Change in responsiveness of rabbit corpus luteum to prostaglandin F-2 α during pregnancy and pseudopregnancy

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Summary. Previous studies have suggested that prostaglandin F-2 α (PGF-2 α) may have a role in luteolysis in rabbits. Rabbits (4–6/group) were given a single injection of saline, or 100, 500 or 2500 μ g PGF-2 α (i.m.) on Day 7, 9, 12 or 15 of pregnancy or pseudopregnancy. Daily blood samples were taken via the marginal ear vein before and for 3 days after the PGF-2 α injection. Concentrations of serum progesterone were determined by radioimmunoassay in pseudopregnant rabbits. There were no significant differences between PGF-2 α -treated and control rabbits on Days 7 or 9. On Day 12 of pseudopregnancy, progesterone concentration was significantly ($P < 0.05$) lower in treated than in control rabbits, the effect being dose dependent. On Day 15 of pseudopregnancy, it was not possible to distinguish between controls and treated groups because luteolysis occurred in all rabbits.

In contrast, on Days 7 and 9 of pregnancy, the concentration of progesterone in treated groups was lower than in the control groups ($P < 0.05$), the effect being dose dependent. This difference was maintained throughout the sampling period and resulted in termination of pregnancy. By Day 12 of pregnancy, the response to PGF-2 α was transient, with a significant decline in progesterone for only 2 days, followed by a return to control concentrations and normal delivery of litters. On Day 15 of pregnancy, no treatment with PGF-2 α significantly altered progesterone concentration. Our results indicate that (i) there is a change in responsiveness to PGF-2 α over time during pregnancy and pseudopregnancy and (ii) there are differences between pregnancy and pseudopregnancy in the pattern of responsiveness.

Keywords: corpus luteum; prostaglandin F-2 α ; pregnancy; pseudopregnancy; rabbit

Introduction

The corpus luteum (CL) of pregnant rabbits produces high concentrations of progesterone for 30–32 days, but in pseudopregnancy the CL is functional for only 16–18 days. Maternal recognition of pregnancy occurs on Day 12 *post coitum* (Nowak & Bahr, 1983), and it is then that the CL of pseudopregnant rabbits begins to regress. The rabbit conceptus prolongs the life of the CL through an unknown mechanism, but probably by production of luteotrophic and/or antiluteolytic factors.

The factors which cause regression of the CL of pseudopregnancy are not fully understood. The uterus may have a role in luteolysis; pseudopregnancy was partially extended for 6–8 days by hysterectomy (Scott & Rennie, 1970; Hilliard, 1973). Uterine PGF-2 α is responsible for luteolysis in many species (see Goldberg & Ramwell, 1975 and Knickerbocker *et al.*, 1988 for reviews). An intraovarian mechanism may also be involved in luteolysis, as the ovary is a source of prostaglandins (Miller *et al.*, 1983; Schlegel *et al.*, 1988). Administration of exogenous PGF-2 α caused luteolysis in pseudopregnant rabbits (Gutknecht *et al.*, 1972; Keyes & Bullock, 1974;

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Carlson & Gole, 1978; Kehl & Carlson, 1981) and pregnant rabbits (Koering & Kirton, 1973). Inhibition of prostaglandin synthesis by indomethacin extended pseudopregnancy (O'Grady *et al.*, 1972) and delayed parturition in pregnant rabbits (Nathanielsz *et al.*, 1972).

Prostaglandin F-2 α has been measured in uterine venous plasma in rabbits (Lytton & Poyser, 1982). Concentrations in the uterine vein are similar in pregnant and pseudopregnant rabbits until Day 17, when the concentrations in pseudopregnant rabbits are significantly greater than those in the pregnant doe (Lytton & Poyser, 1982). On Day 15, concentration of PGF-2 α in the ovaries is significantly higher in pseudopregnant than in pregnant rabbits (Schlegel *et al.*, 1988). Concentrations of progesterone and PGF-2 α during pseudopregnancy are inversely related except that the decline in progesterone occurs before the increase in PGF-2 α , which might suggest that PGF-2 α itself does not initiate luteolysis. Alternatively, there may be a change in the responsiveness of the CL to the effects of PGF-2 α over time, such that even low concentrations of PGF-2 α can initiate luteolysis.

This experiment determined whether the responsiveness of the CL to PGF-2 α changes during pseudopregnancy and pregnancy in rabbits, especially in relation to the time of maternal recognition of pregnancy.

Materials and Methods

Animals and treatment

Does were housed individually in an indoor facility. Oestrous does were mated (designated Day 0) to either an intact buck to induce pregnancy or to a vasectomized buck to induce pseudopregnancy. Animals were randomly grouped (4–6 rabbits/group) in a 4 $\times$ 4 $\times$ 2 factorial design, with day of injection, dose of PGF-2 α and pregnancy status as the factors. On the day of treatment, a blood sample was collected via the marginal ear vein followed by a single intramuscular injection of 0 (saline), 100, 500 or 2500 μ g PGF-2 α (Lutalyse: UpJohn Company, Kalamazoo, MI, USA). These doses were chosen because previous studies in rabbits have demonstrated that injection of 1–2 mg PGF-2 α induces luteolysis (Gutknecht *et al.*, 1972; Koering & Kirton, 1973; Keyes & Bullock, 1974; Carlson & Gole, 1978). Blood samples were taken at intervals of 24 h for 3 days. Each of the doses of PGF-2 α was given on Days 7, 9, 12 and 15 of pregnancy and pseudopregnancy. Effects of treatment were determined by changes in concentrations of serum progesterone.

Hormone measurement

Serum was assayed for progesterone by radioimmunoassay, according to the procedure of Bahr *et al.* (1980). Progesterone was extracted from the serum with petroleum ether and the efficiency of the extraction procedure was monitored by addition of [3 H]progesterone. Each sample was corrected for recovery of label. Intra- and interassay coefficients of variation were 8.6 and 10.4%, respectively.

Statistical analysis

Significant differences were determined by multivariate repeated-measures analysis of variance. Progesterone concentrations in response to each dose of PGF-2 α were compared with controls on each day of treatment. Differences were considered significant if $P < 0.05$. Multiple linear regression of dose on progesterone was also done to determine whether effects of PGF-2 α were dose dependent. Regression lines with a negative slope and correlation of > 0.3 and $P < 0.05$ were considered significant. All statistics were performed using the Systat statistics package (Wilkinson, 1988).

Results

Pseudopregnancy

On Days 7 and 9 of treatment (Fig. 1a, b), there was no effect of PGF-2 α on progesterone ($P > 0.05$) in pseudopregnant rabbits; on Day 12 (Fig. 1c), progesterone concentrations were significantly lower than in controls 24 h after injection with 2500 μ g of PGF-2 α ($P < 0.1$). This

Fig. 1. Concentrations (mean $\pm$ SEM) of serum progesterone after treatment of rabbits with 0 ($\square$), 100 (▨), 500 ($\blacksquare$) or 2500 (▩) μg prostaglandin F-2a on Day 7 (a), 9 (b), 12 (c) or 15 (d) of pseudopregnancy; significant difference from control at * $P < 0.05$ and ** $P < 0.01$, $n = 4-6$.

difference was also observed on Day 2 after treatment ($P < 0.05$), but was absent by Day 3, because control CL were also regressing at this time. There was a significant relationship between dose of PGF-2a and response of progesterone concentration on Day 12 after treatment ($P < 0.05$). By Day 15 of pseudopregnancy (Fig. 1d), there was no longer a significant effect of PGF-2a on progesterone, which declined in all groups over time because of inherent luteolysis.

Pregnancy

On Day 7 of pregnancy (Fig. 2a), concentrations of progesterone were lower in rabbits treated with PGF-2a than in the controls. Progesterone concentration was significantly lower than in controls ($P < 0.05$) on all days after treatment in rabbits given 2500 μg PGF-2a and on Days 2 and 3 after treatment in those treated with 500 μg of PGF-2a. The effect of dose of PGF-2a on progesterone was highly significant ($P < 0.005$). Similarly, treatment with 2500 μg PGF-2a on Day 9 of pregnancy (Fig. 2b) significantly lowered progesterone concentration compared with controls ($P < 0.05$). Regression analysis showed that progesterone concentrations were dependent on dose of PGF-2a ($P < 0.001$). On Days 7 and 9, significant decreases in progesterone resulted in termination of pregnancy within 24–48 h. Termination of pregnancy was observed as expulsion of fetal membranes or presence of bloody discharge either in the cage or on the doe. On Day 12 of pregnancy (Fig. 2c), 2500 μg PGF-2a transiently reduced progesterone concentration for 2 days after administration ($P < 0.05$). However, by 3 days after treatment, progesterone had returned to control

Fig. 2. Concentrations (mean $\pm$ SEM) of serum progesterone after treatment of rabbits with 0 ($\square$), 100 ($\square$), 500 ($\blacksquare$) or 2500 ($\boxtimes$) μ g prostaglandin F-2 α on Day 7 (a), 9 (b), 12 (c) or 15 (d) of pregnancy; significant difference from control at * $P < 0.05$ and ** $P < 0.01$, $n = 4-6$.

concentrations ($P > 0.05$) and all does delivered normally 30–32 days *post coitum*. By Day 15 of pregnancy (Fig. 2d), no treatment with PGF-2 α significantly altered progesterone.

Discussion

This study confirms previous reports of the ability of exogenous PGF-2 α to induce luteolysis in pseudopregnant rabbits (Gutknecht *et al.*, 1972; Keyes & Bullock, 1974; Carlson & Gole, 1978; Kehl & Carlson, 1981). In three previous reports, when PGF-2 α treatments were initiated on Day 9 of pseudopregnancy, daily injections of 1–2 mg PGF-2 α for 3 days (Keyes & Bullock, 1974), injections of 500 μ g PGF-2 α twice a day for 4 days (Gutknecht *et al.*, 1972) or constant infusion of 25 μ g PGF-2 α into the jugular vein for 6 h (Kehl & Carlson, 1981) induced luteolysis after 2 days of treatment. Unlike the present study, these authors observed luteolysis in Day 9 pseudopregnant does. However, in each of these studies, duration of exposure to PGF-2 α was potentially much longer than in the current study. Higher effective doses of PGF-2 α may be needed for an effect early in pseudopregnancy because the lungs are capable of metabolizing $>90\%$ of the prostaglandins in a single pass (Ferreira & Vane, 1967; Piper *et al.*, 1970). There is evidence that the PGF-2 α metabolite, 13,14-dihydro-PGF-2 α , is capable of inducing luteolysis in pseudopregnant rabbits (Kehl & Carlson, 1981). If the metabolite is the factor directly responsible for luteolysis, this may explain why a high dose of PGF-2 α may be necessary for luteolysis in rabbits, compared with farm animals such as sheep, in which PGF-2 α itself is known to be the luteolytic agent.

The pattern of response to PGF-2 α in pseudopregnant rabbits supports our original hypothesis that an increase in responsiveness to PGF-2 α might mediate luteolysis. The pseudopregnant rabbits were unresponsive to PGF-2 α before Day 12, when there was a significant dose-dependent effect of PGF-2 α on progesterone concentration. Pigs are also unresponsive to PGF-2 α before Day 12 of the oestrous cycle and this can be correlated with a lack of luteal prostaglandin receptors (Gadsby *et al.*, 1990). Further experiments must be done to determine whether the CL of pseudopregnancy develops responsiveness to PGF-2 α as a result of an increase in PGF-2 α receptors.

The pattern of response to PGF-2 α in pregnant rabbits develops very differently. PGF-2 α had significant dose-dependent effects on Days 7 and 9 of pregnancy and resulted in termination of pregnancy. On Day 12 of treatment, there was a transient decline in progesterone and all does delivered litters normally. By Day 15, there were no effects of PGF-2 α treatment. In effect, pregnant rabbits became less responsive to PGF-2 α and pseudopregnant rabbits more responsive over time. Further experiments must be done to ascertain why pregnant does were responsive to PGF-2 α before Day 12. The conceptus may decrease responsiveness through an effect on the number of PGF-2 α receptors. Alternatively, PGF-2 α may have a deleterious effect on the fetus before complete implantation on Day 12. However, we would expect that fetal death prior to maternal recognition of pregnancy would not induce luteolysis, as removal of the conceptus at these times does not cause a rapid decline in progesterone (Nowak & Bahr, 1983).

By Day 15 of pregnancy, there was no effect of PGF-2 α on progesterone, suggesting that after maternal recognition of pregnancy, the fetal-placental unit protects the CL from luteolysis. In other species, there is evidence that the fetal-placental unit maintains the CL by suppressing either the production or the responsiveness to PGF-2 α , or both. In ewes, the conceptus produces a trophoblastic protein that suppresses production of PGF-2 α by the uterus (Fincher *et al.*, 1986; Knickerbocker *et al.*, 1986). The sheep conceptus also decreases the responsiveness to PGF-2 α , administration of PGF-2 α being less effective in pregnant than in nonpregnant ewes (Inskeep *et al.*, 1975; Silvia *et al.*, 1986) and the CL being less responsive to PGF-2 α after maternal recognition of pregnancy (Silvia & Niswender, 1986). Recently, a protein other than oTP-1 was identified in the sheep trophoblast that is able to inhibit the luteolytic action of PGF-2 α in large luteal cells *in vitro* (Wiltbank *et al.*, 1990). The rabbit conceptus may act through a similar mechanism to protect the CL from PGF-2 α after maternal recognition of pregnancy.

Our data support the hypothesis that one role of the rabbit conceptus may be to modify ovarian responsiveness to PGF-2 α . Our results also suggest that one possible mechanism for luteolysis in pseudopregnant rabbits is an increase in responsiveness to PGF-2 α . The conceptus appears to change the responsiveness of the CL as early as Day 7, even though its presence is apparently not necessary for maintenance of the CL until maternal recognition of pregnancy on Day 12. It may be necessary to redefine maternal recognition of pregnancy, because CL function in rabbits differs in pregnant and pseudopregnant rabbits before Day 12. Maternal recognition of pregnancy may involve gradual physiological changes, the expression of which is most evident on Day 12 of pregnancy.

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