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# THE INFLUENCE OF OESTROGEN AND PROGESTERONE ON SPERM CAPACITATION IN THE REPRODUCTIVE TRACT OF THE FEMALE RABBIT

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SUMMARY

Using the techniques of sperm transfer into the oviduct of recipient females, or ovum transfer to the site of capacitation, it has been shown that the capacitating activity of the female genital tract may be modified by oestrogen and by progesterone. The normal capacitating potential of the Fallopian tube is diminished after ovariectomy, and is restored by oestrogen injection. In the absence of oestrogen (in the ovariectomized and adrenalectomized female) this activity is still maintained at a basal level sufficient to capacitate the numbers of sperm which normally enter the Fallopian tube after coitus. Capacitation is not significantly disturbed in the Fallopian tube of the intact progesterone-dominated female (Chang, 1958), and the innate basal capacitating activity persists in the Fallopian tubes of ovariectomized females treated with progesterone.

The ability of the uterus to bring about complete capacitation is lost in the progesterone-dominated female. Sperm are completely capacitated in about 11 hr. in the oestrous uterus, but only partial capacitation occurred during this period in the uterus of the ovariectomized rabbit. As judged by the time of penetration of eggs placed into the uterus at different times after insemination, sperm in the ovariectomized uterus did not acquire the capacity to fertilize for approximately 20 hr. Injection of up to 1000 i.u. human chorionic gonadotrophin at the time of insemination did not result in significant depression of capacitation in the uterus.

It is concluded that the rabbit oviduct has a greater innate potential for capacitation than the uterus, though this potential is increased in both sites by oestrogen stimulation. In contrast to its depressive effect on capacitation in the uterus, progesterone apparently does not significantly affect capacitation in the oviduct, even in the absence of the ovaries. This implies that modification of the endocrine status of the female rabbit would not produce a total contraceptive block of capacitation *per se*.

* Present address.

## INTRODUCTION

There is evidence in several species of mammals that spermatozoa passing through the female tract undergo a functional change which confers upon them the capacity to penetrate the egg. Since the discovery of this phenomenon by Austin (1951) and by Chang (1951), several attempts have been made to clarify the nature of the factors responsible for sperm capacitation. In the hamster, substances present in the ovarian follicle bring about functional capacitation (Barros & Austin, 1967) but the requirement for capacitation in the rabbit appears to be somewhat more rigorous. Some initial phase of capacitation of rabbit sperm can be achieved in several different situations (Noyes, Walton & Adams, 1958; Bedford & Shalkovsky, 1966; Hamner & Sojka, 1966; Bedford, 1967) but complete or functional capacitation seems to require factors present in the rabbit uterus or oviduct (Bedford, 1967). Since the secretory activity of these organs is regulated by the ovary, it seemed pertinent to find out whether the capacitating activity of the uterus and oviduct is innate, or whether it depends on stimulation by ovarian steroids. Previous evidence is conflicting. There is little doubt that progesterone depresses the capacitating activity of the uterus (Chang, 1958; Soupart, 1967; Bedford, 1967; Hamner, Jones & Sojka, 1968), though carefully timed inseminations have shown that some initial phase of capacitation may still be achieved in the progesterone-dominated uterus (Bedford, 1967). Chang (1958) has concluded that, 'capacitation of sperm in the uterus or Fallopian tube is independent of gonadotropic or oestrogenic stimulation'. Notwithstanding this, it has been claimed more recently that capacitation in the rabbit uterus can be influenced by either human chorionic gonadotrophin (HCG) or luteinizing hormone (LH) injected intravenously at the time of insemination (Soupart, 1966, 1967). Furthermore, other investigators have reported only a very poor degree of capacitation in the uterus of the ovariectomized rabbit (Soupart, 1967; Hamner *et al.* 1968). According to Soupart, the capacitating potential of the uterus may be restored by oestrogen, whereas Hamner *et al.* found no such improvement after oestrogen administration and concluded that oestrogen alone cannot maintain optimal conditions for capacitation in the rabbit. Later, however, Hamner & Sojka (1968) obtained an 86% fertilization rate among ova exposed *in vivo* to rabbit sperm capacitated in the uterus of ovariectomized oestrogen-treated does.

With regard to the Fallopian tube, Noyes *et al.* (1958) obtained fertilization in the tube of ovariectomized rabbits, and Chang (1958) reported a satisfactory fertilization rate after tubal insemination of epididymal sperm in progesterone-dominated females some 12 hr. before HCG-induced ovulation. This last result indicates that capacitation activity is maintained in the oviduct under conditions of progesterone-domination which serve to inhibit similar activity in the uterus. However, Hamner *et al.* (1968) obtained no fertilization when capacitated sperm were introduced soon after ovulation into the progesterone-dominated Fallopian tube, and have interpreted this to mean that the latter secretes some factor(s) which inhibit or reverse the capacitated state; this would imply also that sperm cannot be capacitated successfully in the progesterone-dominated tubal environment.

The present report describes experiments performed to investigate the importance

of hormones in the regulation of capacitation activity in the uterus and Fallopian tube of the rabbit. Part of this work has been discussed previously (Bedford, 1968).

## MATERIALS AND METHODS

The first series of experiments (Table 1) was performed to study the effect of oestrogen and progesterone on capacitation in the Fallopian tube. Adult New Zealand White rabbits were ovariectomized unilaterally or bilaterally by way of a flank incision, and were allowed to recover for at least 3 weeks before use. In four out of 11 animals which had been ovariectomized 1–7 months previously, both adrenal glands were removed successfully. These animals were used 12–14 days after adrenalectomy, the completeness of which was checked on termination of the experiment.

Epididymal sperm, suspended in 0·025 ml. Ringer solution containing 10% serum at 37°, were inseminated via a flank incision into the tubal ampulla, to a depth of 3–4 cm. In the first three groups (Table 1), relatively large numbers (more than 10 million sperm) were inseminated, but in later experiments (groups 4–7 Table 1) a more dilute suspension containing only 0·1–0·4 million sperm in 0·025 ml was used. Twelve to 18 hr. after tubal insemination of the capacitators, fresh ova were recovered from donor females, 12 hr. after injection of 50 i.u. HCG, and were carefully instilled with a fire-polished pipette into the tubal ampulla. These ova were recovered by flushing the oviduct some 9–12 hr. later, and were examined for evidence of fertilization after fixation and staining with lacmoid. Fertilization in the one-celled eggs was judged by the presence of the male and female pronucleus, and the first and second polar bodies; the occurrence in some eggs of one or more supplementary sperm in the peri-vitelline space of the fresh ovum, and detection of the fertilizing sperm tail in the ooplasm of many of the fixed ova provided additional evidence of fertilization. In one or two cases the eggs had cleaved and, in several others, the metaphase chromosomes of the first mitosis were present in the centre of the fertilized ovum. These procedures were repeated in groups of ovariectomized females which had received injections of oestradiol cypionate (Upjohn) (group 3, Table 1) or progesterone (groups 6 and 7, Table 1), for several days.

The second series of experiments was undertaken to investigate the influence of ovarian steroids on sperm capacitation in the rabbit uterus. These were performed using two methods of approach, involving either the recovery of sperm from the uterus and their insemination into the oviducts of recipient does at various times after ovulation, or, conversely, the instillation of ova into the uterine capacitation site at various times after insemination. Using the first method, 2–4 million sperm in 0·2 ml. Hanks's solution were injected through the uterine wall into each horn in ovariectomized females. The uterine sperm were recovered approximately 12 hr. later by flushing the uterine horns with 3–4 ml. Ringer solution + 10% heated rabbit serum. After concentration of these sperm by centrifugation at 1000 rev./min. for 2–3 min., aliquots were inseminated in 0·025 ml. into one Fallopian tube of a normal animal which had received an ovulation injection either 12 or 15 hr. previously. Uterine sperm samples recovered similarly from intact females about 12 hr. after natural mating, were instilled as controls into the contra-lateral Fallopian tube of each recipient. About 10 hr. later the ova were recovered and were examined for fertilization and for the presence of sperm.

Rabbit eggs can be fertilized normally in the oestrous uterus (Chang, 1955; Bedford, 1969). In the second method of approach, fresh ova in cumulus were injected through the cervix into one uterine horn of ovariectomized animals either 12, 15, 17½ or 20–24 hr. after direct uterine insemination of 5–10 million epididymal spermatozoa in 0·2 ml. Ringer solution. A ligature was placed around the cervix to prevent loss of the ova. The uterine ova were recovered 7–8 hr. later. To collect these ova, 1–2 ml. Ringer solution was injected through the wall near the uterotubal junction to produce a slight distension of the uterine lumen; the cervical ligature was then cut and the uterine contents allowed to flow on to a watch glass. The ova were examined for fertilization, and particularly for the stage of development of the male and female pronuclei as an approximate indication of the time of sperm penetration.

Some experiments were also carried out to test the effect of HCG on capacitation in the uterus of the normal female. Oestrous females were either mated, or 0·2–0·5 ml. semen, containing 150–180 million freshly ejaculated spermatozoa, was inseminated into the anterior vagina. The animals were then immediately injected with either 500 or 1000 i.u. HCG, i.v. Sperm were flushed from their uteri 11–12 hr. later, and were inseminated tubally into recipients 12½–13½ hr. after an ovulation-inducing injection of HCG. The tubal eggs were collected after 10–12 hr. and were examined for fertilization. In an alternative approach, two females were injected intravenously with 1000 i.u. HCG at the time of mating, and fresh ova were placed in their uteri 9 hr. later. The ova were recovered by flushing the uterus after 6–7 hr. and were examined for fertilization.

## RESULTS

Fertilization failed to occur when ova were placed into the Fallopian tubes of ovariectomized females either 12 or 18 hr. after tubal insemination of more than 10 million sperm in 0·025 ml. (group 1, Table 1); in these cases motile and non-motile sperm were adherent to the zona surface of most of the ova recovered 10 hr. later. By contrast with these results in oestrogen-deficient females, most eggs were fertilized and supplementary sperm were often present when the same procedure was performed using the Fallopian tube on the operated side of unilaterally ovariectomized animals (group 2). Similarly, a very high fertilization rate was obtained in bilaterally ovariectomized rabbits which had been treated with oestradiol for 5 days (group 3).

The experiments on totally ovariectomized untreated females were repeated, but with only 0·1–0·4 million sperm being inseminated. Eighty-two per cent of 17 ova were fertilized normally when introduced 12 hr. after tubal insemination (group 4). Fertilization was also obtained in the oviducts of three out of four ovariectomized animals which had been adrenalectomized 12–14 days previously (group 5). Administration of progesterone for 7 (group 6) or 10 (group 7) days in doses of approximately ten times the amount required to maintain pregnancy in the rabbit (Courrier & Kehl, 1938), did not depress the innate basal capacitating activity of the Fallopian tube in the ovariectomized female, since fertilization was obtained in all the experimental females. Furthermore, contrary to the findings of Hamner *et al.* (1968), 13 out of 18 ova were fertilized in two experiments in which uterine sperm, flushed from oestrous donors 12 hr. after natural mating, were inseminated tubally into 9-day

pseudopregnant females, approximately 13½ hr. after an ovulation-inducing injection of 50 i.u. HCG.

The results of experiments concerned with capacitation in the uterus are shown in Tables 2 and 3. Sperm recovered from the uterus of ovariectomized females 12 hr. after insemination fertilized a significant proportion of eggs when transferred into the

Table 1. *Influence of oestrogen and progesterone on capacitation in the rabbit Fallopian tube*

Sperm in group 1 were incubated for 12–18 hr. in capacitating tube before transfer; in all other groups sperm were resident for about 12 hr. in capacitating female.

| Group no. | Treatment of capacitating female | No. of expts | Ova recovered | % of ova fertilized |
|---|---|---|---|---|
| 1 | *Bilateral ovariectomy | 11 | 53 | 0 |
| 2 | *Unilateral ovariectomy | 3 | 30 | 90 |
| 3 | *Bilateral ovariectomy and 50 μg. oestradiol cypionate for 5 days | 4 | 20 | 100 |
| 4 | †Bilateral ovariectomy | 4 | 17 | 82 |
| 5 | †Bilateral ovariectomy and adrenalectomy 12–14 days previously | 4 | 9 | 44 |
| 6 | †Bilateral ovariectomy and progesterone s.c., 25 mg. for 4 days, 50 mg. for 3 days | 4 | 18 | 66 |
| 7 | †Bilateral ovariectomy and progesterone s.c., 25 mg. for 7 days, 50 mg. for 3 days | 2 | 12 | 66 |

* More than 10 million sperm inseminated into the tube in 0·025 ml. Ringer solution containing 10 % serum.

† 0·1–0·4 million sperm inseminated into the tube in 0·025 ml. Ringer solution containing 10 % serum.

Table 2. *Comparison of the fertilizing ability of rabbit spermatozoa recovered 12 hr. after insemination from ovariectomized animals and the oestrous uterus of intact animals and assayed by tubal insemination into recipients at different times after injection of human chorionic gonadotrophin (HCG)*

| | Tubal insemination (12 hr. after HCG) | | Tubal insemination (15 hr. after HCG) | |
|---|---|---|---|---|
| Group no. | Sperm from ovariectomized uterus | Sperm from oestrous uterus | Sperm from ovariectomized uterus | Sperm from oestrous uterus |
| 1 | 3/9* | 2/2 | 0/4 | 8/8 |
| 2 | 5/7 | 3/4 | 0/4 | 1/2 |
| 3 | 2/8 | 2/2 | 0/4 | 1/6 |
| 4 | 0/6 | 4/6 | 0/3 | 1/3 |
| 5 | 4/9 | 3/3 | 0/6 | 3/3 |
| Total | 14/39 | 14/17 | 0/21 | 14/22 |

* Number of recovered eggs fertilized.

oviducts of recipients 12 hr. after injection of HCG (i.e. about 2 hr. after ovulation) (Table 2). However, these sperm could not fertilize when tubal insemination was delayed until about 5 hr. after ovulation (15 hr. after HCG), though uterine sperm collected from oestrous females 12 hr. after coitus fertilized 63% of eggs when inseminated coincidentally into the contra-lateral (control) tube.

Table 3. *Time required for completion of capacitation in the uterus of ovariectomized rabbits: judged by the ability of uterine sperm to fertilize rabbit eggs placed in the uterus at different times after insemination*

| No. of ovariectomized females in each expt | Time of ovum instillation into the uterus (hr. after insemination) | No. of recovered ova fertilized* | Comments |
|---|---|---|---|
| 3† | 12 | 13/16 | Mature pronuclei in apposition |
| 3 | 12 | 0/12 | Moving sperm on egg surface |
| 4 | 15 | 0/16 | Moving sperm on egg surface |
| 2 | 17½ | 2/22 | Fertilizing sperm head showed early swelling phase |
| 2 | 20 | 2/6 | No moving sperm round eggs |
| 2 | 24 | 4/7 | Pronuclei well formed in fertilized eggs |

* Eggs recovered from uterus after 7 hr.
† Control experiments in intact females.

The results of egg transplantation to the uterine capacitation site are given in Table 3. Of the eggs injected into the oestrous (control) uterus 12–13 hr. after uterine insemination, 13 out of 16 were fertilized when collected 6–7 hr. later, and these had fully developed pronuclei which in most eggs had come into apposition in the centre of the vitellus. By contrast, no eggs were fertilized when placed into the uterus of ovariectomized females 12–13 hr. or 15 hr. after insemination, though, on recovery, they showed spermatozoa associated with the surrounding granulosa cells or attached to the naked zona surface. When introduced into the uterus at 17½ hr. after insemination, only two of the recovered eggs (8%) were fertilized which, after 7 hr. in the uterus, showed the fertilizing sperm head in the early swelling phase. A rather higher percentage of the eggs were penetrated when instilled 20–24 hr. after uterine insemination; eggs fertilized in these groups showed fully-formed pronuclei in central apposition, but there were very few or no sperm adherent to the egg surface and no peri-vitelline sperm were observed.

There was an obvious difference in the reaction of ova in the uterus of the oestrous and ovariectomized females respectively. Several of the ova recovered after 6–7 hr. from the ovariectomized uterus were still surrounded by cumulus cells, and in hardly any had the coronal cells become detached from the surface of the zona. By contrast, in the oestrous uterus the granulosa cells were almost always lost from around the ova within 6 hr., and a distinct thinning of the zona and deterioration of

the ooplasm was observed if eggs were left for more than about 7 hr. This change in the consistency of the zona made it more difficult to anchor the uterine egg between coverglass and slide during fixation and staining.

There was no indication that administration of 500–1000 i.u. HCG caused a depression of sperm capacitation in the uterus (Table 4). A high percentage of eggs were fertilized in recipients inseminated tubally with sperm from these uteri $12\frac{1}{2}$ or $13\frac{1}{2}$ hr. after an ovulation injection. Furthermore, nine of the ova placed 9 hr. after insemination into the uteri of HCG-treated females were recovered after 6–7 hr.; five of these had been fertilized and showed early stages of the formation of male and female pronuclei.

Table 4. *Effect of human chorionic gonadotrophin (HCG) on capacitation in the uterus of the normal rabbit**

| Units of HCG† given to capacitating female | No. of recipients | Time of tubal insemination (hr. after HCG) | Eggs fertilized |
|---|---|---|---|
| 500 | 2 | $12\frac{1}{2}$ | 8/14 |
| 500 | 2 | $13\frac{1}{2}$ | 10/14 |
| 1000 | 4 | $13\frac{1}{2}$ | 21/25 |

* Sperm recovered from uterus 11–12 hr. after mating or vaginal insemination.
† Injected i.v. at time of insemination.

## DISCUSSION

In the rabbit, the uterus has been regarded as the most favourable site for capacitation, the impression being that this change in sperm can be accomplished in the uterus in about 6 hr. (Chang, 1955), and thus more rapidly than the 10–11 hr. required for capacitation in the Fallopian tube (Adams & Chang, 1962*b*). More recent evidence obtained by studying the time after insemination at which eggs placed in the uterus can first be penetrated indicates, however, that sperm exposed to the uterus alone do not reach the fully capacitated state before 10–11 hr. Furthermore, this interval is prolonged to about 15 hr. when the utero-tubal junction is blocked, suggesting that some tubal influence hastens the time of appearance of capacitated sperm in the uterus (Bedford, 1969). Only when rabbit sperm are exposed to both uterus and oviduct can capacitation be completed in 6 hr. (Adams & Chang, 1962*b*).

The inference that the Fallopian tube is of primary importance for capacitation is strengthened in the present study. Apparently there is a reduction in oviducal capacitating activity in the absence of some source of oestrogen, such that the oestrogen-deficient tube can only capacitate moderate numbers of sperm (Table 1). Nevertheless, the innate basal level which persists in the tube of ovariectomized and adrenalectomized females is still sufficient to achieve capacitation, within 12–13 hr., of greater numbers of sperm than normally reach this site (Braden, 1953). Hamner *et al.* (1968) have concluded that the fertilizing ability of capacitated sperm is inhibited in the Fallopian tube of pseudopregnant females. Repetition of their experiments (J. M. Bedford unpublished observations) and the results in groups 6 and 7 (Table 1) contradict this conclusion and show that capacitated sperm can fertilize after insemination into a progesterone-dominated oviduct, where capacita-

tion may still occur in the absence of oestrogenic stimulation. Thus, the rabbit oviduct has an innate ability for capacitation which can be enhanced by oestrogen, but which cannot be depressed significantly by progesterone, even in the absence of both ovaries.

Both oestrogen and progesterone seem to influence capacitating activity in the uterus to a greater degree than in the oviduct. It is noteworthy, moreover, that a state of progesterone dominance causes a greater depression of uterine capacitating activity than does insufficiency of oestrogen. Complete capacitation cannot occur in the progesterone-dominated uterus, and sperm exposed to this environment for up to 24 hr. fertilize a significant proportion of eggs only when tubal insemination is made as early as 9 hr. after HCG (Bedford, 1967). By contrast, sperm present for only 12 hr. in the oestrogen-deficient uterus will fertilize if inseminated tubally $11\frac{1}{2}$–12 hr. after HCG (Chang, 1958; this study, Table 2). Nevertheless, it is probable that the rabbit sperm are only partially capacitated after 12 hr. in the oestrogen-deficient uterus, since they cannot fertilize when inseminated tubally later (i.e. more than 13 hr. after HCG) in the fertile life-span of the egg (Soupart, 1967; this study, Table 2), and are not yet competent to penetrate eggs in the uterus (Table 3). This draws attention again to the importance of the relationship between the time of tubal insemination and the post-ovulatory age of the ovum in the assessment of capacitation *in vivo* (Bedford, 1967). Differences in the times of tubal insemination in relation to ovulation seem likely to underlie the apparent conflict between the results of Chang (1958), on the one hand, and those of Soupart (1967) and Hamner *et al.* (1968) on the other, with respect to the role of oestrogen in capacitation. Functionally capacitated sperm ultimately appear in the oestrogen-deficient uterus, but not until about 20 hr. after insemination, as judged by the time of penetration of eggs in this environment (Table 3). In a biological or temporal sense, therefore, the oestrogen-deficient uterus must be considered inadequate for capacitation, since the interval between insemination at coitus and termination of the fertile life of the ovum is only about 18 hr. (Hammond, 1934; Chang, 1952; Adams & Chang, 1962*a*). The results of Soupart (1967) indicate that oestrogen is the important variable factor in the maintenance of the normal capacitating potential of the uterus, and there is no evidence to support the suggestion of Hamner *et al.* (1968) that an additional factor is required. Indeed, these latter workers (Hamner & Sojka, 1968) later reported an 86% fertilization rate after exposure *in vivo* of rabbit ova to sperm previously resident in the uterus of ovariectomized oestrogen-treated does.

Inhibition of capacitation is an obvious approach to the question of contraception and this might be accomplished by alteration of the hormonal environment. It has been claimed that the injection of 400 i.u. HCG, or 400 $\mu$g. luteinizing hormone (NIH-LH-S11, ovine) at the time of insemination inhibits capacitation in the uterus though not in the Fallopian tube (Soupart, 1966, 1967). By contrast, Wetteman & Hafs (1970) failed to find any depression of capacitation after i.v. administration of up to 2 mg. LH. However, they did find a significant depression of uterine capacitation after the injection of high doses of HCG. In apparent contradiction to these findings there was no evidence in the present study that HCG inhibits capacitation in the uterus; the i.v. injection of 500 or 1000 i.u. HCG at the time of mating or artificial insemination into the vagina did not depress capacitation in the uterus as judged by

the assay involving sperm transfer from the uterus to a recipient oviduct containing fresh ova, or by egg transfer to the uterine capacitation site. Sperm recovered from the uteri of females which had received 500 or 1000 i.u. HCG fertilized a high proportion of eggs when inseminated into the oviducts of recipients given an ovulating injection $12\frac{1}{2}$ or $13\frac{1}{2}$ hr. previously (Table 4). Likewise, when ova were introduced into the uterus 9 hr. after insemination, with coincidental i.v. injection of 500 or 1000 i.u. HCG, approximately 50 % of these recovered 6 hr. later had been fertilized; this last result is comparable to that obtained in the normal mated female (Bedford, 1969). The reason for the difference between these results and those of Soupart (1966) and Wetteman & Hafs (1970), is not clear; it may be relevant that in the present instance insemination was always vaginal, whereas, in the reports quoted, sperm were instilled in relatively large numbers directly into the uterine lumen.

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