Effect of age and season on the Epididymal Sperm and testosterone level in camel (*camelus dromedarius*)

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Summary

The aim of this study was to evaluate the effect of age and season on the epididymal sperm and level of testosterone in camel. A total 103 camel testes samples were collected from Al-Najaf slaughter house during a cold season (December 2012, January and February 2013) and moderate-hot season (March, April and May, 2013). Testes were divided into 3 Gs according to camel age, G1 included the testes of 2-3years, G 2 (3 – 4years) and over 4 years (G 3). Blood samples were collected for determination of serum testosterone level**.** The sperms were obtained from the tail of epididymis from all animals groups and the results of the sperms individual motility percentage was increased at the level of (P< 0.05) significantly with age progress in both seasons. Also, sperm motility of G3 recorded a significantly higher than those of G1 and G2 in cold and moderate-hot seasons .The live percentage of G 3 animals was 90.01% in cold season with a significantly higher than those of other Gs and in both seasons. However, the live sperm percentage of G 3 during moderate-hot season was 87.82% and G 2 during moderate-hot and cold seasons were 88.58 and 88.72% respectively, showed significantly higher than those of G1 during cold and moderate-hot seasons .The concentration of sperm obtained from epididymis tail of bulls camel significantly increase with age progress in both seasons. However, the mean of the sperm concentration in cold season showed significance higher than those in moderate-hot season in animals of G 1 and 3, respectively. The abnormal morphologically sperm percentage of animals G 1 in both cold and moderate-hot seasons were significantly higher than those of G3.The testosterone levels of the young animals (under 4 years) increased gradually and reached its peak in February 2.28 ng/ml and March 2.27ng/ml. In the same trend older animal (more than 4 years) was showed 8.14 and 7.35 ng/ml, respectively. The older animals showed a significantly monthly, higher values than those of the younger animals in their testosterone level started from January up to May. In conclusions during cold months the camel over 4 years shows higher percentage of epididymal sperms parameters (live and individual motility) and sperms concentration as well as serum testosterone level.

Keywords: Camel, Season, Age, Sperm, Testosterone.

Introduction

Camels play a vital socio-economic role and support millions of human beings in the dry and arid zones of Asia and Africa (1). Camels are known to be seasonal breeders, and their reproductive efficiency under natural pastoral condition is low (2). The age is an important aspect in considering the potential fertility of a camel. Young and old dromedary bulls may have problems with tacking on a full breeding labor with consistent success rates (3). Based on the testicular morphometry and rutting behavior, a young bull may be sexually active and used for service as early as 3 years of age as reported in Saudi Arabia (4), Egypt (5) and India (6). By 4.5-5 years of age the males are capable of producing adequate number of spermatozoa to mate as many females as an adult bull but full fertilizing capacity is not attained until 6 years of age on average (7). Conversely, old age may be a problem due to an age related decline in fertility over 18 years of age (8). The fertility rate in camels is extremely low (50%) when compared to other domestic animals (9).

The lack of consistency information on fertility of Iraqi dromedary camel highlighted the need for studying. The present study aimed to determine the effects of age and seasons of the year on the tail epididymis sperm traits and serum testosterone concentration in dromedary camels.

Materials and Methods

The present study was conduct on 103 healthy camel testes collected from Al-Najaf abattoir, from December 2012 to May 2013. The cold season December 2012, January and February 2013, while the moderate-hot season March, April and May, 2013. General examination of males was performed before slaughtering. Testes samples were taken from the animals immediately after slaughter, the samples divided depended on age into three Gs, (G1 (2-3years), G2 (3-4years) and G3 (over 4years). All testes samples used were grossly normal and macroscopically free from pathological lesions. Before slaughtering blood samples taken from male animals and classified depended on age into two Gs, G1 (2-3years), consider as prepuberty and G2 (over 4years), as mature camel.

Spermatozoa were collected from the tail of the left epididymis by slicing and rinsing-pressing technique (rinse-press) each pieces of tail tissue in 5 ml of the physiological saline at 37 ^{0}C in a small glass dish and sperms were immediately evaluated (10).

Sperm motility percentage was assessed according to the method reported (11). The method of Chemineau (12) was used to evaluate the abnormal sperm morphology. Live and dead sperms were carried out according to (13).

Sperms count was done according to (10) those sperm count in the cauda of the left epididymis by taking 1 g from the cauda and let in the blender in 30 ml of normal saline for 2 minutes to homogenize the contents then 0.1 ml was taken from sperm suspension by pipetting and diluted by adding 19.9 ml of normal saline. One drop of this solution and let in Hemocytometer (Neubauer Type) to calculate sperms according to (14). When finished sperms count is by using formula (Sperm conc/g=sperm conc/weight of epididymis) for deducing sperm per-gram of epididymis according to (15).

$$\text{Sperm conc/g} = \frac{\text{sperm conc/ ml}}{\text{weight of epididymis}}$$

Blood samples (5ml) were collected from Jugular vein before slaughtering by using 10 mls syringe and 18 gage needle. After that serum was separate by centrifugation at 3000(rmpXg) for 15 minutes, serum samples was collected and stored at-20°C until analysis. The testosterone hormonal level was analysis by using of Gamma counter .

Data were analyzed statistically by a complete randomized design in two-ways ANOVA. G differences were determined using Duncan Test at (P<0.05) (16 and 17). In the statistical analyzed, SPSS (18) program was used. G differences were determined by using the least significant difference (LSD) test at P<0.05 (18).

Results and Discussion

The sperms individual motility percentages were increased with age progress in both seasons. Animals of G3 showed 75.83% in cold season and 73.0% in moderate-hot seasons which was significantly (p<0.05) higher than those of G1 and G2. Also, animals of G2 had a significantly (p<0.05) higher sperm individual motility than those of G1 in both seasons (Table, 1).

The individual motility percentage of this study was higher than those found by (19). But similar to the results revealed by (20). The sperm motility percentages were 50.5 % and 51% when semen was collected by artificial vagina (21 and 22), respectively. The sperms individual motility percentage was 50% and 68 % as recorded by (21 and 22) respectively, when semen was collected by electro-ejaculator.

The live sperm percentage of G3 animals was 90.00 % in cold season significantly (p< 0.05) higher than those of G1 and G2 in both seasons. However, the live sperm percentage of G3 (87.82%) and G2 (88.58%) of moderate-hot season .While animals of G2 during cold season showed a significant (p< 0.05) higher than those of G1 in both seasons (table, 1).

The live sperm percentage of this study (table, 1) during both seasons was higher than those reported by (21, 23 and 24) by using electro-ejaculation or artificial vagina. Present results were similar to the live sperm percentage obtained from cauda epididymis during breeding and non-breeding seasons (25). No differences in the live sperm percentage between different seasons, but the older camel showed significantly P<0.05

higher values than in the percentage of younger animals (26).

The sperm concentration obtained from epididymis tail of bulls camel (table, 1) was significantly ($p<0.05$) increased with age progress in both seasons. However, the sperm concentration of cold season showed a significant difference higher than those of moderate-hot season in each age G. Animals of G3 exhibited significantly ($p<0.05$) higher than those of G1 and G2 in both seasons. While animals of G2 showed a significantly ($p<0.05$) higher sperm concentration than those of G1 in both seasons (table, 1).

The sperm concentrations in this study were higher than those found by (19 and 20) whom recorded the highest values at age 5-10 years and the lowest values at age 2.5-5 years. But Chenoweth *et al.*, *(*27) showed that sperm cell concentration was 5.7 $X10^8$ /ml during breeding season and 4.7 $X10^8$ /ml during the non-breeding season, which were higher than the values recorded in this study. The differences between the seasons and ages could be attributed to the size and weight of the testis and the level of testosterone in different age and seasons, as well as nutrition and individual variation.

The total sperms abnormalities percentage recorded in present work was recorded in (Table, 1). Ten different sperm abnormalities was taken in consideration, sperms of G1 showes a significant higher abnormalities when compared to that found in animals of G2 and G3 in both season and age.

In dromedary camel the abnormal sperm was 22% using electro-ejaculation and 19% when using artificial vagina (28). While in Dromedary and Bactrin camel when semen was collected by artificial vagina were 28 and 24%, respectively (22). These findings were lower to that reported by present results (table, 1) this could be attributed to the age of animals and seasons, present results agreed with suggestion that the younger animals produce high percentage of abnormal sperms as a result of immature animals (23).

Table, 1: Effect of age and season on the characteristics of caudal epididymis sperms camel (Mean ±S.E).

Age and season	No. of animals	Individual Motility %	Live sperm %	Concentration $X10^6$/gm	Abnormal %
2-2.5years/ cold	33	61.82±0.81 d	84.09±1.16 c	139.33±12.87 e	44.61±1.39 a
3 years/ cold	12	70.00±1.74 bc	88.72±1.24 b	237.42±8.89 c	40.96±1.75 ab
4 years and over/ cold	12	75.83±1.49 a	90.01±0.78 a	360.00±17.82 a	37.33±1.70 b
2-2.5years/ hot	28	61.07±0.79 d	83.37±1.04 c	95.18±5.61 f	44.79±1.40 a
3years/ hot	8	67.50±1.64 c	88.58±1.16 b	197.38±12.38 d	39.63±1.32 ab
4years and over/ hot	10	73.00±3.35 ab	87.82±1.07 b	281.90±20.40 b	36.80±4.47 b
L.S.D		4.5	1.00	37.2	5.6

Different letters showed significant differences among different means ($p<0.05$) (L.S.D)

The same trend was shown for more than 4years, serum testosterone levels showed significantly higher values than those of the younger animals during the period of this study (Table, 2).

In present study the older animals showed higher serum testosterone values than those found by (29) who reported that the testosterone concentration was 4.8±0.7ng/ml while 5.5 ng/ml during breeding season was reported (29 and 30) . The testosterone hormone level during non-breeding season ranged between 1.3±1.1 to 3.8±1.8ng/ml as reported by (31-33) which, could be one of the main reasons responsible for failure of male to copulate and fertilize a female during non-breeding season. The same trend of testosterone values (table, 2) was also, conducted by (32 and 33). The corresponding with these changes, there were also changes in activity of poll glands (31 and 34). The significant increase in serum testosterone concentration of dromedary camel during the breeding season may be attributed to an increase in the volume and number of leydig cells during rutting period (35) also weight of testes (19 and 20) and the number of spermatozoa in the epididymis (36).

Table, 2: Effect of age and season on the testosterone level (ng/ml) in blood of bull camel (Mean ±S.E).

Different letters showed significant differences among different means (p<0.05) (L.S.D 1.2).

Age	No. of animals	Month					
		December	January	February	March	April	May
2-3years	4	0.53±0.10 e	1.05±0.14 e	2.28±0.36 de	2.27±0.33 de	0.88±0.23 e	0.61±0.17 e
4 years and over	4	1.31±0.17 e	4.81±0.95 cd	8.14±0.73 a	7.35±0.55 ab	7.00±0.71 ab	6.48±0.46 bc

In conclusions during cold months the Iraqi mature camel over 4 years shows higher percentage of epididymal sperms parameters live and individual motility and sperms concentration as well as serum testosterone level.

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تأثير العمر والموسم على نطف البربخ ومستوى الهرمون الخصوي في الجمال

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الخلاصة

لقد كان الهدف من الدراسة الحالية هو تحديد تأثير العمر ومواسم السنة المختلفة على صفات النطف في ذيل البربخ وتركيز الهرمون الخصوي في مصل الدم للجمال الوحيدة السنام. جمعت 103 نماذج من خصى الجمال من مجزرة النجف خلال الموسم البارد (كانون الأول 2012, كانون الثاني وشباط 2013) وخلال الموسم المعتدل الحار (آذار,نيسان,أيار 2013) ثم قسمت الخصى إلى ثلاث مجاميع اعتماداً على عمر الجمال: المجموعة الأولى (2-3 سنوات) , المجموعة الثانية (اكثرمن 3-4 سنوات) والمجموعة الثالثة (4 سنوات فما فوق). تم جمع نماذج الدم لتحديد مستوى هرمون التيستيرون في مصل الدم. كذلك تم جمع النطف من ذيل البربخ من جميع حيوانات التجربة واظهرت النتائج زيادة معنوية في الحركة الفردية للنطف عند مستوى ($p<0.05$) مع تقدم العمر وفي كلا الموسمين. كما سجلت المجموعة الثالثة زيادة معنوية للحركة الفردية للنطف ($p<0.05$) اكثر من المجموعة الأولى والمجموعة الثانية في الموسم البارد والمعتدل الحار على التوالي. كما ازدادت نسبة النطف الحية في المجموعة الثالثة بصورة معنوية ($p<0.05$) وكانت 90,01 % في الموسم البارد أكثر من باقي المجاميع وفي كلا الموسمين. بينما أظهرت نسبة النطف الحية في المجموعة الثالثة خلال الموسم المعتدل الحار 87,82 % , والمجموعة الثانية خلال الموسم المعتدل الحار والموسم البارد 88,58% و 88,78 % على التوالي وبنسب معنوية ($p<0.05$) اعلى من المجموعة الاولى خلال الموسم البارد والمعتدل الحار 83,37 % و 84,09 على التوالي.ان تركيز النطف المأخوذة من ذيل بربخ الجمل اظهرت زيادة معنوية مع تقدم العمر بكلا الموسمين. وقد اظهر تركيز النطف خلال الموسم البارد 139,33، 237.42 و 360.00 % فرق معنوي ($p<0.05$) اعلى من النسب المشاهدة في الموسم المعتدل الحار (95.18 و 197.38 و 281.90 x 10^6 /غرام) لاعمار المجموعة الاولى والثالثة على التوالي. وكانت نسبة النطف المشوهة في حيوانات المجموعة الاولى في كلا الموسمين البارد والحار المعتدل اعلى معنوياً($p<0.O5$) من المجموعة الثالثة. ان مستوى الهرمون الخصوي في الحيوانات الصغيرة العمر (اقل من 4 اعوام) ازداد تدريجياً ووصل ذروته في شباط واذار وانخفض بعدها. وقد تبعت الحيوانات الاكبر عمراً (اكثر من 4 اعوام) نفس النمط من التغيير لمستوى الهرمون الخصوي ولكنها اظهرت شهرياً مستويات اعلى معنوياً من الحيوانات الصغيره بدءاً من كانون الاول وحتى ايار. ويمكن ان نتستنتج من هذه الدراسة ان الجمال فوق 4 سنوات تمتاز بنسب عالية للنطف بالبربخ ونسب عالية للنطف الحية والحركة الفردية بالاضافة الى تركيز النطف مع ارتفاع مستوى الهرمون الخصوي.

الكلمات المفتاحية : تأثير العمر ، هرمون الخصوي ، الجمال.