Blood Flow to the Uterine and Ovarian Vascular Beds of Gilts During the Estrous Cycle or Early Pregnancy

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ABSTRACT

Blood flow to uteri, ovaries and other selected maternal tissues of 3 nonpregnant (NP) and 4 pregnant (P) gilts was determined on Days 9, 11, 13 and 15 postestrus or post-mating (Day 0=first day of estrus or mating) using gamma-labeled microspheres. On either Day 6, 7 or 8, catheters were inserted into the left ventricle and the left femoral artery for infusion of microspheres and simultaneous withdrawal of blood, respectively. At random, 7 × 10⁶ 25 μm spheres with one of 4 gamma labels (141Cr, 89Sr, 131I or 148Yb), in a volume of 10 ml, were infused on each of Days 9, 11, 13 and 15 such that each gilt received all 4 isotopes. Nonpregnant gilts were sacrificed on Day 15 and P gilts were sacrificed on Day 18. Each uterine horn from NP gilts was divided into 10 sections of equal length and their position in the uterine horn noted. Uteri of P gilts were divided into sections in contact with and devoid of conceptus tissue. Each section of uterus, as well as the cervix, vagina, liver, kidneys, lungs, and fibularis tertius muscles were weighed, homogenized and aliquoted prior to determination of radioactivity. Corpora lutea (CL), and the extraluteal component of each ovary, utero-ovarian lymph nodes, oviducts and the pituitary were weighed and placed directly into vials for counting. Blood flow (BF; ml/min per g of tissue) to uterine segments was greater (P<0.05) in P than NP gilts on each of the days measured. In P gilts, BF to uterine segments was highest (P<0.05) on Days 11 and 13 than Days 9 and 15 (2.05 ± 0.11 and 1.92 ± 0.10 vs. 1.18 ± 0.05 and 1.48 ± 0.06, respectively). On Day 13, BF to uterine segments in contact with conceptuses was elevated (P<0.01) compared to segments between conceptuses (2.13 ± 0.04 vs. 1.76 ± 0.04). Corpora lutea BF was elevated (P<0.01) in P gilts on Day 13 (23.97 ± 2.51) compared to P gilts on Days 9, 11 or 15 (15.01 ± 1.26) or NP gilts across all Days (15.20 ± 1.21). These data demonstrate that by Day 13 of gestation, uterine segments in contact with conceptuses have greater blood flow than segments between conceptuses. In addition, this conceptus-associated increase in uterine BF on Day 13 occurs concomitantly with a transient rise in BF through the CL.

INTRODUCTION

Patten (1948), Dhindsa et al. (1967), Waite and Day (1967), Polge and Dziuk (1970) and Anderson (1978) determined that intrauterine migration of porcine embryos occurs between Day 7 and Day 12 (Day 0=1st day of estrus). By Day 13, most embryos had elongated extraembryonic membranes and were spaced at regular intervals. Embryonic spacing within the uterus of the pig occurs coincident with increased blood flow to the gravid uterine horns (Ford and Christenson, 1979), and the signal which initiates luteal maintenance (Dhindsa and Dziuk, 1968; Ford et al., 1982a). The conceptus appears to mediate these changes in uterine and luteal function, since Ford and Christenson (1979) observed that blood flow increased only to the gravid uterine horn in unilaterally pregnant pigs and progesterone concentrations were greater in corpora lutea (CL) on the ipsilateral ovary. In agreement with these data is the observation by Anderson et al. (1966) and Christenson and Day (1971) that in unilaterally pregnant sows that remain pregnant there is a high incidence of unilateral maintenance of CL adjacent to the gravid horn. The quantity of blood flowing though a CL is highly correlated with its ability to secrete progesterone (Niswender et al., 1975; Nett et al., 1976). During normal, as well as prostaglandin F₂α (PGF₂α)-induced luteal regression, decreased ovarian progesterone secretion is

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associated with decreased blood flow through the CL (Ford et al., 1979; Nett and Niswender, 1981). It has been suggested that at least a portion of the luteolytic activity of PGF\(_2\alpha\) may be manifested via a reduction in blood flow through the CL (Pharriss, 1970; Nett and Niswender, 1981). A substance produced by the early ovine and bovine conceptus or early pregnant uterus appears to reduce uterine vascular responsiveness to PGF\(_2\alpha\) (Ford et al., 1976), with subsequent increases in uterine blood flow (UBF; Greiss and Anderson, 1970). It is conceivable that this vasodilatory effect of the conceptus is also functioning at the luteal vascular bed to prevent luteolysis.

This study was conducted to: 1) determine the association, if any, between hyperemia within the microcirculation of the porcine uterus and embryo spacing and 2) determine if this vasodilatory effect of the conceptus occurred at the level of the ovarian vascular bed. To test these hypotheses, radioactive microspheres were used to determine the flow of blood through the uterine and ovarian vascular beds of gilts on Days 9, 11, 13 and 15 of the estrous cycle or pregnancy.

**MATERIALS AND METHODS**

Seven Yorkshire gilts with at least 2 estrous cycles of normal duration (19–22 days) were utilized in this study. Four gilts were mated to a boar and the remaining 3 served as nonmated controls. Mated and nonmated gilts were assigned to surgery on either Day 6, 7 or 8 after mating or estrus, respectively (first day of estrus or mating = Day 0). Food was removed from gilts 24 h before surgery. General anesthesia was induced by intravenous infusion of 1.0 g of Surital (sodium thiamylal, Parke Davis Labs., Detroit, MI), regardless of body weight, while surgical anesthesia was maintained with a mixture of oxygen and halothane (Fluothane, Ayerst Labs., New York, NY) administered in a closed-circuit system with soda lime for removal of CO\(_2\). At surgery, the left femoral artery was catheterized 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; Ford et al., 1979). Following surgery, gilts were housed individually in small pens for the duration of the study.

Microspheres in the present study were 25 ± 5 μm in diameter, labeled with either \(^{89}\)Cr, \(^{89}\)Sr, \(^{141}\)Ce or \(^{199}\)Yb (3M Nuclear Products Div., St. Paul, MN) and suspended in 20% dextran solution (82,000 mol. wt.). At random, 7 × 10\(^5\) spheres with one of the 4 gamma labels, in a volume of 10 ml, were infused into the ventricular cannula on Days 9, 11, 13 and 15 of the estrous cycle or pregnancy, such that each gilt received all 4 isotopes. Microspheres were injected over a 60-sec period.

Withdrawal of a reference femoral arterial blood sample was begun 30 sec before microsphere injection and continued at a rate of 6.40 ml/min with a Harvard infusion-withdrawal syringe pump for at least 150 sec after the end of microsphere injection. The reference arterial blood sample was withdrawn with a 50-ml syringe containing 20 ml of heparinized sterile physiological saline into a 250-cm segment of polyethylene tubing (3.2 mm i.d., 6.4 mm o.d.) also filled with saline. At the end of the sample withdrawal, the blood sample was expelled into plastic counting tubes (16 × 85 mm). The saline in the withdrawal syringe and tubing was used to flush residual blood from the tubing. Nonpregnant gilts were sacrificed within 2 h of the last microsphere injection (Day 15 of the estrous cycle), and pregnant gilts were sacrificed on Day 18 of pregnancy after firm attachment of the conceptus to the uterine wall. Gils were sacrificed with an intraventricular injection of a euthanasia solution (T-61, American Hoechst Corp. Animal

**TABLE 1. Blood flow to selected tissues of gilts.**

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Tissue wt (g)</th>
<th>Blood flow (ml/min per g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior vagina</td>
<td>...b</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Liver</td>
<td>2314.11 ± 44.27</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>Fibularis terti mus</td>
<td>72.25 ± 3.86</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>Cervix</td>
<td>...b</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Lung</td>
<td>...b</td>
<td>1.26 ± 0.24</td>
</tr>
<tr>
<td>Ovary (minus corpora lutea)</td>
<td>3.51 ± 0.12</td>
<td>1.64 ± 0.12</td>
</tr>
<tr>
<td>Utero-ovarian lymph nodes</td>
<td>0.97 ± 0.10</td>
<td>2.23 ± 0.15</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>0.34 ± 0.03</td>
<td>3.74 ± 0.22</td>
</tr>
<tr>
<td>Kidney</td>
<td>203.26 ± 7.71</td>
<td>7.22 ± 0.29</td>
</tr>
</tbody>
</table>

*There was no effect of day of microsphere injection (9, 11, 13 or 15), condition (pregnant vs. nonpregnant) or side of removal (right or left) on these measurements (n=7).

*Total wts of these tissues could not be determined since only a portion was removed, weighed and homogenized for blood flow measurement.*
Health Division, Somerville, NJ). At necropsy, the uterus, ovaries, kidneys, lungs, fibularis tertius muscles, utero-ovarian lymph nodes, cervix, vagina, liver and pituitary were removed and weighed. Corpora lutea were dissected from the remainder of the ovary and weighed separately. The CL, the extratalutal component of the ovary, pituitaries and lymph nodes were placed individually in counting tubes for determination of radioactivity using a Tracor Analytic Model 1197 automatic gamma system. Each uterine horn was opened longitudinally from the uterotubal junction to the uterine body. Each uterine horn of nonpregnant gilts was divided into 10 sections of equal length, and the position of each section was noted (10-oviductal end of uterine horn; 1-cervical end of uterine horn). Uterine horns of pregnant gilts were divided into sections in contact with conceptus tissue (n=50) and sections devoid of conceptus tissue (n=67). A conceptus was defined as a viable embryo and its associated extraembryonic membranes as observed with a dissecting microscope. An average of 5 conceptuses (5.0 ± 0.7) were found dispersed throughout each uterine horn of pregnant gilts on Day 18. Each section of uterus was weighed, homogenized in a blender (B6, Model 91-215, Waring Product Division, New Hartford, CT), and aliquots of each were counted. The kidneys, lungs, fibularis tertius muscles, cervix, vagina and liver were also homogenized, aliquoted and counted. Triplicate samples of each homogenate were obtained for counting. Data were corrected for background and channel overlap.

Blood flow to the uterus, ovaries and other body tissues was determined by the proportion of microspheres that were transported to the arterial system and became lodged in the capillary bed of the tissue. The number of microspheres in the tissue (M_t) was determined by the following equation:

\[
M_t = \frac{R_b}{R_m} \times \left( W_t + WH_2O \right) \times \left( TW_2 \right)
\]

where \( R_b \) = counts per minute (CPM) per gram of homogenized or nonhomogenized tissue, \( R_m \) = mean CPM per microsphere determined from standard samples, \( W_t \) = weight of coarse ground tissue, \( WH_2O \) = weight of H_2O added to ground tissue and \( TW_2 \) = total weight of tissues. Total blood flow to tissue was determined by the equation:

\[
\text{Total blood flow (ml/min)} = \frac{M_t}{M_{ar}} \times W
\]

where \( M_t \) = number of microspheres in tissue; \( M_{ar} \) = number of microspheres in arterial reference blood sample and \( W \) = withdrawal rate (ml/min) of arterial reference blood sample.

**Statistical Analysis**

Blood flow to uterine segments of pregnant and nonpregnant gilts was adjusted by covariate analysis for a gradient increase in blood flow from the cervical to the oviductal end of each uterine horn. Differences in blood flow within pregnancy status were then determined by using a split-plot analysis for a randomized block design across days (Kirk, 1968). The average blood flow for uterine segments, CL, the extratalutal component of the ovary, oviducts, utero-ovarian lymph nodes and other maternal tissues of pregnant versus nonpregnant gilts was analyzed as a completely randomized split-plot design with day as a subplot. There were no differences due to side of sampling in any of the analyses performed. Differences between means or adjusted means were evaluated by use of orthogonal contrasts.

**RESULTS**

Blood flow (ml/min per g of tissue) to selected maternal tissues of gilts in this study are presented in Table 1. There was no effect of day of microsphere injection (9, 11, 13 or 15), condition (pregnant vs. nonpregnant) or side of removal (right or left) on blood flow to or weight of any of these tissues. Blood flow to the kidneys and lungs in this study are closely related to those reported for pregnant ewes by Christenson and Prior (1978) who utilized similar blood flow measurement techniques.

Weight of and total blood flow to each uterine horn of nonpregnant gilts did not differ across days, averaging 350.53 ± 19.08 g and 189.2 ± 10.35 ml/min, respectively. When blood flow to individual segments of uterine horns was examined, however, it was determined that blood flow increased (P<0.01) from the cervical (segment 1) to the oviductal end (segment 10) of each uterine horn (y=0.013X + 0.678, where y=blood flow in ml/min per g of tissue and x=segment number, Fig. 1).

A gradient increase in blood flow from the cervical to the oviductal end of uterine horns was also observed for pregnant gilts (y=0.032X + 0.431). As observed in nonpregnant gilts, blood flow (ml/min per g of tissue) was significantly higher (P<0.01) at the oviductal when
compared to the cervical end of each gravid uterine horn. In addition, blood flow to all uterine segments (in contact with and devoid of conceptuses) was greater (P<0.05) in pregnant sows than nonpregnant sows on each of the days studied (Fig. 2 and Fig. 1, respectively). Blood flow to uterine segments of pregnant gilts was higher (P<0.01) on Days 11 and 13 than on Days 9 and 15 (2.05 ± 0.11 and 1.92 ± 0.10 vs. 1.18 ± 0.05 and 1.48 ± 0.06 ml/min per g of tissue, respectively; Fig. 2). On Day 13, blood flow to uterine segments in contact with conceptuses was greater (P<0.01) than to uterine segments between conceptuses due mainly to a decreased blood flow to segments of uterine horn between conceptuses (2.13 ± 0.04 vs. 1.76 ± 0.04, ml/min per g of tissue). In addition, blood flow (ml/min per g of tissue) to the oviduct was significantly elevated (P<0.01) on Day 13 of pregnancy when compared to blood flow on other days of pregnancy or across all days in nonpregnant gilts which did not differ (Fig. 3).

There was no effect of condition (non-pregnant vs. pregnant) or day of sacrifice (15 or 18) on CL weight or the weight of the extra-luteal component of the ovary which averaged 0.47 ± 0.02 and 3.51 ± 0.12 g, respectively. In addition, average numbers of CL on each ovary of nonpregnant gilts were similar to those of pregnant gilts averaging 7.7 ± 0.7. Blood flow to the extraluteal component of the ovary, as stated in Table 1, was unaffected by day, condition or side of removal. Corpora lutea blood flow (ml/min per g of tissue), however, was elevated (P<0.01) in pregnant gilts on Day 13 (23.97 ± 2.51) when compared to CL blood flow of pregnant gilts on Days 9, 11 or 15 which averaged 15.01 ± 1.26 or nonpregnant gilts across all 4 days post-estrus which averaged 15.20 ± 1.21 (Fig. 4).

**DISCUSSION**

Data presented here demonstrate an effect of the porcine conceptus on increasing blood flow to the uterine vascular bed as early as Day 9 of pregnancy, coincident with the migration of conceptuses from just below the tubouterine junction to the uterine body. During the time when embryos are migrating within the uterine lumen (Days 9 and 11), blood flow was uniformly elevated throughout each uterine horn of pregnant gilts. Only when the spacing of embryos was complete (Day 13) was vaso-dilation associated preferentially with uterine segments in contact with conceptuses at necropsy. These data suggest that the vasodilatory effect emanates from each conceptus to the endometrium in contact with it and argues against the presence of preferred highly vascularized attachment sites within a uterine horn. Placentation in the pig is diffuse; it is a gradual process beginning as early as Day 13 and is well advanced by Day 18 of pregnancy (Perry et al., 1976). It thus appears that the initial stages of attachment in the pig may be
Maximal conceptus-stimulated increases in UBF of pregnant gilts first occurred on Day 11 which was 48 h earlier than the transient increase in blood flow to CL observed on Day 13. Blood flow through the extraluteal component of the ovary, however, remained constant. These data suggest that the conceptus produces a vasodilatory factor which acts first at the level of the uterus and then may diffuse to the ovary where a selective increase in luteal blood flow is stimulated. This increase in blood flow to porcine CL (Day 13) occurs on a day when CL become susceptible to the luteolytic effects of PGF₂α (Douglas and Gitner, 1975; Hallford et al., 1975; Moeljono et al., 1976). An increase in blood flow to the oviduct of pregnant gilts occurred concomitantly with the transient rise in luteal blood flow, suggesting a rather diffuse transfer of the conceptus-associated vasodilatory substance from the uterine lumen. Each uterine horn in the pig has prominent lymphatics which drain fluid from the horn and anastomose with the ovarian and tubal lymphatic systems (Yoffey and Courtice, 1970; Andersen, 1926, 1927). Concentrations of estrogens in uterine lymph from Day 11 to 15 of pregnancy in gilts are elevated when compared to uterine lymphatic concentrations of these steroids on similar days of the estrous cycle (Magness and Ford, 1981). Thus, one route whereby estrogen of embryonic origin leaves the uterus is via the lymphatic drainage, which may function in the transfer of this substance to the ipsilateral ovary and oviduct.

Elevated levels of PGF₂α are found in the utero-ovarian vein of sows (Gleeson et al., 1974) coincident with declining plasma progesterone concentrations during the estrous cycle, and exogenous PGF₂α has a luteolytic action in this species (Moeljono et al., 1976). The luteolytic properties of PGF₂α were first described over a decade ago (Pharriss and Wyngarden, 1969), yet the mechanisms by which PGF₂α induces regression of the CL are not well defined. Some investigators have suggested that the luteolytic activity of PGF₂α is mediated via its vasoconstrictive properties, resulting in a reduced flow of blood to the CL (Pharriss, 1970; Novy and Cook, 1973; Nett and Niswender, 1981). In contrast, others have found no change in total ovarian blood flow after administration of PGF₂α even though secretion of progesterone was greatly reduced (Baird,
In the rat, PGF₂α causes a decrease in receptors for luteinizing hormone (LH) and an associated decrease in secretion of progesterone (Grinwich et al., 1976; Hichens et al., 1974). More recently, Behrman et al. (1978) demonstrated that in vivo accumulation of human chorionic gonadotropin (hCG) in ovarian tissue of pseudopregnant rats was decreased within 2 h after administration of PGF₂α, but the capacity of the isolated membranes to bind hCG was not changed. These data suggest the possibility that PGF₂α may have decreased luteal perfusion, and therefore, the quantity of hCG available to bind to receptors. In agreement with this hypothesis, Nett and Niswender (1981) observed a 90% reduction in luteal blood flow within 2 h after administration of PGF₂α to ewes. This dramatic reduction in luteal blood flow in response to PGF₂α preceded the decreases in total ovarian blood flow and systemic concentrations of progesterone which occurred within 6 h. Thus at least a portion of the luteolytic activity of PGF₂α may be manifested via a reduction in blood flow through the CL.

As previously discussed, increased concentrations of estrogens are found in uterine venous plasma and uterine flushings as early as Day 11 of gestation in pigs (Ford et al., 1982a). These steroids appear to be derived from the conceptus, since high concentrations of both estrone and estradiol-17β (E₂β) are found in porcine conceptus tissue on Days 12 and 13 of gestation (Gadsby and Heap, 1978). Estrogens are luteotrophic in the pig when administered systemically (Gardner et al., 1963) or into the uterine lumen (Ford et al., 1982b) between Days 11 and 15 after the onset of estrus. In addition, Ford et al. (1982b) demonstrated a local effect of E₂β in the stimulation of luteal function. These researchers observed that the injection of E₂β into one isolated uterine horn of gilts, preferentially stimulated luteal function on the ipsilateral ovaries. This local effect of E₂β on stimulating progesterone secretion by CL on the ipsilateral ovaries was not due to decreased uterine PGF₂α secretion, since PGF₂α concentrations were similar in utero-ovarian venous blood draining both uterine horns of treated gilts. The presence of an embryonic luteotropin has also been demonstrated by Ball and Day (1982a,b), during early pregnancy in the pig.

It has been suggested by Bazer and Thatcher (1977) that luteal maintenance during early pregnancy in the pig may result from an estrogen-induced sequestering of PGF₂α in the uterine lumen resulting in a reduced secretion of PGF₂α into the uterine vein. Since CL of most gilts become susceptible to the luteolytic effects of PGF₂α by Day 12-post-estrus (Douglas and Ginther, 1975; Halford et al., 1975) or post-mating (Guthrie and Polge, 1978), the decreased uterine venous concentrations of PGF₂α observed on Day 13 (Moeljono et al., 1977) may be an important event in luteal maintenance during early pregnancy. This proposal is supported by the observation that the presence of embryos in the uterus of the pig only until Day 13 post-mating will result in an extension of luteal life span (Ford et al., 1982a). Since UBF of pregnant gilts is significantly higher than for nonpregnant gilts on Days 12 and 13 (Ford and Christenson, 1979), the reduced concentrations of PGF₂α in uterine venous blood during this period of early pregnancy may result from dilution of PGF₂α by the increased quantities of uterine venous blood associated with the transient rise in uterine blood flow. In agreement with this hypothesis Schille et al. (1979) have demonstrated elevated concentrations of 15-keto-13, 14-dihydro-prostaglandin F₂α in systemic blood of pregnant gilts when compared to nonpregnant gilts on Days 11 to 13 of gestation or the estrous cycle, respectively. A conflicting report exists, however (Guthrie and Rezroad, 1981). It has been demonstrated that 15-keto-13, 14-dihydro-prostaglandin F₂α is the major blood plasma metabolite of PGF₂α in systemic blood (Kindahl et al., 1976) and it has been suggested that measurement of this metabolite is the best means of following uterine PGF₂α release in vivo. If this premise is true, a greater amount of PGF₂α may actually be secreted by uteri of pregnant than nonpregnant gilts on Days 11 to 13, days critical for pregnancy recognition in this species. Thus, the conceptus-mediated vasodilatory effect may function to reduce blood concentrations of PGF₂α, as well as to prevent its vasoconstrictor effects on the utero-ovarian vascular bed.

Of interest is the observation that blood flow increased from the cervical to the oviductal end of each uterine horn of nonpregnant and pregnant gilts in this study. These data agree with those of Ford et al. (1979) who observed a similar gradient in blood flow from the cervical to the oviductal end of each uterine horn of estrous ewes.
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REFERENCES


