Plasma concentrations of progesterone and 13,14-dihydro-15-keto prostaglandin F-2 α in pregnant, pseudopregnant and hysterectomized pseudopregnant mice

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Summary. Hysterectomized pseudopregnant mice had plasma progesterone concentrations on Days 6, 8, 10 or 12 after mating that were greater ($P < 0.05$) than those of intact pseudopregnant mice and lower ($P < 0.01$) than those of pregnant mice. Concentrations of 13,14-dihydro-15-keto-prostaglandin F-2 α on Days 6, 7, 8, 9 or 10 after mating were lower ($P < 0.05$) in pregnant and higher ($P < 0.01$) in intact pseudopregnant mice than in hysterectomized pseudopregnant mice.

The results support the conclusion that there are uterine and extra-uterine luteolytic mechanisms which are mediated by prostaglandin and are perhaps functional by Day 6 of pregnancy.

Introduction

Functional corpora lutea (CL) are essential throughout gestation for the maintenance of pregnancy in the mouse (Parkes, 1928). Both uterine (Bartke, 1970; Critser, Rutledge & French, 1980) and extra-uterine (Critser *et al.*, 1981) luteolytic mechanisms have been proposed which limit the functional lifespan of the CL. A successful pregnancy therefore requires a means of blocking these luteolytic effects and prolonging the duration of luteal function: this role has been attributed to factors from the pituitary (Gardner & Allen, 1942; Choudary & Greenwald, 1969a, b; Mednick, Barkley & Geschwind, 1980) and from the conceptus (Deanesly & Newton, 1940; Critser *et al.*, 1980). It has been suggested that by the 8th day of gestation a factor from the conceptus prevents the effect of the uterine luteolytic mechanism (Critser *et al.*, 1980); and that by the 10th day of gestation the conceptus produces a luteotrophin which prolongs the lifespan of the CL beyond that seen when the uterine luteolytic mechanism is blocked (Critser *et al.*, 1980). Previous reports have presented profiles of progesterone levels throughout pregnancy (Murr, Stabenfeldt, Bradford & Geschwind, 1974; Virgo & Bellward, 1974) but little information is available comparing progesterone concentrations in pregnant, pseudopregnant and hysterectomized pseudopregnant mice. Further, although prostaglandin (PG) F-2 α has been implicated as being the uterine luteolysin (Bartke, Merrill & Baker, 1972; Critser *et al.*, 1981) there are few data available concerning the production of prostaglandins *in vivo* during these reproductive states.

The present experiments were designed to evaluate plasma progesterone and 13,14-dihydro-15-keto-prostaglandin F-2 α (PGFM) concentrations in pregnant, pseudopregnant and hysterectomized pseudopregnant mice.

Materials and Methods

Six-week-old virgin mice, obtained from a local randomly bred colony (derived from the Institute of Cancer Research), were used in two experiments. Standard husbandry procedures under a controlled light:dark regimen (14 h light/24 h) were followed. Individual females (hysterectomized females and those to be pregnant with intact males; those to be pseudopregnant with vasectomized males) were caged with males for the duration of the experiment and checked daily for vaginal plugs. The day of vaginal plug was designated as Day 1 (for both pregnancy and pseudopregnancy). The interoestrous interval, an endpoint for both experiments, was determined from the finding of vaginal plugs. The interval included the first day of oestrus (Day 1 of pseudopregnancy) but did not include the day on which the subsequent vaginal plug was found. Animals were hysterectomized 1 week before the onset of an experiment. The surgery was performed under methoxyflurane anaesthesia through a midline abdominal incision under clean but not sterile conditions. Both oviducts were ligated, as was the cervix, and the entire uterus was removed.

Experiment I

In an experiment of 3×4 factorial design, 96 mice were randomly assigned to one of 12 groups to evaluate plasma progesterone levels. The factors tested were reproductive status (pregnant, pseudopregnant, or hysterectomized pseudopregnant) and day after mating (6, 8, 10 or 12). On the assigned day of blood sampling animals were lightly anaesthetized with methoxyflurane and mice were exsanguinated by cardiac puncture using 3 ml heparinized syringes. Plasma samples were stored frozen until assayed for progesterone (Critser *et al.*, 1980). In addition, 10 mice of each reproductive state were used to determine the interoestrous interval.

Experiment II

Plasma levels of PGFM, the primary metabolite of PGF-2 α , were measured in 120 mice in an experiment of 3×5 factorial design. The factors tested were reproductive status (pregnant, pseudopregnant, or hysterectomized pseudopregnant) and day after mating (6, 7, 8, 9 or 10). On the assigned day of sampling animals were lightly anaesthetized with methoxyflurane and exsanguinated by cardiac puncture using heparinized 3 ml syringes. Blood samples were immediately placed on ice, centrifuged and plasma was stored frozen until assayed for PGFM (Haning, Kieliszek, Alberino & Speroff, 1977). In addition, 10 mice of each reproductive state were used to assess the interoestrous interval.

Progesterone assay

Progesterone concentrations were measured by double antibody radioimmunoassay (Critser *et al.*, 1980) using a highly specific antiserum (Staigmiller, Short, Bellows & Carr, 1979). Plasma samples were extracted with hexane and not subjected to further purification. There were no reagent blanks and the efficiency of extraction averaged 81%. All samples were analysed at two dose levels with two tubes per dose. Validation of the assay was based on failure to find non-parallelism among unknowns and on the quantitative recovery of various amounts of progesterone (11.25 to 90.0 ng/ml) added to male mouse plasma (correlation between amount added and measured $r = 0.98$, $n = 4$). The sensitivity of the assay was 60 pg/tube; when 0.1 ml samples were used the lower limit of the assay was 3 ng/ml. In 21 assays a standard mouse plasma pool gave a progesterone value of 33.6 ng/ml with a coefficient of variability of 6.9%.

PGFM assay

PGFM was measured by radioimmunoassay using antibody specific for the metabolite and a modification of the procedures of Haning *et al.* (1977). Plasma samples were acidified (with 1 N-HCl) and extracted with ethyl acetate (average efficiency of extraction was 83%). The PGFM concentrations in samples subjected to column chromatography (SilicAR-CC4) were not lower than those of extracted, unchromatographed samples, indicating that there was no interference by PGEM, and routine chromatography was not employed. All samples were analysed at two dose levels with two tubes per dose. Validation of the assay was based on failure to find non-parallelism among unknowns and on the quantitative recovery of various amounts of PGFM (50 pg/ml to 1 ng/ml) added to charcoal-treated mouse plasma (correlation between amount added and measured $r = 0.96$, $n = 5$). The sensitivity of the assay was 20 pg/tube; when 0.4 ml samples were used the lower limit of the assay was 45 pg/ml. In 9 assays a standard mouse plasma pool gave a PGFM value of 526 pg/ml with a coefficient of variability of 8.8%.

Statistical analysis

The data were assessed by one- or two-way analysis of variance with preplanned orthogonal comparisons.

Results and Discussion

The concentrations of plasma progesterone in the three groups of mice are presented in Text-fig. 1. Hysterectomized pseudopregnant mice had plasma progesterone values which were greater ($P < 0.05$) than those of uterine intact pseudopregnant mice. The plasma progesterone values obtained from pregnant mice were similar to those reported by others (Murr *et al.*, 1974; Virgo & Bellward, 1974) and were greater ($P < 0.01$) than in mice of the other two groups. These data differ from the profiles of progesterone production reported for the rat (Pepe & Rothchild, 1974). Pseudopregnant hysterectomized rats have interoestrous intervals which are comparable to the length of gestation. Progesterone production in the hysterectomized pseudopregnant rat continues for nearly 20 days, similar in length (though not in magnitude) to progesterone production during pregnancy. In contrast, hysterectomized pseudopregnant mice had interoestrous intervals (15.3 ± 0.7 days) which were greater ($P < 0.01$) than those for pseudopregnant mice (11.8 ± 0.3 days) but less ($P < 0.01$) than those of pregnant mice

Text-fig. 1. Plasma progesterone concentrations of pregnant (■), pseudopregnant (▲) and hysterectomized pseudopregnant mice (●) on different days after mating (Day 1).

(19.3 ± 0.4 days) (see also Critser *et al.*, 1981). This is reflected in the pattern of plasma progesterone for these three reproductive states (Text-fig. 1) and is consistent with the existence of uterine (Bartke, 1970; Critser *et al.*, 1980, 1981) and extra-uterine (Critser *et al.*, 1981) luteolytic mechanisms.

Previous studies utilizing hysterectomy (Critser *et al.*, 1980) or injection of inhibitors of prostaglandin synthesis (Critser *et al.*, 1981) showed that the uterus exerted a luteolytic effect by the 8th day after mating and suggested that a factor from the conceptus must prevent the uterine luteolytic function before this time. The plasma progesterone levels in pregnant mice were greater ($P < 0.01$, determined by least significant difference test within day) than in pseudopregnant mice (both uterine intact and hysterectomized) as early as Day 6. This suggests that the conceptus had already influenced CL function by the 6th day of pregnancy. An effect of the blastocyst on luteal function before Day 6 has been attributed to an action of the trophoblast (Zeilmaker, 1968). Blastocysts (Day 3 and Day 4) transferred to the kidney capsule of cyclic mice prolonged the oestrous cycle, and induced spontaneous pseudopregnancies (9–13 days). However, Kirby (1965) failed to find an effect of the ectopically developing blastocyst on luteal function. Clarification of this contradiction and further characterization of plasma progesterone values during the first 6 days of pregnancy and pseudopregnancy is necessary to determine exactly when the influence of the conceptus on the CL is first manifested.

The plasma PGFM concentrations showed differences ($P < 0.01$) among treatment groups but no significant differences among days or any significant treatment $\times$ day interactions. The lack of significant differences among days was attributed in part to the large variation between animals within day. The data across days were pooled and the mean values are given in Table 1. Pseudopregnant, uterine-intact mice had plasma concentrations of PGFM which were greater ($P < 0.01$) than those of the other two groups and this difference is consistent with the interoestrous intervals obtained (Table 1) and with a PG-mediated uterine luteolytic mechanism in mice (Bartke, 1970; Bartke *et al.*, 1972; Critser *et al.*, 1980, 1981). However, even in the absence of the uterus (hysterectomized pseudopregnant) PGFM concentrations were greater ($P < 0.05$) than those found during pregnancy. These data support the premise that the extra-uterine luteolytic mechanism exerts its effect through prostaglandin mediated events (Critser *et al.*, 1981). Furthermore, the results suggest that the conceptus inhibits both uterine and extra-uterine PGF- 2α production. The source of the extra-uterine prostaglandin in mice is unknown. However, in primates, in which there is no uterine luteolytic mechanism, an intra-ovarian source of prostaglandins has been implicated in regression of CL (Challis *et al.*, 1976; Auletta, Agins & Scommegna, 1978). Even in species in which the uterus has a luteolytic function, intra-ovarian production of prostaglandins has been demonstrated (pig: Patek & Watson, 1976; cow: Shemesh & Hansel, 1975; guinea-pig: Sharma, Wilson & Pugh, 1976; rat: Poyser & Scott, 1980; Carminati, Luzzani, Soffientini & Lerner, 1975). It is tempting to

Table 1. Plasma levels of PGFM and interoestrous interval in different groups of mice

	Interoestrous interval (days)	PGFM (pg/ml)
Pregnant	19.4 ± 0.4 (10)	$\$209 \pm 19$ (40)
Hysterectomized pseudopregnant	$\dagger*15.6 \pm 0.6$ (10)	$\$ \dagger 389 \pm 28$ (40)
Pseudopregnant	$\dagger 12.1 \pm 0.2$ (10)	595 ± 45 (40)

Values are mean $\pm$ s.e.m. for the no. of mice indicated in parentheses.

* Value greater than that for pseudopregnant mice, $P < 0.01$.

$\dagger$ Value less than that for pregnant mice, $P < 0.01$.

$\dagger$ Value greater than that for pregnant mice, $P < 0.05$.

$\$$ Value less than that for pseudopregnant mice, $P < 0.01$.

speculate that the extra-uterine luteolytic mechanism in the mouse involves ovarian production of PGF-2 α .

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