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Effects of the anti-progestin and anti-glucocorticoid steroid RU486 on cell proliferation in oviductal embryos of lactating mice

A. C. McRae

Department of Physiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0

The effects of the progestin and glucocorticoid antagonist RU486 on cleavage rate of oviductal embryos from lactating mice was studied. Preliminary experiments suggested that, owing to suppression of follicular activity by lactation, the effects of RU486 during early embryo development could be studied independently of overt oestrogen dominance in mice mated at *postpartum* oestrus. After treatment with RU486 on days 1–3 of pregnancy in lactating mice, embryos recovered on day 4, but not on day 3, had significantly fewer cells than did those from control animals. However, neither exogenous progestins nor dexamethasone could reverse this effect of RU486. Nevertheless, embryos recovered from lactating mice treated with progestins alone had significantly more cells on day 4 than did those from control animals. In contrast, embryos recovered from mice treated with dexamethasone alone had significantly fewer cells per embryo on day 4 than did those from control mice. These results provide further evidence that the growth rate of oviductal embryos in mice is influenced by the maternal hormone milieu.

Introduction

It is well established that ovarian progesterone secretion is essential for blastocyst implantation and the successful establishment of pregnancy in mammals. However, whether maternal progesterone is important during preblastocyst development of oviductal embryos is less certain and remains controversial.

Three approaches have been used to examine the importance of progesterone during early embryo development in mice. These are ovariectomy (or hypophysectomy) carried out shortly after mating, passive immunization against progesterone using monoclonal antibodies and treatment with progesterone antagonists which compete with progesterone for its receptor. In general, studies using these approaches have reported that progesterone withdrawal results in (i) arrest of embryo development before cavitation (Wang *et al.*, 1984), (ii) delayed transformation of morulae to blastocysts (Wu *et al.*, 1971; Roblero and Garavagno, 1979; Roblero *et al.*, 1987; Vinijsanun *et al.*, 1990; Yang and Wu, 1990; Vinijsanun and Martin, 1991) or (iii) significantly fewer cells per embryo (Roblero, 1973; Roblero and Garavagno, 1979; Roblero *et al.*, 1987).

However, Vinijsanun and Martin (1991) clearly demonstrated that early ovariectomy causes profound non-specific effects of tissue trauma on embryo development and survival. Abrogation of the action of progesterone by passive immunization has the distinct advantage over early ovariectomy of avoiding this confounding influence of tissue trauma. However, immunoneutralization of progesterone using monoclonal antibodies either arrested embryo development or caused only a slight delay in the transformation of morulae to blastocysts, depending upon whether the DB3 monoclonal (Wang *et al.*, 1984) or the 11P27 monoclonal antibody (Vinijsanun *et al.*, 1990), respectively, was used. Moreover, Heap *et al.* (1988) reported an inability to confirm their earlier findings (Wang *et al.*, 1984). Furthermore, the effectiveness of monoclonal antiprogesterone antibodies in blocking pregnancy in mice is strain specific (Rider *et al.*, 1986; Vinijsanun *et al.*, 1990), and is highly effective only in mice syngeneic to those in which the antibodies are generated, suggesting an immunological, rather than anti-progesterone, mode of action (Vinijsanun *et al.*, 1990). Conflicting results have also been obtained using the steroid progesterone antagonist RU486 (17β-hydroxy-11β-(4-dimethylaminophenyl-1)-17α(prop-1-ynyl)-estra-4,9-dien-3-one): several studies have reported either retarded embryo development (Roblero *et al.*, 1987; Yang and Wu, 1990) or relatively little effect (Vinijsanun and Martin, 1990). However, in two of these studies (Yang and Wu, 1990; Vinijsanun and Martin, 1990), treatment with RU486 was associated with embryo losses by day 4 of pregnancy in excess of 60–80%. These losses were probably due to uterine expulsion caused by excessive oestrogen action that was unopposed by progesterone (Vinijsanun and Martin, 1990; Ortiz *et al.*, 1991). It is therefore not entirely clear whether altered embryo development in response to RU486 treatment is due to progesterone withdrawal, excess of oestrogen, both or neither.

The importance of progesterone secretion during preimplantation embryo development remains crucial to our understanding of maternal factors influencing embryo viability and successful establishment of pregnancy. The objective of this study was therefore to develop an animal model in which the

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effects of progesterone withdrawal on preimplantation embryo development can be studied specifically and to reassess the effects of the progesterone antagonist RU486 on early embryo development in mice.

Materials and Methods

Animals

Swiss CD1 mice were used in this study. Female mice were obtained at 6–8 weeks of age from the colony at the University of Saskatchewan. In initial experiments, virgin females were mated randomly to adult males purchased from Charles River (Quebec, Canada). Females were separated from males on day 1 of pregnancy, the day of finding a vaginal plug. In subsequent experiments, embryo development was assessed in lactating mice. Pregnant mice (first pregnancy) were placed individually with adult males approximately 1 week before whelping. Each cage was inspected twice daily (09:00 h and 17:00 h) for the presence of pups and to determine whether whelping occurred before or after 17:00 h. Females with pups were checked for vaginal plugs on the morning after whelping. The presence of a vaginal plug confirmed that *postpartum* mating had occurred and denoted day 1 of pregnancy. Lactating females and litters were left with males until the females were killed for embryo recovery.

Hormones and treatments

All hormone treatments were administered as s.c. injections of 0.1 ml and were given between 08:00 h and 10:00 h. RU486 (provided by Roussell UCLAF, Romainville), LHRH antagonist (Ac-3, 4-dehydro-Pro-D-p-F-Phe, D-Trp: Sigma, St Louis, MO) and progesterone (Sigma) were dissolved or suspended in sesame oil. Medroxyprogesterone acetate (Depo-Provera: UpJohn, Don Mills, Ontario) was given as a suspension in 0.9% saline and dexamethasone (Sigma) was given as a suspension of 1% ethanol (v/v) in 0.9% saline (w/v).

RU486 was given in doses ranging from 62.5 μg to 250 μg (2.5–10 mg kg^{-1}) beginning on day 1 of pregnancy and repeated daily. LHRH antagonist (Gallo, 1981) was given either as 20 μg or 100 μg per day on days 1–3 of pregnancy. Exogenous progesterone was given as 2 mg medroxyprogesterone acetate on day 1 plus 1 mg progesterone on days 1–3 of pregnancy (Vinijsanun and Martin, 1990). Dexamethasone was given as 200 μg on days 1–3 of pregnancy (Vinijsanun and Martin, 1990).

Embryo collection and analysis

Mice were killed by asphyxiation in carbon dioxide. Embryos were recovered from individual mice by flushing excised oviducts and uteri with M2 medium (Miller and Schultz, 1983) containing 0.4% (w/v) BSA. Embryos were recovered from flushings, transferred into fresh medium and held at 4°C until the number of cells or stage of development was determined.

The numbers of cells in embryos with 4–5 cells or fewer were counted directly during examination under magnification with a Nikon TMS inverted microscope. Other embryos were subjected to a modified Tarkowski (1966) procedure for visualizing cell nuclei. Briefly, embryos were incubated in 0.8% sodium citrate (w/v) for 15–25 min at room temperature. They were then transferred in small droplets to a microscope slide coated with gelatin. Several drops of freshly prepared fixative (ethanol:glacial acetic acid 3:1, v:v) were permitted to fall from the pipette onto the droplets containing embryos. The fixative was allowed to dry and the preparation was stained with 2% aceto–orcein or 2% lactic–acetic–orcein. The nuclei were counted at × 400.

Statistical analysis

Data are presented as proportions of the total number of embryos or as the mean and SD. Proportional data were analysed for statistical significance using the χ^2 test. Differences between means were tested for statistical significance using Student's t test, rather than analysis of variance, because of the considerable variability between numbers of embryos per treatment group in each experiment.

Results

Primiparous mice

Of 15 mated mice treated on days 1 and 2 with RU486, embryos were recovered on the afternoon of day 4 from only six mice and none was from animals treated with the highest dose tested (250 μg) (Table 1). This low incidence of embryo recovery was in marked contrast to that of mice treated with vehicle. When a dose of 62.5 μg RU486 was given on days 1 and 2 of pregnancy, the number of mice with embryos and the average number of embryos per mouse with embryos were less than those of control mice, but the difference was not significant. Treatment with RU486 on day 1 only (125 μg) resulted in significantly fewer mice having embryos on day 4 ($\chi^2 = 7.0$, $P < 0.05$), although the average number per mouse with embryos was not significantly different from that of control mice ($t = 2.09$, $P > 0.05$). Notably, the uteri of those RU486-treated mice from which no embryos were recovered were short and distended in appearance and were therefore entirely reminiscent of those from oestrous mice. Furthermore, the presence of cumulus masses containing oocytes in the ampulla of 11 of 16 mice with 'oestrous' uteri suggests that spontaneous ovulation had also occurred. RU486 given post-coitally to primiparous mice therefore resulted in these characteristic effects of oestrogen, presumably owing to the absence of progesterone antagonism.

From the 12 control mice (Table 1), 112 embryos were recovered of which 109, or 97%, were blastocysts and three were considered degenerate. By contrast, of a total of only 30 embryos recovered from the 22 mice treated with RU486, 23 were blastocysts, four were morulae and three were considered degenerate. The proportion of normal embryos from RU486-treated animals that were blastocysts was significantly smaller (23 of 27 versus 109 of 109, $\chi^2 = 16.3$, $P < 0.05$).

Table 1. Embryo recovery and reproductive status on day 4 of pregnancy in primiparous mice treated post-coitally with RU486

Treatment (μg RU486)	Mean (± SD) number of embryos per mouse[a]	Number of mice		
		With embryos	With oestrous uteri	With ampullary oocytes
Days 1 and 2				
0	8.2 ± 2.2	6/6	0/6	0/6
62.5	3.7 ± 2.9	3/5	2/5	2/5
125	8	1/4	3/4	1/4
250	0	0/6	6/6	5/6
Day 1 only				
0	10.5 ± 2.9	6/6	0/6	0/6
125	5.5 ± 0.7	2/7	5/7	3/7

[a]Includes only mice with embryos.

Table 2. Embryo recovery and stage of development on day 4 of pregnancy in primiparous mice

Treatment	Number of mice with embryos	Total embryos recovered (mean ± SD)	Stage of development (%)		
			Morula	Early blastocyst	Expanded blastocyst
Vehicle	4/4	45 (11.3 ± 1.3)	1 (2)	15 (33)	29 (64)
RU486[a]	0/6		0 (0)	0 (0)	0 (0)
RU486 + LHRH antagonist[b]	7/12	25[c] (3.6 ± 2.4)	15 (60)	5 (20)	4 (16)

[a]250 μg RU486 on days 1 and 2.
[b]250 μg RU486 on days 1 and 2 plus 20 μg or 100 μg LHRH antagonist on days 1–3.
[c]Includes one two-cell embryo and five embryos recovered from the oviduct.

In the subsequent experiment, an attempt was made to lower ovarian oestrogen secretion in RU486-treated mice by decreasing LH secretion through concurrent treatment with an LHRH antagonist. Primiparous mice were either given vehicle alone (controls) or treated on days 1 and 2 with RU486 (250 μg) and 0, 20 or 100 μg of LHRH antagonist on days 1–3 of pregnancy. On day 4 of pregnancy, the proportions of RU486 plus LHRH antagonist-treated mice having embryos and the mean number (± SD) of embryos per mouse having embryos did not differ significantly between doses of 20 μg and 100 μg LHRH antagonist day^{-1} (3 of 6 versus 4 of 6; 4.0 ± 3.6 versus 3.3 ± 1.5, respectively). The data from mice treated with both RU486 and LHRH antagonist were therefore pooled (Table 2). When RU486 was given to primiparous mice without concurrent treatment with LHRH antagonist, embryo loss was complete, that is, 0 of 6 mice with embryos. However, when LHRH antagonist was given in addition to RU486, the proportion of mice with embryos was not significantly different from that of the untreated control mice (7 of 12 versus 4 of 4; $\chi^2 = 2.4$, $P > 0.05$). Nevertheless, significantly fewer embryos were recovered per mouse with embryos (3.6 ± 2.4 versus 11.3 ± 1.3; $t = 5.3$, $P < 0.05$). Notably, vaginal lavages taken from all mice from which no embryos were recovered were entirely dominated by cornified cells. In contrast, lavages from all untreated pregnant mice were unequivocally characterized by leucocytes. Vaginal lavages from mice treated with RU486 and LHRH antagonist from which embryos were recovered were, however, neither clearly cornified cells nor leucocytes but mixtures of both. In addition, ampullary oocytes were present in only two of 12 mice treated with both antagonists as opposed to five or six mice treated with RU486 alone ($\chi^2 = 7.5$, $P < 0.05$).

Of the embryos recovered from RU486-treated mice, most (60%) had developed only to the morula stage by day 4 of pregnancy. In contrast, most (97%) embryos from control mice at this time were blastocysts (Table 2).

Lactating mice

In a preliminary experiment, mice were mated at the *post-partum* oestrus following their first pregnancy and were treated on days 1 and 2 with 250 μg RU486 or vehicle. These animals were killed on day 5 as opposed to day 4, as embryo development is reportedly slower in lactating mice (Menke and McLaren, 1970). On day 5 of pregnancy, all of the five RU486-treated mice had embryos, all had leucocytic vaginal lavages and none had ampullary oocytes or early cleavage embryos. However, significantly fewer embryos were recovered from the RU486-treated mice (control: 13.6 ± 2.1 versus RU486: 6.4 ± 2.4, $P < 0.05$). Nevertheless, whereas 50% (34 of 68) of blastocysts from control animals had hatched from their zonae pellucidae, only 18% (6 of 32) of those from RU486-treated mice had done so. The uterine weights from RU486-treated mice were also significantly less than those of control

Table 3. Number of cells (mean ± SD) per embryo on the morning of day 3 or 4 of pregnancy from mice mated following whelping before or after 17:00 h on day 0 and treated on days 1–3 with RU486

Dose of RU486 (μg)	Day 3		Day 4	
	Pups before 17:00 h	Pups after 17:00 h	Pups before 17:00 h	Pups after 17:00 h
0	5.9 ± 1.6 (47,4)[a]*	3.8 ± 0.7 (60,4)[a]	34.5 ± 10.5 (57,6)[a]	22.3 ± 6.5 (33,5)[ab]
125	6.5 ± 1.4 (44,4)[a]	3.9 ± 0.5 (31,2)[a]	26.9 ± 3.2 (37,5)[b]	20.6 ± 5.5 (45,5)[bc]
250	5.6 ± 1.8 (48,4)[a]	3.9 ± 0.8 (52,4)[a]	23.2 ± 5.5 (45,5)[c]	19.1 ± 4.4 (54,6)[c]

*(Number of embryos, number of mice).
[abc]Values with different superscripts within columns are significantly different ($P < 0.05$).

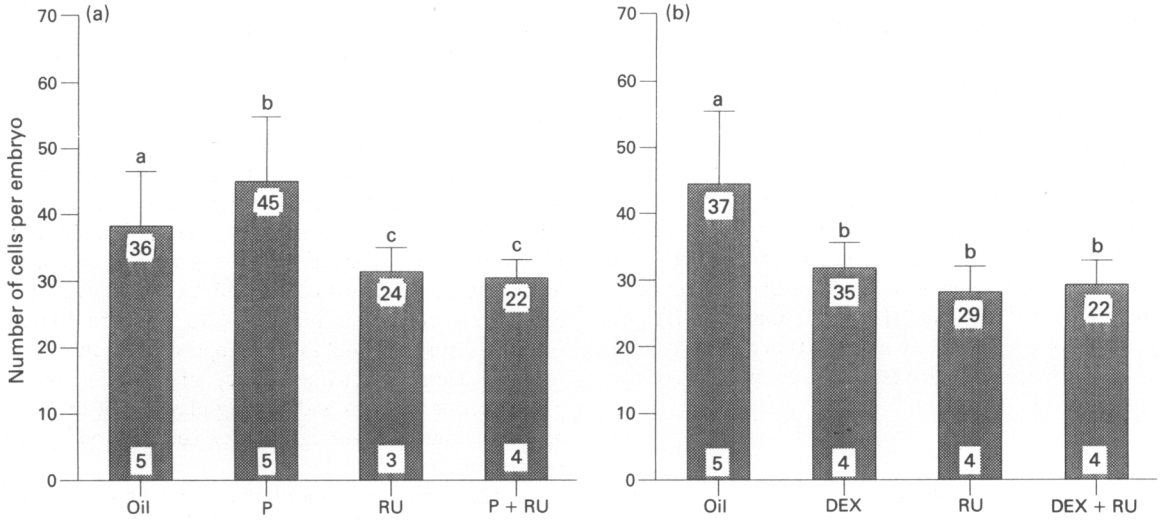

Fig. 1. Effects of RU486 antagonist and competing agonists alone and in combination on number of cells per embryo (mean ± SD) in mice mated at *postpartum* oestrus: (a) embryos recovered on the morning of day 4 of pregnancy in females whelping before 17:00 h of day 0, (b) embryos recovered on the afternoon of day 4 of pregnancy in females whelping after 17:00 h on day 0. Numbers at top and bottom of bars are the numbers of embryos and animals, respectively. Different letters above bars within panels indicate significant differences ($P < 0.05$). P: progestin; RU: RU486; DEX: dexamethasone.

animals (control: 191 ± 18 mg versus RU486: 154 ± 15 mg, $P < 0.05$).

In a subsequent experiment, the effects of RU486 on the number of cells per embryo from lactating mice on days 3 and 4 of pregnancy were examined (Table 3). From these data, it is clear that the time of whelping is a significant factor determining the numbers of cells in early embryos from lactating animals. Nevertheless, treatment with RU486 was associated with significantly fewer cells per embryo on day 4 of pregnancy, but not day 3, regardless of the time of whelping. Furthermore, the lower dose of RU486 (125 μg) was effective in reducing the numbers of cells in embryos from dams whelping before 17:00 h on day 0.

To evaluate whether the effect of RU486 on cell numbers was due to an antagonism of the action of progesterone, exogenous progestins were given in an attempt to reverse this negative effect. The results of this experiment are summarized (Fig. 1a). The mean number of cells per embryo on the morning of day 4 of pregnancy of lactating mice whelping before 17:00 h on day 0 was significantly lower in those animals receiving 125 μg of RU486 on days 1–3 than in control mice. Exogenous progestins given concomitantly with RU486 failed to overcome the negative effect of the antagonist. However, in the absence of RU486, exogenous progestins significantly increased the mean number of cells per embryo. In a concurrent experiment, the ability of dexamethasone, a synthetic glucocorticoid, to reverse the effect of RU486 was also tested. These results are summarized (Fig. 1b). The mean number of cells per embryo on the afternoon of day 4 of pregnancy in lactating mice whelping after 17:00 h on day 0 was significantly lower in those animals receiving 125 μg RU486 on days 1–3 than that in control mice. Exogenous dexamethasone given concomitantly with RU486 failed to reverse its inhibitory effect. Furthermore, dexamethasone alone significantly decreased the mean number of cells per embryo. Since the embryos with fewer cells came from mice whelping earlier and not later on day 0, it seems likely that this difference was due instead to the earlier time of embryo recovery (morning versus afternoon of day 4). Thus, a delay of several hours in embryo recovery appears sufficient to alleviate the effect of time of whelping. This result in turn suggests that most embryos recovered on the morning of day 4 from mice whelping after

Table 4. Number of cells (mean ± SD) per embryo on day 3 or 4 of pregnancy from primiparous mice or mice mated following whelping before 17:00 h on day 0

Animals	Day 3	Day 4
Primiparous	6.8 ± 1.3 (66,6)[a]*	42.8 ± 14.2 (40,6)[a]
Postpartum	5.9 ± 1.6 (47,4)[b]	34.5 ± 10.5 (57,6)[b]

*(Number of embryos, number of mice).
[ab]Values with different superscripts within columns are significantly different ($P < 0.05$).

17:00 h on day 0 (Table 3) were about to enter mitosis. Thus, it is possible that an effect of RU486 in mice whelping after 17:00 h on day 0 (Table 3) was masked because the time of recovery (Goldbard and Warner, 1982) was such that the embryos from oil-treated mice were about to divide, whereas those from RU486-treated mice were at an earlier stage in their fifth cell cycle.

The influence of lactation on cell division in preimplantation mouse embryos was also investigated. The numbers of cells per embryo on the mornings of day 3 and 4 of pregnancy in primiparous mice were compared with those of lactating mice whelping before 17:00 h on day 0 (Table 4). On both days of pregnancy, there were significantly more cells per embryo from primiparous mice than from mice mated at *postpartum* oestrus and actively lactating.

Discussion

This study was carried out to investigate early embryo development in mice treated with the steroid antiprogestin RU486 under conditions in which oestrogen dominance, resulting from inhibition of the anti-oestrogen action of progesterone by RU486, was less likely to occur. Lactating mice were used in which tonic LH secretion and follicular activity is suppressed by suckling, as it is in rats (Taya and Sasamoto, 1980). Although post-coital treatment of lactating mice with RU486 was associated with significantly fewer embryos on day 4, oestrous uteri, cornified vaginal smears and spontaneous ovulation were not observed in these animals. Furthermore, this loss of embryos on day 4 may have been due to RU486 antagonism of progesterone-dependent closure of the uterine lumen (Martin *et al.*, 1970), in contrast to oestrogen-induced expulsion. It is therefore less likely that the effects of RU486 on early embryo development observed in lactating mice were due primarily to adverse effects of oestrogen; however, this possibility cannot be entirely ruled out.

Treatment of lactating mice mated at *postpartum* oestrus with the progestin and glucocorticoid antagonist RU486 was associated with growth retardation of mouse oviductal embryos. That is, oviductal embryos recovered on day 4 from lactating mice treated with RU486 had markedly fewer cells than did those of control mice. Furthermore, for mice whelping before 17:00 h on day 0, both doses of RU486 were effective, but the higher dose caused greater reduction in the number of cells than did the lower dose. These findings suggest that the retarded embryo development observed in primiparous mice in response to RU486 in this study and others (Roblero *et al.*, 1987; Yang and Wu, 1990; Vinijsanun and Martin, 1990) was not due to oestrogen dominance but was due instead to a direct effect of RU486.

RU486 clearly affected the cleavage rate of oviductal embryos in the present study; however, whether this effect was due to specific antagonism of progesterone action remains uncertain. That is, exogenous progestins, given at physiological doses, were unable to restore the number of cells per embryo to control values of RU486-treated mice. Consequently, the reduced rate of cell proliferation observed with RU486 treatment may have been due to a nonspecific effect of this steroid, such as membrane stabilization (Seeman, 1966). However Vinijsanun and Martin (1990) reported that exogenous progestins reversed the effects of a lower (100 μg), but not a higher (200 μg), dose of RU486 on embryo implantation, although they were also unable to reverse the effects of RU486 on embryo development. Furthermore, even pharmacological doses of progesterone (120 mg kg^{-1}) were unable to reverse the effects of RU486 on the peri-ovulatory FSH surges in rats (Knox and Schwartz, 1992). Thus, RU486 antagonism of uterine and hypothalamic progesterone receptors is not invariably reversed by concurrent treatment with progestins. Exogenous progestins might fail to override RU486 antagonism because of continued receptor occupancy by RU486, resulting from the strong affinity of this compound for the progesterone receptor and its long plasma half-life (Chrousos *et al.*, 1988), thus preventing effective competition by exogenous progestins.

RU486 is also an antagonist of both peripheral and central type II glucocorticoid receptors (Philibert, 1984). However, in the present study, exogenous dexamethasone (at the dose given) was also unable to restore the number of cells per embryo to control values in RU486-treated mice. Consequently, it cannot be concluded that the decrease in the number of cells observed in embryos from RU486-treated mice was caused by an antiglucocorticoid effect of this steroid. Treatment with dexamethasone (in the absence of RU486), however, was associated with significantly fewer cells per embryo. Van Der Schoot *et al.* (1990) reported that RU486 treatment of ovariectomized rats at 10 mg kg^{-1}, similar to those used in this study 2.5–10 mg kg^{-1}, was associated with increased synthesis and secretion of adenocorticotrophic hormone, presumably because of inhibition of glucocorticoid-induced negative feedback. This finding suggests that because of enhanced ACTH secretion, plasma glucocorticoid concentrations were higher in RU486-treated mice, as has been observed in rats (Okada *et al.*, 1988). There are many glucocorticoid effects, many of which exhibit differing dose–response relationships (Chrousos *et al.*, 1988). Differential dose–response relationships among central and peripheral glucocorticoid receptors could therefore lead to paradoxically enhanced expression of less sensitive glucocorticoid-responsive genes resulting from high concentrations of glucocorticoids in plasma (caused by RU486 antagonism of central receptors). Thus, depending upon the sensitivities of the glucocorticoid-responsive genes involved, RU486 and dexamethasone might have altered embryo growth rates, at least in part, through identical mechanisms. Moreover,

because both exogenous progestins and dexamethasone altered embryo growth, it may be that the effect of RU486 on embryo development resulted ultimately from antagonism of both progestin and glucocorticoid receptors. If this is the case, exogenous progestins and glucocorticoids given separately would not be expected to override the effects of RU486.

The addition of supraphysiological concentrations of D-glucose, but not L-glucose, to the culture medium has been shown to impair the *in vitro* development of mouse preimplantation embryos (Diamond *et al.*, 1990). The observed response to dexamethasone might therefore be due to its hyperglycaemic effects. It is relevant that lactation in rats is associated with high concentrations of plasma corticosterone (Voogt *et al.*, 1969), as well as suppressed oestrogen secretion. Whether the delayed embryo development in lactating mice observed in this study and others (Menke and McLaren, 1970) was due to the hormonal milieu during lactation or to timing of ovulation is currently unknown.

Finally, an anti-uterotrophic effect of RU486 in oestrogen-treated rats was reported by Van Der Schoot *et al.* (1990). This observation suggests that RU486 may, under some circumstances, exert anti-oestrogenic effects and that the present findings are due to oestrogen deficiency. However, RU486 in post-menopausal women treated with oestrogen appeared to act upon the endometrium as a progestin agonist in the absence of progestins and as a progestin antagonist in their presence (Gravanis *et al.*, 1985). The anti-uterotrophic effect of RU486 in rats may therefore be due to a progestagenic action and not due to an anti-oestrogenic action. Furthermore, Vinijsanun and Martin (1990) did not observe any oestrogenic or gestagenic activities of RU486 on mouse uterus.

The effect of exogenous progestins (in the absence of RU486) observed in this study to increase number of embryo cells nevertheless supports an anti-progestin effect of RU486 on embryo growth. Furthermore, exogenous progestin (medroxyprogesterone acetate) given on day 2 of pregnancy to primiparous mice undergoing either ovariectomy or sham-ovariectomy on day 2 was associated with significantly more cells per blastocyst on day 4 (D. A. Palmer and A. C. McRae, unpublished observations). Notably, insulin is mitogenic in mouse embryos *in vitro* (Gardner and Kaye, 1991) and early embryo development is retarded in experimental diabetes (Diamond *et al.*, 1989; Beeb and Kaye, 1991). Furthermore, peripheral insulin resistance and compensatory hyperinsulinaemia are typical of pregnancy in humans and rats (reviewed in Leturque *et al.*, 1987) and can be induced in rats by progesterone treatment (Sutter-Dub *et al.*, 1981). Thus, progesterone might alter cell proliferation in oviductal embryos indirectly by increasing concentrations of insulin in plasma.

In conclusion, these results provide further evidence that the growth rate of oviductal embryos in mice is influenced by the maternal hormone milieu. The mechanisms through which this milieu might affect growth rates require further investigation. However, the reproductive significance of this milieu is suggested by several studies in which a relationship between cleavage rate in preimplantation embryos and embryo survival has been observed (Bowman and McLaren, 1970; Warner *et al.*, 1991; Walker *et al.*, 1992).

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