

## وصف الحيوانات المنوية من الجهاز التناسلى لديوك اندقى - ٤ أثناء نموها

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تم فى هذا البحث دراسة مورفولوجية للحيوانات المنوية الموجودة فى الخصية والوعاء الناقل لعدد ٨٩ من ديوك الدقى - ٤ ذبحت أثناء نموها عند أعمار ١٢ ، ١٦ ، ٢٠ ، ٢٤ أسبوعا . ولقد لوحظ أن تفاعل الخصية المقطوعة يميل الى الحموضة ولكن تفاعل السائل المنوى المخزون فى الوعاء الناقل كان قلويا .

ويتقدم العمر من ١٢ الى ٢٤ أسبوعا زادت النسبة المئوية للحركة الطبيعية للحيوانات المنوية وكذا نسبة وجود الحيوانات المنوية السليمة زيادة ذات قيمة احصائية عالية . وهكذا كان الحال أثناء انتقال الحيوانات المنوية من الخصية الى الوعاء الناقل .

وقد وصف الباحثون حركة الحيوانات المنوية على أنها تعبانية وتم مناقشة كيفية وجود هذه الحركة بنسبة ملحوظة فى الخصية وبنسبة عالية جدا فى الوعاء الناقل . وقد استخدم فى هذا البحث صبغة الأيوسين الحيوى لفحص الأشكال المختلفة للحيوانات المنوية المتطورة فى الخصية (الاسبرماتيد) وتم رسمها بجوار الأشكال المختلفة من الحيوانات المنوية كاملة النمو السليمة والغير سليمة .

## SPERM MORPHOLOGY IN THE REPRODUCTIVE TRACT OF GROWING DOKKI-4 COCKERELS

(With one table and one figure)

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### SUMMARY

Morphological studies were made on the sperm reserve in the testis and vas deferens of 89 slaughtered growing Dokki-4 cockerels. The pH of the incised testis was slightly acidic, while that of semen reserve in the vas deferens was slightly alkaline. The percentages of motility and normal spermatozoa increased significantly from 12 to 24 weeks of age as well as during their transportation through the excurrent ducts. The reverse of these findings was reported with the percentages of sperm with secondary abnormalities. The motility of testicular spermatozoa was discussed in the text, while the different forms of spermatids, normal and abnormal spermatozoa were illustrated.

### INTRODUCTION

Sperm morphology is one of the most important criteria for semen evaluation and male fertility either in mammals or in birds. PARKER, Mc KENZIE and KEMPSTER (1942), WILCOX and SHAFFNER (1957), WILCOX (1958), KAMAR (1959 & 1960), KAMAR and BADRELDIN (1959), SCHINDLER and NEVO (1962), MANN (1964) and LAKE (1971) have studied certain characteristic features of semen collected from the domestic fowl.

Moreover, the ultra structure of spermatozoa in male fowl have been investigated by GRIGG and HODGE (1949), NAGANO (1962), Mc INTOSH and PORTER (1967), LAKE, SMITH and YOUNG (1968) and TINGARI (1973). Apart from the publications of MINRO (1938) who studied the fertilizing capacity of cock sperm taken from the testis and excurrent ducts, no informations appear in the literature about the motility and morphological

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appearance of normal and abnormal sperm present in the male genitalia of the fowl. Therefore, the present work was conducted to study the morphology of sperm reserve in the testis and vas deferens of growing Dokki-4 cockerels.

## MATERIALS AND METHODS

A group of 89 Dokki-4 cockerels were used in the present investigation. At 12, 16, 20 and 24 weeks of age variable numbers of these cockerels were selected at random and slaughtered. After evisceration, the freshly picked testis was cut with a scalpel and the pH of its interior structure was measured by a special indicator paper (E. MERCK DARMSTADT pH RANGE 6.4—8.0).

Thereafter, a portion of each testis was cut into small pieces and mixed carefully with one of the following solutions : Ringer saline or citrate buffer (2.9%  $\text{Na}_3 \text{C}_6 \text{H}_5 \text{O}_7 \cdot 2\text{H}_2\text{O}$ ). A drop of the prepared suspension was examined microscopically to evaluate the sperm motility. Another drop was mixed with two drops of 0.5% eosin (dissolved in phosphate buffer pH 7.4) to distinguish the different forms of spermatids and spermatozoa, since the alive and dead spermatozoa, could not be differentiated by this stain as domestic animals. From the prepared testicular mixture, 2—3 thin films were made and stained with alkaline methyl violet according to BLOM (1943) to examine the typical and atypical forms of spermatozoa.

At 20 and 24 weeks of age, the semen squeezed from the vas deferens of each slaughtered cockerels was similarly treated. The obtained results were statistically analysed using the t-test after SNEDECOR (1956).

## RESULTS

The data of present work were tabulated according to the age and organs as shown in Table 1. The different forms of the examined spermatids and spermatozoa are clearly illustrated in Fig. 1.

It is of interest to mention that the motility of cock spermatozoa which was observed in the testes and vas deferens resembled snake movement but more vigorous and rapid. This movement begins at the anterior portion of the sperm head and propagates posteriorly along the remaining portion of its structure. Occasionally, some samples obtained from the testis were completely immptile. On the other hand, low dilution of semen reserved in the vas deferens revealed as strong mass activity in most cases.

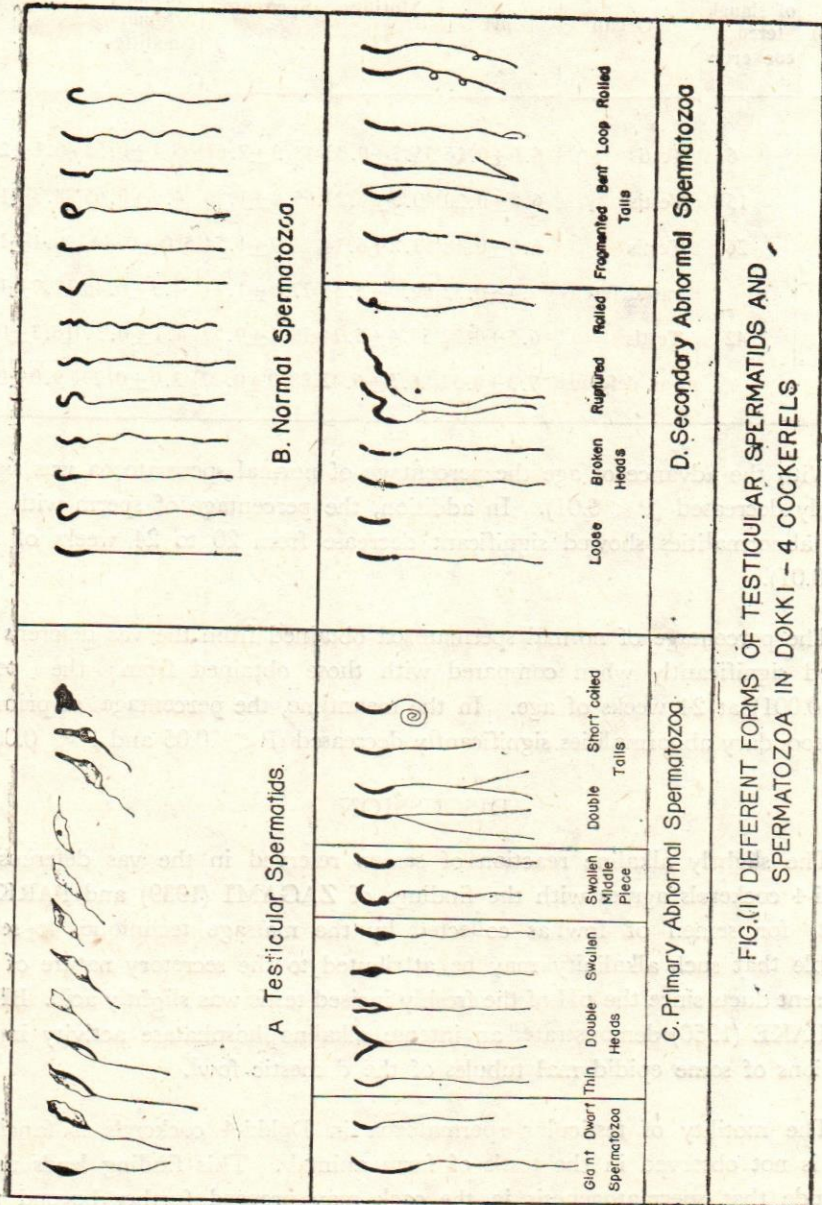


FIG.(1) DIFFERENT FORMS OF TESTICULAR SPERMATIDS AND SPERMATOZOA IN DOKKI - 4 COCKERELS

TABLE 1. Morphology of sperm reserve in testis and vas deferens of growing Dokki-4 cockerels

Age (weeks)	Number of slaughtered cockerels	Organ	pH	Motility %	Normal Spermatozoa %	Primary Abnormalities %	Secondary abnormalities %
12	6	Testis	6.6±0.16	35.3±8.02	48.5±2.61	5.4±0.52	46.1±2.12
16	15	Testis	6.5±0.20	40.5±6.23	66.4±1.80	4.9±0.65	28.7±1.83
20	26	Testis	6.6±0.18	53.2±6.14	74.9±1.54	5.0±0.44	20.1±1.48
		vas deferens	7.4±0.32	82.0±5.51	77.6±1.41	4.7±0.46	17.7±1.31
24	42	Testis	6.5±0.22	58.4±5.24	79.1±0.79	4.1±0.37	16.3±0.77
		vas deferens	7.3±0.32	86.5±6.42	88.0±0.77	3.0±0.20	9.0±0.75

With the advance of age the percentage of normal spermatozoa was significantly decreased ( $P < 0.01$ ). In addition, the percentage of sperm with primary abnormalities showed significant decrease from 20 to 24 weeks of age ( $P < 0.01$ ).

The percentage of normal spermatozoa obtained from the vas deferens increased significantly when compared with those obtained from the testis ( $P < 0.001$ ) at 24 weeks of age. In the meantime, the percentages of primary and secondary abnormalities significantly decreased ( $P < 0.05$  and  $P < 0.001$ ).

#### DISCUSSION

The slightly alkaline reaction of semen reserved in the vas deferens of Dokki-4 cockerels agrees with the findings of ZAGAMI (1939) and PARKER (1968) for semen of fowl as collected by the massage technique. It seems possible that such alkalinity may be attributed to the secretory nature of the excurrent ducts since the pH of the freshly incised testis was slightly acid. BELL and LAKE (1960) demonstrated an intense alkaline phosphatase activity in the secretions of some epididymal tubules of the domestic fowl.

The motility of testicular spermatozoa in Dokki-4 cockerels is fanciful as it is not observed in the testis of farm animals. This finding leads us to conclude that spermatogenesis in the cock may proceed further beyond that occurs in domestic animals and the end result of which is the formation of

spermatozoa with fair maturity and certain motility. This is the case since it is clearly understood that the process of sperm maturation is in close association with their motility. Recently, TINGARI (1973) showed that the morphological differentiation of the fine structure of spermatozoa is virtually complete when they are released from the seminiferous epithelium of the fowl. He concluded from this finding that the fertilizing capacity of cock spermatozoa reported by MUNRO (1938) may be attained in the excurrent ducts earlier than mammals. MAUNY (1968) and TAKEDA (1969) reported that spermatozoa in the cock took 3—4 days to traverse the epididymis and vas deferens. This period is very short when compared to 2—3 weeks in farm animals as reported by WHITE (1968). This big difference as well as the observation of PARKER (1968) that male chicken could mate 35—40 females per day may support our previous conclusion.

The tremendous increase in the motility of cock spermatozoa during their passage through the excurrent ducts coincide with the results of MUNRO (1938) who found that sperm taken from the vas deferens of fowl have the highest fertilization rate than those taken from the epididymis and testis. Moreover, the percentages of sperm motility in semen reserved in the vas deferens of Dokki-4 cockerels at 20 and 24 weeks of age are slightly higher than that reported by PARKER (1968) for semen collected from mature cocks. Thus, we are in accordance with the consideration of LARK (1957 a & b) that the content of the vas deferens is a normal semen in the domestic fowl.

The snake movement of cock spermatozoa described in this work differs distinctly from that observed in domestic animals. Through the insight of this movement, it is clear that the head of sperm participates in such action as did its tail. On the ground of this observation, it seems likely that the movement of cock spermatozoa may be initiated at the tip of its head rather than at its base as reported in mammals by MANN (1964). z

One of the most valuable results of the present study is the descriptive morphology of testicular spermatids which are much variable than those illustrated by KUMARAN and TURNER (1949) who studied the histology of the testis in growing White Plymouth Rock. Moreover, the different forms of normal and abnormal spermatozoa in Dokki-4 cockerels differ greatly from those published by PARKER *et al.* (1942) in male domestic fowl.

The significant decrease in the percentage of secondary abnormalities due to age and their transportation through the excurrent ducts may indicate that

both these factors are involved in the increased resistance of mature spermatozoa to the adverse condition of smearing. In buffalo, OSMAN and EL-AZAB (1973) attributed the high percentage of this abnormality in the testis to the fragility of such unripe spermatozoa. However the severe narrowness of cock sperm head, which resembled greatly the middle piece of bull sperm, may be responsible for the impossible differentiation between the alive and dead spermatozoa when using the eosin stain. It is necessary to mention that this dye could stain clearly the spermatids and spermatozoa as well as certain other testicular cells than did the alkaline methyl violet.

As a major conclusion from the present investigation, it is advisable to use Dokki-4 cockerels for successful breeding at 20 to 24 weeks of age when the percentages of their normal spermatozoa and motility reach their maximum values.

#### REFERENCES

- Bell, D.J. and Lake, P.E. (1960) : Phosphatases in seminal plasma of the domestic cock *Biochem. J.* 75,15.
- Blom, E. (1943) : *Skand. Vet. Tidskr.* 33, 428. Cited by D.R. Metrose & J.A. Laing. In : *Fertility and Infertility in the Domestic Animals.* J. A. Laing (Ed), 2nd Ed. Bailliere, Tindal and Cassel, London.
- Grigg, G.W., and Hodge, A.J., (1949): Electron microsoopic studies of spermatozoa. I. The morphology of the spermatozoon of the common domestic fowl (*Gallus domesticus*). *Aust. J. Sci. Res. B.*, 2,217.
- Kamar, G.A.R. (1959) : Semen characteristics of foreign and native fowls under Egyptian conditions. *Ind. J. Vet. Sct.* 29,19.
- Kamar, G.A.R., (1960) : Studies on fowl sperm. *Emp. J. Exp. Agric.* 28, 16.
- Kamar, GA.R. and Bedreldin, A.L. (1959) : Seasonal variations in semen characteristics of adult Fayoumi cocks. *Poult. Sci* 38,302.
- Kumaran J.D.S. and Turner, C.W. (1949) : The normal development, of the testes in the White Plymouth Rock. *J. Poult. Sci.* 28, 511.
- Lake, P.E. (1957 a) : Fowl semen as collected by the massage method. *J. Agric. Sci.*, 49, 120.
- Lake, P.E., (1957 b) : The male reproductive tract of the fowl. *J. Anat.* 91,116.
- Lake, P.E. (1971) : The male in reproduction. In : *Physiology and Biochemistry of the Domestic Fowl.* D. J. Bell & B.M. Freeman (Eds) Academic Press, New York.
- Lake, P.E., Smith, W. and Young, D. (1968) : The ultrasturcture of the ejaculated fowl spermatozoon. *Q.J. Exp. Physiol.* 53,356.
- Mauny, R. (1968) : 4 th Int. Congr. Anim. Reprod. A.I., & Paris, 1, 183. Cited by Lake, P.E. (1971)

- Mann, T.** (1964) : Biochemistry of Semen and of the Male Reproductive Tract. Methuen and Co. Ltd, London.
- Mc Intosh, J.R. and Porter, K.R.**, (1967) : Microtubules of the spermatids of the domestic fowl. *J. Cell. Biol.* 35,153.
- Munro, S.S** (1938) : Functional changes in fowl sperm during their Passage through the excurrent ducts of the male *J. Exp. Zool.* 79,71.
- Nagano, T.** (1962) : Observations of the fine structure of the developing spermatid in the domestic chicken. *J. Cell. Biol.* 14, 193.
- Osman, A.M. and El Azab, E.A.** (1973) : Studies on the morphology of testicular and ampullar spermatozoa in Egyptian buffalo bulls. *Zbl. Vet. Med. A.* 20,22.
- Tarker, J.E.** (1968) : Reproductive physiology in poultry. In : *Reproduction in Farm Animals.* E.S.E.Hafez (Ed) 2nd Ed. Lea and Febiger Philadelphia.
- Parker, J.E., Mc Kenzie, F.F., and H.L., Kempster** (1942) : Fertility in the male domestic fowl. *Mo. Agric. Expt. Sta. Res. Bull.* No. 347.
- Schindler, H. and Nevo, A.** (1962) : Reversible inactivation and agglutination of fowl and bull spermatozoa under anaerobic conditions. *J. Reprod. Fertil.* 4, 251.
- Snedecor, G.W.** (1956) : *Statistical Methods.* Iowa State College Press. Ames 5th Ed.
- Takeda, A.** (1969) : *Jap. J. Zootech. Sci.* 40,413. Cited by Lake, P.E. (1971).
- Tingari, M.D.**, (1973) : Observations on the fine structure of spermatozoa in the testis and excurrent ducts of the fowl, *Gallus Domesticus.* *J. Reprod Fert.* 34,255.
- White, I.G.** (1968) : Mammalian semen. In : *Reproduction in Farm Animals.* E.S.E.Hafez (Ed) 2nd Ed Lea and Febiger, Philadelphia.
- Wilcox, F.H.**, (1958) : Changes in the pH of semen of the domestic cock as affected by temperature and frequency of collection, *Poult. Sci.* 37,444.
- Wilcox, F.H. and Shaffner, C.S.** (1957) : The effect of differences in salt and hydrogen ion concentration on the fertilizing ability of avian sperm. *J. Appl. Physiol.* 11,429.
- Zagami, V.** (1939) : Sulla composizione chimica e sulla proprieta chimicofisiche del liquido sminale. *Arch. di Sci. Biol.* 25, 208. Cited by Mann, T. (1964).
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