PITUITARY GONADOTROPIN LEVELS, ENDOCRINE GLAND AND REPRODUCTIVE ORGAN WEIGHTS OF GILTS FED A DITHIOCARBAMOYLHYDRAZINE (ICI 33828) 1, 2, 3

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Summary

The experiment was designed to test the effects of feeding gilts various levels of ICI 33828 for 16 days beginning on day 3, 13 or 18 of the estrous cycle. On the last day of feeding or on day 3 or 25 of gestation following withdrawal of the compound, the uterus, ovaries and various endocrine glands were weighed and the anterior pituitary glands were stored for measurement of FSH and LH.

Gilts slaughtered on the last day of ICI 33828 feeding had heavier (P < .05) adrenals if feeding began on day 18 as compared with those fed on day 3 or 13. The ovaries were heavier (P < .05) on the last day of feeding when ICI 33828 feeding was started on day 3 as compared with those started on day 18.

Relative concentration of anterior pituitary FSH was the same (P > .05) for the control gilts on day 18 as it was for the gilts slaughtered on the last day of feeding when ICI 33828 feeding started on day 13 or 18, indicating that FSH release was suppressed. However, the gilts fed ICI 33828 commencing on day 3 had a relative lower concentration of (P < .05) anterior pituitary FSH on the last day of feeding as compared with the control gilts slaughtered on day 18, indicating that synthesis of FSH was inhibited. There were no follicles > 6 mm diameter or corpora lutea on the ovaries of any of the gilts slaughtered on the last day of ICI 33828 feeding, and there was a tendency for uterine weights to decline after luteal regression which supports the contention that the release of FSH was suppressed. Release of LH was suppressed in gilts fed ICI 33828 regardless of the day feeding started since relative LH concentrations were similar to (P > .05) those found in the control gilts on day 13 or 18. Suppression of LH release is supported by the lack of ovulation and estrus in all of the gilts fed ICI 33828.

Of the traits studied on day 3 of gestation, only relative LH concentration was lowered (P < .005) by the 116 mg dose level when compared with 58 or 232 mg levels fed. There were no effects (P > .05) of ICI 33828 feeding prior to mating on any traits measured at day 25 of gestation.

Introduction

A dithiocarbamoylhydrazine, ICI 33828, inhibits estrus and ovulation in some species (Paget, Walpole and Richardson, 1961; Gerrits and Johnson, 1964; Polge, 1964; Stratman and First, 1965, 1969) and ovulation in the human (Bell et al., 1962; Meats, 1962). It is also known to inhibit thyroid function (Tulloch, Crooks and Brown, 1963). The addition of iodinated casein containing thyroxine to a ration containing ICI 33828 failed to alleviate its estrus inhibiting characteristics (Stratman, 1970). The dithiocarbamoylhydrazine, ICI 33828, fed to the adult male rat reduced the pituitary content of FSH (Brown, 1963). Total gonadotropin content was reduced in the same proportion as was the FSH content; however, there was no direct measurement of LH content. ICI 33828 has been reported (Bell et al., 1962) to reduce human urinary gonadotropins. Stormshak et al. (1970) and Garbers and First (1969) reported that ICI 33828 increases the pituitary content of FSH and LH in gilts and sows. These experiments and the side effects of ICI 33828 suggests that it acts on the hypothalamus as indicated by Malven (1969).

The purpose of this experiment was to evaluate the effect of ICI 33828 fed at various levels for 16 days, commencing at day 3, 13 or 18 of the estrous cycle, on the pituitary and other endocrine glands and the reproductive organs of gilts.

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2 Dithiocarbamoylhydrazine (ICI 33828) Ayerst Laboratories Incorporated, New York, New York.
3 Supported in part by funds from Tri-State Breeders Cooperative, Westby, Wisconsin; The American Meat Institute, Chicago, Illinois and Murphy Products Company, Burlington, Wisconsin.
4 Fellow, N.I.H. during experiment. Present address: Institute for Enzyme Research, University of Wisconsin, Madison.
5 Department of Meat and Animal Science, Paper No. 557.
Materials and Methods

Data were gathered from the same experimental animals as reported on by Stratman and First (1965, 1969). One supplementary group (Group VIII, Subgroups A, B, C) of gilts, taken at random from the same population at the same time as the other groups was slaughtered on day 3, 13 or 18 of the estrous cycle to establish a control level of pituitary gonadotropins. These control gonadotropins were compared with pituitary gonadotropins of gilts slaughtered on the last day of feeding 116 mg of ICI 33828 per day for 16 days starting on day 3, 13 or 18 of the estrous cycle (Group VII, Subgroups A, B, C) (Experiment I). In Experiments II and III, gilts were started on 16 days feeding of various levels of ICI 33828 on day 3, 13 or 18 of the estrous cycle and slaughtered on day 3 and day 25 of gestation, respectively. Statistical analyses of data from these groups were on the basis of multiple experiments (table 1).

The pituitary, thyroid and adrenal glands were trimmed and weighed and the anterior lobes were dissected from the pituitary glands, weighed, frozen and stored at --10°C for future gonadotropin assays. Prior to assay, glands were partially thawed, homogenized in distilled water, lyophilized and stored in a desiccator. Ovaries and uteri were weighed.

The Steelman and Pohley (1953) HCG-S assay as modified by Kirkpatrick et al. (1967) was used to estimate follicle stimulating hormone (FSH) activity of the anterior pituitary glands. Doses of 2.25, 4.50 and 9.00 mg of each lyophilized anterior pituitary gland were used and 20 IU of HCG augmented each dose. Covariance analysis was used to adjust the ovarian weight to the average body weight. Relative FSH activity was obtained by adding the adjusted ovarian weight increments of the three dosage levels (ovarian weight increment = ovarian weight of individual rat receiving anterior pituitary powder and HCG minus average ovarian weight of all rats receiving HCG alone).

The OAAD assay of Parlow (1961) as modified by Kirkpatrick et al. (1967) was used to estimate the luteinizing hormone (LH) activity of the anterior pituitary glands. Doses of 0.15, 0.30 and 0.60 mg of each lyophilized anterior pituitary gland were assayed. Multiple covariance analysis was used to adjust the ascorbic acid content of the ovaries to the average ovarian weight (Sakiz and Guillemin, 1963) and to the average body weight. The adjusted ascorbic acid content of the rat ovaries for the three dosage levels was used as an estimate of relative LH content of each individual pituitary. Ascorbic acid content of the ovary is inversely related to LH content of the pituitary.

Validity of these bioassays was tested according to Bliss (1952) and Emmens (1948). These procedures were previously described by Howland et al. (1966), Kirkpatrick et al. (1967), Short et al. (1968a, b) and Stormshak et al. (1970). Linear and quadratic components of the main effect of dose and any other dose interaction with other main effects were determined.

All other data were tested by analysis of variance.

Results and Discussion

Experiment I—Control Estrous Cycle and the Last Day of Feeding

The endocrine gland and reproductive organ mean weights and relative concentrations of the anterior pituitary FSH and LH of gilts slaughtered on day 3, 13 or 18 of the control estrus cycle (Group VIII, Subgroups A, B, C) are compared with the gilts slaughtered on the last day of feeding ICI 33828 for 16 days (Group VII, Subgroups A, B, C) in table 2.

Endocrine Gland Weights. Neither the total wet pituitary weight nor the wet weight of the anterior lobe was significantly (P> .05) influenced by the day on which the control gilts (Group VIII) were slaughtered nor by the day when the feeding of ICI 33828 was
### TABLE 2. MEAN ENDOCRINE GLAND AND REPRODUCTIVE ORGAN WET WEIGHTS AND GONADOTROPIN CONCENTRATIONS OF GILTS FED ICI 33828 FOR 16 DAYS AND OF CONTROL GILTS

<table>
<thead>
<tr>
<th>Group</th>
<th>ICI 33828 mg/day</th>
<th>Subgroup</th>
<th>Day of estrus cycle started on treatment</th>
<th>Autopsied</th>
<th>Pituitary weight (g)</th>
<th>Anterior pituitary weight (g)</th>
<th>Thyroid weight (g)</th>
<th>Adrenal weight (g)</th>
<th>Ovaries weight (g)</th>
<th>Uteri weight (g)</th>
<th>HCG-S (FSH)</th>
<th>OAAD (LH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIII</td>
<td>0</td>
<td>A</td>
<td>day 3 of cycle</td>
<td>0.25</td>
<td>0.17</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>298</td>
<td>440</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>day 13 of cycle</td>
<td>0.29</td>
<td>0.21</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>460</td>
<td>339c,e,t</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>day 18 of cycle</td>
<td>0.23</td>
<td>0.17</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>579</td>
<td>365c,e,t</td>
</tr>
<tr>
<td>VII</td>
<td>116</td>
<td>A</td>
<td>last day of feeding</td>
<td>0.26</td>
<td>0.20</td>
<td>6.1</td>
<td>3.7</td>
<td>7.8b</td>
<td>428</td>
<td>...</td>
<td>469</td>
<td>338c,e,t</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>last day of feeding</td>
<td>0.24</td>
<td>0.18</td>
<td>9.8</td>
<td>3.5</td>
<td>7.1</td>
<td>192</td>
<td>...</td>
<td>648*</td>
<td>347c,e,t</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>last day of feeding</td>
<td>0.25</td>
<td>0.17</td>
<td>5.7</td>
<td>4.7*</td>
<td>4.6</td>
<td>88</td>
<td>...</td>
<td>586*</td>
<td>291</td>
</tr>
</tbody>
</table>

- **Significantly (P<.01) different from day 13 or 3.
- **Significantly (P<.05) different from only day 18.
- * Values having different superscripts are significantly (P<.05) different.
- A Mean rat ovarian weight increments of three dose levels.
- B Mean rat ovarian ascorbic acid content of three dose levels. Ascorbic acid content of the ovary is inversely related to LH content of the pituitary.

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Stratman and First (1969) studied the effect of ICI 33828 on various endocrine organs of gilts. The weights of the pituitary, thyroid, and adrenals were measured, and the relative concentration of FSH was determined.

- **Relative Concentration of FSH:** The relative concentration of FSH increased from day 3 to day 13 to day 18 in the control gilts (Group VIII) and in the gilts fed 116 mg of ICI 33828 and slaughtered on the last day of treatment (Group VII). The relative concentration of FSH was significantly higher in the pituitaries of gilts fed ICI 33828 on day 18 than in the control gilts on day 13.

- **Reproductive Organ Weights:** The weights of the ovaries were influenced by the stage of regression of corpora lutea. Gilts slaughtered on the last day of treatment (Group VII) had heavier ovaries than those slaughtered on day 3 (Group VIII) when ICI 33828 feeding started on day 3. Those gilts started on day 18 had ovaries of intermediate weight.

- **FSH Concentration:** There was a significant decrease in the relative concentration of FSH in the anterior pituitaries of gilts fed ICI 33828 on day 3 as compared with those fed on day 13 or day 18. The relative concentration of FSH in the anterior pituitaries of gilts fed ICI 33828 on day 3 was significantly lower than in the control gilts (Group VIII) on day 3.

- **HCG-S and OAAD Levels:** The levels of HCG-S and OAAD were not significantly different between the groups.

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The study concluded that ICI 33828 at 116 mg/day significantly inhibited the synthesis of FSH and LH, as evidenced by the reduction in relative concentration of FSH and the decrease in ovarian weight. The results suggest that ICI 33828 may be a potential supplement for controlling the estrous cycle in gilts.
feeding (Group VII) than the control gilts on day 13 (Group VIII). There was no difference (P > .05) in relative concentration of FSH of pituitaries from control gilts slaughtered on day 18 (Group VIII) and those gilts started on ICI 33828 treatment on day 13 (Group VII) and day 18. Synthesis of FSH was probably nearly completed by the time an effective physiological level of ICI 33828 was reached in the gilts started on ICI 33828 treatment on days 13 and 18.

Although there are no control ovarian weights or ovarian structures available for comparison, we (Stratman and First, 1969) reported that there were no follicles (dia. 6 mm or > ) or corpora lutea on the ovaries of any of the gilts slaughtered on the last day of feeding ICI 33828 indicating that FSH release was suppressed. All ovaries of those gilts contained a large number (40.2) of secondary or visible follicles <6 mm in diameter (average 2 to 3 mm). Significantly (P < .01) fewer corpora albicantia (2.6) were evident on the last day of feeding when gilts were started on treatment on day 18 than on either day 3 (12.3) or day 13 (11.0). The tendency for uterine weights (table 3) to decline after the regression of corpora lutea supports the contention that release of FSH was suppressed.

One can postulate from these data that release of FSH was effectively suppressed in the gilts started on ICI 33828 treatment on days 3, 13 and 18 of the estrous cycle. Synthesis was partially inhibited when treatment started on day 3 and additional FSH was not synthesized when treatment started on day 13 or 18.

Walpole (1968) reported an inhibition of synthesis of both FSH and LH in the intact male rat dosed with ICI 33828, 100 mg/kg, once daily for 28 days. Our results are not in agreement with Garbers and First (1969) or Stormshak et al. (1970), who reported no inhibition of FSH synthesis; however, the feeding level and length of treatment were different than those reported here.

Evaluation of the bioassay for validity projected a linear response (P < .005) as dose level increased.

Relative Concentration of LH. Relative concentration among the control gilts (Group VIII) increased significantly (P < .05) from day 3 to day 13 and decreased significantly (P < .05) from day 13 to day 18.

Day of the estrous cycle when feeding of ICI 33828 started had no effect (P > .05) on the relative concentration of LH on the last day of feeding (Group VII).

Treatment with ICI 33828 effectively suppressed the release of LH (Group VII) on day 3 (relative concentration, 338), day 13 (relative concentration, 347) and day 18 (relative concentration, 291). Suppression of LH release was substantiated further since the gilts started on ICI 33828 feeding on day 18 failed to ovulate. At that time maximal response of follicle growth to FSH should have occurred and LH release would have caused ovulation. However, the relative concentration of LH at day 18 (365) in the control gilts (Group VIII) was lower (P < .05) than the LH level at the last day of feeding ICI 33828 only in those gilts started on treatment on day 18 (Group VII). In this population of gilts LH release may have been initiated earlier than in the gilts reported by Parlow et al. (1964). Thus, the assumption is made that the relative concentration of LH at day 13 in control gilts (Group VIII) reflects the higher level prior to release. Synthesis of LH was not inhibited by treatment with ICI 33828 and there was a tendency for additional LH to be synthesized when gilts were started on treatment on day 18. Malven (1969) concluded that ICI 33828 implants in the hypothalamus of the female guinea pig blocked the release of only those pituitary hormones responsible for follicle rupture.

Bioassay evaluation for validity projected a linear response (P < .005) as dose level increased. There was a dose x ICI 33828 treatment interaction (P < .01) resulting in a quadratic response (P < .005). Evaluation of this interaction indicates that relative concentration of LH in the control gilts' was probably underestimated since the dose response curve plateaued on the high dose while the gilts slaughtered on the last day of feeding ICI 33828 were probably overestimated. A dose x ICI 33828 treatment x day of estrous cycle treatment-started interaction (P < .05) occurred which showed a linear response (P < .025).

Experiment II—The Third Day of Gestation

Table 3 shows the mean weights of the endocrine gland and reproductive organs and the relative concentrations of FSH and LH of gilts slaughtered on the third day of gestation after having been fed ICI 33828 for 16 days (Groups I, II, III, IV; Subgroups A, B, C).

Endocrine Gland Weights. The pituitary
TABLE 3. MEAN ENDOCRINE GLAND AND REPRODUCTIVE ORGAN WET WEIGHTS AND GONADOTROPIN POTENCIES AT THE THIRD AND 25TH DAY OF GESTATION FOR GILTS FED ICI 33828

<table>
<thead>
<tr>
<th>Group</th>
<th>ICI 33828 mg/day</th>
<th>Subgroup</th>
<th>Day of estrous cycle started on treatment</th>
<th>Pituitary g</th>
<th>Anterior pituitary g</th>
<th>Thyroid g</th>
<th>Adrenals g</th>
<th>Ovaries g</th>
<th>Uteri g</th>
<th>Relative concentration</th>
</tr>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>HCG-S (FSH) *</td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>A</td>
<td>3</td>
<td>0.27</td>
<td>0.20</td>
<td>8.2</td>
<td>4.4</td>
<td>9.5</td>
<td>378</td>
<td>313</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>13</td>
<td>0.26</td>
<td>0.19</td>
<td>9.0</td>
<td>5.6</td>
<td>13.4</td>
<td>298</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>18</td>
<td>0.24</td>
<td>0.17</td>
<td>7.0</td>
<td>4.6</td>
<td>5.9</td>
<td>317</td>
<td>286</td>
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<td>II</td>
<td>58</td>
<td>A</td>
<td>3</td>
<td>0.25</td>
<td>0.18</td>
<td>6.5</td>
<td>4.1</td>
<td>6.0</td>
<td>235</td>
<td>263</td>
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<td></td>
<td></td>
<td>B</td>
<td>13</td>
<td>0.27</td>
<td>0.20</td>
<td>6.6</td>
<td>5.3</td>
<td>6.6</td>
<td>252</td>
<td>314</td>
</tr>
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<td></td>
<td></td>
<td>C</td>
<td>18</td>
<td>0.32</td>
<td>0.23</td>
<td>8.0</td>
<td>5.3</td>
<td>18.0</td>
<td>312</td>
<td>280</td>
</tr>
<tr>
<td>III</td>
<td>116</td>
<td>A</td>
<td>3</td>
<td>0.22</td>
<td>0.18</td>
<td>7.3</td>
<td>3.9</td>
<td>9.6</td>
<td>308</td>
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<td>9.2</td>
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<td>0.30</td>
<td>0.24</td>
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<td>5.9</td>
<td>9.8</td>
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<td>242</td>
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<td>232</td>
<td>A</td>
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<td>0.23</td>
<td>7.2</td>
<td>6.1</td>
<td>28.6</td>
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<td>244</td>
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<tr>
<td></td>
<td></td>
<td>B</td>
<td>13</td>
<td>0.28</td>
<td>0.21</td>
<td>7.2</td>
<td>5.0</td>
<td>9.3</td>
<td>251</td>
<td>323</td>
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<tr>
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<td></td>
<td>C</td>
<td>18</td>
<td>0.29</td>
<td>0.22</td>
<td>6.4</td>
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<td>10.5</td>
<td>326</td>
<td>388</td>
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</tr>
<tr>
<td>V</td>
<td>0</td>
<td>A</td>
<td>3</td>
<td>0.31 c</td>
<td>0.23 d</td>
<td>10.8</td>
<td>6.0</td>
<td>12.2</td>
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<td>301</td>
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<td></td>
<td>B</td>
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<td>0.18</td>
<td>5.7</td>
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<td>11.5</td>
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<td></td>
<td>C</td>
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<td>0.22</td>
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<td>4.0</td>
<td>12.0</td>
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<td>446</td>
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<tr>
<td>VI</td>
<td>116</td>
<td>A</td>
<td>3</td>
<td>0.34</td>
<td>0.25</td>
<td>11.3</td>
<td>4.7</td>
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<td>7.2</td>
<td>11.4</td>
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<td>0.20</td>
<td>7.6</td>
<td>5.3</td>
<td>9.4</td>
<td>588</td>
<td>272</td>
</tr>
</tbody>
</table>

Day 3 of gestation

* Significant (P<.005) day x dose interaction.

b Significant (P<.005) reduction in LH potency of 116 mg group compared to 0, 58 and 232 mg groups.

Day 18 group significantly (P<.005) greater than 3. Significantly (P<.005) day x dose interaction.

Day 25 of gestation

* Significant (P<.025) heavier day 3 pituitary weight as compared to day 13 and 18. Significantly (P<.005) day x dose interaction.

a Mean rat ovarian weight increments of three dose levels.

b Mean rat ovarian ascorbic acid content of three dose levels. Ascorbic acid content of the ovary is inversely related to LH content of the pituitary.
and anterior pituitary weights were not altered (P>-.05) on day 3 of gestation following inhibition of estrus by the feeding of ICI 33828. However, an interaction (P<.005) occurred in anterior pituitary weights between the day on which treatment started and the level of ICI 33828 feeding. The reason for this interaction is unknown.

The thyroid or adrenal weights on day 3 of gestation were not significantly influenced (P>.05) by the day treatment started and the feeding of ICI 33828.

Reproductive Organ Weights. The day of the estrous cycle treatment was started and the feeding of ICI 33828 did not affect (P>.05) the ovarian and uterine weights of gilts autopsied on day 3 of gestation.

Relative Concentration of FSH. The feeding of ICI 33828 or the day when treatment started did not affect (P>.05) the relative concentration of FSH on day 3 of gestation, i.e., approximately 10 days after withdrawal.

Bioassay evaluation for validity showed a linear and quadratic response (P<.005) as dose level increased indicating that the assay was not valid. Relative concentration of FSH was probably underestimated since the dose response curve plateaued on the high dose.

Relative Concentration of LH. The feeding of 116 mg per day of ICI 33828 to gilts prior to withdrawal and mating reduced (P<.005) the relative concentration of LH (Group III) when compared with the gilts fed 58 mg (Group II), 232 mg (Group IV) and the gilts not receiving ICI 33828 (Group I). The reason for this ICI 33828 dose response is unexplainable.

Relative concentrations of LH were decreased (P<.005) when gilts were started on treatment on day 13 when compared to day 18 and day 3. Concentrations on day 18 were also greater (P<.005) than on day 3. However, an interaction (P<.005) occurred among days when treatment started and the level of treatments. The gilts fed 0 mg, 116 mg and 232 mg of ICI 33828 per day had less relative concentration of LH when started on day 13 (Groups I, III, IV) while those fed 58 mg (Group II) had less when started on day 3. These responses are unexplainable.

Evaluation for validity of the bioassay resulted in a linear response as dose level increased (P<.005). A dose × ICI 33828 treatment × day of estrous cycle treatment-started interaction (P<.01) occurred and showed a linear response (P<.01).

Experiment III—The 25th Day of Gestation

The mean weights of the endocrine gland and reproductive organs and the relative concentrations of FSH and LH of gilts slaughtered on the 25th day of gestation after having been fed ICI 33828 are shown in table 3 (Groups V, VI; Subgroups A, B, C).

Endocrine Gland Weights. The pituitary gland weights were heavier (P<.05) in gilts started on treatment on day 3 than in the gilts started on treatment on day 13 or on day 18. However, an interaction (P<.005) occurred between the day treatment started and the level of treatments.

The anterior pituitary weights of gilts started on treatment on day 3 were heavier (P<.05) than in those gilts started on treatment on day 13.

There were no effects (P>.05) of the day treatment started or the feeding of ICI 33828 on the thyroid or adrenal weights of gilts on day 25 of gestation.

Reproductive Organ Weights. The ovarian and uterine weights of gilts on day 25 of gestation were not altered (P>.05) by any of the treatments.

Relative Concentrations of FSH and LH. The feeding of ICI 33828 or the day when treatment started did not affect (P>.05) the relative concentrations of FSH or LH of anterior pituitaries of gilts on day 25 of gestation, i.e., approximately 32 days after withdrawal.

Evaluation for validity of the bioassay showed a linear response (FSH, P<.005) (LH, P<.025) as dose level increased.

Literature Cited


Howland, B. E., R. L. Kirkpatrick, A. L. Pope and
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