluous number of capacitated sperm. Some of these deteriorate in the Fallopian tubes, others pass into the peritoneal cavity, but one capacitated sperm, in most cases, collides by chance with an ovum and initiates the block to polyspermy before collision with another capacitated sperm occurs. As far as we know the meeting of an ovum with an uncapacitated sperm is of no consequence.

It is now unclear what benefit results from the rapid post-mating sperm transport which, at first sight, had seemed important.

IV. THE TIMING OF INSEMINATION RELATIVE TO OVULATION

It is well known that in species such as the rabbit, ovulation is induced by, and follows at a fairly constant time after, coitus. The interval allows capacitated sperm to be reaching the Fallopian tubes at the same time as recently shed ova. In spontaneously ovulating species, the mechanism appears less efficient. Ovulation in these usually occurs at about the end of oestrus, and chance apparently governs the stage in oestrus when insemination occurs and in consequence its relation to the optimum time for achieving conception. The work of Trimberger and Davis (1943) and others suggested that this was true for artificially inseminated dairy cows and that cows inseminated early in oestrus had a lower conception rate than those inseminated later. Subsequently, Olds and Seath (1954) obtained data which

indicated that considerable latitude in time of mating is permissible, and in results obtained by officers of the New Zealand Dairy Board from many thousands of inseminations, the expected differences in conception rate between cows apparently inseminated too early and those inseminated later in oestrus did not occur. This led Shannon and Bishop (personal communication) to examine the phenomenon experimentally and to obtain evidence that the handling of the genitalia at artificial insemination during oestrus advanced the time of ovulation. Variations in the acceptability to the cow of ways of handling the genitalia may account for otherwise unexplained differences in conception rates obtained by different inseminating technicians.

It is not known, but it should surely be a matter for study, if natural mating also affects the time of ovulation. Marion *et al.* (1950) found that sterile copulation advanced ovulation in dairy heifers, and such a modifying factor may improve the time relationship between insemination and ovulation, where this departs from the optimum in animals otherwise ovulating spontaneously. Conception rates should be improved if all cows can be induced to ovulate within 24 hours of insemination.

V. EFFECT OF RAMS ON ONSET OF BREEDING SEASON

Another example of the modification of a physiological timing phenomenon, falling within the wide definition of fertilization, is seen in the influence of the presence of the ram on the onset of the breeding season in ewes. The latter are stimulated to breed in the autumn by the changing ratio of daylight to darkness, but the exact date of onset is influenced by the time of joining the ram. Australian workers have shown that when Merino ewes are approaching their breeding season but before they experience the ovulation without heat (silent heat) which often precedes their first oestrus, many of the ewes are stimulated by the introduction of the ram to ovulate in about five days and come into heat about 17 days later; that is about 22 days after joining the ram.

It is only recently that this situation has been found to apply to Romney ewes in New Zealand and it presumably also occurs in other breeds. Wallace (1951) noted that a high proportion of ewes commenced their breeding season during the course of four or five days but that this peak of tupping was independent of, and occurred fairly soon after, the introduction of rams. It has since been shown (Edgar and Bilkey 1963) that the peak of tupping in Romney ewes in New Zealand, as in Merinos in Australia, falls about three weeks after rams are joined. We now know that the teaser rams, which Wallace had put out before the rams, had influenced the occurrence of first oestrus in the ewes. One puzzling feature has been the finding that vasectomised teaser rams are less effective than fertile rams and sometimes have no influence on the ewes. Previously accepted information on the onset of the breeding season in ewes is suspect as we now know that the ram who detected the first oestrus himself influenced its time of occurrence.

Some method of detecting oestrus other than acceptance of service would be needed to follow the onset of the breeding season in ewes uninfluenced by the presence of rams.

Watson and Radford (1960) have commenced the elucidation of the way in which rams influence ewes and appear to have eliminated sight and contact, but further clarification of this and the difference between teasers and rams would

make an interesting study. The possibility has been suggested that if sexual odour is the factor involved, the farmer armed with an aerosol bomb of distillate might initiate breeding in his flock more or less at will.

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VII. REFERENCES

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