EFFECT OF ZEARALENONE ON DAYS 7 TO 10 POSTMATING ON INTRAUTERINE ENVIRONMENT AND MIGRATION OF EMBRYOS IN SOWS

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ABSTRACT

On d 7 to 10 postmating, first-litter sows were fed either a control diet or a diet containing zearalenone (ZEN; 1 mg/kg body weight). Surgery was performed on either d 9, 11 or 13 postmating to collect blastocysts and uterine flushings. The rostral and caudal portion of each uterine horn was flushed with phosphate buffered saline, and the blastocysts were separated from the recovered solution. Uterine flushings were analyzed for total Ca, Mg, Zn, estradiol-17β (E217β) and progesterone (P4). Administration of ZEN did not affect the number of blastocysts recovered or the position of embryos within the uterus on d 9 or 11. Blastocysts recovered on d 13 were filamentous and could not be enumerated. Total Ca in uterine flushings of control sows was higher (P<.001) on d 11 than on d 9 or 13, but intrauterine Ca of ZEN-treated sows did not vary by sampling day (P>.05) and was lower (P=.01) than that of controls on d 11. Total intrauterine Mg of ZEN sows was greater (P=.002) than of control sows on d 11 and 13, and total intrauterine Zn of ZEN sows was greater than that in control sows on d 13. There were no differences in total intrauterine P4 or E217β among ZEN-treated and control sows on d 9, 11 or 13 postmating. Serum concentrations of 13, 14-dihydro-15-ketoprostaglandin F2α (PGFM) increased from d 9 to 13 in control and ZEN-treated sows, but there were no differences between treatment groups. These data indicate that treatment with ZEN on d 7 to 10 postmating did not alter spacing of blastocysts or inhibit E217β secretion by blastocysts through d 11 postmating. Intrauterine environment in ZEN-treated sows was altered on d 11 and 13 postmating, as evidenced by changes in concentrations of intrauterine cations, but no changes were detected in P4 or E217β.

(Key Words: Sows, Zearalenone, Blastocysts, Uterus.)

Introduction

Zearalenone (ZEN) is a mycotoxin with estrogenic activity that is produced by the grain mold Fusarium roseum (Mirocha et al., 1977; Christensen, 1979). Swine are the domestic species most commonly affected by consumption of ZEN. Prepubertal gilts exhibit signs of hyperestrogenism when they consume feed contaminated with ZEN (Kurtz et al., 1969; Mirocha et al., 1977). ZEN fed to mature female swine has been reported to cause nymphomania, anestrus, ovarian atrophy and pseudopregnancy (Vanyi et al., 1976; Mirocha et al., 1977; Chang et al., 1979; Long et al., 1982). Recently, we showed that sows fed ZEN on d 7 to 10 postmating had loss of blastocysts and corpora lutea, whereas sows fed the same daily dosage of ZEN on d 2 to 6 or d 11 to 15 postmating maintained pregnancy (Long and Diekman, 1986).

Intrauterine migration and spacing of porcine blastocysts occurs during d 7 to 12 postmating (Anderson and Parker, 1976; Anderson, 1978). Profound morphologic and metabolic changes occur in blastocysts d 11 to

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14 postmating (Perry and Rowlands, 1962; Perry et al., 1973; Anderson, 1978; Geisert et al., 1982b). Interaction between the establishment of pregnancy in the sow (Perry et al., 1976; Geisert et al., 1982a). The purpose of this study was to investigate the effect of ZEN consumed by sows d 7 to 10 postmating on intrauterine environment and spacing of blastocysts.

Materials and Methods

Experimental Design. Each of 31 first-litter crossbred sows in naturally occurring postweaning estrus was mated to two fertile boars within a 16-h period. Purified ZEN was added to a corn-soybean swine gestation ration on an individual basis to give each ZEN-treated sow 1 mg/kg body weight per day. The ZEN was dissolved in ethanol and poured onto .5 kg of feed. The ethanol was allowed to evaporate. The sow consumed the ZEN-treated feed before an additional 1.5 kg of feed was given. Fifteen sows were fed ZEN on d 7 and 8 postmating (surgery on d 9), or on d 7 to 10 (surgery on d 11 or 13). Five control sows for each day of surgery (9, 11 and 13) were fed the same ration to which ethanol without ZEN had been added. One sow was not pregnant on d 9 postmating and was eliminated from the study.

Collection of Blastocysts and Uterine Flusions. Blastocysts and uterine flushings were obtained at surgery on d 9, 11 or 13 postmating. Sows were initially anesthetized with ketamine and xylazine, intubated, and a surgical plane of anesthesia was maintained with halothane and nitrous oxide. The uterus was exposed via a midventral abdominal incision. A blunt incision was made at the midpoint of each uterine horn and a glass cannula (diameter=1cm) with a smooth, flared end was inserted. The uterus was secured around the cannula with umbilical tape. The segments of each uterine horn rostral (proximal to ovary) and caudal to the inserted cannula were flushed separately with 40 ml of phosphate buffered saline (PBS). For each flushing the fluid was injected via a blunt-tipped needle at either the cervical or ovarian end of the uterine horn with the collecting cannula directed toward the end being injected. Blastocysts were separated from the flush solution by a pipette. A blood sample was taken from the jugular vein after sows were anesthetized, but before the abdominal incision was made, to determine serum concentrations of 13, 14-dihydro-15-ketoprostaglandin F2α (PGFM).

Measurement of Minerals and Hormones. Concentrations of calcium (Ca), magnesium (Mg) and zinc (Zn) in uterine flushings were determined by atomic absorption spectrometry. Concentration of estradiol-17β (E217β) in uterine flushings was quantified by radioimmunoassay (RIA; Kesler et al., 1977; Long and Diekman, 1984). Recovery of 3H-E217β added to uterine flushings before extraction with ethyl ether averaged 93 ± 2%. Recovery of five quantities of E217β (.01 to .25 ng) added to 500 µl of charcoal-stripped uterine flushings was 98.5 ± 3.1%. Inhibition curves generated by uterine flushings in volumes ranging from 250 to 1,000 µl were parallel to standard curves. Sensitivity of the assay was 4 pg/tube. Intra-assay coefficient of variation was 10.3%.

Concentration of progesterone (P4) in uterine flushings was determined by RIA (Niswender, 1973; Long and Diekman, 1984). Recovery of 3H-P4 added to uterine flushings before extraction with ethyl ether averaged 90 ± 2%. Recovery of seven quantities of progesterone (.10 to 1.0 ng) added to 500 µl of charcoal-stripped uterine flushings was 96.4 ± 3.8%. Inhibition curves generated by uterine flushing volumes ranging from 250 to 1,000 µl were parallel to standard curves. Sensitivity of the assay was 5 pg/tube. Intra-assay coefficient of variation was 9.8%.

Serum concentration of PGFM was measured by RIA, as described by Guthrie and Rexroad (1981). Recovery of 3H-PGFM added to porcine serum before double extraction with ethyl ether averaged 83 ± 4%. Recovery of five quantities of PGFM (20 to 320 pg) added to 200 µl of charcoal-stripped serum was 92.0 ± 4.2%. Inhibition curves generated by serum volumes ranging from 50 to 300 µl were parallel to standard curves. Sensitivity of the assay was 2 pg/tube. Intra-assay coefficient of variation was 11.2%.

Statistical Analyses. Total contents of Ca, Mg, Zn, E217β and P4 in uterine flushings were determined by multiplying concentration of the substance in each uterine quadrant by volume of flush recovered from the respective quadrant. The four values determined for each constituent for each sow were combined after initial analysis of variance showed values were similar among quadrants of the uterus. Values for each variable in uterine flushings and distribution of
TABLE 1. NUMBER OF BLASTOCYSTS COLLECTED FROM ROSTRAL AND CAUDAL HALVES OF UTERINE HORNS ON DAY 9 TO 11 POSTMATING IN SOWS FED ZEARALENONE (ZEN) DAY 7 TO 10 POSTMATING

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>Portion of uterine horn</th>
<th>Blastocysts collected</th>
<th>Rostral</th>
<th>Caudal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>8.4 ± 1.1 b</td>
<td>2.8 ± 1.0 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>5.6 ± 0.5 b</td>
<td>7.4 ± 1.0 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZEN</td>
<td>9</td>
<td>7.0 ± 1.7 b</td>
<td>3.0 ± 2.0 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>5.0 ± 1.3 b</td>
<td>6.6 ± 1.1 b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SE for five sows in each treatment x day group.

b,cMeans within a row without a common superscript differ (P<.03).

Results

Administration of ZEN did not affect the number or distribution of blastocysts within the uterus on d 9 and 11 postmating (table 1). Filamentous blastocysts were recovered from each quadrant on d 13. However, the blastocysts were fragmented by the collection process and could not be enumerated. On d 9, a greater number of blastocysts (P=.03) was recovered from the rostral than from the caudal portion of the uterine horn in both control and ZEN sows. On d 11, blastocysts were evenly distributed between the rostral and caudal portions of the uterine horn in both groups of sows.

Total Ca in the uterine flushings of control sows increased from d 9 to d 11 postmating (P<.001) and then decreased (P=.001) from d 11 to 13 (table 2). Total intrauterine Ca from ZEN-treated sows did not vary by sampling day (P>.05). When ZEN-treated sows were compared with controls, Ca content was the same on d 9 and 13 and was less (P=.01) on d 11. Mean recovery volume of PBS was 89.4, 87.5 and 78.1% on d 9, 11 and 13, respectively. No differences in mean recovery volume were found between ZEN and control treatment, but less PBS was recovered on d 13 than on d 9 or 11 (P=.02).

Total intrauterine Mg of control sows did not change (P>.05) from d 9 to d 11, but it increased (P=.002) between d 11 and 13.

TABLE 2. TOTAL CALCIUM, MAGNESIUM AND ZINC (µg) RECOVERED IN UTERINE FLUSHINGS OF SOWS TREATED WITH ZEAORALENONE (ZEN) ON DAY 7 TO 10 AFTER MATING

<table>
<thead>
<tr>
<th>Days after mating</th>
<th>Calciuma</th>
<th>Magnesiuma</th>
<th>Zinca</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>ZEN</td>
<td>Control</td>
</tr>
<tr>
<td>9</td>
<td>386 ± 168 bd</td>
<td>460 ± 200 bd</td>
<td>51 ± 14 bd</td>
</tr>
<tr>
<td>11</td>
<td>1687 ± 430 cd</td>
<td>745 ± 284 be</td>
<td>170 ± 44 bd</td>
</tr>
<tr>
<td>13</td>
<td>421 ± 51 bd</td>
<td>531 ± 164 bd</td>
<td>716 ± 36 cd</td>
</tr>
</tbody>
</table>

Mean ± SE for five sows in each treatment x day group.

b,cMeans within a column for each cation without a common superscript differ (P<.01).

d,eMeans within the same row for each cation without a common superscript differ (P<.03).
TABLE 3. TOTAL ESTRADIOL-17β AND PROGESTERONE (NG) RECOVERED IN UTERINE FLUSHINGS OF SOWS TREATED WITH ZEARALENONE (ZEN) ON DAY 7 TO 10 AFTER MATING

<table>
<thead>
<tr>
<th>Days after mating</th>
<th>Progesteronea</th>
<th>Estradiol-17βa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>ZEN</td>
</tr>
<tr>
<td>9</td>
<td>30 ± 3b</td>
<td>21 ± 6b</td>
</tr>
<tr>
<td>11</td>
<td>51 ± 9bc</td>
<td>31 ± 7b</td>
</tr>
<tr>
<td>13</td>
<td>67 ± 11c</td>
<td>72 ± 14c</td>
</tr>
</tbody>
</table>

aMean ± SE for five sows in each treatment X day group.
b,cMeans within a column for each steroid without a common superscript differ (P<.05). No differences (P>.05) in intrauterine P4 or E2 between control and ZEN sows were found on any of the sampling days.

postmating (table 2). Intrauterine Mg from ZEN-treated sows increased (P<.001) from d 9 to 11 and remained high on d 13. Sows consuming ZEN had the same amount of intrauterine Mg as control sows on d 9, but increased amounts were found on d 11 (P<.001) and d 13 (P<.03) postmating.

Total intrauterine Zn in control sows did not change (P>.05) from d 9 to 13 (table 2). Total intrauterine Zn of the ZEN-treated sows increased (P<.01) between d 11 and 13 and was greater (P<.01) than that in control sows on d 13 postmating.

Total intrauterine P4 of control sows did not differ between d 9 and 11 or d 11 and 13 (P>.05), but it was higher on d 13 than on d 9 (P<.01; table 3). Intrauterine P4 in ZEN-treated sows was similar between d 9 and 11 (P>.05), but it was higher on d 13 than on d 11 or on d 9 (P<.01). There were no differences in intrauterine P4 between control and ZEN sows on any of the sampling days (P>.05).

Intrauterine content of E217β in control sows increased (P<.01) from d 9 to d 11 postmating, and then it decreased (P<.03) from d 11 to d 13 (table 3). Intrauterine content of E217β of sows that received ZEN also increased (P<.05) from d 9 to 11, but no changes occurred from d 11 to 13 (P=.10). Intrauterine E217β did not differ between control and ZEN sows on any of the sampling days.

Serum concentrations of PGFM increased from d 9 to 13 (P=.025; table 4). However, serum concentrations of PGFM on d 9, 11 or 13 postmating were similar (P>.05) in ZEN and control sows on each of the sampling days.

Discussion
In a previous experiment, sows consuming feed containing 1 mg ZEN/kg body weight on d 7 to 10 postmating were not pregnant at d 30 to 32 postmating (Long and Diekman, 1986). Profiles of serum concentrations of progesterone indicated that corpora lutea (CL) in these gilts regressed between d 20 and 28 postmating. Sows consuming ZEN on d 2 to 6 or d 11 to 15 postmating had normally developed fetuses at d 30 to 32 of gestation, indicating that the period from d 7 to d 10 postmating is the "critical" period for ZEN to exert detrimental effects on early pregnancy in swine (Long and Diekman, 1986). This is consistent with results of E2 treatment of mated gilts; treatment on d 9

TABLE 4. SERUM CONCENTRATIONS OF PGFM (NG/ML) IN SOWS TREATED WITH ZEARALENONE (ZEN) ON DAY 7 TO 10 POSTMATING

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days postmatinga</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Control</td>
<td>.12 ± .01</td>
</tr>
<tr>
<td>ZEN</td>
<td>.16 ± .02</td>
</tr>
</tbody>
</table>

aMean ± SE for five gilts in each treatment X day group.
to 10 of estrus was much more effective in causing embryonic death than treatment on d 12 to 13 (Pope et al., 1986). In another study (Long and Diekman, 1984), sows fed approximately the same dosage of ZEN from d 2 to 15 postmating had failure of embryonic development even though remnants of embryonic membranes were present in the uterus. These sows also retained their CL, most likely due to the estrogenic effects of ZEN on d 11 to 15 postmating, because treatment of sows with estrogens on d 11 and 12 of the estrous cycle can cause retention of CL (Kidder et al., 1955; Gardner et al., 1963). Prolonged CL function may require estrogenic stimulation on d 11 and d 14 to 16 of the estrous cycle (Geisert et al., 1987).

In the sow, the intrauterine process of blastocyst migration and spacing begins d 7 to 10 postmating (Perry and Rowlands, 1962; Perry et al., 1973; Anderson, 1978; Geisert et al., 1982b). Rapid changes in structure and metabolism of blastocysts associated with establishment of pregnancy occur on d 11 to 13 postmating (Perry and Rowlands, 1962; Anderson, 1978; Geisert et al., 1982a,b). Thus, it seemed likely that ZEN on d 7 to 10 might alter migration of blastocysts. In the current study, however, ZEN did not alter the number of blastocysts recovered or their caudal or rostral position within the uterus.

Another possible mechanism whereby ZEN is detrimental to development of blastocysts on d 7 to 10 postmating may be that exposure of blastocysts to ZEN inhibits estradiol synthesis on d 11 and 12 (Heap et al., 1975; Perry et al., 1976; Gadsby et al., 1980). Total recoverable E$_2$17β in uterine flushings from pregnant gilts increased with blastocyst elongation and then decreased by d 14 (Geisert et al., 1982a). If we assume that the intrauterine E$_2$17β measured in our current study originated from blastocysts, then treatment with ZEN had no effect on E$_2$17β secretion by the blastocysts.

In the present study, intrauterine Ca peaked on d 11 in control sows, which agrees with previous work (Geisert et al., 1982a). In ZEN-treated sows intrauterine Ca was lower than in controls on d 11, which is consistent with observations in pregnant gilts treated with E$_2$ on d 9 to 10 (Morgan et al., 1987). In contrast to changes in Ca, treatment with ZEN increased intrauterine content of Mg and Zn, suggesting enhancement of secretion of these elements. Administration of E$_2$ on d 9 of pregnancy appears to advance the uterine secretory response (Morgan et al., 1987), but blastocysts survive and elongate in this abnormal environment through d 12. Blastocysts from sows treated with E$_2$ on d 9 were degenerate on d 16 (Morgan et al., 1987), suggesting that blastocysts cannot continue to survive in the abnormal environment, or that changes in endometrial secretion essential for embryonic survival do not occur. In rodents, estrogen induces a state of refractoriness in the endometrium to further estrogenic stimulation during the decidual process (Yoshinaga, 1980). In swine, it appears that endometrial secretion of Ca and of specific proteins does not respond positively to an estrogenic stimulus until d 11 after estrus (Geisert et al., 1987), but that premature estrogenic stimulation may prevent secretory changes that normally occur during d 11 to 12 postmating (Morgan et al., 1987).

No differences in intrauterine content of E$_2$17β or P$_4$ were noted between control and ZEN-treated sows. A change may have occurred in the relative estrogen to progesterone ratio, if the estrogenic effect of the ZEN is taken into account. In previous studies, we have shown that serum E$_2$17β in ZEN sows was normal during the interval investigated in the current study (Long and Diekman, 1984, 1986), but there may have been a reduction in serum concentrations of P$_4$. Because ZEN has a short half-life in blood (Christensen, 1979), the additive estrogenic activity would likely occur during the treatment period (d 7 to 10), or shortly thereafter (d 11).

In the present study, ZEN did not alter serum concentrations of PGFM. A decrease in the amount of PGF2α reaching the ovary is hypothesized to be caused by pregnancy-induced changes in endometrial metabolism and to be responsible for retention of CL in the pregnant sows (Gleeson et al., 1974; Moeljono et al., 1977). If the changes in endometrial metabolism associated with loss of the CL in ZEN-treated sows are similar to those in untreated, nonpregnant sows, one would expect the amount of PGFM secretion to be increased above that in untreated pregnant sows on d 13 postmating (Shille et al., 1979; Guthrie and Rexroad, 1981). In a previous study (Long and Diekman, 1986), pregnant gilts treated with ZEN on d 7 to 10 after mating had regression of CL between d 20 and 28. Treatment with E$_2$ on a single day increased the interestrus interval to 30 d (Geisert et al., 1987), but treatment
with estradiol on d 11 to 15, or d 11 and d 14 to 16, was required to increase the interestrus period to more than 60 d. If a change in PGFM occurred because of ZEN treatment, it may have been after our last sampling time on d 13.

In summary, treatment of sows with ZEN on d 7 to 10 postmating did not influence migration of blastocysts within the uterine horns, intrauterine content of E2 17β or P4 or serum concentrations of PGFM on d 9, 11 or 13 postmating. Intrauterine content of Ca, Mg and Zn indicated that qualitative differences existed in the uterine environment of ZEN-treated and control sows on d 11 and 13 postmating.

Literature Cited