Sperms in the Interstitial Tissue of the Domestic Fowl Testis

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The authors ('61) observed sperm-head-like bodies in the enlarged interstitial tissues of the P₃₂-administered fowl testes which had been stained with Heidenhain's iron hematoxylin and light green. Since these bodies were DNA-reaction (Feulgen) positive and sometimes tail could also be observed, they were regarded as sperms. In addition, the enlarged interstitial tissues contained many germ cells at various stages. Such pictures as these were seen in specimens collected not only from P₃₂-administered domestic fowls but also from intact control ones both by biopsy and autopsy. In later studies these pictures seemed to be characteristic of some strains of fowls. In order to determine whether there was any relationship between the appearance of these bodies and the strain of fowls or not, fifteen independent inbred strains of fowls were examined in the present study.

Materials and Methods

The animals used are shown in Table 1. They were 184 in all and from 7 months to 3 years old. They consisted of 161 White Leghorns (W.L.) of thirteen strains produced by eight breeders, 8 Rhode Island Reds (R.I.R.) of one strain by one breeder, and 15 New Hampshires (N.H.) of one strain by one breeder. Materials for histological examination were taken from the middle portion of the left testis of each bird (materials were cut into 2 to 3 mm thick blocks, with razor blade), fixed in Allen-Bouin's fluid, cut into 7µ paraffin sections, and stained with Heidenhain's iron hematoxylin and light green. Some materials were examined in 7µ serial sections. All the materials were collected by autopsy, except those from three N.H. which were harvested by biopsy.

Table 1. Materials

<table>
<thead>
<tr>
<th>Breeder</th>
<th>A</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>I</th>
<th>Si</th>
<th>Su</th>
<th>T</th>
<th>U</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>W.L.</td>
<td>10</td>
<td>20</td>
<td>21</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>R.I.R.</td>
<td>8</td>
<td></td>
<td>20</td>
<td>21</td>
<td>20</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>N.H.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>(3 by biopsy)</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>82</td>
<td>10</td>
<td>10</td>
<td>21</td>
<td>15</td>
<td>10</td>
<td>15</td>
<td>3</td>
<td>184</td>
</tr>
</tbody>
</table>

Remarks.
Observations

In general, testes were well developed. In 7-month-old N.H. and R.I.R., however, the testis varied widely in weight (1.1-11.0 g. unilaterally). In 7-month-old W.L. of breeder A, the testis showed no good development (1.0-3.9 g. unilaterally). The testis showed a greater variation in weight among 7-month-old fowls than among those 1 to 3 years old. Some of the testes of 7-month-old fowls were inadequate as materials, and these were discarded from the present data.

Histological observations: In 3-year-old fowls, the seminiferous tubules were very long in diameter, the germinal epithelium was thick, the columnar structures of the germinal epithelium with Sertoli cells were very clear, spermatogenesis was very flourishing, and many transformed and mature sperms were seen. A large number of sperms attached to the top of the column in bundles. In some cases, a few seminiferous tubules which had a somewhat thinner germinal epithelium than the others contained decidual epithelial cells and a small amount of colloidal substance in the lumen. Pyknotic polynuclear giant cells were also seen among the decidual epithelial cells. These abnormal figures, however, were very few. Almost all tubules were normal in appearance and spermatogenesis was very active.

Fowls 1 and 2 years old showed the same figure of testis as 3-year-old ones. Cytolysis was found in one of the 2-year-old fowls. Chromosome bridge and tripolar division were observed in one of the 1-year-old fowls. These two birds were of the B and C strain, respectively, of breeder I. These abnormal figures were seen in a very few fowls. In general, the testis can be regarded as normal in these birds. The testis was histologically the same in most of the 7-month-old fowls as in the 3-year-old ones. The seminiferous tubules and the lumen were great in diameter. Spermatogenesis was active. The columnar structures of the germinal epithelium and sperm bundles were seen clearly. Abnormal mitotic figures were also observed, although they were very small in number. In some cases, seminiferous tubules which were relatively short in diameter (below one-half of normal) showed weak spermatogenesis, containing a small number of mitotic cells and a few sperms. In such tubules, the columnar structures of the germinal epithelium were very obscure. Various grades of development were observed between the two types of testes mentioned above. In materials of all ages, various combinations of germ cells were present in the epithelial columns according to the portion of even the same tubule.

The interstitial tissue was narrow and consisted of only a small amount of connective tissue and a few interstitial cells in the normal fowl testis (Fig. 1). It was, however, enlarged with loosened connective tissue and large spaces in various portions of the materials examined. Sometimes, sperms were observed in these spaces of the enlarged interstitial tissue (Fig. 3). Tail could be seen only in a few of them, as mentioned in the previous report ('61). There were various degrees in the number of sperms contained in that tissue, i.e., from a single one to more than ten aggregated in a mass (Figs. 2, 3). In the neighborhood of the enlarged interstitial tissue, the basement membrane became loose (Fig. 4) and sometimes disappeared (Fig. 5), and germ cells exhibited irregular arrangement in the seminiferous tubules. These figures were the same as those mentioned in the previous report ('61). Sperms in the interstitial tissue (interstitial sperms) were not only in one place but in several portions of the same section (expressed with +). In many materials in which a solitary interstitial sperm was
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Table 2. Sperms in interstitial tissue of fowl testis

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Breed</th>
<th>No. of strains</th>
<th>Age</th>
<th>No. of fowls</th>
<th>Sperm in interstitial tissue*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>W.L.</td>
<td>2</td>
<td>10m</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R.I.K.</td>
<td>1</td>
<td>10m</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>W.L.</td>
<td>4(A)</td>
<td>7m</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1y</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7m</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1y</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7m</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1y</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7m</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1y</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>W.L.</td>
<td>1</td>
<td>1y</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>W.L.</td>
<td>1</td>
<td>1y</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>W.L.</td>
<td>3(A)</td>
<td>7m</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1y</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2y</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7m</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2y</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7m</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1y</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Si</td>
<td>W.L.</td>
<td>1</td>
<td>1y</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Su</td>
<td>W.L.</td>
<td>1</td>
<td>3y</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>N.H.</td>
<td>1</td>
<td>7m</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1y</td>
<td>3(biopsy)</td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>W.L.</td>
<td>1(D)</td>
<td>3y</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>15</td>
<td>184</td>
<td></td>
<td>7(3.80%) 4(2.17%) 5.98</td>
</tr>
</tbody>
</table>

* For the symbols, + and ±, see the text.

found in a place, careful examination revealed that sperms were also present in several other places of the same section. If only a solitary sperm was observed in any one of the serial sections, the result was expressed with ±. As shown in Table 2, ± was found only in four of the 7-month-old fowls of the B strain on the farm of breeder E. The presence of sperms expressed with + was found in five fowls from breeder E and one from breeder T (N.H.), 7 months old, and in one from breeder I 1 year old. Of the five birds showing +, four were of the B strain and the other of the C strain from breeder E. One bird belonged to the A strain from breeder I. Total percentage was 5.98 in the present study. The birds presenting + and ± were nine in number and derived from the farms of breeder E, which showed the highest percentage (10.98) of those of the nine breeders. The fowls used in the previous study ('61) were also derived from the farm of breeder E.

The fertility rate was higher than 80% among four strains of breeder E (the fertility was 91.67, 84.37, 98.46, and 100.00% in the E-A, E-B, E-C, and E-D strains, respectively). It was in a normal range among the birds derived from the farms of breeder I and T (the fertility was 90.40% in the T strains, and no accurate data from the breeder I could be received).

Discussion

Such abnormalities as decidual epithelial cells, cytolysis, pyknotic polynuclear giant cells, tripolar division, and chromosome bridge were observed in the present study. Since they were found in fowls of all ages, no relationship could be thought to exist between them and the age
of birds. They were very few in number in the present study. These abnormal figures were reported by Nishida in the testes of normal domestic ducks ('53), domestic fowls ('64) and by Sneider ('40) in the ovaries of prepubertal rats and cats. Therefore, it can be thought that the present materials were in the normal stage. These figures were seen in materials, regardless of the presence or absence of interstitial sperms. Therefore, they had no relations to the appearance of interstitial sperms.

The testes of the fowls more than 1 year old were in good development and were essentially the same in weight and histological figures. On the contrary, there was a great variation among the testes of the 7-month-old fowls. The testes of the fowls from breeder E which contained the most numerous interstitial sperms of all the birds showed good development with almost the same size.

In the testes of the normal adult fowl, the interstitial tissue was very narrow and consisted of a small amount of connective tissue and a few interstitial cells. The columnar structures of germinal epithelium and sperm bundles were very clear. The combinations of germ cells in the epithelial columns varied from column to column in the fowl testes. From their series of studies on mammalian testes, Clermont et al. ('59-'66) reported that the combinations of germ cells from base to lumen were almost uniform in one section of seminiferous tubule, and that the same combinations were observed at a certain length of the tubule. These combinations varied according to the stage of spermatogenesis both in one portion and in the longitudinal area of the tubule. Those authors proposed the name “cycle” for the changes in one portion and “wave” for the longitudinal changes. They also mentioned that there were five kinds of spermatogonia, A1, A2, B1, B2, and B3. According to them, primitive A1 proliferates and differentiates into A2, which begin to differentiate into spermatozoa (through B1-B3); primitive A1 is the origin of a derivative A1, which differentiates into A5. In the present study on the fowl testis, however, the combinations of germ cells varied even in the same section of the seminiferous tubule. From these results, it is doubtful that longitudinal waves of spermatogenesis exist in the seminiferous tubules of the fowl testis. Since many kinds of combinations of germ cells are found in the fowl testis, it may be considered that there are cycles in the germinal epithelium of the fowl testis. This view is in accordance with that of Clermont et al. As no detailed statistical examination was performed in the present study, the duration of a cycle and the kind of spermatogonia in the fowl seminiferous tubule could not be explained clearly. These points require further studies.

Only Heidenhain’s iron hematoxylin and light green staining method was employed in the present study, as in the previous study of authors ('61). It can be thought that the filamentous bodies found in the interstitial tissues are true sperm head.

The cases showing + and ± numbered nine (5 cases of + and 4 of ±) among 7-month-old fowls, two among 1-year-old ones, and none among those 2 to 3 years old. Since there were no fowls 2 to 3 years old among those from the B strain of breeder E and a few among those from all breeders in the present study, the relationship between interstitial sperms and age of the bird cannot be explained clearly. Interstitial sperms were found in fowls of three of the nine breeders, and in fowls of four of the fifteen strains used. Most of these fowls were found in a single strain, i.e., four fowls of + and four fowls of ± were among those of the B strain from breeder E. The percentage of sperm-positive birds was the highest (10.98%) among the birds from breeder E of those from all the breeders. When the number of strains was regarded as one for one breeder, interstitial
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sperms were found in three of the ten strains. Total percentage of appearance of interstitial sperms was 5.98 (11 in 184). When broken into strains, the percentage of + cases was 19.05, 4.76, 14.29 and 6.66 in the E-B, E-C, I-A, and T strains, respectively, and that of ± was 19.05 in the E-B strain. The percentage of + plus ± was the highest in the E-B strain (38.09%). From the results obtained, it may be assumed that there is a relationship between the appearance of interstitial sperms and the strain of fowls, although it is possible that there may be changes in percentage according to the method of collecting materials, to be mentioned below. Since interstitial sperms were found in W.L. and N.H., it may be thought that there is no relationship between them and the breed of fowls. However, this cannot be asserted because of the small numbers of N.H. and R.I.R. used for the present study.

Histological materials were collected only from the middle portion of the left testis. Three to six sections were examined for each material. Serial sections were prepared only from some ± cases. Thus materials were taken from extremely limited portions. If the bilateral whole testes had been examined, the percentages would have been changed. For these reasons, the percentage of appearance of interstitial sperms cannot be asserted. Changes in percentage, however, may not be so great, because interstitial sperms could be detected from several portions of the same section. In such materials as this, characteristic enlarged interstitial tissues were also observed.

In the fowls of the strain from breeder E which showed the highest percentage of appearance of interstitial sperms, fertility was in a normal range. Accordingly, it cannot be assumed that interstitial sperms lowered fertility extremely. In the present study, fertility was examined only on the basis of strain, and not on the basis of individual which was found to contain interstitial sperms. Therefore, further studies are required to be performed on individual birds. The physiological significance of these phenomena is unknown at present.

The authors ('61) already made discussion on the mechanism of these phenomena. In his electron-microscopic study, CLERMONT ('58) stated that smooth-muscle-like cells were contained in the limiting membrane of the rat seminiferous tubule, the movement of which was produced by their contraction. If such cells exist also in the fowl testis, it may be thought that the contents of the tubule can be pressed out from the weak portions of the tubule by their contraction. Irregularity of arrangement of germ cells can also be explained by the contraction of such cells. There are no reports, however, on the presence of smooth-muscle-like cells in the fowl tubule. The question how the weak portions were produced in the tubule cannot be solved. Therefore, the mechanism remains in doubt.

Summary

The middle portion of the left testis was examined in 184 adult male fowls of fifteen independent inbred strains (from nine breeders). It was fixed in Allen-Bouin's solution, cut into 7μ paraffin sections—serial sections in some cases—and stained with Heidenhain's iron hematoxylin and light green. The results obtained are summarized as follows.

1. When sperms could be found in the enlarged interstitial tissue, a few or more than ten sperms were aggregated into a mass. In the majority of them the structure of tail was not clear. In some cases, a solitary sperm was also observed in the interstitial tissue.

2. In many cases, either solitary or aggregated sperms could be seen in several portions of the same sections.
3. Interstitial sperms were observed in four of the fifteen strains, or in one New Hampshire and three White Leghorn strains. They appeared most frequently in the birds of a particular strain (the B strain of breeder E). When the density of interstitial sperms was graded by symbols, four of seven + cases and all of four ± cases belonged to this strain.

4. So far as the number of interstitial sperms is concerned, there were seven + cases, of which six were White Leghorns (of three strains) and the other was a New Hampshire, and four ± cases, which were all White Leghorns of one strain. The rate of appearance of + cases was 3.80%, that of ± cases 2.17%, and that of + plus ± cases 5.98%. When broken in strains, the rate of appearance of + cases was 19.05 and 4.76% in the B and the C strain of breeder E, respectively, 14.29% in the A strain of breeder I, and 6.66% in the strain of breeder T, and that of ± cases 19.65% in the B strain of breeder E. The rate of appearance of + plus ± cases was 38.09 in the B strain of breeder E. These results seem to indicate that there is a relationship between interstitial sperms and the strain of fowls, although there remains a question about the rate of appearance because of incomplete determination of this rate. Probably, there is no relationship between the appearance of interstitial sperms and the breed of fowls.

5. Almost all these phenomena were observed in materials collected from all the 7-month-old fowls and two of the 1-year-old ones, but in no materials from the fowls 2 to 3 years old examined. Since the materials from the fowls 2 to 3 years old were small in number, the relationship between interstitity sperms and age of fowls could not be explained clearly from the results of the present study.

6. Fertility was more than 80% in all the strains. It can be assumed that these phenomena did not lower the fertility of the male fowl extremely, although fertility was not studied in any individual positive case. The physiological significance of these phenomena was not clear from the results of the present study.

7. The mechanism of these phenomena was discussed, but there remained many questions on these phenomena.

Acknowledgments

The authors are indebted to Messrs. T. MAKITA, H. MORI, H. MIYAKAWA, N. OGAWA, K. MAMBA, and other members of their laboratory for assistance in collecting materials. This work was supported partially by grant-in-aid No. 67034 of the Ministry of Education in 1965.

References

Fig. 1-a, b. Normal structure of testis of 3-year-old fowl. Note narrow interstitial tissue, a few interstitial cells, columnar structure of germinal epithelium, and sperm bundles. White Leghorn (W.L.) No. 38S-196 of Su strain. a, ×100; b, ×400.

Fig. 2-a, b. Solitary interstitial sperm (+). Note enlarged interstitial tissue and large spaces in it. W.L. No. 1102T of E-B strain. a, ×100; b, ×400.

Fig. 3-a, b. Interstitial sperms in mass (+). Note enlarged interstitial tissue and large spaces in which sperms are in mass. W.L. No. 1124T of E-B strain. a, ×100; b, ×400.
Fig. 4-a, b. Loosened basement membrane of seminiferous tubule. Same material as in Fig. 3.  
   a, ×100; b, ×400.

Fig. 5-a, b. Lack of basement membrane of seminiferous tubule. Same material as in Fig. 3.  
   a, ×100; b, ×400.

Fig. 6-a, b. Sperm crosses with basement membrane. W.L. No. II43T of E-B strain.  
   a, ×100; b, ×400.
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鶏精巢の間質内精子について

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著者らはさきに(61)鶏精巢の間質内精子が存在することを報告した。その後の研究によれば、その出現は鶏の種類と関係があるとし、考えられた。今回はこの点を確かめるため、独立した15組織(3品種)羽の材料について検査して、次の結果を得た。

1. 間質内精子を認めうる場合には、多くは単独でなく、数個ないし数個の集団をなして存在する(+)が、尾部の構造を確認できるものは少数であった。通常切片で精巣しても、単独精子のみの場合は例であった(+)。

2. 単独、集団いずれの場合も、多くは同一の切片中数か所以上で認めることができた。

3. (+)、±を合せれば、15系統中4系統で認められた。品種的には殆どが白色レグホーン(W.L.)で、ニューハンブシャー(N.H.)で1例が認められたにすぎない。

4. 出現例数は、+はW.L.で6例(3系統),N.H.で1例の計7例,±はW.L.1系統で4例がみられ
た。出現率: + は3.80%, ± は2.17%, 両者を合わせると5.98%であったが、系統別にみると、+はE種
鶏場B,C系でそれぞれ19.05, 4.76%を示し、I種
鶏場A系では14.29%, T牧場では6.66%であり、
±は19.05%(E-B系)であった。E種鶏場B系で
は+、±を合せると38.09%の高率を示した。

5. 日令的には、+、±いずれも殆んどが7ケ月令
にみられ、1カ月令で2例, 2~3年鶏では皆無であ
った。2~3年鶏は数例であるから、出現と日令の関
係は明確ではない。

6. 本現象と品種間には特殊な関係はないように考えら
れる。

7. 本現象の最も多くみられたE種鶏場の系統の授精
率は80%以上であった。従って授精率を極端に低下さ
せるものではないであろう。その他の生理的意義も不明
である。

8. 間質内精子の成因について、2~3の可能性が考
えられたが、やはり疑問は残された。

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