THE EFFECTS OF INJECTED OESTRADIOL-17\beta, PROGESTERONE AND DIETARY ICI 33828 ON OVARIAN AND PITUITARY FUNCTIONS IN THE SOW AND GILT

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(Received 24th October 1968, revised 15th April 1969)

Summary. Two experiments using twenty-four sows and forty-eight gilts were designed to study ovarian and pituitary functions after treatment with oestradiol-17\beta (3 mg/day), progesterone (150 mg/day) or ICI 33828 (50 or 100 mg/day). The dose levels were chosen so that oestrus and ovulation would be inhibited.

None of the treatments blocked total synthesis of FSH or LH within the 10-day treatment period. ICI 33828 at 100 mg blocked FSH release early in the cycle but 50 mg did not. Oestrogen also blocked FSH release early in the cycle. All follicle growth greater than 4 mm diameter was effectively inhibited by oestrogen and the corpora lutea were maintained. Both progesterone and ICI 33828 reduced the number of follicles growing larger than 5 mm as compared to the control gilts. All treatments blocked FSH release late in the cycle.

There were increased levels of pituitary LH in ICI 33828-treated gilts compared to progesterone-treated gilts early in the cycle, but none of the treatments caused differences in LH levels from those found in the control gilts early in the cycle. All treatments blocked pituitary LH release late in the oestrous cycle.

The pituitary content of prolactin did not differ significantly between treatments.

INTRODUCTION

Progesterone and progesterone derivatives cause a high rate of cystic follicle formation in gilts during or after treatment depending on dose level (Baker, Ulberg, Grummer & Casida, 1954; Nellor, Ahrenhold, First & Hoefer, 1961; First, Stratman, Rigor & Casida, 1963; Kirkpatrick, First & Casida, 1963). Oestrogen, however, causes a marked reduction in follicular growth (Foote, Waldorf, Self & Casida (1958) and causes maintenance of the corpora lutea (CL) in the cycling gilt (Gardner, First & Casida, 1963). The ICI 33828 compound, if started during the luteal phase, blocks oestrus and ovulation, as do oestrogen and progesterone; however, the CL are not maintained and neither do cystic follicles develop (Polge, 1964, 1965; Gerrits & Johnson, 1964, 1965a, b; Stratman & First, 1965).
Since the ovarian responses to oestrogen, progesterone and the ICI 33828 compound are different, an experiment was designed to study pituitary FSH and LH levels coincident with ovarian information.

Kirkpatrick, Howland, First & Casida (1967) and Parlow, Anderson & Melampy (1964) have reported that pituitary FSH and LH levels are low until about Day 4 of the oestrous cycle, then rise rapidly until Days 10 to 14, when the levels remain high until about Day 19. These facts provide the basis for an in vivo system which can be used to judge the effects of various treatments on synthesis and release of FSH and LH.

Progesterone appears to block LH release in the gilt without an inhibition of LH synthesis (Foote et al., 1958; Rigor, 1961). Foote et al. (1958) further revealed that a single dose of oestrogen to gilts under progesterone inhibition did not cause LH release. Kidder, Casida & Grummer (1955), however, showed that stilboestrol given to progesterone-treated gilts during the luteal phase of the cycle caused luteinization of follicles. Pituitary LH and FSH levels are reportedly greater in ICI 33828-treated gilts compared to controls at two different stages of the oestrous cycle (Stormshack, Leverage, Kelly & Gerrits, 1968).

Pituitary prolactin levels have been studied by Day, Anderson, Hazel & Melampy (1959). They found a linear increase in prolactin with advancing stages of the oestrous cycle and pregnancy and attributed increased pituitary levels in the control gilts to an increased secretion rate of prolactin.

It was the purpose of this experiment to determine if oestrogen, progesterone, or the ICI 33828 compound were capable of blocking FSH and LH synthesis and/or release over a 10-day period in the gilt or sow and to study pituitary prolactin levels in response to these treatments.

MATERIALS AND METHODS

Experiment 1

Twenty-four, primiparous, Poland China sows were assigned within replicates to either 0, 50 or 100 mg/day of ICI 33828 at either the 3rd or 13th day of the oestrous cycle (day of oestrus = Day 1). Therefore, six sows comprise a replicate in this factorially designed experiment. All sows were penned separately and checked for heat daily. The ICI 33828 drug (carbowax extender) was mixed with a basal ration so that each sow received 1.8 kg feed/day. The animals started at Day 13 were killed on Day 23 of the oestrous cycle, and those started at Day 3 were killed on Day 13. At autopsy, the ovaries and pituitary glands were removed and placed on ice. Usually within 1 hr, the anterior and posterior parts of the glands were weighed separately and frozen at -10°C. The ovaries were weighed separately and diced on dry towelling, after follicle diameters had been measured and CL counted. The difference between the original and the diced ovarian weight was denoted as the follicular fluid weight.

Experiment 2

Forty-eight, sexually mature gilts (20 to 26 months of age), with recorded cycle lengths for two previous oestrous cycles, were randomly assigned to a factorial experiment of progesterone, oestradiol-17β, ICI 33828 and control.
treatments with treatments starting at Day 4 or Day 14 of the oestrous cycle. Subcutaneous injections of 3 mg oestradiol-17β/day or 150 mg progesterone in corn oil/day, or of corn oil alone were given daily at 05.00 hours. (ICI 33828-treated and control gilts). The gilts beginning treatment on Day 4 were killed on Day 14 and those started on Day 14 were killed on Day 24. Control animals were killed on Day 4 of their subsequent oestrous cycles. All animals received 1·8 kg of the same basal ration as those in Exp. 1, but the ICI 33828-treated animals had 100 mg of the drug added per 1·8 kg of feed. All methods at autopsy were the same as those followed in Exp. 1 with the exception that CL were dissected out and weighed.

**Bio-assay**

Relative FSH activity was estimated by the hCG-synergism (hCG-s) method of Steelman & Pohley (1953) as modified by Howland, Kirkpatrick, Pope & Casida (1966) and later by Short (1967). Relative LH activity was estimated by the ovarian ascorbic acid depletion (oaad) method of Parlow (1958) as modified by Kirkpatrick et al. (1967). Ovarian ascorbic acid content was adjusted by covariance for the rat ovarian weight by the method of Sakiz & Guillemin (1963). The validity of the bio-assays was tested according to the methods of Bliss (1952) and Emmens (1948).

In Exp. 1, pituitary doses of 0·1, 0·2 and 0·4 mg of dry pituitary powder from the sows, was used per rat in a three-dose (two rats/dose) assay for oaad activity. Doses of 4, 8 and 16 mg dry pituitary powder from sows was used in the same assay design as used in the oaad bio-assay for hCG-s activity. Analysis of variance was accomplished on the adjusted ascorbic acid values and on the total sum of the rat ovarian weights.

In Exp. 2, 0·15, 0·30 and 0·60 mg of dry pituitary powder from each gilt was used per rat in a three-dose (two rats/dose) assay for oaad activity. Doses of 3, 6 and 12 mg of dry pituitary powder per rat were used in a three-dose (one rat/dose) assay for hCG-s activity. The analysis of variance was accomplished on relative and relative total FSH and LH potency. hCG-s activity and oaad activity were converted to relative potency using the experimental mean as the standard. The relative potencies were converted to relative total potency by multiplying by the pituitary dry weight.

Both oaad assays were entirely valid in that there was a significant linear dose response and no treatment interactions with dose existed. Both hCG-s assays were also valid with respect to a significant linear dose response and no significant interactions with dose. In addition, a significant quadratic dose response occurred in both assays. The quadratic effect appeared to be due to a plateau in rat ovarian weight response between the medium and high doses of pituitary powder in both assays.

The prolactin assay was similar to that described by Bates, Garrison & Cornfield (1963). Exceptions were the use of 10-week-old pigeons and 0·5-ml injections for 6 days. Dry pituitary doses of 40 and 20 mg were used for the high and low doses respectively. Two pituitary glands were randomly pooled per treatment group because of the small amount of powder remaining after FSH and LH assays. Only one pigeon was used per dose in the assay. Because only
two doses were used, a test for linearity was not possible, although parallelism between treatments existed. The pigeons were killed on Day 7 and the crop-sacs removed, cleaned and weighed. The calculated mean slope was 5.6 g for a ten-fold increase in the dose of the unknown. The mean control crop-sac weight was 2.9 g. Bates et al. (1963) calculated a slope of 5.4 g increased crop-sac weight per ten-fold increase in the dose of a prolactin standard in young pigeons. No standard was used in this bio-assay.

Statistical analyses

Data were tested for homogeneity of variance using the F-ratio (Steel & Torrie, 1960). Data not homogeneous were transformed to logs. In Exps. 1 and 2, data were then subjected to analysis of variance with pre-selected orthogonal comparisons. These orthogonal comparisons in Exp. 2 were IC versus OP, O versus P and I versus C where I = ICI 33828, O = Oestrogen, P = progesterone and C = control. The same comparisons were also selected for the hormone × day interaction to give three orthogonal comparisons. The analysis of variance with the pre-selected orthogonal comparisons for Exp. 1 are presented in Table 2. Since further within-day comparisons were required in Exps. 1 and 2, Duncan’s multiple range test within-day was accomplished on LH and FSH data. These analyses are not presented in any table, but are discussed.

RESULTS

Experiment 1

Sows treated with ICI 33828 had greater levels of pituitary FSH than the control sows (P < 0.01; Text-fig. 1). Both the 100- and 50-mg levels caused greater values of FSH at Day 23, but only the 100-mg level caused greater values at Day 13 (P < 0.05; Table 2). Not only was there a significant day × drug versus control
interaction \((P<0.05)\), but also a day \(\times\) 100 versus 50 mg interaction \((P<0.05)\). Inverse mean ascorbic acid values are presented in Text-fig. 2. Again, a day \(\times\) drug versus control interaction existed \((P<0.05)\). In this case, however, the drug caused increased LH levels at Day 23, but had no effect at Day 13 of the cycle \((P<0.05)\). No differences existed \((P<0.05)\) between the 50 or 100 mg/day levels (Table 2). It will be noted in Table 1 that anterior pituitary and follicular fluid weights were not different between treatments \((P>0.05)\) at Day 13. Cystic follicles (>14 mm) occurred in sows in all treatment groups at Day 13 of the oestrous cycle. Some of the mean weights are misleading, since a high mean was often caused by the data for a single animal. One hundred mg ICI 33828/day caused a reduction in the number of follicles greater than 7 mm diameter at Day 23, compared to either sows on the control or 50 mg/day treatments \((P<0.05)\). Oestrus only occurred in one animal receiving 100 mg ICI 33828/day, and in no animals at 50 mg/day.

**Table 1**

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Day of cycle killed</th>
<th>Dry anterior pituitary wt (mg)</th>
<th>Corpora lutea no.</th>
<th>Follicular fluid wt (g)</th>
<th>Posterior pituitary (mg)</th>
<th>Follicles &gt; 7 mm</th>
<th>No. of animals with cystic follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23</td>
<td>92.9</td>
<td>10.5</td>
<td>2.9</td>
<td>123</td>
<td>2.8</td>
<td>0</td>
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<tr>
<td>50</td>
<td></td>
<td>99.5</td>
<td>0.0*</td>
<td>3.4</td>
<td>128</td>
<td>4.0</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>89.4</td>
<td>0.3*</td>
<td>1.4*</td>
<td>113</td>
<td>0.3*</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>13</td>
<td>91.1</td>
<td>15.0</td>
<td>6.4</td>
<td>112</td>
<td>1.0</td>
<td>2</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>98.4</td>
<td>9.0*</td>
<td>73.3</td>
<td>160*</td>
<td>8.3</td>
<td>4</td>
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<tr>
<td>100</td>
<td></td>
<td>82.1</td>
<td>7.8*</td>
<td>9.9</td>
<td>140*</td>
<td>3.8</td>
<td>2</td>
</tr>
</tbody>
</table>

* Values in same column with asterisks differ from those without asterisks \((P<0.05)\).
Table 2

ANALYSIS OF VARIANCE FOR THE EFFECTS OF ICI 33828 ON HCG-S AND OAAD ACTIVITY IN EXPERIMENT 1

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>HCG-S activity mean square</th>
<th>OAAD activity mean square</th>
</tr>
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<tbody>
<tr>
<td>ICI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control versus drug</td>
<td>1</td>
<td>58926**</td>
<td>114</td>
</tr>
<tr>
<td>100 versus 50</td>
<td>1</td>
<td>13144*</td>
<td>188</td>
</tr>
<tr>
<td>Day</td>
<td>1</td>
<td>190</td>
<td>28</td>
</tr>
<tr>
<td>Replicate</td>
<td>3</td>
<td>2670</td>
<td>144</td>
</tr>
<tr>
<td>D × I</td>
<td>2</td>
<td>6989*</td>
<td>325</td>
</tr>
<tr>
<td>D × C versus drug</td>
<td>1</td>
<td>9470*</td>
<td>640*</td>
</tr>
<tr>
<td>D × 100 versus 50</td>
<td>1</td>
<td>4508*</td>
<td>10</td>
</tr>
<tr>
<td>R × D</td>
<td>3</td>
<td>1206</td>
<td>47</td>
</tr>
<tr>
<td>R × I</td>
<td>6</td>
<td>1144</td>
<td>63</td>
</tr>
<tr>
<td>R × D × I</td>
<td>6</td>
<td>717</td>
<td>99</td>
</tr>
</tbody>
</table>

* P<0.05; **P<0.01.

Experiment 2

Table 3 presents mean pituitary and ovarian weights. Oestrogen caused increased anterior pituitary weight (P<0.05) and decreased log follicular fluid weight (P<0.05) when compared to the other treatments. The control gilts showed an increase in log number of follicles of 5 to 7 mm diameter, and in log follicular fluid weight between Days 4 and 14 of the oestrous cycle. There existed an ICI 33828 versus control × day interaction in the analysis of log number of follicles of 5 to 7 mm diameter (P<0.05). Both oestrogen and progesterone were shown to cause a greater number of follicles less than or equal to 4 mm (P<0.05) compared to the controls. Because of the possibility of unilateral effects of the treatments on the ovary, an analysis of variance was accomplished on the left minus right ovarian follicular fluid weights (Table 3).

Table 3

MEAN PITUITARY AND VARIOUS MEAN OVARIAN MEASUREMENTS AFTER PROGESTERONE, ICI 33828 AND OESTROGEN TREATMENT*

<table>
<thead>
<tr>
<th>Day of cycle killed</th>
<th>Treatment</th>
<th>Ant. pit. wt (mg)</th>
<th>Follicle fluid wt (g)</th>
<th>Mean CL wt (g)</th>
<th>No. of CL</th>
<th>No. of follicles &lt; 4 mm</th>
<th>No. of follicles 5 to 7 mm</th>
<th>Follicle fluid wt (L-R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 (4)</td>
<td>Control</td>
<td>310</td>
<td>2.0</td>
<td>0.26</td>
<td>14-8</td>
<td>38</td>
<td>0.5</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Oestrogen</td>
<td>353</td>
<td>1.7</td>
<td>0.30</td>
<td>16-7</td>
<td>117</td>
<td>12-5</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Progesterone</td>
<td>271</td>
<td>2.4</td>
<td>0.00</td>
<td>0</td>
<td>104</td>
<td>6-5</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>ICI 33828</td>
<td>306</td>
<td>2.5</td>
<td>0.16‡</td>
<td>1-7‡</td>
<td>78</td>
<td>6-5‡</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Control</td>
<td>298</td>
<td>3.1</td>
<td>0.49</td>
<td>14-3</td>
<td>84</td>
<td>15-3</td>
<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>Oestrogen</td>
<td>333</td>
<td>1.6</td>
<td>0.43</td>
<td>16-7</td>
<td>76</td>
<td>0-0</td>
<td>-0.22</td>
</tr>
<tr>
<td></td>
<td>Progesterone</td>
<td>292</td>
<td>2.9</td>
<td>0.55</td>
<td>14-7</td>
<td>108</td>
<td>6-5</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>ICI 33828</td>
<td>288</td>
<td>3.5</td>
<td>0.74†</td>
<td>15-0</td>
<td>93</td>
<td>2-0</td>
<td>-0.45</td>
</tr>
</tbody>
</table>

* See Results for significant differences.
† Different from oestrogen or control (P<0.05) with Day 14.
‡ One animal ovulated and had ten corpora lutea.
A highly significant main effect of 'day' on this difference was noted. In all treatments, the left follicular fluid weight tended to be heavier at Day 24 (4) of the oestrous cycle, however only the ICI 33828 and treated control gilts had significantly larger differences between the left and right follicular fluid weights at Day 24 (4) as compared to Day 14 (P<0.05). At Day 14, all treatments had (left minus right) follicular fluid differences near zero. Even though oestrogen caused follicular fluid weight to be lower than levels found in all other treatments (P<0.05), no differences existed between the left minus right follicular fluid weights within Day 24 or 14, or across both days due to treatment. Therefore, although unilateral ovarian differences exist, none of the treatments significantly altered the normal unilateral condition as it occurred in the control gilts.

**FSH and LH relative potencies**

As observed in Exp. 1, ICI 33828 caused greater levels of FSH at Days 24 (P<0.01) and 14 (P<0.05), but caused greater LH levels only at Day 24 (P<0.01) compared to controls (Text-figs. 3 and 4). Oestrogen and progesterone were not shown to be different from each other (P>0.1) in effects on relative pituitary LH or FSH. No significant treatment x day interactions existed (P>0.1). Both oestrogen and progesterone caused greater levels of FSH and LH at Day 24 than were found in the controls (P<0.05). Previous evidence supports these results with progesterone (Rigor, 1961; Foote et al., 1958). Nothing regarding the effect of continuous oestrogen injection on LH and FSH levels in swine has been reported previously. ICI 33828 caused greater levels of LH at Day 14 than were found in the progesterone-treated gilts at Day 14 (P<0.05). Relative total potencies which represent gland content are not reported in any table, but the analysis on total potency was accomplished.

Oestrogen caused greater levels of total FSH at Day 14 than were found in the controls (P<0.05). Except for this important difference, the relative total potency analysis revealed the same results as those found in the analysis of relative potency (concentration).
Text-fig. 4. Mean relative LH potency at two oestrous cycle days at the end of progestosterone, oestradiol-17β or ICI 33828 treatment. a, Treatments with different superscript within day differ (P<0.05).

Prolactin assay

The analysis of variance of the crop-sac weights revealed no differences between the various treatments. Duncan’s test was also accomplished within-day and no differences were observed. Due to the limited pituitary material, only one bird/dose with three observations per treatment subgroup were made and variation between birds in the same treatment group was large (Text-fig. 5).

Text-fig. 5. Mean ± S.E. crop-sac weights in bio-assay for prolactin levels in the pig.

DISCUSSION

It seemed probable that some of the questions regarding the synthesis of gonadotrophins might be answered by starting a group of control and treated
pigs at oestrous cycle Day 3 or 4 where pituitary FSH and LH levels are low, and examining these animals at Day 13 or 14 where FSH and LH levels are normally high. If pituitary FSH or LH was low in the treated group compared to the controls at Day 13 or 14, a definite synthesis block would have occurred. If the levels in treated gilts were the same or higher at Day 13 or 14, no synthesis block would have occurred. A higher level in the treated group compared to the controls could be explained by either enhancement of synthesis or by a block of some basal release of FSH or LH which occurs during the first half of the oestrous cycle.

By starting another group of treated and control pigs on Day 14 when the pituitary levels of FSH or LH are high and then killing these pigs on Days 3 or 4 when pituitary levels in the control gilts fall to a low level, answers concerning FSH and LH release are possible under various treatments. High levels of pituitary FSH or LH on Day 24 (4) and no ovariian response compared to controls would indicate that release is blocked.

Paget, Walpole & Richardson (1961) have demonstrated that the ICI 33828 compound does not affect the ovary or testes directly in the rat, and Polge & Day (1969) have shown that PMSG is capable of stimulating ovulation in gilts under ICI 33828 treatment. Mitchell (1966, 1967) showed that avian gonadotrophins could stimulate the bird ovary in the presence of ICI 33828 treatment. Garbers & First (1969b) have shown that ICI 33828 does not reduce CL weight in oestrogen-treated gilts, indicating that the drug appears to act at some site other than the gonads. Brown (1963) presented pituitary information suggesting that the compound acted at the pituitary or hypothalamic levels. Two groups have shown that the compound can block milk let-down in the sow, but that this inhibition can be overcome by injection of oxytocin, which suggests a pituitary or hypothalamic inhibition (Gerrits, Johnson & Kraeling, 1965; Garbers & First, 1968). The authors of this paper, therefore, have assumed that the compound does not affect the ovarian ability to respond to gonadotrophins, although the direct site of action (pituitary, hypothalamus or other) has not been proved.

The ICI 33828 compound did not inhibit pituitary FSH synthesis, since the levels of FSH were greater or equal in the ICI 33828-treated gilts compared to the control gilts in both experiments. The equal pituitary LH levels of treated versus control gilts also demonstrated that LH synthesis was not inhibited. The higher levels of pituitary FSH at Day 13 or 14 can be explained if the ICI 33828 compound blocks a basal release of FSH during the first half of the cycle or enhances synthesis. Brown (1963) has shown that the compound will inhibit production of the gonadotrophins in the rat, and therefore enhanced synthesis is not likely. Since follicular growth occurs during the first half of the oestrous cycle in the pig, Anderson (1966) has postulated that FSH release occurs during the first half of the cycle. It seems most likely, therefore, that the release of FSH has been blocked during the first half of the cycle and that the synthesis of gonadotrophins is unaffected.

The interaction (50 versus 100 mg x day) in Exp. 1, indicates that lower levels of the drug act differently from high levels on pituitary FSH. Low drug levels do not effectively block a basal release of FSH during the first half of the
cycle, but do block FSH release near the end of the cycle whereas high levels (100 mg), block the release of FSH on both Days 13 and 23. The interaction (drug versus control x day) in the analysis of LH and FSH levels indicates that the ICI 33828 drug caused a differential effect at Days 13 and 23 compared to the controls. Both LH and FSH levels are greater than the control levels at Day 23; this suggests a block of FSH and LH release. At Day 13, however, the LH levels were not different from controls, and only the 100 mg/day level of ICI 33828 caused increased FSH compared to the controls. These data suggest that the pituitary is more susceptible to inhibitory action near the end of the cycle than during the first half, when a 'tonic release' may occur. Stormshack, Leverage, Kelly & Gerrits (1968) have also reported that LH and FSH levels are significantly increased in ICI 33828-treated gilts.

It should be further noted that the work of Brown (1963) and the report by Walpole (1968) are not necessarily in disagreement concerning the mode of action of the ICI 33828 compound. Although they have shown that the synthesis of FSH and LH is inhibited by the ICI 33828 compound in the rat, the treatment period in terms of oestrous cycle lengths was longer and the drug level different. Stratman & First (1965) have indicated that higher doses of ICI 33828 progressively increase the time to oestrus upon drug withdrawal, after a 20-day treatment period. This may indicate some effects of the ICI 33828 compound on LH or FSH synthesis at higher dose levels in the pig.

A dose-response relationship between the hormone levels in the pituitary gland and ICI 33828 was predicted previously by Garbers & First (1969a). They found that gilts on lower levels of the ICI 33828 compound were capable of showing oestrus while only partially ovulating, and suggested that lower levels of the drug allowed only a partial release of gonadotrophin. These data support that hypothesis.

The marked decrease in follicles greater than 4 mm in diameter, and the maintenance of CL by oestrogen have been previously reported by Foote et al. (1958) and Gardener et al. (1963), respectively. These data extend their information since the gilts started on oestrogen as late as Day 14 consistently maintained their CL. Oestrogen and progesterone appeared to act similarly when considering the relative concentrations of pituitary FSH and LH. This might be expected since the oestrogen was acting in the presence of endogenous progesterone. The ovarian responses, however, indicated that secretion rates were different, or that the two hormones had some direct effects on the ovary. Blocking of pituitary LH and FSH release by oestrogen has been reported in the pig (Foote et al., 1958), in the rat (Sidki, Badawi & Soliman, 1958; Gans, 1959a, b) and in the sheep (Cooper & Ellington, 1967). The work in the sheep and rat estimated blood levels of LH, but reports by Dierschke & Clegg (1968), Pelletier (1964) and Grighton (1968) have seriously questioned use of the Parlow blood LH assay in species other than the rat. Direct stimulatory effects of oestrogen on the rat ovary have also been reported (Pencharz, 1940; Desclin, 1949).

That oestrogen, in fact, blocked FSH release early in the cycle is shown in the analysis of relative total potency (pituitary content). Oestrogen and ICI 33828 caused greater levels of FSH at Day 14 than were found in control gilts. It is interesting to note that, although none of the three compounds differed in
their effects on relative or total FSH, the ovarian follicle growth was different. The pituitary concentration of LH at Day 24 was greater in oestrogen-treated gilts compared to Day 4 controls, which suggests the blocking of an LH surge. Since the ovaries were incapable of response to LH because follicles were absent, it is not known whether LH release was actually blocked. Work by Anderson, Dyck, Mori, Henricks & Melampy (1967) has suggested that LH is the luteotrophin in the pig. They also found oestrogen incapable of maintaining corpora lutea in the stalk-sectioned gilt. Therefore, the pituitary gland is necessary for oestrogen to cause maintenance of the CL, but the mechanism is not understood.

The effects of progesterone on the pituitary gland and ovary generally correspond to effects reported earlier by Foote et al. (1958), Rigor (1961) and Short (1967). Progesterone does not appear to have an effect on pituitary FSH or LH synthesis within 10 days. Although some suppression of follicle growth occurs (no follicles > 7 mm), growth of small follicles continues. McCann (1962) and Kaufman & Rothchild (1966), in the rat, have indicated that progesterone in the absence of oestrogen is not potent as an inhibitor of LH release. Short (1967) has clearly shown that progesterone can block ovarian hypertrophy after unilateral ovariection. He also demonstrated increased FSH levels in progesterone-treated gilts compared to control gilts at Day 14, when the gilts were placed on treatment at Day 2 of the same cycle. A similar increase was not observed in this experiment (Text-fig. 3). It appears that progesterone may block the 'LH surge', but not greatly affect 'tonic' discharge of LH. It seems plausible that the development of cystic follicles after progesterone-withdrawal may be due to some damage of the 'LH cyclic centre' in the pig. Gorski (1966) describes in some detail the so-called 'tonic' and 'cyclic' centres for LH in the rat. Other possible explanations for the action of progesterone are the block of some LH synthesis at Day 14 compared to the absence of any such block from ICI 33828 treatment, enhancement of LH synthesis by ICI 33828, or direct effects on the ovary. In a similar experiment to this one, Keever & Greenwald (1967) reported results which nearly paralleled the data reported in this paper. They concluded that progesterone blocks an 'LH surge' but not 'tonic LH' release. Both oestrogen and progesterone caused increased levels of pituitary FSH at Day 4 of the oestrous cycle in the hamster, when the controls showed pituitary depletion. FSH centres similar to those described for LH, would explain the differential effect of 50 and 100 mg of ICI 33828 on pituitary FSH levels at Days 13 and 23 (3). Goldman & Mahesh (1968) suggested that the FSH surge which occurs in the rat may be necessary for normal ovulation. They concluded that more than a steady, tonic FSH release occurs during the oestrous cycle of the rat, and have also implied that the sheep and cow may be similar to the rat in these respects.

These data support the hypothesis of Anderson (1966) that FSH release occurs during the first half of the oestrous cycle in the pig, but are not clear as to whether LH release occurs during this period of the cycle. Anderson & McShan (1966) postulated, on the basis of blood LH assays, that some basal release of LH occurred throughout the oestrous cycle. It is possible that a small release rate would not be measurable as a pituitary depletion by the oAAD bio-assay. Since
ICI 33828 caused greater levels of LH at Day 14 than those found in progesterone-treated gilts, a basal release of LH throughout the cycle may be indicated.

It must be mentioned that gilts (20 to 26 months) and weighing 182 to 240 kg were used in Exp. 2. The level of oestrogen, ICI 33828 or progesterone used may be relatively low or relatively high depending on the effect of age and body weight on drug response. Garbers & First (1967) reported that a lower absolute dose of ICI 33828 was required for oestrus inhibition in sows than in gilts, but no reports exist on the age of gilts and ICI 33828 dose requirements. Since one gilt on ICI 33828 showed oestrus while being treated, it appears that the 100 mg/day level of ICI 33828 was less effective in gilts of this weight and age than in smaller and younger gilts. Therefore, the dose of progesterone and oestrogen may be biologically lower than the same doses given to younger gilts. This may, in part, explain the greater levels of FSH found at Day 14 by Short (1967) after gilts received 150 mg progesterone, whereas the pituitary levels of FSH at Day 14 for gilts in this experiment did not differ from those for the controls.

The study of pituitary prolactin levels was inconclusive (Text-fig. 5). Oestrogen, which caused maintenance of the corpora lutea, may cause lowered prolactin levels. Since secretion rates of prolactin are not known, the lowered pituitary levels would be difficult to interpret.

ACKNOWLEDGMENTS

This paper was published with the approval of the Director of the Wisconsin Agricultural Experiment Station, Madison, as manuscript No. 517 from the Department of Meat and Animal Science. Supported by a grant from Ayerst Laboratories, New York.

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