Possible Significance of Accessory Reproductive Fluid in Exhibition of Fertilizing Ability of Spermatozoa in the Domestic Fowl, Gallus Domesticus

Takato Terada, Moriyuki Watanabe
and Yoshio Tsutsumi

Animal Reproduction Laboratory, Faculty of Applied Biological Science, Hiroshima University, Fukuyama, Hiroshima 720

(Received August 1, 1983)

Abstract To determine the significance of the accessory reproductive fluid in the cock, semen collected from the ducts deferens was diluted with blood serum, seminal plasma or phosphate buffer and deposited in different regions of the hen's vagina. When semen was deposited in the anterior part of the vagina, both the ductal semen diluted with the biological fluids or phosphate buffer and the undiluted semen gave excellent fertility during the first week after insemination. When semen was deposited in the mid part of the vagina, the fertilizing ability of the ductal spermatozoa diluted with the biological fluids was greater than that of the undiluted semen. This was determined from the fertilization rate of eggs during the first and second week after insemination and from the duration of fertility. When semen was deposited in the posterior part of the vagina, differences in fertilization rate and in duration of fertility between the ductal semen diluted with the biological fluids and the undiluted semen were statistically significant. The more posterior was site of semen deposition, the more critical was the effect of the biological fluids on fertility. From these results, it is concluded that cock accessory reproductive fluid plays an important role in fertilization in chickens.


The lymph-like fluid which flows out of the lymph-folds in the cock cloaca at the time of ejaculation has been designated as transparent fluid1,2, blood exudate3 or accessory reproductive fluid (ARF)4. The ARF is ejected during natural copulation, and constitutes one of normal seminal elements4. In semen collection by the "Hiroshima Method", a "no squeezing" operation, Yamane et al.5 also demonstrated that the ARF is a constant component of cock semen. With respect to various biochemical components, a close resemblance between the ARF and the blood serum or plasma has been documented by Nishiyama6, Takeda7, Schindler and Scharp8, Nishiyama and Fujishima9 and Lake and Hatton10. As little information is available on the role of the ARF in natural copulation, we conducted the present study to clarify it.

Materials and Methods

Eighteen cocks and 36 hens used for the present study were White Leghorns, housed in individual cages. Blood was drawn from the brachial veins of the cocks,
Significance of Cock Accessory Reproductive Fluid

and centrifuged at 1,050 g for 20 min, after coagulation at room temperature. The supernatant was frozen at -20°C and stored until use. Semen was collected from the cocks by abdominal massage11) and pooled. The pooled semen was centrifuged at 7,500 g for 45 min and the supernatant was filtered twice through 0.65 μm filters (Toyo Roshi Ltd.) and 0.22 μm filters (Millipore Filter Corporation). The filtrate was stored in the same manner as the blood serum.

Semen from the ductus deferens was collected by squeezing the posterior portions of ducts which had been removed from decapitated cocks. The ductal semen obtained from three or four cocks was mixed thoroughly and the sperm concentration was determined using a hemocytometer. The mixed semen was divided into two to four equal aliquots. Except for the undiluted control semen, each aliquot was diluted two-fold with either blood serum, seminal plasma or phosphate buffer (α = -0.60°C, pH = 7.0).

Immediately after dilution, diluted or undiluted semen was deposited in the vagina by the method of MOULTRE12), using a graduated 1.5-mm-bore glass cannula connected to 1-ml syringe with rubber tubing. The volume of the undiluted semen deposited per hen was one-half the volume of diluted semen deposited. Semen was deposited at one of three sites: the anterior (about 5 cm from the vent), mid (about 3 cm from the vent), or posterior (about 0.5 cm from the vent) part of the vagina. Six to nine hens holding no hard-shelled egg in their oviducts were randomly assigned to each treatment. The hens were used repeatedly, but they did not receive subsequent insemination until they had laid five successive infertile eggs after the previous insemination.

Eggs were collected daily from the 2nd to the 23rd day after a single insemination. They were stored at 15°C and set weekly in an incubator. On the fourth day of incubation, fertility of each egg was inspected by candling and all questionable eggs were examined macroscopically by breaking out. Fertilization rates of eggs (percent fertility) were calculated on the basis of total eggs laid by each hen and were transformed to angles for statistical analysis. When significant treatment effects were detected by analysis of variance, differences among treatment means were further evaluated by Duncan's multiple range test13). Duration of fertility was represented as the number of days from the 2nd day after insemination to the last day of laying of fertilized eggs.

Results

Semen Deposition in the Anterior Part of the Vagina

As for percent fertility in the first week after insemination, the semen diluted with blood serum and phosphate buffer showed the maximum, although the undiluted semen and the semen diluted with seminal plasma also gave an excellent percent fertility (Table 1). In the second week, semen diluted with blood serum showed a higher percent fertility than that obtained with the other semen samples. However, the differences between the percent fertility of the semen diluted with blood serum
Table 1. Fertility of semen collected from ductus deferens, diluted with blood serum, phosphate buffer or seminal plasma and deposited in anterior part of vagina

<table>
<thead>
<tr>
<th>Additive</th>
<th>Volume of semen deposited (ml/hen)</th>
<th>No. of spermatozoa deposited (million)</th>
<th>No. of hens inseminated</th>
<th>Egg production (%)</th>
<th>Mean percent(^{(a)}) fertility</th>
<th>Mean(^{(b)}) duration of fertility (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood serum</td>
<td>0.02</td>
<td>86.0</td>
<td>9</td>
<td>80.2</td>
<td>100.0±0.0</td>
<td>54.9±7.2</td>
</tr>
<tr>
<td>Seminal plasma</td>
<td>0.02</td>
<td>86.0</td>
<td>9</td>
<td>86.5</td>
<td>98.1±1.3</td>
<td>41.8±4.6</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>0.02</td>
<td>86.0</td>
<td>9</td>
<td>84.9</td>
<td>100.0±0.0</td>
<td>40.0±6.1</td>
</tr>
<tr>
<td>Control 1(^{(1)})</td>
<td>0.01</td>
<td>86.0</td>
<td>9</td>
<td>87.3</td>
<td>96.2±1.5</td>
<td>43.2±9.7</td>
</tr>
</tbody>
</table>

\(^{(1)}\)Undiluted semen collected from ductus deferens.  \(^{(a)}\)Mean±S.E.

and that of the other semen samples were not significant.

Semen Deposition in the Mid Part of the Vagina

In the first week’s percent fertility, there was a difference of about 18 percentage points between the semen diluted with blood serum and the undiluted semen (Table 2, Trial 1). When phosphate buffer was used as a diluent, no difference between the percent fertility of the diluted semen and that of the undiluted semen was realized in the first week, but the value of the former became more than twice that of the latter in the second week. The duration of fertility obtained with the semen diluted with blood serum was longer, but not significant, than for the other two treatments. In addition, the fertilizing ability of the spermatozoa diluted with seminal plasma was better than that of undiluted semen, as measured by percent fertility in the first and second weeks and by the duration of fertility (Table 2, Trial 2).

Semen Deposition in the Posterior Part of the Vagina

The percent fertility in both the first and second week was markedly higher for semen diluted with seminal plasma than for undiluted semen (Table 3, Trial 1). Also the duration of fertility obtained with diluted semen was significantly longer than with undiluted semen (P<0.05). Furthermore, semen diluted with either seminal plasma, phosphate buffer or seminal plasma and deposited in mid part of vagina

<table>
<thead>
<tr>
<th>Additive</th>
<th>Volume of semen deposited (ml/hen)</th>
<th>No. of spermatozoa deposited (million)</th>
<th>No. of hens inseminated</th>
<th>Egg production (%)</th>
<th>Mean percent(^{(a)}) fertility</th>
<th>Mean(^{(b)}) duration of fertility (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood serum</td>
<td>0.050</td>
<td>238.1</td>
<td>8</td>
<td>86.6</td>
<td>81.8±6.7</td>
<td>27.3±4.3</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>0.050</td>
<td>238.1</td>
<td>8</td>
<td>91.0</td>
<td>66.0±5.9</td>
<td>26.5±4.1</td>
</tr>
<tr>
<td>Control 1(^{(1)})</td>
<td>0.025</td>
<td>238.1</td>
<td>8</td>
<td>89.3</td>
<td>64.2±7.3</td>
<td>12.8±3.5</td>
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<tr>
<td><strong>Trial 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminal plasma</td>
<td>0.050</td>
<td>212.5</td>
<td>6</td>
<td>84.5</td>
<td>97.4±1.1</td>
<td>42.4±6.5</td>
</tr>
<tr>
<td>Control 2(^{(2)})</td>
<td>0.025</td>
<td>212.5</td>
<td>6</td>
<td>82.1</td>
<td>88.2±3.6</td>
<td>28.8±4.3</td>
</tr>
</tbody>
</table>

\(^{(1)}\), \(^{(2)}\)Undiluted semen collected from ductus deferens.  \(^{(a)}\)Mean±S.E.
plasma or blood serum gave a markedly higher percent fertility in the first week as compared to undiluted semen (Table 3, Trial 2). There were significant differences in the first week's percent fertility between semen diluted with each biological fluid and undiluted semen (P<0.05). In the second week, no fertilized eggs were obtained from deposition of undiluted semen, although the semen diluted with biological fluids resulted in relatively high percent fertility. Furthermore, the duration of fertility associated semen diluted with seminal plasma or blood serum was significantly longer than that obtained with undiluted semen (P<0.05).

**Discussion**

In studies on the accessory reproductive organ of the drake, FUIHARA and NISHIYAMA concluded that fluid obtained from the ejaculatory groove region exerted a favorable effect on the fertilizing ability of spermatozoa at the time of mating. In comparative studies on the fertilizing ability of turkey spermatozoa ejaculated or collected from the ductus deferens, SAEKI and BROWN reported that secretions of the posterior part of the ductus deferens and/or the phallus may enhance the fertilizing ability of spermatozoa. OGAWA et al. also studied the role of the frothy fluid which is secreted by the cloacal gland of male Japanese quails at copulation. They found that fertility in female quails mated with males whose cloacal glands had been removed was markedly lower than in females mated with normal males, and that semen mixed with the frothy fluid gave a higher percent fertility during the first week after insemination, in comparison with unmixed semen. They inferred that the frothy fluid took part in the mechanism of fertilization in the Japanese quail. NISHIYAMA speculated that the ARF in the cock might be involved in a shift in pH of ejaculated semen and of the reproductive tract at mating. However, a favorable effect of the fluid on the fertilizing ability of cock spermatozoa has not previously been demonstrated.
In the present study, when semen was deposited in the posterior part of vagina, the percent fertility obtained with semen collected from the ductus deferens, diluted with blood serum or seminal plasma, was significantly higher than that following use of undiluted ductal semen. Also, the difference between the duration of fertility obtained with semen diluted with biological fluids and that obtained with undiluted semen was statistically significant. These results indicate that blood serum of seminal plasma improves the fertilizing ability of spermatozoa collected from the posterior part of the ductus deferens. Furthermore, it has been shown that the ARF is quite similar to blood serum or plasma with respect to some minerals, some free amino acids, glucose, pH and electrophoretic pattern of proteins. It has also been observed that 66% of the seminal plasma in ejaculated semen consists of the ARF. Therefore, it is clear that the ARF, as well as blood serum and seminal plasma, will improve the fertilizing ability of spermatozoa collected from the ductus deferens.

When ductal semen was deposited in the anterior part of the vagina, an excellent percent fertility was attained during the first week following insemination, whether we used diluted or undiluted ductal semens (Table 1). This indicates that spermatozoa collected from the ductus deferens under our experimental conditions are fully capable of fertilizing ova. Thus, the efficacious effect of blood serum and seminal plasma on the percent fertility and duration of fertility, when added to ductal semen deposited in the posterior part of the vagina, may be related to sperm transport through the vagina.

Takeda studied the sperm distribution in the oviducts of hens by depositing spermatozoa in different regions of the vagina, and found that living cock spermatozoa deposited in the posterior part of the vagina were detected in the anterior part of the vagina and in the uterus after 90 minutes. But dead cock spermatozoa and living heterologous spermatozoa deposited in the same region were never detected in the anterior part of the vagina or in the uterus. He concluded that the motility of spermatozoa was a main factor in sperm transport through the total length of the vagina. In addition, in our previous studies, we found that both cock blood-serum and seminal plasma markedly stimulated the motility, oxygen consumption, and glucose utilization of cock spermatozoa. In reviewing these evidences, it seems that the most likely reason for an improvement in the fertility of ductal semen, due to dilution with blood serum or seminal plasma (Table 3), is stimulation of the motility and metabolism of the spermatozoa. This in turn might enhance the ascent of spermatozoa through the vagina, with more spermatozoa reaching the upper part of the oviduct.

Differences in percent fertility in the first week, between ductal semen diluted with the biological fluids and undiluted semen, were 2~4% when semen was deposited in the anterior part of the vagina, 9~18% when deposited in the mid part, and 35~57% when deposited in the posterior part. In other words, the more posterior is the site of semen deposition, the more critical is the effect of the biological.
Significance of Cock Accessory Reproductive Fluid

fluids on fertility. It may be that semen ejaculated during natural copulation is deposited in a more posterior site than "the posterior part" of the vagina in the present study, because of the copulatory organ in the cock. Therefore, we may conclude that the ARF, one of main components of semen naturally ejaculated, plays an important role in the fertilizing ability of spermatozoa in the chicken.

Acknowledgements

The authors would like to acknowledge Prof. H. NISHIYAMA, Department of Animal Science, Kyushu Tokai University, Kumamoto 869-14, Japan, for useful suggestions and Prof. W. J. MELLEN, Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, U. S. A., for reviewing the manuscript.

References

鶏精子の受精能力に対する副生殖器官液の意義について

寺田隆登・渡辺守之・堤義雄
広島大学生物生産学部，福山市720

本実験は鶏の射精時に排出される副生殖器官液の意義を検討する目的で実施した。

精液は雄鶏から採取した精管後部から採取した。この精液は無希釈の对照区を除いて雄鶏血清、精漿あるいはリン酸緩衝液で2倍希釈した。希釈後ただちに雌の陰茎部、膣中間部あるいは膣浅部に注入し、受精率および受精期間を調査した。

膣深部に精液を注入した場合には、対照区を含めたすべての区の受精率は著しく高い値を示した。膣中間部に注入した場合には、雄鶏血清あるいは精漿で希釈した精液区の1および2週目の受精率が対照区のそれらよりも高い値を示し、長い受精期間を示した。さらに膣浅部に注入した場合には、雄鶏血清あるいは精漿で希釈した精液区が対照区に比較して有意に高い受精率（1週目）と長い受精期間を示した。

さらに、雄鶏血清あるいは精漿で希釈した精液区の受精率と対照区のそれとの差は精液の注入部位が後方になる程大きくなった。

以上の結果から、鶏の射精時に排出される副生殖器官液は精子の受精能力を充分に発揮させるのに重要であると結論された。

日畜会報，55（1）：52-58，1984