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ANALYSIS OF SPERM MOTILITY, VELOCITY AND MORPHOMETRY OF THREE EGYPTIAN INDIGENOUS CHICKEN STRAINS

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ABSTRACT: Sperm quality is a principal determinant of its fertilizing potency. The current study was conducted in an attempt to link some morphometric measures (sperm and flagellum length) and the concentrations of some ions in seminal plasma on the one hand to sperm swimming velocity on the other hand in three Egyptian local chicken strains (Dandarawi, Sharkasi and Fayoumi). Ten adult males (28 weeks old) from each strain were housed in individual cages. Semen samples were collected twice weekly for a period of 16 weeks. Some physical and chemical characteristics of semen including ejaculate volume (mL), sperm concentration, motility (%), and the concentrations of calcium, potassium, sodium and manganese in seminal plasma were measured. Sperm curve linear velocity (VCL), average path velocity (VAP), straight line velocity (VSL) and straightness (STR) were measured using computer assisted sperm analysis (CASA) software. The lengths of entire sperm, head plus mid-piece and flagellum (µm) were measured using image J software. There were no significant differences among strains in the percentages of total motile sperms and the percentages of sperms demonstrating progressive motility. Ejaculates of Dandarawi roosters had higher sperm concentration/ml ($p<0.01$), greater percentages of rapid swimming sperms ($p<0.001$) and higher values of VCL, VAP and VSL ($p<0.0001$) compared to those of Sharkasi and Fayomi strains. Significant differences were observed in sperm morphometry among the different strains where Dandarawi and Sharkasi had longer sperm and flagellum compared to those of Fayoumi ($p<0.001$). Chemical composition of seminal plasma revealed higher potassium concentrations in Sharkasi samples compared to those of Fayomi ($p<0.05$); while the concentrations in Dandarawi ejaculates were intermediate. In conclusion, Dandarawi sperm showed higher swimming velocity compared to both Sharkasi and Fayomi. Slower sperm velocity can be attributed to shorter flagellum and to higher potassium concentrations in seminal plasma in Fayomi and Sharkasi strains, respectively.

Key words: Indigenous strains - Sperm morphometry - Swimming velocity – CASA.

INTRODUCTION

The local chicken strains are used by a broad sector in the Egyptian poultry industry as alternatives for foreign strains which are less tolerant to the prevalent thermal and pathological challenges. Improving the productive and reproductive abilities of the local chicken strains is considered one of the priorities the Egyptian poultry industry should take into account to maintain the genetic diversity of these strains and to protect them from being lost. Semen quality is a major factor affecting the resultant percentage of fertility and hatchability in poultry because it determines the reproductive ability of the males (Peters et al., 2008). Sperm morphology is considered an important parameter when assessing semen quality (Alkan et al., 2002; Tabatabaei et al., 2009). Although normal sperm morphology is poorly correlated with fertility, it is unlikely that morphologically abnormal sperms could possess good fertilizing capacity (Etches, 1996). Furthermore, sperm morphometric measures are thought to be correlated with sperm fertilizing ability. For instance, it is thought that longer sperms are more competitive because of their ability to swim faster. This assumption makes sense as longer sperm has proportionally longer midpiece containing more mitochondria, the site of ATP production, which might provide the sperm with more energy (Cardullo and Baltz 1991). Another hypothesis correlates between the length of the head and the flagellum of the sperm and its swimming velocity (Humphries et al., 2008). In other words, the shorter the head and the longer the flagellum, the greater the swimming velocity will be (Humphries et al., 2008; Lüpold et al., 2009). The absolute or relative length of sperm parts were reported to have an effect on sperm swimming velocity in many species, such as in Iberian deer (Malo et al., 2006)، the zebra finch (Mossman, 2008), cichlid fishes (Fitzpatrick et al., 2009) and in passerine birds (Lüpold et al., 2009). Furthermore, flagellum length was reported by some to be positively correlated to the midpiece length in mammals (Cummings and Woodall, 1985; Gage, 1998) and in birds (Immler and Birkhead, 2007; Lüpold et al., 2009). Seminal plasma, a complex fluid where sperms are suspended in, contains several organic components such as, proteins, steroids, glucose, enzymes, glutamate, and inorganic ions including Na^{+}, K^{+}, Ca^{++} and Mg^{++}. These inorganic ions play an important role in regulating osmotic balance and are also necessary for maintaining sperm function (Ilori et al., 2012). Previous work has proven that increased Ca^{++} uptake (influx) by the mitochondrion augment energy production (ATP) by modulating the activity of Ca^{++} sensitive enzymes (Forman and Feltmann, 2005). However, increased Ca^{++} concentration inside the mitochondrial matrix may cause cell necrosis. Therefore, Ca^{++} influx must be faced by its efflux which is accomplished through the sodium-calcium exchanger (Na^{+}-Ca^{++}exchanger). This means that Ca^{++} uptake and release is dependent on Na^{+} (Forman and Feltmann, 2005; Finkel et al., 2015). In other words, Ca^{++} is necessary for flagella motility and sperm respiration where any defect in these functions will negatively affect sperm viability and fertility (Barna et al., 1998; Karaca et al., 2002). In addition, high concentrations of K^{+} in seminal plasma decreases sperm metabolism and

therefore negatively affects sperm motility (Aghaei et al., 2010).The outcomes of subjective evaluation of semen analysis depend upon the experience and skill of the observer which may substantially vary from person to another (Den Daas, 1992) and the laboratory assays accuracy which evaluate semen (Graham, 2001). Therefore, computer assisted sperm analysis (CASA) has almost replaced subjective analysis in human and veterinary studies to minimize human erroneous. The use of CASA enabled the researchers to extract data from several computed motility parameters which accurately and rapidly characterize sperm motions (Klimowicz et al., 2008). This system works by capturing a sequence of images of the sperm and simultaneously acts to detect and track the sperm to characterize its motion. To our knowledge, the data concerning studying sperm morphometry and its relation to sperm velocity in the local chicken strains are not available. Therefore, the purpose of this study was to evaluate sperm swimming velocity and its relation to sperm morphometry and other semen characteristics in Dandarawi, Sharkasi and Fayoumi strains.

MATERIALS AND METHODS

Experimental birds

The present study was conducted at the Poultry Research Farm, Poultry Production Department, Faculty of Agriculture, Assiut University. Thirty male chicks from each strain (Dandarawi, Fayoumi and naked neck Sharkasi) were obtained from the Poultry Research Farm Hatchery, Assiut University and were reared on floor in a deep litter shed until reaching the sexual maturity. Cocks were reallocated into individual battery cages and given 2 weeks to acclimatize before starting the experiment. Ten adult roosters from each strain were selected, well trained for semen collection and used as semen donors in this experiment. Roosters were exposed to 11- h light at sexual maturity then, the photoperiod was increased 30 minutes weekly until 16 -h photoperiod was reached. Birds were fed on a commercial diet containing 3 % Ca, 0.68 % P, 17% crude protein, 2.80% crude fat, 3.13% crude fiber and 2750 kilo calorie/ kg diet.

Semen collection and preparation

Semen was collected by the abdominal massage method according to (Cole and Cupps, 1977; Mosenene, 2009). Care was taken during the process of semen collection to avoid semen contamination by blood or fecal material.

Individual semen samples were obtained from all strains twice a week for a period of 16 weeks to assess the ejaculate volume (using graduated collection tube) and sperm concentration (using Neubauer hemocytometer).

Sperm motility and velocity

Thirty two semen pools from each strain were analyzed over the experimental period (16 weeks) to assess sperm motility and velocity. Sperm motility and swimming velocity were assessed using a new CASA plugin for Image-J, an image processing soft ware provided by National Institution of Health (http://imagej.nih.gov/ij/), where various features were added to the original plugin to enhance sperm tracking (Elsayed et al., 2015).

A drop of diluted semen (1: 40 v/v Lake and Ravie diluent) was placed on a microscope slide, and covered with a glass cover, then placed on a microscope for examination. A magnification of (400

x) was used and several fields were examined to evaluate motility %, curvilinear velocity (µm/sec; VCL), average path velocity (µm/sec; VAP), straight line velocity (µm/sec; VSL) and straightness (STR = VSL/VAP). Videos of sperm movement were recorded using Tucsen ISH 1000 camera at 30 frames/second mounted on Optika XDS-3 inverted microscope with phase contrast. A minimum 3 fields and 500 sperm tracks were examined. Spermatozoa with VAP < 10 µm/s and VSL < 5 µm/s are considered immotile. Spermatozoa were classified according to their velocity to slow (10 < VAP < 20 µm/s), medium (20 < VAP < 50 µm/s) and rapid (VAP > 50 µm/s). The CASA defined thresholds were 20 VAP and 80% STR. The percentage of spermatozoa demonstrating progressive motility were calculated as the number of spermatozoa exceeding 20 µm/s VAP and 80% STR divided by the number of motile spermatozoa.

Sperm morphometry:

On the eighth week of the experimental period, a number of five samples of semen were collected randomly from 5 roosters each strain, and diluted to 1:200 using Lake and Ravie diluent. The diluted samples were fixed by adding formalin to a final concentration of 5%. A drop of formalin-fixed semen was deposited on a glass slide (two slides were made per rooster) and a smear was made and air-dried. The slide was then rinsed with distilled water (Helfenstein et al., 2010). Photographs of spermatozoa were taken with a digitalcolor video camera (Sony-CCD-IRIS/ RGB) mounted on a microscope at ×400 magnification. From these pictures, ten intact spermatozoa from each slide were selected (no broken tail, midpiece correctly coiled around the flagellum).All pictures were analyzed by the software Image-J.

For each spermatozoon, the entire sperm, sperm head and flagellum lengths were estimated. The head and the flagellum relative lengths and the head : flagellum ratio were calculated.

Ca^{++}, Na^{+}, K^{+} and Mg^{++} concentrations in seminal plasma:

On the eighth week, calcium, magnesium, potassium and sodium concentrations in seminal plasma were measured in seven samples from each strain. seminal plasma were separated gradually from semen in three consecutive centrifugations according to the method of (Blesbois et al., 1997). Calcium and magnesium were measured by ICP emission spectrometer (Model: iCAP 6200 dual-view) whereas the concentrations of potassium and sodium were measured using a flame photometer (Jenway, Bibby Scientific Limited, Beacon Road, Stone, Staffordshire, ST15 OSA, UK).

Statistical analysis:

Data of semen characteristics and sperm morphometry were subjected to analyses of variance using the ANOVA procedure of SAS software (SAS institute, 2009) and the differences among strain means were tested by using Duncan's new multiple range test (Duncan, 1955) at 5% and 1% level of probability. The statistical model for semen quality characteristics was used as follows:

$Y_{ijk} = \mu + S_i + E_{ij}$

Where Y_{ijk} is the observation (semen volume, sperm concentration, sperm morphometric measures and ions' concentrations in seminal plasma) by strain Si for i = 1, 2 and 3.

μ = Overall mean.

S_i = strain effect (i = 1, 2, 3).

E_{ij} = experimental error.

The association between chicken strain and sperm motility and velocity parameters was assessed using the Chi-squared test.

RESULTS

The obtained data of ejaculate volume, sperm concentration, motility, swimming speed and sperm velocity in Dandarawi, Fayomi and Sharkasi chickens are presented in Table

No significant differences were found among strains in the percentages of motile spermatozoa and in percentages of sperms showing progressive motility ($p>0.05$).

Compared to Fayomi and Sharkasi strains, Dandarawi roosters had significantly higher sperm concentration per ml in their ejaculates ($p=0.007$), and higher percentages of rapid spermatozoa ($p=0.001$). They also had smaller percentages of slow sperms ($p=0.001$) and showed significantly higher values of VCL ($p<0.0001$), VAP ($p<0.0001$) and VSL ($p<0.0001$).

Sharkasi roosters returned significantly higher ejaculate volume ($p=0.011$) and higher percentages of slow swimming spermatozoa ($p=0.001$). While, Fayomi roosters returned higher percentages of sperms with medium swimming speed ($p=0.001$).

Data regarding sperm morphometric measures in Dandarawi, Fayomi and Sharkasi chickens are presented in Table 2.

It was observed that Fayomi chickens had ejaculates with shorter spermatozoa compared to those of Dandarawi and Sharkasi ($p=0.0003$), and that this observation is actually attributed to having sperms with shorter flagellum.

As mentioned above, it was difficult for us to distinguish the junction between the head and midpiece and therefore, their lengths were recorded together as (head plus midpiece), and we could not find any differences in their lengths among different strains ($p>0.05$).

There were no significant differences in Ca^{++}, Na^{+} and Mg^{++} concentrations in seminal plasma among different strains ($p>0.05$; Table 3). However, ejaculates from Sharkasi roosters had significantly higher K^{+} concentrations in plasma than those of Fayomi chickens ($p=0.036$; Table 3). The concentrations of K^{+} in Dandarawi seminal plasma were intermediate.

DISCUSSION

Despite the lack of statistical differences in the percentages of immotile, motile and sperms showing progressive motility, the ability of spermatozoa to reach the fertilization site may differ among the studied strains because it does not rely only on the pattern of motility.

In order to ascend the oviduct and reach the fertilization site, sperm must be mobile. To be mobile, sperm must show progressive motility with a certain minimal limit of velocity. Spermatozoa that move linearly but with slow speed might not eventually achieve significant mobility. Furthermore, sperms that swim in circles and those showing increased curvature in their path are unlikely competitive (Sloter et al., 2006). Sperm mobility is therefore defined as the net forward movement of sperm against resistance at body temperature (Forman, 2006). Forman and Mclean (1996) developed an assay to measure sperm mobility in chicken using Accudenz solution in a cuvette with the spermatozoa being layered on top of the solution and incubated at 41°C for 5 minutes. It was reported that sperm straight line velocity

VSL must exceed 30 µm/s in order to penetrate the Accudenz solution (Forman, 2007). This may indicate that spermatozoa must demonstrate progressive motility with certain VSL to be considered as mobile sperm capable of reaching the ova. In the current study, it was found that Dandarawi ejaculates returned significantly higher VSL values than those of both Fayomi and Sharkasi which gives superiority to Dandarawi semen in regard to sperm mobility. Sperm mobility is a fundamental determinant of fertility in poultry (Forman et al., 1999; Bowling et al., 2003). Furthermore, spermatozoa of Dandarawi roosters showed significantly higher values of VCL compared to those of Fayomi and Sharkasi strains. Curvilinear velocity is considered by some a more realistic measure of sperm speed than VAP and VSL under in vitro conditions because of the absence of sperm guidance mechanisms such as thermotaxis and chemotaxis (Rowe et al., 2013). In addition, 31.55 % of Dandarawi spermatozoa had average path velocity > 50 µm/s which was double that of Fayomi and Sharkasi. We could not find any statistical differences in the percentages of motile sperms, pattern of motility and sperm velocity between Fayomi and Sharkasi ejaculates. We studied some sperm morphometric measures such as the length of the whole sperm, flagellum, and head plus midpiece in an attempt to justify the observed differences in sperm velocity between Dandarawi and the other two strains. The obtained results showed that Dandarawi roosters had longer spermatozoa with longer flagellum compared with those of Fayomi. Since the drag caused by the head is compensated by the thrust produced by the flagellum, sperm with longer flagella tend to have higher swimming velocity (Bennison et al., 2016). This may explain the higher swimming velocity of Dandarawi spermatozoa compared to those of Fayomi and agrees with the reports of (Gomendio and Roldan, 2008; Lüpold et al., 2009). On contrary, the lengths of the whole sperm and its flagella did not differ between Dandarawi and Sharkasi; while significant differences in sperm swimming velocity existed between the two strains. These differences in swimming velocity might be attributed to variations in the size of midpiece and consequently in the volume of mitochondria which is responsible of the intracellular ATP concentrations (Cardullo and Baltz, 1991). It is a pity we could not distinguish the junction between the head and the midpiece to explain the observed differences in sperm velocity. Nonetheless, it seems that the relationship between the size of midpiece, energy reserves and sperm swimming velocity is rather complex and differs among taxa. For example, a negative relationship between intracellular ATP concentrations and the length of midpiece was found in zebra finch's spermatozoa (Bennison et al., 2016). In passerine birds, Rowe et al. (2013) found that despite longer midpiece is associated with higher ATP concentrations; the increased energy reserves do not essentially give rise to increased swimming velocity. Although Sharkasi roosters had longer spermatozoa with longer flagellum compared to those of Fayomi, there were

no significant differences in sperm swimming velocity between the two strains. An interesting result is that Sharkasi ejaculates had significantly higher potassium concentrations in seminal plasma than those of Fayomi. Low potassium levels in seminal plasma were necessary for maintaining sperm motility; whereas, high concentrations were reported to have detrimental effects on sperm metabolism (Wales and White, 1958). Increasing potassium concentrations in bull semen inhibited both sperm oxygen uptake and glycolysis (Cragle and Salisbury, 1959). These deleterious effects of high potassium concentrations on sperm metabolism are also reflected on sperm motility. This may explain the absence of differences in sperm swimming velocity between Sharkasi and Fayomi in spite of that the former having longer spermatozoa with longer flagellum. It is known that sperm motility is empowered through calcium cycling which requires both calcium and sodium ions (Forman, 2007). However, there were no significant differences in calcium, sodium and magnesium concentrations in seminal plasma among different strains. Sodium concentrations were comparable to those reported by Hammond et al. (1965); while calcium concentrations were a little bit higher. The obtained results highlighted some differences that exist in sperm and seminal plasma among the studied chicken strains which may be responsible for the observed variations in sperm swimming velocity. These variations along with other factors can affect fertility rates. Therefore, more studies need to be conducted to investigate whether selection for longer spermatozoa in Fayoumi and altering potassium concentrations in extenders used in extending Sharkasi semen would benefit sperm velocity and fertility. Another approach is applying sperm competition experiments using the tested chicken strains.

CONCLUSION

Dandarawi roosters showed higher curve linear and straight line velocity and higher proportion of rapid spermatozoa when compared to Sharkasi and Fayomi. These differences can be attributed to dissimilarities in sperm size (length) and potassium concentration in seminal plasma.

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Table (1): Ejaculate volume, sperm concentration per ml, motility, swimming speed and sperm velocity in Dandarawi, Fayomi and Sharkasi chickens

	Dandarawi	**Fayomi**	**Sharkasi**	**P-value**
Ejaculate volume ml	0.54^{b}	0.46^{b}	1.04^{a}	0.011
Sperm Concentration $\times 10^{7}$/ml	322.92^{a}	285.21^{b}	289.58^{b}	0.007
Motility				
Immotile %	14.50	8.29	13.84	0.33
Motile %	85.50	91.71	86.16	0.33
Progressive sperm %	27.08	23.36	24.81	0.55
Non-progressive %	58.42	68.34	61.22	0.55
Swimming speed				
Slow %	9.81^{b}	15.49^{b}	24.56^{a}	0.001
Medium %	44.1^{b}	61.95^{a}	46.63^{b}	0.001
Rapid %	31.55^{a}	14.26^{b}	14.84^{b}	0.001
Sperm velocity & straightness				
VCL (µm/sec)	79.13^{a}	68.17^{b}	63.00^{c}	0.0001
VAP (µm/sec)	38.33^{a}	32.31^{b}	29.40^{c}	0.0001
VSL (µm/sec)	27.37^{a}	22.44^{b}	21.35^{b}	0.0001
STR	71	68	72	0.06

$^{a\text{-}b\text{-}c}$For main effects, means within a row without common superscripts differ significantly $p<0.05$.

Abbreviations: VCL, curvilinear velocity; VAP, average path velocity; VSL, straight line velocity; STR = VSL/VAP.

Table (2): Lengths of total sperm, head plus midpiece and flagellum in different chicken strains

	Fayoumi	**Dandarawi**	**Sharkasi**	**P-value**
Total Sperm Length (µm)	90.18±1.67 [b]	98.28±1.66 [a]	98.63±1.66 [a]	0.0003
Head + midpiece Length (µm)	22.20±0.68	23.31±0.68	23.1±0.68	0.466
Flagellum Length (µm)	67.98±1.65 [b]	74.97±1.65[a]	75.49±1.65 [a]	0.001
Proportion of the Head + midpiece (%)	24.61	23.71	23.42	0.333
Proportion of the Flagellum (%)	75.38	76.28	76.53	0.333
Head + midpiece : Flagellum Ratio	0.326±0.11	0.311±0.11	0.306±0.11	0.202

[a-b] For main effects, means within a row without common superscripts differ significantly $p<0.05$.

Table (3): Concentration of K^+, Ca^{++}, Na^+ and Mg^{++} in seminal plasma(mg/ 100 ml) of different chicken strains

	Fayoumi	**Dandarawi**	**Sharkasi**	**p-value**
Potassium (K^+)	37.0 ± 5.1 [b]	52.51± 5.1[ab]	61.89 ± 5.1 [a]	0.036
Calcium (Ca^{++})	10.81 ± 1.5	11.52 ± 1.5	14.42 ± 1.5	0.285
Magnesium (Mg^{++})	8.92 ± 2.2	8.24 ± 2.2	13.21 ± 2.2	0.302
Sodium (Na^+)	275.21± 17.0	269.19 ± 17.0	304.09 ± 16.97	0.362

[a-b] For main effects, means within a row without common superscripts differ significantly $p<0.05$.

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Indigenous strains - Sperm morphometry - Swimming velocity – CASA.

الملخص العربي

تحليل حركة و سرعة و قياسات مورفومترية للحيوانات المنوية في ثلاثة سلالات دجاج محلية مصرية

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تعتبر جودة الحيوان المنوي من المحددات الرئيسية التي تحدد قدرته الإخصابية. أجريت هذه الدراسة في محاولة لربط بعض القياسات المورفومترية (طول الحيوان المنوي و طول الذيل) و أيضاً تركيز بعض الأيونات في بلازما السائل المنوي من ناحية و بين سرعة سباحة الحيوانات المنوية من ناحية أخرى في ثلاثة سلالات دجاج محلية (دندراوي – شركسي – فيومي). تم إيواء عشرة ذكور بالغين (28 اسبوع) من كل سلالة في أقفاص فردية. تم جمع عينات السائل المنوي مرتين اسبوعياً لمدة 16 اسبوع. تم قياس بعض الخصائص الطبيعية و الكيميائية للسائل المنوي بما في ذلك حجم السائل المنوي، تركيز الحيوانات المنوية، الحركة، تركيز الكالسيوم، البوتاسيوم، الصوديوم و الماغنيسيوم في بلازما السائل المنوي. تم قياس السرعة المنعطفة و المستقيمة و متوسط سرعة المسار و مدى استقامة حركة الحيوانات المنوية بإستخدام برنامج لتحليل حركة الحيوانات المنوية بمساعدة الحاسوب (كاسا). تم أيضاً قياس طول الحيوان المنوي ككل، الرأس و القطعة الوسطى، الذيل (بالميكرومتر) بإستخدام برنامج لتحليل الصور. لم تكن هناك فروق معنوية بين السلالات في نسبة الحيوانات المنوية المتحركة ولا في تلك التي تظهر حركة تقدمية. احتوى السائل المنوي لذكور الدندراوي علي تركيز أكبر من الحيوانات المنوية في المللي و نسبة أكبر من الحيوانات المنوية سريعة السباحة و قيم أعلى لسرعة الحيوانات المنوية المنعطفة و المستقيمة بالمقارنة بذكور الشركسي و الفيومي. و لقد لوحظت اختلافات معنوية في أطوال الحيوان المنوي و الذيل بين السلالات المختلفة حيث كانت أطول في ذكور الدندراوي و الشركسي بالمقارنة بالفيومي. أيضاً تم ملاحظة احتواء بلازما السائل المنوي لذكور الشركسي علي تركيز أكبر من البوتاسيوم بالمقارنة بتلك المنسوبة لذكور الفيومي. من هذه النتائج يمكن إستنتاج أن السرعة البطيئة للحيوانات المنوية لكل من ذكور الفيومي و الشركسي بالمقارنة بذكور الدندراوي يمكن إرجاعها إلى قصر الحيوان المنوي و ذيله و أيضاً إلى إرتفاع تركيز البوتاسيوم في بلازما السائل المنوي على الترتيب.