A STUDY OF EGG TRANSPORT IN THE RABBIT
USING A FREEZING–CLEARING TECHNIQUE

G. R. HOWE

Department of Veterinary and Animal Sciences,
University of Massachusetts, Amherst, Massachusetts, U.S.A.

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Summary. The utilization of a freezing–clearing technique with rabbit oviducts produced data which differed from those obtained by tubal flushings or autoradiography. The current study indicated that the primary site for sphincteric activity was the tubo-uterine junction rather than the ampullary–isthmic junction.

Previous studies have suggested that ova are retained in the ampullary portion of the oviduct before continuing through the isthmus (Burdick & Pincus, 1935; Alden, 1942; Black & Asdell, 1958). Attempts by Greenwald (1961) to reveal the existence of a sphincter between the ampulla and isthmus have failed. In the earlier studies, ovum transport data were obtained by such techniques as tubal flushing (Greenwald, 1959) and autoradiography (Harper, Bennett, Boursnell & Rowson, 1960), both of which required manipulation of the oviduct. More recently, Hafez (1963) has suggested that tubal secretions may play a rôle in egg transport. If this is correct, it is possible that manipulation of the oviducts during flushing or autoradiography could result in the displacement of ova from their normal position. The purpose of this study was to reveal the position of eggs within the oviduct through the utilization of a technique that minimizes tubal manipulation.

Sexually mature, New Zealand does were killed 15 to 65 hr after ovulation (induced by an intravenous injection of 75 i.u. hCG, Squibb, Follutein) by an overdose of Nembutal. Within 1 min, the animals were opened and liquid nitrogen was gently poured over both uterine horns and oviducts. Following gradual thawing, the entire oviducts with a 5-mm segment of the proximal uterine horn were dissected free of fat and subjected to a benzyl benzoate clearing technique described by Orsini (1962). The exact positions of the ova with respect to the ampullary–isthmic junction (AIJ) and the tubo-uterine junction (TUJ) were determined by microscopic examination.

The length of the different tubal segments of seventy cleared oviducts was as follows: ampullary portion, 30 to 80 mm (average 58 mm); isthmic segment, 40 to 85 mm (average 54 mm); total oviduct length including the TUJ, 70 to 160 mm (average 112 mm). The position of all AIJ was 40 to 65% (average 52%) of the distance from the fimbria to the TUJ. Such variation was noted not only among animals but between oviducts in the same doe.

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The position of ova within the oviduct was recorded with reference to the AIJ. The data are shown in Table 1 for intervals ranging from 12 to 65 hr after ovulation. An examination of fifty-one ova at 12 to 26 hr following ovulation revealed that 51% were proximal to the AIJ, 14% along the AIJ, and 35% distal to the AIJ. Subsequently, the eggs showed a gradual, progressive advancement through the isthmus towards the uterus. With reference to the number of ovulation points, it was assumed that the ova not identified within the oviduct for the 38- to 65-hr interval had passed into the uterus, since all ova were accounted for in the previous groups. Analysis of the data reveals that ova were rapidly transported to a site approximately 15 mm proximal to and 18 mm distal to the AIJ within 26 hr of ovulation. Not until 65 hr were eggs completely absent from the oviducts and assumed to be in the uterus. In no instance were ova actually observed within the TUJ. In an attempt to see if the TUJ allows ova to pass through it in one direction only, unfertilized eggs were injected into the upper segment of uterine horns ligated 5 mm distal to the TUJ at 10 hr after ovulation in five artificially inseminated does. A comparison of fertilized and unfertilized ova within the cleared oviducts and the number of ovulation points suggested that ova had migrated from the uterus into the oviduct.

It normally requires 3 to 4 days for eggs to be transported through the rabbit oviduct (Burdick & Pincus, 1935; Greenwald, 1959); the rate of progression, however, is not uniform throughout the entire oviduct (Harper, 1961). Observations by different investigators have suggested that ova are retained at the site of the AIJ for several hours before advancing through the tubal isthmus (Greenwald, 1961). These observations are in agreement with the postulation (Brundin, 1964) of a functional occlusion between the isthmus and ampulla. By contrast, the present study indicated that ova traverse the ampullary portion within a relatively short period of time (12 hr or less) and are distributed both proximally and distally to the AIJ by 26 hr after ovulation. Subsequently, the eggs progressively advance through the tubal isthmus during the next 19 to 36 hr until they are all in the uterus by 65 hr. This is in agreement with histological

### Table 1

**OVUM TRANSPORT IN THE RABBIT OVIDUCT USING THE BENZYL BENOATE FREEZING TECHNIQUE**

<table>
<thead>
<tr>
<th>Hours after ovulation</th>
<th>No. of does</th>
<th>No. of ova</th>
<th>Mm proximal to AIJ</th>
<th>No. of ova at AIJ</th>
<th>Mm distal to AIJ</th>
<th>No. of ova in uterus</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>5</td>
<td>11</td>
<td>2 to 15 (7)</td>
<td>4</td>
<td>2</td>
<td>7 to 10 (8)</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>3</td>
<td>6 to 13 (9)</td>
<td>3</td>
<td>9</td>
<td>2 to 18 (8)</td>
</tr>
<tr>
<td>26</td>
<td>5</td>
<td>12</td>
<td>2 to 8 (5)</td>
<td>0</td>
<td>7</td>
<td>1 to 18 (9)</td>
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<tr>
<td>38</td>
<td>5</td>
<td>0</td>
<td></td>
<td>0</td>
<td>19</td>
<td>13 to 45 (23)</td>
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<tr>
<td>45</td>
<td>3</td>
<td>0</td>
<td></td>
<td>0</td>
<td>5</td>
<td>27 to 55 (33)</td>
</tr>
<tr>
<td>57</td>
<td>3</td>
<td>0</td>
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<td>0</td>
<td>8</td>
</tr>
<tr>
<td>62</td>
<td>5</td>
<td>0</td>
<td></td>
<td>0</td>
<td>2</td>
<td>42 to 46 (44)</td>
</tr>
<tr>
<td>65</td>
<td>5</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
</tbody>
</table>


observations which have failed to demonstrate any sphincteric structure at the AIJ site (Lisa, Gioia & Rubin, 1954). On the other hand, the freezing–clearing technique suggests that the TUJ regulates the time of entry for ova into the uterus. It is obvious that the specific techniques utilized for studying ovum transport have a significant bearing upon the experimental interpretation (Longley & Black, 1968).

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REFERENCES