OXYTOCIN-PRODUCED CHANGES IN THE BOVINE OVARY BEFORE AND AFTER UNILATERAL OVARIECTOMY

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Summary. Exogenous oxytocin in intact heifers shortened the oestrous cycle and decreased luteal progesterone content and in vitro synthesis both with and without the addition of LH to the incubation medium. Unilateral ovariectomy decreased the proportion of shortened oestrous cycles following oxytocin treatment. Both the removal of one ovary and oxytocin injection are thought to upset the normal hypophysial–gonadal balance of hormones leading to increased variability in cycle length.

INTRODUCTION

Exogenous oxytocin shortens the bovine oestrous cycle (Armstrong & Hansel, 1959) though the subsequent untreated cycle length appears normal. Corpora lutea (CL) from treated animals are usually smaller and contain fewer lutein cells as well as an increased proportion of connective tissue elements than those from normal animals (Armstrong & Hansel, 1959; Donaldson & Hansel, 1965). Luteal progesterone content has been studied in cows throughout the normal oestrous cycle (Armstrong & Black, 1966; Hafs & Armstrong, 1968) and in vitro luteal synthesis of progesterone has been studied in the same animals under the influence of various stimulatory agents in an attempt to carry the understanding of progesterone biosynthesis in vivo a stage further. Purified bovine LH has been found to increase in vitro synthesis of this steroid.

Various mechanisms have been postulated to explain the ability of oxytocin to shorten the bovine oestrous cycle. Most of the current evidence indicates that it acts indirectly upon the hypothalamus and alters gonadotrophin synthesis or release (Armstrong & Hansel, 1959; Black & Duby, 1965; Donaldson, Hansel & Van Vleck, 1965). The resulting imbalance appears to upset the normal cycle of events causing early luteal regression and enhancing subsequent oestrus.

In this study, luteal progesterone content and in vitro synthesis in both normal and oxytocin-treated heifers were studied to examine the changes in the bovine corpus luteum induced by this hormone. The effect of oxytocin upon cycle length before and after removal of one ovary was also examined.
MATERIALS AND METHODS

Sequence of hormone treatments

Twelve heifers, previously examined for normal oestrous cycles, were used in this study. Each was checked daily for signs of heat. All animals were injected subcutaneously with 100 U.S.P. units of oxytocin (Jansen-Salsbery Laboratories) daily from the 3rd to 6th day after oestrus (Cycle 1) and this was followed by a rest cycle (Cycle 2). For the purposes of measuring and comparing changes in progesterone synthesis, six of the animals were injected with the same dose of oxytocin in the third cycle (Cycle 3). On either the 5th or 8th day of this cycle, the ovary containing the corpus luteum was removed. Six untreated animals were ovariectomized on either the 5th or 8th day after oestrus to obtain the CL-bearing ovary. All heifers with one ovary were injected with oxytocin on Days 3 to 6 following oestrus during the fourth cycle (Cycle 4). Before slaughter, two heifers were treated with oxytocin on Days 1 to 4 and four were treated on Days 1 to 6 after oestrus. Those injected on Days 1 to 4 were killed on Day 4, while two animals treated on Days 1 to 6 were killed on Day 6 and the last were killed on Day 8. The remaining six heifers were used as 4-, 6- and 8-day controls.

Tissue incubation

Ovaries obtained during the third cycle were removed through a flank incision, immediately placed into ice-cold saline, and kept there until dissection. Each CL was removed from the ovary and sliced with a razor blade to an approximate thickness of 0.5 mm. The slices were distributed randomly into one of three different incubation vials. Three samples, each weighing from 100 to 200 mg, were prepared from every CL in order to measure: (1) the initial progesterone content; (2) progesterone synthesis during 2-hr incubation; and (3) progesterone synthesis with LH added for the 2-hr incubation period. Sample 1 was frozen immediately and stored until extraction. Samples 2 and 3 were frozen after incubation and also refrigerated until extraction. The incubation medium consisted of 4 ml Krebs-Ringer bicarbonate buffer with 1 mg/ml glucose. The medium was gassed before use with 95 O₂ : 5 CO₂ (Umbreit, Burris & Stauffer, 1957). Twenty micrograms of NIH-LH-B-1 (5 µg/ml) were added to the third flask to measure the influence of LH on progesterone formation. Flasks 2 and 3 were incubated for 2 hr at 37°C.

Analysis of progesterone

For progesterone analysis, the luteal tissue was homogenized, placed into a separating funnel, and extracted according to the method of Armstrong, O'Brien & Greep (1964). Progesterone was measured with a recording spectrophotometer at a wavelength of 240 nm and 0.08 µCi of [³H]progesterone were added to each sample for estimation of recovery losses. The data were analysed by analysis of variance.

RESULTS

Effects of oxytocin upon cycle length in intact and unilaterally ovariectomized heifers

From Table 1, it can be seen that 75% of the intact animals exhibited a
Oxytocin and progesterone synthesis

Table 1
LENGTH OF TREATED AND CONTROL OESTROUS CYCLES

<table>
<thead>
<tr>
<th>Animal</th>
<th>Length of oestrous cycle in days*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycle 1</td>
</tr>
<tr>
<td>Y16</td>
<td>8</td>
</tr>
<tr>
<td>Y22</td>
<td>9</td>
</tr>
<tr>
<td>Y23</td>
<td>21</td>
</tr>
<tr>
<td>Y27</td>
<td>22</td>
</tr>
<tr>
<td>Y29</td>
<td>8</td>
</tr>
<tr>
<td>Y30</td>
<td>19</td>
</tr>
<tr>
<td>Y31</td>
<td>8</td>
</tr>
<tr>
<td>Y32</td>
<td>9</td>
</tr>
<tr>
<td>Y35</td>
<td>8</td>
</tr>
<tr>
<td>Y39</td>
<td>14</td>
</tr>
<tr>
<td>X239</td>
<td>9</td>
</tr>
<tr>
<td>X288</td>
<td>9</td>
</tr>
</tbody>
</table>

* The first day of heat was called Day 0 post-oestrus.
Cycle 1—the first oxytocin-treated cycle.
Cycle 2—a rest cycle.
Cycle 3—the cycle in which the CL-bearing ovary was removed.
Cycle 4—the second oxytocin-treated cycle.

shortened oestrous cycle following oxytocin administration. After removal of one ovary, only 50% of the animals had shortened cycles. Furthermore, cycle length during oxytocin treatment was more erratic following unilateral ovariectomy. For example, three of the six animals exhibiting shortened cycles had abnormally short di-oestrous periods (3 to 6 days); two animals displayed unusually long cycles (31 and 40 days).

Effects of oxytocin upon progesterone content and synthesis in intact heifers

In Cycle 3, the CL-bearing ovary was removed and analysed for progesterone (Table 2). Differences between the control and oxytocin-treated groups in progesterone content and in vitro synthesis were much greater at Day 8 than at Day 5 post-oestrus. Progesterone synthesis without LH in the 5-day oxytocin-

Table 2
PROGESTERONE CONTENT (µg/g TISSUE) OF CORPORA LUTEA FROM 5- AND 8-DAY CONTROL AND OXYTOCIN-TREATED HEIFERS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean progesterone content ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>5-Day control</td>
<td>53.4±13.7</td>
</tr>
<tr>
<td>5-Day oxytocin</td>
<td>45.4±9.9</td>
</tr>
<tr>
<td>8-Day control</td>
<td>65.5±19.2</td>
</tr>
<tr>
<td>8-Day oxytocin</td>
<td>35.1±9.4</td>
</tr>
</tbody>
</table>

A—Initial progesterone content.
B—Progesterone present after 2 hr of incubation.
C—Progesterone present after 2 hr of incubation with LH.
* Different from control P<0.05.
** Different from control P<0.01.
treated group was approximately 57% of the synthesis found in control tissue. A similar comparison at Day 8 reveals that the tissue from treated heifers synthesized only 40% of the progesterone synthesized by the 8-day controls. It is clear that corpora lutea from treated animals are less efficient at pro-
gesterone synthesis.

The addition of LH to the incubation tissue decreased the difference in progesterone synthesis between treated and control tissue. Approximately 68% as much progesterone was synthesized by 5-day-old corpora lutea from oxytocin-treated animals as by CL from untreated cows, but, by Day 8, CL from the treated group produced only 55% as much progesterone as CL from control animals. Although CL from oxytocin-treated animals were still able to respond to LH in vitro, the response was less.

**Effects of oxytocin upon luteal development in unilaterally ovariectomized heifers**

In the final cycle, half of the animals were injected with 100 U.S.P. units of oxytocin, while the remaining half served as controls. Both groups were killed on Day 4, 6 or 8 post-oestrus. The remaining ovary was removed and examined for luteal development. Four of the six heifers treated with oxytocin failed to develop a corpus luteum; but five of the six control heifers manifested a distinct CL.

**DISCUSSION**

Oxytocin injection shortens the bovine oestrous cycle and inhibits CL function. Luteal progesterone content and in vitro synthesis per gram of tissue decreased throughout the treated cycle, although CL from oxytocin-treated animals were still capable of synthesizing progesterone and responding to LH stimulation on Days 5 and 8 post-oestrus. This response indicated that the luteal tissue excised at either Day 5 or Day 8 from oxytocin-treated heifers is not functionally similar to the 'inactive' CL studied by Armstrong & Black (1966). They found that luteal tissue removed at the end of the normal oestrous cycle synthesized little if any progesterone during incubation and their study also indicated that CL removed at this time were insensitive to LH stimulation. By contrast, luteal tissue removed from intact, oxytocin-treated animals exhibited more than a two-fold increase in progesterone synthesis during incubation with LH.

Quantitative differences in progesterone synthesis between control and oxytocin-treated heifers may be the result of morphological changes within the CL (Armstrong & Hansel, 1959; Donaldson & Hansel, 1965). Decreased progesterone synthesis may result from a decreased number of functional lutein cells since tissue from treated animals was still capable of responding to LH in vitro.

The treated cycle lengths following unilateral ovariectomy were more variable than in intact animals and there were fewer shortened cycles (8 to 12 days). Oxytocin-induced luteal regression does not appear to be dependent on local changes brought about by the presence of a large Graafian follicle on the ovary containing the CL. Instead, these results indicate a hypophysial–gonadal imbalance of hormones. It seems that one change is caused by the
removal of an ovary and that a second variable is added by the injection of oxytocin. Unilateral ovariectomy may alter the normal feedback of ovarian hormones on the hypothalamic–hypophysial axis. Subsequent oxytocin injection appears to aggravate this imbalance leading to a wide range of cycle lengths. Post-mortem examination revealed a greater inhibition of luteal development in the hormone-treated, unilaterally ovariectomized heifers.

Donaldson et al. (1965) have presented evidence to indicate that exogenous oxytocin causes release of pituitary gonadotrophins, particularly LH. In the present study, this hypothesis may partially explain the variable cycle length in many of these heifers. Early gonadotrophin release may also explain the appearance of oestrus during the injection period in three of the six treated animals.

ACKNOWLEDGMENTS

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REFERENCES


