Effect of oestradiol-17 β and progesterone on oviductal transport and early development of mouse embryos

L. S. Roblero and Anita C. Garavagno

*Laboratory of Endocrinology, Institute of Biological Sciences,
Pontifical Catholic University of Chile, Santiago, Chile*

Summary. Transport of embryos through the oviduct, cleavage rate and transformation of morulae to blastocysts, were delayed in females ovariectomized on Day 2 of pregnancy. Oestradiol-17 β in doses of 60 to 6000 pg/day for 3 days did not normalize the transport of embryos, but the transformation of morulae to blastocysts reached values near or equal to those of the controls, in spite of a lowered rate of cleavage. Progesterone at a dose of 100 μ g/day, resulted in normal transport, rate of cleavage and rate of differentiation. Treatment with both hormones had synergistic effects on transport and the rate of cleavage and differentiation. These results give further support to the concept that ovarian hormones are the controlling factors for these processes in early pregnancy.

Introduction

Before implantation, the mammalian embryo lies free in the genital tract for several days. During this period it is bathed by oviductal or uterine fluids which constitute a microenvironment for exchanging substances between the embryo and the mother (Biggers & Borland, 1976). The occurrence of ovarian steroids of maternal origin has been demonstrated in the fluids of the genital tract during early pregnancy (Cowan, Manes & Hagerman, 1976; Borland, Erickson & Ducibella, 1977; Fowler, Johnson, Walters & Eager, 1977). However, little is known about the interactions of those hormones with the embryos during the early development. Obviously, such information is necessary for a full understanding of the mother–embryo relationship in this period, and to assess the role of the microenvironment of the genital tract on embryonic development.

It is possible to achieve normal development of embryos *in vitro* up to blastocyst stage in a hormone-free medium (Brinster, 1963). However, under *in-vitro* conditions, embryonic development takes longer than *in vivo* (Bowman & McLaren, 1970). It has also been demonstrated that in mice ovariectomy performed soon after fertilization leads to a decrease in the rate of cleavage (Roblero, 1973) and in the transport of embryos through the oviduct (Whitney & Burdick, 1939). However, the cleavage rate of embryos in ovariectomized mice treated with progesterone increases to values nearly equal to those of intact animals (Roblero, 1973) and better results are obtained when the embryos are cultured in medium containing progesterone, in the presence of blood serum or its macromolecular fraction (Roblero & Izquierdo, 1976). Therefore, ovarian hormones could be playing a regulatory role at early stages of development in synchronizing the embryonic development with the maternal physiological state.

In this work we have studied the effect of oestradiol-17 β and progesterone on transport and development of embryos in female mice, ovariectomized on the 2nd day of pregnancy.

Materials and Methods

Sexually mature (4–6 weeks old), virgin, Swiss-Rockefeller mice were randomly selected at different stages of the oestrous cycle and injected with 4 i.u. PMSG (Gestyl: Organon, Oss,

Netherlands). After 44 h they were injected with 4 i.u. hCG (Pregnyl: Organon) and then each female was placed with a male of proven fertility. Mating was confirmed by the presence of a vaginal plug on the following morning (Day 1 of pregnancy). On Day 2 the animals were randomly assigned to 5 experimental groups.

Group 1. The mice were left intact as controls.

Group 2. The mice were ovariectomized and injected with 0.1 ml olive oil.

Group 3. These mice were ovariectomized, divided into 3 subgroups, and injected as follows: (A) 60 pg oestradiol-17 β /animal/day; (B) 600 pg oestradiol-17 β /animal/day; (C) 6000 pg oestradiol-17 β /animal/day.

Group 4. The mice were ovariectomized and injected with 100 μ g progesterone/animal/day.

Group 5. These mice were ovariectomized, divided into 3 subgroups, and injected as follows: (A) 60 pg oestradiol-17 β plus 100 μ g progesterone/animal/day; (B) 600 pg oestradiol-17 β plus 100 μ g progesterone/animal/day; (C) 6000 pg oestradiol-17 β plus 100 μ g progesterone/animal/day.

The number of animals assigned to each treatment is shown in the 'Results'.

The animals in Groups 2 to 5 were bilaterally ovariectomized between 10:00 and 12:00 h on the 2nd day of pregnancy. Immediately after ovariectomy the animals in Groups 3 to 5 were injected subcutaneously with oestradiol-17 β (Calbiochem), progesterone (Calbiochem), or both. The hormones were dissolved in 0.1 ml olive oil and injections were repeated at 10:00 h on Days 3 and 4.

All animals were killed 100 h after the hCG injection. The embryos were recovered by flushing the genital tract with BMOC-2 (Brinster, 1963). The numbers of morulae and blastocysts from the oviduct and uterus of each animal were recorded. The embryos were then treated with sodium citrate (1%, w/v), fixed with ethanol-acetic acid (3:1, v/v) and then stained with orcein to count the number of cells (Tarkowski, 1966).

For statistical purposes, analysis of variance and the test of Scheffé (Glass & Stanley, 1973) were used to compare the mean number of embryos and their distribution in the genital tract, Wilcoxon's test (Geigy, 1965) was used to compare the mean number of blastomeres per embryo, and the test of significance for difference of proportion (Spiegel, 1961) was used for comparing the percentages of morula to blastocyst transformation.

Results

All the results are shown in Table 1.

All the embryos of the control animals (Group 1) were in the uterus, but in Group 2 only a few had reached the uterus by Day 4 and most were still in the oviduct. This distribution of embryos was significantly different ($P < 0.001$).

Embryonic transport in ovariectomized females, treated with oestradiol alone (Group 3), remained slow, since a high proportion of the embryos was recovered from the oviduct, but was nevertheless faster than that in Group 2 since fewer embryos were present in the oviducts of Group 3 than Group 2 mice ($P < 0.01$). Treatment with progesterone (Group 4) produced the same effect as the two higher doses of oestradiol upon oviductal transport, but the mean number of uterine embryos was significantly ($P < 0.05$) greater than in Group 3. The distribution of embryos in Groups 5B and 5C was similar to that in Group 1, but the mean number of embryos in the uterus was significantly different.

The total recovery rate of embryos in Groups 4 and 5 was not significantly different from that in Groups 1 and 2 but injections of oestradiol (Group 3) caused a decrease.

The effects of oestradiol and progesterone on the cleavage rate of the embryos are also shown in Table 1. The number of blastomeres per embryo was less, particularly in blastocysts, in Group 2 than in Group 1. Oestradiol-17 β treatment had no effect upon cleavage rate and the

Table 1. Mean $\pm$ s.e.m. number of blastomeres and distribution of embryos on Day 4 of pregnancy in mice ovariectomized (Ovex) and treated with oestradiol (E_2) or progesterone (P)

Group	Treatment	No. of mice	Distribution of embryos in:		Total embryos in genital tract	Mean number of blastomeres per embryo (no.)						
			Oviduct			Uterus		Oviduct		Uterus		Total %
			Morulae	Blastocysts		Morulae	Blastocysts	Morulae	Blastocysts	Morulae	Blastocysts	
1	Controls	8	0.0 ± 0.0	^a 10.5 ± 0.74	^b 10.5 ± 0.74	0.0 ± 0.0 (0)	0.0 ± 0.0 (0)	34.0 ± 2.85 (9)	^c 50.9 ± 1.47 (75)	17.7	^d 89.3	
2	Ovex	11	^a 6.9 ± 0.75	^a 4.4 ± 0.48	^b 11.3 ± 0.63	20.1 ± 0.71 (56)	29.2 ± 1.99 (19)	25.8 ± 1.78 (21)	^c 36.1 ± 1.47 (28)	62.1	^d 137.9	
3	Ovex											
(A)	60 pg E ₂	8	^a 4.4 ± 0.64	^a 3.6 ± 0.53	^b 8.0 ± 0.78	20.5 ± 1.17 (26)	27.6 ± 1.21 (9)	25.5 ± 2.15 (10)	^c 37.2 ± 2.44 (19)	56.2	^d 143.8	
(B)	600 pg E ₂	9	^a 2.8 ± 0.57	^a 4.2 ± 0.33	^b 7.0 ± 0.50	21.1 ± 0.98 (9)	27.3 ± 1.62 (16)	24.8 ± 3.13 (8)	^c 38.3 ± 1.66 (30)	27.0	^d 173.0	
(C)	6000 pg E ₂	8	^a 2.5 ± 0.42	^a 3.7 ± 0.35	^b 6.2 ± 0.60	21.0 ± 1.08 (4)	30.1 ± 1.04 (16)	0.0 ± 0.0 (0)	^c 39.3 ± 1.30 (30)	8.0	^d 192.0	
4	Ovex											
	100 µg P	8	^a 2.3 ± 0.53	^a 6.9 ± 0.53	^b 9.2 ± 0.85	30.6 ± 1.78 (11)	34.1 ± 2.17 (7)	23.7 ± 1.85 (8)	^c 57.1 ± 1.54 (47)	26.0	^d 174.0	
5	Ovex											
(A)	60 pg E ₂ + 100 µg P	8	^a 4.0 ± 0.78	^a 5.3 ± 0.18	^b 9.3 ± 0.85	20.2 ± 1.61 (17)	33.5 ± 1.67 (15)	22.5 ± 2.22 (13)	^c 47.1 ± 2.53 (29)	40.5	^d 159.5	
(B)	600 pg E ₂ + 100 µg P	8	^a 1.5 ± 0.39	^a 7.5 ± 0.85	^b 9.0 ± 0.85	27.0 ± 1.78 (0)	36.7 ± 3.47 (8)	0.0 ± 0.0 (0)	^c 75.7 ± 2.31 (60)	5.6	^d 194.4	
(C)	6000 pg E ₂ + 100 µg P	8	^a 1.5 ± 0.39	^a 7.0 ± 0.60	^b 8.5 ± 0.85	0.0 ± 0.0 (0)	27.7 ± 1.97 (12)	27.2 ± 2.34 (8)	^c 60.5 ± 2.49 (48)	11.8	^d 188.2	

The *P* values for the interactions were as follows: s-t, t-v, t-w, t-x, t-z, t-*, *P* < 0.001;a-b, a-c, d-e, l-m, l-n, l-q, m-o, m-q, m-r, n-o, n-q, n-r, o-q, p-q, t-y, *P* < 0.01;b-j, d-f, d-g, e-f, h-i, h-j, j-k, l-r, m-p, o-p, p-r, q-r, s-v, u-y, v-w, v-z, x-z, x-*, *P* < 0.05.

blastomere number per morula or blastocyst was similar to that in embryos of Group 2. Treatment with progesterone (Group 4) and oestradiol + progesterone (Group 5) had no effect on the rate of cleavage in morulae and blastocysts located in the oviduct or on morulae in the uterus, but did have a pronounced effect upon uterine blastocysts which had more blastomeres than did those in Groups 2 and 3. The uterine blastocysts in Groups 5B and 5C also had more blastomeres than did those in Group 1. Ovariectomy resulted in a decrease in the transformation of morulae into blastocysts. Treatment with oestradiol increased the proportion of blastocysts and higher doses had greater effect, values in Group 3C being similar to those in Group 1. Progesterone and oestradiol + progesterone (Group 5A) also stimulated differentiation into blastocysts, but the transformation in Groups 5B and 5C was similar to that in Group 1.

Discussion

The present results are consistent with previous findings with regard to the stimulatory effect of progesterone *in vivo* upon the cleavage rate of embryos (Roblero, 1973). The retention of embryos in the oviduct of ovariectomized females (Whitney & Burdick, 1939), and the delay of transformation of morulae to blastocysts provoked by ovariectomy (Wu, Dickmann & Johnson, 1971) were also confirmed.

Although the control mice in this study were intact, laparotomy on Day 2 of pregnancy has no effect on the tubal transport or development of embryos (unpublished). The present results indicate that oestradiol-17 β has a double effect on the transport of embryos through the genital tract. The slower tubal transport provoked by ovariectomy was partly corrected by oestradiol but oestradiol also produced a significant loss of uterine embryos which could be counteracted by progesterone. However oestradiol also affected the transformation of morulae into blastocysts and these results indicate a specific effect of oestradiol upon blastocyst formation.

Under the effect of progesterone, the transport of embryos through the oviduct was faster than in ovariectomized females and very similar to the transport provoked by the higher dose of oestradiol. But in spite of this increase in the rate of transport through the oviduct, the number of embryos recovered from the uterus was greater than for mice injected with oestradiol. Progesterone also had an effect upon the cleavage rate, blastomere number being normal after treatment with this hormone, and upon the morula to blastocyst transformation, which was improved.

In spite of the fact that oestradiol alone had no effect on the cleavage rate and only a small effect on the transport of the embryos, it did potentiate the action of progesterone in these processes. Thus, a synergistic effect of these hormones, when given in combination, was observed for the transport and recovery of embryos, differentiation of blastocysts, and rate of cleavage and the values obtained were similar to those for intact mice. This finding suggests that normal functioning of these reproductive processes requires both hormones, although progesterone appears to be capable of exerting a greater control than oestradiol.

Although oestrogen and progesterone do stimulate transport, cleavage rate and differentiation, they are not essential, because even in ovariectomized animals the embryos reach the blastocyst stage and also the uterus, in spite of some delay. It is known that both hormones affect not only the concentrations of some inorganic ions present in the genital tract fluids (Tantayaporn, Mallikarjuneswara, De Carlo & Clemeston, 1974), but also the quality of their organic components (Beir, 1968; Arthur & Daniel, 1972). These fluids would then constitute a changing microenvironment for the constantly changing requirements of the embryo on its way from the ampulla to the uterus. Accordingly, both ovarian hormones could then act at two levels: directly, upon the embryo, which would be a target tissue, and indirectly, through the stimulation of the maternal genital tract, which would provide the proper microenvironment by secreting the optimum fluid for development, and by maintaining the appropriate motility for embryonic

transport. Oestradiol and progesterone could therefore act synergistically to control the synchrony between the embryonic stage of development and the physiological state of the maternal genital tract.

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