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H. Elsharnoby
Embryology, Zoology and Entomology Department, Faculty of Science, Al-Azhar University (Girls)., ha.elsharnoby@gmail.com

O. M. Kandil
Embryo Biotechnology, Department of Animal Reproduction & Artificial Insemination, Veterinary Research Division, National Research Centre, omaima_mk@yahoo.com

H. A. Abu-Elnaga
Embryology, Zoology and Entomology Department, Faculty of Science, Al-Azhar University (Girls)., nehal.61@hotmail.com

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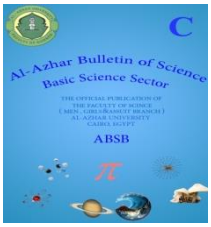

DROMEDARY CAMEL EPIDIDYMAL SPERM CHARACTERISTICS AT BREEDING AND NON-BREEDING SEASONS

Elsharnoby, H. A[1]., Kandil, O. M. ,[2*] Abu-Elnaga, N. A.[1]

[1]*Embryology, Zoology and Entomology Department, Faculty of Science, Al-Azhar University (Girls).*
[2*]*Embryo Biotechnology, Department of Animal Reproduction & Artificial Insemination, Veterinary Research Division, National Research Centre (NRC), Embryo And Genetic Resources Conservation Bank, NRC.*
*Corresponding Author Omaima Mohamed Kandil: Omaima_mk@yahoo.com
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ABSTRACT

Testicles from 30 clinically healthy male mature dromedary camels were gathered from the El-Basatin slaughterhouse all the breeding season (Nov. 2018 to Apr. 2019) and the nonbreeding season (May 2019 to Oct. 2019). Within one year the epididymal sperm were obtained twice per month. This manuscript aimed to investigate the influence of seasonality on dromedary camel epididymal sperm characteristics (motility, viability, sperm cell concentration, morphology and membrane integrity). The results depicted that during the breeding period, the rate of motility, concentration, live spermatozoa, normal morphology and the intact membrane of dromedary camel epididymal sperm showed a high significant increase ($P<0.05$) when compared to the nonbreeding period. While rate of death, abnormalities and membrane damage of the dromedary camel epididymal sperm were highly increased ($P <0.05$) during non-breeding when compared to the breeding period. In conclusion, the dromedary epididymal camel's sperm characteristics, viability and quality improved during breeding season.

Keywords: Dromedary camel; epididymal sperm; morphology; viability; season.

1. Introduction

Throughout history, dromedary camels have been used in many works that serve humanity for instance, transportation, producing meat and milk. Recently, it has been commonly used for entertainment in the Middle East. Additionally, it has a commercial significance role[1,2]**.** The dromedary camel is considered seasonal breeders. .Many variations of seasonal semen quality have been reported in many males of several domestic species, like bulls[3,4]**,**stallions [5], bucks [6] and rams [7]**.** Seasonality maintain the productivity in most species, to make sure the breed is born in optimal conditions for survival [8]**.** The Seasonality of camels varies geographically; there are many factors that effect on the seasonal patterns of reproduction such as temperature and climate change and the food quality. Changing in photoperiod length is the main climate factor which affects the seasonal physiological and biochemical changes [9]**.**The secretion of the neuro-hormone and melatonin from the pineal gland stimulated by decreasing the day length. Melatonin functions to regulate the excretion of gonadotropin-releasing hormone (GnRH) from the hypothalamic reproductive axis and the release of reproductive androgens from the gonads [10]. The epididymis is the most important organ for completing sperm maturation and generating spermatozoa that have the ability to fertilize the oocyte. The epididymis increases the chances of male gametes surviving as well as preserving and storing them before ejaculation. This organ facilitates a microenvironment as well as an appropriate pH, ion and solute concentrations for the development of spermatozoa which are regulated by androgenic hormones [11]**.** The use of epididymal spermatozoa from slaughtered or recently died animals may be

alternative for ejaculated semen and increase the opportunities to improve camel productivity for the conservation of camel genetic resources [12]**.** Our motivation was studying the influence of breeding and non-breeding periods on the qualities of epididymal semen of the male dromedaries'.

2. Materials and methods

2.1. Testes collection and transportation:

Testicles from mature dromedary camels were collected at El-Basatin slaughterhouse (According to availability of males in day of collection) over the course of the breeding season from the onset of November to the end of April, also the non-breeding season from the onset of May to the end of October. Both testicles were isolated on average 1-2 hr for each animal[13], immediately after slaughter, the sample is transported in ice inside a thermal container to the laboratory.

2.2. Method of sperm recovery from the epididymis

Approximately 180 min after collection, testes and epididymis are isolated from the scrotal sac and then the caudal epididymis were separated from the testes and from the surrounding connective tissue[14]**.** Then washed three times by saline and eventually by ethyl alcohol (70%). Epididymal sperm were collected using the retrograde flushing technique as performed by **Zarazaga *et al.*** [15]**.** Epididymis (Tail) and vas deference were dissected till become straight, injected (insulin syringe) with Sperm -Tyrode's Albumin Lactate Pyruvate (SP-TALP) supplemented with heparin (10 µg/mL)) in the vas deferens to collect spermatozoa from the tail of epididymis in Eppendorf **(Figs 1,2).**

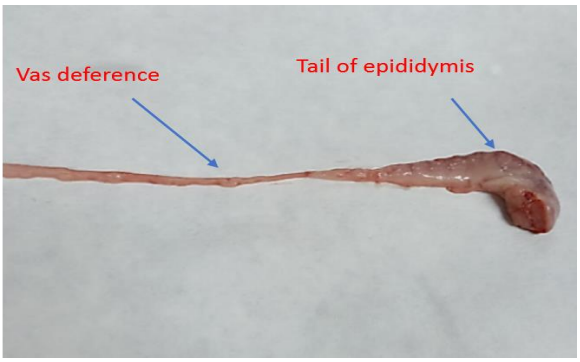

Fig. 1. Tail of epididymis and vas deference of the Dromedary camel

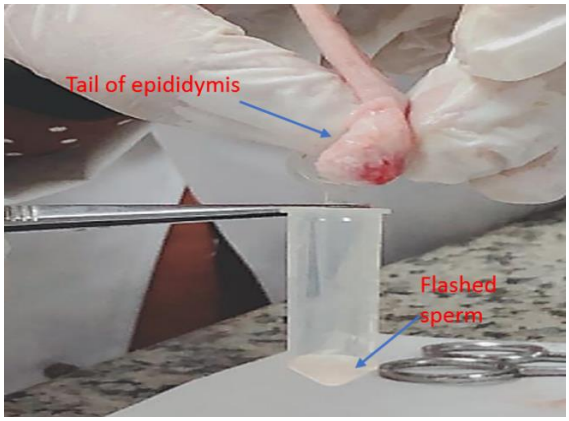

Fig. 2. Flushing of Dromedary camel epididymal sperm

Experimental design: the collected semen was employed to estimate characteristics of camel semen at breeding and non-breeding periods.

Percentage of sperm motility (%): due to the viscous materials of the camel semen, the motility (%) of the camel spermatozoa was recognized as an oscillatory motion not progressive. To evaluate sperm motility, one drop of the camel epididymal sperm diluted by physiological saline (0.9% NaCl) was added on a dry clean glass slide prepared in pre-warmed (37°C). In addition, it was determined under an inverted microscope (400x) by record the percentage of the forward and normal vigorous swimming motion of epididymal spermatozoa via the field of vision[16].

2.3. Evaluation of sperm live/ dead ratio and sperm-cell concentration

All semen samples were evaluated immediately for live /dead count and sperm cell concentration ($\times 10^7$/ml) in breading and non-breading season using automated cell counter (Thermo Fisher) **(Fig. 3)**using trypan blue dye [17]**.** The epididymal sperm was suspended in 1ml SP-TALP for assessment of epididymal sperm concentration and the percent of live and dead sperm using trypan blue stain and automated cell counter. By mixing well 10 µL of epididymal sperm to 10 µL of 0.4% trypan blue dye. Then loading 10 µL of the mixed specimen to each chamber of the slide.Let the specimen stabilized for 30 seconds. Insert the glass slide into the cell counter. The instrument will automatically focus and set bright field illumination intensity. The device took the

image and presented the results (sperm cell concentration, live and dead cell percentage).

2.4. Evaluation of sperm abnormality (morphological analysis)

Sperm abnormalities in breading and non-breading season were determined by morphological analysis Using triple stain according to [18]. Smears from each semen sample during breading and non-breading season were made after ½ hr from incubation in an atmosphere of 5% CO2 incubator at 38.5 ºC, using (swim up technique). After a preliminary fixation of the slide in ethanol for 10 minutes and dyed by the method of the differential dyeing with the following mechanism: 0.5% aqueous solution of eosin for 6 minutes, the saturated aqueous solution of kongorot for 5 minutes, and 0.5% aqueous solution of gentian violet for 3-5 seconds. After washing the slide many times and drying it, each smear was evaluated by counting 200 spermatozoa under the inverted microscope (Olympus, objective linse100×) and counting the abnormal spermatozoa head (swallow and separated), abnormal tails (multiple, broken, coiled, absent, bent) **(Fig.4).** The percent of sperm abnormality was determined by the equation = (Number of sperm counted / 200) x 100.

Evaluation of sperm membrane integrity: after ½ hr from incubation the membrane integrity was determined by using triple stain as mentioned before according to **Gradinarska *et al.*** [18]. Approximately 200 spermatozoa counted under inverted microscope (Olympus, objective linse100×)

1) Sperm intact membrane (membrane intact cover the sperm head and without staining)
2) Sperm damage membrane (membrane staffing or damage and stained) **(Fig.5).**

Fig. 3. Evaluation of epididymal Dromedary camel sperm viability and Sperm-cell concentration by Automated Cell Counter

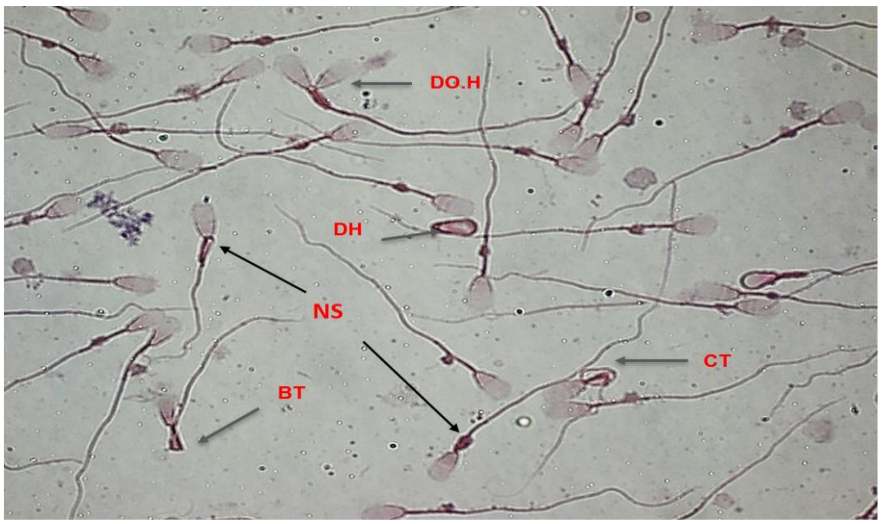

Fig. 4. Dromedary epididymal sperm morphology (abnormality). NS= normal sperm, DO.H= Double Head, DH= Detached Head, BT= Bent Tail, CT= Coiled Tail.

The percent of membrane integrity determine by the equation = (Number of sperm counted / 200) x 100.

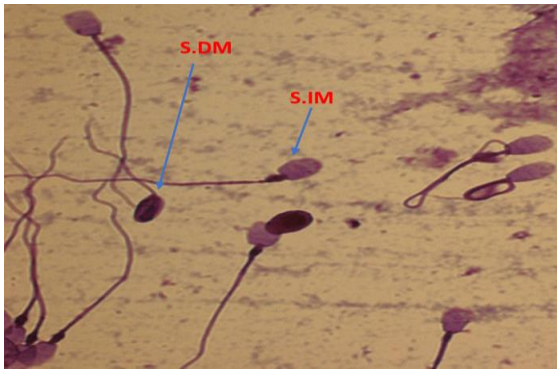

Fig. 5. Dromedary epididymal spermmembrane integrity. S.DM = sperm damage membrane (dead), S.IM=sperm intact membrane (live).

Statistical Analysis: The obtained data for sperm viability, membrane integrity, Sperm abnormality, individual motility percentage (IM%), were analyzed statistically using version 3.03 of Costat statistical software; Copyright Cottort Software.

3. Results

3.1. Effect of seasonality on Dromedary camel epididymal sperm motility, viability, and sperm cell concentration:

Table 1 and Figs. 6-8, illustrated the effects of breeding and non-breeding seasons on the properties of the epididymal spermatozoa. The sperm motility percentage, mean of sperm concentrations and percentage of live sperm in the breeding seasons group (75±2.24 %, 10.88±0.31 10^6/ml suspension, and 80±1.18% respectively) with very high significant increase (P<0.01) than the non-breeding seasons group (55±2.24 %, 9.44±0.19 10^6/ml suspension and 64.5±1.26 respectively). On the other hand the percent of dead sperm in the breeding seasons group was 20±1.18) with very highly significant decrease (P<0.01 when compared to the non-breeding seasons group (35.5±1.26).

3.2. Effect of seasonality on Dromedary camel epididymal sperm morphology

Table 2 and fig. 9 illustrated the influences of breeding and non-breeding periods on the epididymal sperm morphology. The mean of normal sperm morphology in the breading seasons group (89.17±1.19 %) with very highly significant increase (P<0.01) than the non-breeding seasons group (80.42±1.68). In contrast the mean of abnormal sperm morphology in the breading seasons group (10.83±1.19%) with very high significantly decreased (P<0.01) than the non- breeding seasons group (19.58±1.68).

Table1: Effect of seasonality on Dromedary camel epididymal sperm motility, concentration and sperm viability

seasons Parameter	Breeding Mean± S. E	Non-breeding Mean± S. E
Sperm motility (%)	75±2.24	55±2.24**
Sperm concentration (10^7/ml)	10.88±0.31	9.44±0.19**
Live sperm (%)	80±1.18	64.5±1.26 **
Dead sperm (%)	20±1.18	35.5±1.26**

Data recorded as mean %± S.E; compared to the breading seasons the **P value<0.01 means highly significant

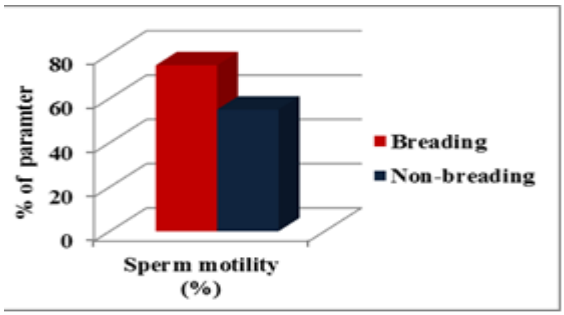

Fig. 6. Effect of season on the Dromedary epididymal sperm motility

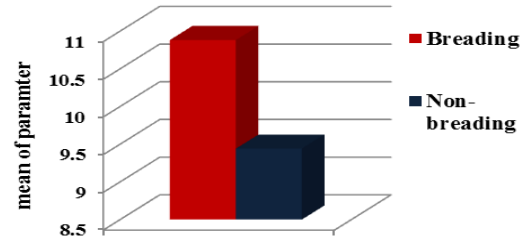

Fig. 7. Effect of season on the Dromedary epididymal sperm concentration

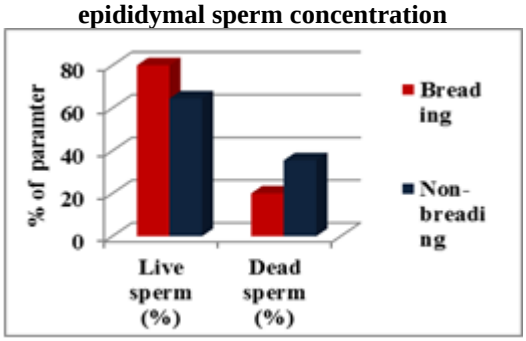

Fig. 8. Effect of season on the Dromedary epididymal sperm viability

Table 2. effect of seasonality on Dromedary camel epididymal sperm morphology

seasons Parameter		Breading Mean %± S.E	Non-breading Mean %± S.E
Sperm morphology	Normal	89.17±1.19	80.42±1.68**
	Abnormal	10.83±1.19	19.58±1.68**

Data recorded as mean%± S.E; the **P value<0.01 means highly significant

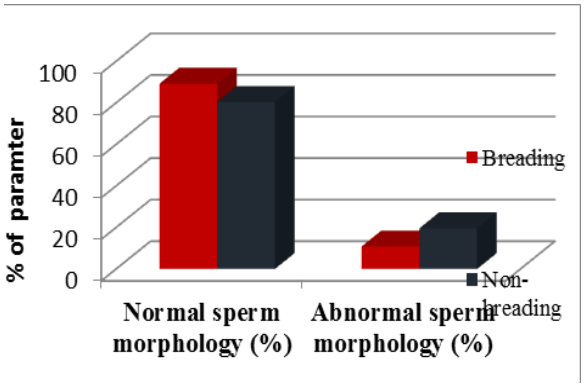

Fig. 9. Effect of season on the Dromedary epididymal sperm morphology

3.3. Effect of seasonality on Dromedary camel epididymal sperm membrane integrity

Table 3 and fig. 10 illustrated the effects of breeding and non-breeding seasons on the epididymal sperm membrane integrity. The mean of intact membrane in the breading seasons group (96.67±0.77%) with very high significant increase (P<0.01) than the non-breeding seasons group (92.33±0.83%). in contrast the mean of damage membrane in the breading seasons group (3.33±0.72%) with very high significant decrease (P<0.01) than the non- breeding seasons group (7.67±0.83%)

Table 3. Effect of seasonality on Dromedary camel epididymal sperm membrane integrity

Season Parameter		Breading Mean %± S.E	Non-breading Mean%± S.E
Membrane integrity (%)	Intact membrane	96.67±0.77	92.33±0.83**
	Damage membrane	3.33±0.72	7.67±0.83**

Data recorded as mean± S.E; **P value<0.01 means highly significant

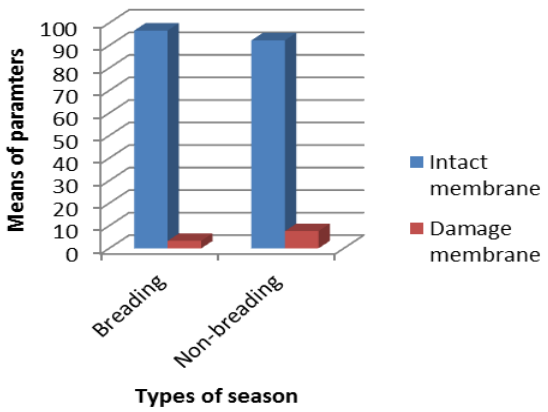

Fig. 10. effect of seasonality on Dromedary camel epididymal sperm membrane integrity

4. Discussion

In most species, seasonality support reproduction and the breeding must be occurring during those times of the year. Changing in daylight ratio is the mostly moderator for seasonality and regulating the seasonal activity[19]. Therefore, in this study, we examined the influence of seasonality on dromedary camel epididymal sperm characteristics (motility, viability, cell concentration, morphology and membrane integrity).

Results obtained from the present work showed that during the breeding season when photoperiod was shorter, the percentage of motility and live epididymal spermatozoa of the male dromedaries were significantly increased (P<0.05) when compared to the other months of the year. These results agree with another authors [9,16], Where the sperm viability and motility increased as a result of the greater quantities of the fluid of the accessory sex glands which contain useful components for sperm viability. The varying ecological stimuli like a shorter daylight period, make the production of testosterone hormone increase, which enhances the libido, sexual behavior and increase the testes and accessory sex glands volume. These changes lead to an increase in the quality and quantity of semen produced during the breeding season (November to April) [9].Most importantly, the increase of motility percentage of the epididymal dromedary camel spermatozoa was detected during the rut period (winter) this is may be because of the development of the mature Leydig cells and increases spermatogenesis process than during the summer. Since the

Leydig cells are principally chargeable to produce testosterone hormone, consequently the semen quality is expected to be improved during rutting time of year [20].

The percentage of dead spermatozoa during non-breeding period was significantly higher when compared to breading period. Our results agree with those published by **Maiada *et al***. [16] who detected that during summer (non-breeding) the dead spermatozoa percentage was significantly higher when compared to breeding periods. Similar results were reported by **Turri *et al***. [21] in camels. These findings may result from the high temperature and the length of daylight during summer which has an influence on the activity of the pituitary gland and spermatogenesis, where the process of spermatozoa formation is inhibited when the temperature increases. Further, when ambient temperatures were greater during the non-breeding period (summer), which produce disorder in the spermatogenesis activity because of degenerative alterations which lower the number of sperm or even the spermatozoa destruction [22].

The present work showed that the concentration of the dromedary camel epididymal sperm was highly significant ($P<0.05$) during the rutting season compared to the non-breeding period. The long day period and heat stress during non-breeding season led to reduction in sperm-cell concentration of the camel semen, this may be due to a decline in the interstitial cells stimulating hormones and therefore, lowering in androgen generation [22]. Further, the rise of epididymal sperm concentration throughout the breeding season may be expected and agree with the results reached by another authors [9,16], who mentioned that FSH concentrations were high during the rutting season in the dromedary camel. A positive correlation between FSH level and the process of spermatogenesis was reported by **Giuliano *et al***. [23]. The sperm concentration is influenced by several factors like the season of the year, virility of the bull and the strength of sexual stimulation, which are responsible for the diversity in the results of this study and other workers [24].

Results obtained from the present work showed that the normal morphology percent of the dromedary camel epididymal spermatozoa was importantly ($P<0.05$) higher throughout the breeding period when compared to the non-breeding, while the percentages of sperm abnormalities were significantly higher through non-breeding period. Sperm abnormalities in breading and non-breading season were determined by morphological analysis using triple stain according to **Gradinarska *et al***. [18]. Estimation of sperm morphology is more difficult to assess, however, estimation of sperm cell-concentration and forward motion is easier and there are differences between separate laboratories because of the individual character of the sperm morphological evaluation [25]. Head, mid-piece and tail of the sperm were investigated for normal morphology. In addition, any abnormality in those parts of the sperm may point to abnormal sperm and makes the sperm unable to achieve the fertilization process.

The present study showed that the epididymal sperm with normal morphology and cell structures were changed throughout the year. As seasonality has a dramatic effect on sperm morphology, these results are in agreement with those notified by **El-Harairy *et al***. [26]. **Maiada *et al***. and **Giuliano *et al***. [16,23] analyzed the effect of season on the seminal characteristics in the llama and they found that the sperm tail abnormalities were different throughout the year, the sperm concentration decreased, moreover the tail abnormalities were increased during the non-breeding season when compared to the breeding season. Current study proved that the proportions of normal live epididymal spermatozoa was ranged from 80.17 % to 89.17 %, this result is similar to that those stated by **Shekher *et al***. [27] but, more than those described by **Buendıa *et al***. [28] (58% to 83%) for alpacas and by **Zeidan *et al***. [29] (71% to 84%) for camels. These variations in the sperm abnormality because of the age of camels used since the older camels are stated to have higher proportion of the abnormalities than the younger ones [30].

Our results disclosed that there were little changes in sperm membrane integrity percent ($P < 0.05$) between breeding and non-breeding season. These results are in agreement with results of **Martí *et al***. [31]. The percentage of membrane integrity of spermatozoa during the non-breeding season was significantly higher than that was exposed during the breeding season. These results may be associated with

the start of the rut which is marked by an improvement in the activity of secreting cells in the anterior pituitary gland, enhance Leydig cells activity and finally, high testosterone levels which consequently improve the spermatogenic process and decrease in the membrane and acrosomal damage[21].

5. Conclusions

This is seen that seasonality has a direct influence on the reproductive function of the dromedary camels. Breeding season improves Dromedary camel epididymal sperm character, motility, viability, sperm concentration, normal morphology, and membrane integrity. Non-breeding season increases sperm abnormalities of epididymal sperm of Dromedary camel.

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خصائص الحيوانات المنوية البربخية للجمل العربي فى مواسم التكاثر وغير التكاثر

هاجر عبد الله الشرنوبي[1], أميمة محمد قنديل[2] ,نهال علي ابو النجا[1]

1 قسم علم الحيوان والحشرات، كلية العلوم جامعة الأزهر (فرع البنات)

2 قسم التكاثر في الحيوان والتلقيح الإصطناعى– شعبة البحوث البيطرية – المركز القومى للبحوث.

الملخص العربي

تم جمع الخصيتين من 30 من ذكور الإبل الناضجة التي تتمتع بصحة جيدة من مسلخ البساتين خلال موسم التكاثر (نوفمبر 2018 إلى إبريل 2019) وموسم عدم التكاثر (مايو 2019 إلى أكتوبر 2019). تم جمع الحيوانات المنوية البربخية مرتين في الشهر لمدة عام. الهدف من هذا العمل هو دراسة تأثيرالفترات الموسمية على خصائص الحيوانات المنوية البربخية الجملية (الحركه، الحيويه ، تركيز خلايا الحيوانات المنوية ، وسلامة الأغشية).

أظهرت النتائج أن نسبة حركة الحيوانات المنوية ، وتركيز الخلايا المنوية ، والحيوانات المنوية الحية ، والشكل الطبيعي للحيوانات المنوية ، والحيوانات المنوية ذات الأغشية السليمة كانت أعلى ($P < 0.05$) أثناء موسم التكاثر (من نوفمبر إلى إبريل) بالمقارنة بموسم غير التكاثر (من مايو إلى أكتوبر). بينما كانت نسبة الحيوانات المنوية الميتة ، تشوهات الحيوانات المنوية وتلف غشاء الحيوانات المنوية أعلى ($P < 0.05$) خلال موسم عدم التكاثر بالمقارنة مع موسم التكاثر. الخلاصه ، تحسنت خصائص الحيوانات المنوية للجمل العربي ، وحيويتها وجودتها خلال موسم التكاثر.