STEROID EFFECTS ON THE REPRODUCTIVE SYSTEM IN THE BOVINE

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IMPROVED methods for producing the various steroid hormones have increased their availability for experiments with large domestic animals and have made it possible to study their effects on the pituitary, on the reproductive organs and on the modification of the estrous cycle. Asdell, de Alba and Roberts (1945) found that estrus could be induced in ovariectomized heifers by injections of 600 R.U. of estradiol over a 3-day period. Dutt and Casida (1948) reported that the estrous cycle of the ewe could be controlled and synchronized by injections of progesterone. Hansel and Trimberger (1952) found that 5 to 10 mg of progesterone injected at the beginning of estrus hastened ovulation and shortened the duration of receptivity in cows. Cole, Hart and Miller (1945) found that injections of testosterone propionate increased the incidence of behavioral estrus in ewes receiving injections of PMS.

Characteristic changes in the composition of semen and in spermatozoa can be produced with exogenous steroid hormones (Cupps, Laben and Rahlmann, 1960a; Cupps et al., 1960b; Cupps and Briggs, 1965; Matsuyama, Richkind and Cupps, 1967; Richkind, Matsu-gama and Cupps, 1967). The objectives of this paper are to review some of the effects of exogenous steroid hormones in the bovine, particularly in the female, and to report some of the changes produced by them.

Estrogens

Following daily injections of graded amounts of estradiol beginning on the third day of the cycle, Rahmann and Cupps (1962a) found that levels from 50 to 150 µg per day had no effect on the length of the cycle, or on ovulation time with respect to estrus. Daily levels of 150 µg interfered with conception during the time the animals were treated but had little or no effect on subsequent fertility. Levels of 200 to 300 µg per day shortened the cycle and delayed ovulation (table 1). Three-hundred-fifty µg of estradiol beginning at the 87th day of pregnancy caused abortion but had no effect when the injections were started after the 150th day of gestation.

The chronic effects of daily injections were different from those reported by Hansel, Trimberger and Bearden (1952) in which a single injection of estradiol did not affect the time of ovulation with respect to estrus. At 350 µg daily, persistent follicles from 20 to 35 mm in diameter were detected in the ovary. These abnormally large follicles usually remained for a week or more and then regressed. They formed at irregular times without relation to behavioral estrus, were thin walled, ruptured easily during palpation, and showed atresia (figure 1). The granulosa was absent and the cells of the theca interna were hypertrophied. At levels of 3.5 mg per day, all follicle growth beyond the secondary stage was inhibited and follicles greater than 1 mm in diameter were atretic. At this level typical proestrous behavior was seen at irregular intervals in the heifers. Chronic injections of estradiol resulted in the accumulation of granules in the small basophilic cells of the pituitary but the significance of this histological change is still unknown. Exogenous estrogen, 250 µg per day, beginning on the day following estrus inhibited the normal development of the corpus luteum (Loy, Zimbelman and Casida, 1960).

Melampy and Rakes (1958) found that the length of estrus in ovariectomized cows increased with increasing amounts of estradiol and that very small doses of progesterone acted synergistically when given with the estrogen. Carrick and Shelton (in press) reported that ovariectomized heifers became refractory to repeated doses of estradiol (10 mg) given at 5-day intervals. For example, fifty-three percent of the animals came into estrus after the first injection as compared to
TABLE 1. EFFECT OF ESTRADIOL ON THE ESTROUS CYCLE AND OVULATION

<table>
<thead>
<tr>
<th>Estradiol levels in µg/day</th>
<th>0</th>
<th>50</th>
<th>75</th>
<th>100-150</th>
<th>200</th>
<th>250-300</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of observations</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Range in days</td>
<td>20-22</td>
<td>18-22</td>
<td>17-20</td>
<td>16-19</td>
<td>17-17</td>
<td>10-18</td>
</tr>
<tr>
<td>Mean cycle length in days</td>
<td>21.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ovulation time&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol levels in µg/day</td>
</tr>
<tr>
<td>No. of observations</td>
</tr>
<tr>
<td>Range in hours</td>
</tr>
<tr>
<td>Mean ovulation time in hours</td>
</tr>
</tbody>
</table>

<sup>a, b</sup> Figures with different superscripts are significantly different.
<sup>c</sup> Ovulation time—the period of time from the onset of standing estrus to ovulation.

33% following the seventh injection. The duration of estrus was shortened from an average of 14 hr. following the first injection to 4 hr. following the seventh injection in the animals showing estrus. All of the animals were refractory to injections of 400 µg of estradiol immediately following the large doses. Partial recovery occurred over an interval of 30 days. Treatment with 10 mg of progesterone per day for 5 days followed by injection of 400 µg of estrogen 3 days after progesterone withdrawal removed the inhibitory effect of

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Figure 1. Wall of 20 mm follicle from cow receiving 300 µg estradiol per day. × 600
the estrogen with 93% of the animals showing estrus. The length of estrus was also increased following pretreatment with progesterone. These investigators did not find a synergistic estrous response with the simultaneous injection of 1 mg of progesterone and the estrogen. Animals receiving 400 μg of estrogen at 21-day intervals did not become refractory to estrogen and their response was similar to those which received progesterone during the interval between estradiol injections. Eighty-nine percent of the animals receiving progesterone and 91% of those not receiving progesterone showed estrus following the injection of estradiol. The duration of estrus was 14 and 13 hr. respectively, for the two groups.

**Progesterone**

Williams and Williams (1921) and Hammond (1927) reported that the length of the estrous cycle in the cow could be modified by enucleation of the corpus luteum. Ulberg, Christian and Casida (1951) were able to modify the length of the cycle in heifers by injections of progesterone. They reported that follicles continued to grow and in many cases to regress although estrus was suppressed. At high doses new follicles developed following cessation of the injections but at lower doses the large follicle that developed during the injections ovulated following withdrawal. During the past 2 years we have been investigating the nature of the changes which occur in the ovary following injections of exogenous progesterone. The results are shown in table 2. Of the 12 animals treated with 12.5 or 25 mg of progesterone per day beginning between the 14th and 19th day of the cycle, seven failed to ovulate and a large follicle, 22 to 28 mm in diameter, persisted. In two instances, these large follicles ruptured and a corpus luteum formed following withdrawal of the progesterone. The ovaries from the animals which failed to ovulate resembled “cystic ovaries” and the condition of the large persistent follicle is shown in figure 2. The granulosa and theca interna were still present in these follicles and appeared to be normal histologically. The blood vessels of the theca interna were larger than those found in the normal preovulatory follicle and the lining was thrown into folds. It might be assumed that the folds seen in follicles following fixation is the result of shrinkage but shrinkage could not account for the changes in these follicles because they were fixed by exchanging equal volumes of fixative with the follicular fluid by simultaneous withdrawal of follicular fluid and injection of the fixative.

When injections of progesterone were begun on the day of estrus, the intermediate sized follicles, 10 to 12 mm in diameter, were atretic on the 10th and 15th day of the cycle (figure 3). The atresia found in these follicles resembles very closely the type that Marion, Gier and Choudary (1968) have named “cystic follicular atresia”.

Five animals ovulated while receiving injections and in all cases the collapsed follicle formed a cystic corpus luteum (figure 4). The luteal cells formed a layer from 3 to 5 mm around the periphery and enclosed about 2 ml of fluid. Not all of the cells in the cystic corpora lutea were luteinized to the same de-

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**TABLE 2. EFFECTS OF EXOGENOUS PROGESTERONE ON THE OVARY**

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Injections</th>
<th>Ovarian activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fem.</td>
</tr>
<tr>
<td></td>
<td>Day* of cycle</td>
<td>Length of treatment days</td>
</tr>
<tr>
<td>4</td>
<td>14–19</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>14–16</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

* The day of the cycle on which the injections were started.
gree and it was not possible to determine their origin with respect to the zonal cells of the follicle. When supplementary progesterone was started on the day of estrus, 12.5 mg per day had no effect on development of the corpus luteum but 50 mg per day completely inhibited its development. The luteal tissue from these cystic corpora had normal ability to convert pregnenolone to progesterone as measured in vitro (Sasser and Cupps, 1969). Smallwood and Sorensen (1969) have reported the formation of similar luteinized cysts in the ovary following the feeding of a synthetic progestogen.

**Androgens**

Although androgens have been isolated from the ovaries of many species of animals, observations on their effects on the reproductive physiology of the female are few in number. Rahlmann and Cupps (1962a, b) found that injections of dehydroepiandrosterone at levels from 15 to 40 mg per day shortened the in-
terval between the onset of estrus and the time of ovulation in dairy cows. It also caused an increase in the number of hyalinized delta cells in the pituitary gland. Its effect on shortening the interval between the onset of estrus and ovulation is similar to that produced by progesterone (Hansel and Trimberger, 1952).

**Summary**

Exogenous estrogen, depending on the levels injected and the time of injection, will cause estrus in ovariectomized cows, shorten the estrous cycle, delay ovulation, interfere with the normal development of the follicle, cause abortion and cause the development of a refractory condition to behavioral estrus.

Exogenous progesterone, also depending on dosage and timing, will modify the length of the estrous cycle, hasten ovulation, cause the development of luteal cysts, and interfere with the normal development of the follicle in cows. Dehydroepiandrosterone acts similar to progesterone in its effect on ovulation.
Figure 4. Cystic corpus luteum from ovary of a cow receiving 12.5 mg progesterone daily. × 900

Literature Cited


Cupps, P. T., R. C. Laben, D. F. Rahlmann and


**DISCUSSION**

**Question: Oxenreider**

Dr. Erb, was there an increased incidence of dystocia and retained placentas in those cows having high levels of progesterone before parturition and then later for those cows having metritis?

**Erb:** We had only one case of dystocia and this cow was not included in the summary. The metritis group did include a high percentage of cows retaining the placenta. All cases of retained placenta were accompanied by metritis postpartum. The six cases summarized 3 to 42 days postpartum had first pyometra and then persistent endometritis during the entire period.

**Question: Eaton**

Dr. Erb, in regard to your reports that urinary estradiol-17 alpha and estrone are proportionately higher compared to estradiol-17 beta, would you infer from this that a similar proportional difference would be found in the plasma and be of physiological importance during the cycle or during pregnancy?

**Erb:** I wouldn't want to be misunderstood on this, I am referring only to the changes in proportion as they appear in the urine. I haven't the slightest idea what this might be in peripheral plasma. It is interesting that we continue to see these rather sizeable changes in proportion as excreted in urine.

**Question: Casida**

I would like to ask Dr. Erb regarding the cows that he had labelled as cystic as having anovulatory follicles. Was some of the variability in steroid levels perhaps traceable to time at which the cystic follicle condition was observed? In this condition there is a series of stages.

**Erb:** One of the things we're going to have to do, whether it's with the cystic animal or some other type of postpartum animal, is to follow the steroid changes each day. I believe it was mentioned in the paper that we occasionally found high urinary estrogen in the animals that were subsequently diagnosed cystic. If one measured urinary estrogen each day I expect all of them would be