Maternal recognition of pregnancy in the rabbit

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Summary. Conceptuses were removed by extrusion through incisions in the uterus on Days 11, 12 and 18 *post coitum* (*p.c.*). Pseudopregnant does at Days 11 and 12 and pregnant does at Day 18 were sham-operated and served as controls. Blood samples were collected before and daily for 3 days after conceptus removal. Serum progesterone profiles of does whose conceptuses were removed on Day 11 *p.c.* were identical to those of intact pseudopregnant and sham-operated pseudopregnant controls. Conceptus removal on Days 12 or 18 *p.c.* resulted in a precipitous decline ($P < 0.01$) in progesterone levels within 48 h. LH levels were low (< 1 ng/ml) in all groups before and after surgery and there were no significant differences between treated and control rabbits. These data demonstrate that the maternal recognition of pregnancy occurs by Day 12 of gestation and that conceptus removal does not result in an alteration in serum LH levels.

Introduction

The precise time at which maternal recognition of pregnancy occurs in the rabbit has not been identified. The corpus luteum of the pregnant doe requires the presence of the conceptus during the second half of gestation to maintain progesterone production for the normal length of pregnancy (Chu, Lee & Yau, 1945; Porter, Becker & Csapo, 1968; Holt & Ewing, 1974; Lanman & Thau, 1979). The rabbit placenta itself does not synthesize progesterone (Wilson & Siiteri, 1973; Holt & Ewing, 1974; Thau & Lanman, 1974). Browning & Wolf (1981) demonstrated that dependence on the conceptus develops between Days 10 and 18 of gestation. Lanman & Thau (1979) showed that treatment with oestrogen or anterior pituitary extracts prevents luteal regression following removal of the fetal placenta on Day 18 of pregnancy and concluded that the fetal placenta synthesizes a substance which is biologically similar to one of the anterior pituitary hormones or has a stimulatory effect on the pituitary.

The purposes of the present study were (1) to identify the day on which maternal recognition of pregnancy occurs in the rabbit; and (2) to determine whether removal of the conceptus causes a change in serum LH concentrations.

Materials and Methods

Animals

Virgin New Zealand or California does weighing 4.0–5.0 kg were used. Rabbits were housed individually in a temperature- and light-controlled building, with 14L:10D (lights on 06:00 h). Pregnancy was induced by mating oestrous does twice to bucks of proven fertility. Pseudopregnancy was induced by injecting (*i.v.*) oestrous rabbits with 50 i.u. hCG (Ayerst Laboratories, New

York, U.S.A.). The day of mating or day of injection was designated as Day 0 of pregnancy or pseudopregnancy, respectively.

Experimental design

Conceptuses were removed from 5 or 6 does on Days 11, 12 and 18 *post coitum* (*p.c.*). Animals were anaesthetized with an i.m. injection of ketamine hydrochloride, 44 mg/kg (Bristol Laboratories, Syracuse, NY, U.S.A.) and acepromazine maleate, 0.5 mg/kg (Ayerst). Ovaries and uterine horns were exposed through a mid-ventral incision and the number of corpora lutea (CL) and fetuses was recorded. Small longitudinal incisions were made along the antimesometrial walls of the uterine horns at the implantation sites. Exposed fetuses were extruded with gentle digital pressure and fetal placentas were removed by gently peeling them from the endometrium.

The controls for the pregnant does on Days 11 and 12 were pseudopregnant does, 5 on Day 11 and 5 on Day 12. These animals were operated on in the same manner as the pregnant animals. Incisions were evenly spaced along the antimesometrial walls of the uterine horns in a number equal to the number of CL. Pseudopregnant does were used as controls to determine whether removal of conceptuses from pregnant does would result in CL function similar to that occurring in pseudopregnancy. In addition, 5 intact pregnant animals were bled daily for measurement of progesterone and LH concentrations during normal gestation. The controls for the Day-18-pregnant does were 5 Day-18-pregnant rabbits in which small evenly spaced incisions were made along the antimesometrial walls of the uterine horns; these animals were allowed to litter. On the day of surgery and daily for 3 days, between 08:00 and 09:00 h, animals were bled via the marginal ear vein. Blood samples were allowed to clot overnight at 4°C. The serum was removed and stored at -20°C until assayed for progesterone and LH.

Assays

Progesterone. Progesterone was measured by the specific RIA described and validated previously (Bahr, Gardner, Schenck & Shahabi, 1980). Progesterone was extracted from serum before assay with petroleum ether. The extraction efficiency was monitored by the addition of [³H]progesterone and the mean recovery was 82%. The progesterone antiserum obtained from Dr O. D. Sherwood (GS-253) was generated against progesterone-11-hemisuccinate:BSA. The antiserum cross-reacts 22% with 11 α -hydroxyprogesterone and <1% with other steroids. The lower limit of sensitivity for the assay was 25 pg per tube. The intra- and interassay variations were 6.2% and 12.3%, respectively.

LH. An homologous rabbit LH assay was used. The iodination preparation was rabbit LH (AFP-559-B) obtained from Dr A. F. Parlow, University of California, Los Angeles, CA. The procedure used for iodination of rabbit LH was a modification of the method of Greenwood, Hunter & Glover (1963). Briefly, 1.0 mCi Na¹²⁵I (New England Nuclear, Boston, MA, U.S.A.) was added to 2.5 μ g rabbit LH in 50 μ l 0.5 M-phosphate buffer (pH 7.5). The reaction was initiated by the addition of 10 μ l chloramine-T solution (250 mg/100 ml 0.05 M-phosphate buffer). After 1 min and 45 sec, 100 μ l sodium metabisulphite (62.5 mg/100 ml 0.05 M-phosphate buffer) were added, followed by 50 μ l 1% KI. Labelled hormone was purified by gel filtration on Sephadex G-75.

The procedure used in the double-antibody radioimmunoassay was similar to that reported by Niswender, Reichert, Midgley & Nalbandov (1969), except that ¹²⁵I was used as the radioactive label. The anti-rabbit LH antiserum (AFP-8-1-28), at a final dilution of 1:1 800 000, exhibited a mean binding of 28.3%. Duplicate samples of 500 μ l serum were assayed. Rabbit LH (AFP-599-B) was used as the standard. When various volumes (100, 200, 300 μ l) of serum from ovariectomized rabbits were assayed in triplicate, the LH values were 2.96 ± 0.37 , 2.39 ± 0.30 , and 2.29 ± 0.12 ng/ml, respectively. Recovery of unlabelled ligand was checked by adding 0.60, 1.25, and 2.50 ng rabbit LH to 100 μ l serum of ovariectomized rabbits. The percentages recovered were 96.6 ± 11.54 , 100.8 ± 7.6 , and 106.6 ± 10.8 , respectively. The lower limit of sensitivity for the assay was 100 pg. Inter- and intra-assay coefficients of variation were 9.7% and 8.1%, respectively.

Statistics

Data were analysed by split-plot analysis of variance using the method of Gill & Hafs (1971). When appropriate, Student's *t* test was employed. Differences were considered significant if $P < 0.05$.

Results

Serum progesterone profiles of animals whose conceptuses were removed on Day 11 *p.c.* were not different from those of sham-operated pseudopregnant control animals (Text-fig. 1a). However, the progesterone values of both groups were different from those of intact pregnant rabbits on Day 14 ($P < 0.01$). When conceptuses were removed on Day 12 (Text-fig. 1b) or Day 18 (Text-fig. 1c), serum progesterone concentrations declined within 48 h from preoperative levels of 10–16 ng/ml to < 2.5 ng/ml. The decline in serum progesterone levels occurred more rapidly in Days 12 and 18 *p.c.* animals with conceptuses removed than in the corresponding control animals ($P < 0.01$).

Serum LH concentrations were similar in all 3 groups (Text-figs 1a, b, c). There were no significant changes in LH levels over time for any of the groups.

The mean number of CL per rabbit did not differ between pseudopregnant or pregnant rabbits or between treated and intact control rabbits. Moreover, treated and intact control rabbits had the same number of conceptuses.

Discussion

Our significant findings are that maternal recognition of pregnancy in the rabbit occurs by Day 12 of gestation and that removal of the conceptus does not result in a change in serum LH levels. Removal of conceptuses on Days 12 or 18 *p.c.* resulted in a dramatic decline in serum progesterone levels to non-pregnant values within 48 h. In contrast, removal of conceptuses on Day 11 *p.c.* did not cause a precipitous decline in serum progesterone levels. Rather, the levels declined at a rate similar to that seen in pseudopregnant does. At 72 h after surgery, progesterone levels were still within normal range for pseudopregnancy and pregnancy (~ 6 ng/ml).

The CL of Day-11-pregnant does apparently have not as yet become dependent on the presence of the conceptus (fetus and placenta), and so removal of the conceptus results in progesterone levels similar to those of pseudopregnancy. On the other hand, the CL of Day-12-pregnant does has apparently become dependent on the presence of the conceptus. The CL of the Day-12-pregnant doe begin to regress almost immediately after removal of the conceptus; progesterone concentrations were equivalent to non-pregnant values by 48 h after surgery.

The day of maternal recognition of pregnancy is coincident with maximum progesterone production by the CL. After ovulation, progesterone levels increase steadily in pseudopregnant and pregnant does until Day 12 when they begin to decline in pseudopregnant does, but remain fairly constant in pregnant does until several days before parturition (Browning & Wolf, 1981). The presence of the conceptus is necessary by Day 12 of gestation to rescue the CL of pregnancy and prevent luteal regression.

Serum LH levels from does in which the conceptuses were removed were low throughout the period of sampling and were not different from those of the controls, suggesting that the conceptus does not alter LH levels. However, the overall pattern of LH secretion may not be revealed by samples taken only once daily because LH is released in a pulsatile manner.

The serum LH levels reported here are in agreement with those reported by Osteen & Mills (1979) for the pregnant doe. They measured an LH ovulatory surge of ~ 12 ng/ml after which the LH levels decreased to basal values of < 1 ng/ml and remained at these levels throughout pregnancy. The LH values in the present study were low (< 1 ng/ml) and similar for pregnant and pseudopregnant does. The very low levels of LH during a time of high progesterone output from the

Text-fig. 1. Serum concentrations of progesterone and LH in rabbits from which the conceptuses have been removed on (a) Day 11, (b) Day 12 and (c) Day 18 of pregnancy. Each bar represents the mean $\pm$ s.e.m. for 5 (a) or 6 (b, c) rabbits. In (a) values for intact pregnant rabbits were higher ($P < 0.01$) than those of the other 2 groups on Day 14. In (b) the progesterone profiles for the pregnant rabbits from which the conceptuses were removed were different ($P < 0.01$) from those of the other 2 groups. In (c) the progesterone profiles of the intact pregnant rabbits were different ($P < 0.01$) from those of the pregnant rabbits from which the conceptuses were removed.

CL support the work of Hilliard, Schally & Sawyer (1971) who found that ovulation in the doe, in response to LH-RH infusion into the anterior pituitary, could be blocked by administration of progesterone. Progesterone appears to suppress pituitary LH secretion during pregnancy and pseudopregnancy.

Nonetheless, the rabbit CL does require LH support. Hypophysectomy causes CL regression after collapse of the ovarian follicles (Foster, Foster & Hisaw, 1937; Robson, 1947; Spies, Hilliard & Sawyer, 1968). While low levels of LH are luteotrophic in the doe (Rennie, 1968; Hilliard, Saldarini, Spies & Sawyer, 1971), high doses of LH are luteolytic (Stormshak & Casida, 1965) because they ovulate the follicles which are the source of oestradiol needed for CL maintenance in the rabbit. Hunzicker-Dunn & Birnbaumer (1976) reported that levels of LH-stimulated adenylyl cyclase activity in the CL and serum progesterone were highly correlated after Day 10 of pregnancy, suggesting that LH may play an important role in regulating progesterone synthesis.

The effect of the conceptus on the rabbit CL by Day 12 of pregnancy may be direct or indirect. The nature of the signal emanating from the conceptus and its site of action are not known. Although LH does not appear to be involved, the conceptus may modify FSH or prolactin secretion or synthesize a gonadotrophin-like substance.

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