EFFECT OF PROGESTERONE *IN VIVO* UPON THE RATE OF CLEAVAGE OF MOUSE EMBRYOS

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The effects of ovarian hormones upon the female genital tract have been extensively studied (see Parkes & Deanesly, 1966a), but very little is known of the effect of ovarian steroids on the early development of the mammalian embryo. It has been shown that oestrogens and progesterone enhance the incorporation of labelled uridine and labelled amino acids in delayed blastocysts of certain mammals (Prasad, Dass & Mohla, 1968; Weitlauf & Greenwald, 1968). On the other hand, studies *in vitro* have shown that progesterone has an inhibitory effect on the early development of mammalian embryos as judged by the arrest of cleavage (Whitten, 1957; Daniel, 1964; Kirkpatrick, 1971). In the presence of macromolecules of the uterine fluid, however, progesterone *in vitro* has a stimulatory effect as measured by the growth of rabbit blastocysts (El-Banna & Daniel, 1972).

In the present work, we have studied the effect of progesterone *in vivo* upon the rate of cleavage during early development of the mouse embryo.

Immature female mice of the Swiss-Rockefeller strain were induced to superovulate with an injection of 5 i.u. PMSG (Gestyl, Organon) followed 44 hr later by an injection of 10 i.u. HCG (Apoidina, Parke Davis). After the HCG injection, the animals were mated with males of proven fertility. The mated females were allocated to three groups: in Group 1, females were left intact and served as controls; in Group 2, females were ovariectomized 34 hr after HCG injection; in Group 3, females were also ovariectomized 34 hr after HCG injection, but beginning at the time of ovariectomy and continuing up to the time of killing, the females of the third group received a daily intraperitoneal injection of 1 mg progesterone. The animals from each group were killed 63, 82 and 87 hr after the estimated time of ovulation. The oviducts and uterine horns were flushed with BMOC-2 (Brinster, 1965) and the recovered embryos were treated with a 0.2% solution of sodium citrate. The embryos were then fixed with a mixture of glacial acetic acid–ethanol (1:3) and finally stained with orcein (Tarkowski, 1966). The cell nuclei of each embryo were counted with the aid of a drawing projector.

The results clearly show (Table 1) that embryos of ovariectomized females have significantly fewer cells when compared to embryos from control animals. The embryos recovered from ovariectomized progesterone-treated females had significantly fewer cells when compared to controls but significantly more cells when compared to embryos of ovariectomized females.

Table 1. Mean number of blastomeres per embryo recovered 63, 82 and 87 hr after ovulation from female mice treated under different conditions

Group	No. of females	Treatment	No. of embryos	Mean no. of blastomeres per embryo
1	8	None (control) ^a	89	12.7
	7	Ovariectomized ^b	85	9.3
	8	Ovariectomized and progesterone-treated ^c	91	10.2
8	8	None (control) ^a	82	39.8
	8	Ovariectomized ^b	87	15.9
	7	Ovariectomized and progesterone-treated ^c	78	25.5
3	10	None (control) ^a	119	57.4
	10	Ovariectomized ^b	99	23.0
	10	Ovariectomized and progesterone-treated ^c	108	40.6

The *P* value for all three Groups and all three 'interactions' (a/b, a/c, b/c) in Wilcoxon's Test was *P* < 0.01, with the exception of b/c for Group 1 where the value was *P* < 0.02.

These results suggest that the decrease in progesterone and other ovarian hormones to the low levels occurring following ovariectomy has a detrimental effect upon development of the mammalian embryo. The corollary of the detrimental effect would be that maternal ovarian hormones control early embryogenesis, such as cleavage rate. This hypothesis is supported by the significant increase in the number of cells in embryos recovered from ovariectomized progesterone-treated females compared to the ovariectomized ones. The uterine secretions could be acting as carriers of progesterone to become incorporated into the developing embryo (El-Banna & Daniel, 1972). This view is supported by the fact that progesterone has a stimulatory effect on the cleavage rate *in vitro* only when the culture medium contains blood serum (L. Roblero, unpublished results).

The synergistic physiological effects of progesterone and oestrogens have been extensively illustrated (Parkes & Deanesly, 1966b). These low levels probably have no effect on embryonic development.

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