Luteotrophic effect of the rabbit blastocyst

M. M. Singh* and C. E. Adam

*A.R.C. Institute of Animal Physiology, Animal Research Station,
307 Huntingdon Road, Cambridge CB3 0JQ, U.K.*

Summary. Progesterone concentrations in peripheral plasma of 27 rabbits were determined by radioimmunoassay daily from Days 1 to 7. The rate of increase was significantly higher in pregnant than in pseudopregnant rabbits from Days 5 to 7 ($P < 0.02$), but not before Day 5. Transfer of Day-4 or -5 blastocysts to synchronous, pseudopregnant recipients resulted in a significant rise in progesterone levels in comparison with those in sham-operated controls ($P < 0.01$) or pseudopregnant does ($P < 0.01$).

Introduction

Fuchs & Beling (1974) reported that plasma progesterone levels were significantly higher in pregnant than in pseudopregnant rabbits from Day 5 up to Day 10. They suggested, therefore, that the ovary recognizes the presence of blastocysts before implantation (Day 7), thereby implying that the blastocyst can exert a luteotrophic influence. This concept gained support from the finding that, when blastocysts were transferred to pseudopregnant recipients on Days 4 or 5, plasma progesterone concentrations were increased significantly compared with levels in intact, pseudopregnant controls (Fuchs, Beling & Park, 1974). However, Holt, Heise, Wilson & Keyes (1976) found that serum progesterone concentrations were not different in pregnant and pseudopregnant does before Day 7, although they were higher 4 or 5 days later.

The present work was undertaken in an attempt to clarify these apparently conflicting findings.

Materials and Methods

Adult female rabbits, weighing 3.5–4 kg and from our own colony, were mated with fertile or vasectomized (sterile) males. All the does were given an intravenous injection of 25 i.u. hCG (Gonadotrophon: Paines & Byrne Ltd, Greenford, Middlesex) shortly after mating (Day 0) and allocated to treatment groups as follows. Group A contained 5 does mated with fertile males. Groups B–F contained 22 does mated with sterile males: Group B consisted of 4 does; the 6 pseudopregnant does in each of Groups C and E each received 4–8 blastocysts from synchronized donors on Day 4 (Group C) or Day 5 (Group E); the 3 pseudopregnant does in each of Groups D and F were treated similarly on Days 4 and 5 respectively but received no blastocysts (sham transfer).

For transfer of blastocysts or saline only the animals in Groups C–F were anaesthetized with sodium pentobarbitone (Nembutal: Abbott Laboratories, Queensborough, Kent) given intravenously and halothane (Fluothane: Imperial Chemical Industries, Macclesfield, Cheshire) in nitrous oxide via a face mask. The ovaries were examined before transfer to verify that ovulation had occurred. The blastocysts were obtained from donors on Days 4 or 5 by flushing the excised uteri with 5 ml 0.9% (w/v) NaCl solution at 37°C and were transferred almost immediately to both uterine horns.

Animals in Groups A and B were examined by laparotomy on Day 10 to establish the presence of conceptuses and/or corpora lutea.

Blood samples (2–3 ml) were collected daily from a marginal ear vein into heparinized tubes from the rabbits in all groups on Days 1–7 inclusive; on the day of laparotomy, samples were taken before any other treatment. After centrifugation of the blood for 10 min at 1600 g, the plasma was removed

* Present address: Division of Endocrinology, Central Drug Research Institute, Lucknow, India.

and stored initially at -20°C and then at -70°C for not more than 3 months before assay. The plasma was extracted with *n*-hexane ($>90\%$ efficiency) and the progesterone content of the extract was measured by the radioimmunoassay method described by O'Grady, Caldwell, Auletta & Speroff (1972). The antiserum was ICI 465/5 and its cross-reactivity, estimated at 50% displacement of labelled progesterone, was less than 1.3% for all the major steroids, except for pregnenolone (3.4%), as described by Newcomb, Booth & Rowson (1977). Each assay contained three blanks and 6 standards (0.1–4 mg progesterone) in duplicate. Approximately 20% of the extracted samples were duplicated. The limit of sensitivity of the assay was 0.01 ng/ml.

The data were examined by carrying out analyses of variance of the progesterone values for Days 1–7 individually, and also by conducting similar analyses on functions of the progesterone values over time, e.g. the rate of increase per day over specified periods.

Results

In the 5 Group-A females the mean litter size was 7.6 (range 3–12) whilst in females of Groups C and E, which received means of 6.3 and 5.3 blastocysts, it was 4.2 (1–8) and 2.1 (1–3) respectively.

The concentrations of plasma progesterone in does in Groups A–F during Days 1 to 7 are given in Table 1. The overall mean value (Days 1–7) for the pregnant does (Group A) was significantly higher than that for the pseudopregnant does (Group B). The estimated difference was 1.89 ± 0.41 pg. The difference remained quite small for the first 5 days but thereafter the values diverged. The rate of increase of progesterone concentration during Days 5–7 was 2.21 ± 0.64 pg/day (Group A) and 0.08 ± 0.08 pg/day (Group B) ($P < 0.02$).

Transfer of blastocysts on Days 4 or 5 (Groups C and E) resulted in a significant increase in plasma progesterone concentration compared with the levels found in the sham-operated (Groups D and F, $P < 0.01$) or pseudopregnant does (Group B, $P < 0.01$). This effect of the presence of blastocysts was discernible within 24 h of transfer (Table 1). A comparison of the pseudopregnant (Group B) with the sham-operated animals (Groups D and F) for Days 5–7 failed to reveal any significant differences.

Table 1. Plasma progesterone concentrations (ng/ml, mean $\pm$ s.e.m.) in pregnant and pseudopregnant rabbits

Group	Treatment	No. of does	Days						
			1	2	3	4	5	6	7
A	Pregnant	5	1.24 $\pm$ 0.16	2.26 $\pm$ 0.25	3.90 $\pm$ 0.37	4.72 $\pm$ 0.26	6.22 $\pm$ 0.38	7.06 $\pm$ 0.69	10.84 $\pm$ 1.23
B	Pseudopregnant	4	0.75 $\pm$ 0.10	1.35 $\pm$ 0.22	2.03 $\pm$ 0.37	4.00 $\pm$ 0.49	4.90 $\pm$ 0.94	4.93 $\pm$ 0.80	5.05 $\pm$ 0.82
C	Day 4 blastocysts transferred	6	1.30 $\pm$ 0.13	2.88 $\pm$ 0.61	3.93 $\pm$ 0.40	4.37 $\pm$ 0.38	7.08 $\pm$ 0.74	7.42 $\pm$ 0.71	8.58 $\pm$ 0.65
D	Sham transfer Day 4	3	1.20 $\pm$ 0.60	1.37 $\pm$ 0.38	2.47 $\pm$ 0.57	4.33 $\pm$ 0.70	5.20 $\pm$ 0.60	5.43 $\pm$ 0.70	5.70 $\pm$ 0.55
E	Day 5 blastocysts transferred	6	1.08 $\pm$ 0.12	1.82 $\pm$ 0.14	2.88 $\pm$ 0.20	4.92 $\pm$ 0.39	5.77 $\pm$ 0.40	8.40 $\pm$ 0.67	8.77 $\pm$ 0.66
F	Sham transfer Day 5	3	0.73 $\pm$ 0.15	2.57 $\pm$ 0.27	3.63 $\pm$ 0.72	3.27 $\pm$ 0.32	5.93 $\pm$ 0.43	6.23 $\pm$ 0.34	6.47 $\pm$ 0.88

Discussion

Our finding that plasma progesterone concentrations in pregnant does were significantly higher than in pseudopregnant does over the period Days 5–7 *post coitum* and that 4- and 5-day blastocysts exerted a luteotrophic effect agree closely with the earlier reports of Fuchs & Beling (1974) and Fuchs

et al. (1974), but not with those of Holt *et al.* (1976). By using a radioimmunoassay rather than a competitive protein binding method, as used by Fuchs & Beling (1974), for measuring progesterone values and by establishing that the 'pseudopregnant' rabbits did have functional corpora lutea and the pregnant rabbits viable embryos, we have accounted for the major sources of variation in their work as advanced by Holt *et al.* (1976). Additionally, we have characterized the role of the blastocyst more specifically by demonstrating the lack of effect of a sham operation in elevating progesterone levels before implantation.

There is disagreement about the existence and nature of the luteotrophic signal from the blastocyst. A gonadotrophin similar to hCG or LH has been found in blood plasma, uterine fluid and blastocysts of the rabbit before implantation (Fujimoto, Woody & Dukelow, 1973; Haour & Saxena, 1973), and assayed in blastocysts (Fujimoto, Euker, Riegel & Dukelow, 1975). However, Sundaram, Connell & Passantino (1975) and Holt *et al.* (1976) have failed to demonstrate hCG-like activity or LH-like hormone in the preimplantation blastocyst. The present findings should serve to stimulate further investigation of this question.

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