BRIEF COMMUNICATION

SURVIVAL OF CAPACITATED SPERMATOZOA IN
THE OVIDUCT OF THE RABBIT

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Spermatozoa attain the ability to fertilize an ovum by incubation in the reproductive tract for a given period of time (capacitation). Recently Bedford & Shalkovsky (1967) and Bedford (1967) have indicated that the final stages of capacitation may be species-specific, normally occur in the oviduct and require from 0.5 to 2.5 hr in the rabbit. Capacitation enables the spermatozoon to penetrate the cumulus oophorus, corona radiata and to initiate fertilization. The work of Dukelow, Chernoff & Williams (1967) suggested that the final stages of capacitation may involve an ovum-sperm interaction occurring on the surface of, or within, the zona pellucida. These workers have suggested that the oviducal environment was detrimental to the fertility of uterine capacitated spermatozoa resident in the oviduct for several hours before ovulation. The objectives of the present study were to determine (1) if the oviduct environment was in fact detrimental to capacitated spermatozoa and (2) whether ligation of the tubo-uterine junction would affect the results by preventing the possible migration of spermatozoa between the uterus and oviduct.

New Zealand White virgin does, 6.5 to 7 months old, were mated three to four times to fertile males. Capacitated spermatozoa were recovered 8 or 12 hr later from the uteri by injection and aspiration of 3 ml of Krebs Ringer phosphate containing 0.25% glucose and 5% heated rabbit serum (KRP-GS). The spermatozoa were washed, counted, centrifuged and resuspended to a concentration of 10^6 spermatozoa/ml. Recipient does received an intravenous injection of 50 i.u. of HCG to induce ovulation. Capacitated spermatozoa (2.5×10^4 in 0.05 ml KRP-GS) were deposited to a depth of 1 cm in the infundibular end of the oviduct either 6 or 10 hr after HCG injection. This was approximately 4 hr before ovulation (-4 hr) and at the time of ovulation (0 hr) respectively. In the ligated series a single ligature was placed on the tubal side of the left tubo-uterine junction in eleven does.

The results of these experiments are shown in Tables 1 and 2. Spermatozoa residing in the uterus for 8 or 12 hr were capable of fertilizing a high percentage of the ova when inseminated 10 hr after HCG injection. When 12-hr uterine spermatozoa were inseminated 6 hr after HCG injection, only 4.5% of the ova

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were fertilized. Spermatozoa residing in the uterus for 8 hr and inseminated 6 hr after HCG fertilized a higher percentage of the ova, but the fertilization level was significantly lower than that observed with 12-hr uterine spermatozoa inseminated at the time of ovulation.

Ligation of the tubo-uterine junction had no effect on the proportion of eggs fertilized when 8-hr uterine spermatozoa were deposited 6 hr after the injection of HCG.

TABLE 1
EFFECT OF UTERINE INCUBATION TIME ON FERTILIZATION BY
CAPACITATED SPERMATOZOA INTRODUCED INTO THE FALLOPIAN
TUBE 4 hr BEFORE, OR AT THE TIME OF OVULATION

<i>Uterine incubation (hr)</i>	<i>Time of insemin. after HCG (hr)</i>	<i>Fertilized ova / Total ova</i>	<i>% Fertile</i>	<i>No. of oviducts</i>
12	10	59/73	80.9	22
8	10	22/24	91.6	11
12	6	1/22	4.5	6
8	6	9/22	40.9	8

TABLE 2
THE EFFECT OF LIGATION OF THE TUBO-
UTERINE JUNCTION ON FERTILIZATION BY
PRE-CAPACITATED SPERMATOZOA*

<i>Ligation</i>	<i>Fertilized ova / Total ova</i>	<i>% Fertile</i>	<i>No. of oviducts</i>
—	24/57	42.1	13
+	14/28	50.0	11

* 8-hr uterine spermatozoa inseminated 6 hr after HCG injection (4 hr before expected ovulation).

In one additional experiment a doe was anaesthetized 8 hr after mating and spermatozoa were aspirated from the left uterine horn. These, after washing, counting and concentration to 10^6 spermatozoa/ml, were inseminated into the left oviducts of three recipients which had received 50 i.u. of HCG 6 hr before. At this time, both the left and right utero-tubal junctions were ligated in the recipients. Four hours later the mated doe was killed, the spermatozoa recovered from the right uterine horn and, after washing, were inseminated into the right oviducts of the recipient does.

When 12-hr old uterine spermatozoa were inseminated into the rabbit oviduct 4 hr before ovulation (i.e. the spermatozoa were at least 16 hr old at fertilization) a fertility level of 4.5% was observed. In contrast, insemination of spermatozoa incubated in the uterus for 16 hr resulted in a fertility level of 77% (Chang, 1955). A similar observation was made when 8-hr uterine spermatozoa were inseminated 4 hr before ovulation (i.e. the spermatozoa were at

least 12 hr old at fertilization) when 41% of the ova were fertilized compared with 81% of ova exposed to spermatozoa which had resided the entire 12-hr period in the uterus. This confirms our earlier observation that a detrimental effect is exerted upon capacitated spermatozoa in the oviduct.

Freshly ejaculated rabbit spermatozoa survive for considerably longer periods of time in the oviduct since 9.5 to 10 hr are required for capacitation in the oviduct (Adams & Chang, 1962; Dukelow *et al.*, 1967). Previous workers have suggested the existence of a coating on the sperm head surface which is removed at the time of capacitation (Austin, 1951; Weinman & Williams, 1964; Dukelow, Chernoff & Williams, 1966a, b). Removal of this coating would increase membrane permeability, and indeed such an increase has been observed in capacitated spermatozoa (C. Norman, personal communication, 1965). This increased permeability would increase the metabolic rate of the spermatozoa (Hamner & Williams, 1963), hence decreasing their survival time.

Since ligation at the utero-tubal junction did not interfere with the level of fertilization, migration of the capacitated spermatozoa between the oviduct and uterus on the control side either did not occur, or, if it did, it would appear that these spermatozoa were incapable of fertilization due to exhaustion of their respiratory and glycolytic metabolic pathways. With ejaculated spermatozoa such migration does occur (Dukelow *et al.*, 1967).

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Authors' note added in page proof, 11th September 1967

Recently Dr Pierre Soupart of Vanderbilt University has demonstrated, by different techniques from those discussed in the present paper, that the acquisition of the ability to penetrate the zona pellucida (capacitation) is associated with a shortening of the life-span and fertilizing ability of rabbit sperm. (SOUPART, P. (1967) Studies on the hormonal control of rabbit sperm capacitation. *J. Reprod. Fert. Suppl.* **2**, 49.)