EFFECT OF PROGESTERONE ON FERTILIZATION IN THE GOLDEN HAMSTER

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The effect of post-ovulatory ageing of mammalian eggs before mating or insemination has been extensively studied, and the variable though relatively short fertilizable life of the egg has been documented for a considerable number of species (for review see Blandau, 1961). It is not clear, however, to what extent the fertilizable life is an intrinsic property of the egg or is under the influence of the conditions prevailing in the Fallopian tubes. Nor is it clear whether the abnormalities associated with ageing eggs, such as polyspermy, spontaneous activation, fragmentation and loss of fertilizability, are to some degree determined by the nature of the tubal secretions.

In the short interval during which post-ovulatory ageing occurs, the secretions of the Fallopian tube are subjected to the influence of ovarian progesterone rather than to that of oestrogen which predominated at the normal time of mating and ovulation. Two recent experiments in the pig have demonstrated that injection of progesterone shortly before the time of ovulation (Polge & Day, 1967) or induction of ovulation in the presence of active corpora lutea (Hunter, 1967) may frequently result in a high incidence of polyspermy, which is a well-known sequel to delayed insemination in this species (Hancock, 1959; Thibault, 1959). At least in the pig, therefore, one anomaly associated with fertilization of ageing eggs may be influenced by ovarian hormones. The present work examined the influence of injected progesterone on fertilization in the golden hamster (*Mesocricetus auratus*).

Mature hamsters, weighing 100 to 150 g, were checked through two or more consecutive 4-day oestrous cycles, and the day of the cycle on which the thick vaginal discharge occurred was designated Day 1, Day 4 being the day of pro-oestrus. A single subcutaneous injection of progesterone in 0.25 to 0.5 ml cottonseed oil was given to individual animals at various times relative to the onset of oestrus and ovulation (see Table 1). Control animals received 0.25 ml oil at a time appropriate for that treatment. Females were tested for oestrus between 20.30 and 21.30 hours on Day 4 and those to be mated were caged individually with two active, fertile males and left for 30 to 90 min. Inseminations were performed under pentobarbitone anaesthesia, approximately 0.2 ml of a freshly prepared suspension of epididymal spermatozoa in medium TC 199 being injected into the lumen of each uterine horn. Animals were killed at various times on Day 1 in order to obtain eggs that would normally have been at the

TABLE 1

THE EFFECT OF A SINGLE INJECTION OF PROGESTERONE GIVEN AT VARIOUS TIMES BEFORE OVULATION ON FERTILIZATION IN THE GOLDEN HAMSTER

Treatment No.	Doses of progesterone (mg/animal)	Time of progesterone injection	Time of mating or insemination*	No. of hamsters		% of hamsters with fertilized eggs	No. of eggs		% of eggs fertilized
				Injected	With fertilized eggs		Examined	Fertilized	
I	1,2,4,10,20	Day 4 20.30 to 21.30 hours	Day 4 20.30 to 21.30 hours	40 (7)	35 (7)	87.5 (100)	510 (94)	394 (94)	77.3 (100)
II	4,10,20	Day 4 15.00 to 17.00 hours	Day 4 20.30 to 21.30 hours	41† (7)	26 (7)	63.4 (100)	516 (73)	295 ¶ (72)	57.2 (98.6)
III	4,10,20	Day 4 17.00 hours	Day 1* 08.00 to 09.00 hours	24 (6)	16 (6)	66.6 (100)	238 (60)	92 (26)	38.6 (43.3)
IV	1,4,10†	Day 3 15.00 to 17.00 hours	Day 4* 18.00 to 21.00 hours	15§	10	66.6	98	38	38.8
Total				120	87	72.5	1362	819	60.2

Results from control animals are in parentheses.

* Animals in these groups were inseminated directly into the uterine lumen.

† Animals in Treatment IV also received an ovulating injection of 30 i.u. HCG given intra-peritoneally between 14.00 and 15.00 hours on Day 4.

‡ Two animals did not ovulate following this treatment.

§ Only those animals ovulating after this treatment are recorded.

|| One animal with unilateral fertilization.

¶ Four animals with unilateral fertilization.

pronucleate stage. The oviducts were dissected under Ringer's solution, and whole-mounts of the recovered eggs examined under a phase-contrast microscope both in the fresh and fixed condition.

The results of all treatments, summarized in Table 1, demonstrate that a significant lowering of fertilization occurred following injection of progesterone. This was due both to a decrease in the proportion of animals yielding some fertilized eggs and a reduction in the number of eggs fertilized in such animals. The incidence of fertilization decreased from 77% to 39% when progesterone was injected at the time of mating or on the afternoon of Day 3, respectively, indicating that a more profound influence upon fertilization was obtained by advancing the time of treatment relative to the time of ovulation. No consistent effect of the dose of progesterone was seen within treatments.

A lowered proportion of successful matings found after Treatments I and II (see Table 1), as evidenced by very few or no spermatozoa in the vaginal plug, was probably a reflection of a diminished incidence of oestrus in these animals. The failure of two animals in Treatment II to ovulate by the late morning of Day 1 may also have been associated with progesterone blockade, resulting in incomplete release of luteinizing hormone. However, unpublished studies indicate that ovulation is not normally prevented by similar doses of progesterone unless given by the afternoon of Day 3, an observation that is supported by the work of Greenwald (1965).

The finding of a reduced proportion of fertilized eggs within fertile animals after progesterone treatment would best be explained in terms of a decreased number of spermatozoa present at the site of fertilization, possibly indicating a diminished rate of sperm transport. The recovery of fertilized eggs from only one oviduct in five animals after Treatments I and II, and the observation that fertilized eggs very rarely had accessory spermatozoa attached to the zona pellucida, would also be in accord with this explanation.

It was reasoned above that injections of progesterone might influence the normal sequence of events at fertilization due to an indirect effect on the eggs mediated through the tubal secretions. No such effect was seen, for, in eggs that were penetrated by spermatozoa, resumption of meiosis and development of pronuclei appeared normal. The total number of polyspermic eggs plus those with supplementary spermatozoa in the perivitelline space was only four among 1362 eggs, an incidence considerably less than that reported by Austin (1956) following normal mating. Nor was the number of eggs showing other abnormalities increased by the treatment, spontaneous activation and cytoplasmic fragmentation being seldom observed in any of the groups.

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