

Evaluation of some spermatological characteristics in Gerze cocks

Pürhan Barbaros TUNCER, Hüseyin KİNET, Neval ÖZDOĞAN

Lalahan Livestock Central Research Institute, Elmadağ, Ankara - Turkey.

Summary: The aim of this research was to prove the principal spermatological characteristics in Gerze cocks in Turkey. In this experiment, 30 Gerze cocks were used. Semen was collected from cocks by abdominal massage method, two times a week during the 9 weeks period. The spermatological characteristics of samples are as following: Ejaculate volume average 0.37 ± 0.006 ml, spermatozoa motility $74.28\pm 0.73\%$, spermatozoa concentration $2.42\pm 0.02\times 10^9$ /ml, percentage of abnormal spermatozoa $6.32\pm 0.10\%$, percentage of dead spermatozoa $19.74\pm 0.73\%$, pH averaged 7.71 ± 0.01 in Gerze cocks. Acrosome, head, middle piece and tail deformations of 540 ejaculates were recorded as average $0.39\pm 0.03\%$, $1.06\pm 0.03\%$, $2.32\pm 0.05\%$ and $2.53\pm 0.04\%$, respectively. This is the first study which was proved some spermatological characteristics of Gerze cocks.

Key words : Abnormal spermatozoa, concentration, Gerze cocks, motility, pH.

Gerze horozlarında bazı spermatolojik özelliklerin değerlendirilmesi

Özet: Bu araştırmanın amacı, Türkiye'ye ait bir ırk olan Gerze horozlarında başlıca spermatolojik özelliklerin ortaya konulmasıdır. Bu çalışmada Gerze ırkı 30 horoz kullanılmıştır. Horozlardan sperma örnekleri 9 hafta süresince haftada 2 kez abdominal masaj yöntemiyle alınmıştır. Horozlardan alınan sperma örneklerinin spermatolojik özellikleri; ejakülat miktarı 0.37 ± 0.006 ml, spermatozoa motilitesi 74.28 ± 0.73 , spermatozoa yoğunluğu $2.42\pm 0.02\times 10^9$ /ml, anormal spermatozoa oranı 6.32 ± 0.10 , canlı spermatozoa oranı 19.74 ± 0.73 ve spermatozoa pH değeri 7.71 ± 0.01 olarak bulunmuştur. Çalışmada elde edilen 540 ejakülatın anormal spermatozoa tiplerinden akrozom, baş, orta kısım ve kuyruğa ait bozuklukların genel ortalama değerleri sırasıyla 0.39 ± 0.03 , 1.06 ± 0.03 , 2.32 ± 0.05 ve 2.53 ± 0.04 olarak belirlenmiştir. Bu çalışma, Gerze horozlarında spermatolojik özelliklerin belirlenmesi için yapılan ilk çalışmadır.

Anahtar sözcükler : Anormal spermatozoa, Gerze horozu, motilite, pH, yoğunluk.

Introduction

Gerze fowl is a local breed, which belongs to the region of Sinop-Gerze province in Turkey. Gerze has got a plain black colour and its crest has got a fork shape like a horn, and also there are no varieties of this species in other parts of the world. This breed of cocks has got tall legs and a huge body. There is a remarkable whiteness behind the ears of the cocks and the chickens as well. The beak and the tibia and the skin of foot is black. By the end of 52nd week the average weight in hens is 1706.32 gr and in cocks 2317.86 gr (1, 2, 23).

The function of the male is to produce semen, and in the case of natural mating, to copulate and ejaculate the semen onto the everted cloaca of the hen. Approximately 3 billion spermatozoa are produced daily by sexually active cock. The testes are located in the centre of the body cavity and, therefore, spermatogenesis proceeds at the internal body temperature of 41°C in birds, as opposed to the scrotal temperature of $24-26^{\circ}\text{C}$ in mammals (10). Both testes are functional in the cocks, as sexual maturity is attained, the weight of paired testes increases from 2-4 g to 25-35 g; the left testis is usually 0.5-3 g heavy than the right. Semen is ejaculated from

phallus by mixing lymph liquid (10, 14). Spermatogonia are produced continuously by mitotic division to yield subsequent generations of spermatogonia and spermatocytes which enter the first meiotic division. After approximately 6 days, the second meiotic division has been completed, and the four haploid spermatids begin to elongate. Spermatogenesis is completed during the next 7 to 8 days, during which the morphology of mature spermatozoa acquired. The sperm cells of domestic birds are long, cylindrical, and tapered at both ends. The spermatozoa are about $0.5\ \mu\text{m}$ at their widest point and approximately $100\ \mu\text{m}$ in length (10). Qualified and high concentration semen of cock is pink-white and consistency is also high. Ejaculation volume, which depends on breed, age, individual, season, light and many environmental factors, is averagely 0.7 ml and spermatozoa concentration is 3.0×10^9 /ml and pH of spermatozoa is 7.5 (8, 10, 11, 24). In 1912, after killing a cock, Ivanof made artificial insemination (AI) with semen collected by pressure from ductus deferens. In the following years, too many studies were conducted on this subject, Burrows and Quinn collected semen by massage method (10, 17). Kono and Hiura (15) found ejaculate

volume for Single Comb White cocks 0.1-1.1 ml. Lake and Stewart (16) and Rouvier et al. (18) found the average ejaculate volume for broiler cocks 0.35 ml, for light-weight egg layer 0.15 ml, for medium-weight egg layer 0.2 ml; Alkan et al. (3) and Keskin et al. (14) found ejaculate volume for Erbro cocks averagely 3.29 ± 1.19 ml and 0.6 ± 0.1 ml, respectively. Chalov A. (7) found this volume for Leghorn cocks 0.3 ml and Kamar et al. (12) found ejaculate volume for Fayoumi, Plymouth Rock and Rhode Island cocks 0.26, 0.62 and 0.48 ml, respectively. Keskin et al. (13) and Tuncer et al. (25) found this volume for Denizli cocks 0.6 ± 0.1 ml and 0.7 ± 0.01 ml, respectively.

Tuncer et al. (25) determined the spermatozoa motility for Denizli cocks $72.32 \pm 0.80\%$. Different researchers have found the spermatozoa motility for Leghorn breed $83.2 \pm 0.6\%$, for New Hampshire breed $77.6 \pm 0.2\%$ (19), for Erbro cocks $79.4 \pm 11.5\%$ and $87.7 \pm 0.9\%$ (14), for Denizli cocks as $65.0 \pm 2.9\%$ (13); but Pakdil (17), Alkan et al. (3), Carvalho et al. (5), Chalah et al. (6), Dube et al. (9) and Schula and Tomar (22) have found 82.2%, 85.83%, 50.8%, 83%, 80% and 86.5%.

Tuncer et al. (25) have found spermatozoa concentration for Denizli cocks $2.38 \pm 0.03 \times 10^9$ /ml. Some researchers have found spermatozoa concentration 2.20×10^9 /ml (17), $1.878 \pm 0.2 \times 10^9$ /ml (19) for Leghorns cocks, 3.32×10^9 /ml, 3.347×10^9 /ml for New Hampshire, 5.0×10^9 /ml for light-weight egg layer and medium-weight egg layer (18); $2.0 \pm 0.2 \times 10^9$ /ml for Denizli cocks (13).

Banarjee and Katpatal (4), who examined the changes in the quality of cock ejaculates, determined the rate of abnormal spermatozoa for White Leghorn, Rhode Island Red, Leghorn X Rhode Island Red and Deshi cocks breeds as 23.3%, 23.2%, 24.2% and 25.9%, respectively. Sevinç et al. (19) fixed the total abnormal spermatozoa rate as $5.44 \pm 0.73\%$ for Leghorns and $6.76 \pm 0.95\%$ for New Hampshire cocks. The abnormal spermatozoa rate for Erbro cocks have found $5.0 \pm 0.06\%$ (14). However, other researchers determined this total rate as $11.83 \pm 0.96\%$ (3), $5.25 \pm 0.55\%$ (17) and $5.4 \pm 0.7\%$ (26). Tuncer et al. (25) have found acrosome, head, middle piece and tail deformations of abnormal spermatozoa for Denizli cocks as average $0.39 \pm 0.03\%$, $1.06 \pm 0.03\%$, $2.32 \pm 0.05\%$ and $2.53 \pm 0.04\%$, respectively.

The semen pH value for Denizli and Erbro cocks have found 7.5 ± 0.1 and 7.4 ± 0.1 (13, 14). Sevinç et al. (20, 21) found this value was 6.9 for White Leghorn and New Hampshire. Pakdil (17) and Tuncer et al. (25) found this rate 7.68 ± 0.01 for Denizli cocks. The dead semen rate was $83.34 \pm 6.43\%$ (3), $21.65 \pm 0.81\%$ (25).

The studies have been still carried on for improving genetic characteristics of Gerze cocks and chickens which are covered in the scheme of indigenous animal species and breeds in Lalahan Livestock Research Center Institute. There is not any research about spermatological

characteristics of Gerze cocks in Turkey. Therefore, the aim of this research was to get some statistical data about spermatological characteristics of Gerze cocks and to contribute the future experiments. This research is an "Informative Study" about Gerze cocks semen.

Materials and Methods

Thirty Gerze cocks aged 44 weeks, from the Gerze conservation flock in Lalahan Livestock Research Center Institute, were used in this study. The cocks were kept in individual battery typed cages under a day length of 16 h lightness program and *ad libitum* nutrition was applied. Semen was collected by abdominal massage method two times a week in the morning during 9 weeks. Ejaculate volume was determined as 'ml' by finding directly from semen collecting tube. Semen was diluted and motility was estimated by a hot plate phase-contrast microscope (Nikon-Labophot, Japan) at x200 magnification. Spermatozoon concentration was calculated by using a digital photometer (Accuell Poultry Photometer, I.M.V., France) and was recorded as $\times 10^9$ /ml. A Hancock solution was employed in morphologic observations. The preparation was examined by light microscope at x1000 magnification by counting 200 cells (total). A drop of fresh semen was mixed with a drop of eosin and it was examined at 400x magnification and, about 200 spermatozoa were counted for dead-live spermatozoa. The pH value of the fresh semen was measured by using a colour-scaled pH meter (5.5 – 9.0, Merck) (10, 11, 13, 25).

Statistical analysis of the spermatological characteristics of fresh semen was performed by the SPSS program and ANOVA for repeated measures. When the F values were significant ($p < 0.05$), Duncans's multiple range test was performed (27).

Results

This informative study aimed to revealing the truth about the semen characteristics of Gerze cocks. In this informative study, general average values of Gerze cocks' spermatological characteristics are shown Table 1. During these nine weeks period, the differences in the ejaculate volume, spermatozoa motility, spermatozoa concentration, abnormal spermatozoa, dead spermatozoa and pH values among the collected semen were considered statistically important ($p < 0.05$ and $p < 0.001$).

The general average values of defect found in the acrosomes, heads, middle parts and tails of totally 30 cocks were $0.39 \pm 0.03\%$, $1.06 \pm 0.03\%$, $2.32 \pm 0.05\%$ and $2.53 \pm 0.04\%$, respectively (Table 2). At the end of the evaluation, a statistical difference was found depending on cocks' spermatozoa's head, middle parts and tail among the weeks ($p < 0.001$). While no difference was found in the defects occurred in acrosomes among the weeks ($p > 0.05$), but some individual differences were determined among cocks ($p < 0.05$ and $p < 0.001$).

Table 1. Some spermatological characteristics for Gerze Cocks (n=60)

Tablo 1. Gerze horozlarında bazı spermatolojik özellikler (n=60)

Week	Spermatological characteristics					
	X ± Sx					
	Ejaculate Vol.(ml)	Motility (%)	Concentration (x10 ⁹ /ml)	Abnormal Sp. (%)	Dead Sp. (%)	pH
1	0.36±0.02 ^a	76.67±1.71 ^a	2.04±0.08 ^a	5.28±0.31 ^a	16.47±1.74 ^a	7.54±0.04 ^a
2	0.37±0.02 ^a	80.08±0.94 ^b	2.35±0.07 ^{ab}	5.71±0.25 ^a	13.93±0.99 ^b	7.69±0.04 ^b
3	0.36±0.02 ^a	71.25±2.79 ^c	2.27±0.08 ^{ab}	6.48±0.32 ^b	22.43±2.86 ^c	7.77±0.03 ^b
4	0.35±0.01 ^b	73.67±2.39 ^d	2.55±0.07 ^{abc}	6.61±0.31 ^b	20.03±2.37 ^c	7.80±0.03 ^b
5	0.35±0.01 ^b	74.17±2.06 ^a	2.47±0.08 ^{abc}	6.78±0.31 ^b	19.55±2.04 ^d	7.70±0.04 ^b
6	0.38±0.01 ^{abc}	70.08±2.78 ^c	2.53±0.09 ^{abc}	6.86±0.33 ^b	24.08±2.77 ^e	7.67±0.04 ^b
7	0.36±0.02 ^{ab}	70.58±2.58 ^c	2.37±0.09 ^{ab}	6.76±0.33 ^b	24.35±2.47 ^e	7.79±0.03 ^b
8	0.39±0.01 ^{abc}	75.25±1.99 ^a	2.58±0.07 ^{abc}	6.18±0.31 ^b	18.88±1.85 ^d	7.75±0.03 ^b
9	0.43±0.02 ^{abc}	76.83±1.75 ^a	2.64±0.06 ^{abcd}	6.23±0.31 ^b	17.93±1.75 ^d	7.72±0.04 ^b
Gen. Ave. n=540	0.35±0.01	74.28±0.73	2.42±0.02	6.32±0.10	19.74±0.73	7.71±0.01
F	4.54 ^{**}	2.01 [*]	3.98 ^{**}	4.35 ^{**}	5.02 ^{**}	2.32 [*]

* : p<0.05 ** : p<0.001

a-e : Values within each column with different superscripts differ significantly.

Table 2. Average rate (%) of acrosome, head, middle part and tail defects at Gerze cocks (n=60).

Tablo 2. Gerze horozlarında akrozom, baş, orta kısım ve kuyruk bozukluklarının ortalama oranı (n=60).

Week	Morphological defects			
	X ± Sx			
	Acrosome (%)	Head (%)	Middle (%)	Tail (%)
1	0.23±0.06	1.06±0.09 ^a	2.21±0.17 ^{ab}	1.78±0.14 ^a
2	0.30±0.06	1.06±0.10 ^a	2.40±0.16 ^{bc}	1.95±0.10 ^{ab}
3	0.38±0.07	1.16±0.09 ^a	2.50±0.18 ^{bcd}	2.43±0.15 ^{bc}
4	0.41±0.08	1.21±0.09 ^b	2.63±0.16 ^{bcd}	2.35±0.14 ^{cd}
5	0.41±0.09	1.23±0.08 ^b	2.60±0.17 ^{bcd}	2.53±0.14 ^{cde}
6	0.53±0.09	1.15±0.09 ^a	2.50±0.16 ^{bcd}	2.66±0.12 ^{bd}
7	0.58±0.11	1.00±0.09 ^c	2.18±0.16 ^{ab}	3.00±0.12 ^{de}
8	0.33±0.09	0.83±0.09 ^d	1.96±0.17 ^e	3.05±0.12 ^e
9	0.38±0.10	0.88±0.10 ^d	1.88±0.13 ^e	3.08±0.13 ^e
General Average (n=540)	0.39±0.03	1.06±0.03	2.32±0.05	2.53±0.04
F	1.01	3.05 [*]	3.65 [*]	7.25 ^{**}

* : p<0.05 ** : p<0.001

a-e : Values within each column with different superscripts differ significantly.

Discussion and Conclusion

Cocks show their mating desire in the late hours in the afternoons. Semen of cocks is collected and AIs are done during those hours in the afternoon (13). But, in this research, the procedure was different because ejaculates were collected from Gerze cocks in the mornings. However, some other researchers had indicated semen-collecting hours were not so effective on the spermatological characteristics. On the other hand, the frequency of collection of semen was effective on spermatozoa (8, 16, 18). Ejaculate volume was different from the values of the physiological limits in Gerze cocks. The maximum ejaculate volume of total 540 ejaculates was 1.2 ml (for cock number 28), minimum ejaculate volume was 0.1 ml (for cocks number 1, 3, 6, 7, 16, 22, 26 and 30). General average spermatozoa motility of 540 ejaculates, that were examined, 74.28±0.73%, and for cocks number 16 this percentage was found 0% (once time) but specially for 2nd cock, this motility is really

very low compared to the other cocks and for other cocks 85% spermatozoa motility was found. The general spermatozoa concentration that depends on various factors such as breed, age, light, season, individual, semen collecting frequency was determined as 2.42±0.02 x10⁹/ml, and the minimum value was found 710x10⁶/ml (for 6th cock) and maximum value was 3.83x10⁹/ml (for 8th cock). Sometimes spermatological characteristics of cocks passed over the normal value and sometimes rested under those limits. Semen collecting hours and different researchers who carried out this experiment might cause the difference.

The morphological defects affect the fertility more than the motility. One of the most important criteria is to designate the spermatozoa having these types of structure (8, 10). Normally, percentage of abnormal spermatozoa found in certain rate in the cock ejaculate increases because of the cold water shock made during keeping-semen operations in short term or by freezing (3).

Abnormal spermatozoa was found Gerze cocks 6.32±0.10 % in this study. The maximum value was 27% for the 6th cock. Many researchers indicate that defects depend on cock semen acrosome from the point of its effect on fertility, are the most important ones (10). Dead spermatozoa rate was determined as Gerze cocks 19.74±0.73%. These were found in normal limits when compared to spermatozoa motility rates. During this experiment, although it was mixed with feces or other substances from outside changed sometimes pH of spermatozoa, general average semen pH was calculated as Gerze cocks 7.71, that was within its normal limits. This is the first study which proved some spermatological characteristics of Gerze cocks, so we couldn't compare the datas same breed cocks, that compared datas on other breed cocks.

In conclusion, the outcomes obtained in the informative study which was considered as a pre-study, will help us do some further researches on freezing of Gerze cocks' semen. This study was to help other experiments about the principal spermatological characteristics of Gerze cocks by revealing their *in vitro* outcomes.

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Address for correspondance

Pürhan Barbaros Tuncer, DVM, PhD.
Lalahan Live stock Central Research Institute
(Lalahan Hayvancılık Merkez Araştırma Enstitüsü)
Ankara, Türkiye.