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Comparison of Glucose, Fructose, Ascorbic Acid and Glucosephosphate Isomerase Enzymatic Activity in Uterine Flushings from Nonpregnant and Pregnant Gilts and Pony Mares[1,2]

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ABSTRACT

In Experiment 1, 40 gilts and 30 pony mares were used to characterize changes in glucose, fructose, ascorbic acid and glucosephosphate isomerase (GPI) enzymatic activity in uterine flushings collected either during the estrous cycle or early pregnancy. Total recoverable glucose was greater ($P<0.01$) in uterine flushings from pregnant gilts, but pregnancy status had no effect on total recoverable glucose in pony mare uterine flushings. Fructose was undetectable in uterine flushings from nonpregnant gilts and pony mares and pregnant gilts and pony mares prior to Day 14, but occurred in increasing amounts between Days 14 and 18 or 20 of pregnancy. In Experiment 2, it was demonstrated that the porcine conceptus is the primary source, if not the sole source of fructose. Total recoverable ascorbic acid in uterine flushings was not affected by pregnancy in gilts, but was greater ($P<0.01$) in pregnant versus nonpregnant pony mares. In both species, total recoverable ascorbic acid was affected ($P<0.01$) by day of the estrous cycle and pregnancy.

The GPI enzyme allows for the interconversion of glucose-6-PO_4 and fructose-6-PO_4. GPI total and specific activities were greater ($P<0.01$) for pregnant than nonpregnant gilts and pony mares. The periods of greatest GPI activity were temporally associated with elevated estrogens of either ovarian or blastocyst origin. Results from Experiment 3 indicated a marked increase ($P<0.01$) in GPI activity in uterine flushings from gilts treated with estradiol valerate.

Results of this study indicate that glucose (gilt only), fructose, ascorbic acid and GPI activity are increased in uterine flushings of gilts and pony mares during early pregnancy. The increase in these constituents may reflect increased carbohydrate metabolism in ways which are uniquely beneficial to conceptus development in ungulates.

INTRODUCTION

Glucose and fructose are present in uterine fluids of several species of animals. Glucose appears to be the major free sugar in uterine flushings from the nonpregnant rat, rabbit, cow, ewe and pig (Bishop, 1956; Lutwak-Mann, 1962; Haynes and Lamming, 1967). However, fructose has also been detected in uterine flushings obtained from pigs during estrus and metestrus (Haynes and Lamming, 1967) and in uterine flushings from pregnant roe deer with developing blastocysts (Aitken, 1976). Fructose has also been identified in the uterus of rabbits (Gregoire and Gibbon, 1965), cows (Suga and Masaki, 1973) and women (Douglas et al., 1970).

Ascorbic acid also was examined because it may be synthesized from glucose (Birney et al., 1976). However, data are not available to indicate how ascorbic acid concentrations in uterine flushings are affected by stage of the estrous cycle or pregnancy.

Glucosephosphate isomerase (EC 5.3.1.9, GPI) enzymatic activity is responsible for interconversion of glucose-6-PO_4 and fructose-6-PO_4. Singhal et al. (1967) reported that

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exogenous estrogen administration resulted in a marked increase in rat endometrial GPI activity. Otherwise, the activity of this enzyme in reproductive tissue has received little attention.

This study was undertaken to characterize changes in glucose, fructose and GPI enzymatic activity in uterine flushings of nonpregnant and pregnant gilts and pony mares. In addition, ascorbic acid was studied since it is a vitamin which can be derived from glucose-6-PO_4 and may also play a nutritional role for developing mammalian embryos.

MATERIALS AND METHODS

Experiment 1

Gilts. Forty sexually mature crossbred gilts of similar age, weight and genetic background were assigned to this study after they had exhibited at least two estrous cycles of normal duration (18 to 22 days). Two gilts were eliminated because of failure to exhibit normal estrous cycles. Day of onset of estrus was defined as Day 0 and gilts were checked for estrus at 12-h intervals with intact boars. Prior to initiation of this study, gilts were assigned randomly to provide uterine flushings on either Day 6, 8, 10, 12, 14, 15, 16 or 18 of the estrous cycle. Gilts were then allowed the subsequent estrous cycle for recovery. During their second post-surgical estrous period, gilts were bred at 12 and at 24 h after detection of estrus. Uterine flushings were collected so that the same gilts were represented within the same respective days of the estrous cycle and pregnancy.

Uterine flushings were obtained as described previously (Bazer et al., 1978) for all days of the estrous cycle and for Days 6, 8 and 10 of pregnancy. Collection of uterine flushings on Days 12, 14, 15, 16 and 18 of pregnancy required that about 0.5 cm of the ovarian end of each uterine horn be removed for collection of uterine flushings containing conceptuses. Any uterine flushing contaminated with blood was discarded, as were uterine flushings from bred gilts not containing embryos. Volume of uterine flushing recovered from each uterine horn was measured. Samples from the two uterine horns were then pooled, centrifuged at 12,000 × *g* for 20 min and the supernatant stored at −20°C until assayed for glucose, fructose, ascorbic acid and GPI enzymatic activity. Pregnancy was confirmed by examining the uterine flushings for the presence of normal blastocysts.

These uterine flushings and plasma samples were also assayed for estrone, estradiol, progesterone, prostaglandin F and protein. Results of these analyses have been published elsewhere (Zavy et al., 1980), and will be used to describe temporal relationships between these steroids and components of uterine flushings described in this paper.

Pony Mares

Thirty cycling pony mares were randomly assigned to six treatment groups. Pony mares were teased daily by a vigorous stallion in order to detect estrus. Ovulations were detected by daily rectal palpation of the ovaries in cases where developing follicles were 25 mm or greater in diameter. Uterine flushings were collected on Days 8, 12, 14, 16, 18 or 20 after ovulation. Mares were allowed one complete estrous cycle as a recovery period and were mated at the next estrus to a fertile stallion. They were then ovariohysterectomized on the same treatment day to which they had been previously assigned to the nonpregnant phase of this study. Using this design, flushings were collected from each mare on equivalent days of the estrous cycle and pregnancy, and as such each animal served as its own control.

Uterine flushings were collected from anesthetized mares by a modification of the nonsurgical, transcervical procedure described by Zavy et al. (1978). Prior to the collection, jugular blood samples were taken. Mares were then tranquilized with Acepromazine,® anesthetized with thiopental sodium (5% solution, i.v.), and maintained with halothane. They were then placed in dorsal recumbency on a padded cart, the rear of which was raised to an angle of 30° with the floor. Sixty milliliters of sterile 0.33 M NaCl were introduced into the uterus via a modified Foley catheter. After mixing the uterine luminal contents for 5 min by repeated back-flushing into a syringe, the flushing was withdrawn and placed in sterile vials, cooled, measured, and frozen until analyzed.

For collection of pregnant uterine flushings, mares were prepared as previously described and then ovariohysterectomized through a midventral incision. The excised uterus was then clamped and chilled on ice for 10 min. A small incision was then made in the isthmal portion of the oviduct near the uterotubal junction through which 60 ml sterile saline were infused via a polyvinyl catheter passing into the uterine lumen. After a mixing period of 5 min, a section of the uterine horn proximal to the uterotubal junction was excised in order to recover the blastocysts with their membranes intact. Uterine flushings were decanted into sterile culture dishes where blastocysts were separated from the flushing. This technique was successful for harvesting Days 8, 12, 14, and 16 blastocysts; however, due to the fragility of the membranes on Days 18 and 20 it was impossible to collect intact embryos at these times, and as such they contributed yolk sac fluid to the uterine flushings. Pure samples of yolk sac fluid were obtained when possible by needle aspiration of the yolk sac cavity. Uterine flushings were processed as previously described for the pregnant pig uterine flushings.

Glucose. Glucose in uterine flushings were measured colorimetrically as described in Sigma Technical Bulletin 510 (1976). This is a quantitative procedure for determining glucose using the enzymes glucose oxidase and peroxidase and is a modification of the procedure reported by Keilin and Hartree (1952) and Keston (1956). Sample size varied between 1.0 and 3.0 ml and was brought to a final volume of 6.0 ml with distilled water. These samples were deproteinized prior to assay with 2.0 ml of 0.3 N BaOH and 2.0 ml of 0.3 N $ZnSO_4$ and were centrifuged at 600 × *g* for 10 min. One-half milliliter of the supernatant was pipetted into new tubes in preparation for assay.

Standard curves were constructed by aliquoting 1.0 ml from glucose stock solutions (0.05, 0.10, 0.30, 0.50, 1.00, and 2.00 mg/ml) into tubes to which 5.0 ml of distilled water were added. Standards were deproteinized and centrifuged along with the sample

unknowns. Standards and samples were assayed by adding 5.0 ml of a glucose oxidase/peroxidase/odianisidine dihydrochloride solution. All tubes were incubated at 37°C for 30 min and the optical density at 510 nm determined on a spectrophotometer.

Fructose. Nonpregnant and pregnant uterine flushings were analyzed for fructose content by the methods of Roe (1934). Sample sizes varied from 8 ml for nonpregnant animals to 1 ml for Day 14–18 pregnant animals. All samples were brought to a final volume of 8.0 ml with distilled water and were deproteinized with 1.0 ml 10% $ZnSO_4$ and 1.0 ml 0.5 N NaOH prior to assay. The assay as described by Roe (1934) is considered to measure only ketohexose sugars, because of the resorcinol step which is performed at pH 1 to 2. When deproteinization is carried out with $ZnSO_4$ and NaOH the assay is relatively specific for fructose; however, if trichloracetic acid (TCA) (6.25%) is used, it appears to measure fructose and fructose-6-PO_4 (Warren R. Clark, unpublished observations). Since the former deproteinization scheme was used, fructose was assumed to be the sugar measured. Following deproteinization, samples were centrifuged and 2.0 ml of supernatant were pipetted from each and placed in new assay tubes. To these tubes 2.0 ml of resorcinol (1 mg/ml) in 95% ethanol were added which was followed by 6.0 ml of HCl (30.0%). All tubes were vortexed, incubated at 80°C for 8 min, cooled with tap water, and read at 490 nm on a spectrophotometer.

With each assay a standard curve was run concomitantly with samples. Standard curves were from standards containing 0, 0.1, 0.3, 0.5, 1.0, 1.5, and 2.0 mg of fructose/ml of stock solution. As with samples, all standards were brought to a final volume of 10.0 ml with distilled water. Tubes containing standards and unknowns were processed in the same manner.

Ascorbic Acid. Ascorbic acid content of uterine flushings was measured according to the procedure described by Maickel (1960). This procedure depends on the reduction of Fe^{+++} by ascorbic acid and estimation of the Fe^{++} as the red-orange, α, α'-dipyridyl complex; other reducing materials in the uterine flushings were inhibited by the presence of orthophosphate and a high degree of acidity, pH 1–2.

For each assay a standard curve was constructed by making up a fresh solution of ascorbic acid in TCA (100 mg ascorbic acid in 100 ml 5% TCA). A standard curve was constructed by adding 5, 10, 20, 40, 80 and 100 μg to conical centrifuge tubes. These samples were then brought to a final assay volume of 1.0 ml by the addition of distilled water. The standard curve tubes were then treated exactly the same as for unknown assay tubes.

Glucosephosphate Isomerase (GPI). The GPI activity was determined by the method detailed in Sigma Technical Bulletin No. 650 (1974). This method is based on the procedure reported by Bodansky and Schwartz (1966) in which the conversion of glucose-6-PO_4 to fructose-6-PO_4 is catalyzed by GPI. The fructose-6-PO_4 formed was determined colorimetrically by means of the Seliwanoff reaction which entails treatment with acidic resorcinol at 80°C. The intensity of the resulting red color, measured at 490 nm on a spectrophotometer, is proportional to GPI activity. This color reaction is specific for ketose sugars and is unaffected by either glucose or glucose-6-phosphate. For each assay, a standard curve was run simultaneously. Data are presented in Bodansky units. One Bodansky unit is defined as the reciprocal of that amount of enzyme that would cause formation of 25 μg fructose-6-PO_4/ml reaction mixture in 30 min at 37°C and pH 7.4.

Statistical Analyses. Data were analyzed by least squares analysis of variance using the Harvey LSML 76 computer program (Harvey, 1977). The mathematical model included effects of day of estrous cycle and pregnancy status (nonpregnant and pregnant) and day by status interactions. To evaluate day trends on a within status basis, a reduced model for least squares regression analysis was used. In this model, variability among days was partitioned into single degrees of freedom by weighted least squares to detect curvilinearity in trends within status and differences in trends when significant day by status interactions were detected.

Gross correlations due to time trends and/or an association between two variables and partial correlations (association between two variables that is independent of time) also were obtained (Harvey, 1977).

When analyzing uterine flushings, data were expressed on a concentration and, in some cases, a total recoverable basis. Total recoverable glucose, for example, was obtained by multiplying glucose concentration by recovery volume of uterine flushings.

Hormone Values. The correlations between estrogens and the various components of uterine flushings are based on data obtained for estrogen and progesterone concentrations in uterine flushings obtained in this study. The results of these hormonal analyses have been published elsewhere for the pig (Zavy et al., 1980) and horse (Zavy, 1979).

Experiment 2

General. The tissue source of fructose found in uterine flushings of pigs in early pregnancy has not been reported. Therefore, this question was examined with pig blastocysts only since equine blastocysts were not available.

In Vitro Culture. Conceptuses were obtained on Day 16 of pregnancy by flushing each uterine horn with 20 ml sterile saline (0.9%). Immediately after blastocysts were recovered in a sterile culture dish, they were transferred into a culture dish containing minimal essential medium (MEM). The conceptuses were then minced to 1 to 2 mm pieces using fine scissors. Endometrial tissue, about 1 gm per uterine horn was dissected from adjacent tissue from the uterus of the same gilts from which conceptuses were obtained and minced in MEM to 1 to 2 mm cubes.

Culture flasks containing 5 ml each of modified MEM (Basha et al., 1980) were set up and received either conceptus tissue alone, endometrial tissue alone or endometrial and conceptus tissues. Approximately 200 mg of each tissue was added to each flask. The conceptus-endometrial incubations contained a total of 400 mg tissues. The incubations were carried out at 37°C under an atmosphere of 5% CO_2:95% O_2 in a Dubnoff Metabolic Shaker. Tissue cultures were terminated after either 0, 2, 4, 12 or 24 h by pouring the contents of the culture flask into a 15-ml plastic storage tube and freezing in liquid nitrogen. Samples were then stored at −20°C until analyzed.

In preparation for analyses, samples were thawed and then centrifuged at 2000 X *g* for 20 min to remove tissue. The supernatant was assayed for glucose, fructose and GPI enzymatic activity, as previously described, and protein concentration was measured by the method of Lowry et al. (1951).

Statistical Analysis. Analysis of variance was performed on data to account for effects of tissue, time of incubation and tissue X time of incubation interaction (Harvey, 1977).

Experiment 3

General. This experiment was to determine the influence of exogenous estrogens on GPI enzymatic activity in uterine flushings obtained on Days 11, 13, 15, 17 and 19 of the estrous cycle and Days 11, 13, 15, 17 and 19 of pseudopregnancy in pigs. Gilts may be rendered pseudopregnant by injection of 5 mg estradiol valerate on Days 11 through 15 of the estrous cycle. The pseudopregnant gilts have prolonged corpus luteum maintenance (Frank et al., 1978) and they maintain uterine secretory function (Basha et al., 1980) for about 120 days.

Treatment. A total of 15 sexually mature crossbred gilts of similar age, weight and genetic background were randomly assigned to provide uterine flushings (Bazer et al., 1978) on either Day 11, 13, 15, 17 or 19 of the estrous cycle, i.e., three gilts/day. The gilts were then allowed one estrous cycle for recovery. On Days 11 through 15 after onset of the second post-surgical estrous cycle, they received 5 mg estradiol valerate to induce pseudopregnancy. Uterine flushings were obtained on Days 11, 13, 15, 17 and 19 of pseudopregnancy with the same gilts represented within each of the respective days of the estrous cycle and pseudopregnancy.

Statistical Analysis. The data were analyzed for effects of day and status (estrous cycle *vs.* pseudopregnancy) and day X status interaction by least squares analysis of variance (Harvey, 1977).

RESULTS

Experiment 1

Recovery Volume of Uterine Flushing

Gilt. Total volume of uterine flushings recovered from nonpregnant gilts was greater ($P<0.01$) than for pregnant gilts, but no status x day interaction was detected. Within status, day effects were not detected for pregnant gilts. Recovery volumes increased after Day 15 for pregnant gilts and this was likely due to some contribution of allantoic and/or yolk sac fluid from conceptuses. These data have been described previously (Zavy et al., 1980).

Mare. Recovery volumes of uterine flushings were greater ($P<0.01$) in pregnant as compared with nonpregnant mares. A day by status interaction ($P<0.01$) was also found. Percent of uterine flushing volume recovered for nonpregnant mares averaged 95.3%, which was about 10% lower than values reported for horse mares (Zavy et al., 1978). No significant day differences were found in nonprenant mares; however, a significant ($P<0.03$) day effect was detected in pregnant mares in which the highest recovery volumes were found on Days 18 and 20. The increase in recovery volume on Days 18 and 20 was probably due to embryonic fluid contamination since, in all cases, the blastocyst membranes ruptured on those days and resulted in release of embryonic fluid into the uterine flushing.

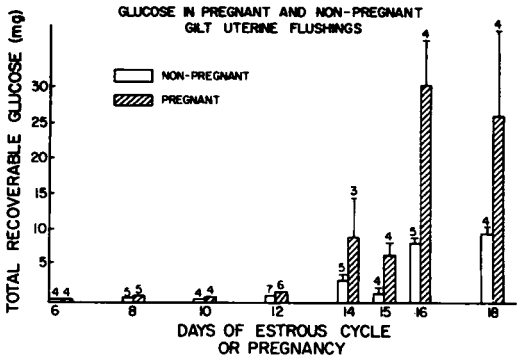

FIG. 1. Comparison of total recoverable glucose in uterine flushings from pregnant and nonpregnant gilts ($\bar{X} \pm$ SEM).

Total Recoverable Glucose

Gilts. Total recoverable glucose was greater ($P<0.01$) in uterine flushings from pregnant compared with nonpregnant gilts and a day by status interaction ($P<0.01$) was detected. Within each status, day trends ($P<0.01$) were detected (Fig. 1) and were similar for both nonpregnant and pregnant gilts. Most notable was a marked increase in glucose between Days 12 and 16 for both groups; however, the magnitude of increase was about threefold greater for pregnant gilts.

In nonpregnant gilts, total recoverable glucose was correlated (gross:residual) with total recoverable ascorbic acid (0.37, $P<0.05$, 0.20, NS) and total recoverable estrone (Zavy et al., 1980) in uterine flushings (0.41, $P<0.05$; 0.003, NS, respectively).

In pregnant gilts, correlations (gross:residual) were detected between total recoverable glucose and total recoverable fructose (0.82, $P<0.01$; 0.55, $P<0.01$, respectively) and total recoverable GPI (0.85, $P<0.01$; 0.55, $P<0.01$)

and total recoverable ascorbic acid (0.48, $P<0.05$; −0.08, NS).

Pony Mares. Total recoverable glucose in uterine flushings of nonpregnant and pregnant mares did not differ and a day by status interaction was not detected. Also within each status, no significant day effects were detected (Fig. 2). Total recoverable glucose in nonpregnant and pregnant mares was consistently around 5 to 6 mg per flushing, with a tendency for higher values during either proestrus or Days 16 and 18 of pregnancy. In pregnant animals, gross correlations were found between total recoverable glucose and uterine fluid estrone (0.481, $P<0.05$, respectively) and uterine fluid estradiol (0.547, $P<0.05$), while a negative residual correlation was found between total recoverable glucose and peripheral plasma estrone.

Total Recoverable Fructose

Gilts. Fructose was undetectable in nonpregnant gilts even when 7.0 ml of uterine flushing were used in the analyses. In pregnant gilts, there was a Day effect ($P<0.01$). Fructose was undetectable until Day 14 and then increased to maximum values on Day 18 of pregnancy (Fig. 3). For pregnant gilts, total recoverable fructose was correlated (gross:residual) with: total GPI (0.96, $P<0.01$; 0.83, $P<0.01$); total recoverable ascorbic acid (0.53, $P<0.05$; −0.29, NS); and total recoverable glucose (0.82, $P<0.01$; 0.54, $P<0.01$).

Pony Mares. Fructose was undetectable in uterine flushings from nonpregnant mares. In pregnant mares, fructose was first detectable on Day 14 and then increased ($P<0.02$) to Day 20 (Fig. 4). Correlations (gross and residual, respectively) were found between total recoverable fructose and uterine fluid estrone (0.58, $P<0.05$; 0.63, $P<0.01$), total ascorbic acid (0.65, $P<0.01$; 0.64, $P<0.01$), and GPI specific activity (0.60, $P<0.01$; 0.54, $P<0.05$).

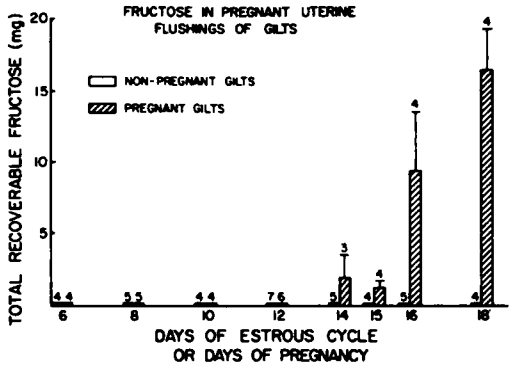

FIG. 3. Comparison of total recoverable fructose in uterine flushings from pregnant and nonpregnant gilts ($\bar{X}$ ± SEM).

Total Recoverable Ascorbic Acid

Gilts. Total recoverable ascorbic acid was not different between nonpregnant and pregnant gilts. However, a day by status interaction ($P<0.01$), as well as day trends ($P<0.01$), for the nonpregnant and pregnant groups,were detected. In both groups, total ascorbic acid values were greatest between Days 12 and 18 of the estrous cycle and pregnancy (Fig. 5).

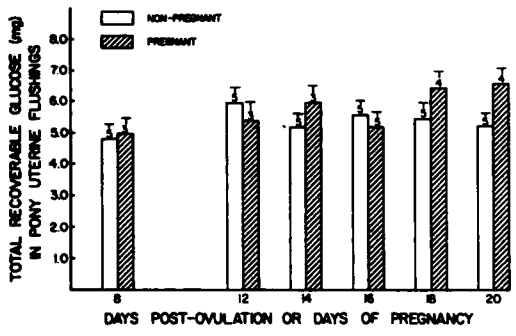

FIG. 2. Comparison of total recoverable glucose in uterine flushings from pregnant and nonpregnant pony mares ($\bar{X}$ ± SEM).

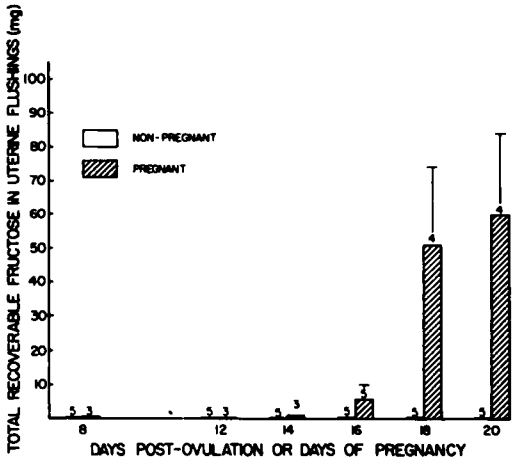

FIG. 4. Comparison of total recoverable fructose in uterine flushings from pregnant and nonpregnant pony mares ($\bar{X}$ ± SEM).

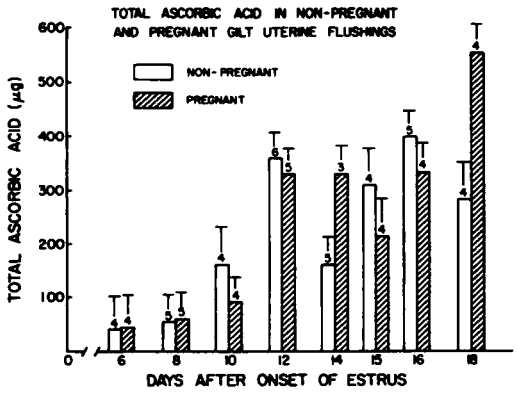

FIG. 5. Comparison of total recoverable ascorbic acid in uterine flushings from pregnant and nonpregnant gilts ($\bar{X}$ ± SEM).

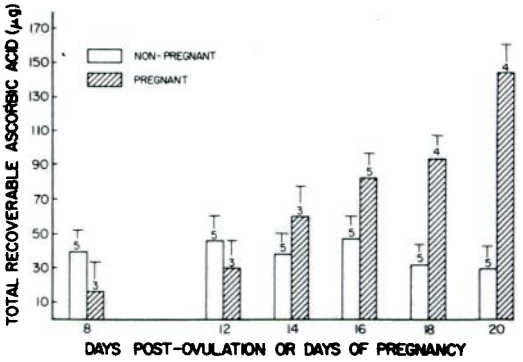

FIG. 6. Comparison of total recoverable ascorbic acid in uterine flushings from pregnant and nonpregnant pony mares ($\bar{X}$ ± SEM).

For nonpregnant gilts, correlations were found between total recoverable glucose and ascorbic acid, as previously noted, and there was a correlation (gross:residual) with plasma estradiol (see Zavy et al., 1980) concentrations (0.38, $P<0.05$; 0.37, $P<0.05$).

In pregnant gilts, correlations between total recoverable glucose, total recoverable fructose, and total recoverable ascorbic acid have been indicated. Total ascorbic acid was also correlated (gross:residual) with total GPI (0.59, $P<0.01$; −0.16, NS) and total recoverable estrone in uterine flushings (0.60, $P<0.01$; 0.44, $P<0.05$).

Pony Mares. Total recoverable ascorbic acid differed ($P<0.01$) between nonpregnant and pregnant mares and a day × status interaction ($P<0.05$) was detected (Fig. 6). Total recoverable ascorbic acid was affected by day of pregnancy ($P<0.01$), but not by day of the estrous cycle. Correlations (gross:residual) were found between total recoverable ascorbic acid and total GPI (0.54, $P<0.01$; 0.52, $P<0.01$) and total protein (0.64, $P<0.01$; 0.52, $P<0.01$) for nonpregnant mares. For pregnant mares, correlations (gross:residual) were found between total ascorbic acid and total GPI (0.69, $P<0.01$; 0.51, $P<0.05$), total fructose (0.65, $P<0.01$; 0.64, $P<0.01$) and GPI specific activity (0.73, $P<0.01$; 0.64, $P<0.01$).

Glucosephosphate Isomerase (GPI)

Gilts. The GPI total (Table 1) and specific activity (Bodansky units/mg protein) in uterine flushings were greater ($P<0.01$) for pregnant gilts and a day by status interaction ($P<0.01$) was detected. Within the nonpregnant and pregnant groups, both total GPI and GPI-specific activity were affected by day ($P<0.01$). In nonpregnant gilts, total GPI and GPI-specific activity were highest on Days 6, 8 and 18. An association between GPI activity and estrogens was suggested by gross (0.62, $P<0.01$) and partial (0.52, $P<0.01$) correlations between GPI specific activity and total estrone in uterine flushings. In pregnant gilts, GPI total and specific activities increased markedly between Days 12 and 18. Although the highest GPI total and specific activities were temporally associated with elevated estrogen content of uterine flushings, neither gross nor partial correlations were significant.

Pony Mares. In pony mares there were differences in total GPI and GPI-specific activity due to status ($P<0.01$), day × status ($P<0.01$) and day trends within each status ($P<0.01$) as illustrated for total GPI in Table 1. For nonpregnant pony mares, GPI total and specific activities were highest on Days 8 and 12 of the estrous cycle. The GPI activities for nonpregnant mares and gilts followed a similar trend, but values were lower for gilts late in the estrous cycle.

GPI activities were similar for nonpregnant and pregnant pony mares prior to Day 18, when GPI activity increased markedly in pregnant mares. Gross correlations were found between total GPI and uterine fluid estradiol (0.61, $P<0.01$), uterine fluid estrone (0.83, $P<0.01$) and total fructose (0.56, $P<0.05$). A residual correlation between total GPI and uterine fluid estradiol (0.54, $P<0.05$) was also detected.

Experiment 2

This experiment compared utilization of glucose and net production of fructose in MEM in which either conceptus, endometrium or conceptus and endometrial tissues were incubated for either 0, 2, 4, 12 or 24 h. The data are summarized in Table 2. Reductions in glucose concentrations were affected ($P<0.01$) by length of incubation when expressed as μg/mg of tissue or μg/ml of MEM. There was also less ($P<0.05$) glucose utilized by endometrium alone. Although glucose (μg/mg tissue) utilization tended to be greater for conceptus than endometrium, the coincubation of conceptus with endometrium resulted in the greatest amount of glucose utilization. This was assumed to be due to the fact that the incubation medium contained twice as much tissue, but the same initial amount of glucose, as for the other two groups.

Fructose concentration in MEM containing endometrium also was not affected by time of incubation, which suggests that endometrium alone either produces no fructose or the production rate was below detectable limits of the assay used. However, MEM containing either conceptus alone or conceptus and endometrium had greater ($P<0.01$) fructose concentrations (μg/ml of MEM and μg/mg tissue) and was affected by length of incubation (μg Fructose/ml of MEM) at 4 ($P<0.05$) and at 12 and 24 h ($P<0.01$).

Fructose (72 ± 5 to 72 ± 11 μg/ml) was present in MEM only, but was unaffected by time of incubation. This fructose may represent some contamination of the glucose source. The slightly higher fructose concentrations in endometrial incubation medium compared with MEM alone may reflect leaching of fructose from the tissue or the activity of conceptus tissue that adhered to the endometrium.

TABLE 1. Glucosephosphate isomerase (GPI) activity in uterine flushings from nonpregnant and pregnant gilts and pony mares.

	Nonpregnant			Pregnant		
Day[a]	N[b]	Total[c]	Specific[d]	N	Total	Specific
			Gilts			
6	4	26[ef]	1.3[ef]	4	22[ef]	2.6[ef]
8	5	20	0.9	5	31	0.9
10	4	6	0.2	4	31	0.7
12	6	0	0.0	5	16	0.5
14	5	3	0.1	3	74	0.5
15	4	5	0.3	4	118	1.0
16	5	8	0.3	4	590	3.8
18	4	22	1.0	4	815	4.4
			Pony Mares			
8	5	73[ef]	6.0[ef]	3	125[ef]	4.8[ef]
12	5	119	6.2	3	119	7.0
14	5	38	1.8	3	71	6.0
16	5	64	2.7	5	38	2.5
18	5	19	0.9	4	269	10.9
20	5	20	2.0	4	579	22.9

[a]Days after onset of estrus or day of pregnancy for gilts and days post-ovulation or day of pregnancy for pony mares.

[b]N=number of observations.

[c]Total GPI/uterine flushing in Bodansky units.

[d]GPI activity/mg protein in uterine flushings expressed as Bodansky units.

[e]GPI total and specific activities differed ($P<0.01$) between nonpregnant and pregnant gilts and pony mares; within each species day effects within status ($P<0.01$) and day by status interactions ($P<0.01$) were detected.

[f]The overall standard errors for total and specific GPI activities were 42 and 0.5 and 52 and 2.0, respectively, for gilts and pony mares.

Tissue weights (mg, $\bar{X}$ ± SEM) and GPI activity/mg protein ($\bar{X}$ ± SEM) in MEM are summarized by tissue and time of incubation (Table 3). The effect of treatment ($P<0.01$) on tissue weight per incubation was by experimental design, i.e., endometrium (approximately 200 mg) plus conceptus (approximately 200 mg) tissues were present in the coincubates. The GPI-specific activity was less ($P<0.01$) for endometrial tissue, but was unaffected by time of incubation.

Experiment 3

In Experiment 1, GPI total and specific activities in uterine flushings were greatest in early diestrus (Days 6 and 8) and proestrus (Day 18) of nonpregnant gilts, and between Days 12 and 18 of pregnancy when estrone concentrations in uterine flushings were increasing. Data from this experiment (Table 4) indicate that GPI-specific activity was increased ($P<0.01$) in uterine flushings from gilts treated with estradiol valerate (5 mg/day) from Day 11 to Day 15.

DISCUSSION

The accumulation of glucose in uterine flushings of pigs was associated with the proestrous period of the estrous cycle and the period of rapid blastocyst development (Anderson, 1978) between Days 12 and 18 of pregnancy. Total recoverable glucose in uterine flushings was greater for pregnant than nonpregnant gilts between Days 14 and 18 when total recoverable estrogens in uterine flushings were increasing (Zavy et al., 1980). These estrogens are of blastocyst origin (Gadsby et al., 1980) and may act to increase glucose transport into the uterine lumen of pigs as has been reported for rats (Roskoski and Steiner, 1967).

TABLE 2. Glucose (GL) utilization and fructose (FR) production during in vitro incubation of conceptus (C), endometrium (ENDO) or conceptus and endometrium tissues from Day 16 pregnant gilts ($\bar{X}$ ± SD).

Item	Incubation period (h)[ab]				
	0	2	4	12	24
FR, μg/mg tissue[c]					
C	2.2 ± 0.2	2.3 ± 0.2	2.5 ± 0.3	3.4 ± 0.4**	4.0 ± 0.6**
ENDO**	1.5 ± 0.7	1.8 ± 0.3	1.6 ± 0.6	1.8 ± 0.3	2.0 ± 0.2
C + ENDO	1.1 ± 0.1	1.3 ± 0.1	1.4 ± 0.2*	1.7 ± 0.2**	2.3 ± 0.5**
FR μg/ml[c]					
C	85 ± 8	96 ± 7	102 ± 11*	139 ± 19**	168 ± 22**
ENDO**	74 ± 6	74 ± 10	75 ± 11	75 ± 9	82 ± 9
C + ENDO**	90 ± 6	100 ± 8	114 ± 9*	142 ± 11**	188 ± 42**
GL, μg/mg tissue[c]					
C	83 ± 3	76 ± 6	76 ± 3	62 ± 9**	42 ± 5**
ENDO	86 ± 4	81 ± 6	79 ± 4	74 ± 4**	64 ± 3**
C + ENDO	41 ± 3	39 ± 6	36 ± 3	28 ± 2**	18 ± 3**
GL, μg/ml[c]					
C	3292 ± 71	3166 ± 144	3086 ± 170*	2510 ± 19**	1780 ± 259**
ENDO	3418 ± 133	3286 ± 156	3308 ± 82	3046 ± 60**	2594 ± 189**
C + ENDO	3308 ± 93	3082 ± 212	2904 ± 146**	2272 ± 197**	1474 ± 282**
Medium, μg/ml					
FR	72 ± 5			72 ± 11	72 ± 5
GL	3693 ± 70			3648 ± 68	3696 ± 76

[a]Each mean represents 5 incubations.

[b]Effect of incubation time was different (*$P<0.05$, **$P<0.01$) from that of 0 hour.

[c]Significantly (*$P<0.05$; **$P<0.01$) different from incubation with conceptus only.

TABLE 3. Tissue weight and glucosephosphate isomerase (GPI) specific activity in medium in which conceptus (C), endometrium (ENDO) or endometrium and conceptus tissues from Day 16 of pregnancy had been incubated in vitro ($\bar{X}$ ± SD).

Item	Incubation period (h)				
	0	2	4	12	24
Tissue weight (mg)[a]					
C	198 ± 4	210 ± 9	203 ± 4	203 ± 12	211 ± 6
ENDO	198 ± 8	203 ± 10	208 ± 7	206 ± 8	203 ± 10
C + ENDO	399 ± 38	417 ± 5	413 ± 3	398 ± 11	401 ± 11
GPI/mg protein					
C	431 ± 97	429 ± 78	451 ± 89	425 ± 86	335 ± 92
ENDO[b]	168 ± 95	217 ± 50	216 ± 46	226 ± 13	205 ± 35
C + ENDO	348 ± 86	459 ± 69	338 ± 85	317 ± 52	389 ± 110

[a]Total tissue weight was greater (P<0.01) due to experimental design, i.e., 200 mg blastocyst tissue plus 200 mg endometrial tissue were coincubated.

[b]GPI/mg protein was less (P<0.01) than in blastocyst culture medium.

Also, in this study, there were significant gross correlations between total recoverable glucose and total recoverable estrone (0.48, P<0.05) and estradiol (0.55, P<0.05).

Glucose can, of course, serve directly as an energy source. However, in ungulates, such as the pig and horse, much of the glucose is converted to fructose within the conceptus (Alexander et al., 1966). As noted in Experiment 1, total recoverable glucose and total recoverable fructose are highly (P<0.01) correlated (gross=0.82, residual=0.55) in uterine flushings from gilts. This relationship was not detected in mare uterine flushings. In both the gilt and pony mare, fructose was detectable only in uterine flushings from pregnant females after Day 12 of gestation. These data plus that from Experiment 2 indicate that the blastocyst, and not the endometrium, is the primary source of fructose production, but fructose production probably depends upon glucose transport from the maternal system. Fructose in uterine flushings of the roe deer also increases markedly during the period of blastocyst elongation and implantation (Aitkin, 1976).

The potential role(s) for fructose has long been a subject for speculation. Huggett et al. (1961) concluded that fructose was important to the sheep conceptus because it could not be transported back to the maternal circulation after having been produced by the placenta. Mann (1946) concurred that fructose may be an important substitute for glycogen and/or glucose in the pregnant uterus and seminal vesicles of the male because it is a sequesterable hexose not readily utilized by somatic cells. Because fructose can be sequestered in the conceptus (1 to 4 mg/ml fetal plasma) and can

TABLE 4. Glucosephosphate isomerase activity (Bodansky units/mg protein) in uterine flushings from control and estradiol valerate-treated gilts ($\bar{X}$ ± SEM).[a]

Treatment	N	Day after onset of estrus				
		11	13	15	17	19
Control	3	0.6 ± 0.4	0.9 ± 0.9	0	6.8 ± 4.5	8.6 ± 8.6
Estradiol valerate	3	3.0 ± 1.5	2.4 ± 0.3	185.0 ± 56.8	201.2 ± 79.1	255.1 ± 66.4

[a]Effects of treatment and treatment X day were significant (P<0.01).

be converted intracellularly from fructose-6-PO_4 to glucose-6-PO_4, it may serve as a "storage" form of carbohydrate for ungulates that have little or no glycogen stored in their placentae (Goodwin, 1956).

Observations by Reitzer et al. (1979) from studies of HeLa cells in vitro may be especially important in considering the role of fructose in the conceptus. They found that HeLa cells cultured in the presence of fructose derived at least 98% of their energy needs from glutamine and only 4 to 5% of the fructose was metabolized through glycolysis. When glucose was the primary sugar in the culture medium, about 50% of cellular energy was derived from glutamine and about 80% of the glucose was metabolized through glycolysis. Almost all of the fructose, when present, was metabolized through the oxidative arm of the phosphogluconate pathway which generates NADP (H) and ribose sugars essential for biosynthesis in rapidly proliferating tissue. The conceptus may rely extensively on amino acids, particularly glutamine (Ferrell and Ford, 1980) as an energy source and fructose may be utilized via the phosphogluconate pathway to provide reducing equivalents for biosynthesis.

Data are not available concerning the metabolic fate of fructose in conceptuses of ungulates; however, White et al. (1978) reported incorporation rates of fructose into fetal pig skeletal muscle DNA and RNA to be about 4 and 3 times greater, respectively, than for glucose. For fetal liver DNA and RNA, there was little difference in incorporation rates for glucose and fructose. It is possible that the preferential utilization of fructose over glucose may reflect differences in the demands of different tissues for nucleic acid precursors and reducing equivalents, e.g., NADPH.

The precise role of ascorbic acid in uterine secretions is not known. In general, however, ascorbic acid is required for: *1)* collagen formation due to its role in hydroxylation of proline and lysine; *2)* synthesis of norepinephrine from dopamine and conversion of tryptophan to 5-hydroxytryptophan in the synthesis of serotonin; *3)* iron absorption and the Fe^{+++} to Fe^{++} transition (Buhi, 1980); and *4)* its antioxidant role and related sparing effect on B-complex vitamins and vitamins A and E.

Glucosephosphate isomerase is an enzyme presumed to allow the intracellular interconversion of glucose-6-PO_4 and fructose 6-PO_4. Data from the present study support that of Singhal et al. (1967) who found that estrogen stimulated endometrial GPI activity. This enzyme has also been found in granulosa and theca cells following initiation of follicle growth (Gearhart and Oster-Granite, 1980). The significance of markedly elevated GPI activity in uterine flushings of pregnant pigs and horses after Day 14 and 16 of pregnancy, respectively, is not clear. There was a temporal relationship between the increase in this enzyme and total recoverable fructose in uterine flushings of both the gilt and mare. However, GPI does not utilize glucose or fructose until they have been phosphorylated upon entering the cell. It is possible that GPI is associated with the epithelial membranes and is released into the uterine lumen in response to destabilization of the membranes involved in secretory activity. This appears to be the case for leucine aminopeptidase enzymatic activity in pig uterine secretions (Mancarella et al., 1981).

In general, establishment of pregnancy in the pig and horse results in increased glucose, fructose, ascorbic acid and GPI enzymatic activity in the uterine lumen. These components of the uterine environment serve to nourish the developing blastocyst and the quantity of each of these compounds appears to be regulated by chemical signals from the blastocysts. Pig embryonic development from the 4-cell to blastocyst stage can take place in a bicarbonate-buffered salt solution such as BMOC-2, supplemented with either glucose and bovine serum albumin (BSA) or BSA alone (see review by Brackett, 1981). These data indicate that an energy source, e.g., glucose, can be derived from amino acids derived from BSA by gluconeogenesis. Production of glucose by gluconeogenesis would involve GPI. Estrogens of blastocyst origin may be especially important embryonic signals in the pig (Zavy et al., 1980) and mare (Zavy et al., 1979) with respect to carbohydrate transport and metabolism in the pregnant uterus.

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