التقرير الفني النهائي المنقح

رقم المشروع: م ص -1- 19

دراسات على صفات السائل المنوي وطريقة تجميده بإستخدام مخففات مختلفة في الإبل ذات السنام الواحد

Studies on Semen Characteristics and Freezing Technique using Different Extenders in the Dromedary Camel

الباحثون

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شكر وتقدير

نشكر كل من ساهم ودعم هذا المشروع ونخص بالشكر الجزيل مدينة الملك عبد العزيز للعلوم والتقنية ممثلة في الإدارة العامة لبرامج المنح لما قاموا به من جهد في تمويل مثل هذه المشاريع الهادفة لخدمة هذا الوطن العزيز كما نشكر جامعة الملك سعود ممثلة بوكيل الجامعة للدراسات العليا والبحث العلمي لما قاموا به من تسهيل في الإجراءات الإدارية ونشكر أيضا مركز البحوث الزراعية بكلية الزراعة وقسم الإنتاج الحيواني على ما وفراه من إمكانيات وأجهزة بحثية سهلت من عملية إجراء البحث وأخيرا وليس آخر نشكر مزارع الفيصلية بالدلم وعلى رأسهم صاحب السمو الملكي الأمير بندر بن عبدالعزيز وبقية منسوبى المزارع على ما قاموا به من توفير حيوانات البحث والخدمات التي قاموا بها تجاهنا .

الملخص العربي

أجريت هذه الدراسة لتقييم السائل المنوي للإبل العربية عن طريق فحص الصفات الطبيعية للنطف وتأثير الاسموزية عليها . إضافة إلى اختيار افضل مخفف لتجميد السائل المنوي . لذا وكما ذكر في التقرير الفني الأول فبعد الموافقة على إجراء البحث تم شراء الأجهزة (ميكروسكوب عاكس ووحدة تجميد السائل المنوي) والكيماويات واختيار الحيوانات حيث تم التنسيق مع إدارة مزارع الفيصلية في تجهيز مكان جمع وفحص السائل المنوي واختيار عدد 10 فحول إبل مجاهيم (أعمارها من 5–10 سنوات) لاستخدامها في البحث . تم جمع السائل المنوي باستخدام المهبل الصنعي أو التنبيه الكهربائي خلال الفترة من سبتمبر إلى ديسمبر لعام 1997م . وتم تحليل البيانات إحصائيا ببرنامج SAS الإحصائي .

أوضحت النتائج أن خواص السائل المنوي (الحجم/مل ، نسب كل من الحيوية والحي والشواذ ، تركيز النطف بالمليمتر) المتحصل عليها عن طريق المهبل الصتعي كانت 6.3 وَ 29.0 وَ 36.9 وَ 12.1 وَ 10X18.5 7 . كما أوضحت النتائج زيادة نسبة حيوية السائل المنوي عند تخفيفه بمخفف السترات مع صفار البيض وسكر الفركنوز (FYC) أو بمخفف التريس مع صفار البيض (TY) ، إضافة إلى انه بالإمكان تجميد السائل المنوي للإبل باستخدام المخففات وكان افضل مخفف أعطى أعلى نسبة حيوية بعد التسييح هو السترات مع صفار البيض وسكر الفركنوز . كما اتضح من اختبار تأثير مستويات مختلفة من الاسموزية على النطف أن زيادة نسبة النطف الملتوية الذيل في المحاليل المنخفضة الاسموزية (60 أسمومول) بينما كان شكل النطف طبيعي في المحاليل ذات الاسموزية المتعادلة (300 أسمومول) . ولوحظ من تحليل عينات بلازما الدم أن مستوى التستستيرون كان في زيادة مستمرة خلال فترة الدراسة (سبتمبر إلى ديسمبر) . إضافة إلأى أن مستوى التستستيرون كان متشابه في عينات بلازما الدم وعينات البلازما المنوية المتحصل عليها عن طريق التنبيه الكهربائي بينما كان مستوى الهرمون منخفضا في البلازما المنوية عند استخدام المهبل الصنعي .

توصي الدراسة بإجراء المزيد من الأبحاث لتحديد انسب المخففات لاستخدامها في تجميد السائل المنوي في الإبل ودراسة تأثير التجميد على خواص السائل المنوي .

English Abstract

This study was conducted to evaluate the semen characteristics of dromedary camel using both the hypo-osmotic swelling test and physical sperm parameters, and to develop a technique for freezing the semen. Semen samples were collected, during breeding season (From September to December, 1997), from ten Arabian camels of good health and maturity (5-10 years old) at Al Faisaliah farms. Following collection, semen characteristics were examined using inverted microscope. The results show that camel semen characteristics (ejaculate volume, sperm motility, the percentage of live spermatozoa, the abnormality and concentration of spermatozoa) collected either by artificial vagina or by electro-ejaculation were 6.2 ml, 29.0 %, 12.1%, 18.5 x 10^4 cells/ml; and 8.2 ml, 40.5 %, 49.2 %, 8.3 %, 7.8 x 10^7 cells/ml , respectively.

Data show that adding the two extenders (TY and FYC) to the semen increased the individual motility, while adding the other two extenders (LYC and GYC) to the semen did not improve the individual motility compared to the initial motility. Furthermore, data show that freezing the semen reduced the individual motility in all extenders. The post thawing sperm motility is very low compared to those ones before freezing. Motility after thawing was best when using FYC and it was lowest when using LYC compared to other extenders. The number of spermatozoa showing curling decreased gradually with the increase of osmolaity solution. Maximum curling (HOS-reactivity) occurred, in most instances, at 60 mosmol. While curling decreased with 300 mosmol (isotonic solution).

There was an increase in the testosterone levels throughout the breeding season (from 2.3 to 2.6 nmol/l). Testosterone concentrations were similar in peripheral blood and in seminal plasma that was collected by electro-ejaculation. While seminal plasma collected by electro-ejaculation contained more testosterone than that one collected by artificial vagina.

In conclusion, more studies are required to assess semen post-thaw in vivo fertilization outcomes. These would elucidate sperm parameters before and after freezing-thawing process using electronic microscopes and biochemical analysis to improve artificial insemination.

Introduction

The Arabian camel (Camelus dromedarius) is an indispensable animal which contributes effectively both as a source of food and welfare to people living under harsh desert condition. It is frugal in habits yet highly productive of milk, meat, wool and work. Opportunities to improve reproductive efficiency of the dromedary camel are limited by inherent characteristics, which include long gestation, a restricted breeding season, and induced ovulation. The continued use of traditional management adds to the practical difficulties in improving reproductive performance as it is often difficult to be certain that females are pregnant at the end of the breeding season. A further factor in the poor reproductive rate is the level of inbreeding in traditional herds. Freezing semen and artificial insemination (AI) could be employed to overcome some of these problems (Chen et al., 1990; Cooper et al., 1990; Zhao et al., 1994). AI with frozen semen is not well developed as a technique for breeding cameilds, compared to its widespread application in other farm animals. In addition, AI with frozen semen could be used to improve genetic traits such as milk, meat and wool production, and racing ability in camels.

The study of spermatozoal morphology has received great emphasis in present day research. It is regarded as an essential factor to be taken into account in analysis of spermatozoa, the morphology of spermatozoa is the spermatozoal parameter such as the sperm motility pattern, sperm morphology, and the number of spermatozoa in an ejaculate (Jouannet et al., 1981; Keel and Webster, 1990). The process of fertilisation involves a complex of biochemical and physiological events that are not measured by the gross physical indicators used in routine semen evaluation (Jeyendran et al., 1984; Pace and Graham, 1970). It seems plausible that better appraisal and prediction of sperm quality can be derived from a combination of microscopical examination of spermatozoa for morphology and motility with test based on the functional characteristics of the spermatozoa (Wood et al., 1986; Hafez, 1993). One such test is the hypo-osmotic swelling test (Kumi-Diaka, 1993) that assesses the integrity of the sperm cell membrane (Spittaler and Tyler, 1985; Zaneveld et al., 1987). The cell membrane plays a central role in determining the architecture of biological systems and provides the basic structural element for complex fluid (Kroll and Gompper, 1992). Membrane integrity is not only important for sperm metabolism, but a correct

change in the properties of the sperm membrane is needed for sperm capacitation, acrosomal reaction, and thus for fertilisation (Keel and Webster, 1990). The hypo-osmotic swelling test could thus be used along with physical sperm parameters to identify the population of infertile male camels.

Frozen semen of camel has not been as extensively studied as that of other species (Chen et al., 1990). The first published work on deep frozen preservation of the sperm of camelidae dates to the late 1970s (Graham and Schmehl, 1978; Seager et al., 1978) using zoo camels and electroejaculated semen frozen in pellets. However, the results achieved on thawing were unsatisfactory. Musa et al. (1992) compared different methods to deep freeze camel semen and tested the motility and morphology of the spermatozoa and their lifespan after thawing. They found that best method was a modification of the technique described by Westendorf et al., 1975. Furthermore, Chen et al.. (1990) and Zhao et al. (1994) conducted a study to evaluate the performance of frozen semen after thawing. They concluded that deep-frozen preservation of camel semen is generally practicable for AI in camel. Therefore, this study will be conducted to evaluate the semen of dromedary camel using both the hypo-osmotic swelling test and physical sperm parameters, and to developed a technique for freezing the semen.

Measurement of testosterone concentrations in peripheral blood has been helpful in evaluating testis activity in animal. Spermatogenesis has become maximal by six years of age in camel and, because the Leydig cells are mainly responsible for androgen production, the male camel can breed when mature Leydig cells are abundant (Matharu, 1966). Fat-Halla and Ismail (1980) reported that FSH concentrations were higher during the rutting season in camel, and FSH acts synergistically with LH to increase testosterone production in males (Johnson and Ewing, 1971).

The objectives

This study was conducted to achieve the following objectives**:**

1- To study the semen characteristics of dromedary camels and selected camels with superior semen to use their semen in AI scheme.

2-To develop a technique for freezing the camel semen to establish camel semen bank which has many benefit such as:

- Overcome low quality of semen out season.
- Increase of conception rate by using superior semen.
- Widespread use of. AI with frozen superior semen that will improve genetic traits such as milk, meat and wool production, and racing ability in camels
- Use frozen semen for in vitro fertilisation technique.

Materials and methods

Semen Collection

Ten dromedary camels (Majaheem, 5-10 years old) of good health and maturity were used during breeding season (from September to December, 1997). The animals were kept in a barn on the Al Faisaliah farms in Al Delm. Camels were fed on concentrates and roughage according to their actual requirements. The concentrates mixture was containing 13.4% digestible protein and 72% total digestible nutrients while the roughage was alfalfa. Water is available at all times in drinking basin and salt licks are provided ad libitum. Blood samples from all animals were collected (using a crate see Figure 1) weekly from jugular vein into 10 ml evacuated heparinized tubes. Then, samples were centrifuged at 3000g for 20 min. and plasma was sorted at –20°C for later analysis. Concentrations of testosterone in the plasma and in the seminal plasma were determined using a direct solid phase ^{125}I radioimmunoassay method (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA, USA) according to the manufacturer (Abraham, 1977).

Semen samples were collected (from September to December, 1997) from all camels using artificial vagina (AV) designed specially for camel (IMV, Cassou, France). This AV constructed in such a way that the semen is ejaculated directly into

the graduated collection vessel because most rubber liners have a deleterious effect on camel spermatozoa. The AV was filled with water at 55-60°C so that temperature inside the inner liner stabilises at 41-43°C a few drops of Vaseline were smeared on the inner liner at the entrance to the AV to provide lubrication. A female in oestrus was secured in the sitting position, preferably with its forelegs tied. The bull was teased by allowing him to smell the female before leading him to her rear. Then, the operator was ready to knell at the right side of the female once the bull had sat upon her. When the bull began to thrust, the operator should grasp the sheath with his left hand and direct the extended penis into the AV that he was holding in his right hand. The ejaculate usually came in fractions and bull made several thrusts, interrupted by period of rest, until ejaculation was complete. The process took 5 to 10 minutes.

Electro-ejaculation was used when collection by AV is impossible, since the characteristics of semen recovered by electro-ejaculation are similar to that collected by AV (Musa et al., 1992 and 1993). A bull electro-ejaculator (Standard Precision Electronics, Colorado, USA) is used with the camel secured in sternal recumbancy and then turned onto its side (Figure 4) as described by Tingari et al. (1986). Electro-ejaculation was achieved by using the rectal probe lubricated with copious amounts of jelly to ensure good electrical contact with the mucosa and giving two sets of stimulation, each of 10 to 15 pulses of 3 to 4 seconds duration at 12 volts and 180mA, with a rest of 2 to 3 minutes between the two sets. The collection is made into a flask held at the preputial orifice, with occasional milking of the prepuce to expel all the semen.

Figure 1. A crate for collecting blood from camels

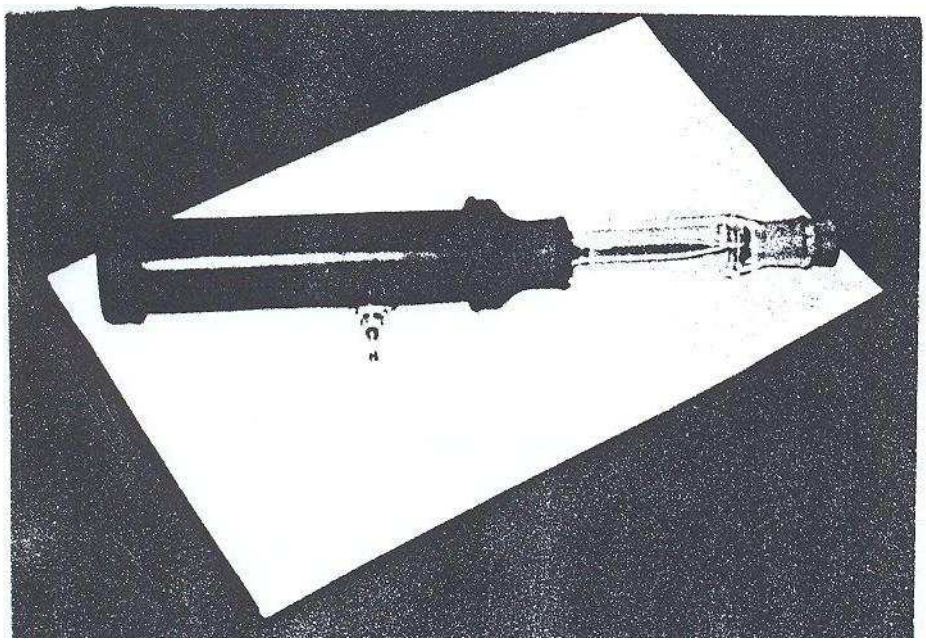


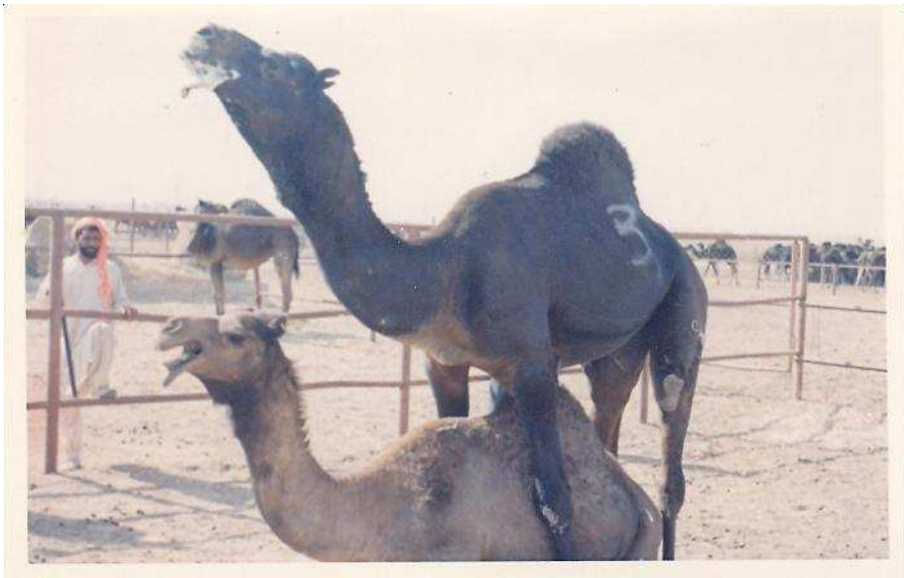
Figure 2. Camel's Artificial Vagina

Semen Evaluation

Following collection, flask containing the semen samples (Figure 5) was placed in a water bath at 30°C for 15 minutes to liquefied the jelly-like semen and allow the spermatozoa to attain motility. Then, semen was examined for volume, percentage of progressive motility, concentration, and percentage of morphologic sperm abnormalities according to standard procedures (Roberts, 1986). Sperm motility was assessed by placing a drop of semen on warm slide, placing a cover slip over it, and examining it under inverted microscope (Nikon TMS-F, Japan) at 100x and 200x magnification for the pattern and percentage of motility. The total number of spermatozoa per ejaculate was determined in a hemocytometer, by first determining the concentration per ml, and multiplying the latter by the volume of the ejaculate. Eosin vital stain (Berthelsen, 1981) was used to study the ratio of live: dead and the abnormality spermatozoa. One hundred spermatozoa per slide were counted for each determination. Nigrosin-eosin stained (Watson, 1975) samples were used to study sperm head morphology under inverted microscope at 1000x magnification and for counting 200 spermatozoa per slide.

(A)

(B)



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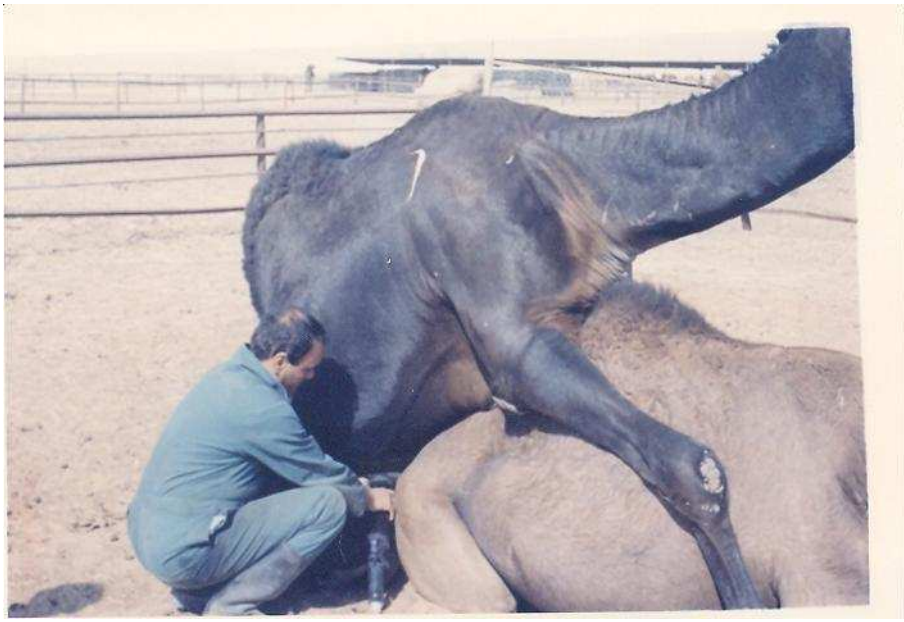

(D)

Figure 3A, B, C and D. Sexual behaviour during collecting semen using artificial vagina.

Figure 4. Collecting semen in camel using electro-ejaculation

Figure 5. Camel's Semen Sample

Hypo-Osmotic Swelling Test

The response of the camel spermatozoa to the Hypo-osmotic Swelling Test (HOS) test was assessed with fructose and sodium citrate (Na-citrate, electrolyte) solution of varying osmolaltities (60, 150, 200 and 300 mosmols). This was done to determine the HOS solution with optimal osmolality that will give maximum HOS-reactivity (sperm curling). Aliquots of the mixtures (semen +solution) were incubated at 37°C at 15 minutes at which the maximum number of spermatozoa curled. After incubation, 1 or 2 drop of the treated mixture in thin slide cover slip preparations by inverted microscope at 200x and 400x. Two hundred spermatozoa per slide were counted at 400x magnification, and the percentage of curling/swelling will be determined (number of spermatozoa with curled tails were divided by the total number of spermatozoa counted, multiplied by 100).

Semen Freezing

Split ejaculations were used to determine the best conditions fpr the cryopreservation of semen that was frozen using four different extenders (Table 1).

Table 1. Composition of different extenders for camel semen

Components	Grams/100 ml distilled water for each extender*			
	TY	LYC	FYC	GYC
Sodium citrate dihydrate	---	2.90	2.90	2.90
Citric acid anhdrous	1.68	0.04	0.04	0.04
Lactose	---	1.25	---	---
Fructose	---	---	1.25	---
Glucose	---	----	---	1.25
Tris aminomethane	3.03	---	---	---
Egg-yolk (ml)	20.0	20.0	20.0	20.0
Glycerol (%)	7.0	7.0	7.0	7.0

*Antibiotics were added to each extender at the rate of 500 iu penicillin plus 500µg streptomycin sulphate/ml.
TY=Tris yolk (Triladin, Dorset, Germany); LYC=Lactose yolk citrate; FYC=Fructose yolk citrate; GYC=Glucose yolk citrate.

A volume of semen was added to 4 tubes containing one of following extenders: TY, LYC, FYC or GYC at 32°C so that the semen was diluted 1:3 (semen: extender). The equilibration time (time the semen was exposed to extender before

freezing) was less than 5 minutes at 32°C. Four 0.5 ml clear straws (I. M.V., L'Aigle, France) were labelled for each of the 4 tubes. The straws were filled and blue-phenyl sealed. Then, 23 straws were loaded into the cryochamber of a Freeze Control Model CL-863 (Cryologic PTY, LTD., Victoria, Australia). The straws of semen were cooled from 32°C to -60°C at the following rate:

32 to 4.0°C the rate was 1°C/min.
4.0 to -10°C the rate was 2°C/min.
-10 to -30°C the rate was 3°C/min.
-30 to -60°C the rate was 4°C/min.

The frozen straws were then plunged into canes for storage in liquid nitrogen. One straw of semen in each of the 4 extenders was thawed by immersion in a 37°C water bath for 30 seconds. The straws were wiped dry and each was emptied into a separate vial. The semen was then examined by inverted microscope in order to determine the individual motility of the sperm. Frozen semen samples showing a post-thaw progressive motility or 30% or higher were packed, labelled and stored in liquid nitrogen to use them in future AI scheme.

All data were statistically analysed using general linear model procedure (Goodnight et al., 1986).

Results and Discussion

Semen Characteristics

Characteristics of camel semen collected by artificial vagina (volume motility, live, abnormal and concentration) are presented in Table 1 and Figures 6,7 and 8. The ejaculate volume was 6.2 ± 1.3 ml, with sperm motility of 29 .0 ± 3.2 %, and the percentage of live spermatozoa was 36.9 ± 4.8, while the abnormality was 12. 1 ± 1.8%. The concentration of spermatozoa was 18.5 ± 2.7x 10^4 cells/ml. Semen collection time (from intermission of the penis into the artificial vagina) was 4.2 ± 1.9 minutes. These results were in agreement with Adel-Raouf and El-Naggar (1976) and Wilson (1984). They reported that the volume of the ejaculate varies from 5-22 ml and the average number of spermatozoa was about 0.4x10^6 cells/ml.

Table 1. Characteristic of camel semen collected by artificial vagina (8 ejaculates per animal).

Animal	Volume/ml	Motility %	Live %	Abnormal %	Conc. $X10^4$
1	3	20	20	15	5
2	9	20	30	8	18
3	4	30	65	4	15
4	7.5	30	25	5	13
5	5	40	45	18	15
6	2	40	52	15	16
7	3.5	45	45	8	37
8	10	20	25	10	18
9	3	15	20	20	19
10	15	30	42	18	26
Mean ± SE	6.2 ± 1.3	29.0 ± 3.2	36.9 ± 4.8	12.1 ± 1.8	18.2 ± 2.7

The characteristics of camel semen collected by electro-ejaculation (volume, motility, live, abnormal and the concentration) are presented in Table 2. The ejaculate volume was 8.2 ± 1.2 ml, with sperm motility of 40.5 ± 4.7 %, and the percentage of live spermatozoa was 49.2 ± 4.8, while the abnormality was 8.3 ± 1.3 %. The concentration of spermatozoa was 7.8 ± 1.2 x 10^7 Cells/ml. Semen collection time (from applying the probe into the rectal) was 12 ± 1.5 minutes. In respect to the volume, motility, live sperm, abnormality and concentration, data show that characteristics of camel semen hat was collected by electro-ejaculation were better than those collected by AV (8.2, 40.5, 49.2, 8.3 and 7.8 $x10^7$ vs. 6.2 ml, 29.0%, 36.9%, 12.1% and 18.2 x 10^4, respectively). These differences were due to the fact that semen collected by AV was contained large amount of seminal plasma, which make semen characteristics low specially the concentration.

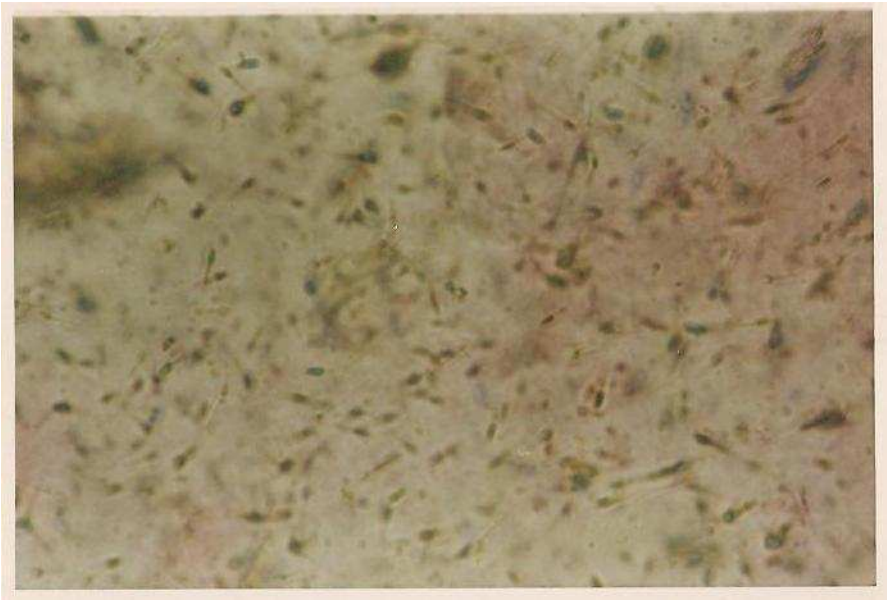

Figure 6. Individual motility of camel spermatozoa.

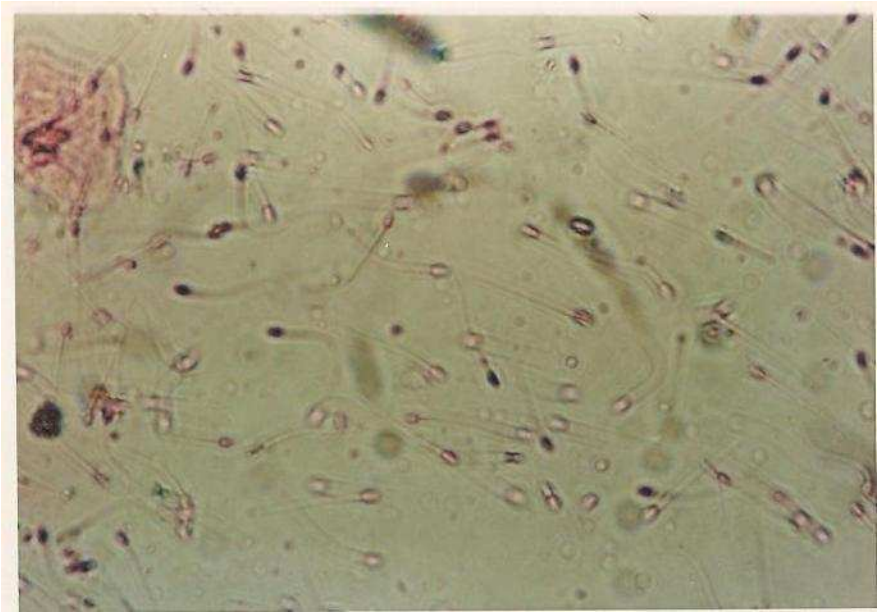

Figure 7. The live and dead camel spermatozoa, note colored sperm head represent the dead spermatozoa, while the non-colored ones represent the live spermatozoa

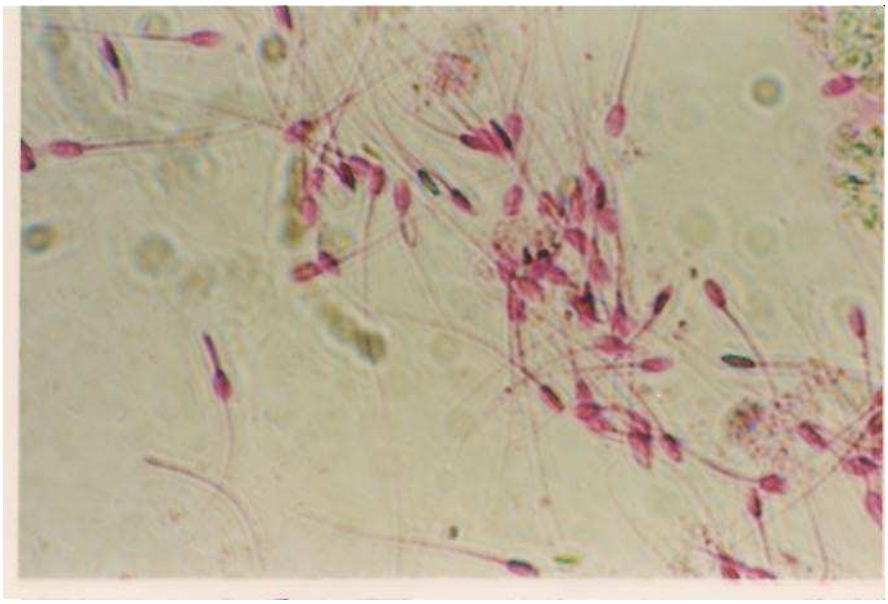

Figure 8. The abnormal spermatozoa of camel semen.

Table 2. Characteristics of camel semen collected by electro-ejaculation (3 ejaculates per animal).

Animal	Volume/ml	Motility %	Live %	Abnormal %	Conc. $X10^7$
1	12	20	20	5	5
2	9	20	30	8	6
3	15	60	65	4	15
4	7.5	30	50	5	13
5	5	40	45	18	6
6	5	50	52	5	10
7	3.5	45	45	8	7
8	12	50	60	10	6
9	4.5	30	55	12	4
10	8	60	70	8	76
Mean ± SE	8.2 ± 1.2	40.5 ± 4.7	49.2 ± 4.8	8.3 ± 1.3	7.8 ± 1.2

Semen Freezing

The results of the percentage of individual motility of sperm before and after freezing using different extenders are presents in table 3. Date show that adding the two extenders (TY and FYS) to the semen increased the individual motility, while adding the other two extenders (LYC and GYC) to the semen did not improve the individual motility compared to the initial motility (42 and 42; 33 and 25 vs 35%, respectively). Furthermore, date show that freezing the semen reduced the individual motility in all extenders. The post thawing sperm motility is very low compared to those once before freezing (10 vs 42; 3.0 vs 25; 15 vs 42; 6.0 vs 33, for extenders TY, LYC, FYC and GYC, respectively). Motility after thawing as best when using FYC and it was lowest when using LYC compared to other extenders. These variations in the post thaw motility between extenders may be due to the fact that the camels were not selected for semen quality; therefore, they varied in their individual semen characteristics. Therefore, the initial motility determines the output results of post thawing motility. Zhao et al. (1994) conducted to freeze camel semen using different extenders. They obtained good results in post thaw motility (53%) when they used semen with high initial motility (78 %). Thus, good quality of camel semen diluted with specific extenders is suitable for freezing and can be used for routine artificial insemination scheme.

Table 3: The percentage of individual motility of sperm before and after freezing using four different extenders (TY, LYC, FYC and GYC).

Initial Motility	TY		TY		TY		TY	
	Before	After	Before	After	Before	After	Before	After
30	40	10	10	00	45	15	35	05
45	50	10	40	05	50	20	35	10
20	25	05	20	00	30	15	20	05
40	45	10	30	05	45	15	34	05
40	50	15	25	05	40	10	40	05
Mean 35± 4.4	42± 4.6	10± 1.5	25± 5.0	3.0± 1.2	42±3.4	15± 1.6	33± 3.4	6.0± 1.0

TY = Tris yolk ; LYC = Lactose yolk citrate ; FYC = Fructose yolk citrate ; GYC= Glucose yolk citrate .

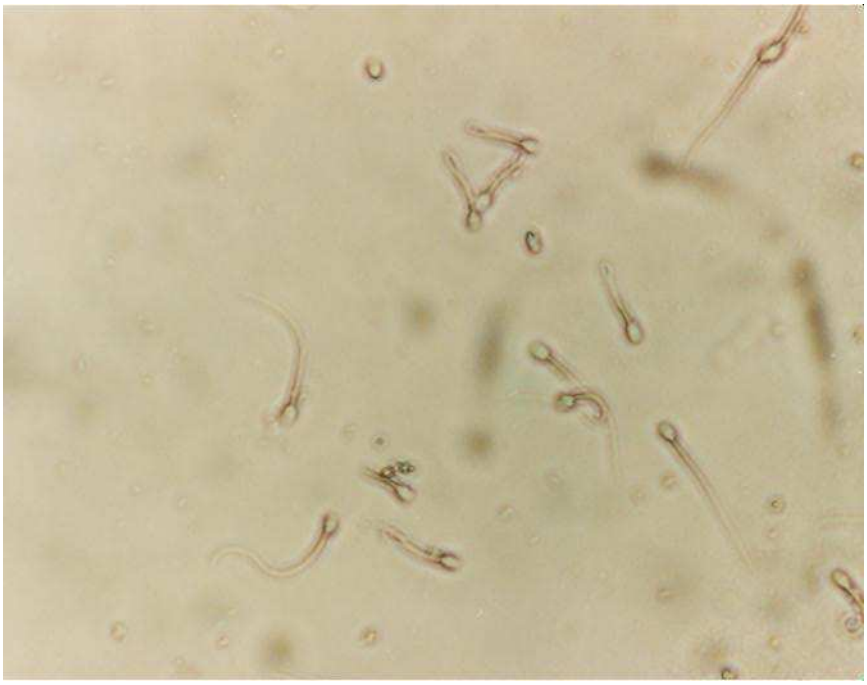

Figure 9. Camel spermatozoa after treatment with HOS-test, note the curling of the spermatozoa.

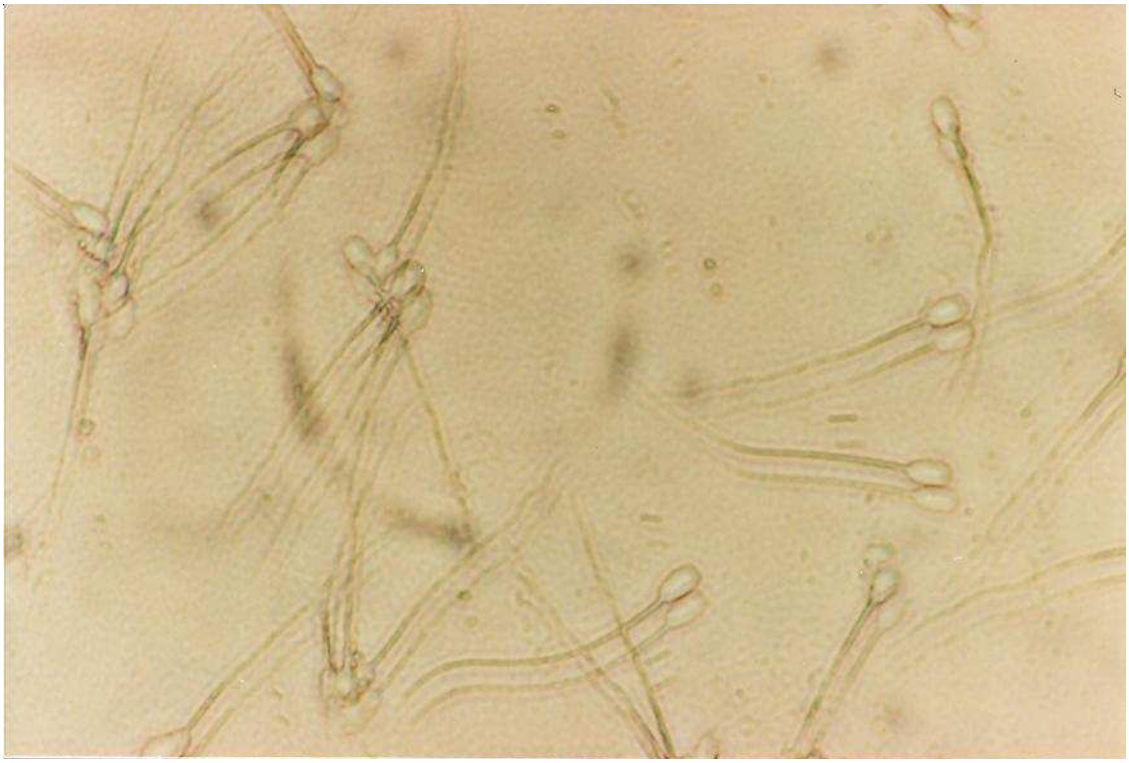

Figure 10. Camel spermatozoa before treatment with HOS-test, note the normal spermatozoa

Hypo –Osmotic Swelling Test

Percentages of curled spermatozoa in response to HOS-test solution with varying osmolalities are summarized in Table 4 . Camel spermatozoa exhibited morphologic change (as evidenced by curling /swelling) when subjected to HOS – test , compared to normal spermatozoa before treatment (Figures 9 and 10) . The mean percentage of curling at 15 minutes of incubation were 57 .2 44 .8 ,22.4 and 10 .8 with solution amorality 60, 150,200 and 300, respectively . The number of spermatozoa solution amorality 60, 150,200 and 300, respectively . The number of spermatozoa showing curling decreased gradually with the increase of Osmolaity solution . Maximum curling (HOS − reactivity (occurred , in most instances , at 60 mosmol . while curling decreased with 300 mosmol (isotonic solution) . This could be due to the fact that sperm motility partly depends on membrane transport and partly on other biochemical activities (Jeyendran et al ., 1984) . The percentage of live sperm before treatment did not significantly (P > 0.05) different from the percentage of curling at 6o mosmol (56 vs 57%). Available data from several human studies indicate that sperm curling in response to the HOS-test is a better endpoint for assessing the ability of spermatozoa to capacitate and effect fertilization than semen analysis (Jeyendran et al ., 1984). The results of the present study indicate the potential usefulness of the HOS-test in the camel, as evidenced by the significant response of the spermatozoa to the test. The HOS-test could be used as an addition to standard semen analysis, to indicate those animals that could be subfertile despite a normal spermiogram. Further clinical evaluation of the HOS-test is needed to assess its diagnostic significance in the camel.

Table 4. Percentage of curled spermatozoa in response to HOS-test solution with varying osmolalities

Live sperm (%) Before treatment	Osmolaity			
	60	150	200	300
60	72	54	32	12
70	75	60	40	22
50	52	35	15	5
40	35	30	10	5
55	52	45	15	10
Mean = ± SE 55.5 ± 5.0	57.2 ± 7.4	44.8 ± 5.6	22.4 ± 5.8	10.8 ± 3.1

HOS-test = Hypo-Osmotic Swelling Test

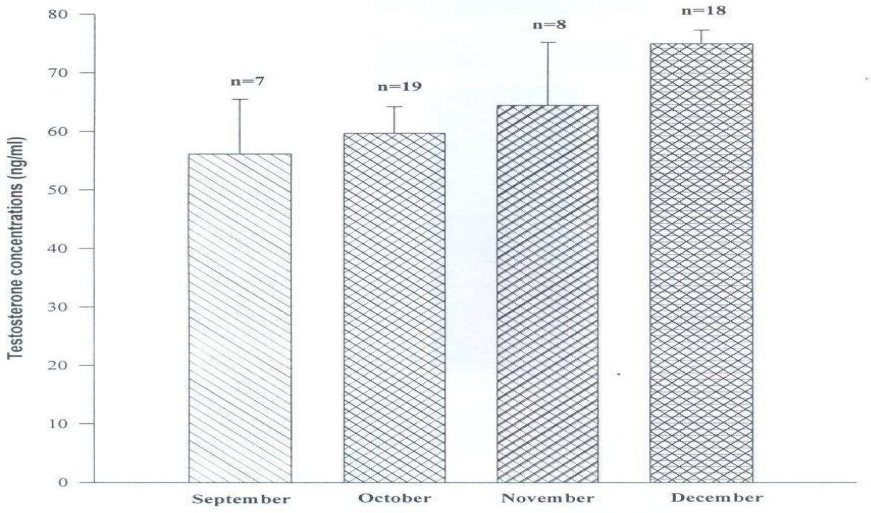

Figure 11. Testosterone concentrations in blood plasma in camel during breeding season.

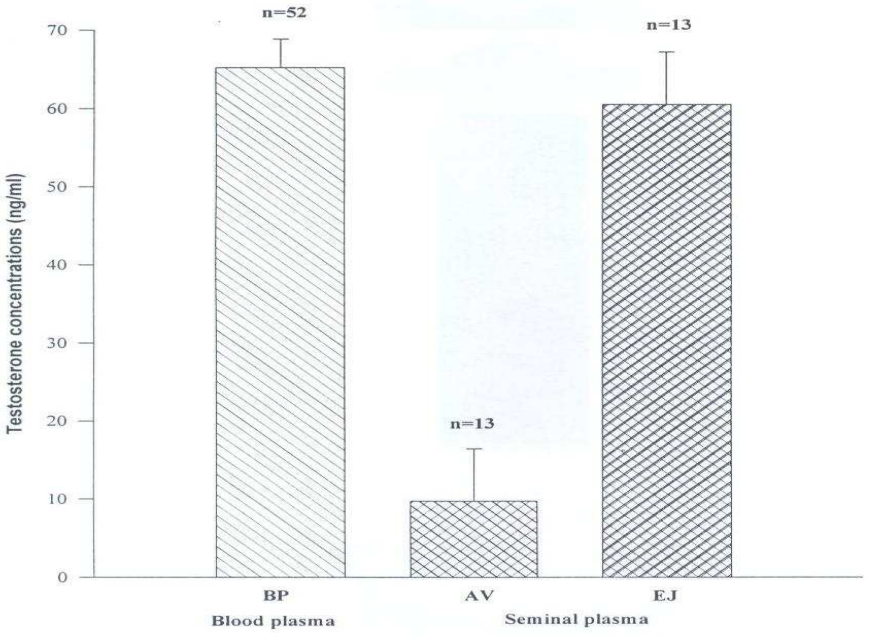

Figure 12. Testosterone concentrations in blood plasma (BP) and in seminal plasma that was collected by artifical vagina (AV) or electroejaculater (EJ) in camel.

Testosterone Profiles

Testosterone concentrations in blood plasma during September, October, November and December are presented in Figure 11. The mean of testosterone concentrations during these months range from 56 to 75 ng/ml. There was an increase in the testosterone levels throughout the breeding season in camel. This increase may reflect the development of the interstitial cells (Abdel-Raouf et al., 1975; Singh and Bharadwai, 1978).

Testosterone concentrations in blood plasma and in seminal plasma that was collected either by artificial vagina or by electro-ejaculation are depicted in Figure 12. From this figure , it appears that testosterone concentrations were similar in peripheral blood and in seminal plasma that was collected by electro-ejaculation (65 vs 60 ng/ml). While seminal plasma collected by electro-ejaculation contained more testosterone than that on collected by artificial vagina (60 vs 10 ng/ml). This may be explained by the fact that semen collected by artificial vagina was more diluted compared to that one collected by electro-ejaculation (Musa et al., 1993).

Conclusions

It can be concluded that testosterone concentrations in male camel are high during breeding season and in semen collected by electro-ejaculation.

It is clear from the results of this study that semen can be readily collected from camel using artificial vagina or electro-ejaculation. Electro-ejaculation is a reliable method for semen collection in camel (since it is easy to perform and gives good semen cry characteristics). This procedure is fundamental to the application of artificial breeding technology, including insemination of freshly collected, short-term preserved and opreserved camel semen.

Data showed that the hypo-osmotic swelling test is efficacious for assessing sperm viability and this test may be used in assessing the quality of both fresh and stored camel semen and in screening out animals that could be subfertile despite normal spermiograms .

Based on these results it was concluded that freezing of camel semen using ordinary extenders did not impair the physical and functional characteristics of the

spermatozoa. Under the condition of this study, examination of extenders showed that FYC is best extender for freezing camel semen and can be used for routine insemination scheme. However, more studies are required to assess semen post-thaw in vivo fertilization outcomes. These would elucidate sperm parameters before and after freezing-thawing process using electronic microscopes and biochemical analysis to improve artificial insemination.

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