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Full Length Research Paper

Scrotal, testicular and semen characteristics of the mountain gazelle (*Gazella gazella*) males

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This study aimed to evaluate seasonal differences in the characteristics of various male reproductive organs and in semen quality. Ten adult males of mountain gazelles (*Gazella gazelle)* were used in this study during summer and winter. The results showed significant differences in July to September versus October to December for volume (*P*≤0.05), motility and viability (*P*≤0.01), progressive motility, and morphologic alteration (*P*≤0.001). Hence it was concluded that sperm characteristics are strongly influenced by season. This study therefore suggested that the suitable period for semen collection is the breeding season (October to December), which has a good quality for manipulation and long-term preservation of *G. gazelle* in Saudi Arabia.

Key words: *Gazelle*, reproduction, semen, sperm, cryopreservation.

INTRODUCTION

The Arabian sand gazelles *(Gazella subgutturosa marica)* is classified as vulnerable (VU) on the IUCN Red List (IUCN Red, 2009) and listed under appendix II of the convention on migratory species (CMS, 2005). One subspecies is listed on the IUCN Red List: the Arabian sand gazelle (*G. s. marica*) is classified as vulnerable (IUCN Red, 2009). Nevertheless, the following sub-species are all considered vulnerable: Hillier's goitered gazelles or Mongolian gazelles (*G. s. hillieriana*), Yarkand or Xingjian goitered gazelles (*G. s. yarkandensis*) and the Persian gazelles (*G. s. subgutturosa*) (East, 1992; Groombridge, 1993). Scientific references on the Arabian Sand Gazelles concern only the ethological aspects, reintroduction and some researches on veterinary and diseases (KKWRC, 2008). No data has been published on reproductive physiology and sperm characteristics accept that was by (Al-Eissa, 1997, 2007a, 2007b).

Moreover, some studies described the reproductive season of Arabian sand gazelles and Compared its reproductive cycles to Persian gazelle (*Gazella subgutturosa subgutturosa*) (Sempere, 2001). However, the length of gestation for Arabian sand gazelles is about 148 to 160 days compared to gestation length between 160 to 170 days for *G. cuvieri*, *G. dorcas*, and *G. leptoceros*, as mentioned by Al-Eissa (in press). In addition, evaluation of spermatozoa is an important factor that must be accurately analyzed to ensure the use of animals with good fertility. These evaluations often include measuring volume, concentration, motility, morphology, and other characteristics that influence sperm functionality.

Since it is important to improve the knowledge on reproductive male characteristics for protection of this species from extinction, this study was undertaken to investigate some testicular and sperm characteristics of the mountain gazelles and to find out which are the suitable months for semen quality collection, manipulation

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and long-term preservation.

MATERIALS AND METHODS

Experimental animals

Ten adult males of mountain gazelles (*Gazella gazelle)* were used in this study. Animals were about three years old and around 14 kg weights. Animals were maintained and managed by the KKWRC, which is located in Riyadh, Saudi Arabia. Animals were transferred to an enclosure of 150 ha, while some animals were kept in small pens (0.5 ha) for research purposes. All animals were fed on dry alfalfa (*Medicago sativa*; min. 15% crude protein) and 1.5 L of water; quantities 10 to 15% above their average daily requirements (Ostrowski, 2006).

Extender

Various cryopreservation media (diluents) and Triladyl, a commercially available extender (Diluent A; Minitub, Tiefenbach, Germany), were used.

Semen collection and processing

The animals were captured by using trap made with canvas around food point, then were caught manually inside the trap. The animal's heads were covered immediately on capture, and no stress was noted during handling. This study was conducted during 2008 in two different periods: July to September and October to December. Semen was collected from the males using the electroejaculation procedure described by Al-Eissa (2007a, 2007b), which typically involved less than 10 stimulations up to maxima of 4.5 to 7.0 volts as described by Holt et al. (1988, 1996). The animals were anaesthetized using xylazine (8.4 ± 1.5 mg/kg body weight) and ketamine hydrochloride (6.9 ± 1.5 mg/kg body weight). Testicular measurements were taken with a caliper and a tape after restraining the gazelle with a single intravenous administration of xylazine (8.4 ± 1.5 mg/kg body weight and ketamine hydrochloride (6.9 ± 1.5 mg/kg body weight).

Testicular length was measured by placing the fixed arm of the caliper at the proximal end and the sliding arm at the distal end of the testis. Care was taken to exclude the epididymides. Thickness or depth was measured by placing the fixed arm of the caliper at the anterior aspect, and the sliding arm at the posterior aspect of each testis, at the point of maximum depth. Width of each testis was measured by sliding the other testis up in the scrotum and placing one arm of the caliper at the medial aspect and other at the lateral aspect, at the point of maximum width. For measurement of scrotal circumference the testicles were pushed firmly into the bottom of the scrotum by placing the thumb and fingers laterally on the side of neck of the scrotum and pushing ventrally. A flexible cloth tape was formed into a loop and slipped over the scrotum, and scrotal circumference was measured in centimeters by pulling the tape snugly around its greatest diameter (Elmore et al., 1976). Testes volume was calculated by using the formula for volume of an ellipsoid as described by Pant et al. (2003). Weight of the testes was calculated by multiplying volume with 1.038, which is the approximate density of testicular tissue (Amann, 1990). The seminal collection was obtained by electroejaculation as mentioned earlier. Volume was measured in a sterile glass tube graduated with 0.1 ml optical visible intervals, then, semen was diluted 1:100 with Triladyl and placed in water bath at 37°C. The evaluation was performed at the laboratory within 1 h.

Semen evaluation

Sperm volume, concentration and subjective scores of motility (wave motion, individual and progressive motility) were determined within 30 to 60 min of collection. The volume was measured by aspirating semen with a micropipette. Concentration was estimated using a haemocytometer. Semen was diluted (1:200) with an eosin solution (Madan et al., 1994). Motility was assessed by placing 10 µl of sperm suspension between a glass slide and a 22 mm × 22 mm cover slip, pre-warmed to 37°C. The proportion of motile spermatozoa and of spermatozoa with progressive motility (among motile ones) in each sample was assessed subjectively. In addition, motility was rated by using a scale 0 to 5 (0: non-motile; 5: highly motile), while the individual and progressive motility) were determined by using eosin solution by placing 10 µl of sperm suspension on the slide without cover slip.

Sperm plasma membrane integrity was referred to viability by using acridine orange (AO), ethidium bromide (EB) and FluroQuench. Fluorescent dye has been used for analysis of DNA integrity of somatic cell nuclei and mammalian sperm nuclei. The AO staining ability of mammalian sperm was investigated to clarify the differences of its fluorescent emissions according to Kosower et al. (1992). However, stainability of sperm nuclei depends on the number of S-S bonds in sperm nuclear chromatin, and nuclear maturation of sperm can be analyzed by using AO fluorescence dye. A semen sample was mixed with 5 L of AO and incubated at 37°C for 10 min, then the sperms were evaluated with an epifluorescence microscope (Nikon, Eclipse E 600 w.), at 1000 × magnification. Green spermatozoa (AO) were considered as alive, while those partially or completely red (EB) were considered dead. The percentage of plasma membrane intact spermatozoa was determined on at least 200 spermatozoa. Percentage of morphologic alterations was determined by analysis of sperm with normal morphology by using Spermac stain Enterprises, Wellington, (South Africa), (Baran et al., 2004; Schafer and Holzmann, 2000). A total of 200 cells were investigated by using the phase contrast microscopy (1000×).

Statistical analysis

Scrotal circumference and testicular diameters were statically analyzed by using *t*-test. Volume, concentration, motility, progressive motility, viability and morphologic alterations were analyzed by using one-way ANOVA test. Correlations were done for these parameters using SPSS version16 (Sendecor and Cachran, 1980).

RESULTS

Testicular size as a proportion of body weight varies widely. In the mammalian of Saudi Arabia, there is a tendency for testicular size to correspond with multiple mates. Production of testicular output sperm and spermatic fluid is also larger in polygamous animals, possibly a spermatogenic competition for survival. Also, testis weight varies in seasonal breeders. In this study, the testicular measurement was summarized in Table 1.

Sperm characteristics, viability and sperm morphologic alterations during July to September and October to December periods are reported in Table 2. The statistical analysis showed significant differences in July to September versus October to December for volume ($P \leq 0.05$), motility and viability ($P \leq 0.01$), progressive

Table 1. Scrotal and testicular measurements in July to September period and October to December period, considered as non-breeding and breeding season, respectively.

Measurement	July to September means (±S.D.)	October to December means (±S.D.)
Scrotal circumference(cm)	10.4 ± 0.14	12.4 ± 0.12
Testis length (cm), right testicle	3.8 ± 0.28	4.0 ± o.24
Testis length (cm), left testicle	3.3 ± 0.31	3.7 ± 0.21
Testis width (cm) , right testicle	1.8 ± 021	2.2 ± 0.31
Testis width (cm), left testicle	1.5 ± 0.41	1.9 ± 0.11
Testicular volume (cm^3)	87 ± 2.1	103.8 ± 1.7

Table 2. Seminal characteristics calculated at 1, 10 and 24 h July to September period and October to December period considered as non-breeding and breeding season, respectively.

Parameters	July to September means (±S.D.)			October to December means (±S.D.)		
Time post-collection (h)	1	10	24	1	10	24
Volume (ml)	0.19 ± 0.026	-	-	0.418 ± 0.077	-	-
Concentration (×106/ml)	574.17 ± 108.2	-	-	910 ± 59.89	-	-
Total motility (%)	49 ± 10.46	20 ± 9.5	0	75.33 ± 4.82	32.1 ± 6.4	53.7 ± 3.5
Progressive motility (%)	37 ± 8.16	5.0 ± 2.1	0	73 ± 4.53	30.2 ± 2.1	43.9 ± 2.9
Viability (%)	48.3 ± 9.03	18.8 ± 6.1	4.7 ± 2.5	85.33 ± 3.3	38 ± 4.2	10.9 ± 4.5

motility, and morphologic alteration ($P \leq 0.001$). Testicular diameters in July to September and October to December periods were determined and a light asymmetry between right and left testis in every single animal was observed. The scrotal circumference was significantly different in July to September than in October to December periods. More also, semen color was watery/opalescent during July September and creamy during October to December. Correlation coefficients of semen characteristics with testicular measurements and live weight are shown in Table 3. Scrotal circumference and width showed the largest coefficients (r = 0.8 to 0.85) with the semen volume and the total sperm number in the ejaculate, followed by the number of live and normal sperm in semen (r = 0.70). Correlation coefficients of these attributes with the testicular length were lower (r = 0.45 to 0.50) and barely reached the 5% significant level.

DISCUSSION

The limitations of this research were the scant number of valued subjects (Chemineau, 1986), the large extension of the protected area and the restricted number of captures (Delgadillo et al., 1991) in comparison with the attempts. According to Karagiannidis et al. (2000), a significant relationship between testicular diameters, ejaculate volume and breeding season exists in male. This correlation indicated that the animals in reproductive activity with repeated coupling showed a reduction in semen volume for ejaculate. Other semen parameters values reported in bibliography in small ruminants were: volume 536 to 3786 µL, concentration 358 to 922 × 10^6 spermatozoa/ml, motility 53 to 81% and sperm abnormalities percentage 19.4 to 30% (Howard et al., 1983; Cassinello et al., 1998; Garde et al., 2003).

In this study, the mountain gazelles *(G. gazella)* semen characteristics were comparable. In fact, semen concentrations in mountain gazelle were 574 ± 108 and 910 ± 59 × 10^6 spermatozoa/ml, respectively, in July to September and October to December periods. Seminal quality parameters in Mountain gazelle during October–December period showed a TM of 49 ± 10.46 % and PM of 37 ± 8.16% after 1 h maintenance in Equitainer which was preserved at 54% after 34 h. In July to September period, the values showed a poor quality of semen, but the decrease was comparable with other studies. Value of progressive motility in October to December (73 ± 4.53) was significantly greater than in July to September period (37 ± 8.16) because some studies reported that Influence of motility was strongly correlated with IVF rates on the outcome of *in vitro* fertilization/ intracytoplasmic sperm injection with fresh vs. frozen testicular sperm (Park et al., 2003). So it could be suitable a semen collection for long-term semen preservation in the months of October to December. The degree of fluctuating asymmetry is positively related to the coefficient of inbreeding and negatively related to the proportion of normal sperm, suggesting that it is a reliable indicator of genetic stress and of ejaculate quality (Roldan et al., 1998).

Table 3. Correlation coefficients of semen characteristics with testicular measurements and live weight in the mountain gazelle.

Parameters	Scrotal circumference	Testis width	Testis length	Live weight
Semen volume	0.85***	0.79**	0.51	0.73**
Sperm concentration	0.32	0.36	0.54*	0.35
Percent live sperm	0.13	0.10	-0.04	0.01
Percent abnormal sperm	-0.16	-0.16	-0.15	0.01
Total sperm number	0.86**	0.83***	0.63*	0.76**
Number of live sperm	0.71**	0.66**	0.45	0.61*
Number of live and normal sperm	0.75***	0.72**	0.52	0.63*

*Significant at P<0.05; **significant at P<0.01; ***significant at P<0.001.

In mountain gazelle there seems to be a clear seasonal influence on both semen production and characteristics as it is also reported in some antelope breeds (Asher et al., 1993). In other antelope like goitered gazelles (*G. subgutturosa subgutturosa)* living in an arid environment conditions, intense sexual activity was observed during the breeding season (November to December), male gazelles control their harem, moving around with it when population density is low, but when population density is high some males become territorial and participate in reproduction, while others remain mobile in non-reproductive bachelor groups (Mambetdjumaev, 1970; Gorelov, 1972; Djevnerov, 1984; Blank, 1985). During the summer, most activity takes place in the late afternoon and early morning, consisting of leisurely walking and simultaneous grazing. At midday, herds take shelter in the shade, where they excavate shallow oval-shaped pits to lie in. During the cooler winter months, this midday break is significantly reduced, and sometimes even eliminated. If disturbed from its shelter, a goitered gazelle rapidly flees for 200-300 meters, pausing to assess the danger from this distance. A broad circular path is then taken back to the original resting spot. Extremely speedy, these gazelles can run up to 60 kmph/36 mph. Each animal generally consumes about 30% of its body weight in green matter per day, and can derive most of its needed moisture from it. In the spring and summer, groups may travel to water sources, but even still they rarely drink daily. Herds cover 10-30 kilometers per day in the winter, with these distances being reduced nearly tenfold in summer. Throughout much of their range, mountain gazelle undergo seasonal migrations. During the breeding season, adult males become territorial, using dung middens placed at strategic locations to indicate ownership. At this time, males emit hoarse bellows, and glandular activity increases significantly, with the result that the male is often seen smearing secretions on objects (Heptner et al., 1989) and (Walther, 1990). In contrary, (Al-Eissa et al., 2007a) reported a breeding season in late summer and autumn with high quantity (volume and concentration) and quality (percentage of motile and progressive spermatozoa, percentage of abnormal spermatozoa).

In the males, the breeding season in temperate areas seems to be different from the mountain gazelle. A possible hypothesis could be related to the Mountain gazelle different sensitivity to photoperiod or other influences on melatonin release. The studied periods (July to September and October to December) represented non-breeding and breeding season respectively, if compared with other study as carried out by Al-Eissa, 2007a), the results were conformable with the analysis of reproductive characteristics during whole year (Al-Eissa, 2007a). Semen characteristics of mountain gazelle are highly influenced by season. The acceptable semen production to try cryopreservation programs was produced in October to December, but more investigations are needed in order to identify the exact breeding period. Frozen semen could be utilized in artificial insemination for genetic studies, so as to increase genotypic variability and resolve the problem of rupicapra species extinction.

Conclusion

Since sperm characteristics are strongly influenced by season, this study therefore suggests that the suitable period for semen collection is the breeding season (October to December), which has a good quality for manipulation and long-term preservation of *G. gazelle* in Saudi Arabia.

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