ACTIONS OF HORMONES ON THE UTERUS AND EFFECT ON CONCEPTUS DEVELOPMENT

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Summary

THE process of reproduction in the mammalian female involves interactions between gonadal and placental steroids and, possibly, protein hormones such as placental lactogen, and the uterine endometrium. These interactions are discussed relative to available data from pigs and with the understanding that the concepts presented may not be directly applicable to other species.

Swine embryos enter the uterus for continued development beyond the early blastocyst stage and, within the uterus, the spherical blastocyst, of about 2 mm diameter on day 12, undergoes rapid elongation to an organism of about 1 m in length by day 15 of pregnancy. During this period of elongation, the blastocyst appears to provide the chemical signal, presumably unconjugated estrogens, that allows for corpora lutea (CL) maintenance (luteostatic effect) and, therefore, continued production of progesterone by the CL which is essential for pregnancy. Estrogens from the blastocyst may exert their luteostatic effect on the uterus by causing prostaglandin F₂α, the presumed porcine uterine luteolysin, to be secreted in an exocrine (toward the uterine lumen) rather than an endocrine (toward the endometrial stroma and vasculature) direction during pregnancy. Consequently, prostaglandin F₂α is sequestered in the uterine lumen and does not become available, via the utero-ovarian vasculature, to exert its luteolytic effect.

The potential steroid precursor(s) available to the trophoblast for estrogen synthesis appear to be produced through endometrial conversion of progesterone to unconjugated androgens, e.g., androstenedione and testosterone, and conjugated estrogens, e.g., estrone-sulfate and estradiol-sulfate. The estrogens may, in concert with progesterone and possibly other hormones, placental lactogen for example, act locally, i.e., at the site of placentation to: (1) result in a luteostatic effect; (2) increase uterine blood flow; (3) enhance water, electrolyte and substrate (carbohydrate and amino acid) transport to the site of placentation and across the placenta; (4) affect synthesis and secretion of macromolecules (histotroph) by the uterine glands that serve as enzymes and (or) carrier molecules and (5) control or modulate physiological and (or) biochemical events essential to placental and fetal development that are not understood.

(Key Words: Conceptus, Uterus, Trophoblast, Placenta, Steroids, Pregnancy.)

Introduction

Critical events associated with intrauterine embryonic/fetal development in mammals include: (1) shedding of the zona pellucida; (2) intrauterine migration of embryos; (3) expansion and elongation of the blastocyst; (4) initiation of steroidogenesis by the blastocyst; (5) production of the luteostatic signal for the so-called "maternal recognition of pregnancy"; (6) water and electrolyte transport associated with expansion of the chorioallantoic membranes and (7) placental transport of nutrients from dam to fetus. The purpose of this report is to summarize data that may help us to understand interactions between the uterus and conceptus which appear to be of special significance for embryonic survival and development. Since the pig has served as the primary experimental animal in
our studies, the concepts discussed can only be directly related to that species at present.

Expansion and Elongation of the Porcine Blastocyst

The pig embryo develops beyond the early blastocyst stage in the uterine environment (Murray et al., 1971; Pope and Day, 1972). Within the uterine lumen, the blastocyst emerges from the confines of the zona pel-lucida ("hatches") between days 6 and 8 of gestation, expands to about 2 mm in diameter by day 12 and then elongates to a thread-like organism of 420 to 1,900 mm in length by day 16 of pregnancy (Perry and Rowlands, 1962). The intrauterine factor(s) associated with expansion and elongation of the blastocyst has (have) not been identified.

Initiation of Steroidogenesis by the Blastocyst

Perry et al. (1973) first demonstrated conversion of tritium labeled androgens, dehydroepiandrosterone and androstenedione, progesterone and estrone-sulfate to free estrogens by day 14 to 16 pig blastocysts. Later, Perry et al. (1976) reported that dehydroepiandrosterone, androstenedione and conjugated estrogens (estrone-sulfate) could be readily converted to estrone and estradiol; however, conversion of progesterone, pregnenolone and testosterone to estrone and estradiol by pig blastocysts was negligible. The conversion of dehydroepiandrosterone and androstenedione to estrone and estradiol has consistently been found (Perry et al., 1976). The source of these estrogen precursors has not been demonstrated; however, they were presumed to be of maternal origin (Heap and Perry, 1974).

Based upon two lines of evidence from our laboratory, we developed the working hy-

pothesis that the porcine endometrium converts progesterone, from corpora lutea (CL), to androgens, primarily androstenedione, which could be used by the blastocyst for conversion to estrogens. Support for this hypothesis was the observation that the uterine artery-uterine vein (A-V) difference for plasma progesterin concentrations was positive at all stages of gestation studied, i.e., between days 20 and 100 of pregnancy (Knight et al., 1977). This observation indicated that progesterone was being taken up and possibly metabolized by the pregnant uterus. Conversely, estradiol and estrone were present in higher concentrations in plasma from uterine vein than uterine artery (Knight et al., 1977). This observation indicated that estrogens were being produced by a component(s) of the pregnant uterus. Previous data have been interpreted to indicate that the trophoblast (chorion) is the source of estrogen in the pregnant uterus (Velle, 1960; Raeside, 1963; Molokwu and Wagner, 1973; Choong and Raeside, 1974; Robertson and King, 1974; Wette mann et al., 1974; Perry et al., 1976; Knight et al., 1977).

Data from our laboratory indicated that gilts bilaterally ovariectomized on day 4 of pregnancy and treated with either 25, 50, 100 or 200 mg progesterone per day to maintain pregnancy had plasma progesterin concentrations that were markedly affected by pregnancy status on day 60 of gestation (table 1). Pregnant gilts (viable conceptuses) or those that had been pregnant (reabsorbing conceptuses) had mean plasma progesterin concentrations ranging from 7.0 to 26.5 ng/milliliter. However, gilts for which there was no indication that pregnancy had been established had progesterin concentrations between 163.4 and 428.0 ng/milliliter. Generally,
plasma progestin concentrations increased as daily dosage of progesterone injected increased. These data suggest that the uterus and fetal-placental unit and, in fact, the uterus alone, in females in which pregnancy had been temporarily established affected plasma progestin concentrations. That is, based on plasma progestin concentrations, a pregnant uterus or a uterus in which pregnancy had been established, was capable of metabolizing or altering the metabolism of progestrone so that plasma progestin concentrations were markedly reduced when compared with plasma progestin levels in gilts in which pregnancy had not been established.

In the course of normal pregnancy in pigs, there is a 30 to 70% decrease in plasma progestin concentrations between days 14 and 30 of gestation (Guthrie et al., 1972; Robertson and King, 1974). This decrease in plasma progestin concentration has been attributed to partial CL regression. However, we propose, based on the type of evidence previously presented, that progesterone is, in fact, rapidly metabolized by the pregnant pig uterus and, as a consequence, plasma progestin levels decrease.

We (T. H. Wise, B. D. Dueben, Fuller W. Bazer and M. J. Fields, unpublished data) have studied metabolism of 3H-progesterone by endometrium from pregnant gilts in vitro. Endometrium was obtained from gilts on days 18 and 25 of pregnancy and incubated in Minimum Essential Medium containing 5.5 μCi 3H-progesterone (30 ng) under an atmosphere of 95%O2:5%CO2 for either 15, 30, 60, 120 or 180 minutes. The incubation was stopped with methanol and frozen (−20°C) until analyzed. The samples were homogenized with a polytron homogenizer/disintegrator and vigorously extracted three times with diethyl ether after a methanol:hexane (1:10 v/v) wash. Then, 14C-labeled androstenedione and testosterone were added to allow assessment of procedural losses and to serve as chromatographic markers. The diethyl ether-soluble material was taken to serve as chromatographic markers. The material under peaks I, II and III was subjected to the Brown reaction (Brown, 1955) to give methyl esters of estrone and estradiol that are soluble in 1 N NaOH in 3M NaCl (pH ≈ 23.0). This solution was then extracted twice with diethyl ether, the ether soluble material was taken to dryness and redissolved in benzene:methanol (85:15, v/v) and subjected to Sephadex LH-20 column chromatography. Again, tritium labeled compounds cochromatographed with 14C-labeled estrone and estradiol. The material in these peaks was recrystallized to constant specific activity and shown to be estrone and estradiol.

In addition to the 3H-labeled steroids that could be extracted from the culture medium with diethyl ether, considerable radioactivity remained in the aqueous phase. Percentage radioactivity (cpm added to medium vs cpm remaining in the aqueous phase) increased from 15 (8%), to 30 (16%) to 60 (18%), to 120 (24%) to 180 (45%) minutes. Because sulfotransferase enzymatic activity has been demonstrated in pig uterine endometrium (Pack and Brooks, 1974) snail (Helix pomatia) enzyme containing glucuronidase and sulfatase activity was added to the water soluble material to give a total of five enzyme units/milliliter culture medium and incubated for 3 hr (Fishman et al., 1948).

The medium was then extracted with diethyl ether. The ether soluble material was taken to dryness, the residue dissolved in benzene:methanol (85:15) and subjected to Sephadex LH-20 column chromatography using the same solvent system. Three tritium peaks resulted. Peaks II and III cochromatographed with 14C-labeled estrone and estradiol, respectively. The material under peaks II and III was subjected to the Brown reaction (Brown, 1955) and peak I was eliminated which indicated that the material in peak I is not a phenolic steroid. The material in peaks II and III was soluble in the alkaline; aqueous phase after the Brown reaction. The
compounds were extracted and rechromatographed, using benzene:methanol (85:15 v/v). Again, tritium peaks co-chromatographed with $^{14}$C-labeled estrone and estradiol and the $^3$H-labeled material under the respective peaks was shown to be estrone and estradiol by recrystallization to constant specific activity.

Collectively, these data indicate that the uterine endometrium of pregnant gilts can metabolize $^3$H-progesterone to free steroids, e.g., androstenedione, testosterone, estrone and estradiol and conjugated estrogens, e.g., estrone-sulfate and estradiol-sulfate. Also Henricks and Tindall (1971) reported that uterine endometrium from nonpregnant gilts can convert $^3$H-progesterone to 5α-pregnane-3,20 dione, 3β-hydroxy-5α-pregn-4-ene-3,20-dione and pregn-4-en-3,20-dione. They found that about 36% of their radioactivity remained in the aqueous phase after ether extraction following a 2-hr incubation.

The conjugated steroids, especially estrone and estradiol sulfates, are of special interest for several reasons. First, the percentage conversion of androstenedione to estrone (12.7%) and estradiol (1.4%) by pig blastocysts is relatively low compared with the conversion of estrone-sulfate to estrone (36%) and estradiol (12%) according to Perry et al. (1976). In an earlier study, Perry et al. (1973) found that day 14 pig blastocysts converted androstenedione to estrone (13.6%) and estradiol (2.4%) less efficiently than they converted estrone-sulfate to estrone (82%) and estradiol (16%). Due to the very active sulfatase activity of the trophoblast of pig blastocysts, estrogen sulfates can be readily converted to free estrogens.

Conjugated estrogens were the major product of endometrial metabolism of $^3$H-progesterone and this observation is consistent with the fact that the pig trophoblast has high sulfatase activity and can readily convert conjugated estrogens to free estrogens. Pig endometrium is known to have 5α-reductase enzymatic activity which allows for the reduction of progestins and androgens (Henricks and Tindall, 1971). These reduced steroids cannot be aromatized to estrogens (Wilson, 1972). Therefore, free androgens may have little significance, in vivo, as compared to conjugated estrogens, as precursors for free estrogen production by the trophoblast. If so, these data indicate that the endometrium is the primary source of estrogen in the pregnant pig and that the trophoblast only converts conjugated estrogens to free estrogens so that the free estrogens may exert their local effect at the site of placentation.

Based on the data of Perry et al. (1976) and data from our laboratory, a theoretical model indicating interactions between the CL, uterine endometrium and trophoblast (chorion), that lead to free estrogen production by the trophoblast, is present in figure 1.

CONTROL OF STEROIDOGENESIS BY PORCINE TROPHOBLAST

Maternal Recognition of Pregnancy

The mechanisms associated with the so-called "maternal recognition of pregnancy" phenomenon in swine remain unclear (Perry...
et al., 1976). However, we have developed a theory relative to the mechanism whereby estrogen, produced by the trophoblast (chorion) prevents luteal regression in swine (Bazer and Thatcher, 1977). In discussing this theory the assumptions made were: (1) that prostaglandin F₂α is the uterine luteolysin in swine; (2) that prostaglandin F₂α can be secreted either toward the uterine lumen or toward the endometrial stroma and vasculature of the uterus and (3) that the direction of secretion of prostaglandin F₂α is determined by the local concentration of estrogen established by the trophoblast (chorion) within the uterine lumen of the pregnant pig.

Prostaglandin F₂α has been shown to be luteolytic in pregnant (Diehl and Day, 1974) and nonpregnant (Moeljono et al., 1976) gilts. Studies of immunoreactive prostaglandin F (PGF) concentrations in the utero-ovarian vein plasma of nonpregnant gilts indicated that increased levels of PGF were temporally related to CL regression and rapidly declining plasma progesterone concentrations (Gleeson et al., 1974; Moeljono et al., 1977; Frank et al., 1977). However, this relationship was not observed in pregnant gilts (Moeljono et al., 1977) or nonpregnant gilts treated with 5 mg estradiol valerate per day on days 11 through 15 after onset of estrus (Frank et al., 1977).

In studies of utero-ovarian vein plasma estrone and estradiol concentrations, a transient rise was detected in one or both of these estrogens between days 13 and 15 in all pregnant, but in none of the nonpregnant gilts (Moeljono et al., 1977). This observation is consistent with evidence that the pig blastocyst begins to produce free estrogen by day 12 of pregnancy (Perry et al., 1976), and that estrone-sulfate is the primary form of estrogen in systemic plasma (Robertson and King, 1974). The administration of exogenous estrogens to nonpregnant gilts or sows also has been found to prolonged CL maintenance (Gardner et al., 1963).

Recent data from our laboratory (Frank et al., 1977) indicate that utero-ovarian vein PGF concentrations and number of PGF peaks (greater than X + 2 SD) are significantly lower, between days 12 and 20 after onset of estrus, in gilts treated with estradiol valerate (5 mg/day) on days 11 through 15 after onset of estrus as compared with controls.

In a related study, Frank et al. (1978) obtained uterine flushings on days 11, 13, 15, 17 and 19 after onset of estrus from gilts during a “control” estrous cycle and then, on the same days after onset of estrus, following estradiol valerate (5 mg/day) treatment on days 11 through 15. Total recoverable protein and total recoverable PGF were significantly greater in uterine flushings from estradiol valerate-treated gilts. Total recoverable PGF per uterine horn during the control vs treatment periods, respectively, was 2.0 vs 1.9 ng, 27.1 vs 18.4 ng, 31.3 vs 2022.6 ng, 210.2 vs 4144.3 ng and 66.2 vs 4616.7 ng on days 11, 13, 15, 17 and 19 after onset of estrus. These data suggest that estrogen did not reduce the synthesis and secretion of PGF, but the direction of movement of PGF was affected, i.e., it accumulated in the uterine lumen. This explanation is consistent with the previous observation that utero-ovarian vein plasma PGF concentrations were reduced in estradiol valerate-treated gilts.

Based on the preceding data, our theory relative to the luteotropic effect of estrogen (Bazer and Thatcher, 1977) can be more clearly stated. The direction of movement of uterine secretions, e.g., PGF can be either toward the uterine lumen (exocrine secretion) or endometrial stroma and associated vasculature (endocrine secretion). During the estrous cycle the endometrium does, by definition, become an endocrine tissue. The uterine luteolytic agent (presumably PGF) is secreted into the uterine venous system and transported, possibly by counter-current exchange in the ovarian pampiniform plexus, to the ovarian artery and eventually to the CL where PGF exerts its luteolytic effect. An explanation for the mechanism of PGF induced luteolysis has been proposed by Henderson and McNatty (1975).

The maintenance of exocrine secretion of PGF in pregnant and estradiol valerate-treated gilts, presumably an estrogen mediated effect, prevents PGF from entering the uterine vasculature and exerting a luteolytic effect. Of comparable significance is the fact that maintenance of exocrine secretion is essential for the accumulation of histotroph in the uterine lumen for nourishment of the developing conceptus.

Expansion of the Chorio-Allantoic Membranes Leading to Placentation

In animals having epitheliochorial and syndesmochorial placentas, placentation (inappropriately called implantation initially involves the achievement of apposition between the
Allantoic fluid volume has been measured at selected stages of gestation (Wislocki, 1935; McCance and Dickerson, 1957). Knight et al. (1977) systematically characterized allantoic fluid volumes between days 20 and 100 of gestation and observed two peaks in allantoic fluid volume. The first peak occurred on day 30 ($\bar{X} \approx 225$ ml) and the second occurred on day 60 ($\bar{X} \approx 325$ ml) of pregnancy. The volume declined after each peak to a volume of about 65 ml on days 40 and 100 of gestation. Goldstein (1977) confirmed this pattern of change in allantoic fluid volume and indicated that the second peak in allantoic fluid actually occurred on day 58 of pregnancy when average allantoic fluid volume was about 600 milliliters.

Associated with the observed changes in allantoic fluid volume are equally dynamic changes in sodium and potassium concentrations in allantoic fluid. There are significant changes in other electrolytes as well, but their relationship to changes in allantoic fluid volume are more difficult to assess (Goldstein, 1977). As shown in figure 2 changes in sodium and potassium concentrations appear to be interrelated with changes in fluid volume. During periods when the rate of allantoic fluid accumulation is greatest, i.e., days 20 to 30 and days 50 to 60, allantoic fluid so-
Sodium concentration is greater than that for potassium. The net transfer of water and sodium across the pig placenta has been demonstrated (Crawford and McCance, 1960).

Crawford and McCance (1960) measured net sodium fluxes on either side of the porcine chorio-allantois and found a net flux from the allantoic side toward the chorionic side, i.e., from a maternal to fetal direction. The net flux of sodium accounted for the measurable short-circuit current (Ussing and Zerahn, 1951), a measure of active electrolyte transport. It is also known that allantoic fluid is hypotonic with respect to maternal plasma (Crawford and McCance, 1960; Goldstein, 1977). Therefore, there is an electrical potential difference across the chorio-allantoic membranes with the fetal (allantoic) side being negative relative to the maternal (chorion) side at all stages of gestation studied (Goldstein, 1977).

Meschia (1955) found that fetal lambs, at 115 days of gestation, produce about 78 g of new tissue each day. About 75% of this new tissue is water; therefore, 60 ml of water are required by a fetus each day at that stage of gestation. If the 15 liters of oxygen utilized by the lamb each day were used for oxidative metabolism of glucose, only 12 g or 20% of the needed water would be produced. Consequently, the transport of water from maternal tissue into the pregnant uterus seems essential. The fact that there is an electrical potential difference (a measure of selective permeability) and a short-circuit current (a measure of active ion transport, usually sodium) across the porcine chorio-allantois indicates that this membrane is similar to other membranes that actively transport water and electrolytes (Diamond and Bassert, 1968).

At this point, we propose a theoretical explanation for the transport of water and electrolytes across the chorio-allantois and the effect of progesterone and estrogen on this phenomenon (Goldstein, 1977). As shown in figure 2 allantoic fluid accumulation occurs when the progesterone:estrogen ratio is high. We propose that between days 10 and 18 of gestation progesterone may promote the synthesis and activation of various transport enzymes, e.g., sodium-potassium activated transport ATPase in the chorio-allantois. This enzyme system would initiate the pumping of sodium out of the allantoic fluid beginning on day 20. The allantoic fluid sodium concentration (X ≈ 140 mEq/liter) which is almost identical to that of maternal plasma (X ≈ 152 mEq/liters at day 20 becomes markedly lower on day 30 (X ≈ 10 mEq/liter) as compared with that for maternal plasma on day 30 (153 mEq/liter). Therefore, sodium is pumped out of the allantoic fluid (fetal to maternal direction) faster than the passive diffusion of sodium into the allantoic fluid (maternal to fetal direction) and a steep concentration gradient is achieved and maintained during the first period of allantoic fluid accumulation. At the beginning of this period (day 20 to 30 of pregnancy) progesterone concentration is high relative to that of estrogen and would allow the chorio-allantois to behave as a "leaky" membrane that would enhance flow of the sodium-H₂O-anion complex down the concentration gradient (maternal plasma to allantoic fluid). The sodium-anion complex would be pumped out of the allantois toward the maternal tissue and the concentration gradient would be maintained.

The accumulation of allantoic fluid to a peak would not occur without the influence of estrogen. Estrogens decrease the permeability of the chorio-allantois and decrease the passive flow of the sodium-water-anion complex across the placenta. As seen in figure 3 the accumulation of water in the allantois ceases on day 30 when the progesterone: estrogen ratio becomes very low.

Estrogen levels increased in allantoic fluid until day 30 to 35 and the electrical potential difference across and the resistance of the chorio-allantois to passive ion diffusion are maximum at this time. After day 35, estrogen levels in plasma and allantoic fluid decrease markedly while plasma and allantoic fluid progestin concentrations remain relatively stable. As with the first period of allantoic fluid accumulation, the second period of allantoic fluid accumulation (days 50 to 60) is preceded by the establishment of a high progesterone:estrogen ratio. The permeability or "leakiness" of the placenta again increases and, since the sodium gradient has been maintained (sodium concentration in plasma (X ≈ 151 mEq/liter) is higher than that for allantoic fluid (X ≈ 15 mEq/liter), the sodium-water-anion complex moves rapidly down the concentration gradient and water accumulates in the allantoic sac. Fluid accumulation continues until about day 60 and then ceases as the progesterone:estrogen ratio decreases rapidly.

It is possible that protein hormones, e.g., prolactin and placental lactogen, may modify...
Figure 3. The relationship between allantoic fluid volume (—) and the ratio between plasma progestin concentration and allantoic fluid estrone + estradiol concentration (o—o).

the responsiveness of the porcine chorio-allantois. Goldstein et al. (1976) reported that addition of human placental lactogen to the chorionic side of the porcine and human placenta increased the electrical potential difference and short-circuit activity of placentae from both species in vitro. Although a precise understanding of the mechanism controlling placental transport of water and electrolytes is not available, it is clear that water does accumulate within the allantois. This observation may provide the primary impetus for expansion of the chorio-allantois and the establishment of apposition between the placenta and endometrium which is necessary for subsequent nutrient exchange between the dam and conceptus.

Protein in Uterine Histotroph

According to Bonnet (1882), the concept that uterine milk exists for nourishment of the conceptus was discussed by Aristotle and William Harvey. This so-called uterine milk or histotroph is believed to serve in nourishment of the conceptus. It is not unreasonable, therefore, to think of uterine secretion as a very sophisticated culture medium that is secreted by the endometrium under the influence of the ovarian and (or) placental hormones progesterone and estrogen. It is also possible that lactogenic hormones may influence uterine secretory activity in some species.

In swine, there is a marked increase in the quantity and composition of protein components of uterine secretion during the estrous cycle (Murray et al., 1972; Squire et al., 1972) and early pregnancy (Zavy et al., 1977). These quantitative and qualitative changes are induced and maintained by progesterone (Knight et al., 1973) and there are positive dose-response relationships between quantity of progesterone or progesterone and estrogen administered and amount of uterine protein recovered (Knight et al., 1974).

One of these progesterone-induced proteins has a purple color and has been purified (Chen et al., 1973). Its properties were investigated in detail by Chen et al. (1973) and later by Schlosnagle et al. (1974, 1976). Much of the early research was reviewed by Bazer (1975). An apparently identical purple protein was later isolated from allantoic fluid of pigs (Bazer et al., 1975). The purple pro-
tein from the nonpregnant uterus and allantoic fluid have the following common properties: (1) acid phosphatase activity (pH optimum 4.9) towards the artificial substrate p-nitrophenylphosphate, with a Km of about 2 mM and a Vmax of 25 to 50 μmole Pi released/min/mg protein; (2) reduction of the protein with mercaptoethanol or ascorbate increases the Vmax to greater than 250 μmole Pi/min/mg protein; (3) visible protein from the nonpregnant uterus and surface and glandular epithelium of the uterus; (4) low, but detectable, phosphoprotein phosphatase activity (Roberts and Bazer, 1976); (5) high pi (about 9.7); (6) molecular weight of 32,000 ± 3,000; (7) a glycoprotein and (8) contains 1 atom of Fe³⁺ per polypeptide in both the purple and pink form.

Chen et al. (1975) purified a specific antibody to the purple protein and used immunofluorescent antibody procedures to demonstrate that the purple protein is: (1) synthesized and secreted by the endometrial surface and glandular epithelium of the uterus; (2) absorbed by the placental areolae which are opposite the uterine glands and (3) sequestered in allantoic fluid (Bazer et al., 1975). In allantoic fluid the purple protein loses its iron and acid phosphatase activity and the polypeptide becomes degraded (W. C. Buhi, R. M. Roberts and F. W. Bazer, unpublished results). This process occurs relatively slowly in fluid up to day 60 of pregnancy, but is rapid in the later stages of gestation. The removal of iron probably involves reduction of a disulfide bridge followed by reduction of the now accessible Fe³⁺ to the ferrous state. Ferrous iron does not bind to protein (Schloesnagle et al., 1976), but would be in the proper valence state for absorption by the allantoic epithelium which is, in fact, derived from the hindgut of the embryo.

Purple protein (Bazer et al., 1975) and total iron (Ducsay, 1977) accumulate in allantoic fluid to maximum amounts between days 30 and 60 of gestation and then decline to day 100 supporting our view that the allantoic sac is a reservoir of nutrients. In addition, Palludan et al. (1969) demonstrated the localization of ⁵⁹Fe in uterine endometrial glands and placental areolae after it was administered intravenously to the dam and he suggested that iron was transported to the conceptus by “embryotroph”. Histotroph and embryotroph are synonymous terms.

It has been traditional to administer iron to pregnant sows during the last one-third of gestation in an attempt to ameliorate the problem of iron deficiency anemia in the neonatal piglet. However, based on the fact that the purple protein and iron accumulate primarily between days 30 and 60 of gestation, studies were conducted to evaluate the effect of administering 7.3 mg iron/kg body weight on days 40, 50 and 60 to sows days 90, 100 and 110 of gestation. Piglets from sows receiving iron during midgestation had higher hemoglobin concentrations at birth in the first experiment and at 7 days of age in the second experiment (Ducsay, 1977). In a related study, gilts were treated with iron dextran (7.3 mg/kg body weight) on days 40, 50 and 60 of gestation and hysterec­tomized on day 90 to compare iron content of the fetal livers, fetuses minus their livers and allantoic fluid between conceptuses from treated and control gilts. Total liver iron and allantoic fluid iron concentration were significantly greater in conceptuses from treated gilts (Ducsay, 1977). These data suggest that management schemes to supplement available iron to sows from day 30 of gestation to term may be most effective in ameliorating iron deficiency anemia in neonatal piglets.

The porcine purple intrauterine glycoprotein is an example of a component of the uterine histotroph which serves a transport role, i.e., to transport iron from the maternal uterine endometrium to the allantoic fluid. In the pig, we have also found a protein which binds retinol and there is tentative evidence for a retinoic acid binding protein (K. L. Adams, R. M. Roberts and Fuller W. Bazer, unpublished data). These proteins may also serve a transport role, i.e., to transport these two forms of Vitamin A to the conceptus.

Proteins of pig uterine histotroph also serve as enzymes. We have presented evidence for the presence of lysozyme and the proteolytic enzymes leucine aminopeptidase and cathepsin, B, D and E activities in pig uterine flushings (Roberts et al., 1976). Phosphohexose isomerase activity has also been found in pig uterine flushings and appears to increase in response to estrogen (M. T. Zavy, R. M. Roberts and Fuller W. Bazer, unpublished results). This enzyme catalyzes the conversion of fructose-6-PΟ₃ to glucose-6-PΟ₃.

In general, the protein components of porcine uterine histotroph have been shown to
serve as carrier molecules and as enzymes that may be involved in nutrition of the conceptus (carrier proteins and proteolytic enzymes), in bactericidal action (lysozyme), in placentation (proteolytic enzymes) and in carbohydrate metabolism (phosphohexose isomerase). It is obvious that considerable research is needed before we can fully assess the role of the numerous proteins present in porcine uterine histotroph. However, it now appears clear that uterine histotroph plays its role for much of pregnancy and is not necessarily associated with a single event, e.g. blastocyst formation (Krishnan and Daniel, 1967) during gestation.

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