EFFECT OF CIS AND TRANS ISOMERS OF CLOMIPHENE ON REPRODUCTIVE PERFORMANCE IN EWES 1, 2

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CLOMIPHENE (Triethylamine, 2-[p-(2-chloro-1-2-diphenylvinyl) phenoxyl]) is a synthetic non-steroidal estrogen antagonist, which has been widely used in the treatment of sub-fertile women (Greenblatt, 1961; Kistner, 1968) and has been found to cause superovulation in PMS-treated immature rats (Coppola and Perrine, 1965). Clomiphene is a mixture of the trans and cis isomers in approximately equal proportions and previous studies in the rat have indicated that these isomers have different effects (Self, Holtkamp and Kuhn, 1967; DiPietro, Sanders and Goss, 1969; Labhsetwar, 1970). In the rat the trans isomer has been found to have a slight estrogenic action, while the cis isomer was found to be antiestrogenic (Self et al., 1967; Labhsetwar, 1970). Lindsay and Robinson (1970) reported that clomiphene induced estrus without ovulation in anestrous ewes. Since previous studies in rats had shown that the two isomers of clomiphene had different effects, it was thought that either the cis or trans isomer of clomiphene, or both, might be useful in synchronizing estrus and/or causing superovulation in estrous ewes.

The specific objectives of this study were: (1) to determine the effects of both isomers of clomiphene on the occurrence of estrus during and following treatment, (2) to study the effects of both isomers on ovulation rate and fertility, (3) to evaluate the gross effects of both isomers on the reproductive organs, and (4) to determine if either isomer had any effect on body weight gain, wool growth or quality.

Experimental Procedure

Pretreatment Period. A group of white-faced western ewes were checked twice daily for estrus with a marked vasectomized ram beginning September 22, 1967. Body weights were recorded at the start of the pretreatment period and at weekly intervals until the beginning of treatment. At the end of the pretreatment period, 50 ewes which had shown at least two consecutive estrous cycles of normal length were divided into four groups according to stage of the estrous cycle (Day 0-Day 4, Day 5-Day 8, Day 9-Day 12, Day 13-Day 19) 5 and randomly assigned to one of 10 groups as shown in table 1.

<table>
<thead>
<tr>
<th>Day 0 = First day of estrus.</th>
<th>Day 0-Day 4</th>
<th>Day 5-Day 8</th>
<th>Day 9-Day 12</th>
<th>Day 13-Day 19</th>
</tr>
</thead>
</table>
| Pretreatment Period.          | Ewes were checked twice daily for estrus as during the pretreatment period. Body weights were recorded at the beginning, middle (day 9) and end (day 17) of the treatment period. Each ewe received a daily intramuscular injection for 16 days of either the cis or trans isomer of clomiphene in sterile water, according to the schedule shown in table 1. The doses used in this experiment were based on preliminary studies conducted in this laboratory and by the research division of Hess and Clark. The treatment period for the ewes receiving cis-clomiphene was commenced 9 days following the beginning of the treatment period for the ewes receiving trans-clomiphene.

Post-treatment Period. A marked vasectomized ram was allowed to run with the ewes following the treatment period and the ewes were checked every 6 hr. for estrus. Those ewes that were marked by the vasectomized ram were served twice by an intact Hampshire ram and 6 hr. later were bred twice to a Dorset ram. All ewes were checked for estrus in this manner for a period of at least 4 weeks following cessation of treatment and were then checked twice daily for estrus until the end of the breeding season (about the middle of January).

The ewes which received trans-clomiphene were laparotomized 8 days following the cessation of treatment, while the ewes which received cis-clomiphene were laparotomized 4 days following cessation of treatment; these times were selected because preliminary

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studies in anestrous ewes indicated that most of the ewes would exhibit estrus before this time. Each ovary was checked for the number of ovulations by counting ovulation points and new corpora lutea. The size of all follicles greater than 4 mm in diameter and corpora lutea on each ovary were recorded and the diameters of each uterine horn were measured. The general vascularity of the ovaries and uteri was noted.

Body weights were recorded at weekly intervals for a period of 8 weeks following cessation of treatment.

Sixty days after the beginning of the treatment period the length of wool in a small area on the left flank, which had been clipped at the beginning of the treatment period, was measured and a small portion of the wool in this area was clipped and weighed. Later, the wool collected from this area was graded according to the Bradford system.

**Lambing Period.** The weight of each lamb at birth, and the sex and number of lambs born per ewe were recorded at the time of lambing. The ewes were weighed 1 week post-partum and the lambs were weighed 4 and 8 weeks after birth.

All data were analyzed by covariance analysis and treatment differences were determined by the least significant difference test (Steel and Torrie, 1960).

**Results**

**Occurrence of Estrus Before, During and Following Treatment.** Trans-clomiphene had no effect on the occurrence of estrus. There were no significant differences in cycle length between pre-treatment (period between last two heats before treatment was begun), treatment (period between last estrus before treatment and first estrus during treatment period) or post-treatment cycle (period between last estrus which occurred during treatment and first estrus following treatment). The mean treatment cycle lengths for the five groups were 17.0, 17.0, 17.0, 16.6 and 17.2 days.

There were no significant differences in the length of the treatment cycles for the ewes receiving cis-clomiphene. The mean treatment cycle lengths for the five groups were 17.5, 16.4, 18.2, 17.8 and 17.2 days. Likewise, there were no significant differences between the length of pretreatment, treatment and post-treatment cycles, although the post-treatment cycle for two groups was longer than normal. This was because one ewe in both groups (II-B and V-B) had cycles at least twice the normal length. Although all the ewes in Group V-B had treatment cycles which were within the normal range, four of the five ewes were detected in estrus a second time within a 48-hr. period near the end, or slightly after the treatment period. These ewes had returned to estrus within a period of 3 to 7 days; in some cases they appeared to be in constant estrus.

**Ovarian and Uterine Morphology.** Trans-clomiphene had no significant effect on any of the ovarian parameters measured (table 2) or on the average diameter of either uterine horn. All ewes receiving this isomer ovulated following the estrus that occurred during or shortly after treatment, as indicated by the presence of functional corpora lutea at the time of laparotomy. All but three of the ewes had one or more follicles greater than 4 mm.

**TABLE 2. EFFECT OF CIS OR TRANS CLOMIPHENE ON OVARIAN MORPHOLOGY AS DETERMINED BY LAPAROTOMIES FOLLOWING TREATMENT**

<table>
<thead>
<tr>
<th>Group</th>
<th>Avg No. of functional corpora lutea/ewe</th>
<th>Avg No. of follicles &gt; 4 mm/ewe</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-A</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>II-A</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>III-A</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>IV-A</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>V-A</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>I-B</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8</td>
</tr>
<tr>
<td>II-B</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6</td>
</tr>
<tr>
<td>III-B</td>
<td>1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;, 2</td>
</tr>
<tr>
<td>IV-B</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;, 2</td>
</tr>
<tr>
<td>V-B</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means with the same subscript number are not significantly different; those with different numbers differ significantly (<i>P</i> < .05); treatment groups for which no subscript numbers are shown are not significantly different.
in diameter. No differences in ovarian or uterine vascularity were observed among the five groups.

The ovaries of the ewes (Group V-B) receiving the highest levels of cis-clomiphene (1.5 mg/day) were markedly different from all other ewes in the experiment (table 2). None of these ewes ovulated at either estrus which occurred during or shortly after treatment. Only one follicle greater than 4 mm in diameter was seen in the five ewes in Group V-B; on the other hand only three ewes in the other four groups lacked follicles greater than 4 mm in diameter. Although no differences in the average diameter of the uterine horns or in uterine vascularity were observed between the five groups, the ovaries of the ewes in Group V-B were noticeably pale and poorly vascularized when compared to the other four groups. The only exception to this observation was the single ewe in Group V-B that had a follicle 7 mm in diameter.

**Fertility.** Although the group which received the highest level of trans-clomiphene (Group V-A) required slightly more services per conception (1.8 services vs. 1.2 services) and had a longer elapsed time between the initiation of treatment and conception (35.8 days vs. 28.3 days) compared to the other four groups, there were no significant differences in fertility between any of the groups. The average length of gestation for the five treatment groups was approximately the same.

The group which received the highest level of the cis isomer (Group V-B) required a significantly greater number of services per conception than the other four groups (P<.05) (table 3). However, there were no significant differences in the length of time from initiation of treatment to conception among the groups. There was a significant difference in average length of gestation between Group II-B and the other three groups which received the cis-clomiphene (P<.05), however all averages were within the normal range.

**Lambing.** There were no significant differences in lambing rate, average weight of lambs at birth (table 4), lamb weight at 4 or 8 weeks of age, or ewe weight 1 week postpartum between the various treatment groups for either the cis or trans isomers. Likewise, the trans isomer had no significant effect on the sex ratio of the lambs; however, the sex ratio for the groups receiving 0.3 mg and 0.06 mg/day of the cis-clomiphene (Groups III-B and IV-B) were significantly different from the expected ratio (P<.05) (table 4).

**Body Weight and Wool Growth.** There were no significant differences in average daily weight gain between the various treatment groups for either the trans or cis isomer for any of the three periods. However, there was a significant treatment period effect for the Group A ewes (P<.01). Most of the ewes receiving trans-clomiphene or the saline control lost a small amount of weight during the treatment period.

Trans-clomiphene had no significant effect on wool growth or quality. The cis isomer also had no effect on wool growth, but a significant difference in wool grade was found between the group receiving the lowest level of the cis clomiphene (Group II-B) and Groups V-B and III-B (P<.05).

**Discussion**

Trans-clomiphene, in doses ranging from 0.17 mg/day to 4.5 mg/day, a 27-fold difference, had no effect on any of the reproductive...
parameters studied. Recent studies in humans (Charles et al., 1969) indicate that transclomiphene has little or no effect in stimulating urinary steroid output or causing ovulation in anovulatory women. In the rat, Labhsetwar (1970) found that trans-clomiphene in doses up to 5 mg/kg had no ovulation blocking action, while 1 mg/kg of cis-clomiphene caused complete ovulation blockage. However, Self, Holtkamp and Kuhn (1967) reported that trans-clomiphene facilitated PMS-induced ovulation in immature rats and had an uterotropie effect in immature castrate rats.

The ability of the high dose of cis-clomiphene (1.5 mg/day) to block ovulation in all ewes is of particular interest. The effects of this dose of cis-clomiphene (1.5 mg/day) are similar to those reported by Hansel (1967) for cycling ewes fed 150 mg of clomiphene/ewe per day, and those reported by Lindsay and Robinson (1970) for anestrous ewes receiving intramuscular injections of 10 mg or more of clomiphene prior to or after progesterone withdrawal. Hansel (1967) found that the ewes continued to cycle during the treatment period. However, 11 of 15 ewes were in estrus within a 24-hr. period after the last feeding; only two of the 15 ewes conceived. Similar to our observations, several of the ewes appeared to be in constant estrus for a period of several days. Therefore, it seems reasonable to conclude that the results obtained by Hansel (1967) were probably due to the cis-clomiphene in the preparation used.

Experiments with rats (DiPietro et al., 1969; Labhsetwar, 1970) have shown that cis-clomiphene has an antiestrogenic effect, while trans-clomiphene has an estrogenic effect. It is suggested that the results obtained with cis-clomiphene in this experiment were due to its antiestrogenic action in blocking estradiol induced preovulatory LH release. Recent studies by Goding et al. (1969) and Scaramuzzi, Caldwell and Moor (1970) indicate that estradiol is probably responsible for inducing the preovulatory LH surge in sheep. Clomiphene was found to reduce markedly the uptake of tritiated estradiol by the anterior hypophysis and anterior hypothalamus in ovariectomized rats (Kato, Kobayashi and Ville, 1968). This hypothesis is also supported by the studies of Labhsetwar (1970), who showed that the ovulation blocking action of clomiphene in the rat was due to its ability to block the positive feedback of estrogens on LH release.

It is difficult to explain the effect of cis-clomiphene on the occurrence and duration of estrus if it is considered to have antiestrogenic activity. The most likely explanation is that the sheep has one hypothalamic center concerned with the regulation of estrus behavior and another regulating ovulation, as shown for the rat by Halasz and Gorski (1967) and that cis-clomiphene has an antiestrogenic action on the ovulation center and an estrogenic effect on the estrous behavior center. Studies in rats by Self et al. (1967) and Labhsetwar (1970) also indicate that cis-clomiphene has some estrogenic, as well as antiestrogenic effects.

The reason for the significant difference in sex ratio obtained with .06 and 0.3 mg of cis-clomiphene per day is not known. There is no evidence from this experiment, or experiments with clomiphene in other mammals (Holtkamp et al., 1960; Kistner, 1968; Labhsetwar, 1970) that would give any reason to expect a difference in sex ratio. Experiments with larger numbers of ewes need to be conducted before any significance can be attributed to these differences.

The results of this experiment indicate that neither the cis or trans isomers of clomiphene have any practical application in promoting body weight gains or wool growth. Although there was a significant difference in wool grade between the groups receiving .06 mg and 1.5 mg of cis-clomiphene per day and the group receiving .012 mg of cis-clomiphene per day, all the wool grades were within the normal range found for western white face ewes. We have no good explanation why the ewes in Group A had significantly lower body weight gains during the treatment period.

Cis-clomiphene would probably not be useful for estrous cycle regulation methods that depend on producing synchronized estrus following withdrawal of the compound. However, it could prove useful if used prior to gonadotropin injections in synchronization techniques designed for inseminating animals at a predetermined date and without regard to the occurrence of estrus.

Summary

Trans-clomiphene injected intramuscularly daily for 16 days in doses ranging from 0.17 mg to 4.5 mg/day did not result in synchronized estrus cycles following withdrawal and had no effect on ovulation rate or fertility. On the other hand, 1.5 mg of cis-clomiphene injected daily for 16 days resulted in 4 of 5
ewe being in estrus within a 48-hr. period at the end of the treatment period. This dose of cis-clomiphene completely blocked ovulation and caused a significant reduction in the number of follicles greater than 4 mm in diameter (P<.01) and a significant increase in the number of services required for conception (P<.01). Doses of .012, .06 and 0.3 mg of cisclomiphene per day had no effect on the occurrence of estrus, ovulation rate or fertility, except that the dose of .06 mg caused a significant increase in the number of functional corpora lutea (P<.05). Neither isomer of clomiphene stimulated body weight gains or wool growth. However, cis-clomiphene could prove useful if used prior to gonadotropin injections in synchronization techniques designed for inseminating animals at a predetermined date without regard to the occurrence of estrus.

Literature Cited


