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Testicular Biometry, Spermigram, and Biochemical Parameters in Male Goats

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THE STUDY is designed to monitor how both the age and body weight of clinically healthy male goats would be related to testicular biometry, semen attributes, and biochemical profiles. Thirty male goats (Crossbreed) aged between 2.5 and 4 years with body weight 41.2±0.83 kg (mean±SEM) were included. The morphometry of the testes and scrota of all males were measured and blood plasma samples were harvested for further analysis. The results showed that there was a strong positive correlation between the body weight of the male goats and testicular measurements; while there was a negative correlation between the body weight and scrotal length measurement. Moreover, positive correlations were found between the body weight and most of the semen parameters except semen pH, a negative correlation was found. Strong positive correlations between the age of the male goats and most of the testicular measurements were recorded. The semen volume and sperm motility were positively correlated with age, while the semen pH was negatively correlated. Moreover, semen volume, spermatozoa motility, viability, and concentration were positively correlated to testicular and scrotal measurements. In contrast, semen pH and primary abnormalities of the spermatozoa were negatively correlated with testicular and scrotal measurements. The levels of testosterone and glucose, globin, and total protein were found to be negatively correlated with spermatozoa abnormalities. In conclusion, besides the clinical findings, the scrotal and testicular measurements and biochemical analysis could be a good mirror for monitoring breeding soundness evaluation and early diagnosis of subfertile male goats

Keywords: Biochemical, Body weight**,** Male bucks, Spermigram, Testicular measurements.

Introduction

The use of low-fertile males in the herd causes a poor conception rate and prolongs the calving interval resulting in large economic losses. Because of that determination of male fertility is important to maintain the sustainability of herd productivity [1]. Variations in the male fertility rate could be addressed by the routine semen parameters evaluation such as in bulls [1], stallions [2], and rams [3, 4]. Breeding soundness evaluations (BSE) include physical examination [5], examination of the external and internal genitalia [6], assessment of libido and mating ability, examination for venereal diseases, as well as evaluation of spermatozoa

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concentration and other semen quality in parameters [2, 7, 8].

In case of male goats, the procedures used to predict fertility are that the male goat has normal libido and matting ability, normal internal and external genital organs, not suffering from any reproductive disorders, free of caprine arthritis-encephalitis virus and caseous lymphadenitis disease infections, and free from gastrointestinal parasitic infestations. [3]. Furthermore, there is no formal standard breeding soundness evaluation for prospective identification of fertile, sub-fertile, and infertile individuals [3, 9]. Usually, the criteria described for rams were used for the classification of a male goat as a satisfactory prospective breeder [3]. However, a morphological difference in male reproductive organs was found between the male goats and rams [10].

Breeding soundness evaluation in male goats plays a crucial role in goat flock fertility. It is a simple, precise, and dependable method of measuring the potential fertility of male goats based on testicular characteristics and biochemical analysis. To our knowledge, there are limited references (studied BSE) of male goats in Egypt. The current study aimed to study the andrological parameters in clinically healthy male goats and the possible relation between them to assess the BSE.

Material and methods

Ethical approval

This research protocol was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt. Approval number (VM/SVU/23(2)-13). Male goat husbandry and experimental procedures were performed under the guidelines for the care and use of experimental animals, Aswan University.

Clinical examination, Sample collection, and animal handling

Thirty male goats (crossbreed) between the ages of 2.5 and 6 years were selected at the Faculty of Veterinary Medicine Animal Farm, Aswan University, Aswan, Egypt. All animals in this study were subjected to precise clinical examination, the heart rate, respiratory rate, rumen motility, rectal temperature, body weight, and scrotal circumference were recorded before semen collection. Rectal temperature was measured using a handheld digital thermometer. Body weight was measured using a sensitive electronic scale with a range up to 100 kg. For each male goat, the scrotum was examined by palpation, and the length, diameter, and height of each testicle, as well as total scrotal circumference, width (cm), and length (cm), were measured. Testicular length, testicular diameter, and scrotal thickness were determined with a metal caliper precision of 0.01 mm [11]. The scrotal circumference, scrotum width, and scrotum length were measured by a tape measure [11, 12].

The blood samples were collected before semen collection. The hematological analysis was performed by an automated hematological analyzer (Veterinary hematology system, Exigo^{Tm}, Boule Medical AB, Sweden) [13]. Approximately 5 mL of whole blood was collected by using vacutainer heparinzed tubes. Plasma was separated from whole blood by centrifugation (1006 xg, 10 min) at room temperature and stored at -20 ° C until analysis. Plasma testosterone was determined by ELISA kit (Cod: 3725-300) with intra and inter-assay coefficients of 4.8% and 9.7% (Monobind, USA) using wavelength 450. About glucose (Cod: GL13 20, Biodiagnostic, Egypt) using a wavelength 510 nm (490-530), total protein (Cod; TP20 20, Biodiagnostic, Egypt) using a wavelength 550 nm (520-570), cholesterol (CHOD-PAP, Cod; 230001, Spectrum®, Egypt) using wavelength 546 nm, albumin (Cod; ALB100250, BIO-TEC Egypt) using a wavelength 580-630 nm, and triglyceride (Medium-Chain Triglycerides, Cod; M4200, Spectrum®, Egypt) using a wavelength 500-550 nm. The biochemical were measured by spectrophotometer (UV/VIS spectrometer- T80) [12].

Semen collection and analysis

Semen samples were collected using artificial vaginas special for male goats. Semen samples were grossly examined for the volume, color, and pH. Spermatozoa motility was estimated by placing one drop of semen and 4 drops of normal saline above a warm slide using a phase- contrast microscope at 400x magnification [8]. Concentration of spermatozoa was determined using a hemocytometer. Assessments of sperm abnormalities were made by placing a drop of fresh semen, two drops of Eosin stain, and 4 drops of nigrosin stain, above a warmed glass slide, we made thin films from the mixture and left till dry and the count of spermatozoa was recorded (alive without stain, dead with pink stained head) [10]. The morphology of 300 individual spermatozoa in each sample was assessed using 1000x magnification under a light microscope (Solo-E3, Prisma Tech-USA) (oil immersion). The percentages of life versus dead sperm as well as normal versus abnormal sperm were determined after counting at least 300 spermatozoa for each buck in different microscopic fields. Stained semen smears were examined and morphological abnormalities were classified into primary and secondary abnormalities [14]. Acrosome integrity was assessed using a semen smear stained with Giemsa as previously mentioned [15]. The diluted semen samples were fixed for 10 min in buffered formal saline and stained for 60 min in a 6% Giemsa stain. Acrosome integrity was evaluated by counting sperm

300/slide in different microscopic fields at 400X magnification. Sperm with swollen or missing acrosomes were considered damaged.

Statistical Analysis

The data were analyzed using Graph Prism (version 5) statistical software. The data were expressed as mean±SEM. Spearman's correlation coefficient (r) with a p-value was used to determine significant differences between variables. $p \leq 0.05$ was considered significant

Results

Measurements of body temperature, heart rate, respiratory rate, rumen motility, and hematological profile of male goats were summarized in Table 1. The average rectal temperature was 39.96±0.02, heart rate was 81.66±1.39, respiratory rate was 27.33±0.79, and rumen motility was 3.33±0.31. White blood cell count was 9.8±1.03 ×109/l, RBCs was 14.8±0.25 ×1012/l, Hg was 9.26±2.7 g/dl, PCV was 25.16±1.14%, MCV was17.03±0.64 fl, MCH was 6.06±0.14 pg, and MCHC was 36.88±0.99 g/dl.

TABLE 1. The clinical examination and hematological analysis of male goats

Parameters	Mean ±SEM
Body temperature ºC	39.96±0.02
Heart rate (beat/minute)	81.66±1.39
Respiratory rate (breath/minute)	27.33±0.79
Rumen motility	3.33±0.31
WBC ($\times 10^9$ / I)	9.8±1.03
RBCs ($\times 10^{12}$ /I)	14.8±0.25
Hg (g/dl)	9.26±0.27
PCV%	25.16±1.14
MCV (fl)	17.03±0.64
MCH (pg)	6.06±0.14
MCHC (g/dl)	36.88±0.99

WBCs: white blood cell count, RBCs: Red blood corpuscle count, PCV: packed cell volume, MCV: Mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration. Values within the normal range that were reported previously [33].

The relationship between the body weight and age with the testicular morphometric parameters was given in Table (2 a). Testicular measurements were taken from 30 male goats including scrotum circumference was 23.2±1.3 cm, scrotum width was 9.6±0.28 cm, scrotum length was 12.6±0.15 cm, scrotum thickness was 0.72±0.42 cm, scrotum height was 29.6±1.06 cm, testicular length was13.1±0.4 cm, and testicular diameter was 12.9±0.7 cm table was given in Table (2b). There were positive correlations between the body weight of the male goats and testicular characteristics; except for scrotal length measurement a negative correlation was found. There were strong positive correlations between the age of the male goats and testicular characteristics; except for scrotal length and scrotal height measurements no correlation was found.

TABLE (2 a). Pearson's correlation coefficients between body weight, age, and testicular characteristics of male goats

Parameters	Age	Body weight
Scrotal circumference Corr.	0.7*	0.9*
Scrotal width Corr.	0.7*	0.7*
Scrotal length Corr.	0.7*	-0.6*
Scrotal thickness Corr.	-0.4	0.6*
Scrotal height Corr.	0.5	0.7*
Testicular length Corr.	0.7*	0.8*
Testicular diameter Corr.	0.6*	0.7*

A correlation coefficient of 0.1- 0.3 was weak, 0.4 - 0.5 was moderate, and .6 - 1 was strong. * $P < 0.05$

TABLE (2 b). Means of age, body weight, and testicular measurements of male goats

Measurement	Age	BW	SC	SW	SL	ST	SH	TL	TD
Mean±SEM	3.8 ± 0.69	41.2± 0.83	23.2 ±1.3	9.6±0.28	12.6±0.15	0.72±0.36	29.6±1.06	13.1±0.4	12.9±0.7

BW: body weight (kg), SC: Scrotal circumference (cm), SW: scrotal width (cm), SL: Scrotal length (cm), SH: scrotal height (cm), TL: testicular length (cm), TD: testicular diameter (cm).

The correlation between age and body weight with semen parameters is listed in Table (3a). Semen variables were taken from 30 male goats including semen volume (1.15±0.12), semen PH (6.43±0.14), mass motility (2.85±0.69), sperm concentration (1749.5±7.54), individual motility (84±0.84), live sperm (82±0.67), and normal motility (90.6±0.32). The first sperm abnormalities were (2.7±0.29), 2nd abnormalities were (6.7±1.1), and the intact acrosome was (89.8±0.18) was given in Table (3b). In contrast to semen PH, which was negatively correlated with body weight, sperm volume, sperm concentration, live sperm, normal sperm, sperm motility, and intact acrosome all showed favorable correlations with body weight. Age was negatively correlated with semen PH but positively correlated with sperm volume and motility.

TABLE (3 a). Pearson's correlation coefficients between body weight, age, and semen variables of the male goat

Parameters	Age	BW
Semen Volume (ml)	0.3*	0.3*
Semen PH	-0.3*	-0.3*
Mass motility (%)	0.3*	0.3*
Spermatozoa concentration (10^6/ml)	0.1	0.3*
Individual Motility (%)	0.3*	0.3*
Live sperm (%)	0.2	0.3*
Normal sperm (%)	-0.02	0.1*
Primary abnormalities (%)	-0.1	-0.2
Secondary abnormalities (%)	0.05	-0.1
Intact Acrosome (%)	0.2	0.3*

A correlation coefficient of 0.1- 0.3 was weak, 0.4 - 0.5 was moderate, and .6 - 1 was strong. * $P < 0.05$.

TABLE (3 b). Means of semen parameters of male goats

Measurements	Mean±SEM
Semen Volume (ml)	1.15±0.12
Semen PH	6.43±0.14
Mass motility (%)	2.85±0.69
Spermatozoa concentration (10^6/ml)	1749.5±7.54
Individual Motility (%)	84±0.84
Live sperm (%)	82±0.67
Normal sperm (%)	90.6±0.32
Primary abnormalities (%)	2.7±0.29
Secondary abnormalities (%)	6.7±1.1
Intact Acrosome (%)	89.8±0.18

Values are expressed as mean±SEM,

The correlation between testicular characteristics and semen characteristics was given in Table (4). Sperm volume, concentration, live sperm percentage, motility, and intact acrosome were all favorably connected with the scrotal circumference; semen PH was inversely correlated with the scrotal circumference. The scrotal width was inversely connected with semen PH and favorably correlated

with both individual and mass motility. None of the semen characteristics were linked with the scrotal length, thickness, or height. Testicular length and diameter were inversely connected with sperm abnormalities and semen PH but positively correlated with sperm volume, concentration, living sperm, normal sperm, and individual and mass motility.

TABLE 4. Pearson's correlation coefficients between variables from fresh semen and testicular biometry of the male goat

Parameters	SC	SW	SL	ST	SH	TL	TD
Semen volume (ml)	0.3*	0.2	0.1	-0.1	0.2	0.4*	0.4*
Semen PH	-0.3*	-0.3*	-0.1	0.1	-0.2	-0.3*	-0.3*
Mass motility (%)	0.2	0.3*	0.1	-0.1	0.2	0.2	0.3*
Spermatozoa concentration (10^6/ml)	0.3*	0.1	0.1	-0.1	0.2	0.4*	0.4*
Individual motility (%)	0.3*	0.3*	0.1	-0.2	0.2	0.3*	0.3*
Live sperm (%)	0.3*	0.2	0.1	-0.1	0.2	0.4*	0.4*
Normal sperm (%)	0.2	-0.1	0.02	-0.03	0.1	0.3	0.2
Primary abnormalities (%)	-0.3*	-0.05	-0.08	0.1	-0.2	-0.4*	-0.3*
Secondary abnormalities (%)	-0.2	0.1	-0.01	0.01	-0.1	-0.3	-0.2
Intact Acrosome (%)	0.3*	0.2	0.1	-0.1	0.2	0.4*	0.4*

SC: Scrotal circumference (cm), SW: scrotal width (cm), SL: Scrotal length (cm), SH: scrotal height (cm) TL: testicular length (cm), TD: testicular diameter (cm), A correlation coefficient 0.1- 0.3 was weak, 0.4 - 0.5 was moderate, and .6 - 1 was strong. * $P < 0.05$.

The relationships between levels of glucose, globulin, total protein, cholesterol, triglyceride, and testosterone with semen parameters were summarized in Table (5a). The average glucose level was 52.5±0.1 mg/dL, globulin was 2.2±0.06 mg/dL, albumin was 4.5±0.04 mg/dL, total protein was 6.8±0.05 mg/dL, triglyceride was 51.8±0.5 mg/dL, testosterone was 5.4±0.02 ng/mL, and cholesterol was 82.8±0.07 mg/dL was given in Table (5b). The morphology of sperm was found to be associated with glucose, globulin, total protein, cholesterol, triglyceride, and testosterone levels. Abnormalities were shown to be inversely associated with glucose, testosterone, globin, and total protein.

TABLE (5 a). Pearson's correlation coefficients between blood biochemical parameters and semen variables

Parameters	Testosterone	Glucose	Albumin	Globulin	Total protein	Cholesterol	Triglyceride
Volume	0.14	0.16	0.01	0.03	0.01	0.17	-0.08
pH	-0.08	-0.11	0.05	0.02	0.06	-0.16	0.07
Mass motility	-0.01	0.03	-0.11	-0.1	-0.15	0.14	-0.05
Spermatozoa concentration	0.25	0.26	0.1	0.17	0.16	0.17	-0.09
Individual motility	0.04	0.08	-0.08	-0.05	-0.09	0.15	-0.06
Live Sperm	0.2	0.22	0.05	0.1	0.09	0.17	-0.09
Normal Morphology	0.31*	0.29*	0.2	0.28*	0.31*	0.1	-0.07
Primary abnormalities	-0.28*	-0.28*	-0.14	-0.21	-0.22	-0.15	0.08
Secondary abnormalities	-0.31*	-0.29*	-0.21	-0.29*	-0.31*	-0.1	0.06
Intact Acrosome	0.18	0.2	0.03	0.08	0.06	0.17	-0.08

A correlation coefficient of 0.1- 0.3 was weak, 0.4 - 0.5 was moderate, and .6 - 1 was strong. * $P < 0.05$.

TABLE (5 b). Means of biochemical analysis of male goats

Measurement	Testosterone (ng/ml)	Glucose (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	Total protein (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Mean±SEM	5.4±0.02	52.5±0.1	4.5±0.04	2.2±0.06	6.8±0.05	82.8±0.07	51.8±0.5

Values are expressed as mean±SEM

Discussion

Breeding soundness evaluation is considered a simple and low-cost tool for decreasing the risk of using subfertile bucks. There is no standardized procedure or protocol for assessing the quality of male goat sperm. The results revealed a positive correlation between testicular weight, testicular volume, and scrotal circumference this agrees with the previous studies in goats [16, 17]. However, body weight and scrotal length in the current study had a negative correlation. Moreover, the link between body weight and scrotal length is positive [16, 17].

It has been reported that male goats aged (19-30 months) have higher performance than younger ages in terms of normal sperm motility and semen volume [18] because of that the minimum age used in this study was 30 months. The results revealed a strong positive relationship between age and testicular measurements such as scrotal circumference, scrotal width, scrotal length, testicular diameter, and testicular length. Other authors have previously documented a positive relationship between age and testicular measurements [16, 17, 19]. Furthermore, semen volume and motility of spermatozoa were found to be positively associated with age. This could be caused by considering that adults produce more testosterone hormones than young animals. More testosterone hormone available for use in spermatogenesis as a result of matured reproductive organs and sex glands will result in higher quality sperm than that produced by young animals [18].

The normal sperm morphology was 90.6%±0.32 which agrees with recent research that found male goat sperm morphology to be 92.83%±6.18% [20] and 71.66%±2.02% [21]. Furthermore, body weight was positively connected with sperm volume, concentration, live sperm, normal sperm, and motility, but negatively correlated with sperm pH.

The current study also found correlations between testicular biometric factors and sperm characteristics. Individual sperm motility, percentage of live sperm, sperm concentration, and intact acrosome were all positively linked with scrotal circumference. This finding was consistent with previous findings that there is a considerable negative connection between scrotal circumference and sperm DNA fragmentation in Brahman bulls [22]. Another study on bulls found a high correlation between sperm shape and fertility [23, 24]. Moreover, in bulls, scrotal circumference is connected to testis growth [8] and good semen quality [1].

In the present study, testicular length and width were positively correlated with the percentage of normal sperm and this could be attributed to that the elongation of the testes may be related to greater thermoregulation which could result in more desirable sperm morphology as proposed by [8]. Testicular length and width were also adversely connected with sperm abnormalities this agrees with previous research in bulls [25]. Sperm production has been linked to testicular size [8].

Testosterone regulates male sexual development and the reproductive system since it is responsible for sperm generation, masculine features development, secretory and cytologic activation in the prepuce, and muscular mass. [26].The data revealed that sperm morphology was positively associated with testosterone levels. Low testosterone levels, for example, may result in small and soft testicles, as well as a decrease in the volume and function of the prostate, resulting in decreased reproductive capacity [27, 28]. Additionally, there was a bad correlation between sperm DNA fragmentation and testosterone levels [22]. However, the plasma testosterone concentration did not have a strong correlation with the morphology of sperm [28].

Furthermore, the findings demonstrated a negative relationship between plasma testosterone content and sperm abnormalities. This is consistent with prior research that found substantial associations between testosterone levels and sperm abnormalities in Osmanabadi bucks [29]. These findings support prior research in bulls that found a link between an increase in sperm abnormalities and a decrease in testosterone concentration [26, 30]. The results

revealed that plasma testosterone concentration did not correlate with sperm motility and concentrations which is consistent with a prior study in crossbred Bulls [26]. Additionally, there was a positive correlation between sperm morphology and blood sugar, globulin, total protein, cholesterol, and triglyceride levels. Glucose, globin, and total protein were all adversely linked with abnormalities. Also, results showed that rectal temperature, rumen motility, and respiration rate were slightly greater than findings in prior research [31, 32] and these changes could be attributed to variances in breeds, regions, and ages.

Conclusion

These findings suggested that selecting clinically healthy male goats based on regular and thorough diagnostic testing on body weight, age, scrotal circumference, scrotal width, testicular length and width, and testosterone level would increase the reproductive performance of the herd

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Conflicts of interest

The authors declare that there is no conflict of interest.

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القياسات الحيوية للخصية، وسمات السائل المنوي، والقياسات البيوكيميائية في ذكور الماعز

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تم تصميم الدراسة الحالية لرصد كيفية ارتباط القياسات الحيوية للخصية مع عمر ووزن جسم ذكور الماعز الأصحاء سريريًا بالقياسات الحيوية للخصية، وسمات السائل المنوي، والمستوي البيوكيميائي لبعض القياسات فى ثلاثين ذكر من الماعز تتراوح أعمارهم بين 2.5 عام و 6 سنوات و كان متوسط وزن الجسم 41.2 ± 0.83 كجم. تم قياس ابعاد الخصيتين والصفن لجميع الذكور وتم جمع عينات من بلازما الدم للتحليل الهرموني والبيوكيميائي. أظهرت النتائج وجود علاقة إيجابية قوية بين وزن الجسم لذكور الماعز وقياسات الخصية؛ بينما كانت هناك علاقة سلبية بين وزن الجسم وقياس طول كيس الصفن. علاوة على ذلك، وجدت علاقة إيجابية بين وزن الجسم ومعظم مؤشرات السائل المنوي باستثناء الرقم الهيدروجيني للسائل المنوي، حيث وجدت علاقة سلبية. تم تسجيل ارتباطات إيجابية قوية بين عمر ذكور الماعز ومعظم قياسات الخصية. كان حجم السائل المنوي وحركة الحيوانات المنوية مرتبطين بشكل إيجابي مع العمر، في حين كان الرقم الهيدروجيني للسائل المنوي مرتبطا سلبا. علاوة على ذلك، فإن حجم السائل المنوي وحركة الحيوانات المنوية وحيويتها وتركيزها ارتبطت بشكل إيجابي بقياسات الخصية والصفن. في المقابل، ارتبطت درجة حموضة السائل المنوي والتشوهات الأولية للحيوانات المنوية بشكل سلبي مع قياسات الخصية والصفن. وجد أن مستويات هرمون التستوستيرون والجلوكوز والجلوبين والبروتين الكلي ترتبط بشكل سلبي مع تشوهات الحيوانات المنوية. في الختام، إلى جانب النتائج السريرية، يمكن أن تكون قياسات الصفن والخصية والتحليل الكيميائي الحيوي مرآة جيدة لمراقبة تقييم سلامة التربية والتشخيص المبكر لذكور الماعز قليلة الخصوبة.

الكلمات الدالة: الكيمياء الحيوية، وزن الجسم، ذكور الذكور، مخطط الحيوانات المنوية، قياسات الخصية.