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Effect of Green Laser Irradiation on Epididymal Camel Spermatozoa Quality Stored at 5°C

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Abstract

Total number of forty testes from twenty Sudani camels (*Camelus dromedarius*) were used in the present study (>5-10 years old and 500-600 kg body weight). The experimental work was executed to define the effect of green laser irradiation with short-wavelength 532 nm and continuous wave from a diode laser light with a total output power of 3 mW on epididymal camel spermatozoa quality at different exposure times of 0 (control, non-irradiated), 2, 4, 6, 8, and 10 min. Following irradiation, the percentages of motile spermatozoa, storagability, viability, and acrosomal damage were assessed of the epididymal camel spermatozoa stored at 5°C for 4 days. Epididymal spermatozoa was diluted with lactose-yolk-citrate (LYC) extender. The obtained results showed that the highest (P<0.05) value of the percentages of motile and storagability of spermatozoa was recorded with spermatozoa exposed to 6 min of laser irradiation and the lowest (P<0.05) value was recorded with the control group. Otherwise, the highest (P<0.05) value of the percentages of dead and acrosomal damage of spermatozoa was recorded with spermatozoa exposed to 10 min and the lowest (P<0.05) value was recorded with 2 min. The advancement of storage time at 5°C decreased (P<0.05) the percentages of motile and storagability of spermatozoa, while increased (P<0.05) the percentages of dead and acrosomal damage of spermatozoa during storage at 5°C for 4 days. Consequently, enhancing the artificial insemination program can be achieved using the laser irradiation which is considered a cost-effective technique for improving semen quality. The profitable effects of laser irradiation on epididymal camel spermatozoa quality raised the motile spermatozoa, storagability, livability, acrosomal integrity which consider an indicator to improve mitochondrial function which extends the survival of spermatozoa.

Keywords: Camels; Epididymal spermatozoa; Green laser irradiation; Viability; Storagability

1 Introduction

About 75% of dromedary camel (*Camelus dromedarius*) population is raised in arid and semi-arid areas, which is considered the strategic stockpile of food security in the Arabian world. Due to its unique adaptive characteristics, it is considered the most versatile animal

which can live and produce under such harsh environmental conditions. However, plentiful and complicated natural restrictions can adversely affect the reproductive capacity of the one-humped camel (El-Hassanein et al 2004). This is resulted in the poor reproductive performance of this species, however great efforts were exerted over the past years to enhance dromedaries' reproductive capacity (El-Bahrawy et al 2015).

Artificial insemination (AI) can be a helpful tool in animal production and the technique can enhance breeding programs and improve productivity when used widely and properly. In camel, some genetic traits such as milk, meat, wool production, and racing ability could be improved by AI. High reproductive activity can be achieved by the success of AI which depends on the semen quality and its capacity for extension and preservation with minimal loss of fertilizing ability (Shekher et al 2012 & Mostafa et al 2014). Generally, sperm viability can be extended for many days by storage at 2-5°C. However, fertilizing capacity could be adversely affected mostly after one day of chilled storage (Al-Bulushi et al 2019).

The term laser stands for light amplification by stimulated emission of radiation. In late 1970s and early 1980s, lasers were presented to the medical field of assisted human reproduction for reconstructive pelvic surgery through operative microscopes and laparoscopes (Tadir et al 1992). In the 1980s and 1990s, laser began to be used in the treatment of infertility. The terms such as *in-vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), and assisted reproductive technologies (ART's) have been evolved effectively. Oocytes and spermatozoa could be treated by IVF, ICSI, and ART technologies through laser beams. The low-power laser irradiation of spermatozoa can improve sperm motility, as well as, its velocity. Irradiation of spermatozoa has been shown to improve sperm motility with a low-intensity helium-neon (He-Ne) laser (Karu, 2012). Thus, the current study aimed to define the effect of green laser irradiation with short-wavelength 532 nm, 3 mW with different exposure times of 0, 2, 4, 6, 8, and 10 min on the epididymal camel spermatozoa quality under chilled storage at 5°C for 4 days.

2 Materials and Methods

The current study was carried out at Embryo Transfer Laboratory, Artificial Insemination and Embryo Transfer Department, Animal Reproduction Research Institute, Giza, Egypt, in cooperation with the Laser Atomic Spectroscopy Laboratory, Department of Laser Applications in Measurements, Photochemistry and Agriculture, National Institute of Laser Enhanced Sciences, Cairo University, Giza, Egypt during the period from June, 2019 to October, 2019.

2.1. Experimental animals

A total number of twenty clinically healthy Sudani camels (*Camelus dromedarius*) with a total number of forty testes aged from >5 to 10 years or more with live body weights of 500-600 kg were used in the present work and provided from automated El-Bassatein slaughterhouse, Cairo, Egypt.

2.2. Epididymal spermatozoa collection

2.2.1. Transportation of the samples

The genitalia (epididymides connected to the testes) were removed from the carcass and transported in a thermos flask including sterile physiological saline (0.9%) with 100 µg/ml streptomycin at 25°C according to the method described by Goto et al (1989) through 2-3 hours after slaughtering.

2.2.2. Sperm recovery

Forty testes were fully cleaned then the blood was wiped off by puncturing the superficial blood vessels of the cauda. By using a sterile scalpel and forceps, the epididymis was sectioned into three respective parts, caput, corpus, and cauda in three sterile Petri dishes of 100 mm diameter containing saline solution.

2.3. Semen extension

Epididymal camel spermatozoa were collected, pooled, and evaluated for each camel and then diluted with lactose-yolk-citrate dilution (2.9 g sodium citrate dihydrate, 0.04 g citric acid anhydrous, 1.25 g lactose and 10 ml egg-yolk, per 100 ml distilled water, 500 I.U/ml penicillin and 500 µg streptomycin sulphate were also added to the extender) according to Musa et al (1992).

2.4. Chilling of semen at 5°C

In order to facilitate frequent checking of the temperature during the cooling duration, the test tubes containing diluted semen were placed in a 500 ml beaker containing water at 30°C with a thermometer which gradually cooled till 5°C during a period of 1.5-2.0 hours (Salisbury et al 1978). The cooled spermatozoa were kept at 5°C for 4 days. Motile, dead and acrosomal damage (%) of spermatozoa exposed to different exposure times of laser irradiation (0, 2, 4, 6, 8, and 10 min) were recorded following chilled storage.

2.5. Semen evaluation

2.5.1. Epididymal sperm motility (%)

Motile spermatozoa (%) were detected as an oscillatory movement of the flagellum due to the highly viscous nature of camel semen according to Tibary and Anouassi (1997).

2.5.2. Storagability (%)

Storagability (%) of the cooled camel spermatozoa refers to the original motile spermatozoa (%) still motile 4 days post storage at 5°C as the method described by Yassen and El-Kamash (1970). Storagability (%) was calculated by dividing the percentage of motile spermatozoa at day 4 of storage on the percentage of motile spermatozoa at day 0 of storage.

2.5.3. Dead spermatozoa (%)

The eosin/nigrosin staining procedure was carried out by dissolving 1.67 gm eosin and 10 gm nigrosin in distilled water up to 100 ml according to Hackett and Macpherson (1965).

2.5.4. Acrosomal damage of spermatozoa (%)

The percentage of acrosomal damage was assessed according to Watson (1975). Spermatozoa were stained with 2% of trypan blue (T-0887 Sigma) for assessment of sperm viability then for 40 min with a 10% solution of Giemsa (Merck, Darmstadt, Germany) in distilled water prepared immediately before use.

2.6. Laser specifications and irradiation parameters

Green laser (λ=532 nm) from a Diode Pumped Solid State (DPSS) laser [LSR-PS-II] with an output power of 3 mW was used for irradiation of spermatozoa with different exposure times (0, 2, 4, 6, 8, and 10 min). Parameters of green laser irradiation were shown in **Table 1** as follows: 532 nm wavelength and average power output 3 mW. The irradiance of 3 mW/cm^2 was calculated according to Calderhead (1990) using the following equation:

$$Irradiance = \frac{Power\ output\ (mW)}{Application\ surface\ (cm^2)}$$

Table 1. Laser fluency and corresponding exposure times

Fluency (J/cm^2)	Power output (mW)	Exposure time (min)	Wavelength (nm), (Colour)
0.38	3	0, 2, 4, 6, 8, and 10	532 (Green)

2.7. Statistical analysis

Two-way ANOVA was used to analyze data statistically, using the procedure of General Linear Model (GLM) of SAS (SAS, 2000). Duncan's Multiple Range Test (Duncan, 1955) was used to detect significant differences among means. Percentage values were transformed into arc-sin values before being statistically analyzed. The statistical model used in the experimental work was as follows:

$$Y_{ijk} = \mu + L_i + S_j + (L_i \times S_j) + e_{ijk}$$

Where,
Y_{ijk} = the observed value of the dependent variable determined from a sample taken of spermatozoa.
μ = the overall mean.
L_i = the fixed effect of laser irradiation durations (min), i= 0, 2, 4, 6, 8, and 10.
S_j = the fixed effect of storage time (days), j= 0, 1, 2, 3, and 4.
$L_i \times S_j$ = the interaction between laser irradiation durations (min) and storage time (days).
e_{ijk} = the residual error.

3 Results and Discussion

3.1. Sperm motility (%)

Table 2 revealed that the effect of green laser irradiation (532 nm, 3 mW) for 6 min ($P<0.05$) increased the percentages of motile and storagability of the dromedary camel spermatozoa during storage at 5°C than the control group. These results were in agreement with Wenbin et al (1996), who found an increase in sperm fructose fermentation, respiration, ^{32}P absorption capacity and Ca^{2+} absorption in buck sperm treated with laser irradiation, which in turn led to increase its motility and survivability. Similarly, Fernandes et al (2015) reported variable progressive motility when comparing the control group with 4 joules group in cryopreserved bovine spermatozoa. It could be attributed to the interaction between the mitochondria and laser irradiation which in turn enhance semen quality (Lone et al 2018).

The present study showed the prolongation of storage time at 5°C for 4 days decreased ($P<0.05$) the percentages of sperm motility and storagability at all exposure times of laser irradiation and the control group **(Table 2)**. These findings may be due to the high metabolic activity of spermatozoa which leads to a toxic effect on the sperm by increasing the production of lactic acid. The current results agreed with Zeidan et al (2013) in the dromedary camel spermatozoa. A similar trend was observed by Morton et al (2009), who reported that sperm motility of alpaca was declined ($P<0.05$) after 24 hours of the beginning of liquid storage, with the highest loss in motility at the first 24 hours of liquid storage. Furthermore, the current study revealed that the interaction effect between storage time at 5°C for 4 days and green laser irradiation on motile spermatozoa was insignificant.

3.2. Dead spermatozoa (%)

The dead spermatozoa (%) during storage at 5°C was higher ($P<0.05$) of spermatozoa exposed to 10 min of laser irradiation than other exposure times and the control group **(Table 3)**. Comparing to the control group (52.56%), the dead spermatozoa (%) decreased ($P<0.05$) with spermatozoa exposed to 2, 4, and 6 min of laser irradiation with an average value of 39.78, 44.56, and 49.67%, respectively. A similar trend was reported by Iaffaldano et al (2010 & 2016), who found that the viable spermatozoa (%) was higher ($P<0.05$) at 24 hours of storage with 6.12 and 9.0 J/cm^2 than the control in rabbit semen. Similarly, Fernandes et al (2015) reported that laser irradiation increased ($P<0.05$) the live spermatozoa (%) in cryopreserved bovine spermatozoa than the control group.

The present study showed that the prolongation of storage period at 5°C for 4 days increased ($P<0.05$) the dead spermatozoa (%) of the dromedary camels at all exposure times of laser irradiation and the control group **(Table 3)**. A similar trend was observed by Zeidan et al (2013) in the camel spermatozoa, in which the lowest dead spermatozoa (%) was reported

Table 2. Mean percentage of motile epididymal camel spermatozoa, with different exposure times (min) of green laser irradiation during storage at 5°C for up to 4 days

Storage time (days)	Laser exposure times (min)						Mean
	Control	2	4	6	8	10	
0	45.56±1.30	48.89±1.11	56.11±1.62	65.00±1.44	62.22±1.46	56.11±1.82	55.65^{A}±1.09
1	31.11±1.11	42.22±0.87	49.44±1.75	58.33±1.44	56.11±1.82	48.33±1.86	47.59^{B}±1.37
2	19.44±1.30	32.78±1.21	40.56±1.00	48.33±0.83	47.22±1.68	39.44±1.30	37.96^{C}±1.43
3	10.56±0.56	26.67±1.44	33.33±1.17	39.44±1.30	37.78±1.21	31.67±0.83	29.91^{D}±1.38
4	10.00±0.00	20.56±1.55	26.11±1.11	31.67±0.83	30.00±0.83	22.78±0.87	23.52^{E}±1.05
Overall mean	23.33^{e}±2.07	34.22^{d}±1.64	41.11^{c}±1.73	48.56^{a}±1.89	46.67^{b}±1.87	39.67^{c}±1.87	38.93
Storagability (%)	21.95	42.05	46.53	48.72	48.22	40.60	41.35

$^{A-E}$ Values with different superscripts within a column are significantly different (P<0.05).

$^{a-e}$ Values with different superscripts within a row are significantly different (P<0.05).

$$Storagability\ (\%) = \frac{\%\ of\ motile\ spermatozoa\ at\ day\ 4\ of\ storage}{\%\ of\ motile\ spermatozoa\ at\ day\ 0\ of\ storage}\ X\ 100$$

Table 3. Mean percentage of dead epididymal camel spermatozoa, with different exposure times (min) of green laser irradiation during storage at 5°C for up to 4 days

Storage time (days)	Laser exposure times (min)						Mean
	Control	2	4	6	8	10	
0	38.89±2.46	26.11±2.32	30.00±1.86	34.44±1.75	36.11±2.00	44.44±1.30	35.00^{E}±1.12
1	45.56±2.12	33.33±2.50	37.78±2.06	42.22±1.88	44.44±1.94	52.22±1.68	42.59^{D}±1.14
2	52.22±1.68	37.78±2.65	43.89±2.46	48.89±2.16	52.78±2.06	58.89±1.62	49.07^{C}±1.25
3	58.89±2.17	45.56±2.27	50.00±2.20	56.67±2.20	61.11±2.46	67.78±1.46	56.67^{B}±1.30
4	67.22±2.52	56.11±2.32	61.11±1.62	66.11±2.61	68.89±2.16	75.00±2.04	65.74^{A}±1.19
Overall mean	52.56^{b}±1.76	39.78^{e}±1.86	44.56^{d}±1.82	49.67^{c}±1.89	52.67^{b}±1.97	59.67^{a}±1.77	49.81

$^{A-E}$ Values with different superscripts within a column are significantly different (P<0.05).

$^{a-e}$ Values with different superscripts within a row are significantly different (P<0.05).

at day 0 of storage and increased significantly with the increase in time of storage at 5°C for 3 days. The interaction effect between storage time and green laser irradiation on dead spermatozoa was insignificant.

3.3. Acrosomal damage of spermatozoa (%)

The percentage of acrosomal damage was significantly (P<0.05) higher of spermatozoa exposed to 10 min of laser irradiation than other exposure times and the control group **(Table 4)**. In respect to the control group (27.73%), acrosomal damage (%) decreased significantly (P<0.05) with spermatozoa exposed to 2 and 4 min of laser irradiation with an average value of 24.69 and 25.76 %, respectively. Similar trends were observed by Iaffaldano et al (2010), who reported that non-irradiated rabbit spermatozoa had significantly lower acrosome intact than those irradiated with 3.96 and 9.0 J/cm^2 (P<0.05) and 6.12 J/cm^2 (P<0.01) at the first day of storage. Moreover, higher percentages (P<0.01) of intact acrosomes were recorded with spermatozoa exposed to 6.12 J/cm^2 than the control.

Table 4. Mean percentage of acrosomal damage of epididymal camel spermatozoa, with different exposure times (min) of green laser irradiation during storage at 5°C for up to 4 days

Storage time (days)	Laser exposure times (min)						Mean
	Control	2	4	6	8	10	
0	20.22±0.57	19.44±0.56	20.11±0.67	21.11±0.75	21.44±0.65	24.33±0.68	21.11^{E}±0.33
1	24.22±0.55	21.67±0.78	22.56±0.86	23.56±0.66	24.67±0.62	27.22±0.60	23.98^{D}±0.36
2	28.11±0.73	24.67±0.79	25.78±0.66	27.44±0.68	28.22±0.68	30.67±0.76	27.48^{C}±0.38
3	31.33±0.55	27.33±0.79	28.67±0.57	30.33±0.67	31.44±0.60	33.67±0.57	30.46^{B}±0.37
4	34.78±0.46	30.33±0.68	31.67±0.57	33.44±0.47	34.67±0.55	36.44±0.41	33.56^{A}±0.35
Overall Mean	27.73^{bc}±0.81	24.69^{e}±0.66	25.76^{d}±0.68	27.18^{c}±0.73	28.09^{b}±0.75	30.47^{a}±0.71	27.32

$^{A-E}$ Values with different superscripts within a column are significantly different (P<0.05).

$^{a-e}$ Values with different superscripts within a row are significantly different (P<0.05).

The present study showed that the prolongation of storage period at 5°C for 4 days increased (P<0.05) the acrosomal damage (%) at all exposure times of laser irradiation and the control group **(Table 4)**. The current results agreed with Ijaz and Hunter (1989). Similarly, Abdalla et al (2011) found that prolongation of storage period at 5°C for 3 days increased (P<0.05) the acrosomal damage (%) of the dromedary camel spermatozoa. The interaction effect between storage time at 5°C for 4 days and green laser irradiation on acrosomal damage of spermatozoa was insignificant.

4 Conclusion

It could be recommended for the collection and storage of the epididymal spermatozoa of the dromedary camels at 5°C for artificial insemination by being exposed to 6 min of green laser irradiation with short-wavelength (532 nm, 3 mW) to reinforce the fertilizing ability of she-camel, especially in the desert regions where liquid nitrogen may not be available for freezing of semen for a long time.

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أجريت هذه الدراسة على عدد 40 خصية من إجمالى عدد 20 من الإبل السودانى (أكبر من 5–10 سنوات، 500–600 كجم وزن حى) لمعرفة تأثير التشعيع بالليزر الأخضر ذات الطول الموجى القصير (532 nm, 3 mW) على جودة الحيوانات المنوية البربخية للإبل عند التعرض لفترات مختلفة 0 (مجموعة المقارنة، غير المشععة)، 2، 4، 6، 8، 10 دقيقة للتشعيع بالليزر. بعد التعرض للتشعيع تم تقدير كلً من النسبة المئوية لحركة الحيوانات المنوية، قابلية التخزين، الحيوانات المنوية الميتة، تلف الأكروسوم فى الحيوانات المنوية المحفوظة على درجة حرارة 5 درجة مئوية لمدة 4 أيام وذلك بعد تخفيف الحيوانات المنوية البربخية بمخفف اللاكتوز سترات. أوضحت النتائج أن أعلى قيمة للنسبة المئوية لحركة الحيوانات المئوية وكذلك قابليتها للتخزين مسجلة فى الحيوانات المنوية المعرضة للتشعيع بالليزر لمدة 6 دقائق وأقل قيمة فى مجموعة المقارنة وذلك أثناء الحفظ على درجة 5 درجة مئوية. من ناحية أخرى كانت أعلى قيمة للنسبة المئوية لكلً من الحيوانات المنوية الميتة وتلف الأكروسوم فى الحيوانات المنوية مسجلة فى الحيوانات المنوية المعرضة للتشعيع بالليزر لمدة 10 دقائق وأقل قيمة مسجلة عند دقيقتين من التشعيع وذلك أثناء الحفظ على درجة 5 درجة مئوية. إنخفضت النسبة المئوية لحركة الحيوانات المنوية وكذلك قابليتها للتخزين إنخفاض معنوى (على مستوى 0.05) مع زيادة النسبة المئوية للحيوانات المنوية الميتة وتلف الأكروسوم بالتقدم فى مدة الحفظ على درجة حرارة 5 درجة مئوية لمدة 4 أيام. يترتب على ذلك، أن الليزر تقنية فعالة من حيث التكلفة ويمكن استخدامها لتحسين برامج التلقيح الاصطناعي وجودة السائل المنوى. تضمنت الأثار الإيجابية للتشعيع بالليزر على جودة الحيوانات المنوية البربخية للإبل زيادة نسبة حركة الحيوانات المنوية، قابليتها للتخزين، القدرة على الحياة وسلامة الأكروسوم وهذا يعتبر مؤشرعلى تحسين وظيفة الميتوكوندريا التي تؤدي بدورها إلى تعزيز بقاء الحيوانات المنوية حية لفترة أطول.