# Effect of pre- and postmating nutritional manipulation on plasma progesterone, blastocyst development, and the oviductal environmentduring early pregnancy in gilts

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# Effect of pre- and postmating nutritional manipulation on plasma progesterone, blastocyst development, and the oviductal environment during early pregnancy in gilts[1,2]

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**ABSTRACT:** Two experiments were conducted to determine mechanisms mediating effects of nutritional manipulation before and after mating on embryonic survival in pigs. Experiment 1 studied the mechanisms by which continued high feeding levels after mating result in differences in plasma progesterone during early pregnancy. Gilts fed 2.0 times maintenance energy requirements either remained on this high level or feed was reduced to 1.5 times maintenance immediately after mating. Ovarian, oviductal, and jugular vein progesterone concentrations were determined 72 h after onset of estrus, and samples taken every 4 h were used to determine LH and progesterone during the periestrous period. Treatment did not affect peripheral progesterone concentrations, the timing or rate of rise of progesterone, or progesterone in ovarian, oviductal, or jugular veins at the time of surgery. Time after the LH peak was highly correlated ($P = 0.0001$) with jugular progesterone concentrations, but not with those in oviductal and ovarian veins, suggesting that responses in the reproductive tract mediated by peripheral progesterone concentrations will be temporally different to effects within tissues supplied by the ovarian and oviductal vasculature. Experiment 2 studied mechanisms mediating nutritional manipulation in the preovulatory period on postovulatory reproductive function, using feed restriction during the first (RH) or second (HR) week of the estrous cycle. Surgeries were performed 12 to 20 h after ovulation, and fertilized oocytes were cultured for 144 h in vitro. Ovulation rate was not affected by previous nutritional regimen. Fertilization rate was higher ($P = 0.056$) in RH vs HR gilts, but development of cultured oocytes was not affected by treatment. There were no treatment differences in peripheral or oviductal plasma progesterone, estradiol, or insulin-like growth factor-I (IGF-I) at surgery, or in porcine oviductal secretory protein abundance and IGF-I concentrations in oviduct flushings, but treatment affected total protein concentration ($P = 0.002$). These results indicate that either previous nutritional treatment does not affect the early developmental competence of fertilized oocytes in vitro or differences in developmental competence of oocytes are not expressed up to the early blastocyst stage. However, the lack of an effect of previous nutrition on steroids in the local oviductal circulation may also be related to the lack of effects on oviductal function and embryonic development.

Key Words: Embryos, Gilts, Nutrition, Oviducts, Reproduction

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## Introduction

Nutritional manipulation in the pig is used to study mechanisms involved in embryonic loss during the preimplantation period (Foxcroft, 1997). Experimental paradigms involving manipulation of feed intake before (Almeida et al., 2000) and after (Jindal et al., 1996) mating in gilts influence embryonic survival, independent of effects on ovulation rate. Nutrition may influence embryonic survival by affecting follicular develop-

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ment, and as a consequence, oocyte quality and subsequent luteal function. Changes in follicular steroidogenesis and circulating steroid concentrations (Almeida et al., 2001; Mao et al., 2001) may also affect both the oviductal and uterine environments.

Embryonic loss caused by overfeeding immediately after mating has been associated with lower plasma progesterone concentrations in early pregnancy in the gilt (Pharazyn et al., 1991a; Jindal et al., 1997). Such effects were reversed with exogenous progesterone injections (Ashworth, 1991; Jindal et al., 1997), implicating progesterone as a mediator of nutritional effects on embryonic survival. In turn, reduced progesterone concentrations in early pregnancy may critically affect the uterine (Roberts et al., 1993) and oviductal (Buhi et al., 1997) environments.

To study the mechanisms involved in nutritionally induced embryonic loss, a first experiment used an established gilt model (Jindal et al., 1996) to determine whether the lower progesterone concentrations observed in gilts fed a high feed intake after mating were a result of decreased ovarian production of progesterone or an increase in metabolic clearance of progesterone, as suggested by Prime and Symonds (1993). A second experiment used a gilt model established by Almeida et al. (2000) that manipulates premating feed intake during the estrous cycle to determine whether steroid-associated differences in embryonic survival in this model can be attributed to effects on oocyte quality or changes in the oviductal environment.

## Materials and Methods

### *Animals*

Camborough × Canabrid terminal-line gilts (Pig Improvement [Canada] Ltd., Acme, Alberta, Canada) were housed at the University of Alberta Swine Research Unit, in barns with a totally controlled environment. Gilts were housed in groups of six (3 $m^2$ of space/pig). Gilts had access to a self-locking stall at the front of the pen, which they could enter by means of a hinged gate that locked behind them. After entering, they were fed the appropriate amount of diet. When a gilt finished eating and exited its stall, any feed remaining was weighed back. All animals were cared for in accordance with Canadian Council for Animal Care Guidelines and with approval from the Faculty Animal Policy and Welfare Committee. Based on metabolic body weights (BW $kg^{0.75}$) within 10-kg BW ranges, and a maintenance (**M**) energy allowance of 461 kJ of DE/kg of metabolic BW (NRC, 1988), all gilts were fed a wheat-barley-soybean diet to 2.0 times M energy requirements, which was nutritionally balanced in terms of amino acids, vitamins, and minerals to meet NRC (1988) recommended nutritional requirements. Gilts had ad libitum access to water.

Estrous detection was carried out every 12 h (Exp. 1) and every 6 h (Exp. 2), starting at d 18 of the cycle using backpressure testing during good fenceline contact with a mature vasectomized boar. The onset of estrus (d 0 of pregnancy) was determined as the time of occurrence of a standing reflex in the presence of a boar, minus 6 (Exp. 1) or 3 h (Exp. 2). To minimize any effect of boar within each experiment, gilts were artificially inseminated with fresh, pooled semen from the same three boars and $3 \times 10^9$ morphologically normal sperm per AI dose (Alberta Swine Genetics Corp., Leduc, Alberta). All inseminations were carried out by the same, trained person, at 12 and 24 h after onset of estrus.

### *Experiment 1: Postmating Nutritional Manipulation*

Twenty gilts were fed at 2.0 times M requirements during their first or second estrous cycle. Immediately after the last insemination (d 1 of pregnancy), gilts were matched for weight and randomly assigned within a weight-pair to one of two feeding treatments. Feed intake was either reduced to normal (**N**) NRC recommendations (1988) for gestation (1.5 times M) or continued at 2.0 times M (H) until d 10 of pregnancy.

On d 16 of the estrous cycle, gilts were surgically fitted with indwelling jugular catheters via the superficial cephalic vein under general anesthesia (Cosgrove et al., 1993). From d 18 until d 5 of pregnancy, or until the time of a second surgery, 5-mL blood samples were taken every 4 h to determine timing of the preovulatory peak in plasma LH and the rise in plasma progesterone concentrations. All blood samples were centrifuged at 2,200 × *g*, 4°C and plasma was separated and stored at –30°C until assayed for progesterone and LH concentrations. To determine whether nutritionally dependent differences in progesterone concentrations during early pregnancy in gilts are due to increased metabolic clearance by the liver or to differences in secretion of progesterone by the ovary, heparinized blood samples were taken from the jugular, uterine, and oviduct veins and from mixed arterial and venous blood from the ovarian pedicle 72 h after onset of estrus under general anesthesia. This is a time when progesterone concentrations were previously shown to be different between the two treatment groups (Jindal et al., 1997). Our rationale was that if progesterone concentrations were different in the ovarian drainage, as well as being different in the peripheral circulation between groups, this would imply that nutritional treatment altered progesterone production by the corpora lutea. However, if progesterone concentrations were similar in the ovarian drainage, but different in peripheral plasma, this would imply that metabolic clearance rate of progesterone was affected.

### *Experiment 2: Premating Nutritional Manipulation*

*Animals.* The procedures of selection and pretreatment of gilts were performed as in our previous study (Almeida et al., 2000). Before treatment, all gilts were

fed 2.8 times M during their first estrous cycle. At the start of their second estrous cycle, 19 pairs of littermate gilts were allocated to one of the following two feeding treatments based on either feed restriction (**R**) or high level feeding (**H**) in the first or second week of the estrous cycle: Feed restriction at 2.1 times M from d 1 to 7 of the cycle, and then high-level feeding at 2.8 times M from d 8 to 15 of the cycle (**RH**), or the inverse—high-level feeding at 2.8 times M from d 1 to 7 and feed restriction at 2.1 times M from d 8 to 15 (**HR**). All gilts were fed 2.8 times M from d 16 until onset of estrus. Body weight and backfat thickness measured at the last rib, 6 cm off the midline ($\mathbf{P_2}$) (Renco Lean-Meter, Renco Corp., Minneapolis, MN), were recorded in all animals at d 0 (onset of second estrus), 7, 15, and at the onset of the third estrus.

Of the 38 gilts initially allocated to treatment, two (one HR and one RH) did not show behavioral estrus after treatment, one (HR) was sick during treatment and six (two HR and four RH) had embryos with more than two cells at collection. Data from these animals were excluded from the final analysis, and treatment effects on developmental competence were therefore based on fertilized oocytes recovered from 16 HR and 14 RH gilts.

In addition to the estrous detection and insemination described previously, the time of ovulation was monitored using transcutaneous ultrasonography (Pie Medical Scanner 200, model 41480, Can Medical, Kingston, Ontario), using a 5.0- to 7.5-MHz multiple-angle transducer to scan for the presence of preovulatory follicles, as described previously (Almeida et al., 2001). Blood samples for progesterone determination were taken by acute venipuncture of an ear vein at ovulation, 12 h after ovulation, 48 h after onset of standing heat, and at surgery. The sample taken 48 h after onset of standing heat enabled comparisons with progesterone concentrations in previous studies that used onset of standing heat rather than time of ovulation as the time point from which to compare progesterone concentrations.

*Zygote Collection and Embryo Development.* Surgery was performed 12 to 20 h after ovulation, under general anesthesia, to recover fertilized oocytes. The surgical procedure involved laparotomy and exposure of the uterine horns, oviducts, and ovaries. Ovulation rates were recorded and each oviduct was flushed twice with 5 mL of Dulbecco's PBS (Sigma Chemical Co., St. Louis, MO), previously warmed at 39°C. Flushings were collected in sterile Falcon dishes (Fisher Scientific, St. Louis, MO) and transported to the laboratory in a Styrofoam box containing a tray and flasks filled with warm water to avoid cooling of the recovered oocytes.

Falcon dishes containing 2 mL of NCSU-23 culture media (Peters and Wells, 1993) supplemented with 4 mg/mL of BSA (Sigma Chemical Co., catalogue No. 8022) were prepared and left in incubators to warm and pregas at least 2 h before surgery. Embryos were immediately transferred into the culture dishes and incubated under standard conditions of 39°C and in an atmosphere of 5% $CO_2$ in air. Embryo development was observed daily for 7 d with a dissecting microscope at 16× and 40× magnification. Indications of fertilization (presence of sperm heads on the zona pellucida) and abnormal features (cells dividing unevenly) were observed under an inverted-stage phase-contrast microscope at 400× magnification (Nikon Corp., Tokyo, Japan).

*Collection of Plasma Samples and Oviduct Flushings.* After recovery of fertilized oocytes, the remaining oviductal flushings were transferred into 15-mL sterile Falcon (Fisher Scientific, St. Louis, MO) centrifuge tubes, immediately frozen, and stored at –30°C until further analysis. Time from flushing of the oviduct to freezing of oviduct flushings was approximately 30 min. During surgery, peripheral blood samples were collected by jugular venipuncture, and oviductal blood samples were taken by venipuncture of a vein draining the midsection of the oviductal vasculature. Heparinized blood samples were centrifuged (2,200 × $g$, 4°C), and plasma was separated and stored at –30°C until assayed for progesterone, estradiol, and IGF-I concentrations. The estradiol:progesterone (**E:P**) ratio was calculated as the estradiol concentration in a particular sample divided by the progesterone concentration in the same sample.

### *Radioimmunoassays*

Plasma LH concentrations were determined in duplicate using the homologous double-antibody RIA previously described (Cosgrove et al., 1991). The intra- and interassay CV were 11.5 and 11.9%, respectively. Assay sensitivity, defined as 96% of total binding, was 0.02 ng/mL.

Plasma progesterone concentrations were determined using an established RIA (Coat-a-Count Progesterone, Diagnostic Products Corp., Los Angeles, CA) previously validated for use with porcine plasma without extraction (Mao and Foxcroft, 1998). The sensitivity of the assay for both Exp. 1 and 2 was 0.098 ng/mL. The intra- and interassay CV were 3.1 and 9.8%, respectively, for Exp. 1 and 9.3 and 12.3%, respectively, for Exp. 2.

Estradiol-17$\beta$ concentrations for peripheral and oviductal plasma samples were determined in duplicate in a single RIA using a double-antibody kit from Diagnostics Products Corp. previously modified and validated for use with porcine plasma (Yang et al., 2000b). Recovery of radiolabelled hormone was 83.7 ± 11.7%, and samples were not corrected for recovery. Assay sensitivity was 0.32 pg/mL and the intraassay CV for the single assay run was 7.2%.

The IGF-I concentration in peripheral and oviductal plasma was determined using the homologous double-antibody RIA described previously (Cosgrove et al., 1992). The anti-human IGF-I antiserum (product name AFP4892898, obtained from A. F. Parlow through the NIDDK National Hormone and Pituitary Program) was

used at a 1/654,000 final dilution, resulting in 38% specific binding. The single assay had an intraassay CV of 6.9% and the sensitivity was 0.015 ng/tube. Recovery efficiency was 86.6 ± 2.5%; samples were not corrected for recovery. IGF-I concentration in oviduct flushings was determined using the same assay with modifications to the extraction procedure (Novak et al., 2002). Recovery efficiency was 98.6 ± 4.4%, and samples were not corrected for recovery. The assay sensitivity for the single assay run was 0.00195 ng/tube, and the intraassay CV was 11.5%.

### *Oviduct Flushing Protein Determination and Western Blotting*

Oviduct flushings were thawed on ice and the volume was recorded. They were then centrifuged (2,200 × *g*, 4°C) for 10 min and dialyzed against 10 m*M* Tris buffer (4 L, 4°C) for 24 h with one change. Samples were then assayed for total protein concentration using the assay from Pierce according to manufacturer's instructions. Five micrograms of total protein from experimental samples and from a positive (pooled oviduct fluid collected at estrus) and negative (pooled oviduct fluid collected at d 28 of pregnancy) control were loaded onto 10% (wt/vol) sodium dodecyl sulfate-PAGE gels in duplicate. After electrophoresis, one gel was silver stained to adjust for protein loading, as described subsequently, and the other was transferred onto an electrochemical luminescence-Hybond (Amersham Life Sciences, Buckinghamshire, U.K.) nitrocellulose membrane. Western blotting was performed as previously described (Novak et al., 2002) with polyclonal antibody against porcine oviductal secretory protein (**pOSP**) (a gift from W. C. Buhi, University of Florida), specific for pOSP 1 to 3 in pigs (Buhi et al., 1996). Protein bands for pOSP 1, 2, and 3 were quantified with densitometric techniques (Molecular Analyst V. 2.01, Bio-Rad Labs, Richmond, CA); values were then grouped together and collectively termed pOSP. The pOSP abundance (per microgram of protein) was expressed as a proportion of the positive control sample density and was corrected for protein loading on the corresponding silver-stained gel. The control sample was run with each blot to allow for standardization of blots and comparison across gels. The average densitometric value of duplicate samples was used for statistical analysis.

### *Statistical Analyses*

Normal distribution of data was checked by the Shapiro-Wilk test, and plasma progesterone concentrations were log transformed to achieve normal distribution. Treatment effects on feed intake and number of corpora lutea for Exp. 1 were analyzed by the GLM procedure of SAS (SAS Inst., Inc., Cary, NC), using treatment as the independent variable and the variation across animals as the error term. For evaluation of time and treatment effects on progesterone concentrations from sequential sampling, repeated-measures ANOVA was used. Plasma progesterone concentrations at each sampling site were analyzed using the GLM procedure, with time after ovulation as a covariate, to eliminate effects of time on progesterone concentrations. The statistical model included treatment as the independent variable, progesterone concentrations at each site as dependent variables, time after LH peak as the covariate, and variance across animals as the error term. Because of unequal sample sizes, a protected LSD test was used to compare differences between means. This test was only performed if the statistical model and treatment were both significant ($P < 0.05$). All correlations were analyzed using linear regression analysis. The data are presented as least squares means (±SEM).

For Exp. 2, data were analyzed as a randomized complete block design, with each block consisting of two littermates representing each treatment. Treatment effects on ovulation rate, fertilization rate, BW and backfat changes, embryo development, and progesterone concentrations after ovulation were analyzed using the GLM procedure of SAS. The analysis of BW and backfat changes during treatment included the effects of block and treatment in the model, with BW and backfat at d 0 of the treatment cycle as covariates. For evaluation of treatment effects on embryo developmental competence in vitro, data were arcsine transformed before analysis. For oviduct data, total protein concentration, IGF-I concentrations and pOSP in oviduct flushings, and estradiol and progesterone concentrations in oviductal veins were tested using treatment and block as the main effects, time after ovulation as a covariate, and variation across animals as the error term. Treatment and block were tested on time of surgery with respect to ovulation, ovulation rate, and volume recovered. Peripheral plasma measurements were tested using treatment and block as the independent variables, time after ovulation as a covariate, and variance across animals as an error term. Gilt was considered the experimental unit in all statistical analyses. Pearson correlation coefficients were used to establish relationships between measurements on individual oviducts, irrespective of gilt. In the event that significant treatment effects were established, comparisons between least square means were performed using the probability of differences, adjusted by Tukey-Kramer. All data are reported as least squares means (±SEM).

## Results

### *Experiment 1*

At the start of treatment on d 1 of pregnancy, gilts weighed 130.2 ± 4.36 kg (H) and 137.4 ± 4.36 kg (N). Average feed intakes during treatment were 2.54 ± 0.19 (range 2.2 to 2.8 kg) and 2.02 ± 0.17 kg (range 1.8 to 2.2 kg) daily for H and N, respectively.

There was no difference in the interval between onset of estrus and the preovulatory LH peak between treat-

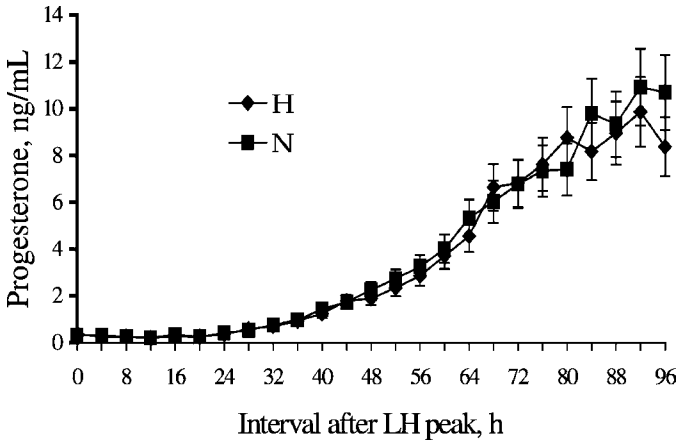


**Figure 1**. Profiles of peripheral progesterone concentrations standardized to time after the pre-ovulatory LH peak. Both groups were fed 2 times energy maintenance requirements (M) during the previous estrous cycle; the H group continued on 2 times M until d 11 of gestation, whereas the N group was reduced to 1.5 times M immediately after mating (24 h after onset of estrus). Least squares means are presented; n = 10 for each group.

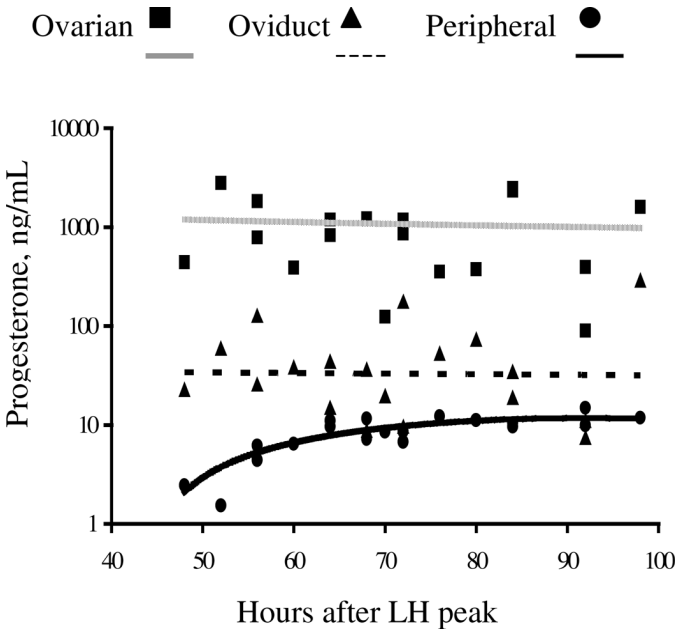


**Figure 2**. Relationships between ovarian, oviductal, and peripheral progesterone concentrations and time after the preovulatory LH peak. Samples were obtained at surgery 72 h after onset of estrus and retrospectively analyzed with respect to time of the preovulatory LH peak determined by RIA. A correlation was observed between peripheral progesterone concentrations and time after LH peak (r = 0.79, $P = 0.0001$), but not between time after LH peak and oviductal or ovarian progesterone concentrations.

ment groups (7.6 ± 4.4 and 10.7 ± 4.7 h for H and N, respectively). However, the H had a lower ($P < 0.03$) ovulation rate (13.6 ± 0.72), compared to the N (16.0 ± 0.72) group.

*Plasma Progesterone Profiles.* The plasma progesterone profiles with respect to LH peak are presented in Figure 1. There was no treatment effect on mean progesterone concentrations at any sampling time or on the timing and rate of rise of progesterone.

*Ovarian, Oviduct, and Jugular Samples at Surgery.* Surgeries were performed at 71.0 ± 4.6 and 71.8 ± 4.9 h after the LH peak for H and N gilts, respectively, and there was no difference in this interval between treatments. There was no treatment effect or time × treatment interaction, on progesterone concentrations obtained from ovarian (1,283.1 ± 263.5 vs 861.9 ± 277.7 ng/mL), oviduct (64.6 ± 23.0 vs 47.2 ± 24.2 ng/mL), and jugular (8.26 ± 0.7 vs 9.11 ± 0.7 ng/mL) venous plasma at surgery for H and N treatments, respectively. However, there was an effect of time (r = 0.79, $P = 0.0001$) on plasma progesterone concentrations in the jugular vein (Figure 2), but not on ovarian and oviductal venous plasma progesterone concentrations, which were already high at 48 h after the LH surge and remained fairly constant during the 50-h period. There was high variation among gilts in both oviductal and ovarian progesterone concentrations.

## *Experiment 2*

*Body Weight and Backfat Changes.* Feed intake, BW and weight change, backfat thickness at $P_2$, and backfat change of gilts are summarized in Table 1.

Both RH and HR gilts had a similar growth rate during the period of d 1 to d 7 and from d 16 until the onset of estrus. The HR group had a lower growth rate ($P = 0.0015$) during the period of feed restriction (d 8 to 15) compared to their RH counterparts. Backfat changes were not different among groups during the experimental period. A litter effect was observed for BW at d 0 ($P = 0.049$) and at estrus onset ($P = 0.02$) and for backfat at d 15 ($P = 0.027$) and at estrus onset ($P = 0.033$).

*Embryo Developmental Competence.* Reproductive characteristics of gilts (Table 2) showed that the ovulation rate and the number of embryos recovered did not differ between treatments ($P > 0.05$). Consistent with our previous study (Almeida et al., 2000), a litter effect was observed for ovulation rate among gilts ($P = 0.039$). Embryo recovery rate was similar for both treatments, but fertilization rate tended to be higher ($P = 0.056$) in oocytes from RH than from HR gilts.

The development of embryos recovered from each treatment group is summarized in Figure 3. There was a gradual decrease in the percentage of embryos reaching successive stages of early development and a marked decrease in the percentage of embryos making the transition from morula to blastocyst. No treatment effects were observed on the development of embryos to the four- to eight-cell and morula stages, nor on the percentage of blastocysts obtained at 96 and at 144 h

**Table 1**. Feed intake, body weight, body weight change, backfat thickness, and backfat change of gilts feed restricted during the first (RH) or second (HR) week of the estrous cycle at d 0, 7, 15, and at estrus (least squares means ± SEM)

| Item | RH | HR | Pooled SEM |
|---|---|---|---|
| Feed intake, kg[x] | | | |
| d 1 to d 7 | 2.4 | 3.0 | 0.06 |
| d 8 to d 15 | 3.6 | 2.7 | 0.05 |
| d 16 to estrus | 2.9 | 3.0 | 0.16 |
| BW, kg | | | |
| d 0 | 134.2 | 130.8 | 2.0 |
| d 7 | 137.0 | 139.0 | 0.9 |
| d 15 | 147.2[a] | 144.4[b] | 0.8 |
| Estrus | 148.8 | 148.0 | 0.7 |
| BW change, kg | | | |
| d 1 to d 7 | 4.5 | 6.3 | 0.9 |
| d 8 to d 15 | 10.2[a] | 5.5[b] | 0.8 |
| d 16 to estrus | 1.6 | 1.4 | 1.3 |
| Backfat, mm | | | |
| d 0 | 11.7 | 10.9 | 0.4 |
| d 7 | 13.0 | 12.5 | 0.3 |
| d 15 | 13.3 | 12.8 | 0.2 |
| Estrus | 13.3 | 13.0 | 0.2 |
| Backfat change, mm | | | |
| d 1 to d 7 | 1.7 | 1.2 | 0.2 |
| d 8 to d 15 | 0.3 | 0.3 | 0.2 |
| d 16 to estrus | –0.01 | 0.15 | 0.3 |

[a,b]Least squares means within rows with different superscripts differ ($P < 0.05$).

[x]Feed offered, kg: d 1 to 7, 2.6 vs 3.5 ± 0.04; d 8 to 15, 3.7 vs 2.7 ± 0.04; d 16 to estrus, 3.8 vs 3.8 ± 0.04, respectively, for RH and HR gilts.

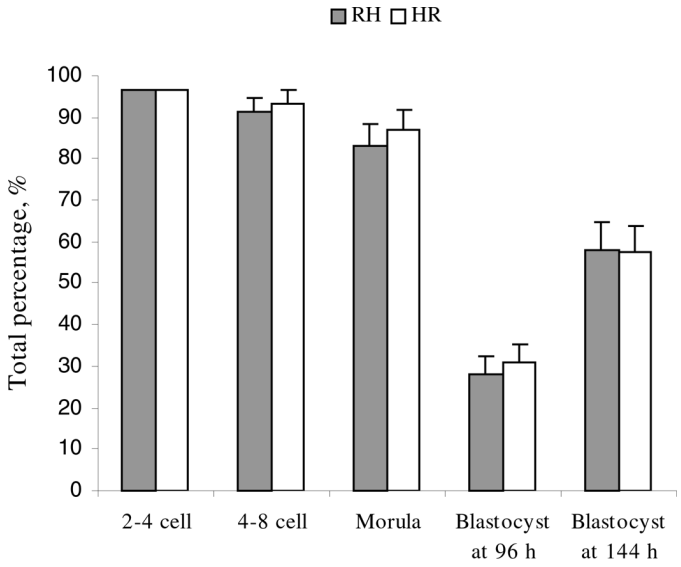


**Figure 3**. Percentage of fertilized oocytes recovered 12 to 20 h after ovulation reaching successive stages of development during culture in vitro for 144 h. RH = feed restriction during the first week of the estrous cycle; HR = restrict fed during the second week of the estrous cycle.

of culture. However, a litter effect ($P = 0.01$) was observed for the percentage of blastocysts at 96 h of culture.

*Peripheral and Oviductal Plasma Progesterone, Estradiol, and IGF-I Concentrations.* Previous nutritional treatments did not affect ($P > 0.05$) progesterone concentrations at 0 (0.7 ± 0.2 and 0.6 ± 0.2 ng/mL for RH and HR, respectively) or 12 h after ovulation (1.4 ± 0.3 and 1.8 ± 0.3 ng/mL for RH and HR, respectively), or at 48h after onset of estrus (1.3 ± 0.3 and 1.0 ± 0.2 ng/mL for RH and HR, respectively).

Peripheral and oviductal plasma progesterone, estradiol and IGF-I concentrations, and the E:P ratio are summarized in Table 3. There were no treatment differences, except for the E:P ratio in peripheral plasma, which was higher ($P = 0.04$) in RH compared to the HR group. Differences were observed in progesterone and estradiol concentrations between oviductal and peripheral plasma, but IGF-I concentrations were not different. There was a littermate effect for IGF-I plasma concentrations ($P = 0.025$) and the E:P ratio ($P = 0.003$).

*Oviduct Flushings.* Samples from a further 10 gilts were excluded from analysis of oviduct flushings because of loss of oviduct fluid samples (four gilts), and unmatched littermates (six gilts). Therefore, only gilts that had littermates remaining in the study, and complete data sets, were included (n = 20).

There was no difference in total flushing volume collected from the oviduct for HR and RH treatment group, but RH gilts had higher ($P = 0.002$) total protein concentrations than their HR counterparts (Table 3).

The IGF-I concentration in oviduct flushings was not affected by treatment (Table 3). However, a relationship was observed between IGF-I concentration in oviduct flushings and the IGF-I concentration in oviductal

**Table 2**. Reproductive characteristics of gilts on treatment (least squares means ± SEM)

| Item | RH | HR |
|---|---|---|
| Number of gilts | 14 | 16 |
| Ovulation rate | 17.1 ± 0.5 | 17.2 ± 0.6 |
| Number of embryos recovered | 14.8 ± 0.6 | 14.4 ± 0.6 |
| Recovery rate, % | 85.0 ± 2.8 | 86.7 ± 3.0 |
| Fertilization rate, % | 99.5 ± 1.4[a] | 95.4 ± 1.3[b] |
| Interval from last insemination to ovulation, h | 14.0 ± 1.5 | 14.7 ± 1.7 |

[a,b]Difference at $P = 0.056$ (analysis based on arcsine transformed data). RH = feed restriction during the first week of the estrous cycle; HR = restricted feeding during the second week of the estrous cycle.

**Table 3**. Least squares means of peripheral and oviductal plasma progesterone, estradiol-17$\beta$, and insulin-like growth factor-I concentrations of RH and HR gilts at surgery

| Item | RH[x] | HR[x] | Pooled SEM |
|---|---|---|---|
| Peripheral plasma | | | |
| Progesterone, ng/mL | 1.97 | 2.43 | 0.21 |
| Estradiol, pg/mL | 0.95 | 0.80 | 0.06 |
| E:P ratio | 1.17[a] | 0.39[b] | 0.45 |
| IGF-I, ng/mL | 114.8 | 114.2 | 3.72 |
| Oviductal plasma | | | |
| Progesterone, ng/mL | 60.9 | 66.9 | 18.8 |
| Estradiol, pg/mL | 8.13 | 9.14 | 2.96 |
| E:P ratio | 0.27 | 0.17 | 0.07 |
| IGF-I, ng/mL | 115.8 | 111.6 | 4.40 |
| Oviductal flushings | | | |
| Volume recovered (mL) | 3.36 | 3.42 | 0.24 |
| Total protein, ng/mL concentrations (pg/mL) | 280.2[a] | 181.7[b] | 19.0 |
| IGF-I, ng/mL | 4.53 | 4.92 | 0.07 |
| pOSP abundance (ng/mL) | 1.12 | 1.07 | 0.06 |

[a,b]Least squares means within row with different superscripts differ ($P < 0.05$). RH = feed restriction during the first week of the estrous cycle; HR = restrict fed during the second week of the estrous cycle; pOSP = porcine oviductal secretory protein; E:P ratio = estradiol:progesterone ratio.

plasma (see Figure 4). There was also a litter effect on IGF-I concentration in oviduct flushings ($P = 0.027$).

The Western blotting technique resulted in immunoreactive protein bands migrating at 88 and 115 kDa (Figure 5A), which correspond to the 85-kDa pOSP 1 and the 100-kDa pOSP 2 and 3 that comigrate as a single 115-kDa band on a one-dimensional sodium dodecyl sulfate-PAGE gel, as described by Buhi et al. (1990). Specificity of the antibody was confirmed by lack of immunoreactive staining in the negative control pool of oviduct flushings obtained from d-28 pregnant gilts (Figure 5B). The pOSP abundance (arbitrary units per microgram of total protein), as measured by densitometry, was not affected by treatment (Table 3), but there was a littermate effect ($P = 0.0001$). The pOSP abun-

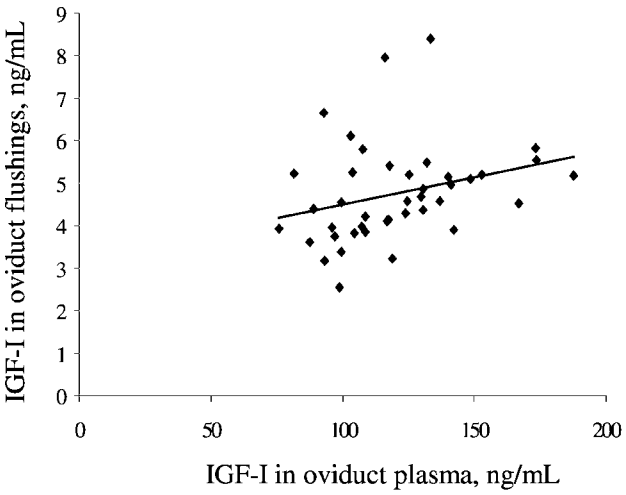


**Figure 4**. Relationship (r = 0.33, $P = 0.03$) between IGF-I in oviduct flushings and oviductal plasma.

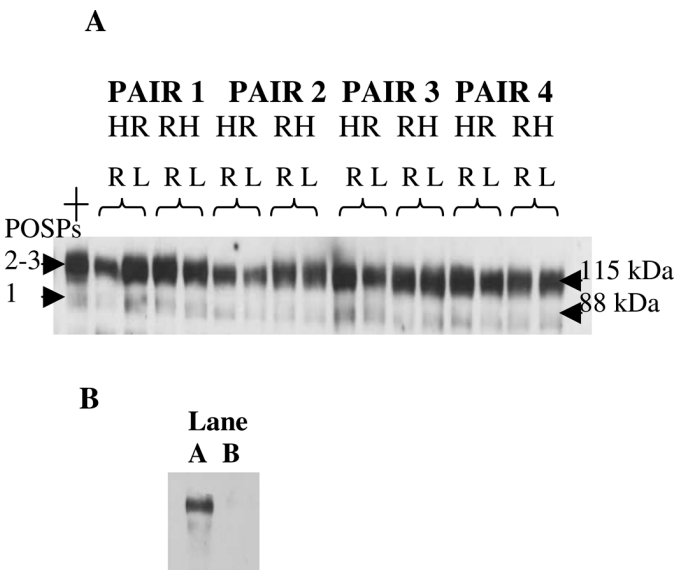


**Figure 5**. A) Respresentative Western blot of pOSP abundance. Immunoreactive bands were detected migrating at approximately 88 and 115 kDa. The 88-kDa band corresponds to porcine oviductal secretory protein (pOSP) 1, and pOSP 2 and 3 are the 115-kDa band as they migrate together (collectively termed pOSP) on one-dimensional sodium dodecyl sulfate-PAGE gels. Oviduct flushings of eight gilts are represented (four littermate pairs), and within animal oviducts are labeled right (R) and left (L). Positive control (+) is indicated. B) Representative Western Blot of positive (lane 1) and negative (lane 2) control pools. The negative control pool is pooled oviduct flushings collected from d-28 pregnant gilts. RH = feed restriction during the first week of the estrous cycle; HR = restrict fed during the second week of the estrous cycle.

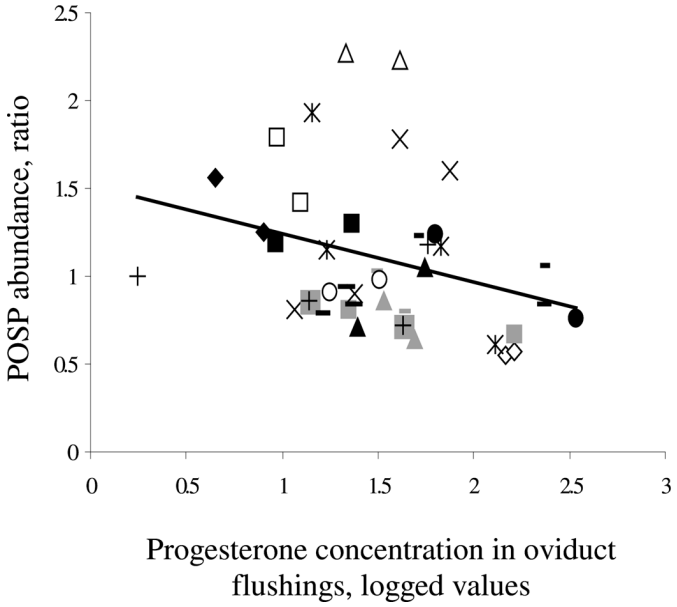


**Figure 6**. Relationship between progesterone in oviductal plasma and porcine oviductal secretory protein (pOSP) abundance in oviduct flushings within each oviduct. The relationship ($r = -0.37$, $P = 0.02$) is negative and independent of gilt. The two oviducts for each gilt are represented by the same symbol, demonstrating that the pOSP abundance in each oviduct is regulated separately by the ipsilateral ovary.

dance was negatively correlated with progesterone concentrations in that oviduct, independent of gilt (Figure 6).

## Discussion

Altering feed intake of gilts after mating in Exp. 1 resulted in no differences in plasma progesterone concentrations or in the timing or rate of rise of progesterone in the first few days of pregnancy. As a result, we were not able to achieve the original goal of determining whether differences in peripheral progesterone concentrations in the first few days of pregnancy were due to changes in metabolic clearance rate or to changes in progesterone secretion by the ovary. These results are not consistent with results from previous studies in our laboratory (Jindal et al., 1996; 1997) using gilts of the same genotype. However, others have also failed to observe changes in progesterone concentrations and/or embryonic survival as a result of nutritional manipulation after mating (Dyck and Strain, 1983; Pharazyn, 1991a; Cassar et al., 1994; Ashworth et al., 1999a).

There is no clear explanation for the lack of consistency between studies. One possibility is that the H gilts in the present study did not experience a change in their feed intake after mating. In the present experiment, we refined the model by Jindal et al. (1996) by feeding gilts at 2 times M before ovulation rather than providing a constant 2.5 kg of feed, irrespective of BW. This resulted in no readjustment of feed intakes for the high-fed group after mating. That may have inadvertently removed a possible effect of increased feed intake after mating on progesterone concentrations that existed in the previous model. In other studies in which gilts were fed a high feed intake before mating and continued on this level after mating, an effect on embryonic survival was also lacking (Ashworth, 1991; Pharazyn et al., 1991a; Ashworth et al., 1999a). Furthermore, no relationship was observed between the timing and rate of rise of plasma progesterone and embryonic survival in sows fed the same nutritional regimen (Soede et al., 1994). Therefore, the absence of an increase in feed intake after mating in these models and in our current study may explain the lack of an effect of these nutritional regimens on peripheral progesterone concentrations in early pregnancy.

In sheep, it has been argued that increases in feed intake after mating result in lower progesterone concentrations during early pregnancy (Parr et al., 1987) due to increases in metabolic clearance rate. In the pig, it is suggested that a high feed intake will also increase progesterone metabolism due to higher portal blood flow (Prime and Symonds, 1993). However, although Ashworth et al. (1999a) found that gilts with high postmating feed intake (3.0 vs 1.25 kg/d) also had higher liver weights, there was no association with either hepatic cytochrome P450 or plasma progesterone concentrations, suggesting that the situation in the pig is much more complex. This does not suggest that lowering feed intake after mating will not have beneficial effects on embryonic survival since there is good evidence to support this in the pig (Den Hartog and van Kempen, 1980; Dyck and Strain, 1983; Jindal et al., 1996); however, it may be too simplistic to assume that peripheral progesterone is the only mediator, of such effects.

We believe the difference in ovulation rate between H and N gilts was not an effect of treatment. All gilts would have been in the same nutritional state before treatment, and treatment was imposed well after the LH surge and around the time of ovulation. The difference between treatments, therefore, seems to be a chance occurrence. Even though this difference existed, progesterone concentrations were not corrected for ovulation rate since there was no relationship between these two measurements.

Although a significant relationship between peripheral progesterone concentrations and time after the LH peak was established, this temporal relationship did not exist for oviductal or ovarian progesterone concentrations. We are therefore able to extend the earlier observations of Pharazyn et al. (1991b) by establishing that the rise in plasma progesterone concentrations in the oviductal circulation occurred earlier than 48 h after the LH peak. After the LH surge, follicles contain high concentrations of progesterone that are reflected in the oviductal circulation even before ovulation (Hunter et al., 1983). Therefore, when nutritional treatments are

imposed after mating, which was about 18 h after the LH surge in these gilts, it is unlikely that the oviduct is involved in mediating associated effects on embryonic loss. In contrast, when nutritional treatments are imposed during the previous estrous cycle, as in Exp. 2, follicular development and luteinization are adversely affected (Almeida et al., 2001; Mao et al., 2001) and steroid-mediated effects on oocyte maturation and oviductal/uterine environments (Novak et al., 2002) may be mechanisms by which changes in metabolic state affect subsequent fertility.

High progesterone concentrations in the oviductal circulation are created by the subovarian countercurrent system (see review by Krzymowski et al., 1990), which transfers high concentrations of steroids in the ovarian vein to the ovarian and oviductal arteries. As a result, the concentration of progesterone in the oviduct veins is 10-fold higher than in peripheral blood (Pharazyn et al., 1991b; Novak et al., 2002). The temporal increase in peripheral progesterone concentrations is likely due to simple dilution of high concentrations of progesterone in the ovarian venous drainage into the peripheral circulation; however, the transfer of progesterone from the ovarian vein to the oviductal vasculature is known to be more complex. There is a large accumulation of steroids in the lymph tissue surrounding the countercurrent exchange system (Kotwica et al., 1981; Krzymowski et al., 1982), which suggests that progesterone concentrations in the oviductal vein may not always immediately reflect progesterone concentrations in the ovarian vein. Another complicating factor is the action of steroids on the vascular bed, regulating blood flow and changing the countercurrent transfer of steroids (Stefanczyk-Krzymowska et al., 1997). These complexities may explain the lack of a close temporal relationship between ovarian and oviductal progesterone concentrations in the present study.

Unlike sheep (Weems et al., 1989), the countercurrent system does not extend to the uterine arteries in the pig (Pharazyn et al., 1991b); therefore, the uterine environment will only be affected by peripheral progesterone concentrations after ovulation and is likely the mediator of embryonic loss when nutritional treatments are imposed after mating. In contrast, if changes in splanchnic clearance of progesterone are indeed responsible for differences in peripheral progesterone in early pregnancy and associated embryonic loss, the oviduct is probably not directly affected by this mechanism.

In Exp. 2, when gilts experienced changes in feed intake before mating, we did not observe the differences in progesterone concentrations previously seen in the same model (Almeida et al., 2000). There is no obvious explanation for the discrepancies between the two studies since they were conducted with strict adherence to the same protocol using the same experimental conditions and facilities. The possibility exists, however, that the model may still have produced differences in embryonic survival rate in absence of differences in progesterone concentrations because the embryos in this study were only cultured in vitro for 144 h. These results are consistent with a subsequent study with the same model (Almeida et al., 2001), where in the absence of differences in absolute progesterone concentrations during the periestrous period, there were also no differences in development to the early blastocyst stage between RH and HR gilts. In contrast, manipulation of lactation feed level in sows did not affect embryonic survival rates in the absence of differences in progesterone concentrations (van den Brand et al., 2000; Yang et al., 2000b). It is evident that nutritional effects on embryonic survival, and their associations with progesterone, are not consistent and that this phenomenon remains controversial.

Although differences in progesterone concentrations were not evident in these studies, the intervals between the onset of estrus to peak estradiol (Blair et al., 1994) and between peak estradiol and the rise in progesterone (Soede et al., 1994) have also been associated with differences in embryonic survival. It has been suggested that differences in periestrous hormone profiles between gilts with high and low embryonic survival could be related to follicular development and oocyte quality (Blair et al., 1994; Jindal et al., 1996; Almeida et al., 2001). This has also been suggested on the basis of data from studies in sows fed different planes of nutrition during lactation (Zak et al., 1997; Yang et al., 2000a).

In Exp. 2, the higher fertilization rate in the RH gilts provided with a high plane of nutrition (2.8 times M) during the late luteal phase of the cycle might be consistent with the concept of higher quality oocytes in this group, although indirect effects of nutritional treatments on oviductal function and sperm maturation may also be a factor. Successful fertilization depends mainly on the time of insemination or mating relative to ovulation (Waberski et al., 1994; Soede et al., 1995). As shown in Table 3, the interval from the last insemination to ovulation in the present study is consistent in both treatment groups, suggesting that previous nutritional regimens affected fertilization rate by some other mechanism. The differences in the oviduct environment created by nutritional treatment may have been a factor in the observed differences in fertilization rate, and if oocytes had remained in the oviduct, they may also have affected early embryonic development.

The absence of treatment effects on developmental competence of early-fertilized oocytes to the blastocyst stage is in agreement with the recent comparable findings of Graham et al. (1999), in which no difference was observed in the percentage of blastocyst formation in gilts at first estrus fed ad libitum and gilts at third estrus fed either ad libitum or restricted diets. Pope (1994) suggested that although the immediate impact of the spread in ovulation time may appear limited, the complex interactions between embryos of different maturity with the uterine environment greatly amplifies developmental differences during the transitional

stage of development on d 11 to 12 and places the lesser developed embryos at risk. Data to support this suggestion was reported by Ashworth et al. (1999a,b), in whose studies higher feeding levels before mating in Meishan gilts resulted in higher embryonic survival. Although there was no effect on blastocyst developmental rate at d 12 of gestation, there was a lower within-litter variation in blastocyst size. It is possible, therefore, that effects of nutritional treatments on the inherent developmental potential of the embryo may not be expressed during the limited period of in vitro culture used in the present study. In contrast, in studies conducted on sheep by McEvoy et al. (1995), in which nutrition was manipulated in the preovulatory period, embryonic development and viability in vitro were compromised in superovulated ewes.

The other goal of Exp. 2 was to determine whether changes in the oviduct environment could be a factor in embryonic loss. Total protein concentration in oviduct flushings was lower in the HR group than in the RH group, which is consistent with our previous study (Novak et al., 2002). However, there were no differences in pOSP abundance or IGF-I concentration in oviduct flushings between the two treatments. Oviduct synthetic ability is greatest during estrus (Buhi et al., 1989), and maximal protein production in the oviduct is reported to be coincidental with highest fluid volume and elevated estrogen concentrations (Wiseman et al., 1992). We suggest that the higher total protein concentrations in the RH group are due to increased oviductal fluid and protein synthesis over the HR group at 12 to 20 h after ovulation. We have previously shown that total protein concentrations in oviduct flushings decrease sharply after ovulation and were associated with the E:P ratio (Novak et al., 2002). A higher E:P ratio was observed in the RH group, possibly reflecting higher estradiol concentrations before ovulation, and could explain the differences in total protein concentration between treatment groups. This is consistent with the higher peak estradiol observed in the RH group as shown by Almeida et al. (2001) in a subsequent study, in which total protein concentrations in the oviduct were also shown to be higher in the RH group (Novak et al., 2002). The HR group was nutritionally restricted during early folliculogenesis and this could have altered follicular growth and, in turn, steroidogenesis (Hunter and Wiesak, 1990), thereby affecting the oviductal environment. Furthermore, steroid-associated regulation of oviduct protein concentrations has been demonstrated in unilaterally ovariectomized gilts (Novak et al., 2002).

We chose pOSP as a marker of the quality of the oviductal environment because it is a protein specific to the oviduct and has been shown to improve embryonic development in vitro (Kouba et al., 2001). In addition, the synthesis and secretion of pOSP has been shown to be estrogen-dependent and is modulated by progesterone (Buhi et al., 1992; 1996). Since there were no differences in peripheral or oviductal estradiol and progesterone concentrations in the present study, we would not expect differences in pOSP abundance. However, there was a negative relationship between pOSP abundance and oviduct plasma progesterone concentrations within each oviduct, which is consistent with pOSP being estrogen-dependent. Also, this relationship was established irrespective of variance among gilts, suggesting that even in intact animals, the oviductal environment is regulated independently by ipsilateral concentrations of ovarian steroids.

Insulin-like growth factor-1 was chosen as the other marker of oviduct quality since it is present in high concentrations during estrus (Wiseman et al., 1992) and enhances human blastocyst development in vitro (Lighten et al., 1998). The IGF-I concentrations in oviduct flushings were not different between RH and HR groups, which suggests either that the treatment does not affect IGF-I concentrations or that the feed restriction was too modest to induce a difference in IGF-I concentrations in flushings. We found, however, a positive relationship between IGF-I concentrations in oviduct plasma and oviduct flushings. Although Wiseman et al. (1992) observed no relationship between IGF-I in plasma and oviduct flushings over time, in the present study all samples were collected within 12 to 20 h after ovulation and some association was established. This may be because oviduct fluid synthesis and secretion is not changing substantially over the collection period and, therefore, not affecting IGF-I concentrations. Wiseman et al. (1992) have shown that IGF-I concentrations in oviduct flushings were highest during estrus, which is coincident with the highest oviduct fluid volume (Wiseman et al., 1992) and highest protein synthesis (Buhi et al., 1989), suggesting that oviduct fluid dynamics play a large role in the IGF-I concentration in oviduct flushings. The association between IGF-I concentrations in oviduct plasma and flushings in this study suggests that some of the IGF-I found in oviduct flushings may originate from plasma. Although in vitro synthesis of IGF-I is highest from oviduct epithelial cells obtained at estrus (Wiseman et al., 1992), we do not know how much of this synthesis contributes to the IGF-I concentrations in oviduct flushings.

The use of littermates in the experimental design substantiates our earlier data on the impact of litter of origin on key reproductive characteristics (Almeida et al., 2001). Blastocyst developmental rate after 96 h in culture was affected by litter of origin, as was as E:P ratio in plasma, and both IGF-I and pOSP concentrations in oviduct flushings, indicating the potential for functional relationships between nutrition, oocyte quality, and oviduct function on embryonic survival.

Lastly, our results confirm that estradiol and progesterone, and not IGF-I, are affected by the subovarian countercurrent multiplier system. In the present study, IGF-I concentrations were not different between oviductal and peripheral veins, suggesting that the ovary is not a significant source of IGF-I and confirming the results of Jesionowska et al. (1990). However, the higher levels of progesterone and estradiol in the ovi-

ductal plasma support the concept of a close relationship between ovarian steroidogeneisis and oviduct function. This conclusion is further supported by the local oviductal relationship between pOSP abundance and progesterone concentrations (this study), demonstrating local regulation of oviduct function. Other studies have also found evidence for local regulation in unilaterally ovariectomized pigs (Nichol et al., 1997; Novak et al., 2002).

## Implications

Improved embryonic survival resulting from lowered feed intake after mating in gilts is probably due to steroid-dependent changes in the uterine, not the oviductal, environment, as the timing of the changes in feed intake is too late to alter the steroid priming of the oviduct. These data suggest that the manipulation of feed intake during the estrous cycle may affect embryonic survival through changes in follicular development by altering oocyte quality, and steroid-dependent priming of the oviductal environment.

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