Embryonic stimulation of ovine and bovine corpora lutea

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Summary. Preimplantation embryos, recovered from sheep on Days 13–15 of pregnancy, were incubated with luteal tissue from cyclic ewes. Extracts of ovine embryos were incubated with luteal tissue from cyclic Holstein cows. Embryos and embryo extracts significantly increased progesterone production by ovine and bovine luteal tissue *in vitro*. The embryos themselves did not produce measurable amounts of progesterone. We suggest that the ovine preimplantation embryo produces a substance with luteotrophic properties which contributes to the maintenance of early pregnancy.

Introduction

The maintenance of pregnancy is dependent upon coordinated interactions between the developing embryo and its dam. Establishment of the short-lived corpus luteum of the oestrous or menstrual cycle as the corpus luteum of pregnancy depends upon the presence of an embryo in the uterus in many species (Moor & Rowson, 1966a, b; Heap, Perry & Rowlands, 1967; Greenwald & Rothchild, 1968; Knobil, 1973). Although the mechanism by which an embryo prevents regression of the corpus luteum is not well defined, it may produce a luteotrophic substance which maintains the corpus luteum in early pregnancy.

Blastocyst fluid from 6-day-pregnant rabbits has been reported to contain a substance similar to hCG and LH (Haour & Saxena, 1974; Fujimoto, Euker, Riegel & Dukelow, 1975), and an hCG-like substance has been detected on cell surfaces of the preimplantation mouse embryo by immunofluorescent staining (Wiley, 1974). In contrast, other investigators using sensitive *in-vitro* bioassay methods report that the rabbit blastocyst or blastocoelic fluid neither produces nor contains any hCG-like or LH-like substance (Sudanram, Connell & Passantino, 1975; Holt, Heise, Wilson & Keyes, 1976).

Embryo transfer experiments have demonstrated that in the sheep an embryo must be present within the uterus by the 12th day of the oestrous cycle for the corpus luteum of the cycle to become the corpus luteum of pregnancy (Moor & Rowson, 1966a, b). Additionally, intrauterine infusion of 14- or 15-day-old sheep embryo homogenates significantly prolongs the length of the oestrous cycle (Rowson & Moor, 1967). In the present study we have examined the effects of preimplantation sheep embryos on ovine and bovine corpora lutea *in vitro*.

Materials and Methods

Adult ewes of mixed breeds were checked daily for oestrus with vasectomized rams. At 12 days after oestrus, the ewes were induced to superovulate by a single intramuscular injection of PMSG (Sigma Chemical Co.) and were mated at the subsequent heat with a fertile ram. The ewes were anaesthetized on the 13th–15th day of pregnancy and the uterus was exteriorized through a midventral incision. The embryos were obtained by flushing each uterine horn with 30 ml sterile 0.9% (w/v) NaCl. Corpora lutea (CL) were obtained from the ovaries of cyclic ewes via midventral

laparotomy 12–14 days after oestrus. The embryos and CL were taken to the laboratory where the embryos were transferred to fresh incubation medium and the CL were trimmed of connective tissue and cut into slices approximately 0.5 mm thick. Random aliquots of luteal tissue were then weighed, transferred to 25 ml Erlenmeyer flasks containing 5 ml Krebs–Ringer–bicarbonate buffer (Krebs, 1950) containing 0.1 mg glucose/ml. Because of the inherent variability of progesterone synthesis by CL tissue *in vitro*, each trial consisted of at least 2 control flasks and 2 flasks containing embryos. The flasks were incubated at 37°C in a shaker bath for 2 h in an atmosphere of 95% O₂:5% CO₂. Tissue metabolism was terminated by placing the flasks in liquid N₂ and the samples were stored at –20°C until extracted. The luteal slices were homogenized with the incubation medium and extracted with anhydrous ether. Progesterone was isolated by submitting the organic residue to 3 one-dimensional thin-layer chromatography systems and quantitated spectrophotometrically according to the procedure described by Hansel (1971). Briefly, sample residues were suspended in known amounts of redistilled ethanol and their absorbance of ultraviolet light was measured at 240 nm in a Bausch and Lomb 600 spectrophotometer. The influence of non-specific contaminants was minimized by taking measurements of each sample at 230 and 250 nm and correcting the reading at 240 nm through the use of the Allen factor (Allen, 1950). The amount of progesterone in a sample was determined by extrapolating the corrected 240 nm reading from a standard curve produced with known amounts of purified progesterone. Standard curves were all linear between 3 and 25 µg/ml and standard deviations were all less than 0.02. Procedural losses were checked by the addition of [³H]progesterone to each flask after homogenization. All samples were corrected for 100% recovery and values were expressed as µg progesterone/g luteal tissue/2 h.

To determine if substances produced by the ovine embryo stimulate progesterone synthesis in other species, ovine embryos were incubated alone in the Krebs–Ringer–bicarbonate incubation medium for 3 h at 37°C, then flash-frozen and stored at –20°C. After thawing the cultures were centrifuged to remove cellular material. Aliquots of the supernatant (embryo extracts) were incubated for 2 h with slices of CL removed *per vaginam* from cyclic Holstein cows on Days 14 or 15 after oestrus.

The significance of differences between the means of treated and control samples was determined by analysis of variance (Steel & Torrie, 1960).

Results

As shown in Table 1, the ovine preimplantation embryo stimulated a dramatic increase in progesterone synthesis in 9 out of 10 trials. The variability of control values between experiments is within the normal range experienced in our laboratory and may be due to differences in initial progesterone concentrations of individual CL. For this reason one CL was used for each experiment. The standard deviation of control values within experiments ranged from 1 to 11%. The variability in the degree or responsiveness by luteal slices to the embryos may be explained by the fact that all flasks did not contain equal amounts of embryonic tissue. Although we attempted to use 1–2 embryos per flask, embryos varied in size and occasionally fragmented during flushing. For this reason, each embryo-treated flask is listed individually in Table 1. No attempt was made to determine the viability of the embryos and this may account for the single failure to stimulate progesterone synthesis. In another experiment, we used embryos which were visibly degenerate; these embryos also failed to stimulate progesterone synthesis. To determine whether the embryos themselves were adding progesterone to the samples, 5 flasks of 1–2 embryos per flask were incubated alone and analysed for progesterone in the same way but no progesterone could be detected by the method used. When bovine luteal slices were incubated with aqueous extracts of bovine (cyclic, Days 12–18) endometrium or intestine there was no change in progesterone synthesis compared to that of controls.

Ovine embryo extracts significantly stimulated progesterone synthesis by bovine luteal tissue (Table 2). Preliminary data indicate that the luteotrophic properties of the extracts are non-dialysable and heat labile.

Table 1. Total progesterone (mean $\pm$ s.e.m.) produced by ovine luteal slices incubated for 2 h with and without ovine embryos

CL	Control		Treated		
	No. of flasks	Progesterone (μ g/g)	No. of flasks	Progesterone (μ g/g)	% increase
A	2	140.0 $\pm$ 15.7	2	203.0 266.6	44 90
B	2	102.3 $\pm$ 10.3	2	155.2 92.8	52 -9
C	2	199.9 $\pm$ 7.6	2	351.7 267.5	76 34
D	2	177.2 $\pm$ 4.7	2	265.3 299.1	50 69
E	2	95.2 $\pm$ 1.1	2	162.5 134.3	71 41
Overall		143.0 $\pm$ 13.8		219.8 $\pm$ 26.0*	51.8 $\pm$ 8.8

* Significantly different from control value, $P < 0.05$.**Table 2.** Total progesterone (mean $\pm$ s.e.m.) produced by bovine luteal slices incubated for 2 h with and without ovine embryo extracts

CL	Control		Treated		
	No. of flasks	Progesterone (μ g/g)	No. of flasks	Progesterone (μ g/g)	% increase
A	2	235.3 $\pm$ 18.2	2	347.7 $\pm$ 15.5*	47.8 $\pm$ 6.6
B	2	242.0 $\pm$ 6.8	4	423.3 $\pm$ 15.5**	75.0 $\pm$ 5.7
C	4	116.9 $\pm$ 5.1	3	178.0 $\pm$ 13.8	52.4 $\pm$ 11.7

Significantly different from the control value, * $P < 0.05$; ** $P < 0.01$.

Discussion

The prolongation of luteal function in sheep before feto–maternal attachment is dependent on the presence of an embryo in the uterus (Moor & Rowson, 1966a, b). Mapletoft, Del Campo & Ginther (1975) have shown that the venous effluent of the gravid uterine horn exerts a luteotrophic effect on the CL. The results of our experiments demonstrate that the ovine preimplantation embryo produces a substance which directly stimulates progesterone synthesis by the corpus luteum. We suggest that this stimulus contributes to the maintenance of pregnancy. In addition, we suggest that the luteotrophic properties of this substance are not species specific and that it may be proteinaceous.

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