Effect of *Fusarium roseum* corn culture containing zearalenone on early pregnancy in swine

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SUMMARY

A corn culture of *Fusarium roseum* was added to a standard corn-soybean swine gestation ration. Low, middle, and high dosage mixed feeds contained 7, 38, and 64 mg of zearalenone/kg of feed (7, 38, and 64 ppm) and 0.5, 2.5, and 4.5 mg of deoxynivalenol/kg, respectively. Control feed was the standard ration without added *F roseum* corn culture. Mature gilts were bred by natural service and fed control or *F roseum* molded feed from 3 to 34 days after breeding.

The main effect of the molded feed was an inhibition of fetal development, with decreased numbers of fetuses present in treated animals at slaughter (38 to 43 days after breeding). Normal litters were present in 7 of 8 control animals, in 2 of 4 gilts given the low-dosage feed, in 1 of 4 gilts given the medium dosage, and in 0 of 4 given the high-dosage feed. Corpora lutea were maintained in all treated animals, as evidenced by serum progesterone concentrations. Serum estradiol concentrations were decreased in gilts in the middle- and high-dosage groups. The genital system of the gilts fed low- and middle-dosage feeds had a gross and microscopic appearance similar to that of the pregnant controls and reflected prolonged progesterone stimulation. Morphologic changes in the genital system of the high-dosage group were intermediate between changes induced by progesterone and those induced by estrogen. Clinical signs of hyperestrogenism and partial feed refusal were noticed in only some of the high-dosage group animals.

Zearalenone, a metabolite of *Fusarium roseum* (Gibberella zea), has been associated with reproductive problems in swine. Zearalenone has estrogenic activity that is most pronounced in prepuberal gilts. The induced hyperestrogenism was initially termed "vulvo-vaginitis" and was associated with the consumption of moldy feed. Changes in immature gilts have included swelling of the vulva, uterine enlargement, ovarian atrophy, mammary development, and, in some gilts, vaginal or rectal prolapse. Clinical signs of hyperestrogenism may be induced in prepuberal gilts with relatively low doses of zearalenone. Immature gilts given 1 mg of zearalenone/day developed tumefaction of the vulva, and 5 mg/day also caused an increase in uterine weight.

Large amounts of zearalenone in the feed (100 mg/kg of feed) have profound effects on cycling animals, including nymphomania, pseudopregnancy, ovarian atrophy, and morphologic changes in the endometrium. Pseudopregnancy was characterized by retention of corpora lutea (CL) and thickening of the endometrium in the absence of fetuses.

Moldy feed in the diet of pregnant sows has been associated with abortion, weak pigs, stillbirths, decreased litter size, and fetal mummification. However, concentrations of zearalenone in the feed were not determined in most of these cases. Zearalenone added to the diet of pregnant sows at 25 and 50 mg/kg of feed (25 and 50 ppm) caused sows to farrow smaller litters and smaller pigs. Lower concentrations of zearalenone in the feed appeared to have little effect on the pregnant sow. A diet with 2.2 mg of zearalenone/kg of feed caused no overt differences in farrowing performance, whereas 3.61 mg of zearalenone/kg caused a decrease in fetal weight at 80 days' gestation, but had no effect on embryonic mortality.

Trichothecene mycotoxins have also been associated with reproductive disorders, especially abortion and infertility, in swine. Trichothecenes are a group of mycotoxins produced by *Fusarium* spp and some other fungi. Those found naturally occurring in foodstuff and associated with disease in man and farm animals include T-2 toxin, deoxynivalenol, diacetoxyscirpenol, and nivalenol. Deoxynivalenol occurs commonly in corn from the Midwest, and is of major economic importance to swine producers because it causes feed refusal.

The purpose of the present study was to characterize the effect of zearalenone on implantation and early pregnancy in swine. To simulate field situations, pigs were exposed to zearalenone by addition of *F roseum* corn culture to the ration.

Materials and Methods

*Fusarium roseum* 'graminearum' (Gibberella zea) No. 1693 was grown on sterile popcorn. Popcorn (410 g) and 220 ml of water were autoclaved for 1 hour at 121 C. Cultures were grown at 23 C for 9 days, followed by 16 C for 4 weeks, followed by 12 C for 3 weeks. Corn cultures were then dried at 38 C with forced aeration and ground in a Wiley mill. Ground cultures were mixed and
divided into sublots, and each sublot was sampled for zearalenone analysis.

Zearalenone concentrations were determined, using a chloroform/water extract of the feed, which was partially purified on a silica gel column and then further purified by liquid-liquid partition, using hexane and acetone. The quantitation was accomplished by thin-layer chromatography plates scanned densitometrically at an excitation wavelength of 327 nm and emission at 455 nm. Deoxynivalenol concentration of the feed was determined, using a methanol extract purified on a silica gel column with chloroform and methanol. Column eluate was analyzed by gas liquid chromatography. Deoxynivalenol was also quantitated by gas chromatography/mass spectrography.

The ground F. Roseum corn culture was added to a standard 14% protein corn-soybean swine gestation ration. Concentrations of zearalenone were 7, 38, and 64 mg/kg of feed for the low (group 1), middle (group 2), and high (group 3) dosage groups, respectively. Deoxynivalenol concentrations were calculated to be approximately 0.4 to 0.5, 2.4 to 2.7, and 4.2 to 5.0 mg/kg of feed for groups 1, 2, and 3, respectively. Feed from the same lot without added F. Roseum culture served as control feed.

Mature 100- to 110-kg cross-bred gilts were bred by natural service. Each gilt was served by 2 boars of proven fertility within a 24-hour period. On postbreeding day (PBD) 3, gilts were randomly assigned to 1 of 3 treatment groups of 4 each or to the control group consisting of 8 gilts. Gilts were given 1.8 kg of feed/day, the infected feed being given from PBD 3 through 34. Blood samples were taken at weekly intervals from PBD 11 to 42 for determination of serum concentrations of estradiol-17β and progesterone.

All gilts were killed between PBD 38 and 43. Reproductive tracts were examined for number and size of CL, number and size of fetuses, and appearance of endometrium. Representative tissues were fixed in 10% formalin, embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin.

Radioimmunoassay procedures were used to determine serum concentrations of estradiol-17β and progesterone. For each steroid, all samples were analyzed in 1 assay. Recoveries of [1H]progesterone and [1H]estradiol-17β added to serum before extraction averaged 92 ± 2% and 91 ± 3%, respectively. Coefficients of variability were < 10% for each steroid. Hormonal data were subjected to 1-way analysis of variance. Differences among means were detected by Newman-Keuls sequential range tests.

Results

Overt feed refusal was not noticed. During the first few days (PBD 3 to 7), gilts in group 3 left small portions (≤ 0.5 kg) of feed. Two gilts in group 3 had clinical signs of hyperestrogenism from approximately PBD 19 through termination of treatment. Clinical signs consisted of vulvar turgescence in gilts Q and R and a standing response to pressure on the loin in gilt R.

Number of CL and fetuses present at slaughter (PBD 38 to 43) are shown in Table 1. Differences in the number of CL were not found among treatments. None of the group 3 gilts was pregnant. There were 2 nonpregnant gilts and 1 gilt with 1 fetus in group 2. The difference in average fetal number between the controls and group 2 approached significance (P = 0.06). Gilts in group 2 had 2 normal and 2 abnormally small fetuses; gilt J with 1 fetus also had 2 sets of degenerate fetal membranes with no associated recognizable fetal bodies. Average weight and length of fetuses from treated gilts fell within the range observed for litters from control gilts.

A precipitous decrease in serum progesterone concentration to < 0.5 ng/ml was measured in gilt B (control) on PBD 19 and 26. Other gilts did not show a distinct decrease in serum progesterone that would have indicated regression of CL. Progesterone values of gilt B were deleted from the control group average value because of this obvious disparity.

Average serum progesterone concentrations for each group are shown in Figure 1. Significant differences from control values were measured for group 1 on PBD 33 (P < 0.01), for group 2 on PBD 20 and 42 (P < 0.01), and for group 3 on PBD 20 (P < 0.01) and 27 (P < 0.05).

A marked divergence of serum estradiol-17β concentrations were not found among gilts in any group (Fig 2). Estradiol concentrations in groups 2 and 3 did not show as high a peak as that noticed in the control group at approximately PBD 27. The decreased estradiol concentrations in groups 2 and 3 were significant on PBD 20 (P < 0.05) and 27 (P < 0.01). Estradiol concentrations were significantly lower in group 3 than were those in controls on PBD 34.

Pregnant control gilts had CL that were solid (cellular) throughout and ranged from 7 to 13 mm in diameter. Fetuses ranged in size up to 5 mm. Developing and atretic follicles were present. Atretic follicles consisted of irregularly collapsed, condensed basement membrane material (250 to 400 µm diameter) surrounded by theca-like cells. Corpora albicantia were not observed. The endometrial

### TABLE 1—Number of CL and fetuses at slaughter (PBD 38 to 43)

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<tr>
<th>Gilt</th>
<th>No. of CL</th>
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<td>Control</td>
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<td>Group 1 (7 mg of zearalenone/kg of feed)</td>
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<td>Group 2 (38 mg of zearalenone/kg of feed)</td>
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<td>Group 3 (64 mg of zearalenone/kg of feed)</td>
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![Graph showing average serum progesterone concentration per group vs days after breeding](image-url)
luminal epithelium in areas of placental attachment was simple columnar, 8 to 20 μm high. The stroma was moderately edematous. Density and total number of glands was relatively low. Glandular epithelium was columnar, 10 to 12 μm high. A few gland ducts were moderately distended with eosinophilic amorphous secretion. Areas of endometrium without adjacent placenta had luminal epithelium that was pseudostratified columnar or stratified and was 25 to 80 μm thick (Fig 3). Occasional small folds of epithelium contained intraepithelial vesicles. A few mitotic figures were observed. Individual epithelial cells were necrotic, and there was a layer of desquamated epithelium on the surface. Gland number and density was about the same as in placental areas, but gland epithelium was lower (6 to 7 μm). The epithelium of the uterine tubes (fallopian tubes) was approximately 10 μm high, with additional cytoplasmic extensions extending past the cilia similar to that described in pregnant swine.21 The mucosal epithelium of the cranial portion of the vagina was 3 to 4 cells thick.

The nonpregnant control gilt had cystic centers in some cot. and focal degenerative changes in others. Corpora albicantia were present. Endometrial luminal epithelium was simple columnar with minimal change to pseudostratified, was 20 to 25 μm high, and had a fairly even ciliated surface.

Morphologic features of uterine and ovarian tissues in group 1 were essentially the same as that of the pregnant controls. A few fetuses were smaller, but tissues from these had the same apparent degree of morphologic differentiation as larger fetuses.

Fig 2—Average serum estradiol-17β concentration per group vs average days after breeding.

Fig 3—Endometrium of pregnant control gilt; area without placental attachment. The endometrium is pseudostratified-to-stratified, with intraepithelial vesicles and desquamated debris on the surface. X 300.

Fig 4—Mucosa of uterine tube of group 2 gilt. The epithelium has cytoplasmic processes extending beyond the cilia, similar to that in pregnant control and group 1 gilts. X 500.

Fig 5—Endometrium of group 3 gilt. The mucosa is simple-to-pseudostratified and not as thick as that of controls. X 300.
Ovaries of group 2 were similar to those of the pregnant controls, except that shrunken degenerate follicles were > 400 μm. Endometrial luminal epithelium appeared similar to that of the controls, 12 to 25 μm high in areas of placental attachment and 25 to 55 μm thick in areas without placental attachment. Endometrial glands from areas without placental attachment had an increased number of glands with epithelium of slightly increased height. Occasional ducts contained pale eosinophilic secretion. An apparent difference between the endometrium of nonpregnant gilts and the nonplacental areas of endometrium of pregnant gilts was not observed. Uterine tube (Fig 4) and vaginal mucosa were similar to that of pregnant controls.

The ovarian morphologic features of group 3 gilts were similar to those of the pregnant controls. Corpora lutea were 6 to 10 μm thick. Epithelial folding, formation of intraepithelial cysts, and desquamated epithelial debris on the surface were similar to nonplacental areas of pregnant controls (Fig 5). Endometrial stroma was relatively edematous. Endometrial gland density was increased in relation to that of controls. Glandular epithelium was cuboidal, 10 to 12 μm high. Gland ducts tended to be dilated. The mucosal epithelium of the uterine tube was 16 to 20 μm high, and cytoplasmic extensions were not obvious. Vaginal and cervical mucosa were similar to that of controls.

Discussion

The main effect of F. roseum toxins in this study was to prevent early fetal development while causing persistence of the CL. The estrogenic effects of zearalenone became obvious only in 2 of the group 3 gilts. Previous experimental studies on the effects of zearalenone in sows involved continuous feeding during the reproductive cycle and/or throughout pregnancy.

In the mature gilt or sow, administration of diethylstilbestrol on day 11 of the reproductive cycle will prolong survival of the CL and lengthen the cycle. Various estrogenic compounds maintained functional CL to 33 days after estrus in mature gilts when treatment was started by day 11 of the cycle. Hence, ingestion of F. roseum-infected corn by bred sows or gilts for even a limited time near implantation could result in loss of the pregnancy or decreased farrowing performance later. The problem would be compounded in a field situation because the persistent CL would delay reproductive cycling. This loss of conception might not be recognized until the pig was pregnancy-tested, returned to estrus, or approached the anticipated farrowing date.

Hormonal patterns in the gilts were apparently dose-related. Serum progesterone was increased at PND 33 in group 1 gilts, but was decreased earlier in groups 2 and 3. Serum estradiol was not significantly affected in group 1, but was decreased midway through the experiment in groups 2 and 3. Hormonal effects observed in the gilts were primarily related to progesterone. The estrogenic effects of zearalenone were obvious only in some of the group 2 and 3 gilts.

Obvious morphologic differences in the reproductive tracts between the controls and gilts in group 1 were not observed. In group 2, reproductive tract morphologic features varied only slightly from that of the pregnant controls and contrasted sharply with that of the nonpregnant control. Morphologic features of the endometrium of group 3 gilts were similar to, but less complex than those in gilts in groups 1 and 2. The endometrial morphologic features were similar to that described previously as "gonadotrophic" effects, rather than changes due solely to the zearalenone.

In that previous study, the height of the uterine tube mucosa (18 to 34 μm) and thickness of the vaginal mucosa (10 to 14 cells) were more suggestive of estrogenic effects. The description of the cytoplasmic blebbing of the uterine tube mucosa was reminiscent of progesterone effect. In the present study, the uterine tube mucosa of group 3 gilts had an appearance intermediate between estrogenic and progestational effect, whereas the vaginal mucosa appeared to be under a progestational influence. The endometrial mucosa in group 3 also appeared to be intermediate in appearance between progestational and estrogenic influence. Squamous metaplasia of the endometrial mucosa described previously was not seen in the present study. It is possible that the morphologic changes in groups 2 and 3 changed after the withdrawal of the zearalenone 10 days before termination of the experiment. Any changes would have been due to a change in concentration of estrogen-like compounds in relation to the relatively stable progesterone concentration that was present. This change would be expected to be more pronounced in the group 2 gilts because they were exposed to more zearalenone and showed more estrogenic effects, whereas serum estradiol-17β concentrations were not different between groups 2 and 3.

A litter of < 4 pigs normally indicates postimplantation fetal death. In the sow, if half of a uterine horn is nongravid, it will cause regression of CL and death of embryos in the opposite horn. The luteolytic effect of the nonpregnant uterus appears to be limited to the early postbreeding period, i.e., before day 12. Because zearalenone can maintain the CL in the nongravid animal, the change in the intratubal milieu preventing implantation or fetal death could have occurred at any time during treatment.

The present effects may not have been due entirely to zearalenone, because deoxynivalenol was also present in the F. roseum corn culture. The concentration of deoxynivalenol in the group 3 feed is consistent with the reduced feed consumption observed. Deoxynivalenol is one of the more commonly occurring trichothecene mycotoxins and is of significant economic importance to swine producers in the Midwest. Certain trichothecene mycotoxins have been associated with reproductive disorders in swine. The T-2 toxin (a trichothecene mycotoxin) administered iv has been shown to cause abortion in pregnant sows. The T-2 toxin given in the feed did not cause abortion, but did cause infertility in subsequent estrous cycles. Effects of deoxynivalenol on pregnancy in swine have not been determined. Considering the widespread occurrence of deoxynivalenol in corn in the Midwest, especially in combination with zearalenone, the effects of this mycotoxin on pregnancy in swine warrant further characterization.

References


