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Steroid concentrations in uterine lymph: possible mediators of uterine and ovarian blood flow during the estrous cycle and early pregnancy in the pig

Ronald Richard Magness
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STEROID CONCENTRATIONS IN UTERINE LYMPH: POSSIBLE MEDIATORS OF UTERINE AND OVARIAN BLOOD FLOW DURING THE ESTROUS CYCLE AND EARLY PREGNANCY IN THE PIG

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Ph.D. 1982
Steroid concentrations in uterine lymph: Possible mediators of uterine and ovarian blood flow during the estrous cycle and early pregnancy in the pig

by

Ronald Richard Magness

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

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GENERAL DISCUSSION

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ABSTRACT

Current investigations strongly suggest a relationship between local concentrations of estrogens and progesterone (P₄) and the function of periarterial adrenergic (vasoconstrictor) nerves innervating uterine and ovarian arteries. During the estrous cycle of the ewe, cow and sow, increases in the estrogen:progesterone (E/P₄) ratio in systemic blood are associated with elevations in blood flow through the uterine vascular bed. In addition, exogenous administration of estrogen has been shown to increase ovarian blood flow in ewes. Lymphatic vessels, which are in close linear apposition to the adventitia of the uterine and ovarian arteries may function to transport steroids to the utero-ovarian periarterial nerves. Techniques were thus developed for the acute and chronic collection of uterine lymph from Yorkshire gilts. Concentrations of estrone (E₁), estradiol-17β (E₂β) and P₄ in uterine lymph were compared to those in systemic blood during the estrous cycle and early pregnancy. Changes in ovarian blood flow were subsequently contrasted to the patterns of these steroids in systemic blood and uterine lymph throughout the estrous cycle and early pregnancy.

Experiment I: Uterine lymphatic catheters were chronically implanted into gilts on Days 0 or 1 (n=5), 5 (n=4), 11 (n=5) or 15 (n=4) of the estrous cycle. Uterine lymph and systemic blood were sampled daily from each gilt until patency of lymphatic catheters was lost, which averaged 5 days. Concentrations of E₁, E₂β and P₄ in
uterine lymph were positively correlated (P<0.01) with the concentrations of these steroids in systemic blood throughout the porcine estrous cycle. The E/P₄ ratio was greater (P<0.01) during the follicular phase than the luteal phase of the estrous cycle in both uterine lymph and systemic blood, however, concentrations of estrogens were greater (P<0.01) and P₄ was lower (P<0.01) in uterine lymph than systemic blood.

Experiment II: Uterine lymph and systemic blood of gilts were sampled under halothane anaesthesia on Days 11 (n=4), 13 (n=4) or 15 (n=4) of the estrous cycle or pregnancy. Although systemic concentrations of E₁, E₂, and P₄ were similar on these days of the estrous cycle and pregnancy, E₁ and E₂ concentrations were greater (P<0.01) in uterine lymph of pregnant compared with nonpregnant gilts. Regardless of pregnancy status, and in agreement with data in experiment I, uterine lymph had greater (P<0.01) concentrations of E₁ and E₂, and less (P<0.01) P₄ than systemic blood resulting in higher E/P₄ ratios in the lymph.

Experiment III: Ovarian blood flow and systemic concentrations of E₁, E₂, and P₄ were quantified in 4 sows throughout the estrous cycle and first 21 days of pregnancy. Blood flow to the ovary was positively correlated (P<0.01) with P₄ concentrations during the estrous cycle and early pregnancy. However, significant negative correlations of ovarian blood flow with E₁ and E₂ were only observed during the estrous cycle. During the estrous cycle, ovarian blood flow and P₄ concentrations were lowest at estrus, highest during the
luteal phase and declined precipitously after Day 14. Patterns of ovarian blood flow during the subsequent pregnancy of each sow were similar to those observed during the estrous cycle until Day 12 post-mating. Between Days 12 and 15 of pregnancy, but not the estrous cycle, ovarian blood flow and P4 concentrations increased transiently by 26% (P<0.01) and 20% (P<0.05), respectively. Luteal function was maintained in the pregnant sows while P4 concentrations and ovarian blood flow declined (P<0.01) abruptly after Day 14 of the estrous cycle.

Thus, the concentrations of E1, E2β and P4 in uterine lymph followed patterns similar to those observed in systemic blood throughout the porcine estrous cycle; however, estrogen concentrations were elevated in uterine lymph of pregnant gilts when compared to nonpregnant gilts between Days 11-15 postmating. This elevation of the E/P4 ratio in uterine lymph of pregnant pigs is associated with a dilatation of the uterine and ovarian vascular beds and these events occur on days when conceptuses initiate luteal maintenance in this species. It is conceivable that steroids in uterine lymph may control dynamic changes in uterine and ovarian blood flow, due to their close proximity to the periarterial nerves of the utero-ovarian vasculature.
REVIEW OF LITERATURE

Vascular Anatomy of the Uterus and Ovaries

Normal physiological function of any mammalian tissue, including the reproductive organs, is dependent upon an adequate blood supply. The uterus and ovary receive their blood supply through the broad ligament (Reynolds, 1949). Each uterine horn in the pig receives blood by way of the middle uterine artery which is derived from a branch of the internal iliac artery (Nunez and Getty, 1969). During the estrous cycle and early pregnancy there is evidence to suggest that the middle uterine artery supplies greater than 90% of the blood to each uterine horn of the pig (Ford and Christenson, 1979; Ford et al., 1982a, 1982d). Another branch of the internal iliac artery, the vaginal or urogenital artery, supplies blood to the vagina, bladder, cervix, vulva, anus and a portion of the uterine body (Nunez and Getty, 1969). Two or three venous channels drain blood from the cervix, uterine body and middle portions of the uterine horn into the middle uterine vein. The middle uterine vein and its branches run in close apposition to the proximal and distal branches of the middle uterine artery. Venous drainage from the cranial third of the uterine horn flows through the uterine branch of the ovarian vein and mixes with venous blood flowing from the ovary via the ovarian branch of the ovarian vein (Ginther, 1976). Blood from the middle uterine vein and the uterine and ovarian branches of the ovarian vein drain into the vena cava or the common iliac vein by way of the common utero-ovarian
vein (Del Campo and Ginther, 1973). In a small number of sows, the middle uterine vein joins the common iliac vein independently from the utero-ovarian vein (Nunez and Getty, 1970).

The main ovarian artery of the pig branches directly from the dorsal aorta and subsequently bifurcates into several convoluted branches which supply blood to the ovary, ovarian bursa, oviduct and tip of the uterine horn (Nunez and Getty, 1969; Del Campo and Ginther, 1973). In the pig, many small communicating rami are present connecting the uterine and ovarian arteries (uterine branches of the ovarian artery; Ginther, 1976) suggesting the possibility of blood flow between the uterine and ovarian vascular beds. In contrast to the many small uterine branches of the ovarian artery in the pig, the cow has only one or two major rami (Lamond and Drost, 1974). Ford and Chenault (1981) recently demonstrated that 20-40% of the blood flow to ovaries bearing fully functional corpora lutea (CL) of cows is of uterine origin, supplied through the uterine branch of the ovarian artery. At estrus, however, blood flow through this branch reversed direction, to supply blood to the ipsilateral uterine horn. Neither the quantity nor the direction of blood flow through the uterine branches of the ovarian artery in the pig has been determined.

Located below the porcine ovary is a prominent utero-ovarian vascular pedicle comprised of highly convoluted sections of the ovarian artery which overlie the point where the uterine and ovarian branches of the ovarian vein join to form the common utero-ovarian vein. The intertwining of the ovarian arterial branches increases the
surface area of contact with the ovarian, oviductal and uterine veins as they traverse between these venous channels in the broad ligament (Ginther, 1976).

Anatomy of the Uterine and Ovarian Lymphatics

The first description of the uterine lymphatic system was reported by Nuck in 1692 (Fabian, 1981), although Hippocrates and Aristotle Galen had described the anatomy of the uterus as early as the fourth century B.C. (Wynn, 1967). In the latter part of the nineteenth century, several investigators characterized the anatomy of the lymphatic vessels of the uterus and their drainage into the lumbar lymph nodes (Fridolin, 1872; Leopold, 1874; Mierzejewski, 1879; Hoggan and Hoggan, 1882; Poirier, 1890). Hoggan and Hoggan (1882) published the most extensive study on the anatomy of the lymphatics of the uterus of both laboratory and farm animal species. These investigators reported that the areas for uterine lymph formation were the main lymphatic plexuses located primarily in the myometrium, but also in the endometrium and perimetrium. These lymphatic plexuses are groups of uterine lymphatic vessels which anastomose between the various tissue layers of the uterus.

In the pig, lymph drains from a uterine horn through collecting lymphatic channels which are in close linear apposition to the
unilateral vasculature in the mesometrium (Hoggan and Hoggan, 1882). Uterine lymphatics function in addition to the venous system to drain fluid from each uterine horn (Yoffey and Courtice, 1970). Prominent uterine lymphatic vessels in sows anastomose with the ovarian and tubal lymphatics in the utero-ovarian vascular pedicle at or near to the utero-ovarian lymph node (Andersen, 1926, 1927) located in the mesometrium where the ovarian branch of the ovarian artery coils around the uterine and ovarian branches of the ovarian vein (Ginther, 1976). A functional pathway for the direct transfer of lymph from the uterus to the ovary has not been demonstrated in either the sow (Andersen, 1927) or ewe (Meckley and Ginther, 1969; Cicmanec, 1972; Staples et al., 1982). Furthermore, lymphatic channels draining lymph away from the uterus and ovaries contain valves which prevent retrograde flow through the lymphatic vessels (Andersen, 1927; Morris and Sass, 1966).

Uterine and Ovarian Perivascular Nerves

Blood flow through the arterial vascular beds in the abdominal viscera of mammals (including the reproductive organs) is controlled by periarterial sympathetic vasoconstrictor nerves (Langley and Anderson, 1896; Bayliss, 1923) which innervate blood vessels from the distributing extrinsic arteries through the arterial resistance vessels (Bevan, 1962; Paterson, 1965). Shabanah et al. (1964) reported that the main sympathetic perivascular plexus surrounding
both the uterine and ovarian vessels is comprised of nonmyelinated postganglionic nerve fibers derived from the periaortic plexus. These fibers become incorporated into the adventitia of the large distributing blood vessels at their points of origin, and enter the uterus and ovary through the broad ligament, by traversing the lengths of the uterine (Medowar, 1928; Keiffer, 1932) and ovarian (Unsicker, 1974) arterial branches. Vasomotor neurons to the uterus and ovaries thus bypass the hypogastric nerves which innervate the smooth muscle of the reproductive organs. In addition, the uterine artery receives additional sympathetic fibers from the pelvic plexus (Reynolds, 1949; Shabanah et al., 1964).

The adrenergic innervation of blood vessels is found specifically within the tunica adventitia at the adventitial-medial junction surrounding the smooth muscle of the tunica media (Dolezel et al., 1975; Bevan, 1979). Dolezel et al. (1975) demonstrated that electrical stimulation of these postganglionic periarterial sympathetic nerves caused release of neurotransmitter (norepinephrine; NE) which diffuses through the tunica media. Vasoconstriction is induced as NE stimulates the membrane bound α-adrenergic receptors, which predominate on the smooth muscle cells in the walls of blood vessels of the abdominal visera including the uterus and ovaries (Innes and Nickerson, 1970).

Data which suggest a vasoconstrictor function of uterine periarterial neurons were supported by the observations that electrical stimulation of these nerves almost obliterated blood flow to the
uterus of the pregnant ewe (Greiss and Gobble, 1967), and the administra-
tion of low doses of NE directly into the uterine artery of non-
pregnant ewes decreased uterine blood flow substantially (Barton et
al., 1974). In addition, in vitro perfusion of NE through uterine or
ovarian arteries or through the entire ovarian vascular bed, augmented
the constrictor response to electrical stimulation of periarterial
nerves. In these studies, vasoconstriction in response to nerve
stimulation was almost completely prevented by prior exposure of the
uterine or ovarian vasculature to phentolamine, again suggesting the
prevalence of α-adrenergic control (Greiss and Pick, 1964; Ladner et
al., 1970; Ford et al., 1977a, 1977b; Reynolds and Ford, 1982). The
presence of β-adrenergic receptors in uterine or ovarian vascular
smooth muscle was not demonstrated in any of these studies and it was
concluded that uterine and ovarian blood vessels are primarily con-
trolled by the presence or absence of adrenergic vasoconstrictor tone.

Contractility of lymphatic vessels also appears to be controlled
by the autonomic perivascular neurons located outside the muscularis
layer of the lymphatic vessels (Vajda, 1966; Todd and Bernard, 1973;
Alessandrini et al., 1981). Florey (1927a; 1927b) reported that the
in vivo spontaneous contractions of intestinal lymphatics of rats and
guinea pigs were enhanced by transmural electrical stimulation of the
sympathetic nerves which supply the lymphatics or by direct applica-
tion of epinephrine to the vessel wall. Roddie et al. (1980) reported
that acetylcholine and atropine did not affect the in vitro contrac-
tility of bovine mesenteric lymphatic vessels. These investigators observed however, that the inherent contractility of these vessels was enhanced by stretch, transmural electrical stimulation or epinephrine and reduced by isoproterenol (a β-adrenergic agonist). The response to electrical field stimulation was abolished by tetrodotoxin, a blocker of nerve action potentials. Furthermore, electrical field stimulation in the presence of the α-adrenergic antagonist phenoxybenzamine caused sympathetic reversal. These data suggest that intrinsic rhythmic contractions of lymphatic vessels are controlled primarily by the sympathetic nerves which innervate both lymphatic and blood vessels, and that alpha and beta adrenergic receptors increase and decrease the rate of contraction of the lymphatics, respectively. No specific studies on the contractility, pressure changes or innervation controlling flow through uterine lymphatic vessels have been reported.

Patterns of Blood Flow to the Uterus

It has long been known that during estrus a state of persistent hyperemia develops in the uterine vascular bed. Markee (1932) observed that intraocular endometrial transplants in rabbits, guinea pigs and monkeys underwent rhythmic changes during the ovarian cycle and were very hyperemic near the time of ovulation. Uterine blood flow was elevated just prior to and at the time of estrus when estrogen is highest and P_4 is lowest, in the ewe (Greiss and Anderson, 1969; Moore et al., 1969), cow (Ford et al., 1979a) and sow (Guthrie
et al., 1972; Ford and Christenson, 1979). In the pig, blood flow to each uterine horn increased from a flow rate of 100 ml/min to 200 ml/min between Days 15 to 16 postestrus, then remained elevated until Day 2 postestrus until declining to a low level again during the luteal phase of the estrous cycle (Ford and Christenson, 1979). Thus, uterine blood flow during the estrous cycle is positively associated with the ratio of estrogen to P₄ (E/P₄) in systemic blood (Ford et al., 1979a; Ford, 1982). Exogenous administration of estrogen, which elevated the E/P₄ ratio in systemic blood, has been shown to increase uterine blood flow in the ewe (Huckabee et al., 1970), cow (Roman-Ponce et al., 1978) and sow (Dickson et al., 1969). In contrast, P₄ has been shown to reduce an estrogen-induced increase in blood flow to the sheep uterus (Caton et al., 1974; Resnick et al., 1977). The magnitude of this antagonism between estrogen and P₄ appeared to be related to the ratio of the two steroids (Caton et al., 1974). These data suggest that the increased uterine blood flow at estrus in the pig (Ford and Christenson, 1979) results from the vasodilatory action of estrogen and that the gradual decline in blood flow after estrus may result from P₄ blockade of estrogen-induced hyperemia, or simply from the withdrawal of estrogen.

During the first 11 days of pregnancy, the profile of blood flow to the uteri of sows is similar to that on the same days of the estrous cycle. On Days 12 to 13 of pregnancy, however, Ford and Christenson (1979) observed a transient 2- to 3-fold increase in
uterine blood flow which occurred in a local manner since the increased blood flow was limited to the uterine horns containing conceptuses in unilaterally pregnant sows (Ford and Christenson, 1979) and was elevated preferentially to the uterine segments adjacent to conceptuses (Ford et al., 1982d). A transient increase in blood flow to the gravid uterine horns of ewes on Days 13 to 15 (Greiss and Anderson, 1970) and cows on Days 15 to 17 (Ford et al., 1979a) of gestation also has been reported. The embryonic signal which initiates luteal maintenance occurs by Day 12 in pigs (Dhindsa and Dziuk, 1968; Ford et al., 1982a), Day 13 in ewes (Moor and Rowson, 1966) and Day 16 in cows (Betteridge et al., 1978; Northey and French, 1980). Thus, the ovine, bovine and porcine conceptuses increase blood flow to the early gravid uterus, during the period when the presence of the embryo is essential to ensure luteal maintenance and the continuation of pregnancy.

Porcine conceptuses develop the capacity to synthesize estrogens in vitro by Day 12 postmating (Perry et al., 1976). In addition, concentrations of estrone (E₁) and estradiol-17β (E₂β) in blastocyst tissue on Days 12 to 13 were greater than those in either the uterine flushings or the endometrium, suggesting that these estrogens are of conceptus origin (Gadsby and Heap, 1978). Concentrations of E₂β were increased in uterine venous blood and uterine flushings of sows coincident with the transient 2- to 3-fold rise in uterine blood flow observed during early pregnancy (Ford et al., 1982a). By Day 15 of
pregnancy, both uterine blood flow and estrogen concentrations had declined significantly, suggesting a cause and effect relationship. In agreement with these data, concentrations of $E_2$ were elevated in the uterine flushings and venous blood draining the gravid uterine horn of cows on Days 14 to 18 of pregnancy, coincident with elevated uterine arterial blood flow only to the horn containing a conceptus (Ford et al., 1979a, 1981). Recently, Reynolds, Magness and Ford (Iowa State Univ., unpublished observations) have observed increased concentrations of $E_1$ and $E_2$ in the uterine flushings of pregnant ewes on Days 13 and 15 postmating, also in association with a doubling of uterine blood flow to the gravid horn. These data suggest an association between the concentration of estrogens of conceptus origin in the uterine lumen and the transient rise in uterine blood flow noted during a time critical for the continuation of pregnancy in these species.

Patterns of Blood Flow to the Ovaries

Ovarian blood flow also fluctuates in a cyclic manner throughout the estrous cycle, but in a manner opposite to the changes noted for uterine blood flow (Ford and Chenault, 1981). Blood flow to the ovary is highest during the luteal phase of the estrous cycle and lowest during proestrus and estrus in the pig (Rathmacher and Anderson, 1968), cow (Ford and Chenault, 1981), ewe (Mattner and Thorburn, 1969; Moore et al., 1972; Niswender et al., 1975; Brown et al., 1976) pseud-
dopregnant rabbit (Novy, 1972), guinea pig (Chaichareon et al., 1976; Sjoquist et al., 1977; Hossain et al., 1979), and rat (Bruce and Dimmitt, 1977). In the cow, ovarian blood flow is positively correlated with \( P_4 \) concentrations and negatively correlated with \( E_2 \) concentrations in systemic blood throughout the estrous cycle (Ford and Chenault, 1981). The cyclic fluctuation in ovarian blood flow occurs only to luteal ovaries and is due primarily to changes in the flow through the CL which receive approximately 90% of the total blood flow to the ovary (Novy, 1972; Bruce and Moor, 1975, 1976; Ford et al., 1982d). Blood flow to the nonluteal ovary in unilaterally ovulating species remains relatively constant throughout the estrous cycle (Niswender et al., 1973, 1975, 1976). Furthermore, luteal \( P_4 \) secretion in the ewe is highly correlated with blood flow through the CL (Niswender et al., 1975; Ford et al., 1979b). Niswender et al. (1976) suggested that the changes in blood flow to the ovary bearing the CL in ewes may be partially explained by changes in the weight of the CL, and thus the size of the luteal vascular bed.

Although the concentrations of estrogen and the \( E/P_4 \) ratio in systemic blood are negatively correlated with ovarian blood flow during the estrous cycle of the cow (Ford and Chenault, 1981), no data exist to suggest that estrogen has a direct effect to decrease ovarian blood flow. These data only indicate that ovarian blood flow is elevated during the luteal phase of the estrous cycle on days that luteal \( P_4 \) secretion and the size of the luteal vascular bed are high.
and estrogens are low. This lack of a vasoconstrictor effect of estrogen is supported by the fact that $E_2\beta$ injections have been shown to increase rather than decrease ovarian blood flow in pregnant (Rosenfeld et al., 1976) and postpartum (Rosenfeld, 1980) ewes. In addition, the $E/P_4$ ratio in systemic blood exhibited a highly significant negative correlation with the ability of the porcine ovarian vascular bed to contract in response to in vitro sympathetic nerve stimulation (Reynolds and Ford, 1982). Elevations of the $E/P_4$ ratio in systemic blood during the estrous cycle are thus associated with a relaxation of the ovarian vascular bed. This relaxation occurs at a time when the size of the ovarian vascular bed, and thus ovarian blood flow, are significantly reduced due to a reduction in the size of the luteal vascular bed. These data are in agreement with those of Novy and Cook (1973) and Ford et al. (1979b) who observed an increased flow of blood to the extra-luteal vascular bed of ovaries during luteal regression in the rabbit and ewe, respectively.

Ovarian blood flow, like $P_4$ secretion, may be partially under pituitary control since hypophysectomy or treatment of luteal phase sheep with luteinizing hormone (LH) antiserum caused a concomitant reduction of $P_4$ concentrations and ovarian blood flow (Hixon and Clegg, 1969; Niswender et al., 1976). In addition, administration of LH increased ovarian blood flow and $P_4$ secretion in luteal phase sheep (Cook et al., 1969; Hixon and Clegg, 1969; Niswender et al., 1976), rats (Wurtman, 1964; Piacsek and Huth, 1971) and estrous rabbits
(Blasco et al., 1975; Janson, 1975; Lee and Novy, 1978). Cook et al. (1969) reported that LH had no effect on ovarian venous blood flow in the pig, however, this study utilized only a limited number of animals. Follicle stimulating hormone did not affect blood flow to the ovaries of gilts (Cook et al., 1969) or rats (Wurtman, 1964), but showed a tendency to increase ovarian venous blood flow in sheep (Cook et al., 1969). Prolactin administration to sheep and pigs (Cook et al., 1969) or treatment of ewes with prolactin antiserum and/or bromocriptine (Niswender et al., 1976) did not significantly alter ovarian blood flow.

Exogenous administration of prostaglandin (PG)F_2α decreases ovarian blood flow and Pu secretion in the ewe (McCracken et al., 1970; Thorburn and Hales, 1972; Baird, 1974; Nett et al., 1976; Nett and Niswender, 1981), pseudopregnant rat (Pharriss, 1970) and rabbit (Bruce and Hillier, 1974; Janson et al., 1975). One mechanism by which PGF_2α is thought to induce luteal regression is by reducing ovarian blood flow with subsequent ischemia of the luteal tissue (Pharriss, 1970). Ovarian blood flow decreased as a result of the reduction of capillary blood flow to the CL following PGF_2α administration in sheep (Thorburn and Hales, 1972; Nett and Niswender, 1981) and pseudopregnant rabbits (Novy and Cook, 1973). In addition, elevations of the concentrations of PGF are found in the utero-ovarian vein coincident with functional luteolysis and declining blood flow to the luteal ovary of the pig (Rathmacher and Anderson, 1968; Gleeson et
al., 1974; Moeljono et al., 1977), cow (Rowson et al., 1972; Shemesh and Hansel, 1975; Ford and Chenault, 1981) and ewe (McCracken et al., 1970; Thorburn and Hales, 1972; Niswender et al., 1975; Ford et al., 1979b). Luteal regression during the estrous cycle, or luteolysis induced by PGF$_2$α, results in a redistribution of blood flow within the ovary by decreasing luteal blood flow and increasing blood flow through the extra-luteal vascular bed (Novy and Cook, 1973; Janson et al., 1975; Pang and Behrman, 1979; Ford et al., 1979b). Recently, Nett and Niswender (1981) demonstrated that intrauterine injections of 6.7 mg PGF$_2$α tham-salt to luteal phase ewes reduced CL blood flow and increased blood flow to the extra-luteal compartment yielding no net change in total ovarian blood flow.

During early pregnancy, when luteal P$_4$ secretion is maintained due to the presence of conceptuses in the uterus, blood flow to the porcine (Rathmacher and Anderson, 1968), ovine (Niswender et al., 1976) and bovine (Ford and Chenault, 1981) ovaries also remained elevated. Recently, a relationship has been observed in gilts between the transient 2- to 3-fold rise in uterine blood flow noted by Ford and Christenson (1979) and a transient 60% rise in luteal blood flow (Ford et al., 1982d), both occurring between Days 11 to 15 postmating. This increase in blood flow was specific to the CL, since blood flow to the extra-luteal portion of the ovary, or the ovaries of nonpregnant pigs on the same days postestrus did not change. Furthermore, this increase in luteal blood flow occurred on the days that porcine
conceptuses produce significant quantities of E₁ and E₂β, as discussed previously (Ford et al., 1982a, 1982d), and porcine CL become susceptible to the luteolytic effects of PGF₂α (Douglas and Ginther, 1975). Systemic, as well as intrauterine, injections of estrogen between Days 11 and 15 postestrus are luteotropic in the pig (Gardner et al., 1963; Ford et al., 1982c), consequently, estrogen has been proposed as the embryonic-luteotropin agent in swine. These data suggest a direct vasodilatory effect of the conceptus on the vascular bed of the uterus as well as the CL during the period of maternal recognition of pregnancy when porcine conceptuses produce significant quantities of estrogen.

Patterns of Lymph Flow from the Uterus and Ovaries

Lymphatic vessels of the female reproductive tract exhibit cyclic changes in diameter during the estrous cycle. In the pig, lymphatic vessels of the uterus (Fabian, 1981) and oviduct (Andersen, 1927) are greatly enlarged in diameter during proestrus and estrus as compared to lymphatic vessels observed during the luteal phase of the estrous cycle. Ford and Christenson (1979) reported that blood flow to the nongravid uterine horn of sows was elevated during the follicular phase of the estrous cycle. Classic responses of the uterus to estrogen administration include increased uterine blood flow (Assali
et al., 1978), induced uterine growth (Hisaw, 1935) and increased permeability of the capillary beds with subsequent water imbibition (Spaziani, 1975). Thus, the hydrated condition of the uterine lymphatic vessels during the follicular phase of the cycle (Wislocki and Dempsey, 1939; Fabian, 1976, 1977, 1978, 1981), was similar to the edematous response noted after the injection of estrogen (Thorn and Harrop, 1937; Aykroyd and Zuckerman, 1938; Astwood, 1939). Recently Staples et al. (1982) estimated that uterine lymph flow from the early pregnant ovine uterus averaged ~0.7 ml/hour which is less than 1% of uterine blood flow as reported by Metcalfe et al. (1959).

During the course of gestation in all species studied to date, the lymphatics of the uterus increase greatly in diameter and length, but not appreciably in number (Hoggan and Hoggan, 1882; Sass, 1964; Wislocki and Dempsey, 1939; Fabian, 1977). Blood flow to the gravid uterus is markedly elevated during pregnancy in sows (Ford and Christenson, 1979; Hard and Anderson, 1982), cows (Ferrell and Ford, 1978; Ford et al., 1982c) and ewes (Rosenfeld et al., 1974). Concentrations of estrogens in these species are also high in both the fetal fluids and maternal circulation throughout gestation (Robertson and King, 1974, 1979; Carnegie and Robertson, 1978).

In contrast to the changes observed for the uterine lymphatics, the lymphatic vessels within the ovaries of sows (Andersen, 1926) and ewes (Morris and Sass, 1966) are greatly developed in size and number when functional CL are present on the ovary. Furthermore, blood flow
to and lymph flow from the ovary is highest during the luteal phase of the estrous cycle and lowest after luteal regression in sheep (Lindner et al., 1964; Niswender et al., 1975) and guinea pigs (Culiner, 1944; Hossain et al., 1979). The rate of lymph flow of ovaries containing an active CL and blood flow to the CL were much higher per unit weight of tissue than any other organ studied in the ewe (Lindner et al., 1964; Christenson and Prior, 1978). These estimates of ovarian lymph flow, however, (Lindner et al., 1964) were only 1-2% of total ovarian blood flow measured on the same days of the ovine estrous cycle (Niswender et al., 1975).

Control of Lymph Flow

Anatomical studies of the lymphatic vessels of the uterus near estrus and during pregnancy, or lymphatics of the ovary bearing functional CL, suggest that their distention parallels the demand for removal of increasing amounts of tissue fluids. Throughout the body, lymph flow increases with the rate of tissue fluid formation (Guyton and Barber, 1980), which results from increases in filtration pressure, blood flow, or capillary permeability (Yoffey and Courtice, 1970). Morris and Sass (1966) increased filtration pressure of the ovine ovary by slight occlusion of the venous outflow. Even with only partial occlusion, they observed higher lymph flows and significant increases in the number of red blood cells in the lymph, suggesting a higher capillary permeability, and that the lymphatic system was func-
tioning in addition to the venous system to remove excess fluids from the capillary bed. Furthermore, estrogen-induced increases in uterine blood flow (Dickson et al., 1969) and increased permeability of the uterine capillary bed (Spaziani, 1975), both elevated capillary tissue fluid filtrate formation, thus causing increased uterine lymph formation.

Lymph is transported through the lymphatic channels, in part by the pumping of the smooth muscle of the lymphatic vessel walls (Nicholl and Taylor, 1977) which undergo spontaneous rhythmic contractions in the intervalvular regions (Ranvier, 1889; Kinmonth and Taylor, 1956; Armenio et al., 1981). Hall et al. (1965) cannulated ovarian, mammary, intestinal, and popliteal lymph nodal ducts of sheep and reported that the intermittent rhythmic flow in these lymphatic vessels was unrelated to respiration, and that the magnitude and frequency of pulse pressure increased as the rate of lymph formation and flow increased. Flow of lymph through the lymphatic vessels is further aided by extrinsic forces (Mayerson, 1962) such as muscular contractions during locomotion (White et al., 1933), peristaltic contractions of viseral organs (Beznák, 1937) and pulsation of blood vessels (Parsons and McMaster, 1938) which all compress the adjacent lymphatic vessels and drive lymph according to the orientation of the valves (Roddie et al., 1980). Although no specific information is available regarding the control of uterine lymph flow, Reynolds (1949)
advanced the idea that at estrus increased capillary permeability, elevated uterine hyperemia and enhanced myometrial contractions all contribute to the movement of uterine lymph.

Steroid Control of Uterine and Ovarian Periarterial Sympathetic Nerves

It can be deduced from these studies that changes in lymph flow are directly related to dynamic changes in blood flow to an organ, including the uterus and ovaries. It appears that ovarian steroids play a primary role in controlling the function of periarterial adrenergic vasoconstrictor nerves, with subsequent alterations in blood flow to the uterus and ovaries. As stated previously, the periarterial adrenergic nerves, which supply the uterine and ovarian arteries, originate from the same periaortic autonomic plexus (Shabanah et al., 1964), and thus the neuronal input is similar.

Progesterone appears to augment the responsiveness of uterine vascular smooth muscle to catecholamines (Ford et al., 1977c) which may partially explain why $P_4$ reduces uterine blood flow (Caton et al., 1974). Uterine and ovarian arteries ipsilateral to the ovary bearing a functional CL from unilaterally ovulating heifers (Kuhl, 1974; Ford et al., 1976) and ewes (Kuhl et al., 1974; Ford et al., 1976, 1977b) responded to in vitro periarterial nerve stimulation following saline
or NE with greater constriction than arteries from the contralateral side. Furthermore, as the secretion of P₄ increased throughout the luteal phase of the estrous cycle in ewes, constriction of uterine arteries ipsilateral to ovaries with CL increased in a parallel fashion (Ford et al., 1977b). Kalsner (1969) reported that P₄ increased smooth muscle contractility in canine aortic strips to NE and epinephrine by inhibiting the action of catechol-o-methyl transferase, thus reducing the rate of catecholamine degradation. Thus, the ability of P₄ to enhance vasoconstriction may involve increased exposure of the tunica media to NE. These data, however, do not preclude the possibility that P₄ might increase NE release from periarterial adrenergic nerves, or that P₄ may increase the number or affinity of α-adrenergic receptors within the smooth muscle of the arterial wall.

In contrast to P₄, estrogen appears to decrease, rather than increase vascular smooth muscle responsiveness to catecholamines (Ford et al., 1977c) with subsequent increases in both uterine and ovarian blood flow (Caton et al., 1974; Rosenfeld, 1980). Barton et al. (1974) reported that prior treatment of ovariectomized ewes with E₂β attenuated the uterine vasoconstrictor response to in vivo intra-arterial NE infusions. Furthermore, uterine arteries from estrogen dominated intact gilts (Reynolds and Ford, Iowa State Univ., unpublished observation) and ewes (Ford et al., 1977b) or estrogen-treated ovariectomized ewes (Ford et al., 1977c) exhibited a reduced
constrictor response to periarterial nerve stimulation following saline or NE. Similarly, Kuhl et al. (1974) observed a tendency for ovarian arteries from estrus ewes (Day 0) to elicit reduced vasoconstriction in vitro in response to nerve stimulation and vasoactive biogenic amines than arteries removed during the luteal phase (Day 13) of the estrous cycle. Reynolds and Ford (1982) reported that ovaries from follicular phase gilts respond with reduced vasoconstriction in vitro to transmural electrical stimulation and NE compared to ovaries from luteal phase gilts. These investigators observed that ovarian vascular contractility was positively correlated ($r=+0.90$) with $P_4$ and negatively correlated ($r=-0.99$) with the $E/P_4$ ratio in systemic blood during the porcine estrous cycle.

Reduced responsiveness of uterine and ovarian arteries from follicular phase or estrogen-treated animals to nerve stimulation may be due in part to an estrogen-induced depletion of neurotransmitter. McKercher et al. (1973) reported that the increased uterine blood flow following estrogen injections in ovariectomized rats was associated with a pronounced reduction in the NE content of the periarterial adrenergic nerves. It is also possible that exposure of uterine and ovarian vascular smooth muscle to estrogen may result in diminished activity of the $\alpha$-adrenergic receptors, which have been shown to modulate NE-mediated vasoconstriction of the uterine and ovarian vascular beds (Greiss and Pick, 1964; Reynolds and Ford, 1982). Recent evidence would suggest that the numbers of $\alpha$-adrenergic receptors in
uterine arteries from periestrus gilts are reduced when compared to arteries from luteal phase gilts (Farley, Van Orden, Univ. of Iowa, and Ford, Iowa State Univ., unpublished observation).

Conceptus Control of Uterine and Ovarian Periarterial Sympathetic Nerves

Evidence by Ladner et al. (1970) and Reynolds and Ford (1982) implicated uterine and ovarian periarterial adrenergic nerves in the physiological regulation of uterine and ovarian blood flow during early pregnancy. These investigators observed that the nonpregnant ovine uterus or porcine luteal ovary had a greater sensitivity to the vasoconstrictor effects of intra-arterial perfusion of NE than the pregnant uterus or ovary. Furthermore, periarterial nerve stimulation, after PGF$_2$α perfusion in vitro, increased the constrictor responses of ovine and bovine uterine arteries collected during the luteal phase of the estrous cycle but not on the same days of pregnancy (Ford et al., 1976). Perfusion of a brei of Day 15 conceptuses through uterine arteries of nonpregnant luteal phase ewes in vitro caused these vessels to lose their responsiveness to PGF$_2$α. Similarly, uterine arteries from luteal phase cows perfused with uterine flushing from Day 16 pregnant cows had decreased arterial constriction while those perfused with uterine flushings from Day 16 nonpregnant
cows exhibited increased constriction to periarterial nerve stimulation following saline, PGF$_2$α or NE (Ford, 1978).

The diminished activity of the uterine and ovarian periarterial adrenergic nerves of pregnant ewes and gilts (Ford et al., 1976; Reynolds and Ford, 1982) resembles the attenuated responses of ovine uterine arteries or porcine ovaries at estrus when estrogen is high and P$_4$ is low (Ford et al., 1977b; Reynolds and Ford, 1982). Exogenous administration of estrogen to ovariectomized ewes reduced constrictor activity of uterine arteries in vitro (Ford et al., 1977c) similar to the perfusion of conceptus brei from pregnant ewes (Ford et al., 1976). In addition, the number of α-adrenergic receptors appears to be similar in uterine arteries of estrus gilts and Day 13 pregnant gilts, which were both less than the receptor number in arteries from luteal phase gilts (Farley, Van Orden and Ford, unpublished observation).

The vasodilatory action of the conceptus may be due to a local effect of estrogen since, as stated previously, blood flow increased preferentially to the segments of uterine horns in contact with conceptus tissue (Ford et al., 1982d) and only to the uterine horn adjacent to conceptuses in sows made unilaterally pregnant (Ford and Christenson, 1979). Furthermore, these researchers observed that the luteotropic effect of the conceptus was manifested by significantly higher CL weights and P$_4$ content of CL on ovaries adjacent to the gravid uterine horn when compared to CL adjacent to the nongravid
When E\textsubscript{2}B was injected into an isolated uterine lumen of nonpregnant sows, in amounts similar to those produced by conceptuses across Days 11 to 15 postmating, P\textsubscript{4} secretion was higher from CL on the ovaries adjacent to the steroid injection (Ford et al., 1982b). Because blood flow through the CL is highly correlated with its ability to secrete P\textsubscript{4} (Niswender et al., 1975, 1976), and since E\textsubscript{2}B increases ovarian blood flow in sheep (Rosenfeld, 1980), it would be logical to conclude that intrauterine E\textsubscript{2}B injections, which are locally luteotropic in the pig (Ford et al., 1982b) also increase ovarian blood flow in this species. A transient rise in blood flow through the luteal and oviductal vascular beds occurred on Day 13 postmating (Ford et al., 1982d) around the time porcine conceptuses are needed in the uterine lumen to maintain the CL of pregnancy (Dhindsa and Dziuk, 1968). This rise in CL and oviductal blood flow occurred 24-48 hours after the initial dilatation of the uterine vascular bed was observed (Ford et al., 1982d). These data suggest a diffuse transport of a conceptus-produced vasodilatory factor from the uterine lumen to the ipsilateral utero-ovarian vasculature. Estrogen is a good candidate for this factor since it is vasodilatory and is elevated in the uterine lumen on these days of pregnancy in pigs.
Possible Role of Uterine Lymph in the Transport of Steroids

Lymphatics draining a uterine horn in the pig function in addition to the venous system to drain tissue fluid from the uterine horn (Yoffey and Courtice, 1970) and are in close linear apposition to the unilateral uterine arterial vasculature (Hoggan and Hoggan, 1882). Because the postganglionic vasoconstrictor nerves which control blood flow to the uterus and ovaries are located in the tunica adventitia (i.e. the outermost layer of the vessels) (Shabanah et al., 1964), they too are associated closely with the uterine lymphatics. It is conceivable that changes in the concentrations of estrogen and P₄ in uterine lymph facilitate local changes in the E/P₄ ratio in the interstitial fluid to expose these periarterial vasoconstrictor nerves of the utero-ovarian arteries to steroids which have been shown to alter the tone and, contractile nature of and flow rate through, the utero-ovarian vasculature (Ford, 1982). In support of this hypothesis, lymph draining the ovary (Lindner et al., 1964) and testis (Morris and McIntosh, 1971) transport very high concentrations of P₄, estrogens and androgens. Due to the great disparity in flow rates between the lymph and blood, the absolute amounts of steroid being transported by the lymph is considerably less than the amount drained by the venous system (Lindner et al., 1964; Morris and McIntosh, 1971). This observation, however, does not preclude the possibility
that high local concentrations of these steroids in the lymph and interstitial fluid are of physiological significance to the function of the organ.

Wislocki and Dempsey (1939) hypothesized that uterine lymphatics may participate in the drainage of fluids from the uterine lumen which are considered an ultrafiltrate of plasma (Ringler, 1961) and distend the uterus near the time of estrus in the pig (Howard, 1933) and cow (Olds and Van Demark, 1957). Drinker and Field (1933) reported that the composition of lymph is that of dilute blood plasma, because the plasma proteins which leave the capillaries to enter the tissue fluid are removed by the lymphatic system. Thus, the lymphatics provide the principal means for the escape of protein-containing interstitial fluid from the tissues. Furthermore, Yoffey and Courtice (1970) reported that lymph within the collecting lymphatics was representative of the tissue fluid from which it was derived.

Lymphatic capillaries of the uterus (Sass, 1964), ovaries (Morris and Sass, 1966), small intestines (Palay and Karlin, 1959) and other tissues contain intercellular gaps which facilitate transport of a variety of large and small molecules. McRae and Kennedy (1979) demonstrated that the lymphatic capillary beds of the rat did not hinder the movement of substances with a molecular weight of less than 5000 from the blood to the lymph. These investigators, however, observed ~50% exclusion of $^{125}$I bovine serum albumin (molecular weight of 60,000) in crossing the capillary beds into the lymphatic system.
During the slow transport of lymph along the collecting and transporting lymphatic channels there is free exchange of molecules of small weight (<600 daltons) between the lymph and blood (Mayerson, 1962; Mayerson et al., 1962; Bashir and Mayerson, 1968). Furthermore, Yoffey and Courtice suggested that the exchange of molecules from the lymph to the blood may be through the vasavasorum located in the tunica adventitia adjacent to the periarterial nerves of the lymphatic (Hoggan and Hoggan, 1883) and blood vessels (Ham, 1969). It is conceivable that a similar exchange mechanism occurs between the uterine lymphatics and the interstitial fluid surrounding the utero-ovarian arteries. This concept is supported by the observation that lymph within the collecting lymphatics is representative of the tissue fluid from which it was derived (Yoffey and Courtice, 1970). Thus, elevated concentrations of estrogen or P₄ in systemic blood (Guthrie et al., 1972) or in the uterine lumen of early pregnant sows (Ford et al., 1982a) may reach the periarterial sympathetic vasoconstrictor nerves locally by way of the uterine lymphatics, since the gram molecular weight of E₁ (270.4), E₂β (272.4) and P₄ (314.5) are well below the exclusion limits of the lymphatic capillary beds (Mayerson et al., 1962).

No research has been conducted to date dealing with the uterine lymphatic transport of steroids and its possible role in mediating physiological changes in uterine and ovarian blood flow. Techniques were thus developed for the acute and chronic collection of uterine
lymph from Yorkshire gilts. The following studies were conducted to compare the concentrations of $E_1$, $E_2\beta$ and $P_4$ in uterine lymph and systemic blood during the porcine estrous cycle (Experiment I) and early pregnancy (Experiment II), and to determine if lymphatic vessels draining uterine horns of pigs act in addition to the venous system for the local transport of steroids. In Experiment III, changes in ovarian blood flow were subsequently compared to the patterns of $E_1$, $E_2\beta$ and $P_4$ in systemic blood and uterine lymph throughout the estrous cycle and early pregnancy.
EXPERIMENT I. ESTRONE, ESTRADIOL-17β AND PROGESTERONE
CONCENTRATIONS IN UTERINE LYMPH AND SYSTEMIC BLOOD
THROUGHOUT THE PORCINE ESTROUS CYCLE

Introduction

Prominent lymphatic vessels drain tissue fluid from each uterine horn in the pig (Andersen, 1926, 1927; Yoffey and Courtice, 1970), and are closely associated with the unilateral uterine arterial vasculature (Hoggan and Hoggan, 1882). Periarterial sympathetic vasoconstrictor nerves, which traverse the length of each uterine artery in the tunica adventitia of the vessels (Shabanah et al., 1964), are in close proximity to these lymphatic vessels. Recent evidence suggests that blood flow to the uterus may be regulated by the presence or removal of adrenergic vasoconstrictor tone (Ford, 1982).

Estrogen increases, while progesterone (P₄) decreases uterine blood flow in many species, and the magnitude of the changes appears to be related to the ratio of the two steroids (Caton et al., 1974). In the pig, the ratio of estrogen to P₄ (E/P₄) in systemic blood and uterine blood flow are elevated from 4 to 5 days prior to estrus and through estrus, before declining to low values for the remainder of the estrous cycle (Ford and Christenson, 1979; Van De Wiel et al., 1981). Estrogen has been observed to reduce both norepinephrine content (McKercher et al., 1973) and α-adrenergic receptor activity (Barton et al., 1974; Ford et al., 1977b, 1977c) of uterine
periarterial sympathetic nerves. In contrast to estrogen, \( \text{P}_4 \) augments rather than reduces the responsiveness of arterial smooth muscle to catecholamines (Kalsner, 1969; Ford et al., 1977c). Thus, steroid-induced changes in uterine blood flow may result from an altered function of uterine periarterial sympathetic vasoconstrictor nerves.

As lymph flows through the collecting and transporting lymphatic vessels, there is free exchange of molecules with weights of <600 daltons between the lymph and blood (Mayerson, 1962; Mayerson et al., 1962; Bashir and Mayerson, 1968). Yoffey and Courtice (1970) reported that the composition of lymph is similar to that of the interstitial fluid from which it originates. Considering the close anatomical association between the uterine lymphatics and periarterial sympathetic vasoconstrictor nerves, changes in the concentrations of steroids in the tissue fluid surrounding the uterine arteries could facilitate the changes in uterine blood flow observed during the estrous cycle. This study was conducted to determine the concentrations of estrone (\( E_1 \)), estradiol-17\( \beta \) (\( E_2 \beta \)) and \( \text{P}_4 \) in uterine lymph throughout the porcine estrous cycle, and to compare the profiles of these steroids in uterine lymph to those in systemic blood.
Materials and Methods

Experimental procedures

Eighteen Yorkshire gilts with 2 previous estrous cycles of normal duration (20.4 ± 0.2 days) were utilized in this study. The first day of estrus was considered as Day 0. Since the duration of catheter patency lasted only 5.0 ± 0.5 days, it was necessary to assign gilts to surgery on Days 0 or 1 (n=5), 5 (n=4), 11 (n=5) or 15 (n=4) so that lymph could be collected on each day of the estrous cycle. Feed was withheld from gilts for 24 h before surgery. General anaesthesia was induced by intra-venous infusion of 1.0 g of surital (sodium thiamylal: Parke Davis Labs., Detroit, MI), while surgical anaesthesia 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 CO₂.

The uterus was exposed through a midventral incision and a uterine lymphatic vessel draining lymph from one randomly selected uterine horn was isolated and ligated with a loop of silk (4-0) to occlude lymph flow (Figure 1). In all cases, the lymphatic vessel became distended on the uterine side of the ligature indicating that lymph was of uterine origin. Each distended lymphatic was then cannulated against the direction of the lymph flow using a polyvinyl catheter (Becton-Dickson Co., I.D.=1.11 mm-O.D.=1.65 mm) with a polyethylene tip (Intamedic ®, I.D.=0.58 mm-O.D.=0.97 mm) which was
Figure 1. Vascular anatomy of a uterine horn of a gilt; lymph was collected from a vessel closely associated with a main branch of the uterine artery and above the anastomosis of the uterine and ovarian lymphatic vessels.
sutured in place. The catheter was exteriorized through the flank, and inserted into a collection tube (Vacutainer; 143 U Na⁺ Heparin/tube). The lymph collection tube was placed in a cloth pouch, which was glued to the side of the gilt. Lymph was allowed to flow continuously into the collection tube which was changed at 12 h intervals (1200 and 2400 h) until loss of catheter patency. Lymph samples from each gilt were subsequently pooled within a day (n=2) and this pool was considered as the observation for that day. Systemic blood was collected by vena cava puncture each day at 1200 h throughout the duration of catheter patency and plasma stored at -20 C until assayed for E₁, E₂β and P₄.

Estrone and E₂β were quantified utilizing a slight modification of a procedure previously reported by Koligian and Stormshak (1977). In contrast to the previous methodology, 2 ml of plasma or 0.25 ml of lymph plus 1.75 ml sterile water (Abbott Laboratories, Chicago, IL) were extracted three times with three volumes of nanograde benzene (Fisher Scientific Co.). The final benzene extract was washed once with 400 µl of sterile water (Abbott Laboratories, Chicago, IL) to remove any polar contaminants prior to Sephadex LH-20-100 (Sigma ®, St. Louis, MO) column chromatography. To determine procedural losses, 3000 dpm [2,4,6,7-3H] (N) -E₁ (92.8 Ci/m mole, NEN) and 3000 dpm [2,4,6,7-3H] (N) -E₂β (90.0 Ci/m mole, NEN) were added to the samples prior to extraction.

The estrogens were measured by radioimmunoassay utilizing the fully characterized antiserum S-310 No. 5/G Abraham (Torrance, CA) as
specified by Tulchinsky and Abraham (1971). Sensitivity of the assay, defined as the $E_1$ or $E_2\beta$ standard which yielded 95% of the counts in the buffer control tubes, was ~2 pg.

Within-assay variability for $E_1$ and $E_2\beta$ was determined from replicates ($n=6$) of a blood plasma pool and a uterine lymph pool from luteal phase sows. The resulting concentrations (pg/ml ± SEM) for the plasma and lymph pools were 15.6 ± 0.4 (coefficient of variation; CV=5.4%) and 21.6 ± 1.5 (CV=17.0%) for $E_1$ and 13.7 ± 5.1 (CV=9.2%) and 10.1 ± 0.6 (CV=14.8%) for $E_2\beta$. Between-assay variation was determined by assaying samples of the same plasma pool ($n=10$) and lymph pool ($n=8$) in each of several assays. Between-assay coefficients of variation for $E_1$ and $E_2\beta$ in plasma were 11.6% and 8.2% and for lymph were 7.2% and 13.7%, respectively. Precision and accuracy were evaluated on a pg/ml basis by the addition of $E_1$ and $E_2\beta$ to plasma from ovariectomized gilts and to a uterine lymph pool (Table 1).

To quantify $P_4$, plasma was extracted with benzene:hexane (1:2 v/v) by a procedure which was a slight modification of the protocol used by Louis et al. (1973). One hundred to 200 µl of plasma or 50 µl of lymph were extracted in triplicate, with one of the replicates receiving 12,000-15,000 dpm of $[1,2,6,7-^{3}H]$-P$_4$ (97.0 Ci/m mole, NEN) to serve as the individual recovery for that set of duplicates. Plasma and lymph extracts were assayed for $P_4$ by a modification of the radioimmunoassay procedure described previously (Anderson et al., 1979), and utilizing the same fully characterized antibody (GDN-337; Niswender, 1973).
Table 1. Precision and accuracy of RIA for estrone (E₁) and estradiol-17β (E₂β) in plasma and lymph

<table>
<thead>
<tr>
<th>E₁ and E₂β added to plasma from ovariectomized gilts (pg/ml)</th>
<th>2.5</th>
<th>5.0</th>
<th>25.0</th>
<th>50.0</th>
<th>75.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₁ quantified</td>
<td>2.1±0.2</td>
<td>7.6±0.3</td>
<td>27.2±0.3</td>
<td>52.7±0.8</td>
<td>73.1±2.6</td>
</tr>
<tr>
<td>E₂β quantified</td>
<td>2.6±0.2</td>
<td>5.5±0.3</td>
<td>27.2±1.6</td>
<td>53.6±1.5</td>
<td>79.4±1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E₁ and E₂β added to a porcine uterine lymph pool (pg/ml)</th>
<th>10</th>
<th>20</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₁ quantified</td>
<td>11.0±0.6</td>
<td>20.2±1.6</td>
<td>56.3±4.7</td>
<td>105.7±3.5</td>
<td>193.9±6.5</td>
</tr>
<tr>
<td>E₂β quantified</td>
<td>11.7±0.7</td>
<td>22.1±0.7</td>
<td>58.1±2.5</td>
<td>100.9±4.6</td>
<td>192.2±9.2</td>
</tr>
</tbody>
</table>

*Mean ± SEM (n=4).*
Mean blank value (± SEM) for plasma from ovariectomized pigs was 0.23 ± 0.01 ng/ml (n=7). The sensitivity of the assay was defined as the amount of P₄ standard which yielded 95% of the counts per minute in the buffer control tubes; this ranged from 50 to 80 pg.

The intra-assay variability for P₄ was determined from replicates of two plasma pools (n=6) and a lymph pool (n=10) from luteal phase sows. The resulting concentrations (ng/ml ± SEM) for utero-ovarian venous plasma, uterine arterial plasma, and uterine lymph were 331.0 ± 7.8 (CV=5.8%), 17.2 ± 0.5 (CV=6.5%), and 24.2 ± 0.7 (CV=9.5%), respectively. Between-assay variation was determined by assaying samples of the same plasma pools in each of several assays. Inter-assay coefficients of variation in the utero-ovarian venous plasma (n=11) and uterine arterial plasma (n=15) were 12.1% and 12.3%, respectively. Due to its limited availability, the inter-assay variation for P₄ (9.2%) in uterine lymph was determined on another lymph pool which averaged 12.7 ± 0.5 ng/ml in 6 independent assays.

Precision and accuracy were evaluated by adding known quantities of P₄ on a ng/ml basis to plasma from an ovariectomized pig or to a pool of uterine lymph (Table 2).

**Statistical analysis**

Data were analyzed by a split-plot analysis of variance using the statistical analysis system (Barr and Goodnight, 1979). Means were compared by orthogonal contrasts (Kirk, 1968). Correlation coefficients were calculated between daily hormone concentrations in systemic blood and uterine lymph throughout the estrous cycle.
Table 2. Precision and accuracy of RIA for progesterone ($P_4$) in plasma and lymph$^a$

<table>
<thead>
<tr>
<th>P$_4$ added to</th>
<th>plasma from ovariectomized gilts (ng/ml)</th>
<th>2.5</th>
<th>5.0</th>
<th>7.5</th>
<th>15.0</th>
<th>30.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_4$ quantified</td>
<td></td>
<td>3.1±0.1</td>
<td>5.7±0.1</td>
<td>7.5±0.1</td>
<td>15.2±0.7</td>
<td>25.0±1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$P_4$ added to a porcine uterine lymph pool (ng/ml)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_4$ quantified</td>
<td>10.4±0.3</td>
<td>21.3±0.8</td>
<td>30.6±0.2</td>
<td>57.8±0.7</td>
<td>117.4±3.8</td>
</tr>
</tbody>
</table>

$^a$Mean ± SEM (n=4).
Results

Patency of lymphatic catheters averaged $5.0 \pm 0.5$ days and ranged from 3-10 days. Surgery and placement of the lymphatic catheters had no significant effect on estrous cycle lengths exhibited by gilts in this study which averaged $20.6 \pm 1.2$ days. To minimize variation, data were adjusted to a 20 day estrous cycle length by analyzing samples collected after Day 14 as days prior to the subsequent estrus.

Concentrations of $E_1$ in uterine lymph and systemic blood followed similar patterns ($r=+0.61; \ p<0.01$) throughout the porcine estrous cycle as illustrated in Figure 2. In both uterine lymph and systemic blood, concentrations of $E_1$ began to increase ($P<0.05$) 5 days prior to estrus and were maximal ($P<0.01$) from Day -2 to Day 1, averaging $47.7 \pm 5.2$ and $17.0 \pm 2.2$ pg/ml, respectively. By Day 3, concentrations of $E_1$ in both uterine lymph and systemic blood had reached basal levels ($P<0.01$) and remained low through Day 15 of the cycle ($24.0 \pm 1.0$ and $9.0 \pm 0.5$ pg/ml, respectively). Estrone concentrations in uterine lymph, however, were 3- to 4-fold higher ($P<0.01$) than concentrations in systemic blood across all days sampled. As observed with $E_1$, concentrations of $E_2\beta$ in uterine lymph were also greater ($P<0.01$) than those in systemic blood and $E_2\beta$ concentrations in uterine lymph and systemic blood were highly correlated ($r=+0.84; \ P<0.01$) throughout the estrous cycle (Figure 3). As observed with $E_1$, concentrations of $E_2\beta$ in uterine lymph and systemic blood began to increase ($P<0.05$) 5 days before estrus, reached peak levels ($P<0.01$) from Day- 2 to Day 1
Figure 2. Concentrations of estrone (pg/ml) in uterine lymph and vena cava blood of gilts throughout the estrous cycle (Mean ± SEM)
Figure 3. Concentrations of estradiol-17β (pg/ml) in uterine lymph and vena cava blood of gilts throughout the estrous cycle (Mean ± SEM)
of the estrous cycle (56.4 ± 4.1, and 17.1 ± 2.6 pg/ml, respectively) then declined (P<0.05) to basal levels (21.3 ± 1.0 and 8.8 ± 0.5 pg/ml, respectively) between Day 2 to 3 of the estrous cycle.

Patterns of \( \text{P}_4 \) in uterine lymph and systemic blood were highly correlated, (r=+0.90; P<0.01) throughout the porcine estrous cycle (Figure 4). Unlike \( E_1 \) and \( E_2 \) levels, however, concentrations of \( P_4 \) were lower (P<0.01) in uterine lymph than systemic blood. Concentrations of \( P_4 \) in uterine lymph and systemic blood began to increase (P<0.05) between Day 3 and Day 4 of the estrous cycle and attained maximal concentrations (P<0.01) by Day 11 (25.0 ± 5.0 and 56.6 ± 12.1 ng/ml, respectively). Progesterone concentrations in uterine lymph and systemic blood remained elevated from Day 11 until 5 days prior to the subsequent estrus then declined (P<0.01) to reach low levels 3 days before estrus (4.2 ± 1.9 and 1.1 ± 0.2 ng/ml, respectively).

Estrogen (\( E_1 + E_2 \))/P(4) ratios (pg/ng) in uterine lymph and systemic blood were greater (P<0.01) during the follicular phase (5 days before estrus to Day 3 postestrus) than the luteal phase (Day 4 to Day 15 postestrus) of the porcine estrous cycle (19 and 3.3 vs. 4.0 and 0.3). In addition, the \( E/P_4 \) ratio was higher (P<0.01) in uterine lymph than systemic blood across all days sampled since the concentrations of \( E_1 \) and \( E_2 \) were higher and \( P_4 \) was lower in the lymph than the blood.
Figure 4. Concentrations of progesterone (ng/ml) in uterine lymph and vena cava blood of gilts throughout the estrous cycle (Mean ± SEM)
Discussion

Concentrations of P_4 in systemic blood followed patterns similar to those reported by Guthrie et al. (1972). Although the patterns of P_4 in uterine lymph were highly correlated with the profile of this steroid in systemic blood, the absolute concentration of P_4 was lower in uterine lymph than in systemic blood. These low levels of P_4 suggest that lymph collected was of uterine origin and not mixed with ovarian lymph. Lindner et al. (1964) reported that the concentrations of P_4 in ovarian lymph of luteal phase ewes contained =100 fold greater concentrations of P_4 than systemic blood and was similar to levels found in ovarian venous blood. The reduced P_4 concentrations in uterine lymph when compared to systemic blood in this study may have resulted from uterine uptake and/or metabolism of P_4. Concentrations of P_4 in uterine venous blood of pigs during the estrous cycle (S. P. Ford, Iowa State Univ., unpublished observations) and early pregnancy (Knight et al., 1977) were also reduced when compared to levels found in uterine arterial blood.

Concentrations of E_1 and E_2β in uterine lymph were highly correlated with the concentrations of these same hormones in systemic blood throughout the estrous cycle, and followed patterns similar to those reported previously for systemic blood (Guthrie et al., 1972; Van De Wiel et al., 1981). Estrone and E_2β, however appeared to be concentrated in uterine lymph since concentrations of these estrogens were 3- to 4-fold higher in uterine lymph than systemic blood. Although specific estrogen binding proteins in uterine lymph have not
been reported, these may account for the elevated estrogen concentrations in lymph when compared to the blood. Morris and McIntosh (1971) observed that the level of protein in testicular lymph, which binds testosterone, determines the concentration of this steroid in the lymph.

Yoffey and Courtice (1970) reported that lymph within the collecting lymphatic vessels was representative of the tissue fluid from which it is derived. Levels of estrogens in uterine lymph could merely reflect tissue concentrations of estrogens because total estrogens were greater in uterine tissue than systemic blood (Peck et al., 1973). These investigators suggested that this disparity in estrogen levels from the systemic blood to the uterine tissue was a function of the accumulation and retention of albumin, a low affinity high capacity estrogen binding protein (Anderson et al., 1974). Furthermore, Peterson and Spaziani (1971) observed an elevated albumin content in the rat uterus in response to E2β administration. The quantity of estrogens in lymph are further increased at estrus due to elevations in lymph flow at this stage of the estrous cycle (Fabian, 1981; Wislocki and Dempsey, 1939). The disparity between the rate of uterine lymph flow and uterine blood flow may also function to magnify the E/P₄ ratio in lymph vs. systemic blood. Staples et al. (1982) estimated that uterine lymph flow from the early pregnant ovine uterus averaged ≈0.7 ml/h which is less than 1% of uterine blood flow (Metcalfe et al., 1959).
Estradiol-17β increases blood flow to the porcine uterus following either systemic (Dickson et al., 1969) or intrauterine (Ford et al., 1982b) administration. Elevations in the E/P_4 ratio in systemic blood during the estrous cycle are positively correlated with increases in uterine blood flow in the ewe (Greiss and Anderson, 1969) and cow (Ford et al., 1979a). The pattern of uterine blood flow to the nonpregnant porcine uterus is negatively correlated (r=-0.94) with the concentrations of P_4 in the systemic blood (Ford and Christenson, 1979) and is temporally associated with the profile of estrogen in systemic blood and uterine lymph in this study. In addition, the proestrus rise in uterine blood flow reported by Ford and Christenson (1979) occurred between Day 15 to Day 16 postestrus coincident with the increase in the E/P_4 ratio in systemic blood and uterine lymph noted in the present experiment. These data suggest that the E/P_4 ratio in systemic blood, which is magnified in uterine lymph, may modulate changes in uterine blood flow during the estrous cycle.

Lymphatic capillaries of the uterus (Sass, 1964), ovaries (Morris and Sass, 1966), small intestines (Palay and Karlin, 1959) and other tissues contain intercellular gaps which facilitate transport of a variety of large and small molecules. During the slow transport of lymph along the collecting lymphatic channels, there is free exchange of electrolytes and small molecules of up to 600 gram molecular weight from the lymph to the blood (Mayerson, 1962; Mayerson et al., 1962; Bashir and Mayerson, 1968). It is conceivable that elevated estrogen or P_4 concentrations in systemic blood may reach the uterine
periarterial vasoconstrictor nerves locally by way of the uterine lymphatics, since the gram molecular weights of E₁, E₂β and P₄ are 270.4, 272.4 and 314.5, respectively. Exchange of estrogen and P₄ by this route would support the hypothesis that local changes in the E/P₄ ratio in uterine lymph during the estrous cycle may expose the peri-arterial nerves within the tunica adventitia of the uterine arteries to steroids which have been shown to alter the tone of, contractile nature of and flow rate through the uterine vasculature.
EXPERIMENT II. STEROID CONCENTRATIONS IN UTERINE LYMPH AND UTERINE ARTERIAL PLASMA OF GILTS DURING THE ESTROUS CYCLE AND EARLY PREGNANCY

Introduction

Each uterine horn in the pig has prominent lymphatic vessels which anastomose with the ovarian and tubal lymphatics at or near the utero-ovarian lymph node located within the broad ligament (Andersen, 1926, 1927). Lymphatics draining a uterine horn in the pig are associated closely with the unilateral uterine arterial vasculature (Hoggan and Hoggan, 1882) and act as one route for the drainage of fluid from the uterine horn (Yoffey and Courtice, 1970). Arteries supplying the uterus and ovaries are innervated by postganglionic sympathetic vasoconstrictor nerves which traverse the length of these arteries in the tunica adventitia, which is the outermost layer of the vessel wall (Shabanah et al., 1964). Recent investigations suggest that steroid-induced changes in uterine blood flow may be controlled by the presence or absence of adrenergic vasoconstrictor tone (Ford et al., 1976, 1977b, 1977c; Ford and Reynolds, 1981). Progesterone ($P_4$) increases while estradiol-17ß ($E_2$ß) decreases the function of uterine periarterial vasoconstrictor nerves (Ford et al., 1977c).

Estrogens cause increased blood flow to the porcine uterus following either systemic (Dickson et al., 1969) or intrauterine (Ford et al., 1982b) administration. Estrogen-induced increases in uterine blood flow are attenuated by subsequent administration of $P_4$, and the
magnitude of the change appears to be related to the ratio of estrogens to $P_4$ (E/P$_4$; Caton et al., 1974). Elevations of the E/P$_4$ ratio during the estrous cycle in uterine lymph and/or peripheral plasma are temporally associated with increases in uterine blood flow (Ford et al., 1979a; Ford and Christenson, 1979; Greiss and Anderson, 1969; Magness and Ford, 1981). On Days 12 and 13 of gestation in the pig, a transient rise in blood flow to the gravid uterus has been reported (Ford and Christenson, 1979), which is temporally associated with elevated concentrations of estrone ($E_1$) and $E_2$ in the uterine luminal fluid (Gadsby and Heap, 1978; Ford et al., 1982a).

Considering the close anatomical association between the uterine lymphatic vessels and the periarterial sympathetic nerves in the tunica adventitia (Hoggan and Hoggan, 1882), it is conceivable that increased estrogens in the uterine lumen may reach the nerves locally by way of the uterine lymphatics. During the passage of lymph along the collecting and transporting lymphatic channels, there is free exchange of electrolytes and molecules of small molecular weight (<600 Daltons) between the lymph and blood (Mayerson, 1962; Mayerson et al., 1962; Bashir and Mayerson, 1968). It is possible that a similar exchange mechanism occurs between the uterine lymph and the interstitial fluid surrounding the uterine resistance arteries. In support of this hypothesis, Yoffey and Courtice (1970) reported that the composition of lymph collected from lymphatic vessels is approximately the same as the interstitial tissue fluid from which it was derived.
Therefore, the following study was conducted to quantify the concentrations of $E_1$, $E_2$, and $P_4$ in uterine lymph draining the uterine horns of gilts during the estrous cycle and early pregnancy.

Materials and Methods

Experimental procedures

Twenty-four Yorkshire gilts were checked daily for estrous activity, and those animals exhibiting at least two consecutive estrous cycles of normal duration (20.0 ± 0.1 days) were assigned to this experiment. On the first day of estrus (Day 0), 12 randomly selected gilts were mated twice to a boar (0700 and 1700 h) and the remaining 12 served as nonmated controls. Mated and nonmated gilts were assigned randomly in equal numbers to laparotomy on Days 11 (n=4), 13 (n=4) or 15 (n=4) postmating or postestrus, respectively. Food was withheld from gilts for 24 h prior to surgery. General anaesthesia was induced by an intravenous injection of 1 g of Surital ® (sodium thiamylal, Parke-Davis, Detroit, MI) regardless of body weight. Surgical anaesthesia was maintained with a mixture of oxygen and halothane (Fluothane; Ayerst Labs, New York, NY) inhalation in a closed-circuit system with soda lime to remove CO$_2$.

The uterus was exposed through a midventral incision and lymph (500 µl) was collected from a vessel which was closely associated with a main branch of the middle uterine artery supplying one uterine horn.
and was above the anastomosis of the ovarian and uterine lymphatic systems as described in experiment I. The uterine lymphatic vessel was isolated and then ligated with a loop of silk (4-0) to occlude lymph flow. In all cases, the lymphatic vessel became distended on the uterine side of the ligature indicating that lymph was of uterine origin. The distended lymphatic vessel was then cannulated against the direction of the flow using a polyethylene cannula (Intramedic®, I.D. 0.76 mm - O.D. 1.22 mm Clay-Adams Inc., New York, NY) with a 23 gauge stainless steel needle tip which was inserted through a hole in the wall of the isolated lymphatic and sutured into place. When needed, a Harvard dual syringe infusion-withdrawal pump was used to apply a slight negative pressure for withdrawal (40-80 μl/min). In most cases, however, uterine lymph moved through the cannulae without the aid of the pump. After the lymph was collected, uterine arterial blood (10 ml) was sampled from a small branch of the middle uterine artery supplying the horn from which lymph had just been collected using the technique of Ford et al. (1979a). Both the uterine arterial blood and uterine lymph samples were treated with a Na* Heparin solution (10 IU/ml) and stored at -20 C until assayed for E₁, E₂β and P₄. Pregnancies were verified in all 12 mated gilts by the presence of conceptuses in uterine flushings. Estrone and E₂β and P₄ were quantified in uterine lymph and uterine arterial plasma utilizing the same radioimmunoassay procedures described in experiment I.
Statistical analysis

Data were analyzed by a 2 x 3 factorial analysis of variance. Comparisons between uterine arterial blood and uterine lymph were performed using a split-plot analysis of variance (Kirk, 1968) using the Statistical Analysis System (Barr and Goodnight, 1979). Means were compared by orthogonal contrasts (Kirk, 1968).

Results

Regardless of pregnancy status (pregnant vs. nonpregnant), there was no effect of Day (11, 13 or 15) on the concentrations of E$_1$, E$_2$B or P$_4$ in uterine arterial plasma (Table 1) or uterine lymph (Table 2). Concentrations of E$_1$, E$_2$B, or P$_4$ did not differ (P>0.05) between uterine arterial plasma entering gravid or nongravid uterine horns throughout the collection period (Table 1). Levels of E$_1$ and E$_2$B were greater (P<0.01) in uterine lymph collected from pregnant gilts, when compared with nonpregnant gilts, during all days sampled (35.5 ± 1.5 vs. 26.7 ± 1.6 and 34.0 ± 3.9 vs. 17.7 ± 2.1 pg/ml, respectively; Table 2). In contrast to the estrogens, concentrations of P$_4$ in uterine lymph were similar between pregnant and nonpregnant pigs (Table 2). Uterine lymph contained greater (P<0.01) concentrations of E$_1$ and E$_2$B than uterine arterial plasma regardless of pregnancy status or day of sample collection (31.1 ± 1.4 vs. 11.5 ± 0.7 and 25.9 ± 2.8 vs. 10.4 ± 0.7 pg/ml, respectively). However, P$_4$ was significantly lower (P<0.01) in uterine lymph than in uterine arterial plasma throughout the sampling period (9.8 ± 1.2 vs. 39.8 ± 3.5 ng/ml).
Table 1. Steroid concentrations in uterine arterial plasma of nonpregnant (NP) and pregnant (P) gilts¹

<table>
<thead>
<tr>
<th></th>
<th>Days Postestrus or Postmating</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Estrone</td>
<td>NP</td>
<td>12.6 ± 1.5</td>
<td>11.8 ± 0.9</td>
<td>9.0 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>9.2 ± 0.8</td>
<td>14.5 ± 1.9</td>
<td>11.9 ± 4.0</td>
</tr>
<tr>
<td>Estradiol-17β</td>
<td>NP</td>
<td>13.1 ± 1.8</td>
<td>9.5 ± 1.6</td>
<td>8.9 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>10.3 ± 1.4</td>
<td>11.6 ± 4.1</td>
<td>9.1 ± 1.9</td>
</tr>
<tr>
<td>Progesterone</td>
<td>NP</td>
<td>48.5 ± 4.2</td>
<td>44.4 ± 6.7</td>
<td>29.0 ± 8.8²</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>39.8 ± 3.2</td>
<td>41.9 ± 9.8</td>
<td>35.4 ± 4.2</td>
</tr>
</tbody>
</table>

¹Mean ± SEM.

²Three of four gilts in this group showed declining P₄ levels on Day 15 of the estrous cycle.
Table 2. Steroid concentrations in uterine lymph of nonpregnant (NP) and pregnant (P) gilts

<table>
<thead>
<tr>
<th></th>
<th>Days Postestrus or Postmating(^2)</th>
<th>11</th>
<th>13</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td>NP</td>
<td>26.6 ± 3.3(^a)</td>
<td>25.1 ± 4.9(^a)</td>
<td>26.2 ± 1.6(^a)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>36.3 ± 2.0(^b)</td>
<td>36.5 ± 2.7(^b)</td>
<td>33.8 ± 3.6(^b)</td>
</tr>
<tr>
<td>Estradiol-17(^β)</td>
<td>NP</td>
<td>17.4 ± 4.9(^a)</td>
<td>18.2 ± 4.0(^a)</td>
<td>17.5 ± 2.5(^a)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>28.4 ± 5.4(^b)</td>
<td>33.1 ± 8.2(^b)</td>
<td>40.5 ± 7.5(^b)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>NP</td>
<td>12.2 ± 2.3(^a)</td>
<td>10.6 ± 1.6(^a)</td>
<td>6.4 ± 1.8(^a,(^3)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>10.3 ± 2.2(^a)</td>
<td>10.2 ± 3.0(^a)</td>
<td>9.1 ± 1.6(^a)</td>
</tr>
</tbody>
</table>

\(^1\)Mean ± SEM.

\(^2\)Pregnancy status and day comparisons were made within a hormone, and means with different superscripts are significantly different (P<0.01).

\(^3\)Three of four gilts in this group showed declining P\(_{4}\) levels on Day 15 of the estrous cycle.
The ratio of estrogens (E₁ + E₂β) to P₄ calculated on a pg/ng basis (E/P₄ ratio) in uterine arterial blood did not differ between pregnant and nonpregnant gilts (Table 3). However, on Days 11 and 13, uterine lymph from pregnant gilts exhibited higher (P<0.05) E/P₄ ratios when compared with lymph from nonpregnant gilts (7.1 ± 1.4 vs. 4.4 ± 1.6 and 9.0 ± 2.9 vs. 4.7 ± 1.5, respectively). By Day 15 post-estrus, the E/P₄ ratio in uterine lymph (10.5 ± 5.1) had increased to a value which tended to be greater (P<0.1) than that observed on Days 11 or 13 of the estrous cycle and similar (P>0.05) to the ratio quantified on Day 15 of pregnancy (9.2 ± 2.1). The E/P₄ ratio was greater (P<0.01) in uterine lymph than uterine arterial plasma across all days sampled and regardless of pregnancy status.

Discussion

Lymph collected in the present study appeared to be solely of uterine origin. If lymph had been of ovarian origin, P₄ concentrations would have been elevated relative to arterial plasma, reflecting luteal P₄ secretion. Lindner et al. (1964) reported that ovarian lymph from pregnant and nonpregnant luteal phase ewes contained approximately 100-fold greater concentrations of P₄ than systemic blood and was similar to levels in ovarian venous blood. Staples et al. (1982) observed that concentrations of P₄ in uterine lymph from ewes
Table 3. Total estrogen:progesterone ratio in uterine arterial plasma and uterine lymph of nonpregnant (NP) and pregnant (P) gilts.

<table>
<thead>
<tr>
<th></th>
<th>Days Postestrus or Postmating&lt;sup&gt;2&lt;/sup&gt;</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>0.5 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>P</td>
<td>0.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lymph</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>4.4 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.7 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.5 ± 5.1&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>P</td>
<td>7.1 ± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.0 ± 2.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.2 ± 2.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Estrone + estradiol-17β (pg)/progesterone (ng).

<sup>2</sup>Mean ± SEM with different superscripts differ (P<0.01).
during early pregnancy were similar to those in systemic blood. The reduced concentrations of $P_4$ in uterine lymph compared to uterine arterial plasma in this study, may have resulted from uterine uptake and/or metabolism of $P_4$. Knight et al. (1977) reported that concentrations of $P_4$ in uterine venous blood of gilts during early pregnancy were reduced as compared with those found in uterine arterial blood.

In contrast to the reduced $P_4$ levels, uterine lymph in this study contained 3- to 4-fold higher concentrations of $E_1$ and $E_2 \beta$ than uterine arterial plasma, regardless of pregnancy status. Although specific estrogen binding proteins in uterine lymph have not been reported, these may account for the elevated $E_1$ and $E_2 \beta$ concentrations in the lymph when compared to the blood. Concentrations of $E_1$ and $E_2 \beta$ in uterine lymph appear to reflect tissue levels of these steroids, since $E_2 \beta$ concentrations were found to be greater in uterine tissue than in systemic blood (Peck et al., 1973). Peterson and Spaziani (1971) observed an elevated content of albumin in response to $E_2 \beta$ by the rat uterus, which may result in the retention of albumin-bound estrogen in uterine tissue fluids. The disparity between the rate of uterine lymph flow and uterine blood flow may further magnify the $E/P_4$ ratio in lymph compared to that observed in systemic blood. Staples et al. (1982) estimated that uterine lymph flow from the early pregnant uterus of the ewe averaged ~0.7 ml/h which represents less than 1% of reported uterine blood flow on the same days of pregnancy (Metcalfe et al., 1959).
Concentrations of $E_1$ and $E_2B$ (estrogens) were significantly higher in uterine lymph of pregnant vs. nonpregnant pigs across all days studied (Days 11, 13 and 15). The elevated concentrations of estrogens in uterine lymph observed in pregnant gilts in this study are temporally associated with increased concentrations of estrogens in the uterine lumen and venous blood (Ford et al., 1982a). These steroids appear to be derived from the conceptus, as suggested by the high concentrations of estrogens in porcine conceptus tissue when compared to endometrium or uterine flushings on Days 12 and 13 of pregnancy (Gadsby and Heap, 1978). In addition, these increased concentrations of estrogens in the uterine lymph and venous blood during early pregnancy occurred coincident with a 2- to 3-fold increase in blood flow to gravid uterine horns (Ford et al., 1982a). The concentrations of estrogens in uterine lymph were 45.2, 43.5 and 53.3 pg/ml higher than those observed in uterine arterial blood on Days 11, 13 and 15 of gestation. In contrast, Ford et al. (1982a) reported only 5.4, 4.7 and 4.6 pg/ml increases in the concentrations of estrogens in uterine venous vs. uterine arterial blood on the same days of gestation. In both of these studies, no differences in $E_1$ or $E_2B$ concentrations were detected in uterine arterial blood between pregnant and nonpregnant pigs. These data demonstrate that the uterine lymphatics function with the uterine veins to transport estrogens from the early gravid porcine uterus. In addition, concentrations of $E_1$ and $E_2B$ in uterine lymph in this study were 3- 4-fold higher than concentrations
of estrogens in uterine venous blood reported previously (Ford et al., 1982a).

Uterine blood flow increased following estrogen administration in the ewe (Huckabee et al., 1970), cow (Roman-Ponce et al., 1978) and sow (Dickson et al., 1969). Caton et al. (1974) observed an antagonistic effect of estrogen and P₄ on uterine blood flow in the ewe. Elevations in the E/P₄ ratio in systemic blood and uterine lymph are temporally associated with increases in uterine blood flow throughout the estrous cycle in the sow (Ford and Christenson, 1979; Magness and Ford, 1981). Recent evidence suggests that estrogen decreases while P₄ increases the vasoconstrictor function of uterine periarterial vasoconstrictor nerves with subsequent alterations in uterine blood flow (Ford, 1982). The uterine periarterial nerves are derived from the periaortic plexus and become incorporated into the adventitia of this vessel at its point of origin (Shabanah et al., 1964). These nerve fibers then traverse the length of the uterine artery in close association with the uterine lymphatics (Hoggan and Hoggan, 1882; Ham, 1969). It is conceivable that elevated estrogen concentrations in uterine arterial blood or uterine luminal fluid may reach the uterine periarterial vasoconstrictor nerves locally by way of the uterine lymphatics. Lymphatic capillaries of the uterus (Sass, 1964), ovaries (Morris and Sass, 1966), small intestines (Palay and Karlin, 1959) and other tissues contain intercellular gaps which facilitate transport of a variety of large and small molecules. Uterine lymph flow is rela-
tively slow compared to uterine blood flow (Metcalfe et al., 1959; Staples et al., 1982), and lymph has been reported to reflect the content of the interstitial fluid from which it was derived (Yoffey and Courtice, 1970). During the slow transport of lymph along the collecting lymphatic channels there is exchange of electrolytes and small molecules of up to 600 gram molecular weight from the lymph to the blood (Mayerson, 1962). The gram molecular weights of $E_1$ and $E_2$ are 270.4 and 272.4, respectively. Exchange of estrogen by this route would support the hypothesis that local increases in the $E/P_4$ ratio in uterine lymph during early pregnancy may expose the periarterial sympathetic vasoconstrictor nerves within the uterine arteries to steroids which have been shown to change the tone and contractility of, and flow rate through the uterine vasculature.

Recently, Ford et al. (1982d) reported a transient 60% increase in blood flow through corpora lutea (CL) of gilts on Day 13 post-mating, 24-48 h after the initiation of the conceptus-mediated rise in uterine blood flow. This delayed increase in luteal blood flow relative to uterine blood flow may result from the slow transport of embryonic estrogens from the uterine horn to the ipsilateral ovary through the uterine lymphatics. Injections of $E_2$ into an isolated uterine horn from Days 11 to 15 in amounts similar to those produced by the conceptuses resulted in elevated uterine blood flow and $P_4$ secretion by CL on the ipsilateral ovary (Ford et al., 1982b). Luteal $P_4$ secretion is highly correlated with blood flow through the CL of the ewe (Niswender et al., 1975) and cow (Ford and Chenault, 1981).
Although a direct anatomical connection for the transfer of lymph from the uterus to the ovary could not be demonstrated in the sow (Andersen, 1927) or ewe (Meckley and Ginther, 1969), recent work in the pig suggests that a mechanism may exist for the transport of PGF$_2$\textalpha{} from the uterine lymph to the ovarian vasculature (Kotwica, 1980).
EXPERIMENT III. OVARIAN BLOOD FLOW THROUGHOUT THE ESTROUS CYCLE AND EARLY PREGNANCY IN SOWS

Introduction

Blood flow to the luteal ovary varies regularly during the estrous cycle in the ewe, cow and other mammalian species, being highest during the luteal phase and lowest during the follicular phase (Niswender et al., 1975; Bruce and Moor, 1975; Hossain et al., 1979; Ford and Chenault, 1981). This cyclic pattern can be attributed primarily to changes in blood flow through the corpora lutea (CL), which receive up to 90% of the total ovarian flow (Abdul-Karim and Bruce, 1973; Niswender et al., 1973; Novy and Cook, 1973; Ford et al., 1979b). Decreased blood flow through the CL is associated with decreased ovarian progesterone (P₄) secretion during natural as well as prostaglandin (PG)F₂α-induced luteal regression (Rathmacher and Anderson, 1968; Niswender et al., 1976; Nett et al., 1976). It has been suggested that a portion of the luteolytic activity of PGF₂α may be manifested by a reduction in blood flow through the CL (Pharriss, 1970; Nett and Niswender, 1981).

The embryonic signal that initiates luteal maintenance in the pig occurs between Days 11 and 13 postmating (Dhindsa and Dziuk, 1968; Ford et al., 1982a) and is temporally associated with transient increases in blood flow to the uterus, oviduct and CL (Ford et al., 1982d). A substance produced by the early ovine and bovine conceptus or early pregnant uterus on days critical for pregnancy recognition
seems to reduce vascular responsiveness to PGF$_{2\alpha}$ (Ford et al., 1976), with subsequent increases in uterine blood flow in each species (Greiss and Anderson, 1970; Ford et al., 1979a). It is conceivable that this vasodilatory effect of the conceptus also is functioning on the sympathetic vasoconstrictor nerves of the luteal vascular bed. Recently Reynolds and Ford (1982) observed a reduced contractility of the ovarian vascular bed of gilts on Day 13 of pregnancy vs. Day 13 of the estrous cycle, which may result from a conceptus-induced reduction in the constrictor activity of the ovarian periarterial sympathetic nerves. Kadowitz et al. (1972) have reported that PGF$_{2\alpha}$ promotes vasoconstriction by facilitating the release of norepinephrine from periarterial sympathetic nerves and thus would be a poor vasoconstrictor during early pregnancy when neuronal activity was low.

The following study was conducted to characterize the pattern of blood flow to ovaries of sows during the estrous cycle and early pregnancy. Correlations of daily changes in ovarian blood flow with the concentrations of P$_{4}$, estrone (E$_1$) and estradiol-17β (E$_2\beta$) also were studied.

Materials and Methods

Experimental procedures

Four multiparous Yorkshire sows, which had exhibited at least 2 consecutive estrous cycles of normal duration (18-23 days), were utilized in this study (Day 0 = first day of estrus). Throughout the
estrous detection and experimental periods, sows were maintained in elevated farrowing stalls, which permitted the animals to stand or lie down.

Feed was withheld from sows for 24 h before surgery, which was performed on Day 8 postestrus. General anaesthesia was induced by intravenous infusion of 1.0 g of Surital (sodium thiamytil: Parke Davis Labs., Detroit, MI), while surgical anaesthesia 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 CO₂. At surgery, ovaries were exposed through a midventral incision, and a pre-calibrated electromagnetic blood-flow transducer (6-8 mm internal circumference) was placed around one randomly selected ovarian artery proximal to its bifurcation into ovarian and tubal branches (Figure 1), in a manner similar to that described previously (Ford and Christenson, 1979).

Beginning on the second day after placement of the probes, blood flow through the ovarian artery was monitored continuously for 10 min once daily between 0700 and 1000 h. Blood flow values (ml/min) displayed by the flowmeter (Model 501D, Carolina Medical Electronics, Inc., King, North Carolina) were recorded at 15-sec intervals during each 10-min monitoring period. Recorded values were averaged over the 10-min period for each artery, and considered an estimate of ovarian blood flow for that day.

Estrus was checked daily with a mature boar throughout the experimental period. Sows were not bred at their first estrus
Figure 1. Schematic drawing of the unilateral utero-ovarian arterial vasculature of the sow. Site of placement of blood-flow transducer (•••••) is indicated.
following placement of the probes to enable each sow to be monitored throughout an entire estrous cycle. At the second post-surgical estrus, sows were artificially inseminated at 12 and 24 h after the first detection of behavioral estrus by using freshly collected semen from a Yorkshire boar. Sows were slaughtered on Day 21 post-insemination to verify the placement of flow transducers. Pregnancy was confirmed by the presence of conceptus tissue.

A polyvinyl cannula was inserted into the femoral artery via the saphenous artery at the time of surgery and used to obtain systemic blood samples from each sow immediately after ovarian blood flow was monitored each day. The concentrations of $P_4$, $E_1$ and $E_2B$ in femoral arterial plasma were determined subsequently by the radioimmunoassay procedures described in experiment I.

**Statistical analysis**

Daily blood flow averages for each artery were considered as single observations for statistical analysis to characterize time trends. Changes in ovarian blood flow and systemic concentrations of ovarian steroids were analyzed by split-plot analysis of variance (Kirk, 1968) using the Statistical Analysis System (Barr and Goodnight, 1979). Differences between means were tested for significance by use of orthogonal contrasts (Kirk, 1968). Correlation coefficients between daily plasma concentrations of $P_4$, $E_1$ and $E_2B$ and ovarian blood flow also were calculated.
Results

Surgery and placement of the blood flow transducers did not significantly affect the lengths of the estrous cycles exhibited by sows in this study. Prior to surgery, the mean (± SEM) estrous cycle was 21.3 ± 0.3 days, (n=4), while the estrous cycle lengths during the surgical and post-surgical cycles averaged 21.5 ± 0.4 days (n=8). To minimize variation, data were adjusted to a 21-day estrous cycle length by analyzing samples collected after Day 14 as days preceding estrus. Three of the 4 sows were confirmed pregnant 21 days after insemination. Data from the fourth sow's post-insemination cycle were not included in any of the statistical analyses.

Patterns of ovarian blood flow (ml/min) were similar (P>0.05) for all 4 sows throughout the experimental period; however, the mean baseline flow rates were significantly different between sows. To reduce variation, data were statistically analyzed as the change in ovarian blood flow from the flow rate observed for each sow on Day 0 of the estrous cycle or pregnancy (Figure 2). Absolute ovarian blood flow averaged 10.9 ± 1.4 ml/min (n=4) and 9.1 ± 1.6 ml/min (n=3) on Day 0 of the estrous cycle and pregnancy, respectively.

Blood flow to ovaries of the 4 nonpregnant sows followed a consistent cyclic pattern during the estrous cycle as illustrated in Figure 2. Ovarian blood flow was low (P<0.01) at estrus, had increased (P<0.05) by Day 2 and was highest from Day 6 to Day 14 of the estrous cycle. Ovarian blood flow remained high until Day 14 when it declined (P<0.01) abruptly to reach low levels 2 days before the
Figure 2. Pattern of blood flow to the ovaries of sows throughout the estrous cycle (n=4) and early pregnancy (n=3). Each point represents the Mean ± SEM. Day 0 = first day of estrus.
•— Nonpregnant Sows (n=4)

— — Pregnant Sows (n=3)

Change in Ovarian Arterial Blood Flow (ml/min)

Days of the Estrous Cycle

Days of Pregnancy
subsequent estrus. The pattern of ovarian blood flow during early pregnancy was similar to that observed during the previous estrous cycle until Day 12 (Figure 2). Between Days 12 and 15 of pregnancy, ovarian blood flow exhibited a transient increase (P<0.01) of 26%, whereas blood flow to the ovaries of nonpregnant sows remained constant. By Day 15 of pregnancy, ovarian blood flow had returned to a flow rate similar to that observed on Day 11, then remained constant through Day 21.

Progesterone concentrations in systemic blood began to increase (P<0.05) on Day 3 of the estrous cycle and were maximal by Day 10 (Figure 3). From Day 10 through Day 14, P4 remained relatively constant before declining (P<0.01) to basal levels 3 days before estrus. The concentrations of P4 in systemic blood from Day 0 to Day 12 of pregnancy followed a pattern similar to that observed during the previous estrous cycle. On Days 13 and 14, however, levels of P4 in systemic blood increased (P<0.05) by 20%. Thereafter, concentrations of P4 declined (P<0.01) gradually through Day 21 post-insemination to approximately 40-50% of the P4 concentrations on Day 12 postmating. This was in contrast to the precipitous decline in P4 observed in the nonpregnant animals after Day 14 postestrus. Plasma concentrations of E1 and E2β were highest (P<0.01) 2 days before estrus to Day 1 postestrus (Figures 4 and 5). By Day 3, concentrations of E1 and E2β had reached low levels (P<0.01) and remained basal through Day -4 of the cycle. As observed in the nonpregnant sows, E1 and E2β concentrations in the systemic blood of pregnant sows were highest (P<0.01) around
Figure 3. Concentrations of progesterone in systemic blood of sows throughout the estrous cycle (n=4) and early pregnancy (n=3). Each point represents the Mean ± SEM. Day 0 = first day of estrus
- Nonpregnant Sows (n=4)

- Pregnant Sows (n=3)
Figure 4. Concentrations of estrone in systemic blood of sows throughout the estrous cycle (n=4) and early pregnancy (n=3). Each point represents the Mean ± SEM. Day 0 = first day of estrus.
Nonpregnant Sows (n=4)

Pregnant Sows (n=3)

Days of the Estrous Cycle

Days of Pregnancy

Esteone Concentrations (pg/ml)
Figure 5. Concentrations of estradiol-17β in systemic blood of sows throughout the estrous cycle (n=4) and early pregnancy (n=3). Each point represents the Mean ± SEM. Day 0 = first day of estrus.
Nonpregnant Sows (n=4)  

Pregnant Sows (n=3)  

Estradiol-17β Concentrations (pg/ml)
estrus and declined (P<0.01) to basal levels by Day 3 postmating. Estrone and $E_2\beta$, however, remained relatively constant through Day 21 of pregnancy.

Daily changes in ovarian blood flow during the estrous cycle were positively correlated (P<0.01) with the concentrations of $P_4$ ($r=+0.63$) and negatively correlated (P<0.01) with $E_1$ ($r=-0.51$), $E_2\beta$ ($r=-0.53$) and the ratio of estrogen ($E_1 + E_2\beta$) to $P_4$ ($E/P_4$; pg/ng; $r=-0.49$) in systemic blood. During pregnancy, however, ovarian blood flow was significantly correlated (P<0.01) only with $P_4$ and the $E/P_4$ ratio ($r=+0.65$ and -0.49, respectively). No significant association between ovarian blood flow changes and the concentrations of $E_1$ ($r=-0.20$) or $E_2\beta$ ($r=-0.19$) in systemic blood could be demonstrated during early pregnancy.

Discussion

Patterns of $P_4$, $E_1$ and $E_2\beta$ in systemic blood during the estrous cycle of sows in this study were similar to steroid profiles reported previously (Stabenfeldt et al., 1969; Henricks et al., 1972). A consistent finding in this study and that of Henricks et al. (1972) was that plasma concentrations of estrogen did not begin to increase during the proestrous period until the day that plasma $P_4$ concentrations began to fall. This negative relationship between the concentrations of estrogens and $P_4$ in systemic blood continued throughout the estrous cycle.

The pattern of blood flow through the ovarian artery observed in this study was similar to results of Niswender et al. (1975) and Ford
and Chenault (1981), who demonstrated decreased blood flow to CL-bearing ovaries during the late luteal phase of the ovine and bovine estrous cycle, respectively. In addition, ovarian blood flow in this study was positively correlated with systemic concentrations of $P_4$ throughout the estrous cycle and early pregnancy. Changes in ovarian blood flow can be attributed primarily to changes in blood flow to the CL, which receive approximately 90% of the blood flow perfusing the porcine ovary (Ford et al., 1982d). These results are in agreement with those of Niswender et al. (1975) and Ford et al. (1979b), who observed that luteal $P_4$ secretion in the ewe is highly correlated with blood flow through the CL. Although the highly significant positive correlation between ovarian blood flow and systemic concentrations of $P_4$ in this study suggests a strong association between the two, no direct cause-and-effect relationship could be determined.

The negative correlation of the concentrations of $E_1$, $E_2$ and the ratio of $E/P_4$ in systemic blood with ovarian blood flow during the estrous cycle in this study are in agreement with the data of Ford and Chenault (1981) for the cow. No data exist to suggest that estrogens have a direct effect to decrease ovarian blood flow; rather, they indicate that ovarian blood flow was temporally elevated only during the luteal phase of the cycle, when luteal $P_4$ secretion and CL weights are elevated and estrogens are low. This lack of a vasoconstrictor effect of estrogen is supported by the fact that $E_2$ injections have been shown to increase, rather than decrease, ovarian blood flow in pregnant (Rosenfeld et al., 1976) and postpartum (Rosenfeld, 1980)
ewes. In addition, the E/P₄ ratio in systemic blood exhibited a highly significant negative correlation (r=-0.99) with the ability of the porcine ovarian vascular bed to contract in response to in vitro sympathetic nerve stimulation (Reynolds and Ford, 1982). Elevations of the E/P₄ ratio in systemic blood during the estrous cycle thus are associated with a relaxation of the ovarian vascular bed. This relaxation occurs at a time when the size of the ovarian vascular bed, and thus ovarian blood flow, are significantly reduced, as a result of a reduction in the size of the luteal vascular bed. These data are in agreement with those of Novy and Cook (1973) and Ford et al. (1979b), who observed an increased flow of blood to the extra-luteal vascular bed of ovaries during luteal regression in the rabbit and ewe, respectively.

Results presented in this experiment clearly demonstrate an association between the presence of embryos and increased ovarian blood flow and P₄ secretion on days previously reported to be critical for the continued maintenance of CL during early pregnancy in the pig (Dhindsa and Dziuk, 1968; Ford et al., 1982a). That this increase in ovarian blood flow was due primarily to increased luteal blood flow was suggested by Ford et al. (1982d), who demonstrated increased blood flow to CL, but not the extra-luteal compartment of the ovary, on Day 13 of pregnancy in gilts. The conceptus appears to mediate these changes in luteal function in a local manner since Ford and Christenson (1979) reported greater P₄ concentrations in CL on the ipsilateral ovaries of unilaterally pregnant sows on Day 16 post-
mating. 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.

Blood flow to the ovaries of sows in the present study was not significantly correlated with the concentrations of $E_1$ and $E_2 \beta$ during early pregnancy, suggesting that the systemic association of estrogen with ovarian blood flow during the estrous cycle was removed because of a local luteotropic effect of the conceptus. Recently, Ball and Day (1982a, 1982b) demonstrated the presence of a luteotropin in extracts of conceptus tissue from early pregnant gilts. Estrogens produced locally by porcine conceptuses have been proposed by numerous researchers as the embryonic luteotropin in this species (Flint et al., 1979). In support of this hypothesis, Ford et al. (1982b) observed that injections of $E_2 \beta$ into one isolated uterine horn of nonpregnant sows, in amounts similar to those produced by conceptuses across Days 11-15 postmating, preferentially stimulated $P_4$ secretion by CL on the ovaries ipsilateral to the steroid injections. In addition, Ford et al. (1982a, 1982d) reported that elevated estrogen concentrations within the gravid uterine horns of sows were temporally associated with maternal recognition of pregnancy as well as increases in uterine, oviductal and luteal blood flow. The initial dilation of the oviductal and luteal vascular beds, however, occurred 24 to 48 h after the initial dilation of the uterine vascular bed, suggesting a delayed transport of a conceptus associated vasodilatory substance
from the uterus to the ovary. Concentrations of estrogens are elevated in uterine lymph from Days 11 to 15 of pregnancy in gilts, when compared with uterine lymphatic concentrations of estrogens on similar days of the estrous cycle (Magness and Ford, 1982). Each uterine horn in the pig has prominent lymphatics which drain fluid from the horn and anastomose with the ovarian and tubal lymphatics in the utero-ovarian vascular pedicle (Hoggan and Hoggan, 1982; Andersen, 1926, 1927). Thus, one route whereby estrogens of embryonic origin leave the uterus is via the lymphatic drainage, which may function in the transfer of this substance to the ipsilateral ovary and oviduct.

Injections of E₂β are luteotropic in nonpregnant sows when administered either systemically (Gardner et al., 1963) or directly into the uterine lumen (Ford et al., 1982b). Contractility of the ovarian vascular bed in response to periarterial nerve stimulation was significantly reduced in Day 13 pregnant gilts as compared with Day 13 nonpregnant gilts (Reynolds and Ford, 1982) coincident with elevated blood flow through the CL (Ford et al., 1982d). Because luteal blood flow is highly correlated with the ability of the CL to secrete P₄, estrogens may maintain luteal function, in part, by preventing PGF₂α-induced contractility of the luteal vascular bed and subsequently prevent luteolysis.
GENERAL DISCUSSION

No differences were observed in the concentrations of estrone ($E_1$), estradiol-17β ($E_2β$) and progesterone ($P_4$) in uterine lymph collected on the same days of the estrous cycle in experiment I and experiment II. This lack of steroid differences suggests that the chronic and acute methods of lymph collection, developed for these studies, did not alter steroid concentrations in the lymph. On Day 15 of the estrous cycle in both studies, the estrogen to progesterone ($E/P_4$) ratio in uterine lymph and systemic blood were elevated ~1 to 2 fold as a result of declining $P_4$ levels associated with luteolysis. This elevation in the $E/P_4$ ratio occurred coincident with declining ovarian blood flow (experiment III) and immediately preceding the proestrus rise in uterine blood flow reported by Ford and Christenson (1979). Many investigators have ascribed the changes in uterine blood flow during the estrous cycle to changes in the $E/P_4$ ratio in systemic blood (Resnik, 1981; Ford, 1982, for review). The work in the present studies extends this treatise to include changes in the $E/P_4$ ratio in uterine lymph. Furthermore, in both experiments I and II concentrations of estrogens were higher and $P_4$ was lower in uterine lymph than systemic blood regardless of pregnancy status. If these steroids have direct access to the walls of the arteries, as hypothesized, then even greater concentrations of estrogen would be in contact with the periarterial sympathetic vasoconstrictor nerves which control blood flow to the reproductive tract.
Exogenous administration of estrogen increases uterine blood flow in the ewe (Assali, et al., 1978), cow (Ford and Reynolds, 1981) and pig (Dickson et al., 1969). A 20- to 30-minute delay occurs between the administration of estrogen and the vasodilatory response of the uterine vascular bed (Killam et al., 1973; Ford and Reynolds, 1981). It is possible that this delay in uterine vasodilation to estrogen may involve the synthesis or release of a secondary vasodilatory intermediate produced by the uterus, or the metabolism of estrogen to another vasoactive form. These hypotheses are supported by the work of Killam et al. (1973) who demonstrated that the dilation of the ovine uterine vascular bed was blocked by prior exposure to cycloheximide, a protein synthesis inhibitor. Furthermore, Van Orden (Univ. of Iowa, unpublished observation) recently showed that pretreatment of rats with the steroid-hydroxylation inhibitor aminogluthethimide, to block estrogen metabolism, prevented estrogen-induced vasodilation. An alternate hypothesis is that this delayed vasodilation may result in part from the uptake and transport of estrogen, its metabolite or a secondary messenger into the uterine lymphatic system for subsequent transport to the utero-ovarian vasculature.

As stated previously, E$_1$ and E$_2$ concentrations were 3- to 4-fold higher in uterine lymph than systemic blood in both experiments I and II. Morris and McIntosh (1971) determined that the level of testosterone binding protein(s) in testicular lymph, determined the
concentration of this steroid in the lymph. From these data it was hypothesized that estrogen levels in uterine lymph may be magnified as a function of an elevation in steroid binding protein content of the lymph. To test this hypothesis, uterine lymph from experiments I and II were analyzed for total protein concentrations by the method of Lowry et al. (1951) using bovine serum albumin standards. Data from this corollary study are presented in Figure A1 and Table A1 of the Appendix.

Concentrations of protein in the uterine lymph of gilts during the estrous cycle and early pregnancy averaged 4.8 g/100 ml which is ~69% of the concentrations of proteins reported previously for blood plasma (Aronson, 1980). Proteins in uterine lymph of early pregnant ewes were 85-90% (Staples et al., 1982) of proteins in systemic plasma. Data presented in Figure A1 and Table A1 do not support the hypothesis that elevations in the protein content of uterine lymph are associated with increased lymphatic estrogen concentrations. In fact, concentrations of proteins in lymph were lowest around estrus, when estrogen concentrations were highest. The proestrus-estrus rise in blood flow to the nongravid porcine uterus (Ford and Christenson, 1979) is temporally associated with the decline in protein observed during these days of the estrous cycle as illustrated in Figure A1. This finding is consistent with those of Sass (1964) who reported that protein concentrations in lymph draining the lumbar lymph nodes of pregnant ewes, which receive increasing quantities of lymph from the gravid uterus during pregnancy, decreased as lymph flow increased.
throughout gestation. A similar negative relationship was observed between protein concentrations in lymph draining the mammary gland, and elevations in mammary gland lymph flow during lactation (Yoffey and Courtice, 1970). Furthermore, these results would explain why the utero-ovarian lymph to plasma protein ratio declined from 85-90% to 47% from early pregnancy (Staples et al., 1982) to late pregnancy (Sass, 1964) in ewes, when uterine blood and lymph flows are increasing.

Although Ford and Christenson (1979) reported a transient 2- to 3-fold increase in uterine blood flow between Days 11-13 of pregnancy in sows, the concentrations of protein in uterine lymph did not decline (Table A1) with this increase in uterine blood flow as observed during the follicular phase of the estrous cycle. These data provide indirect evidence for increased protein secretion from the gravid uterus into the uterine lymph to offset any decline in protein concentrations associated with increased lymph flow on these days. These data also suggest the possibility that pregnancy specific estrogen-induced proteins may be transported in uterine lymph. In support of this concept, protein concentrations (Geisert et al., 1980) and estrogen concentrations (Ford et al., 1982a) are elevated in the uterine lumen of pigs between Days 11-13 of pregnancy. Protein concentrations in uterine lymph of Day 15 pregnant gilts were reduced when compared to lymph collected on Days 11 and 13 of pregnancy (Table A1). Ford et al. (1982a) reported that although uterine blood flow declined by ~50% from Day 13-15 of gestation in sows, the blood flow
to the gravid uterus was still 2-fold greater than on Day 15 of the estrous cycle. Frank et al. (1978) demonstrated that the concentrations of proteins in the uterine lumen of gilts increased on Day 15 of pregnancy when compared to Days 11 and 13. Taken together, these results suggest that on Day 15 of pregnancy, either protein secretion from the uterine lumen into the lymph is declining, or there is a decreased permeability of the uterine capillary bed to proteins. This problem could be investigated in the pig by evaluating the molecular exclusion of the uterine capillary bed to intra-arterial or intra-uterine infusions of I$^{131}$ albumin and quantifying the specific activity in the uterine lymph.

Elevated concentrations of estrogens in uterine lymph, observed from Day 11 to Day 15 in pregnant gilts (experiment II), were temporally associated with the time Ford et al. (1982d) demonstrated that porcine conceptuses initially stimulate a dilation of the uterine vascular bed. This transient rise in blood flow to the gravid porcine uterus peaked on Days 12-13 and declined to 50% of peak flows by Day 14 postmating (Ford and Christenson, 1979). In contrast, ovarian blood flow in experiment III was maximal on Days 13-14 and declined by 50% on Day 15 of pregnancy. Ford et al. (1982d) reported that luteal and oviductal blood flow in gilts were maximal on Day 13 of pregnancy, 24-48 h after the initiation of the conceptus-mediated rise in uterine blood flow. This delayed increase in ovarian and luteal blood flow relative to uterine blood flow may result from the slow transport of
estrogens of conceptus origin from the uterine horn to the ipsilateral ovary through the uterine lymphatics.

This hypothesis is supported indirectly by the observation that in unilaterally pregnant pigs that remain pregnant, there is a high incidence of unilateral maintenance of CL adjacent to the gravid uterine horn (Anderson et al., 1966; Christenson and Day, 1971). The transient rise in uterine blood flow associated with maternal recognition of pregnancy in pigs (Ford et al., 1982a) was observed only to the gravid uterine horn in unilaterally pregnant sows also in association with increased P₄ concentrations of corpora lutea (CL) on ovaries adjacent to the gravid uterine horn (Ford and Christenson, 1979). Furthermore, injections of E₂β into the lumen of an isolated uterine horn of sows from Days 11 to 15 postestrus, in amounts similar to those produced by the conceptuses, resulted in elevated uterine blood flow and P₄ secretion by CL on the ovaries ipsilateral to the steroid injections (Ford et al., 1982b). Since luteal P₄ secretion is highly correlated with blood flow through the CL (Niswender et al., 1975), it would be logical to conclude that intrauterine estrogen is not only luteotropic (Ford et al., 1982b) but also vasodilatory to the CL of the pig. In support of this, E₂β injections have been shown to increase ovarian blood flow in pregnant (Rosenfeld et al., 1976) and postpartum (Rosenfeld, 1980) ewes.

Although a direct anatomical connection for the transfer of lymph from the uterus to the ovary could not be demonstrated in the sow
(Andersen, 1927) or ewe (Meckley and Ginther, 1969; Staples et al., 1982), these lymphatic vessels anastomose with the ovarian and tubal lymphatics in the utero-ovarian vascular pedicle below the ovary. This vascular pedicle is a coiled array of arteries, veins and lymphatics from the uterus, ovaries and oviduct. It has been proposed that the utero-ovarian vascular pedicle is an area for the local counter-current (veno-arterial) transfer of luteotropic and luteolytic substances from the uterine veins of the early gravid and nongravid uterine horn to the adjacent ovarian artery in sheep, cows and possibly the pig (Ginther et al., 1973; Ginther, 1974; Mapleton et al., 1975, 1976; Ginther, 1976). Recent work in the pig suggests that a mechanism may exist for the transport of prostaglandin (PG)F$_2$$_\alpha$ from the uterine lymph to the ovarian vasculature (Kotwica, 1980). Numerous investigators have proposed PGF$_2$$_\alpha$ to be the uterine luteolysin (Gleeson et al., 1974) and estrogen to be the embryonic luteotropin (Flint et al., 1979) in the pig. Thus, it is possible that the lymphatics are involved in the local counter-current transfer of PGF$_2$$_\alpha$ or estrogen from the uterine horn to the ipsilateral ovarian artery to initiate luteolysis or luteal maintenance in the pig.
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Finally, to my bride, Susie, a very special "thank you" for your valuable assistance and cooperation, and above all your patience and love.
Figure A1. Concentrations of protein (g/100 ml) in uterine lymph throughout the porcine estrous cycle (Mean ± SEM)
Protein Concentration in Uterine Lymph (g/100ml)
Table A1. Protein concentrations (g/100 ml) in uterine lymph of nonpregnant and pregnant gilts

<table>
<thead>
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<th>Days Postestrus or Postmating</th>
<th>11</th>
<th>13</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant</td>
<td>5.87 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.75 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.02 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnant</td>
<td>6.14 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.89 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.65 ± 0.49&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>1</sup>Mean ± SEM with different superscripts differ (P<0.05).