DISTRIBUTION OF SPERMATOZOA IN THE OVIDUCT AND FERTILITY IN DOMESTIC BIRDS

II. TRANSPORT OF SPERMATOZOA IN THE FOWL OVIDUCT

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Summary. The distribution of spermatozoa in the oviducts of domestic hens, at various times after artificial insemination or copulation, confirmed and extended previous observations regarding the speed with which spermatozoa may traverse the uterovaginal junction and ascend the oviduct. These spermatozoa disappear within less than 24 hr. Thereafter, spermatozoa are ordinarily found only in the uterovaginal glands except about the time of oviposition or ovulation. They presumably re-enter the oviduct lumen, under the influence of oviposition or ovulation. Spermatozoa were found in considerable numbers on the vitelline membranes of eggs and occasionally in egg albumen.

INTRODUCTION

If uterovaginal glands are the normal residence sites of spermatozoa in the hen’s oviduct, as suggested by Bobr (1962) and Bobr, Lorenz, & Ogasawara (1964), mechanisms must exist for their subsequent release from the glands and transport to the infundibular site of fertilization. This paper presents evidence of such release and movement without commenting at length on the physiological mechanisms involved.

Spermatozoa rapidly become distributed throughout the oviduct after copulation or artificial insemination (A.I.), (e.g. Mimura, 1939; Van Drimmelen, 1946; Allen & Grigg, 1957) but they disappear, at least from the lumen, long before production of fertile eggs ceases (e.g. Warren & Kilpatrick, 1929; Walton & Whetham, 1933; Van Drimmelen, 1946). Mimura (1939) observed that the ascent of spermatozoa deposited in the posterior uterus is blocked by any egg in the oviduct, but that the blockade (at least if the egg was in the uterus) is removed at oviposition. Mimura (1941) also reported that the uterovaginal junction delays upward movement of spermatozoa and reduces the number that reach the upper oviduct.

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MATERIAL AND METHODS

Birds and methods of insemination were as described previously (Bobr et al., 1964) except that large volumes (0.15 to 1.3 ml) were used in some initial intrauterine inseminations so that recovery and semi-quantitative estimation of sperm numbers in the oviduct lumina would be easier. Determination of egg fertility, autopsy procedure, and preparation of histological sections were also as described previously, except that segments of the oviduct were first washed for recovery of luminal spermatozoa. The oviduct was quickly removed from the body cavity and cut to separate the infundibulum, anterior and posterior magnum segments, isthmus uterus, and vagina. Each segment, ligated at its posterior end, was filled with physiological saline; the anterior end was then clamped with a haemostat. The distended segment was tilted several times with gentle squeezing and emptied into a centrifuge tube by cutting the posterior ligature. The washings were centrifuged for 20 min at 1500 rev/min; the supernatant fluid was gently sucked off and the sediment was examined microscopically in a hanging drop for the presence and motility of spermatozoa. The interval between killing the bird and examining the sample did not exceed 40 min.

Since it was at least possible that the washing technique would dislodge spermatozoa from oviduct glands, somewhat as described by Grigg (1957) but through distension produced by the saline instead of by an artificial ovum, the glandular uterovaginal junction was first removed. The rather extensive glandular region in the infundibulum, however, because of its central position, could not be removed before washing.

In some samples, numbers recovered were estimated from triplicate haemocytometer counts, and percentage recovery was calculated from the total number of spermatozoa inseminated as estimated from the optical density of the semen (Carson, Lorenz & Asmundson, 1955).

If the ovarian region was to be explored, the carcass was fastened in an upright position to preserve the spatial relationship of the abdominal organs. The fluid from the ovarian pocket was collected with a Pasteur pipette, through a window made in the abdominal wall above the posterior part of the ovary, before the abdominal cavity was exposed for removal of the oviduct. An ovum, if freshly ovulated, was removed from the ovarian pocket; if one was found further posterior, the oviduct was clamped above and below the ovum to preserve its position during removal of the oviduct.

Spermatozoa were also sought in laid eggs and in eggs removed from the oviduct, as follows: pieces of vitelline membrane fixed in formalin or acetic alcohol, and representing 1 to 8% of the entire membrane, were stained with the modified Mallory stain used for oviduct sections (Bobr et al., 1964) and examined in whole mount. The two layers of the membrane proper (McNally, 1943) stained different intensities of blue. A third external layer, which stained yellow, was present in most of the formalin-fixed preparations from laid eggs, but was present only occasionally on those fixed in acetic alcohol; it probably represents the very first oviduct secretion deposited on the yolk since it was seen only rarely on vitelline membranes from ova intercepted in the infundibulum. The spermatozoa stained purple and were readily identified under ×210.
magnification. Smears of thick albumen and chalazae were air dried, fixed in formalin, and stained with hematoxylin.

RESULTS

Initial distribution of spermatozoa

Several experiments demonstrated the speed with which spermatozoa deposited in the vagina may traverse the uterovaginal junction and ascend the oviduct, at least if inseminated near the time of ovulation. Many spermatozoa were found in the uterus of a hen 45 min after an intravaginal A.I. performed 15 min after oviposition. Another hen copulated 1 min after laying, and was killed 10 min later; considerable numbers of spermatozoa were washed out of the uterus, and a few had reached the magnum. Also five out of seven hens subjected to intravaginal A.I. 3 to 15 min after oviposition laid fertile eggs 24 to 25 hr later. There is no doubt that these birds ovulated near the time of insemination, and that spermatozoa traversed the oviduct within a few minutes.

To study the early distribution of spermatozoa in the oviduct additional inseminations were made directly into the uterus so as to ensure large enough numbers to permit semi-quantitative estimations. Results are recorded in Table 1. All oviducts that were empty at the time of A.I. and were examined within 5 hr thereafter had sperm in washings from all parts examined, except for the infundibulum of one hen killed at 1 hr. Spermatozoa had not penetrated the infundibular glands of the birds killed after 1 hr, but were in those glands in birds killed 2 hr or more after A.I. After a lapse of a day or longer, no spermatozoa were present in washings from any part of the oviduct except the infundibulum; since spermatozoa were observed in infundibular glands of one of these hens, and were presumably present in those of the other as judged by previous observations (Bobr et al., 1964), it is likely that the spermatozoa in infundibular washings came from the glands in these birds (see Discussion, below). All oviducts examined contained spermatozoa in the uterovaginal glands.

Forty-five minutes to 2 hr after intrapreneurine A.I. into an ovum-containing oviduct (Table 1) spermatozoa could be found up to the site occupied by the egg at the time of autopsy but not above it. The one exception resulted from a large (1 ml) dose of semen in a bird with an ovum that reached the isthmus before it was killed. Albumen already deposited around a yolk also contained spermatozoa; apparently these spermatozoa had reached further than the position of the ovum at autopsy and were swept downwards with it. In birds killed on the day after insemination, spermatozoa were present only in the infundibular washings; they were also found in infundibular glands in one of these birds but not in the other.

Spermatozoa recovered by washing were morphologically normal and motile, though percentage motility showed variations (from less than 50 % to nearly 100 %) that, except as specified below, were unrelated to the experimental situation. Uteri and vaginae contained the greatest number of spermatozoa, and infundibula usually contained the fewest. The greatest number recovered from an entire oviduct was $22 \times 10^6$, obtained 2 hr after insemination, of which
Table 1
DISTRIBUTIONS OF SPERMATOZOA IN THE VIDUCT AFTER INTRAUTERINE A.I.

<table>
<thead>
<tr>
<th>Bird No.</th>
<th>Semen vol. (ml)</th>
<th>Autopsy after A.I. (hr)</th>
<th>Condition of oviduct at time of:</th>
<th>Spermatozoa in washings† from:</th>
<th>Spermatozoa in infundibular glands‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A.I.</td>
<td>Autopsy</td>
<td>Vagina Uterus Isthmus Post-magnum Ante-magnum Infundibulum</td>
</tr>
<tr>
<td>1</td>
<td>0.35</td>
<td>1</td>
<td>Empty</td>
<td>Empty</td>
<td>+ + + + + + + + + + (+) / 0</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>1</td>
<td>Empty</td>
<td>Empty</td>
<td>+ + + + + + + + + + (+) / 0</td>
</tr>
<tr>
<td>3</td>
<td>1.30</td>
<td>2</td>
<td>Empty</td>
<td>Empty</td>
<td>+ + + + + + + + + + (+) / 0</td>
</tr>
<tr>
<td>4</td>
<td>0.65</td>
<td>5</td>
<td>Empty</td>
<td>(S) in uterus</td>
<td>+ + + + + + + + + + (+) / 0</td>
</tr>
<tr>
<td>5</td>
<td>0.20</td>
<td>1</td>
<td>(O) in magnum</td>
<td>(O) in post-magnum</td>
<td>+ + + + (+) / (+)§ 0 + 0 +</td>
</tr>
<tr>
<td>6</td>
<td>1.00</td>
<td>1</td>
<td>(O) in magnum</td>
<td>(S) in isthmus</td>
<td>0 + (+) / 0 + 0 + +</td>
</tr>
<tr>
<td>7</td>
<td>0.20</td>
<td>2</td>
<td>(O) in magnum</td>
<td>(O) in post-magnum</td>
<td>+ + + + + + + + + + (+)§ 0 + 0 +</td>
</tr>
<tr>
<td>8</td>
<td>0.20</td>
<td>1</td>
<td>(S) in uterus</td>
<td>(S) in uterus</td>
<td>+ + + + + + + + + + (+) / 0</td>
</tr>
<tr>
<td>9</td>
<td>0.80</td>
<td>1</td>
<td>(H) in uterus</td>
<td>(H) in uterus</td>
<td>+ + + + + + + + + + (+) / 0</td>
</tr>
<tr>
<td>10</td>
<td>0.15</td>
<td>24</td>
<td>Empty</td>
<td>Empty</td>
<td>0 0 0 0 0 (+) / + + + + +</td>
</tr>
<tr>
<td>11</td>
<td>0.20</td>
<td>168</td>
<td>Empty</td>
<td>(S) in isthmus</td>
<td>0 0 0 0 (+) / + + + + +</td>
</tr>
<tr>
<td>12</td>
<td>0.20</td>
<td>25</td>
<td>(O) in magnum</td>
<td>Empty</td>
<td>0 0 0 0 (+) / + + + + +</td>
</tr>
<tr>
<td>13</td>
<td>0.15</td>
<td>22</td>
<td>(S) in uterus</td>
<td>Empty</td>
<td>0 0 0 0 (+) / + + + + +</td>
</tr>
</tbody>
</table>

* Estimated positions: (O)—ovum with or without albumen, but no membrane; (S)—ovum with membrane but little or no calcification; (H)—ovum with hard shell.
† Relative density only; numbers of spermatozoa per high power field (×400) in centrifuged sediments from entire washings: (+), <1; +, 1 to 10; ++, 11 to 30; ++++, too many to count; ++++, too many to count, very dense.
‡ All accumulations in infundibular glands (indicated by +) were 'dense' as defined by Bobr et al. 1 (964). All birds examined had dense accumulations in uterovaginal glands.
§ Including spermatozoa in a strand of albumen.
¶ Spermatozoa were non-motile.
approximately $6 \times 10^6$ were in the infundibulum; the infundibulum yielded an average of $1 \cdot 3 \times 10^6$ spermatozoa 24 hr after insemination. (These figures respectively represent recoveries of about 2-8, 0-75 and 0-16% of the average of $8 \times 10^8$ spermatozoa inseminated.) After 7 days a calculated average of $6 \times 10^8$ spermatozoa were recovered from two hens, but this figure is less reliable than those quoted above; it was arrived at from two spermatozoa actually found in each hen in the total of eight and four haemocytometer fields, respectively.

The obvious conclusion from these initial results is that spermatozoa, which may distribute themselves rapidly throughout the oviduct after insemination, disappear completely from all parts of the oviduct lumen, except in the infundibulum, within less than 24 hr. The situation in the infundibulum is not entirely clear, but from the results recorded in Table 1 (and also in Table 2; see next paragraph) it appears very likely that spermatozoa found in infundibular washings of hens killed 1 day or later after intrauterine A.I. were not usually free in the lumen but had been released from the glands through distension during washing. However, the significance of these results for the present experiment is that any spermatozoa found in oviduct washings (of magnum, isthmus, or uterus, at least) more than 24 hr after insemination must have been released from oviduct glands. Subsequent experiments were designed to search for such spermatozoa.

**Table 2**

**Distribution of spermatozoa in oviducts of intravaginally inseminated fertile hens in relation to the ovulation cycle**

<table>
<thead>
<tr>
<th>Bird No.</th>
<th>Autopsy interval after</th>
<th>Ovum position at autopsy</th>
<th>Vagina</th>
<th>Uterus</th>
<th>Isthmus</th>
<th>Magnum</th>
<th>Infundibulum</th>
<th>Ovarian pocket</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>7*</td>
<td>20</td>
<td>Follicle</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>4*</td>
<td>25</td>
<td>Follicle</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>7*</td>
<td>25</td>
<td>Ovarian pocket</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>10*</td>
<td>90</td>
<td>Fimbria</td>
<td>0</td>
<td>+ $$</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>1*</td>
<td>30</td>
<td>Infundibulum</td>
<td>0</td>
<td>+ $$</td>
<td>0</td>
<td>(+)</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>6*</td>
<td>30</td>
<td>Infundibulum</td>
<td>0</td>
<td>+ $$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>5*</td>
<td>45</td>
<td>Anterior magnum</td>
<td>0</td>
<td>0</td>
<td>+ $$</td>
<td>+ §</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>9*</td>
<td>60</td>
<td>Magnum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>8*</td>
<td>120</td>
<td>Magnum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>23</td>
<td>8*</td>
<td>180</td>
<td>Isthmus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>4*</td>
<td>1500</td>
<td>(H) in uterus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>3*</td>
<td>1320</td>
<td>(H) in uterus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Following separation from the male.
† Following intravaginal A.I.
$$ Spermatozoa were dead.
§ Spermatozoa found in magnum distal to, but not anterior to, ovum.
¶ Including spermatozoa in albumen.

Distribution of spermatozoa presumably released from uterovaginal glands

According to previous results (above and Bobr et al., 1964) hens killed 1 to
10 days after intravaginal insemination should have spermatozoa in utero-vaginal glands but not in infundibular glands, and none free in the oviduct lumen. Out of twelve hens so inseminated (through copulation or A.I., see Table 2), there were no spermatozoa in the oviduct washings of four which were not killed near the time of an ovulation; two had eggs in the uterus and one an egg in the isthmus; one was killed immediately after oviposition of what should have been the terminal egg of the clutch.

In the remaining eight birds the situation was entirely different. These were all killed near to the time of an ovulation, and the oviduct washings of all contained spermatozoa. All were killed shortly after oviposition of a mid-clutch egg; one (Bird 15) had not yet ovulated, though ovulation should have been imminent. In Bird 16 the freshly ovulated ovum was still in the ovarian pocket, and in the remainder (Birds 17 to 22) the ovum was in the infundibulum or magnum, all within 2 hr, and most within 30 min after ovulation. In all these birds, spermatozoa were found in oviduct washings below and at the site of the ovum but not above it. Only Bird 18 also had spermatozoa in ovarian-pocket fluid, the only case of spermatozoa above the ovum; this bird was killed only 24 hr after separation from the male and had an egg in the infundibulum. No spermatozoa were found in vaginal washings from these birds (not including the glandular junction region) or in uterine washings from two birds with the ova furthest advanced (into the magnum); in two others the uterine spermatozoa were dead.

All these spermatozoa could have come only from uterovaginal glands, since initial spermatozoa free in the oviduct lumen would long since have disappeared and these birds presumably had no spermatozoa in infundibular glands. The data in Table 2 suggest that spermatozoa are released from these glands during or immediately after oviposition, and perhaps (as suggested by the negative washings from Bird 14) only in association with an ovulation. Once released, spermatozoa appear to have a very short life span indeed; in Bird 23 they had already disappeared from oviduct washings 3 hr after oviposition.

Presumptive release of spermatozoa from infundibular glands

It is difficult to demonstrate beyond doubt the release of spermatozoa from infundibular glands since they have only been found in these glands when they were also present in uterovaginal glands. However, any spermatozoa in oviduct washings anterior to a descending egg in a bird killed more than 1 day after intrauterine A.I. should have come from infundibular glands. Those found in infundibular washings might have been dislodged from the glands by the washing process, but those found elsewhere must have been released after passage of the ovum. Fluid in the ovarian pocket should be an especially suitable place to search since this can be obtained directly (i.e. without manipulation of the oviduct) and will be anterior to any oviducal egg.

Of six birds killed 1 to 8 days after intrauterine A.I., and with sperm in the infundibular glands, four had spermatozoa in the ovarian pocket fluid. One had just ovulated, with the ovum still in the ovarian pocket; one had an ovum in the magnum; one had a membranous egg in the uterus; and one was presumably about to ovulate a mid-clutch ovum. The remaining two, which had empty
Transport of spermatozoa in fowl oviduct

oviducts when killed several hours after laying, had no spermatozoa in the ovarian pocket. The release of spermatozoa from the infundibular glands therefore appears to be associated with ovulation, or at least with the presence of an ovum in the oviduct.

Spermatozoa in eggs

Spermatozoa were found on the vitelline membrane of nearly all the fertile eggs produced in these experiments. They appeared to be imbedded in or even penetrating the two layers of the membrane proper; additional spermatozoa were frequently found imbedded in the yellow-staining outer layer. In both places the sperm heads were usually seen as bent in half-circles but were otherwise normal. Distribution of spermatozoa over the yolk surface bore no relation to the position of the germinal disc.

As many as 5000 spermatozoa per yolk were estimated from sperm counts, the greatest numbers (1200 to 5000) occurring in the first two ovulated after intrauterine A.I., but the number was usually much lower. Yolk membranes of whole sequences of eggs from three hens following intravaginal A.I. contained a few hundred spermatozoa each. The number per yolk did not change consistently with time after insemination until the end of the fertility period (the 11th or 12th day), when a yolk membrane contained an estimated seventy spermatozoa; spermatozoa were never found on the membranes of infertile eggs laid 15 or more days after insemination. Earlier infertile eggs had at least as many membrane spermatozoa as adjacent fertile eggs. There were very few spermatozoa on the yolk membranes of hens inseminated by the intraperitoneal route (see Bobr et al., 1964).

Only a few observations were made on spermatozoa in the albumen. Both normal-appearing and distorted spermatozoa were found, but their numbers were not large, being estimated at no more than 200 per egg and probably less, except in one egg that had large numbers on the vitelline membrane; this egg contained ten times as many spermatozoa in the albumen as were present in that of other eggs examined.

DISCUSSION

Mimura (1939) demonstrated that spermatozoa deposited in the uterus of a hen with an empty oviduct would reach the infundibulum within a few minutes. This observation has been confirmed, notably by Allen & Grigg (1957), who estimated from the radioactivity of 32P-labelled spermatozoa that some 0.07% reached the infundibulum in 15 min, and 1.3% in an hour. In the present investigation, some 0.75% were recovered 2 hr after insemination. This last figure is certainly a conservative estimate of the number present since additional spermatozoa must have remained in the infundibular glands.

In a later paper Mimura (1941) reported that spermatozoa deposited in the vagina failed to penetrate to the uterus for periods of 2 to 4 hr. This observation was not confirmed by Allen & Grigg (1957), who found labelled spermatozoa in the uterus 15 min after intravaginal A.I. and a small but significant number in the infundibulum after 1 hr. Mimura's observation was certainly not
confirmed in the present investigation. Unfortunately, neither Mimura nor Allen & Grigg described the ovulatory status of their birds, and the seven birds in which rapid transport was observed here (see Results, paragraph 1) were all inseminated near the time of ovulation. It is possible that this is the only time that recently-inseminated spermatozoa can traverse the uterovaginal junction without delay. The observations described here do agree with those of Mimura (1939) regarding the inhibiting effect that the presence of an egg in the oviduct has on sperm transport, except for one detail and its interpretation. He observed no migration of spermatozoa beyond the isthmus and implied that the action of the egg is to inhibit the transport mechanism (presumably antiperistalsis). The results described here lend themselves better to the interpretation that the ovum is merely a mechanical obstruction. Admittedly, the choice of interpretation rests on inadequate information; only one of Mimura’s and two of our birds received intrauterine A.I. (presumably) following ovulation, and were killed with the ovum still in the magnum. He found no spermatozoa in the magnum, either above or below the ovum, but in both of our birds spermatozoa were found up to but not beyond the ovum. In one bird, spermatozoa were caught in the surrounding albumen, suggesting that they had ascended to meet the ovum and had been swept back down with it.

The presence of spermatozoa in this and other eggs, and the distribution of spermatozoa in Bird 20 (Table 2), suggest that each descending egg sweeps the oviduct clean of luminal spermatozoa. However, Bird 4 (Table 1) is consistent with this hypothesis only if spermatozoa were still ascending from the uterus 3 to 5 hr after A.I., or if the spermatozoa found in washings from the infundibulum and magnum had come from infundibular glands.

The results (Table 1) suggest that the naked albumen surrounding an ovum anterior to the isthmus is an absolute obstruction, whereas an egg covered with a membrane and shell is only a partial obstruction. The presence of spermatozoa in the anterior oviduct of Bird 8 can be accounted for only in this way. Those in the infundibulum of Bird 6 may have ascended after the membrane was completed on the descending egg but, if so, spermatozoa should also have been found in the magnum. It is possible that when large numbers are present a few may be left behind by the descending egg.

Mimura’s (1939) results led him to postulate that egg-induced inhibition to cranial migration of spermatozoa is abruptly released at oviposition. This, however, would only account for luminal spermatozoa following the first oviposition after separation from the male or after A.I. Release from uterovaginal glands, as postulated here, accounts for the luminal distribution observed up to 10 days after separation. One of Mimura’s own birds points up the alternate interpretations. This bird (J56) had an egg in the uterus when it received intrauterine A.I.; the egg was laid on the next morning and an ovum was found in the magnum some hours later. Spermatozoa were found in the upper magnum and infundibulum but not in the magnum distal to the ovum. Mimura (1939) felt that these spermatozoa must have ascended during the interval between oviposition and ovulation. If they had ascended so recently, however, they should have been present distal to the ovum and not anterior to it. According to the interpretation postulated here, spermatozoa must have ascended past the
uterine egg and entered infundibular glands (just as must have occurred with Bird 13 here), and have been released again following passage of the ovum. Bobr et al. (1964) found at least a few spermatozoa in infundibular glands of eight of ten birds that received intrauterine A.I. when either a membrane or hard-shelled egg was in the uterus.

We conclude that the presence of spermatozoa in the oviduct lumen is even more transitory than Van Drimmelen (1945) suspected. All luminal spermatozoa that we found as late as 22 hr after insemination had, in all probability, been released from oviduct glands. This very probably applies to the spermatozoa Van Drimmelen found in the infundibular lumen some 72 hr after intraperitoneal A.I.

One matter of technique is important in the interpretation of certain results. Grigg (1957) recovered spermatozoa by washing an infundibular lumen after but not before distending the segment with an 'artificial ovum' made of a cellophane bag filled with warm Ringer's solution. He concluded that no spermatozoa had been present in the lumen prior to distension, and that those he obtained afterwards had been forced out of infundibular glands. His experimental animal, however, was a hen that had been inseminated by an unspecified method 3 hr previously, and at that time there should have been spermatozoa throughout the oviduct lumen. Consequently, the actual situation of the spermatozoa he 'dislodged' remains uncertain. The same uncertainty remains with regard to spermatozoa found in some of the infundibular washings in this investigation. The experiments reported here lead to a conclusion, similar to Grigg's, that spermatozoa in infundibular glands are released into the oviduct at the time of ovulation. Evidence of this has been found in at least one hen but, considering the known activity of the infundibulum just before ovulation, a pressure or distension mechanism such as Grigg postulated could have been activated in this bird by the grasping of the follicle.

If spermatozoa are discharged from oviduct glands by mechanical distension of the oviduct, discharge from uterovaginal glands should coincide with oviposition (as suggested). This would yield spermatozoa for the fertilization of most eggs, but would not account for the fertilization of ova after an interval of several days without insemination. Bird 14 (Table 2) was killed 20 min after oviposition, with no subsequent ovulation expected or observed, and no spermatozoa could be found in the oviduct lumen, as if distension by oviposition was ineffective in the absence of an impending ovulation. Yet Bird 12 (Table 1) did have spermatozoa in the infundibular lumen 4 hr after oviposition. There being no spermatozoa in the infundibular glands, those in the lumen had probably ascended from uterovaginal glands. But, as these spermatozoa had been deposited in the uterus, they may have been still present at oviposition and may have ascended from there.

Whatever the details of these processes, however, the major conclusions appear to be clear and unequivocal; in so far as could be determined by Bobr et al. (1964) and from the present results, any residence of spermatozoa in infundibular glands has nearly always been an artifact of abnormal experimental insemination techniques, though a few spermatozoa rarely enter these glands after copulation. The usual residence sites following copulation or intravaginal
A.I. are the uterovaginal glands. Spermatozoa discharged from these glands, by a mechanism yet to be elucidated but close to the times of ovipositions and/or ovulations, ascend the oviduct rapidly and are presumably responsible for the sequential fertilizations.

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