BLOOD FLOW, STEROID SECRETION AND NUTRIENT UPTAKE OF THE GRAVID UTERUS DURING THE PERIPARTURIENT PERIOD IN SOWS[1]

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Summary

Blood flow to one uterine horn of six Yorkshire sows was measured daily from d −22 to 0 (d of parturition) using an electromagnetic blood flow transducer surgically implanted around the middle uterine artery. Immediately after measurement of uterine arterial blood flow (UABF), samples of femoral arterial (FA) and uterine venous (UV) blood were collected via indwelling catheters and concentrations of progesterone, estrone and estradiol-17β, oxygen, glucose, total α-amino acid N and urea N were determined. Throughout the experimental period, sows were maintained in farrowing stalls. Surgical procedures used in this study had no effect on length of gestation, litter size, number of live piglets born or average weight of live piglets when compared with noninstrumented littermate controls. The UABF remained constant from d −22 through −1, then declined dramatically on the day of parturition with delivery of the fetuses and placentae. Concentration of progesterone in FA and UV blood of sows remained constant from d −22 to −3, but was higher (P<.01) in FA (12.68 ± .48 ng/ml) than in UV (7.56 ± .20 ng/ml) blood. Progesterone concentrations in FA and UV blood began to decline 2 d before parturition to reach low levels on d 1. Estrone and estradiol-17β concentrations were greater (P<.01) in UV than in FA blood, and increased progressively from d −22 to reach peak levels on d −4 through −1 which averaged 7,245 ± 655 and 1,001 ± 88 pg/ml in UV blood and 3,923 ± 157 and 547 ± 35 pg/ml in FA blood, respectively. Concentrations of both estrogens then declined rapidly to reach low levels by d 1. The FA concentration and FA − UV difference in oxygen, glucose, α-amino acid N and urea N did not vary from d −22 to parturition, suggesting that both oxygen and nutrient uptake by the gravid uterus remains constant during the last 22 d of gestation even though fetal weight increases dramatically during this period.
(Key Words: Sow, Gravid Uterus, Blood Flow, Steroid Production, Nutrient Uptake.)

Introduction

A more thorough understanding of hormonal and nutrient requirements for normal development of the porcine fetus and placenta is essential if we are to understand the factors contributing to stillbirths and reduced piglet viability at parturition. Despite recognition of this need, little information is available describing the net uptake of oxygen and nutrients and production of hormones by the gravid porcine uterus. Recently, techniques have been developed that allow continuous measurement of blood flow to a uterine horn in conscious sows over long periods of time (Ford and Christenson, 1979). Also, chronic techniques

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are now available for simultaneous collection of blood from a femoral artery (systemic blood) and the main uterine vein draining a gravid uterine horn.

The objectives of this study were to determine uterine arterial blood flow (UABF) and femoral arterial (FA) and uterine venous (UV) concentrations of progesterone, estrone, estradiol-17β, oxygen, glucose, total α-amino acid N and urea N from 90 d of gestation until parturition.

Experimental Procedure

Six Yorkshire sows were assigned to surgery on d 90 of gestation. Feed was withheld from sows for 24 h before surgery. General anesthesia was induced by infusion of 1.0 g of Surital (Sodium thiamylal)[4], and surgical anesthesia was maintained with a mixture of oxygen and halothane (Fluothane)[5] administered in a closed-circuit system with soda lime for removal of CO_2.

The uterus was exposed through a midventral incision and a precalibrated electromagnetic blood flow transducer[6] (18, 20 or 25 mm internal circumference) was interiorized via a flank incision and placed around the middle uterine artery, supplying one randomly selected uterine horn, proximal to its first bifurcation in the mesometrium as described previously (Ford and Christenson, 1979). The number of fetuses in the selected uterine horn was then determined and a polyvinyl cannula (.86 mm id, 1.57 mm od) treated with a heparin complex[7] was inserted through a small branch of the uterine vein into the main uterine vein draining the horn 5 to 10 cm proximal to its confluence with the ovarian vein. The cannula and the electrical connector of the blood flow transducer were sutured to an elastic patch glued to the flank over the site of entry. An additional polyvinyl cannula (1.12 mm id, 1.65 mm od) treated with heparin complex[7] was positioned in the femoral artery via the saphenous artery and used to obtain arterial blood from each sow throughout the experiment (Magness et al., 1983).

After surgical recovery, sows were placed in farrowing stalls where they remained throughout the experimental period. Beginning on the second day after surgery, UABF was monitored continuously for 10 min once daily between 0700 and 1000 h until the day of parturition (d 0). The measurement of UABF on d 0 occurred within 2 h after the completion of farrowing. Only five of the six sows could be monitored due to a nonfunctional blood flow probe on one of the animals. Blood flow values (ml/min) displayed by the flowmeter[8] were recorded at 15-s intervals during each 10-min period for each artery and were considered an estimate of UABF for that day.

Immediately after UABF was determined each day, samples of blood from the femoral artery and uterine vein were collected simultaneously. Femoral arterial blood continued to be collected daily from d 1 to 7 after parturition. The resulting plasma was frozen at −20 C until assayed for progesterone, estrone and estradiol-17β. Radioimmunoassays for progesterone, estrone and estradiol-17β were exactly as described and validated previously in this laboratory (Magness and Ford, 1982). Day-to-day assay variations for progesterone, estrone and estradiol-17β were determined by including a uterine venous plasma pool from a late pregnant sow in each of several assays. The resulting concentrations (mean ± SE; n = 4) and interassay coefficients of variation were 21.10 ± .98 ng/ml and 2.3% for progesterone, 2,771 ± 108 pg/ml and 6.8% for estrone and 414 ± 15 pg/ml and 6.4% for estradiol-17β.

Plasma concentrations of glucose were determined by the use of the hexokinase and glucose 6-phosphatase dehydrogenase catalyzed reactions. Urea N concentrations were determined by the use of the urease and glutamate dehydrogenase reactions[9]. Concentrations of reduced nicotinamide adenine dinucleotide (NADH) were determined by absorbance at 340 nm in both systems. An autoanalyzer system using reagents previously described[10] was used to determine total α-amino acid N concentrations in plasma. Briefly, .23 ml plasma was mixed with 1.0 ml normal saline and dialyzed (61 cm path length premounted dialysis membrane[11]) into 1.0 ml saline. A solution of .002

[4] Parke-Davis Laboratories, Detroit, MI.
[5] Ayerst Laboratories, New York, NY.
[6] Carolina Medical Electronics, Inc., King, NC.
[7] TDMAC Heparin complex, Polysciences, Inc., Warrington, PA.
[8] Model 501D, Carolina Medical Electronics, Inc., King, NC.
[9] Gilford Diagnostics, Cleveland, OH.
[10] Technicon Res. Bull. No. 20, 1968, Technicon Corp., Ardsley, NY.
[11] Evergreen Scientific, Los Angeles, CA.

TABLE 1. REPRODUCTIVE CHARACTERISTICS[a]

Item	Sows in this study (n = 6)	Littermate controls (n = 18)
Length of gestation, d	114.5 ± .5	115.0 ± .2
Litter size	11.7 ± 1.1	9.2 ± .7
No. live piglets born	8.7 ± 1.0	8.3 ± .5
Avg weight of live piglets, kg	1.30 ± .08	1.46 ± .05

[a]Means ± SE.

M hydrazine sulfate (.32 ml) was added to .6% ninhydrin solution (.8 ml) and subsequently mixed with the dialysis product. The ensuing reaction was at 95 C, and total α-amino acid N was quantified by absorption at 570 nm wavelength. Leucine solutions of 0 to 1.0 mg/ml were prepared in .1 N HCl for use as standards.

Femoral arterial and UV blood samples from four sows were also collected daily into heparinized 2.5 ml disposable glass syringes, which were sealed and transported on ice to the laboratory within 30 min of collection for determination of oxygen content[12]. Hematocrits also were determined for these four sows.

Daily blood flow averages for each artery were considered as single observations for statistical analysis to characterize time trends. Changes in UABF, as well as uterine venous and arterial concentrations of steroids, were analyzed by split-plot analysis of variance (Kirk, 1968) using the Statistical Analysis System (SAS, 1979). Differences between means were tested for significance by use of orthogonal contrasts (Kirk, 1968). Nutrient data were evaluated by analyses of covariance. Sow and day within sow were included in the model. Correlations between UABF, daily plasma concentrations and uterine uptakes of progesterone, estrone, estradiol-17β, glucose, α-amino acid N and urea N were also calculated.

Results and Discussion

As depicted in table 1, surgical procedures used in this study, as well as daily monitoring of UABF and blood collection had no significant effect (P>.05) on length of gestation, litter size, number of piglets born alive or average weight of live piglets when compared with noninstrumented littermate controls. The slightly higher incidence of still births exhibited by gilts in this study when compared with littermate controls (3.0 vs .9) was not significant (P>.05) due to the large variation between gilts.

Blood flow to a uterine horn or uterine blood flow per fetus (figure 1) remained relatively constant during the last 22 d of gestation, averaging 1,571 ± 25 and 243 ± 13 ml/min, respectively, then decreased dramatically within 1 h after delivery of fetuses and placental tissue. These results were consistent with those of Ferrell and Ford (1980), who reported that uterine blood flow did not change significantly during the last one-third of gestation in the cow. Ford et al. (1982) reported that blood flow to the gravid uterine horn was

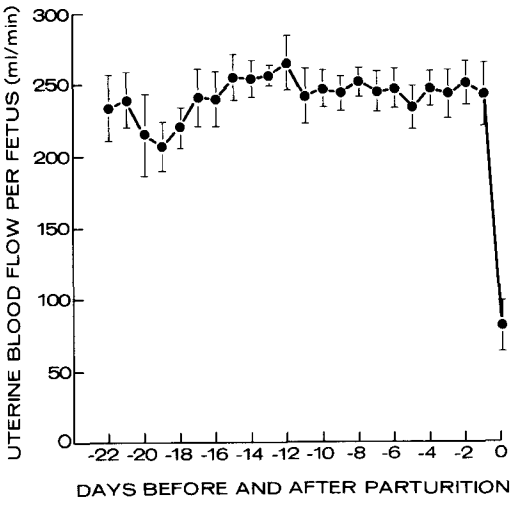

Figure 1. Blood flow to a gravid uterine horn of each of five gilts divided by the number of fetuses per horn, from day −22 through the day of parturition (mean ± SE). Day 0 samples were obtained within 2 h after farrowing.

[12]Blood Gas Analyzer Model 513, Instrumentation Laboratories, Lexington, MA.

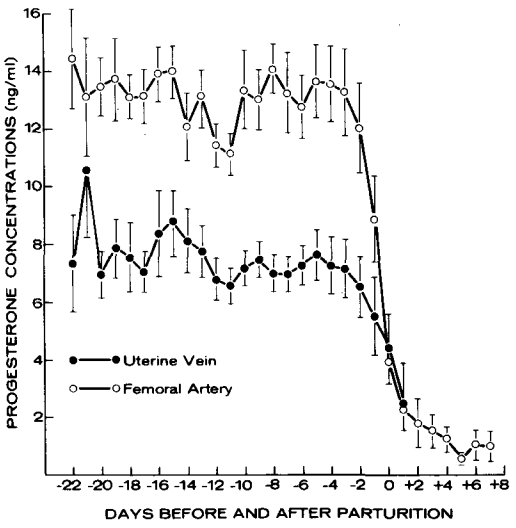

Figure 2. Concentrations of progesterone (ng/ml) in femoral arterial and uterine venous blood of sows during the periparturient period (mean ± SE).

constant during the last 30 d of gestation in the cow. Rosenfeld et al. (1976) reported a substantial increase in uterine blood flow during gestation in ewes. In that study, however, only one blood flow measurement was made on each of seven ewes during the interval of 30 to 78 d of gestation or on each of six ewes 120 to 139 d of gestation. No pattern of change in uterine myometrial, endometrial or placental blood flow with gestational ages is detectable from the six observations reported by Rosenfeld et al. (1976) during the last trimester.

From d −22 to −3 before parturition, the concentration of progesterone in FA and UV blood of gilts remained relatively constant, but was significantly higher ($P<.01$) in FA than UV blood, averaging 12.68 ± .48 and 7.56 ± .20 ng/ml, respectively (figure 2). Daily uterine uptake (FA progesterone − UV progesterone × UABF × 1 − hematocrit) of progesterone throughout the period averaged 7.70 ± .63 mg. Progesterone concentrations in FA and UV blood began to decline 2 d before parturition to reach low levels on d 1 (2.24 ± .67 and 2.46 ± 1.17 ng/ml, respectively). The pattern of progesterone secretion over the last 22 d of gestation differed from that reported by Robertson and King (1974) in that progesterone levels did not begin to decline until 2 d before parturition in the present study, while Robertson and King observed a marked decline in progesterone beginning on d 98 of gestation. The relatively late decline in progesterone beginning 2 d before parturition in the present study is consistent, however, with data previously reported by Molokwu and Wagner (1973) and First and Bosc (1979). The observation that progesterone is markedly higher in FA than in UV blood suggests metabolism by the uterus and(or) fetal placentae in agreement with previous studies (Knight et al., 1977; Kephart et al., 1981), and is consistent with the fact that the pregnant sow is dependent upon the corpora lutea for the maintenance of pregnancy (Du Mesnil du Buisson and Dauzier, 1957).

Estrone and estradiol-17β concentrations were greater ($P<.01$) in UV than in FA blood (figures 3 and 4) and increased progressively from d −22 to reach peak levels on d −4 through −1 which averaged 7,245 ± 655 and 1,001 ± 88 pg/ml in UV blood and 3,923 ± 157 and 547 ± 35 pg/ml in FA blood, respectively. Femoral arterial estradiol-17β concentrations did not increase significantly during the last 10 d of gestation, while UV concentrations were doubling during this same period. In contrast, estrone concentrations in FA and UV blood increased in parallel during the last 10 d of gestation. Uterine production (FA estrone or

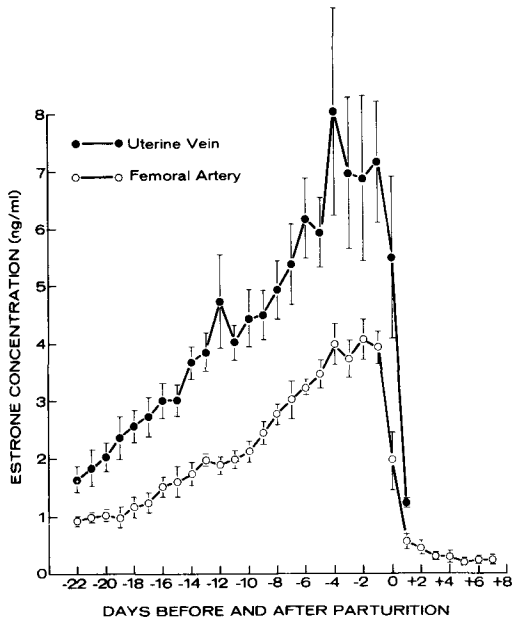

Figure 3. Concentrations of estrone (ng/ml) in femoral arterial and uterine venous blood of sows during the periparturient period (mean ± SE).

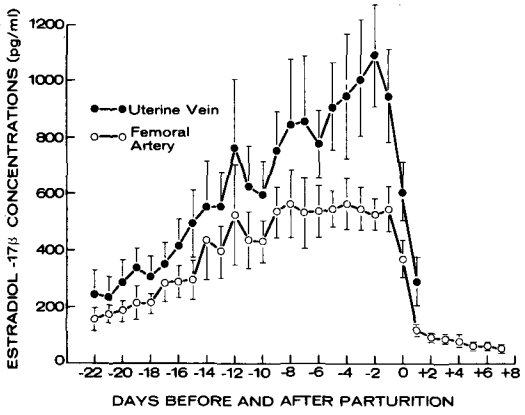

Figure 4. Concentrations of estradiol-17β (pg/ml) in femoral arterial and uterine venous blood of sows during the periparturient period (mean ± SE).

estradiol-17β − UV estrone or estradiol-17β × UABF × 1 − hematocrit) of estrone and estradiol-17β averaged 3.01 ± .27 and .33 ± .04 mg/d, respectively, from d −22 to −1. Concentrations of both estrogens then declined rapidly to reach low levels by d 1. From d 2 to 7, concentrations of progesterone, estrone and estradiol-17β in FA blood remained at very low levels. There was no significant correlation between UABF and steroid concentrations in FA or UV blood or uterine uptake and(or) secretion of steroids during the experimental period. The patterns and relative concentrations of unconjugated estrone and estradiol-17β in UV and FA blood throughout the last 22 d of gestation and the first 7 d postpartum are similar to those reported previously (Molokwu and Wagner, 1973; Robertson and King, 1974). The plasma levels of both estrogens rise over the last 20 d of gestation and attain a peak just before parturition. The fact that significantly higher concentrations of estrone and estradiol-17β are found in UV than in FA blood confirm previous studies, suggesting estrogen production within the gravid porcine uterus (Fèvre et al., 1968; Lunaas et al., 1973; Knight et al., 1977). Uterine uptake of progesterone coincides with the secretion of estrogens in almost equivalent amounts, suggesting conversion of progesterone to estrogens by the gravid uterus and(or) fetus.

Fetal weights on the different days of gestation were calculated by use of the equation reported by Salmon-Legagneur (1967). Estimated weights were adjusted, within litter, by mean weight relative to that estimated by the prediction equation. Blood flow to the gravid uterine horn relative to estimated fetal weight in that horn (figure 5) decreased ($P<.001$) quadratically during the 24-d interval before parturition. Cotter et al., (1969) indicated that blood flow rate per kilogram gravid uterine water was constant during the last one-half of gestation in goats. Fetal weight, as a proportion of gravid uterine weight, increases from about 20% at midgestation to 60% at term (Salmon-Legagneur, 1967; Rattray et al., 1974; Ferrell et al., 1976). Thus, the data reported by Cotter et al. (1969) are not inconsistent with those observed in this study. That uterine blood flow relative to fetal weight decreases during the last trimester of gestation may not imply a decreased nutrient supply to the fetus, relative to its weight. Ferrell et al. (1983) reported that the bovine fetus consumed about 29% of the oxygen taken up by the gravid uterus at 165 d of gestation, whereas Meschia et al. (1980) reported that the ovine fetus consumed about 57% of the oxygen taken up by the gravid uterus during the last 2 wk of gestation. These values are remarkably similar to the ratio of fetal weight to gravid uterine weight at these stages of gestation.

Neither arterial concentrations nor arterio-venous concentration differences of oxygen, glucose, α-amino acid N and urea N changed significantly with advancing gestation. As a result, mean values for each metabolite have been reported in table 2. Arterial oxygen concentrations and arterio-venous differences were slightly higher than that reported in cows (Ferrell et al., 1983) at 165 d of gestation, but

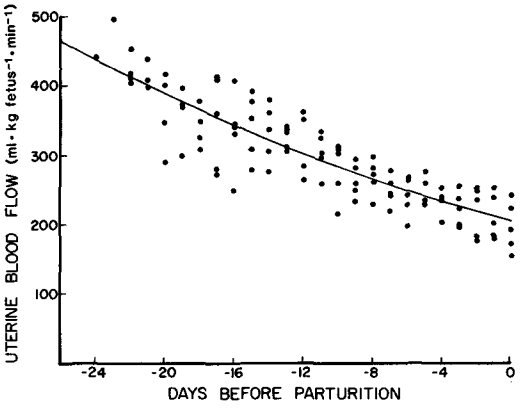

Figure 5. Relationship of uterine blood flow per kg fetus (Y) to days before parturition (X). Least-squares regression was: $Y = 203 \pm 9 - 6.6 \pm 1.8X \pm .13 \pm .08X^2$, $r = .87$, SE = 3.4.

arterio-venous differences were lower than in ewes during late gestation (Meschia et al., 1980). Arterial glucose and α-amino acid N concentrations were greater, but arterio-venous concentration differences were similar to those reported in the cow (Ferrell and Ford, 1980; Ferrell et al., 1983) and ewe (Christenson and Prior, 1978; Meschia et al., 1980). Arterial urea N and arterio-venous differences were similar to values observed in the cow (Ferrell et al., 1983). No significant correlations ($P<.10$) were observed between arterial concentrations and arterio-venous concentration differences for any of these metabolites even though a relatively wide range of arterial concentrations were observed. In this study, arterial concentrations of glucose and α-amino acid N ranged from 2.74 to 8.51 mM and from 28.8 to 60.6 meq/liter, respectively. These results are in agreement with those of Christenson and Prior (1978) and Ferrell and Ford (1980) and support their observations that, within normal ranges of arterial concentrations, arterio-venous differences of these nutrients appear to be essentially constant. These results also support the observation of Ferrell and Ford (1980) that throughout gestation in the normal animal, blood flow appears to be a primary determinant of nutrient availability to the gravid uterus and extend that observation to the periparturient period.

Uterine uptake values (table 2) were expressed on a per fetus basis to remove variation associated with differing numbers of fetuses per uterine horn. Because neither uterine blood flow per fetus nor arterio-venous concentration differences changed with gestational age, uterine uptake of oxygen, glucose, α-amino acid N and urea N did not change significantly during the last 22 d of gestation. Mean values suggested there was a net uptake of oxygen, glucose and α-amino acid N and a small net excretion of urea N.

That glucose is a major energy substrate of gravid uterine tissues has been well documented in the sheep (Meschia et al., 1980), cow (Ferrell et al., 1983) and other species. The data obtained in this study indicate that the glucose respiratory quotient (RQ - 6X glucose uptake/O_2 uptake) for the gravid uterus was .86, suggesting that up to 86% of gravid uterine energy expenditures in the sow can be met by absorbed glucose, assuming that no glucose is used as a carbon source of tissue synthesis. Ferrell and Ford (1980) and Ferrell et al. (1983) reported that 30 to 50% of the α-amino acid N was catabolized to urea by the gravid bovine uterus, indicating that amino acids were a major energy substrate of the gravid uterus in the cow. In contrast, these data indicate that about 13% of the α-amino acid N absorbed by the gravid porcine uterus was catabolized to urea. Oxygen required for urea synthesis (Ferrell et al., 1983) was .041 mmol/min, which yields an RQ value of .19. These results suggest that amino acid catabolism to urea supplies about 19% of the energy consumed by the gravid uterus of the

TABLE 2. ARTERIAL CONCENTRATION, ARTERIO-VENOUS CONCENTRATION DIFFERENCE AND UTERINE UPTAKE OF OXYGEN, GLUCOSE, α-AMINO ACID NITROGEN AND UREA NITROGEN IN THE SOW DURING LATE GESTATION[a]

Metabolite	No. of sows	Arterial concentration	Arterio-venous concentration difference	Uptake[b]
Oxygen	4	7.51 ± .04	.813 ± .002	.210 ± .005
Glucose	6	5.33 ± .08	.22 ± .04	.030 ± .009
α-amino acid nitrogen	6	44.2 ± .6	.55 ± .24	.086 ± .054
Urea nitrogen	6	6.79 ± .20	−.09 ± .07	−.011 ± .011

[a]Means ± SE; arterial concentrations and arterio-venous concentration differences of oxygen and glucose are expressed in mM and α-amino acid N and urea N in meq/liter.

[b]Oxygen uptake was calculated as: (arterio-venous concentration difference × blood flow to a uterine horn/number of fetuses in that horn). Other uptakes were calculated as: [arterio-venous concentration difference × blood flow to a uterine horn × (1-hematocrit)/number of fetuses in that horn]. Values are expressed as $mmol \cdot fetus^{-1} \cdot min^{-1}$ (oxygen and glucose) or $meq \cdot fetus^{-1} \cdot min^{-1}$ (α-amino acid N and urea N). Number of fetuses per horn averaged 5.9 ± .5 and ranged from 4 to 8.

sow. If one assumes that all the urea excreted from the gravid uterus resulted from fetal catabolism of amino acids, the data reported in table 2 suggest that the porcine fetus produced urea at a mean rate of .25 $g \cdot kg^{-1} \cdot d^{-1}$. This value is about 30% lower than reported in sheep (Gresham et al., 1972). These results suggest amino acids to be of lesser importance as energy substrates of the porcine gravid uterine tissues than in gravid uterine tissues of the cow or ewe. Conversely, catabolism of amino acids and excretion of N in other forms such as ammonia may be of greater relative importance in the sow than in ruminant species.

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