A COMPARISON OF EFFECTS OF ZEARALENONE AND ESTRADIOL BENZOATE ON REPRODUCTIVE FUNCTION DURING THE ESTROUS CYCLE IN GILTS

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ABSTRACT

Effects of estradiol benzoate (EB) and zearalenone (Z) on luteal maintenance and plasma hormone concentrations were studied in 45 gilts. Gilts were allocated to receive either 20 mg Z, 2 mg EB or no treatment (C) on d 1 to 5 (T1), 6 to 10 (T2) or 11 to 15 (T3) of an estrous cycle (five per treatment). Onset of estrus was designated as d 0 of the estrous cycle. Zearalenone was added to the daily ration and EB was administered via an intramuscular injection. Blood samples were collected every 10 min over a 4-h period on the first 2 d prior to onset of treatment; the first, third and fifth days of treatment; and the first two and the fifth day after the end of the treatment periods. Gilts receiving EB and Z during T2 and T3 had longer (P<.05) inter-estrous intervals than C gilts. The range in inter-estrous intervals for Z and EB treatments was 28 to 74 and 27 to 63 d, respectively. Mean plasma progesterone concentrations were elevated (P<.05) during T2 and T3 in EB and Z-treated gilts when compared with C females. Estradiol benzoate treatment during T2 and T3 reduced (P<.05) mean plasma luteinizing hormone (LH) concentrations more than C or Z treatments. Mean plasma concentrations of 13, 14-dihydro-, 15-keto-prostaglandin F2α (PGFM) during T3 were higher (P<.05) in C and Z gilts on d 13 and 15 post-estrus when compared with EB gilts. These results indicated that 2 mg EB or 20 mg Z from d 6 to 10 or 11 to 15 of the estrous cycle extended the inter-estrous interval in swine. Estradiol benzoate during the same periods decreased mean plasma LH concentrations while Z did not. Additionally, EB treatment during T3 prevented the increase in PGFM concentrations of d 13 and 15 observed in both C and Z gilts.

(Key Words: Corpus Luteum, Estrogens, Zearalenone, Figs.)

Introduction

Zearalenone is a non-steroidal, estrogenic metabolite of Fusarium roseum (Mirocha et al., 1977). High concentrations of zearalenone have been measured in wheat, barley and corn stored under high moisture conditions (Mirocha et al., 1971). When contaminated grain or purified zearalenone is fed to animals, particularly swine, this phytoestrogen can interfere with normal reproductive processes (Nelson et al., 1973; Chang et al., 1979).

One of the effects of zearalenone in mature cyclic swine is a prolongation of the estrous cycle. Gilts fed purified zearalenone from d 5 to 20 of the estrous cycle exhibited extended inter-estrous intervals of 30 to 70 d (Cantley et al., 1982; Edwards et al., 1984). However, neither the specific effective time nor the manner in which zearalenons is luteotropic in swine is clear. The present study was undertaken to characterize luteotropic properties of zearalenone by examining its effects on lutea maintenance and plasma concentrations of progesterone (P4), luteinizing hormone (LH) and 13, 14-dihydro-, 15-keto-PGF2α (PGFM) during different periods of the estrous cycle.

Additionally, it has been well documented that steroidal estrogenic compounds administered between d 9.5 and 12 post-estrus extend periods of luteal maintenance (Kidder et al., 1955; Gardner et al., 1963; Saunders et al., 1983; Geisert et al., 1987). A comparison between two different estrogenic compounds, zearalenone and estradiol benzoate, in terms of their effects on plasma hormone concentrations and luteal maintenance was made. This may help to clarify further the role estrogens play in maintenance of corpora lutea.
Materials and Methods

Forty-five sexually mature crossbred gilts of Duroc, Yorkshire and Landrace breeding were used in this study. All gilts exhibited at least one estrous cycle of normal length (18 to 23 d) and normal estrous behavior before onset of the experiment. All gilts were housed in individual pens and were fed a complete corn-soybean-based maintenance ration throughout the study.

Approximately 5 d before their respective treatments began, gilts were given an initial anesthetic of 1 g of sodium thiopental and anesthesia was maintained with a closed circuit system of halothane and oxygen. A tygon catheter was inserted into a jugular vein according to the procedures of Ford and Maurer (1978).

Gilts were randomly allocated to receive 2 mg estradiol benzoate (EB)4, 20 mg zearalenone (Z)5, or no treatment (C) from d 1 to 5 (T1), 6 to 10 (T2), or 11 to 15 (T3) of an estrous cycle (five gilts per treatment in each period). The first day a gilt exhibited lordosis behavior in the presence of a boar was designated as d 0 of the estrous cycle. EB was administered via a single intramuscular injection in 2 ml of sesame oil. The 20 mg dose of Z was added to .5 kg of the base ration, which was added to the animal's daily ration as a top dressing. Treatments were administered 2 h before blood sampling began.

Gilts were bled every 10 min for 4 h. Gilts in T1 were bled on d -2, -1, 1, 3, 5, 6, 7 and 10 of the estrous cycle. Gilts in T2 were bled on d 4, 5, 6, 8, 10, 11, 12 and 15 of the estrous cycle and T3 gilts were bled on d 9, 10, 11, 13, 15, 16, 17 and 20 of the estrous cycle. Blood samples were collected in heparinized tubes and stored on ice. At the end of each 4-h bleeding period, blood samples were centrifuged at 1,200 rÂ·g for 10 min and plasma frozen at -20 C.

Gilts were observed daily for estrus with a mature boar. The inter-estrous interval was calculated by determining the number of days between pre-treatment and post-treatment estrus.

Hormonal Analysis. Plasma concentrations of progesterone (P4) were determined by a radioimmunoassay for non-extracted porcine plasma described by Cantley et al. (1981). The inter- and intra-assay coefficients of variation were 10.7 and 8.0%, respectively.

Plasma concentrations of LH were determined by a double antibody radioimmunoassay described by Niswender et al. (1970) using anti-porcine LH (#556)6 as first antibody, sheep anti-rabbit gamma globulin as second antibody and purified porcine LH (LER-783-3)7 as labeled tracer and standard. Inter- and intra-assay coefficients of variation were 15 and 12%, respectively.

Plasma concentrations of PGFM were determined according to Marengo et al. (1986). The PGFM used in standard solutions and PGFM antibody were purchased from Seragen8. The assay was validated in the following manner: Dose-response tests showed parallelism between the standard PGFM inhibition curve and 50-, 100-, 150-, 200- and 250-Âµl samples of porcine plasma. Addition of 502, 998, 2,015 or 3,890 pg of PGFM per ml of ovarietomized-hysterectomized porcine plasma (n = 10 per dose) resulted in recoveries (%) of 98 Â± 7, 101 Â± 6, 90 Â± 6 and 79 Â± 4, respectively. The intra- and inter-assay coefficients of variation were 7.1 and 14.0%, respectively. Technical data from Seragen indicated cross-reactivity of the PGFM antibody with PGF2Âµ, PGE2 and 6-keto-PGF1Âµ was less than 2% and cross-reactivity with 13, 14-dihydro-, 15-keto-PGE2 was 9.3%.

Statistical Analysis. Mean inter-estrous intervals were analyzed by a least-squares analysis of variance within each period with treatment mean comparisons evaluated by protected least significant difference test (LSD; Snedecor and Cochran, 1980). Within each period, comparisons of daily mean concentrations of LH, P4 and PGFM were analyzed by an analysis of variance for a split-split-plot design (Snedecor and Cochran, 1980). Least significant difference tests were performed to detect differences among treatments.

Results

Mean inter-estrous intervals for C, Z and EB treatments are presented in table 1. Gilts receiving Z or EB during T2 (d 6 to 10 of the estrous cycle) and T3 (d 11 to 15 of the estrous cycle) had longer inter-estrous intervals than C gilts.
During T1 (d 1 to 5 of the estrous cycle), inter-estrous intervals were similar (P>.06) for C, Z and EB treatments.

Changes in plasma concentrations of P4 and LH in T1 gilts are shown in figures 1 and 2, respectively. Progesterone profiles were similar (P>.05) in C, Z and EB gilts. In all three treatments P4 increased in a linear fashion from d 1 through 7 of the estrous cycle. After d 7, P4 concentrations appeared to plateau because d 7 concentrations were similar (P>.05) to d 10 values. Mean plasma concentrations of LH were not different (P>.05) among C, Z and EB treatments. However, LH in EB-treated gilts was consistently lower on d 3 through 10 compared with C and Z gilts, and approached significance (P<.07) on d 5 through 10.

Plasma concentrations of P4 in C gilts assigned to T2 increased on d 4 through 6, and remained relatively constant through d 11 of the estrous cycle (figure 3). After d 11, P4 declined (P<.05) through d 15 in C gilts. Progesterone concentrations in EB and Z-treated gilts were similar (P>.05) to those of gilts through d 11. However, on d 12 and 15, Z and EB treatments resulted in higher (P<.05) mean plasma P4 concentrations compared with C gilts. Progesterone profiles of EB and Z gilts were similar (P>.05). Mean LH concentrations were not different (P>.05) between C and Z gilts assigned to T2 (figure 4). However, administration of EB reduced (P<.05) plasma LH concentrations on d 8 through 15 when compared with C- and Z-treated gilts.

**Figure 1.** Mean ± SE plasma concentrations of progesterone in gilts receiving zearalenone (Z), estradiol benzoate (EB) or no treatment (C) from d 1 to 5 of the estrous cycle.

**TABLE 1. INTER-ESTROUS INTERVALS FOR CONTROL AND TREATED GILTS**

<table>
<thead>
<tr>
<th>Periodb</th>
<th>Control</th>
<th>Zearalenone</th>
<th>Estradiol benzoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1–5)</td>
<td>19.8 ± .8c</td>
<td>19.0 ± .9c</td>
<td>22.6 ± 1.5c</td>
</tr>
<tr>
<td>2 (6–10)</td>
<td>20.2 ± .6c</td>
<td>44.2 ± 8.1d</td>
<td>34.4 ± 4.4d</td>
</tr>
<tr>
<td>3 (11–15)</td>
<td>19.6 ± .5c</td>
<td>36.0 ± 6.6d</td>
<td>42.4 ± 7.3d</td>
</tr>
</tbody>
</table>

aMeans ± SE.

bDays of the estrous cycle treatments were administered.

c,dMeans with different superscripts within rows are different (P<.05).
Changes in plasma concentrations of P4, LH and PGFM of T3 gilts are presented in figures 5, 6 and 7, respectively. Progesterone concentrations in C gilts declined (P<.05) from d 11 through 20 of the estrous cycle. EB and Z-treated gilts did not exhibit a reduction (P>.05) in P4. When compared with C animals, those receiving EB and Z had higher (P<.05) P4 concentrations on d 16, 17 and 20. In C animals assigned to T3, LH concentrations were similar (P>.05) through d 16. On d 17, LH increased (P<.05) and remained elevated through d 20.
Figure 4. Mean ± SE plasma concentrations of luteinizing hormone (LH) in gilts receiving zearalenone (Z), estradiol benzoate (EB) or no treatment (C) from d 6 to 10 of the estrous cycle. This increase during the latter stages of the estrous cycle occurred because three of the C gilts returned to estrus in 20 d or less and portions of their preovulatory LH surge were included in mean values for d 17 and 20. When compared with C gilts, gilts receiving Z had similar (P>.05) concentrations of LH on d 9 through 16 and lower (P<.05) plasma concent-

Figure 5. Mean ± SE plasma concentrations of progesterone in gilts receiving zearalenone (Z), estradiol benzoate (EB) or no treatment (C) from d 11 to 15 of the estrous cycle.
trations on d 17 and 20. Gilts receiving EB exhibited lower (P<.05) plasma concentrations of LH than C and Z gilts from d 13 through 20.

PGFM remained between 100 and 300 pg/ml during d 9 to 20 of the estrous cycle in EB gilts assigned to T3. In C and Z gilts, PGFM concentrations were similar (P>.05) to those of EB gilts on d 9 through 11. In C gilts, PGFM concentrations were higher (P<.05) on d 13 through 20 compared with EB gilts and on d 16 and 17.

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**Figure 6.** Mean ± SE plasma concentrations of luteinizing hormone (LH) in gilts receiving zearalenone (Z), estradiol benzoate (EB) or no treatment (C) from d 11 to 15 of the estrous cycle.

**Figure 7.** Mean ± SE plasma concentrations of 13, 14-dihydro-, 15-keto-prostaglandin F$_{2\alpha}$ (PGFM) in gilts receiving zearalenone (Z), estradiol benzoate (EB) or no treatment (C) from d 11 to 15 of the estrous cycle.
when compared with Z gilts. Gilts receiving EB had lower PGFM concentrations on d 13 through 15 compared with Z gilts.

Discussion

Days 6 to 10 and 11 to 15 of the estrous cycle are periods during which the daily consumption of 20 mg of Z by mature cyclic gilts extends inter-estrous intervals due to maintenance of functionally competent corpora lutea. Similarly, 2 mg of EB daily during the same time periods prolongs the estrous cycle. These results are in agreement with previous studies, which have demonstrated the luteotrophic effects of estrogenic compounds between d 9.5 and 12 post-estrus in swine (Kidder et al., 1955; Gardner et al., 1963; Cantley et al., 1982; Saunders et al., 1983; Geisert et al., 1987). Additionally, if length of inter-estrous intervals is an accurate index of the relative effectiveness of each treatment as a luteotrophic stimulus, then non-steroidal estrogenic compounds (Z) and steroidal estrogens (EB) at the doses investigated appear to be similar.

The inhibitory effects of EB on LH secretion observed in this study are consistent with those reported in ovariectomized (Cox and Britt, 1982; Diekman et al., 1986) and cyclic (Guthrie and Rexroad, 1981) pigs. Regardless of time of administration during the estrous cycle prior to d 16, a daily dose of 2 mg of EB consistently lowered mean plasma LH concentrations relative to those in C animals.

Conversely, Z treatment had no significant effect on mean plasma concentrations of LH. Diekman et al. (1986) demonstrated that the suppressive action of a 1 mg/kg of body weight dose of Z on LH concentrations was similar to that of a .1 mg/kg body weight dose of EB. This dose of Z is approximately 10 times higher than that utilized in the present study. Differences in the results of the two studies may, therefore, be attributed to wide variation in doses of Z administered.

Establishment of pregnancy and extended luteal function in swine after d 12 post-breeding depends, at least partially, on LH. This is evident in that removal of LH after d 12 of gestation, either by the administration of an LH antisera (Spies et al., 1967) or by disruption of the hypothalamo-hypophyseal axis (du Mesnil du Buisson, 1966; Anderson et al., 1967), results in luteal regression and abortion in pregnant gilts. This can be prevented by administration of LH and estrogens. In the present study, corpora lutea were maintained in EB-treated gilts whose mean plasma LH concentrations were .1 to .3 ng/ml from d 8 to 15 and 11 to 20 of the estrous cycle. Corpora lutea maintained in the presence of these low LH concentrations exhibited lifespans similar to those that were maintained with LH concentrations of .6 to 1.0 ng/ml (Z gilts). This suggests that the amount of LH from the pituitary gland needed for maintenance of luteal tissue in the pig is small. Consequently, it seems doubtful that alterations in mean plasma LH concentrations is a vital component of Z or EB-induced luteal maintenance in the pig.

The increase in plasma PGFM concentrations from d 13 through 18 of the estrous cycle observed in C gilts was associated with luteolysis and supports numerous studies that have demonstrated the luteolytic influence of PGF₂α in the pig during this time (Diehl and Day, 1974; Moeljono et al., 1977). Guthrie and Rexroad (1981) demonstrated that measuring PGFM in peripheral circulation provides an accurate estimation of PGF₂α secretion by the uterus. If PGFM concentrations are representative of uterine PGF₂α secretion, then results of the present study suggest that EB prevents release of PGF₂α from the uterus, while Z appears only to reduce the amount which is secreted. Similar results concerning PGFM concentrations in gilts receiving no treatment or EB have been reported by Guthrie and Rexroad (1981), and support the concept that one way in which estrogens cause luteal maintenance is by preventing an increase in peripheral plasma concentrations of PGF₂α (Bazer and Thatcher, 1977; Frank et al., 1978).

Conversely, Hunter and Poyser (1982) observed similar concentrations of PGF₂α in the uterine vein of pregnant and non-pregnant gilts from d 7 to 21 post-breeding. These authors suggested that exocrine secretion of PGF₂α into the uterine lumen of pigs under the influence of estrogens does not provide a sufficient explanation for maintenance of luteal tissue.

Although PGFM concentrations in Z gilts were not as great and did not remain elevated as long as those observed in C animals in the T3 group, plasma concentrations of PGFM from the uterus increased substantially and corpora lutea were maintained. One possible explanation for this observation is that Z, due to its lower estrogenic activity (Katzenellenbogen et
al., 1979), was not as efficient as EB in preventing increased secretion of PGF\(_{2\alpha}\). Thus, PGFM concentrations observed in the peripheral circulation might represent an amount of PGF\(_{2\alpha}\) that was not luteolytic. Admittedly, whether concentrations of 1,100 pg/ml of PGFM are able consistently to cause luteolysis in untreated cyclic gilts is not known. However, two of five gilts in the C group during T3 exhibited plasma PGFM concentrations of 890 to 1,100 pg/ml from d 13 to 17 of the estrous cycle and a mean inter-estrus interval of 19.5 d.

Another explanation of how Z prevents luteolysis is a local luteotrophic effect on the ovary. Kraeling et al. (1975) demonstrated that estradiol injections can maintain corpora lutea of hysterectomized gilts in the presence of a luteolytic dose of PGF\(_{2\alpha}\). Additionally, implants of embryonic extracts containing high amounts of estrogens placed directly into the corpora lutea result in maintenance of lutea tissue (Ball and Day, 1982). These studies suggest that estrogens can have a direct effect at the ovary in causing luteal maintenance. Perhaps, Z caused luteal maintenance primarily by a direct luteotrophic effect, while EB, at the dose investigated, protected luteal tissue from prostaglandins by preventing their release from the uterus and by a direct effect at the ovary.

In summary, Z, a non-steroidal estrogen, and EB, a steroidal estrogen, both caused luteal maintenance in the pig when administered from d 6 to 10 and 11 to 15 of the estrous cycle. A 2-mg dose of EB suppressed LH secretion, while a 20-mg dose of Z had no effect on plasma LH during the periods when both compounds cause luteal maintenance. During the period when porcine luteal tissue is sensitive to PGF\(_{2\alpha}\), a 2-mg dose of EB prevented increased secretion of PGF\(_{2\alpha}\), while the 20-mg dose of Z did not. Based on these observations, we speculate that alterations in mean plasma concentrations of LH do not play a major role in EB and Z induced luteal maintenance. Zearalenone, at the dose investigated, might primarily cause luteal maintenance by a direct effect at the ovarian level, while EB may extend the lifespan of the corpora lutea through actions at both uterine and ovarian levels.

**Literature Cited**


