INVESTIGATION ON INDUCTION OF LIMITED MULTIPLE OVULATIONS IN CATTLE
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During the past few years substantial progress has been made in the induction of multiple ovulation in cattle by hormone treatment. The practical results, however, have been unsatisfactory. Hitherto, only about 30% of treated cows ovulated the limited number of eggs (two or three) likely to lead to the birth of twins or triplets. In many cases only one egg could be obtained or the animals failed to ovulate; in others, four or more eggs were ovulated, and normal embryonic development did not ensue.

The following investigations deal with the problem of inducing twin pregnancies by the ovulation of only two or three eggs. Forty-seven cows of the Max Planck Institute were treated with different sex hormones. All cows had been shown to be fertile in tests extending over the previous two or three years. They were kept under the same environmental conditions and fed individually. During the trial, the treatments were changed in different ways (see Table 1).

First experimental group
Thirteen cows received two intramuscular injections of pregnant mare’s serum (PMS) (Anteron—Schering AG, Berlin) on the 17th and 18th day after the last heat. The total dose amounted to 2000 to 2500 i.u. Three different preparations containing this hormone were tested. The animals were slaughtered between the 17th and 156th day after conception. One of these animals failed to ovulate and eight had only one corpus luteum. Four showed multiple ovulations; two of these had ovulated three eggs, one five, and the other seven. In all the ovaries the number of small (<1-cm) and ripe (>1-cm) follicles was larger than in untreated animals.

Second experimental group
In this group it was intended to standardize the cows with respect to their hormonal status. The corpus luteum was enucleated between the 14th and 16th days of the cycle and 100 mg progesterone was injected daily for the next 5 to 7 days, until the expected time of the next oestrus. Twenty-four hours after the last injection of progesterone, four animals were given two injections of 2000 to 3000 i.u. PMS. The other four cows of this group received 1000 i.u. PMS in a single injection.
**Table 1**

**RESULTS OBTAINED BY TREATING COWS WITH VARYING PROPORTIONS OF PROGESTERONE, PMS AND LH**

<table>
<thead>
<tr>
<th>Group</th>
<th>Enucleation (day of cycle)</th>
<th>Progesterone injected (days)</th>
<th>PMS (i.u.)</th>
<th>LH (i.u.)</th>
<th>No. animals</th>
<th>No. animals with different ovulation rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. eggs ovulated</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>None</td>
<td>2000 to 2500</td>
<td>None</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>14 to 16</td>
<td>5 to 7</td>
<td>(a) 2000 to 3000</td>
<td>None</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>12 to 14</td>
<td>14</td>
<td>1500</td>
<td>1000 to 3000</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>16 to 18</td>
<td>None</td>
<td>(a) 1000</td>
<td>(b) 2000</td>
<td>4000</td>
<td>11</td>
</tr>
</tbody>
</table>
Of the four cows which received the higher dose of PMS, two ovulated more than one egg (three and nine respectively). Two cows which received the lower dose of PMS failed to ovulate, and in the remaining four animals there was only one corpus luteum. The average number of small follicles was sixteen, and of ripe follicles four, per animal. The largest numbers of follicles were found in animals which did not ovulate.

**Third experimental group**

The corpora lutea of twelve cows were enucleated on the 12th to 14th day of the cycle. They received a daily dose of 100 mg progesterone during a period of 14 days. Following this, two injections totalling 1500 i.u. PMS were given. During oestrus, either one or two doses of 1000 to 3000 i.u. luteinizing hormone (LH) (Primogonyl—Scherling) or 25 to 30 mg LH (PIH—Armour) were injected.

Only two out of twelve cows ovulated two or three eggs and two ovulated only one. The other eight animals failed to ovulate; they all had two to six ripe follicles. The average number of small follicles was also found to be high (average 9.5).

**Fourth experimental group**

The hormone treatment applied in this group was based on the following argument. It was assumed that a ripe follicle begins to develop during or just after the last oestrus. There is also reason to believe that this follicle grows with others, which become atretic. Our aim was to prevent the process of atresia, or to stimulate the growth of new follicles, by an additional dose of PMS during the postoestrous period. This dose of PMS was not intended to lead to oestrus or ovulation. The method of treatment was as follows:

On the 5th day after heat, a single dose of 1000 to 1500 i.u. PMS was given to eleven cows. (Only one animal showed a weak heat.) The corpora lutea were enucleated between the 16th and 18th day followed by a dose of 2000 i.u. PMS. Progesterone was not given. After 3 to 5 days all the animals came in heat, and they were injected with a total of 4000 i.u. LH (Primogonyl) intravenously. They were slaughtered 48 to 96 hr after ovulation. Out of the eleven cows of this group, four ovulated two eggs and four three eggs. One animal produced only one egg while the remaining two failed to ovulate. In this trial, therefore, more than 70% of the treated cows had developed the desired number of two or three eggs. An average of only three small follicles was found on the ovaries. Ripe follicles, not more than four in number, were found only in cows which failed to ovulate.

Out of three cows treated by the same method but omitting the injection of PMS on the 5th day of the cycle, only one showed a multiple ovulation of three eggs. An average of sixteen small follicles could be counted on the ovaries.

We believe that the method used in Group 4 closely follows the natural process of follicular development. No histological defects were observed. The fertility of the eggs in this experiment was found to be normal; of fifteen eggs, fourteen were fertilized.

These investigations will be continued with a view to finding means of regularly producing twin pregnancies in cattle.
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