OVULATION RATES AND LITTER SIZES IN SEXUALLY RECEPTIVE AND NONRECEPTIVE ARTIFICIALLY INSEMINATED RABBITS GIVEN VARYING DOSAGES OF LUTEINIZING HORMONE


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Summary. At any one time a large proportion of individually caged, sexually mature female rabbits failed to copulate. Intravenous administration of purified pituitary luteinizing hormone (PLH) (Armour) produced ovulations in sexually receptive and nonreceptive does alike. Does with histories of infertility ovulated after the injection of PLH. The routine administration of 2.5 mg of PLH to fifty-seven virgin and multiparous Dutch-Belted does averaging 2 to 3 kg, followed by artificial insemination, resulted in 91% of the does kindling and 307 young being born. Administration of either 0.5 mg or 1 mg of PLH per kg to random members of fourteen pairs of does, accompanied by artificial insemination, resulted in no difference between the number of ovulations or the number of young per treatment (P > 0.05). Fertility and litter size were normal when sixteen does were re-injected with PLH and inseminated after weaning their previous litters.

INTRODUCTION

Because an appreciable percentage of does will not mate at any one time experimental designs to study reproductive problems with rabbits may become unworkable, unless it is established that artificial induction of ovulation leads to normal pregnancy, and post-kindling nursing care of the young. Hammond & Marshall (1925) reported that not all does would copulate at any one time, a majority being nonreceptive in the autumn. Similar phenomena were observed by Venge (1950). Hammond & Asdell (1927) found that insemination without sterile copulation resulted in 3.6% conception, and insemination plus an attempted mating of does that refused to copulate resulted in 13.3% conception. Insemination and sterile mating of receptive does resulted in 90% conception. The low conception rate in the first two groups was believed to be

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due to failure to induce ovulation. Experience in the authors’ laboratory confirmed the poor response obtained by forced mating, although Menzies & Moss (1960) have obtained favourable results. While rabbits usually do not ovulate as a result of mechanical stimulation of the vagina alone, Sawyer & Markee (1959) produced ovulations in 45% of receptive rabbits and 40% of non-receptive rabbits by oestrogen priming followed by stimulation of the vagina with a glass rod.

The ability of luteinizing hormone (LH) to cause ovulation in the rabbit has long been recognized (Pincus, 1940; Parkes, 1943). These workers observed that not all the treated does would mate and that not all young does which mated after being primed with follicle-stimulating hormone (FSH) would ovulate and form functional corpora lutea. In many reports crude extracts and/or superovulation-priming-treatments plus sterile matings were used in addition to artificial insemination or natural matings, to obtain fertilized eggs (Adams, 1954, 1956, 1960; Beatty, 1958; Chang, 1951, 1958; Hafez, 1961). Kawakami & Sawyer (1959) have found encephalographic changes following LH injection similar to those following coitus. Several studies have involved pseudopregnancy (Austin, 1949; Boyarsky, Baylies, Casida & Meyer, 1947; Murphree, Warwick, Casida & McShan, 1947) but few experiments have allowed the does to go to term. Warwick, Murphree, Casida & Meyer (1943) found that the number of young born following hormonal treatments was smaller than the number normally expected.

Chang (1951) reported that only three out of thirteen nonstimulated does receiving transferred eggs and exogenous progesterone made a nest at parturition. Edwards & Gates (1959) found that fertilized mouse eggs could be readily obtained by artificial insemination following pregnant mares’ serum (PMS) and human chorionic gonadotrophin (HCG) injections. However, mating apparently was required to produce functional corpora lutea for maintenance of pregnancy.

There is little evidence in the literature to indicate whether or not an injection of purified LH simultaneous with artificial insemination (without sterile mating or further stimulation) in receptive and nonreceptive rabbits would result in a high percentage of normal pregnancies and the production and maintenance of normal litters. This study was undertaken to provide such evidence, to investigate the LH dosages required, and to determine whether successive injections would result in a similar response. Since this work was undertaken Harper (1961) has reported that LH produces ovulation at the normal time, and Adams (1961) has reported that LH injections and artificial insemination result in efficient reproduction.

MATERIALS AND METHODS

The first experiments to determine the proportion of does which would copulate were carried out with Dutch-Belted, New Zealand and crossbred does. Subsequently, only Dutch-Belted does colony-bred in the department were used. The animals were maintained on a high quality complete pelleted diet containing a minimum of 17% protein. During most of the study, carried out at various times of the year, supplementary lighting to ensure 12 hr of light per
day was provided, thus minimizing possible seasonal effects. All does were caged individually for at least 18 days before being used experimentally.

Each doe was placed in a cage with a vigorous vasectomized buck. The does which exhibited lordosis were considered to be receptive, and those which did not do so after several copulation attempts by the buck were considered to be nonreceptive. In experiments where does were not classified as to receptivity they were assigned at random to treatment groups.

The hormone used to produce ovulation was a highly purified pituitary luteinizing hormone (PLH) (Armour). Just prior to use it was dissolved at the rate of 5 mg/ml in sterile 0·9% sodium chloride. Each doe was injected intravenously into the marginal ear vein, immediately before insemination. In one experiment, 0·2 mg of follicle stimulating hormone (FSH) (Armour) was injected subcutaneously twice daily for 5 days before intravenous injection of PLH the following morning.

Semen was collected from bucks in which there had been no selection or culling for fertility. The collection procedure, semen evaluation, and artificial insemination was performed as described by Gregoire, Bratton & Foote (1958), except that a small amount of glycerol replaced the K-Y lubricant for semen collection. Insemination was performed about 1 hr after semen collection by placing 0·1 to 0·2 ml of semen in the anterior portion of the vagina. The semen was used either undiluted, or diluted with a few drops of 0·9% saline solution, depending upon the needs. Each insemination was made with at least three million motile spermatozoa. Each sample of semen was distributed over all treatments to avoid confounding of ejaculates and treatments. Some does were killed, and the ovaries examined, 16 hr after insemination; others were examined during later stages of pregnancy or allowed to go to term.

RESULTS

PROPORTION OF RECEPTIVE AND NONRECEPTIVE DOES AND THEIR RESPONSE TO PLH

Experiment 1

Ninety-one ‘mature’ does of mixed breeding were purchased and tested for lordosis. Does which accepted vasectomized males were mated or artificially inseminated. Half of the nonreceptive does were killed and examined within 24 hr, and the remainder were re-tested for lordosis 50 days later. The results are shown in Table 1. Only 41% of the does permitted copulation. In agreement with Parkes (1943), there was no relationship between copulatory behaviour and PLH.
Experiment

Mated hormone quite shown. The reproductive organs of ten of these twenty-seven does killed appeared to be infantile. Fifty days later 31% of the previously nonreceptive does accepted a male and ovulated. These data substantiate earlier work (Hammond & Asdell, 1947; Hammond & Marshall, 1925) on the high proportion of nonreceptive does obtained at any one time.

Subsequently, it was found that twenty nonreceptive or immature does receiving a single intravenous injection of 5 mg of PLH ovulated. Other nonreceptive does given priming dosages of follicle-stimulating hormone (FSH) plus PLH ovulated, but whether or not these would have borne normal young was not tested. Also, it has been observed recently that many nonreceptive females in the colony become receptive the same day when repeatedly exposed to males.

Experiment 2

Thirty-nine New Zealand does were tested for receptivity with vasectomized males. Only twelve does showed willingness to accept the males, but these were not allowed to copulate. Thereupon seven of the twelve receptive does were mated with normal males and five were given a priming series of FSH injections, then 5 mg of PLH, and mated, or artificially inseminated if they refused to mate. The twenty-seven nonreceptive does were given the same FSH and PLH treatment and mated (one responded), or artificially inseminated. The results are shown in Table 2.

<table>
<thead>
<tr>
<th>Classification of does</th>
<th>No. does</th>
<th>Pregnant</th>
<th>Corpora lutea*</th>
<th>Foetuses*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptive, bred naturally</td>
<td>7</td>
<td>2</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>Receptive, given FSH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Still receptive, mated</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nonreceptive + PLH + A.I.</td>
<td>4</td>
<td>1</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>Nonreceptive, given FSH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Then receptive, mated</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>19</td>
</tr>
<tr>
<td>Nonreceptive + PLH + A.I.</td>
<td>26</td>
<td>12</td>
<td>46</td>
<td>171</td>
</tr>
</tbody>
</table>

* Data for pregnant does.

The same males used for the natural matings supplied the semen for artificial insemination. Unfortunately, the fertility of the bucks used was apparently quite low. However, the results in Table 2 show that although most of the does not receptive to males they were as fertile and useful when given the hormone injections and inseminated artificially as were the receptive does mated naturally.

Experiment 3

Since Dutch-Belted rabbits are about half the weight of New Zealands, 2-5
mg of PLH usually was administered to the smaller breed with satisfactory results. Animals were killed within 60 hr of mating and the corpora lutea counted. As can be seen by the results in Table 3, all does ovulated regardless of their breeding history.

**Table 3**

<table>
<thead>
<tr>
<th>History of does</th>
<th>No. does</th>
<th>No. does with corpora lutea</th>
<th>Average No. corpora lutea</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertile</td>
<td>8</td>
<td>8</td>
<td>8.7</td>
<td>2.78</td>
</tr>
<tr>
<td>Infertile</td>
<td>10</td>
<td>10</td>
<td>6.7</td>
<td>2.29</td>
</tr>
</tbody>
</table>

The average number of corpora lutea was larger for the fertile group but the difference was not statistically significant ($P > 0.05$). Ovulation failure did not appear to be responsible for breeding failure in the infertile group, because eggs were flushed from the oviducts of most does. However, two does had bilateral occlusions of the oviducts.

**Experiment 4**

To determine whether or not receptive and nonreceptive Dutch-Belted does not primed with FSH would respond as expected to PLH injections, thirty-two mature virgin Dutch-Belted does were checked, and fourteen were found to be receptive to vasectomized males. Copulation was not permitted. Fourteen does were randomly chosen from the eighteen that would not accept the male and paired with the fourteen receptive does. Following intravenous PLH injections the does were artificially inseminated. The results are shown in Table 4. The level of 5 mg of PLH was included to ensure that, should nonreceptive does fail to respond, the possibility of failure due to too low a dosage would be minimized.

**Table 4**

<table>
<thead>
<tr>
<th>Classification and treatment of does</th>
<th>No. does</th>
<th>No. kindled</th>
<th>Total No. young</th>
<th>Average litter size</th>
<th>Average weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptive</td>
<td>14</td>
<td>9</td>
<td>46</td>
<td>5.1</td>
<td>2.24</td>
</tr>
<tr>
<td>Nonreceptive</td>
<td>14</td>
<td>7</td>
<td>30</td>
<td>4.3</td>
<td>2.22</td>
</tr>
<tr>
<td>2.5 mg PLH</td>
<td>16</td>
<td>9</td>
<td>39</td>
<td>4.3</td>
<td>2.16</td>
</tr>
<tr>
<td>5.0 mg PLH</td>
<td>12</td>
<td>7</td>
<td>37</td>
<td>5.3</td>
<td>2.30</td>
</tr>
<tr>
<td>Total or average</td>
<td>28</td>
<td>16</td>
<td>76</td>
<td>4.8</td>
<td>2.23</td>
</tr>
</tbody>
</table>
ROUTINE AND REPEATED USE OF PLH

The routine use of 2.5 mg of PLH was checked by inseminating each of fifty-seven does with 0.1 ml of semen containing at least three million motile spermatozoa, after injection of PLH. Thirty-one of the does were virgins, at least 8 months old. The remaining twenty-six does were parous. Whether or not the does would copulate was not tested. The results are presented in Table 5.

Table 5

<table>
<thead>
<tr>
<th>Classification of does</th>
<th>No. inseminated</th>
<th>No. kindled</th>
<th>Total No. young</th>
<th>Average litter size</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgins</td>
<td>31</td>
<td>27</td>
<td>150</td>
<td>5.6</td>
<td>2.32</td>
</tr>
<tr>
<td>Previously kindled</td>
<td>26</td>
<td>25</td>
<td>157</td>
<td>6.3</td>
<td>2.38</td>
</tr>
<tr>
<td>Total or average</td>
<td>57</td>
<td>52</td>
<td>307</td>
<td>5.9</td>
<td>2.35</td>
</tr>
</tbody>
</table>

The percentage of does kindling as a result of the single insemination and injection of 2.5 mg of PLH was 91%. This equals or exceeds results obtained by naturally-mated receptive does in the colony (Gregoire et al., 1958).

Sixteen of the females which kindled were again given 2.5 mg of PLH and were re-inseminated as soon as they had weaned their young. These sixteen does produced ninety-one young the first time and the second time fifteen conceived and produced eighty-nine young. The results suggest that the procedure can be repeated at least once in the same animals with equal response. Many does have been injected up to four times over a period of 2 years without problems, such as might develop due to antihormone effects. However, Adams (1961) has recently shown that repeated frequent injections lead to greatly reduced conception rates.

DOSAGE OF PLH

Other does, including some larger than 4 kg, ovulated when given 2.5 mg of PLH. Thus, this particular preparation of purified PLH appeared to give results similar to those obtained with chorionic gonadotrophic (Parkes, 1943). Several does given as little as 1.25 mg of PLH, and killed 24 hr later had ovulated.

Experiment 5

Twenty-eight Dutch-Belted does were paired on the basis of body weight. In random order one number of the pair was given 1 mg and the other 0.5 mg of PLH per kg of body weight. Within each hormone level half of the does were killed after 15 days of pregnancy and half were allowed to go to term. All does had previously been inseminated on at least one occasion, and all but two had kindled. A summary of the results is given in Table 6. Three of the does in the group which received the high level of PLH were killed, and one doe in each of the other three groups failed to conceive. However, none of the differences between hormone levels were statistically significant ($P > 0.05$).
One doe in each group that was allowed to go to term failed to kindle. The two were re-injected with the same hormone dosage given previously and sacrificed 18 hr later. The doe receiving 1 mg of PLH per kg had nine recently ruptured follicles, and the doe receiving 0·5 mg of PLH per kg had seven ruptured follicles.

<table>
<thead>
<tr>
<th>Hormone level</th>
<th>Killed</th>
<th>Kindled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. does</td>
<td>No. corpora lutea</td>
</tr>
<tr>
<td>1 mg PLH/kg</td>
<td>7</td>
<td>61</td>
</tr>
<tr>
<td>0·5 mg PLH/kg</td>
<td>7</td>
<td>61</td>
</tr>
</tbody>
</table>

**DISCUSSION AND CONCLUSIONS**

While the rabbit is very useful for many reproductive studies, the failure of a significant proportion of rabbits to mate at a particular time may complicate an experiment, lead to increased variability and possibly to biased results. The data reported confirm those of Hammond & Marshall (1925), that a relatively high proportion of does, although not pseudo-pregnant, may refuse to copulate at any particular time. Supplementary lighting to provide at least 12 hr of light each day throughout the year does not eliminate the problem.

Body weight, previous history, appearance of the vulva, and receptivity of the female to a male are not certain indicators that ovulation will occur (Parkes, 1943; Hammond & Marshall, 1925). However, most of the does that accept a male ovulate, while most of the does that refuse the male do not ovulate.

The data presented in this report indicate that a purified PLH is capable of inducing normal ovulation, with subsequent corpus luteum formation and maintenance of pregnancy, in mature sexually receptive does as well as non-receptive does. Intravenous administration of 0·5 mg of PLH per kg of body weight produced equally as satisfactory a response as when twice this concentration was used. Two successive injections of the purified PLH and infrequent injections up to four did not result in infertility. However, Adams (1961) has produced infertility in rabbits by frequently repeated injections. This difference in results may have been due to the different hormone preparation used, or the larger number of frequent re-injections studied by Adams (1961).

This procedure of inducing ovulation by injection of PLH combined with artificial insemination is convenient, economical, and useful in carrying out experiments with sound statistical design. One hundred per cent of the does examined surgically after intravenous injection of PLH ovulated. A high proportion of those inseminated became pregnant, and produced normal litters. Because of this high reproductive efficiency and the opportunity to extend the use of outstanding bucks, it may be of practical value to the rabbit breeder as well.
ACKNOWLEDGMENTS

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REFERENCES


BEATTY, R. A. (1958) Variation in the number of corpora lutea and in the number and size of 6-day blastocysts in rabbits subjected to superovulation treatment. J. Endocrin. 17, 248.


