Usefulness of a Simplified Artificial Insemination Technique in the Rabbit for Teratology Studies

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We describe usefulness of our simplified artificial insemination technique in rabbits for teratology studies. Our technique includes 1) semen collection from 2 or 3 bucks with a simple artificial vagina, 2) deposition of 0.5 mL of the diluted semen mixture in the vagina with a glass pipette, and 3) injection of 25 U. of hCG through the auricular vein. Applying these techniques and procedures and keeping about 10 proven bucks would enable 2 operators to inseminate 10-15 does daily by only 1 hour work. This artificial insemination technique should be useful for rabbit teratology studies, especially for safety evaluation studies of toxicants, where a relatively large number of pregnant are needed to be prepared in a short period.

In teratology studies with rabbits, pregnant does are usually obtained by natural matings. This procedure, however, has great disadvantages in time and hands as well as in space for keeping a large number of bucks. Although an artificial insemination technique in the rabbit has been recognized to be useful for resolving these problems, most of the laboratories hesitate to apply it since the improvement of instruments and procedures have been made on an individual-laboratory basis and few published references giving a full detail of the entire technique are available. In this report, we describe our simplified artificial insemination technique in the rabbit.

Male and female SPF Japanese White (Kbl: JW, KITAYAMA LABES Co., Ltd.) rabbits were used at 22 and 18 weeks or more of age, respectively. Equipments used for artificial insemination include an artificial vagina, test tubes (5 and 20 mL), pipettes (1-20 mL), syringes (1 mL), inseminating pipettes, hCG, sterile physiological saline, an incubator, and a microscope (Figs. 1-3).

For semen collection, the chamber of an artificial vagina is first filled with hot water (45-50°C) and capped, and a test tube for semen collection is fixed into a hole of the cap (Figs. 1 and 2). Then, 2 bucks are placed in a pen. When one buck mounts another and demonstrates mating behavior, an operator holds the artificial vagina with his/her hand, puts it under the lower abdomen of the buck between the hind legs, and introduces the penis into it to collect ejaculated semen (Fig. 4). When the buck does not come into ejaculation after several intromissions, hot water has to be exchanged to keep the temperature of an artificial vagina between 45 and 50°C. Mean (and range) volume of semen collected from bucks by these techniques was 0.56 (0.1-1.6) mL.

Collected semen is checked for volume, contaminants, and sperm motility. If semen is as little as 0.1 mL or less in its volume, is contaminated by the urine, or has low sperm motility or sperm count, then it is discarded. Semen collected from 2 or 3 different bucks are mixed and diluted 10-fold with sterile physiological saline. A clear gelatinous plug in semen should be removed before dilution, if present. The dilution is reexamined microscopically for semen motility before insemination.
Fig. 1. An artificial vagina (A) (and its components: a: rubber band b: plastic pipe c: plastic cap d: surgical drainage tubing and e: test tube) and inseminating pipettes (B)

Fig. 2. A schematic drawing of the artificial vagina for rabbits

Fig. 3. A set of equipments for artificial insemination in the rabbit: a: incubator b: test tubes c: microscope d: inseminating pipettes e: syringes and needles f: hCG g: sterile physiological saline and h: pipettes

Fig. 4. Semen collection from a buck

Fig. 5. Deposition of semen in the vagina of a doe

For deposition of semen, an assistant operator holds a doe on his/her knees with its back down. An operator takes 0.5 ml of diluted semen with a glass inseminating pipette having an angle of 150°C at a point of 4 cm from the end (Fig. 1), and inserts a pipette into the vagina (Fig. 5). The pipette is first inserted to the point of its angle into the pronaus with its inner angle to the back of a doe. Then a pipette is turned 180°C so that the inner angle can face the ventral side of the doe. After inserting a pipette 12 cm more, semen is deposited in the
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Table 1. Reproductive and teratological data collected from artificially inseminated female rabbits

<table>
<thead>
<tr>
<th>Index</th>
<th>No. of does examined</th>
<th>Mean (minimum–maximum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception rate (%)</td>
<td>376</td>
<td>93.7 (71.9–100)</td>
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<tr>
<td>Abortion rate (%)</td>
<td>376</td>
<td>1.4 (0–5.6)</td>
</tr>
<tr>
<td>% of pregnant does with live fetuses</td>
<td>370</td>
<td>85.7 (53.6–100)</td>
</tr>
<tr>
<td>Number of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpora lutea</td>
<td>303</td>
<td>11.4 (10.3–12.6)</td>
</tr>
<tr>
<td>Implants</td>
<td>303</td>
<td>9.1 (8.0–10.1)</td>
</tr>
<tr>
<td>Live fetuses</td>
<td>303</td>
<td>7.9 (6.7–9.2)</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>303 (2400)</td>
<td>0.519 (0.481–0.563)</td>
</tr>
<tr>
<td>Fetal mortality (%)</td>
<td>303</td>
<td>13.2 (7.6–17.5)</td>
</tr>
<tr>
<td>Fetal weight (g)</td>
<td>303 (2400)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38.2</td>
<td>(35.0–42.5)</td>
</tr>
<tr>
<td>Female</td>
<td>37.1</td>
<td>(34.4–41.0)</td>
</tr>
<tr>
<td>Placental weight (mg)</td>
<td>303 (2400)</td>
<td>5390 (4917–6359)</td>
</tr>
<tr>
<td>Fetal malformation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>External malformation (%)</td>
<td>303 (2400)</td>
<td>0.25 (0–0.88)</td>
</tr>
<tr>
<td>Visceral malformation (%)</td>
<td>265 (2146)</td>
<td>0.82 (0–1.90)</td>
</tr>
<tr>
<td>Skeletal malformation (%)</td>
<td>264 (2136)</td>
<td>1.95 (0–3.80)</td>
</tr>
</tbody>
</table>

* Data were collected from 11 studies conducted during the period from 1985 through 1991. *b* Number of does with implants/number of does inseminated × 100. *c* Number of does aborted/number of does inseminated × 100. *d* Number of does with live fetuses/number of does examined at cesarean sectioning × 100. *e* Total number of male fetuses/total number of live fetuses. *f* Number of fetal resorptions and deaths/number of implants × 100.

Cervical area of the vagina by squeezing a bulb with drawing a pipette. Semen should be handled at a constant temperature (ca. 36°C) as much as possible from collection through insemination in order to avoid a temperature shock. For this purpose, all instruments that may have direct contact with semen are kept warm during procedures.

After semen deposition, the doe is given an injection of 25 U. of hCG through the auricular vein to stimulate ovulation. Finally the semen dilution is examined for sperm count (average and range: 465, 195–885, x 10⁶/m⁶) as well as sperm motility to secure successful results.

Applying these techniques and procedures and keeping about 10 proven bucks would enable 2 operators to inseminate 10–15 does daily by only 1 hour work.

Reproductive results obtained from artificially inseminated does are shown in Table 1. These values were comparable to those of natural breeding (e.g. conception rate, 83.9%; mean litter size, 8.0; personal communication from Mr. Takeiri). The incidences of fetal malformations (Table 1) were also comparable to those in other laboratories [8].

Thus the mating of rabbits can be perfectly controlled by using this artificial insemination technique, and this indicates that our simplified artificial insemination technique should be useful for rabbit teratology studies, especially for safety evaluation studies of toxicants, where a relatively large number of pregnant are needed to be prepared in a short period.

We wish to thank Drs. Kozaburo Esaki and Tatsuo Umemura at the Central Institute for Experimental Animals for their technical instruction and their valuable suggestions regarding rabbit artificial insemination. We also wish to thank Mr. Shuji Takeiri, Manager, Minowa Breeding Center, KITAYAMA LABES Co., Ltd., for his kind presentation of reproductive data on natural breeding of Kbl: JW rabbits.

References


ウサギを用いる催奇形性試験における簡便な人工授精法の有用性

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催奇形性試験のためのウサギにおける簡便な人工授精手技の有用性について検討した。人工授精の手順は、1）簡単な人工陰道を用いた2～3匹の雄からの精液採取、2）生理食塩液による混合精液の10倍希釈液0.5 mlへの注入、および3）排卵誘発のためのhCG (25単位) の皮下静脈内投与である。これらの手技と手順を用い、約10匹の妊娠のある雌を維持することによって、2人の術者の1時間の作業で1日当たり10～15匹の雌に人工授精が可能である。ウサギにおけ るこの簡便な人工授精手技は、短期間に比較的多数の妊娠動物を準備する必要のある化学物質の安全性評価のための催奇形性試験に特に有用である。