Influence of vitamin A injection before mating on oocyte development, follicular hormones, and ovulation in gilts fed high-energy diets

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ABSTRACT: Previous research revealed that treatment with vitamin A approximately 5 d before ovulation may increase litter size in weaned sows and improve embryonal survival in gilts fed high-energy diets that reduced embryonal survival. For the current study, the hypothesis was that administration of vitamin A before ovulation would alter development of follicles and oocytes in a way favorable to enhanced embryonal survival. (Landrace × Large White) × (Duroc × Hampshire) gilts (n = 44) were fed 11.0 Mcal ME\mbox{gilt}^{-1}\mbox{d}^{-1} beginning 7 d after second estrus and given (i.m.) corn oil or 1 × 10^6 IU of vitamin A (retinyl palmitate) on d 15 after second estrus. Gilts were checked for estrus every 4 h, mated naturally at third estrus, and assigned randomly to undergo midventral laparotomy beginning at 24 to 28, 28 to 32, 32 to 36, or 36 to 40 h after onset of third estrus. At laparotomy, ovulated oocytes and early-stage embryos were recovered from oviducts, and ovaries were removed for aspiration of oocytes and granulosa cells from unovulated follicles. Oocytes and embryos were stained for assessment of stage of development. Granulosa cells were cultured to assess their ability to secrete progesterone. Follicular fluid was assayed for progesterone, estradiol-17β, IGF-I, and PGF_2α. Treatment with vitamin A altered development of oocytes and embryos by decreasing the percentage at the germinal vesicle stage and increasing the percentage at advanced stages. Mean stage of development was increased by vitamin A, but variation in stage was decreased. Among follicles matched by meiotic stage of oocyte, follicular fluid concentrations of progesterone, IGF-I, and PGF_2α were greater in vitamin A-treated gilts than in controls, but treatment with vitamin A in vivo did not affect LH-stimulated or unstimulated secretion of progesterone by granulosa cells in vitro. These data provide evidence that vitamin A may influence embryonic development by advancing resumption of meiosis and altering follicular hormonal environment during follicle maturation.

Key Words: Embryonic Development, Follicles, Oocytes, Pigs, Vitamin A

Introduction

Treatment of sows with vitamin A at weaning has been reported to increase subsequent litter size in some, but not all, studies (Coffey and Britt, 1993; Pusateri et al., 1999). We reported evidence that injection of vitamin A may improve embryonal survival in gilts intentionally fed a high-energy diet before and after estrus to reduce embryonal survival (Whaley et al., 1997). In that study, there was evidence that variation in embryo size was reduced in gilts treated with vitamin A. This finding is consistent with the hypothesis that embryonal survival is greater if embryos within a litter develop synchronously (Pope et al., 1990). Embryonal survival may be associated with differences in oocyte development before ovulation. Oocytes of Meishan gilts are at a more advanced stage 7 h before predicted ovulation than oocytes of white hybrid gilts, and embryonal survival rate in the Meishan gilts is greater than in the hybrids (Faillace and Hunter, 1994). The current study assessed whether treatment with vitamin A influenced preovulatory changes in hormone concentrations in follicular fluid, patterns of ovulation,
oocyte and embryo development, and secretion of progesterone by granulosa cells in gilts fed a high-energy diet intentionally to lower embryonal survival.

Materials and Methods

Animals and Treatments

Prepubertal (Landrace × Large White) × (Duroc × Hampshire) (n = 60) gilts were injected with PG600 (400 IU eCG + 200 IU hCG; Intervet, Millsboro, DE) to stimulate pubertal estrus. Gilts averaged 156 ± .5 d of age and 106 ± 2 kg BW on the day of injection. Upon injection, gilts were moved from finishing pens to breeding pens in a curtain-sided building and were penned together (n = 6 per pen) in close proximity to mature boars. Gilts were checked for estrus twice daily by direct contact with a boar.

The dietary treatment has already been described (Whaley et al., 1997). Briefly, gilts were given ad libitum access to a commercial finishing diet before injection of PG600. From first estrus until d 7 after second estrus, gilts were group-fed once daily to provide 5.4 Mcal ME-gilt⁻¹-d⁻¹. On d 7 of the second estrous cycle, gilts were moved to individual stalls and fed an 8% fat-supplemented diet supplying 11.0 Mcal ME-gilt⁻¹-d⁻¹.

Among gilts that showed a second estrus, 44 were selected for subsequent treatment based on an inter-estrus interval of at least 18 d and tight synchrony of second estrus to accommodate scheduling of experimental procedures. On d 15 after second estrus, 21 gilts were given (i.m., in the neck) 1 × 10⁶ IU vitamin A (retinyl palmitate dissolved in 5 mL corn oil; Sigma Chemical Co., St. Louis, MO; Vit A).

Gilts were checked individually for estrus in the presence of a mature boar every 4 h beginning 4 d prior to expected third estrus. Gilts were mated by natural service to two boars at detection of third estrus and 12 h later. Boars were used equally among control and Vit A gilts to avoid biases in embryonic development associated with boar.

Surgical Procedures

All procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee. At third estrus, gilts were assigned randomly to undergo midventral laparotomy beginning 24 to 28, 28 to 32, 32 to 36, or 36 to 40 h after onset of estrus. Laparotomies for gilts assigned to a specific 4-h surgical window were always completed before the end of the designated period, so there was no overlap between periods. The surgical approach was similar to that already reported (Whaley et al., 1997). The proximal reproductive tract of each gilt was exteriorized through a midventral incision, and ovaries were examined for presence of preovulatory follicles and corpora hemorrhagica. Preovulatory follicles were counted, and their diameter was measured using a calibrated instrument. Gilts bearing any preovulatory follicles (≥ 6 mm in diameter) were ovarioctomized after oviducts were flushed to recover oocytes and embryos.

Oocyte and Embryo Recovery and Processing

Ovulated oocytes and early-stage zygotes were recovered by inserting a sterile glass tube into the proximal ampulla and then flushing each oviduct in a retrograde manner using 15 mL of a modified Tyrode’s medium containing HEPES (Parrish et al., 1986; Farin and Yang, 1994). The medium was injected through the uterotubal junction using a 20-mL sterile glass syringe and 27-gauge blunt needle, and the flush medium was collected into a 25-mL culture flask.

Immediately after ovarioectomy, follicular fluid and oocytes were aspirated from individual preovulatory follicles ≥ 6 mm in diameter using a 1-mL syringe containing .5 M PBS and fitted with an 18-gauge short-bevel needle (Becton-Dickinson, Rutherford, NJ) according to methods described by Xie et al. (1990b). The diameter of each follicle was recorded, and the volume of follicular fluid collected from each follicle was measured. Oocytes recovered from oviducts and follicular fluid were denuded of cumulus cells using hyaluronidase and manual pipetting, fixed in ethanol-acetic acid (3:1; vol/vol) for 20 to 24 h, stained with 1% orcein in 25% acetic acid, and destained with ethanol-acetic acid (Motlik and Fulka, 1976; Xie et al., 1990a; Farin and Yang, 1994). After oocyte removal, follicular fluid was immediately centrifuged at 100 × g for 3 min to remove granulosa cells and debris. The supernate was frozen in liquid nitrogen and stored at −80°C until it was assayed for concentrations of estradiol, IGF-I, progesterone, and PGF₂α.

Classification of Oocytes and Embryos

Stained oocytes were visualized at 600× using differential interference contrast optics and evaluated for stage of development according to established criteria (Hunter and Polge, 1966; Motlik and Fulka, 1976; Xie et al., 1990a). Oocytes and embryos recovered at any time between 24 and 40 h after onset of third estrus were classified according to one of five developmental stages (DS) for purposes of statistical analysis: DS₁, germinal vesicle (GV); DS₂, germinal vesicle breakdown (GVBD); DS₃, ovulated and unfertilized; DS₄, ovulated and fertilized (sperm in cytoplasm, but no pronucleus); and DS₅, pronuclear. Oocytes aspirated from follicles of ovaries removed between 24 and 35 h after onset of estrus also were classified according to a more definitive meiotic stage (MS): MS₁, GV I or II; MS₂, GV III; MS₃, GV IV/early diakinesis; MS₄, metaphase I or anaphase I; MS₅, telophase I; and MS₆, metaphase II.
Granulosa Cell Culture and Progesterone Secretion

All reagents were purchased from Sigma (St. Louis, MO) unless otherwise stated. Following aspiration of follicles, ovaries were transported 8 km to the laboratory in cold .01 M PBS (pH = 7.4) supplemented with 200 U/mL penicillin, 200 μg/mL streptomycin,.75 μg/mL amphotericin B, and 100 IU/mL heparin. Granulosa cells were recovered by methods modified slightly from Haney and Schomberg (1978). Briefly, aspirated follicles were bisected and follicle linings were grasped with surgical forceps, everted, and suspended in medium DME-F12 containing 200 U/mL penicillin, 200 μg/mL streptomycin, and .75 μg/mL amphotericin B. Follicle linings from each individual ovary were combined and agitated using a sterile glass stir rod to dislodge granulosa cells. After filtration through a nylon mesh filter (52 μm) and centrifugation (5 min at 100 × g), granulosa cells were resuspended in fresh medium containing 10% fetal bovine serum (HyClone, Logan, UT) and 5 μg/mL ITS (insulin, transferrin, sodium selenite). Cells were plated (0 h; eight wells/ovary) in 48-well culture plates (previously coated with fibronectin) at a concentration of 5 × 10^5 cells per well (5 mL/well) and were incubated in 5% CO2, 95% air at 37°C. After 48 h, media were replaced with serum-free media containing 10 μg/mL ITS. At 72 h, media were removed and replaced with .5 mL of media containing 10 μg/mL ITS with or without 100 μg/LH/mL (NIH-LH-S20). At 96 h, media were collected and frozen. Cells were incubated at room temperature for 10 min with 100 μL of a 1.0% cholic acid, .1% laurel sulfate solution. Cells were ruptured using 100 μL of DNA assay buffer (2 M NaCl). After the addition of 100 μL of 2 M NaCl, cell lysates were harvested and frozen at −20°C until they were analyzed for DNA content according to methods of LaBarca and Paigen (1980).

Assays

DNA. Amounts of DNA (μg/well) were quantified using a TKO 100 Mini-fluorometer (Hoefer Scientific Instruments, San Francisco, CA) according to the manufacturer’s instructions. Stock DNA prepared from calf thymus (Worthington Biochemical, Lakewood, NJ) and Hoechst dye H33258 (Sigma) were used to generate standard curves. Intraassay CV was 1.8%.

Progesterone. Progesterone in culture media and follicular fluid was analyzed with a RIA (Shaw and Britt, 1995). Inhibition curves generated for either charcoal-stripped follicular fluid or charcoal-stripped culture media diluted in assay buffer and dosed with increasing concentrations of progesterone paralleled standard curves generated in assay buffer alone. All media samples (unextracted, diluted 1:300 in assay buffer) were analyzed in three assay runs, and follicular fluid was diluted 1:100 and assayed in one run. Intra- and interassay CV averaged 2.7 and 6.2%. Sensitivity of the assay was .6 ng/mL.

Estradiol. Estradiol-17β was measured with a RIA (Howard et al., 1990) using antibody provided by N. R. Mason (Mason and March, 1975). Samples were diluted 1:1,100 in .01 M PBS, and all samples were analyzed in a single assay. Intra-assay CV was 6.3%, and sensitivity was 7.8 pg/mL.

Insulin-Like Growth Factor I. Follicular fluid concentrations of IGF-I were measured with a RIA (Houseknecht et al., 1988; Jones et al., 1991) using rabbit anti-IGF-I serum (UB3-189, provided by the National Hormone and Pituitary Program). For this assay, follicular fluid samples were incubated with glycylglycine to free IGF-I from its binding proteins (Houseknecht et al., 1988) and there was no correction for extraction efficiency. Samples (50 μL) of follicular fluid were analyzed in duplicate, and all samples were quantified in a single assay. The intraassay CV was 4.3%, and sensitivity of the assay was 3 ng/mL.

Prostaglandin F2α. Concentrations of PGF2α were quantified with a RIA (Richards and Almond, 1994) using a rabbit anti-PGF2α-BSA antibody (ICN, Costa Mesa, CA) that has a low affinity (cross-reactivity < .1%) for prostaglandins other than PGF2α except for PGF2β, which exhibits a cross-reactivity of 60% at 50% inhibition of maximum binding. Samples were analyzed in duplicate in two assays, and intra- and interassay CV were 21 and 23%, respectively.

Statistical Analyses

Distributions of oocyte and embryonic development were evaluated using chi-square analyses. Differences in ovulation rate between treatments were tested with ANOVA.

A subsample of 56 follicles from ovaries excised 24 to 35 h after onset of estrus were matched between treatment groups based on meiotic stage of the oocyte recovered from the individual follicles. These follicles were distributed similarly between the two treatment groups among the three surgical periods (24 to 28 h control = 12, Vit A = 14; 28 to 32 h, control = 10, Vit A = 8; and 32 to 35 h, control = 7 and Vit A = 5). Each time period included follicles from which oocytes of the same meiotic stage(s) were recovered, and all meiotic stages were represented in each treatment group. Differences in follicular fluid concentrations of progesterone, estradiol-17β, IGF-I, and PGF2α between treatment groups were determined using least squares ANOVA (SAS, 1987). Correlation analyses were performed among meiotic stage of follicular oocytes and follicular concentrations of progesterone, estradiol-17β, IGF-I, and PGF2α. Regression analyses of progesterone, estradiol, IGF-I, and PGF2α on meiotic stage of follicular oocytes was conducted using general linear models (SAS, 1987). Parallelism of regression lines was tested by comparing slopes of control and Vit A regression lines for each hormone.

Granulosa cell DNA content and progesterone secretion per unit of DNA were analyzed using least squares
Vitamin A, oocytes, and follicles

Figure 1. Mean stage of development and mean SD of stage of development for oocytes and embryos from control and vitamin A-treated gilts. Developmental stage was scored (DS1 = 1 to DS5 = 5) for statistical analyses. *Values differed between control and vitamin A groups (P < .05).

ANOVA models that included effects of vitamin treatment, LH treatment, gilt within vitamin × LH treatment, and the vitamin × LH interaction.

Results

Recovery rates for follicular oocytes and ovulated oocytes/embryos were 50.3 and 62.6%, respectively, based on number of corpora hemorrhagica and follicles > 5 mm in diameter. A total of 251 oocytes and embryos were recovered. Recoveries of oocytes or embryos per gilt averaged 5.67 ± .65 and 5.74 ± .62 for control and Vit A groups, respectively.

Oocyte and Embryo Development

Treatment with vitamin A increased average developmental stage (P < .05, Figure 1) and decreased variation in developmental stage (P < .05, Figure 1). Distributions of oocytes and embryos by developmental stage differed between treatments (Figure 2; P < .001). There were fewer oocytes at the germinal vesicle stage and more ovulated and fertilized oocytes from Vit A gilts than from controls.

When oocytes and embryos were grouped by time of collection after onset of estrus, distributions also differed (P < .05) between treatments (Figure 3). Specifically, vitamin A decreased the number of oocytes arrested at the GV stage and increased the number of oocytes at more advanced developmental stages at 24 to 28 and 28 to 32 h; however, at 36 to 40 h, there were more oocytes at GVBD in the Vit A group than in the control group, because two gilts in the Vit A group had not yet ovulated (Figure 4).

Ovulation rate did not differ between treatment groups (control = 13.8 vs Vit A = 13.6; P > .10); however, pattern of ovulation among individual gilts was altered by vitamin A (Figure 4). Specifically, vitamin A increased the percentage of gilts exhibiting incomplete ovulation at time of surgery (control = 4.8% vs Vit A = 30.4%; P < .05).

Follicular Fluid Hormones

Diameter (control = 8.9 ± 1.5 mm vs Vit A = 9.0 ± 1.2 mm) and follicular fluid volume (control = .24 ± .1 mL vs Vit A = .25 ± .07 mL) of follicles > 5 mm in diameter did not differ between treatments. Results of hormone analyses for paired follicles based on equivalent stages of oocyte maturation are presented in Figure 5. Progesterone was approximately threefold greater (P < .01) in follicles of Vit A gilts (Figure 5A). Estradiol-17β did not differ (P > .10) between treatment groups (Figure 5B), but the estradiol-17β:progesterone ratio was lower (P < .05) in follicles from Vit A gilts. The IGF-I concentration was greater (P < .05) in follicles from the Vit A group (Figure 5C). Concentration of PGF2α was 15-fold greater (P = .05) in follicles of Vit A gilts (Figure 5D).

Correlations among oocyte meiotic stage and concentrations of progesterone, estradiol-17β, IGF-I, and PGF2α are illustrated in Figure 6. A positive correlation between progesterone and meiotic stage was observed for both control (P < .05) and Vit A (P < .01) gilts. Estradiol-17β was negatively correlated with oocyte stage in control gilts (P < .01), but not in Vit A gilts (P > .05). Meiotic stage was correlated positively (P < .01) with PGF2α in Vit A, but not in control, gilts. Progesterone and PGF2α concentrations were correlated positively (P < .01) in both groups, as were IGF-I and progesterone. A strong correlation (P < .01) between IGF-I and PGF2α, concentra-

Figure 2. Distribution of oocytes and embryos by developmental stage. *Values differed between control and vitamin A groups (P < .001). GV, germinal vesicle; GVBD, germinal vesicle breakdown; Ov/Unfert, ovulated and unfertilized; Ov/Fert, ovulated and fertilized.
Figure 3. Distribution of oocytes and embryos by developmental stage and time from onset of estrus. Values differed between control (top panel) and vitamin A (bottom panel) groups at 24 h (**P < .001), 28 h (*P < .05), 32 h (°P = .07), and 36 h (**P < .001).

Figure 4. Pattern of ovulation for gilts subjected to laparotomies at various times after onset of estrus. For each gilt, the percentage of follicles > 5 mm in diameter that had ovulated is shown.
Figure 5. Concentrations of hormones in follicular fluid for follicles paired according to meiotic stage for control and vitamin A-treated gilts. Bars represent means ± SEM. *Values differed between control and vitamin A groups (P < .05).

Figure 6. Correlations among meiotic stage of oocyte and hormones in follicular fluid.

Vitamin A treatment in vivo did not influence (P > .05) granulosa cell proliferation in vitro, as assessed by amount of DNA per well after culture, or progesterone secretion per unit of DNA (Figure 8). Treatment with LH decreased granulosa cell proliferation, as shown by 50% less (P < .05) DNA per well, and increased progesterone secretion twofold (P < .05) per unit of DNA (data not shown). There was no LH × treatment interaction.

Discussion

In this study, gilts fed a high-energy diet and treated with vitamin A 15 d after second estrus had altered
oocyte and embryo development and altered follicular hormonal environment 24 to 36 h after onset of third estrus. Meiotic and developmental stages of oocytes and embryos were more advanced, and follicular concentrations of progesterone and PGF$_{2\alpha}$ were increased and

![Image](image-url)

**Figure 7.** Curves representing regression of hormone concentration in follicular fluid on meiotic stage of oocyte from the follicle.

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**Figure 8.** Progesterone and DNA concentrations in granulosa cell cultures from control and vitamin A-treated gilts. Bars represent means ± SEM.

estradiol-17β decreased in preovulatory (> 5 mm in diameter) follicles of vitamin A-treated gilts. This occurred despite no difference between treatment groups in apparent time of ovulation after onset of estrus. In fact, the data imply that time of ovulation was more variable or duration of ovulation was greater in vitamin A-treated gilts. These potential differences in variability or duration of ovulation mean that results must be interpreted with caution. Nevertheless, these data suggest that treatment with vitamin A advances follicular and oocyte maturation when provided during the period of final follicle maturation in gilts fed high-energy diets. The high-energy diets were used to decrease embryonal survival as a model for studying effects of treatment with vitamin A, so caution is warranted in extrapolating these results to gilts fed normal diets. Nevertheless, in some field studies, treatment with vitamin A has been shown to improve litter size in sows fed normal diets (Coffey and Britt, 1993).

There is evidence that vitamin A influences oocyte and embryo development. Rabbits that had higher blood levels of vitamin A produced more oocytes and embryos in response to superovulation than those that had lower levels (Besenfelder et al., 1996), and cattle treated with vitamin A at the onset of superovulation produced more transferable embryos (Shaw et al., 1995). Retinol concentrations were higher in healthy bovine follicles than in atretic follicles (Schweigert and
Zucker, 1988). Cellular retinol-binding protein is localized to oocytes and nearby granulosa in the rat ovary, providing evidence that vitamin A may be important in terms of oocyte development (Wardlaw et al., 1997).

In an earlier study (Whaley et al., 1997), we found that embryonal survival to d 11 or 12 of pregnancy was greater and uniformity of embryo development was enhanced in vitamin A-treated gilts. Similarly, in the current study, we found that embryonal developmental stage was more advanced and more uniform in vitamin A-treated animals. In both studies, we used a model in which gilts were fed high-energy diets known to lower embryonal survival rate; therefore, the findings could indicate that vitamin A restored the follicular environment to normal. However, there is additional evidence that treatment with vitamin A before estrus affects follicular development in normally fed gilts (Robertson, 1997; Robertson et al., 1997). In one experiment, treatment of gilts with $1 \times 10^6$ IU of vitamin A on d 16 of an estrous cycle increased the proportion (92 vs 68%) of preovulatory-sized follicles (> 5 mm in diameter) 2 d later and increased retinol in follicular fluid (39 vs 34 μg/mL). Follicles from vitamin-A treated gilts had a lower estradiol:testosterone ratio, indicating a more advanced stage. This would be similar to the lower estradiol:progesterone ratio noted in the current study. Robertson (1997) noted that treatment with vitamin A on d 16 increased the size of follicles on d 19, but the shift in size was associated with an increase in mid-sized (4 to 6 mm) rather than larger follicles, and, in this case, the estradiol:testosterone ratio was higher in vitamin-A treated animals. These results indicate that vitamin A moves follicles into a larger size class earlier in their development, and this leads to more “mature” follicles at the time of ovulation.

Progestosterone concentration in follicular fluid has been positively correlated and estradiol concentration negatively correlated with stage of oocyte maturation in pig follicles prior to ovulation (Xie et al., 1990b). Similar correlations among progesterone, estradiol, and stage of oocyte maturation were observed in the present study, but vitamin A influenced the magnitude of the correlations. For example, there was a greater correlation between progesterone and meiotic stage in vitamin A-treated gilts than in controls, but the opposite was true for estradiol (Figure 7). Progesterone treatment after ovulation enhances embryonal survival in gilts fed high-energy diets before estrus (Ashworth, 1991), and high intrafollicular levels of progesterone may exert a similar response through advancing meiosis.

The correlation between meiotic stage and PGF$_2\alpha$, was greater in vitamin A-treated gilts than in controls, and the correlation between IGF-I and PGF$_2\alpha$, was also greater in vitamin A-treated gilts. These correlations indicate that either vitamin A strengthened relationships one would expect to see as ovulation nears (Xie et al., 1990b) or that it restored normal relationships that were altered by the high-energy diet.

Energy intake before and after estrus influences embryonic development. For example, feeding excess energy for approximately 2 wk before mating lowers embryonal survival, but this effect can be overcome by treatment with progesterone beginning approximately 4 d after mating (Ashworth, 1991). Exogenous progesterone after mating is probably effective because it replaces the progesterone deficiency caused by high energy intake (Jindal et al., 1997). It is unlikely that increased energy intake can cause a progesterone deficiency during the periestrus period, because progesterone is already low (Jindal et al., 1997), but energy intake may affect oocyte development directly. For example, Zak et al. (1997) reported that nuclear maturation of oocytes recovered from follicles of weaned sows was advanced in sows that had 50% restricted feed intake for the first 21 d of lactation and then were fed to appetite for the last 7 d, compared with sows that were fed to appetite for the first 21 d and restricted during the last 7 d of lactation. Thus, altering the metabolic status of the animal may influence oocyte development through metabolic hormones such as IGF-I. In the present study, IGF-I was elevated in vitamin A-treated gilts, even though the control and Vit A gilts were being fed high-energy diets.

The effect of vitamin A is probably mediated indirectly through alteration in granulosa cell function, including the cumulus cells surrounding the oocyte. Follicles of Vit A pigs seemed endocrinologically more mature and promoted earlier resumption of meiosis in oocytes even though the size of follicles was similar to that seen in control gilts. This is reminiscent of the situation in which oocytes cultured in follicular fluid from Meishan donors matured more in vitro than oocytes cultured in follicular fluid from Large White donors (Xu et al., 1998) and in which oocytes cultured in follicular fluid of sows fed to appetite showed greater nuclear maturation in vitro than oocytes cultured in follicular fluid from sows that had a 50% restriction in energy intake (Zak et al., 1997). Thus, treatment with vitamin A may alter follicles in a way that favors oocyte maturation.

Follicular concentrations of IGF-I were positively correlated with progesterone concentrations in follicles from the control ($r = .55; P < .01$) and Vit A ($r = .75; P < .01$) gilts, and similar correlations between IGF-I and progesterone have been previously reported for bovine follicles (Spicer and Enright, 1991). Concentrations of IGF-I were also highly correlated ($r = .79; P < .01$) with PGF$_{2\alpha}$ concentrations in follicles from Vit A gilts but were not correlated with PGF$_{2\alpha}$ concentrations in follicles from controls. Insulin-like growth factor I is a potent mitogen produced by granulosa cells (Hammond, 1991) and has been shown to increase both granulosa cell proliferation and progesterone synthesis and secretion by these cells (reviewed by Guidice, 1992). Therefore, it might be expected that vitamin A, acting through an IGF-I stimulatory mechanism, may advance
follicular maturation, resulting in increased follicular progesterone production.

Pope et al. (1988) and Xie et al. (1990b) provided evidence that an increase in the duration of ovulation increased within-litter embryonal diversity, which is thought to increase embryonal mortality (Pope et al., 1990; Roberts et al., 1993). However, other studies (Soede and Kemp, 1992; Soede et al., 1993) have not detected a relationship between duration of ovulation and early embryonal diversity. In the present study, the within-treatment variation of stage of oocyte maturation and embryonic development was significantly decreased in Vit A gilts, but this seemed to occur without an observable effect on duration or time of ovulation. The percentage of gilts in midovulation at the time of surgery was greater among gilts treated with Vit A than among controls (30.4 vs 4.8%; P < .05), and there was no indication that pattern of ovulation time was shifted relative to the time of onset of estrus (Figure 4). The low recovery rates for follicular oocytes and ovulated oocytes/embryos may have been related to timing surgery around the peri-ovulational period. Ovulated oocytes may have still been in the oviductal fimbria or proximal ampulla and, therefore, not flushed efficiently from the oviducts.

Treatment with vitamin A may alter pattern of follicular hormone production and stimulate earlier resumption of meiosis so that more oocytes have reached an advanced meiotic stage by the time of ovulation. It is not possible to determine cause and effect relationships from correlation analyses, but the regression analyses in Figure 7 show that the rate of increase in progesterone and PGF2α seems to be accelerated in vitamin A-treated gilts, and production of estradiol-17β must have decreased sooner in these gilts. These and associated endocrine or metabolic changes may advance resumption of meiosis.

Implications

Some previous research has indicated that treatment of sows with vitamin A increased litter size. To study the mechanism of action of this effect, an experimental model in which gilts were fed a high-energy diet to induce surgery was greater among gilts treated with Vit A than among controls (30.4 vs 4.8%; P < .05), and there was no indication that pattern of ovulation time was shifted relative to the time of onset of estrus (Figure 4). The low recovery rates for follicular oocytes and ovulated oocytes/embryos may have been related to timing surgery around the peri-ovulational period. Ovulated oocytes may have still been in the oviductal fimbria or proximal ampulla and, therefore, not flushed efficiently from the oviducts.

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