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Effects of intrauterine infusion of oestradiol-17β and prostaglandin E-2 on luteal function in non-pregnant heifers

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Summary. Non-pregnant heifers (4/group) received intrauterine infusions of vehicle, oestradiol-17β (150 ng), PGE-2 (250 μg), or oestradiol + PGE-2 every 6 h from 12:00 h on Day 13 to 06:00 h on the day of subsequent oestrus or 06:00 h on Day 21 (day of oestrus = Day 0). Ten of 12 heifers receiving vehicle, oestradiol or PGE-2 returned to oestrus by Day 21, whereas none of the heifers receiving oestradiol + PGE-2 returned to oestrus by Day 21. Jugular venous progesterone concentrations of vehicle- and PGE-2-treated heifers declined rapidly after Day 15 and were basal (<1 ng/ml) by Day 20. For heifers receiving oestradiol infusions, systemic progesterone levels did not decline until after Day 18, but were again basal by Day 20. Heifers treated with oestradiol + PGE-2 maintained elevated systemic progesterone levels until Day 21 after oestrus. In addition, the corpora lutea of the heifers treated with oestradiol + PGE-2 were heavier ($P < 0.01$) and contained more ($P < 0.05$) progesterone than did corpora lutea of the heifers in the other 3 groups on Day 21 (3·4 g and 19·52 μg/g and 1·2 g and 1·65 μg/g, respectively). It is concluded that oestradiol-17β and PGE-2, both of which are produced by the bovine conceptus and secreted from the gravid uterus, may act synergistically to maintain luteal function during early pregnancy in the cow.

Introduction

The embryonic 'signal' that results in the maintenance of luteal function occurs on about Day 16 after mating in the cow (Northey & French, 1980) and Day 12 after mating in the ewe (Moor & Rowson, 1966) and sow (Ford, Christenson & Ford, 1982a). However, the identity of the molecule(s) that comprises this embryonic signal remains unknown. The bovine conceptus is capable of synthesizing oestradiol-17β *in vitro* by Day 13 after mating (Shemesh, Milaguir, Ayalon & Hansel, 1979; Chenault, 1980), while porcine conceptuses can produce oestrone and oestradiol-17β by Day 12 (Perry, Heap, Burton & Gadsby, 1976). In addition, oestrogens are elevated in uterine flushings and venous blood of cows (Ford, Chenault, Christenson, Echternkamp & Ford, 1981) and sows (Ford *et al.*, 1982a) at the time of maternal recognition of pregnancy for each species. Intrauterine infusion of near physiological concentrations of oestradiol-17β results in an extension of the functional lifespan of corpora lutea (CL) in the sow (Ford, Magness, Farley & Van Orden, 1982b). Prostaglandin (PG) E-2 is produced by cultured bovine blastocysts as early as Day 13 after mating (Shemesh *et al.*, 1979; Lewis, Thatcher, Bazer & Curl, 1982), and by ovine blastocysts by Day 14 (Lacroix & Kann, 1982). Uterine luminal and venous concentrations of PGE-2 are elevated on Day 15 in pregnant ewes (Ellinwood, Nett & Niswender, 1979). In addition, infusion of PGE-2 into the uterus of non-pregnant ewes prolongs the functional lifespan of the CL (Magness *et al.*, 1981).

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The purpose of this experiment was to determine the effects of intrauterine infusion of oestradiol-17β, PGE-2, or both hormones on the function of CL in non-pregnant heifers.

Materials and Methods

Experimental procedures

Sixteen non-pregnant Angus or Hereford × Angus heifers exhibiting oestrous cycles of normal duration (18–23 days) and of similar age and weight (1½–2 years; 320–360 kg) were used. Heifers were checked for oestrus twice daily throughout the experiment (07:30 and 16:30 h), using a vasectomized bull, and were trained to stanchions at least 1 month before surgery to adjust them to handling and confinement. Food and water were removed from heifers 24 h before surgery, which was performed on Day 9, 10 or 11 after oestrus (day of oestrus = Day 0). Induction and maintenance of general anaesthesia were as described by Ford, Chenault & Echternkamp (1979). The uterus and ovaries were exposed through a midventral incision, and the size and location of ovarian structures were recorded. A catheter was inserted into the lumen of the uterine horn ipsilateral to the ovary bearing the CL, through a small incision at the tip of the horn. The intrauterine catheter was similar to one previously used in this laboratory (Ford *et al.*, 1982b). The catheter had a 15-cm Silastic (Dow Corning Corp., Midland, MI) tubing tip (o.d. = 1·65 mm) that was sealed at the end and perforated at 3-cm intervals to ensure delivery of hormones to the entire uterine horn (simulating hormone delivery by an elongated blastocyst). The catheter was exteriorized through a small flank incision and maintained in a cloth pouch glued to the flank area.

After surgery, heifers were assigned randomly, in equal numbers, to receive intrauterine infusions of vehicle (Group 1) consisting of 2% (v/v) ethanol in sterile 0·9% (w/v) NaCl plus 1% (v/v) Combiotic (Pfizer, Inc., New York, U.S.A.), 150 ng oestradiol-17β (Sigma, St Louis, MO; Group 2), 250 μg PGE-2 (free acid; Upjohn Co., Kalamazoo, MI; Group 3), or oestradiol-17β + PGE-2 (Group 4) every 6 h from 12:00 h on Day 13 to 06:00 h on the day of subsequent oestrus if it occurred before Day 21 or 06:00 h on Day 21. Each intrauterine infusion consisted of 0·8 ml vehicle or hormone solution (150 ng oestradiol-17β and/or 250 μg PGE-2 per 0·8 ml) followed by a 0·8-ml vehicle flush (volume of catheter = 0·8 ml). A stock solution of PGE-2 in ethanol was stored at −20°C, and solutions for intrauterine infusion were prepared daily (06:00 h) in a sterile vial. The daily dose of oestradiol-17β was derived by multiplying the maximal venous–arterial difference in oestradiol-17β (Ford *et al.*, 1981) across a gravid uterine horn by the daily uterine arterial blood flow (Ford *et al.*, 1979), for cows on Days 14–18 of pregnancy. The daily dose of PGE-2 was the minimum intrauterine dose which has been shown to have no effect on the lifespan of the CL in heifers (Dalla Porta & Humblot, 1983).

For each heifer, a sample of jugular blood was obtained by venepuncture once daily (07:00–08:00 h) from Day 13 to Day 21 after oestrus. Plasma was obtained and stored at −20°C until assayed for progesterone. All heifers receiving PGE-2 treatment plus 1 animal from the remaining 2 treatment groups (vehicle and oestradiol-17β) were killed on Day 21 (08:00–10:00 h), and the uterus and ovaries were obtained. To minimize expense, the remaining 6 heifers were ovariectomized by midventral laparotomy between 08:00 and 10:00 h on Day 21. Placement of intrauterine catheters was verified at death or laparotomy. The diameters of ovarian structures were measured at the surface of the ovary, and CL were dissected from the ovary and weighed.

Progesterone radioimmunoassay

Plasma. The radioimmunoassay was identical to that previously reported and validated for progesterone in cow plasma in this laboratory (Ferrell, Ford, Prior & Christenson, 1983). Plasma (200 μl) was extracted in triplicate using benzene:hexane (1:2, v/v) with one of the replicates receiving 12 000–15 000 d.p.m. [1,2,6,7-^{3}H]progesterone (sp. act. 97·0 Ci/mmol; New England

Nuclear Corp., Boston, MA) to serve as the individual recovery for that set of duplicates. Recovery of [^{3}H]progesterone averaged 86·5 ± 0·3% (s.e.m.) and all values were corrected for procedural losses. Plasma extracts were assayed for progesterone using a fully characterized antibody (GDN-337; Gibori, Antczak & Rothchild, 1977). Sensitivity of the assay was defined as the amount of progesterone standard that yielded 95% of the counts/min in the buffer control tubes and ranged from 50 to 80 pg. With this method, mean blank value for plasma from an ovariectomized cow was 0·21 ± 0·02 ng/ml (s.e.m., n = 10). The precision and accuracy of the procedure were evaluated by adding 0·10 (n = 4), 0·25 (n = 4), 1·00 (n = 4), 2·50 (n = 4), 5·00 (n = 4), and 10·00 (n = 4) ng progesterone to plasma from the same ovariectomized cow. These standard plasmas were assayed and the progesterone concentration of the plasma blank was subtracted. The resulting concentrations (±s.e.m.) were 0·15 ± 0·01, 0·30 ± 0·01, 1·17 ± 0·03, 2·55 ± 0·09, 4·87 ± 0·17, and 10·81 ± 0·52 ng/ml, respectively. Within-assay variability was determined from replicates (n = 10) of a plasma pool from luteal-phase cows. The resulting concentration (±s.e.m.) was 13·97 ± 0·21 ng/ml and coefficient of variation (CV) was 4·9%. All plasma samples were assayed in a single assay.

CL. Corpora lutea were homogenized in 5·0 ml 0·9% (w/v) NaCl using a Polytron (Brinkmann Instruments, Inc., Westbury, NY), and the resulting homogenate was diluted with saline to a final volume of 15·0 ml. Aliquants of these homogenates were diluted 1:4 and 1:400 (v/v) with sterile bottled H_2O (Eli Lilly and Co., Indianapolis, IN), and 50-μl samples of these diluted homogenates were extracted and assayed as described above. Mean recovery (±s.e.m.) of [^{3}H]progesterone was 87·9 ± 0·3% and all values were corrected for procedural losses. The mean blank value for an homogenate of a corpus albicans from a cow on Day 4 after oestrus was 0·79 ± 0·04 ng/ml (s.e.m., n = 8). The precision and accuracy were evaluated by adding 1 (n = 6), 5 (n = 6), 10 (n = 6), and 20 (n = 6) ng progesterone to samples of the same luteal homogenate. Homogenate plus standards were assayed, and the resulting progesterone concentrations (after subtraction of the homogenate blank) were 0·97 ± 0·08, 6·17 ± 0·10, 12·27 ± 0·21, and 22·78 ± 0·27 ng/ml, respectively. Within-assay variability was determined from replicates (n = 8) of a luteal homogenate from a luteal-phase cow. The resulting concentration (±s.e.m.) was 12·92 ± 0·23 ng/ml (CV = 5·1%). All CL were assayed in a single assay. The concentration of progesterone in each sample was adjusted from ng/ml homogenate to μg/CL and μg/g luteal tissue.

Statistical analysis

Changes in jugular venous progesterone concentrations were analysed by split-plot analysis of variance for repeated measures (Kirk, 1968). Content and concentration of progesterone in CL, as well as diameters and weights of CL on Day 21 after oestrus, were analysed by factorial analysis of variance. Differences between means were evaluated using orthogonal contrasts (Kirk, 1968). Differences between treatment groups in the proportion of heifers exhibiting oestrus by Day 21 were analysed by a χ^2 test, and the lengths of the pretreatment and treatment cycles were compared using a paired t test (Steel & Torrie, 1960). All data are reported as the mean ± s.e.m.

Results

All intrauterine catheters were still in place on Day 21 after oestrus. For those heifers from which uteri were obtained, no infection or inflammation of the endometrium was apparent by gross observation, and the treated and non-treated horns were similar in appearance.

Oestrous activity

For the heifers in Groups 1, 2 and 3, 3 of 4, 3 of 4 and 4 of 4 were observed in standing oestrus by Day 21, respectively. For heifers exhibiting oestrus, there was no difference ($P > 0·10$) between the

lengths of the pretreatment and treatment cycles (20·3 ± 0·4 versus 19·2 ± 0·4 days). The proportion of heifers in Group 4 that exhibited oestrus by Day 21 (0 of 4) was less ($P < 0·03$) than for the other 3 treatment groups.

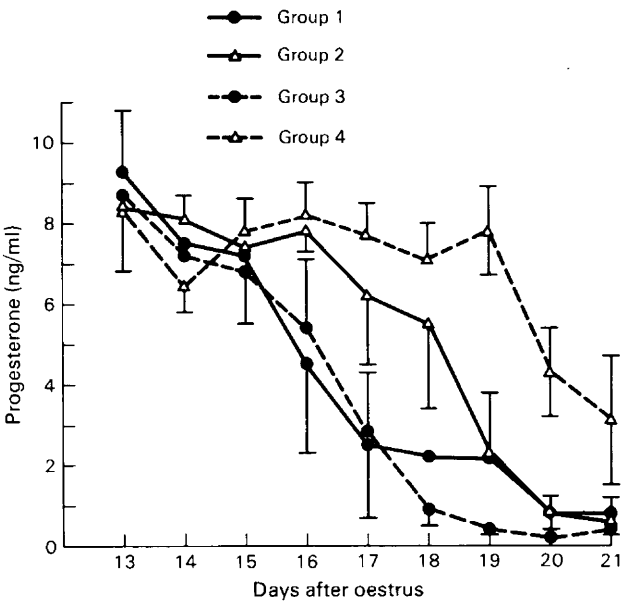
Ovarian structures

All heifers used in this study had a single CL at surgery on Days 9, 10 or 11 after oestrus, which averaged 24·2 ± 1·0 mm in diameter. Diameters and weights of CL of heifers in Group 4 were greater ($P < 0·01$) than for those of the other 3 groups at ovariectomy on Day 21 (Table 1). Three of 4, 2 of 4 and 2 of 4 heifers in Groups 1, 2 and 3, respectively, had a corpus haemorrhagicum (12·1 ± 0·4 mm) present on Day 21. The remaining 5 heifers had a large (15·2 ± 1·0 mm) preovulatory follicle. None of the heifers in Group 4 had a corpus haemorrhagicum and their largest follicle averaged 10·2 ± 0·8 mm.

Table 1. Characteristics of corpora lutea on Day 21 of heifers in Groups 1 (vehicle), 2 (oestradiol-17β), 3 (PGE-2) and 4 (oestradiol-17β + PGE-2)*

Group	No. of heifers	Diameter (mm)	Weight (g)	Progesterone content (μg/CL)	Progesterone conc. (μg/g tissue)
1	4	13·0 ± 1·2[a]	1·1 ± 0·4[a]	2·22 ± 2·06[a]	1·12 ± 0·94[a]
2	4	15·8 ± 1·3[a]	1·6 ± 0·3[a]	4·61 ± 2·06[a]	2·92 ± 1·60[a]
3	4	13·0 ± 1·1[a]	0·8 ± 0·1[a]	0·82 ± 0·32[a]	0·93 ± 0·25[a]
4	4	21·2 ± 1·3[b]	3·4 ± 0·4[b]	64·60 ± 33·58[b]	19·52 ± 10·83[b]

* Means (±s.e.m.) within a column with different superscripts differ ($P < 0·01$ for CL diameter, weight, and progesterone content; $P < 0·05$ for CL progesterone concentration).



Text-fig. 1. Concentrations of progesterone in jugular venous blood of heifers receiving intrauterine infusions of vehicle (Group 1), oestradiol-17β (Group 2), prostaglandin E-2 (Group 3), or oestradiol-17β + PGE-2 (Group 4) from Day 13 to Day 21 after oestrus (N = 4 per group).

Progesterone

Luteal concentration and content of progesterone were greater for CL of heifers in Group 4 than for heifers in the other 3 groups (Table 1). The concentrations of progesterone (ng/ml) in jugular venous plasma were similar ($P > 0{\cdot}10$) for all treatment groups from Days 13–15 after oestrus (Text-fig. 1). From Days 16–18, plasma progesterone concentrations for Group 1 and Group 3 heifers declined to basal levels and were less ($P < 0{\cdot}01$) than for the heifers in Groups 2 and 4 which were similar. Jugular progesterone concentrations of Group 2 heifers decreased on Days 19 and 20 to levels similar to those observed for heifers in Groups 1 and 3 and were less ($P < 0{\cdot}01$) than those in Group 4 heifers. Although systemic progesterone concentrations of Group 4 heifers had declined by Day 21, they remained elevated ($P < 0{\cdot}05$) when compared to the other 3 groups ($3{\cdot}12 \pm 1{\cdot}57$ versus $0{\cdot}58 \pm 0{\cdot}12$; Text-fig. 1).

Discussion

Data from this experiment clearly demonstrate a synergism between oestradiol-17β and PGE-2 in maintaining luteal function in heifers. Progesterone in systemic blood of the heifers treated with vehicle and PGE-2 began to decrease 3–4 days before oestrus, which is similar to previous reports for cyclic cows (Henricks, Dickey & Hill, 1971; Ford *et al.*, 1979). Intrauterine infusion of oestradiol-17β in the present study was able to extend luteal progesterone secretion for 3 days even though the occurrence of oestrus in oestradiol-treated heifers was similar to that of the vehicle-treated heifers. When both oestradiol-17β and PGE-2 were infused, luteal weights and progesterone levels on Day 21 were similar to values reported previously for cows during the luteal phase of the oestrous cycle and pregnancy (Erb & Stormshak, 1961). In addition, jugular venous progesterone concentrations in heifers treated with oestradiol-17β and PGE-2 were maintained for an additional 5–6 days compared to those in vehicle-treated heifers, averaging 3·12 ng/ml on Day 21 after oestrus. Northey & French (1980) observed a similar maintenance of systemic progesterone levels when bovine embryos were flushed from the uterus on Days 17–19 after mating or when embryonic homogenates were infused into the uterus of non-pregnant cows from Days 14 to 18 after oestrus. Thus, intrauterine infusion of oestradiol-17β plus PGE-2, both of which are produced by the bovine conceptus and secreted from the gravid uterus, was able to extend luteal function in the absence of the conceptus.

The mechanism of action of oestradiol-17β and PGE-2 in maintaining luteal function remains unknown. Del Campo, Mapletoft, Rowe, Critser & Ginther (1980) demonstrated that the luteotrophic effect of the bovine conceptus was exerted through a local vascular pathway. A local increase in blood flow to gravid uterine horns occurs coincident with maternal recognition of pregnancy in the sow, ewe and cow, and oestrogen administration has been shown to increase uterine blood flow in these species (see Ford, 1982, for review). Elevated uterine blood flow is associated with an increase in the amount of lymph draining the uterine horn (Fabian, 1981). The concentrations of steroid hormones in uterine lymph have been shown to reflect concentrations in the uterine lumen and uterine venous blood (Magness & Ford, 1982; Ford *et al.*, 1982a). Kotwica (1980) suggested that uterine lymphatics may be involved in the transport of prostaglandins from the uterine horn to the adjacent ovary. Therefore, oestrogens produced by the conceptuses may stimulate increased uterine blood flow, which could be important for the transport of substances such as prostaglandins from the lumen of the gravid uterus to the ovary.

Administration of oestrogen also stimulates increased ovarian blood flow in ewes (Rosenfeld, 1980). Infusion of oestrogen into an isolated uterine horn of non-pregnant sows preferentially stimulates progesterone secretion from the ipsilateral ovary (Ford *et al.*, 1982b), and intrauterine infusion of oestradiol-17β in the present study extended systemic progesterone levels for 3 days. Oestrogens of embryonic origin may therefore enhance luteal function indirectly by causing increased blood flow to the ovary, with a subsequent increase in progesterone secretion. In support

of this hypothesis, a transient increase in ovarian blood flow and progesterone secretion has been observed at the time of maternal recognition of pregnancy in sows (Magness, Christenson & Ford, 1983) and cows (Ford *et al.*, 1979; Ford & Chenault, 1981). A direct effect of oestrogens on luteal function is unlikely because oestradiol-17β has been shown to inhibit LH-induced progesterone secretion by cultured bovine luteal cells (Williams & Marsh, 1978).

Prostaglandin E-2 directly stimulates progesterone secretion and cyclic AMP production of bovine luteal cells *in vitro*, in a manner similar to that of luteinizing hormone (LH) (Speroff & Ramwell, 1970; Marsh, 1971). In addition, PGE-2 has been shown to block PGF-2α-induced luteolysis when both prostaglandins were infused simultaneously into the ovarian artery (Henderson, Scaramuzzi & Baird, 1977) or ovarian vascular pedicle (Reynolds *et al.*, 1981) of non-pregnant ewes. This may be a direct luteotrophic effect on the CL because PGF-2α inhibits LH-induced, but not PGE-2-induced, accumulation of cyclic AMP by cultured luteal cells (Khan *et al.*, 1979). In addition, PGE-2 was able to inhibit PGF-2α-induced loss of luteal LH receptors in non-pregnant ewes (Reynolds *et al.*, 1981).

Although the present study indicates that both oestradiol-17β and PGE-2 are necessary for the maintenance of luteal function during early pregnancy in cows, the role of each hormone remains unknown. As suggested, oestradiol-17β may stimulate an increase in lymph and venous blood draining the gravid uterus and thus enhance the transport of substances such as PGE-2 from the uterine lumen to the ovary. Prostaglandin E-2 may then have a direct effect on the luteal cells to maintain progesterone secretion.

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