PATTERNS OF BLOOD FLOW TO THE UTERUS AND OVARIANS OF EWES DURING THE PERIOD OF LUTEAL REGRESSION

S. P. Ford3, R. K. Christenson2 and J. R. Chenault2

US Department of Agriculture4
Clay Center, Nebraska 68933

Summary

Patterns of blood flow to the uterus and ovaries of 27 mature Finn-cross ewes were determined during the normal period of luteal regression using radioactive microspheres. Ewes were randomly assigned to equal numbers to receive an intrauterine implant on day 9 of an estrous cycle (day of estrus = day 0) containing either 1) cholesterol (control, C), 2) estradiol-17β:cholesterol (E2:C, 2:98% by wt), or 3) α-methyl tyrosine (α-MT). The nine ewes in each group (C, E2:C or α-MT) were randomly assigned to equal numbers to receive microspheres at 0800 hr, and were sacrificed within 2 hr, on days 14, 15 or the first day of estrus following placement of the implants. Venous blood was collected from all ovaries bearing corpora lutea (CL) prior to placement of intrauterine implants on day 9 and within 1 hr following microsphere injection on day 14, 15 or estrus for determination of progesterone by radioimmunoassay. The study failed to demonstrate any effect of uterine implants on estrous cycle length, unilateral uterine or ovarian blood flow or luteal progesterone secretion, regardless of treatment group.

Blood flow to uteri of ewes sacrificed at estrus was greater (P<.05) than blood flow to uteri of ewes sacrificed on day 14 or 15 of the estrous cycle. No differences in uterine blood flow were observed between ewes sacrificed on days 14 or 15 postestrus. At estrus, blood flow to the tip of each uterine horn was greater (P<.05) than blood flow to the cervical end of the uterine horns. There was no effect on day of sacrifice (14, 15 or estrus) or treatment (C, E2:C or α-MT) on ovarian weight. However, ovaries with CL were heavier (P<.05) than ovaries without CL (2.5 ± .09 g, n = 39; 1.67 ± .16 g, n = 15, respectively). Corpora lutea weights (g) remained constant from day 14 (.54 ± .04 g, n = 20) to day 15 (.45 ± .02 g, n = 22) before declining (P<.05) at estrus (.24 ± .02 g, n = 17). Blood flow to the extraluteal component of ovaries with CL was constant on days 14 to 15 but had increased (P<.05) at estrus. Blood flow to ovaries without CL did not differ on the days studied and averaged .37 ± .09 ml/min/g of tissue. The amount of blood flow to the CL decreased (P<.01) from day 14 to 15, as well as from day 15 to estrus when only a minimal flow rate was observed. As observed from luteal blood flow, progesterone concentration in venous blood from ovaries with CL decreased (P<.05) from day 14 to 15, and from day 15 to estrus. Evidence that ovarian progesterone secretion is strongly associated with blood flow through the CL is provided by the overall significant correlation (r = .79; P<.01) found to exist between the two measurements.

(Key Words: Ewe, Blood Flow, Uterus, Ovaries, Microspheres.)

Introduction

The ovine conceptus must be present in the uterus by day 13 postmating for maintenance of the corpus luteum (CL) and establishment of pregnancy (Moor and Rowson, 1966). Greiss and Anderson (1970) observed transient increases in blood flow to the uterine horn.
containing embryos on days 13 to 15 following mating in ewes and suggested their possible importance in luteal maintenance. These data are in agreement with observations from our laboratory (Ford et al., 1979) which demonstrated a transient increase in blood flow to only the gravid uterine horn of cows on days 15 to 17 of pregnancy, the critical time for pregnancy recognition in that species (Betteridge et al., 1978). The presence of the preimplantation conceptus in these species mimics the effects of estrogen by increasing blood flow to the gravid uterine horn. Injection of estrogen into the uterine lumen of the ewe (Greiss and Miller, 1971) causes a rapid unilateral increase in uterine blood flow and it has been demonstrated that the day 12 porcine conceptus can synthesize estrogens in vitro (Perry et al., 1976). In association with the ability of the porcine conceptus to synthesize estrogens, a transient increase in uterine blood flow has been observed on days 12 and 13 of gestation in sows (Ford and Christenson, 1979). Thus estrogens of embryonic origin may act locally and directly to increase uterine and (or) ovarian blood flow. Data published by McKercher et al. (1973) demonstrated that estradiol increased uterine arterial blood flow is associated with a decreased content of norepinephrine in periarterial adrenergic nerves.

Concentrations of prostaglandin F are increased in uterine venous blood of ewes during the period of luteal regression (Thorburn et al., 1972). Prostaglandin F2α (PGF2α) may be the active luteolytic agent in the ewe since its administration results in premature luteal regression (McCracken et al., 1970; Thorburn and Nicol, 1971). During normal, as well as PGF2α induced luteal regression, decreased progesterone secretion is associated with decreased blood flow through the CL (Niswender et al., 1975; Nett et al., 1976). Prostaglandin F2α has been implicated in regulation of adrenergic neurotransmission (Kadowitz et al., 1971), and may decrease blood flow by increasing the synthesis and (or) release of norepinephrine from blood vessels.

Therefore, the following study was conducted to determine the patterns of blood flow to the uterus and ovaries of ewes during the period of luteal regression and to evaluate the effects of continuous intrauterine release of minute (ng/day) quantities of estradiol-17β or α-methyl tyrosine (a depletor of neurotransmitter from sympathetic nerves (Goodman and Gilman, 1970) on the normal pattern of uterine and ovarian blood flow, and upon luteal life span. In addition, correlation of blood flow through the uterus and ovaries with concentrations of progesterone in ovarian venous blood was determined.

Materials and Methods

Twenty-seven mature Finn-cross (1/2) ewes with at least three consecutive estrous cycles of normal duration, as determined by twice daily (0700 and 1700 hr) checks for estrus with vasectomized rams, were used in the following study. Feed and water were removed from ewes 24 hr prior to surgery which was performed on day 9 of the estrous cycle (day of estrus = day 0). On the day of surgery, ewes were preanesthetized with 15 ml of a 5% sodium thiopental solution and surgery was performed under closed circuit anesthesia; oxygen, nitrous oxide and halothane were used. The uterus and ovaries were exposed through a midventral incision and CL number and location on each ovary noted. A 2 ml blood sample was collected into a heparinized syringe via a 23-gauge needle from an ovarian vein draining each ovary bearing a CL. Plasma was then frozen until assayed for progesterone by radioimmunoassay (Ford et al., 1979). Of the 27 ewes utilized in this study, 15 had ovulated unilaterally and 12 bilaterally.

Following collection of ovarian venous blood samples, ewes were assigned at random, in equal numbers (N = 9), to receive a silastic implant containing either 1) cholesterol (control, C), 2) estradiol-17β: cholesterol (E2 :C,2:98% by wt) or 3) α-methyl tyrosine (α-MT). Unilaterally and bilaterally ovulating ewes were distributed equally across treatments (C,E2 :C or α-MT). Thus, each treatment group had 5 unilateral ovulators and four bilateral ovulators. An implant was inserted through a 10 mm incision in the uterine wall about 3 cm from the uterotubal junction and into the lumen of one uterine horn ipsilateral to an ovary with a CL, whether ewes had CL on one or both ovaries. The implant was fastened to the uterine wall with a loop of silk thread inserted through the end of the implant and uterine wall, and the incision closed with a single suture.

Implants consisted of silastic tubing 2.16 mm internal diameter by 20 mm in length (wall thickness = .57 mm) containing a 5 mm column of cholesterol, estradiol-17β: cholesterol or α-methyl tyrosine. Prior to placement in ewes,
all implants were placed individually in 2 ml of a .5% BSA solution and preincubated in a water bath (37 C) for 5 days during which the BSA solution was changed at 24 hr intervals. The rational for this preincubation scheme was based on a preliminary study which demonstrated that the release rate of estradiol-17β, as determined by radioimmunoassay (Ford et al., 1979), stabilized by the sixth day of in vitro incubation and remained constant for at least 7 days thereafter (figure 1). As illustrated in figure 1, release of estradiol-17β from the implants into the incubation media from days 6 to 12 averaged 174.5 ± 8.0 ng/day. Although no assay was available for α-MT, its release from the implants was verified by an observed decrease in column height from 5 to 1 to 2 mm over a similar 12-day in vitro incubation.

Following placement of the silastic implant, the femoral artery was cannulated and a catheter was implanted into the left ventricle of the heart via the left carotid artery for measurement of blood flow to the uterus, ovaries and other tissues by use of radioactive microspheres as described previously (Christenson and Prior, 1978). Microspheres in the present study were 52.4 ± 3.1 μm in diameter, labeled with 85 SR (3M Nuclear Products, Div., St. Paul, MN) and suspended in 20% dextran solution (82,000 MW). The nine ewes in each treatment group (C,E2: C or α-MT) were randomly assigned in equal numbers to receive a 1 ml injection of 8 × 105 microspheres into the ventricular cannula at 0800 hr on either day 14 or day 15 of the estrous cycle or the first day of estrus following placement of the uterine implants. An attempt was made to distribute unilateral and bilateral ovulators equally across days of microsphere injection.

Within 1 hr of microsphere injection, ewes were anesthetized, the uterus and ovaries exposed through a midventral incision and a 2 ml ovarian blood sample was again collected as described previously. Ewes were then sacrificed with an overdose of thiopental and the uterus, ovaries, kidneys and semitendinosus muscles removed. The kidneys and semitendinosus muscles were weighed, coarse ground and homogenized prior to determination of radioactivity (Christenson and Prior, 1978). Each uterine horn was divided into nine sections of equal length, each section was weighed and its position in the uterine horn noted (1-tip of uterine horn; 9-cervical end of uterine horn). Sections were then placed in scintillation vials for determination of radioactivity. Ovaries were weighed and CL, if any, were dissected from the remainder of the ovary and weighed individually. The ovarian components were then placed individually in scintillation vials for determination of radioactivity.

Blood flow, tissue weight and progesterone data were subjected to factorial analysis of variance to determine any significant effect of treatment (C,E2 :C or α-MT) or day of the estrous cycle (14, 15 or estrus) on these measurements. Consideration was given in the analysis to whether ewes were unilaterally or bilaterally ovulating. There was no effect of number of CL per ovary on weights or blood flows of individual CL on that ovary. Thus, when dealing with ewes with more than one CL on an ovary, an average luteal weight and blood flow was calculated for that ovary and used as a single statistical observation. Differences between means were tested for significance by utilizing orthogonal contrasts (Kirk, 1968). Correlations between ovarian venous blood concentrations of progesterone and blood flow to the uterus and ovaries also were determined.

**Results**

There was no effect of treatment (C,E2 :C or α-MT) on estrous cycle lengths (X = 17.6 ± .3 days: n = 9) of ewes in each treatment group sacrificed at estrus. The average estrus cycle length for these nine ewes prior to treatment was 17.1 ± .4 days. Blood flow (ml/min) to uterine horns containing silastic implants, regardless of treatment, did not differ from blood flow to contralateral uterine horns on
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Days 14, 15 or estrus (11.45 ± 2.65 vs 11.93 ± 2.65, 7.16 ± 1.14 vs 6.67 ± 1.39 and 54.64 ± 4.48 vs 53.90 ± 5.97, respectively) whether ewes had CL on one or both ovaries. When blood flow to each segment of uterine horns of E₂: C treated ewes on days 14 and 15 of the estrous cycle was examined, blood flow to the segment of uterine horn containing the E₂: C implant (segment 2) was elevated (P<.01) when compared to blood flow through the remaining segments of that horn or segments of the contralateral horn which exhibited no differences in blood flow (figure 2). With the exception of elevated blood flow to the segment of uterine horn containing the implant of E₂: C treated ewes, blood flow to all segments of uteri from day 14 and 15 ewes was similar regardless of treatment group.

Weights of uteri from ewes sacrificed at estrus were greater (P<.05) than weights of uteri of ewes sacrificed on days 14 or 15 of the estrous cycle (table 1). Uterine weights of ewes sacrificed on days 14 or 15 of the estrous cycle did not differ significantly. Blood flow (ml/min, as well as, ml/g of tissue/min) to uteri of ewes sacrificed at estrus was elevated (P<.01) when compared to blood flow to uteri of ewes on days 14 or 15 of the estrous cycle (table 1). Total blood flow (ml/min) to uteri of ewes on day 14 of the estrous cycle was higher (P<.05) than uterine blood flow on day 15, due to the trend for increased weight of uteri from day 14 ewes. When blood flow was corrected for uterine weight, however, no significant differences (P>.05) in uterine blood flow were observed between ewes sacrificed on days 14 or 15 postestrus. At estrus, the E₂: C containing implant had no observable effect on increasing blood flow to the segment of uterine horn containing the implant. Blood flow to the tip of both uterine horns (segment 1) of estrous ewes, regardless of treatment group, was greater (P<.05) than blood flow to segments 4 to 9 (figure 3).

There was no effect of day of sacrifice (14, 15 or estrus) or treatment on weights of ovaries with or without corpora lutea. However, ovaries with CL were heavier (P<.05) than ovaries without CL (2.54 ± 0.9, n = 39; 1.67 ± 0.16 ng, n = 15, respectively). Weights of corpora lutea (g) remained constant from day 14 (.54 ± 0.04, n = 20) to day 15 (.45 ± .02, n = 22) before declining (P<.05) at estrus (.24 ± .02, n = 17). Blood flow to the extraluteal component of the ovaries with CL was constant from days 14 to 15, before increasing (P<.05) at estrus (figure 4). Blood flow to ovaries without CL did not differ on the days studied and averaged .37 ± .09 ml/min/g of tissue (n = 15). Six unilaterally ovulating ewes were sacrificed on day 14, 5 on day 15, and 4 at estrus. The amount of blood flow to CL decreased (P<.01) from day 14 to 15, as well as from day 15 to estrus when only a minimal flow rate was observed (figure 4).

**Table 1. Uterine Weights and Blood Flow of Ewes During the Period of Luteal Regression**

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Weight (g)</th>
<th>Blood flow (ml/min)</th>
<th>Blood flow (ml/g of tissue/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 14</td>
<td>63.91 ± 4.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.00 ± 1.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.36 ± .04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 15</td>
<td>54.40 ± 2.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.00 ± .75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.26 ± .02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Estrus</td>
<td>99.95 ± 8.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>115.94 ± 10.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.16 ± .07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>Means ± SE without common superscripts, within column, differ (P<.01).
Evidence that ovarian progesterone secretion is associated with blood flow through the CL is provided by the overall significant correlation ($r = .79; P<.01$) found to exist between the two measurements. Progesterone concentration in venous blood of ovaries with CL decreased ($P<.05$) from day 14 to 15, as well as from day 15 to estrus (figure 5).

No effect of side of removal, treatment ($C,E_2:C$ or $\alpha$-MT) or day within treatment (14, 15 or estrus) was observed on blood flow or tissue weights of kidney or semitendinosus muscle. Blood flow to both kidneys and semitendinosus muscles in this study agree with those reported for pregnant ewes by Christenson and Prior (1978).

Discussion

Data presented herein failed to demonstrate any effects of estradiol or $\alpha$-methyl tyrosine in altering estrous cycle length or unilateral uterine or ovarian blood flow. This lack of treatment effects may have been due to improper timing of administration or insufficient dosages of treatment compounds. Evidence for the latter hypothesis, at least in the case of estradiol, is the observation that only the segment of uterine horn containing the estradiol implant exhibited increased blood flow rather than the entire uterine horn. As stated previously, Greiss and Anderson (1970) observed transient increases in blood flow to the entire gravid uterine horn on days 13 to 15 of pregnancy.

The quantity and pattern of blood flow to the uterus of the ewe on days 14, 15 and estrus in this study were similar to those reported by Greiss and Anderson (1969) who utilized electromagnetic flow probes placed around uterine arteries. Uterine blood flow in both studies was low on days 14 and 15 of the estrous cycle before increasing to a high level at estrus. Evidence suggesting differential effects of ovarian secretion of estrogen and progesterone on altering uterine blood flow in the nonpregnant ewe was presented by Huckabee et al. (1968). These investigators observed that uterine arterial blood flow was highest when ovaries contained large follicles without CL and lowest in the presence of one or more well developed CL and minimal follicular development. In the ewe, the major estrogen secreted by the ovaries during the estrous cycle is estradiol-17$\beta$ (Moore et al., 1969). The concentration of estradiol in ovarian venous blood was found to be low (40 pg/ml) about 48 hr before the onset of estrus (Saramusetti et al., 1970) with maximal levels of this steroid (1 ng/ml) present either just prior to or at the onset of estrus. Concentrations of progesterone in utero-ovarian venous blood of the ewe are low (12 ng/ml) for the first 2 days following estrus at which time they increase rapidly between days 3 and 6, then remain constant (200 ng/ml) for approximately 6 days before
declining to a low level on the day before estrus (Thorburn and Mattner, 1971). Thus, the abrupt increase in uterine blood flow observed at estrus may have resulted from a vasodilatory action of estradiol while the reduced blood flow on days 14 and 15 of the estrous cycle may be a result of progesterone-induced vasoconstriction. Further evidence suggesting a role of progesterone in reducing uterine blood flow was the observed overall significant correlation between ovarian venous progesterone concentrations and uterine blood flow in the present study \( r = -.71; P < .01 \).

Of interest is the observation that blood flow to the tip of the uterine horn is greater than that to the remainder of the horn at estrus. Although the significance of this finding is not known, it may be important for transport or survival of ova and (or) sperm and warrants further investigation.

Rates of ovarian blood flow in conscious ewes reported herein are similar to those obtained by Niswender et al. (1975) using a Doppler ultrasonic blood flow technique and by Brown et al. (1974) who utilized radioactive microspheres. Blood flow to ovaries without CL and to the extraluteal component of ovaries with CL of ewes in this study was similar on days 14 and 15 of the estrous cycle averaging \( .30 \pm .03 \) ml/g of tissue/min and agrees with results reported by Bruce and Moor (1976) and Niswender et al. (1976). At estrus, however, blood flow to the extraluteal component of ovaries with CL increased \( (P < .05) \) when compared to levels of blood flow to those ovaries on day 15 \( (.26 \pm .02 \) and \( .59 \pm .05 \) ml/g of tissue/min, respectively), while blood flow to the extraluteal component of ovaries with CL was increasing at estrus, blood flow to the CL was declining markedly from \( 5.54 \pm 1.12 \) to \( .47 \pm .07 \) ml/g of tissue/min, respectively. These data are in agreement with those of Novy and Cook (1973) who demonstrated a redistribution of blood flow from CL to the ovarian stroma in the rabbit ovary during luteal regression. These data disagree with those of Bruce and Moor (1976), however, who found no corresponding increase in blood flow to the extraluteal component of ovaries with CL as luteal blood flow declined from \( 11.22 \) to \( 1.16 \) ml/g of tissue/min on days 15 and 16, respectively. The data of Bruce and Moore (1976) do not correspond exactly with the present study since ewes killed on day 16 in that study were referred to as "oestrus ewes" but were not checked for estrous activity. The highly significant positive correlation between ovarian venous concentrations of progesterone and luteal blood flow during luteal regression suggests a strong association between these two phenomena. However, this experiment did not elucidate if blood flow through the CL influences the secretion of progesterone or if progesterone secretion by the CL regulates luteal blood flow.

**Literature Cited**


