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Determination of the Arabian Sand Gazelle Sperm and Acrosomes Defects by Using the Spermac Staining Technique

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ABSTRACT

This study aimed to investigate the normality and rate of acrosome, sperm head, mid-piece and sperm tail by using the Spermac staining technique. Semen from 10 males of Arabian sand gazelle was collected under anesthesia by electro-ejaculator once a month for 12 months during 2009. The morphological features (acrosome, head, mid-piece and tail) and total morphological defect were investigated by using Spermac stain technique under light microscope. The results showed that the mean of total morphological defects rate was 22.20±2.62%, while the acrosomal defect rate was 12.30±2.55%. The most observed acrosomal defect types were swollen, missed and dissolved acrosome. The most frequently defect seen were microcephalic, mid-piece defect, coiled tail and rarely double tails. There were considerable reduction in the number of sperm abnormalities. It was concluded at the end of this study the Spermac staining technique could provide a detailed observation for gazelle spermatozoa, especially the acrosomal region and could be employed in the determination of all types of defects.

Key words: Sperm defects, acrosom, morphology and artificial insemenation

INTRODUCTION

The Saudi Wildlife Commission (SWC) in Saudi Arabia make huge efforts in terms of keeping and breeding endangered species, with a view to preserve them from extinction and, ideally, to reintroduce them back in their natural habitats. The distribution of sand gazelles (*Gazella subgutturosa marica*) has declined dramatically during recent decades, apparently due to excessive hunting and habitat degradation. Today, sand gazelles survive only in small numbers in a few isolated parts of their former range. The SWC a research centers stand for doing some research on endangered species, one of them is the sand gazelles. In addition, this research centers exert some activities includes captive breeding, habitat protection and reintroduction (Haque and Smith, 1996).

The Arabian sand gazelle known also by goitred gazelle known locally as rheem, found in Saudi Arabia is thought to be a subspecies (*G. subgutturosa rnarica),* different from the one found in Persia (*G. subgutturosa subgut turosa)* (Harrison and Bates, 1991). However, captive populations

managed by the King Khaled Wildlife Research Center (KKWRC) near Riyadh. Some of the reproductive biotechnologies for endangered mammalian species are mentioned (Comizzoli *et al.*, 2000). In 1970, domestic livestock and deer have been successfully used in the experimental stages of biotechnological studies (IVF, ICSI and cloning) and represent a model for endangered gazelle species (Curry, 2000; Harnal *et al.*, 2001; Soler *et al.*, 2003; Purdy, 2006).

The success of artificial insemination is directly related to the quality of the collected semen and the microscopic morphological examination of spermatozoa is one of the most important tests to evaluate the potential fertility of semen. Morphologically and characteristics of the semen of three endangered species of gazelles (*Gazella dama mhorr, G. dorcas neglecta* and *G. cuvieri*) were studied (Cassinello *et al.*, 1998). The abnormal spermatozoa rates in the semen of some domestic and wild animal species are relatively high (Suwanpugdee *et al.*, 2009). There are no study on the Arabian sand gazelle and few studies on the spermatozoa morphology of the other gazelles (Cassinello *et al.*, 1998).

Ram with a normal spermatozoa rate higher than 70% are considered normospermic (producing spermatozoa normal in number and motility) and those with a rate lower than 20-50% are considered asthenospermic (the loss or reduction of spermatozoan motility). The abnormal spermatozoa morphology in (*Gazella gazella, Gazella dorcas* and *Gazella gazelle acaiae*) were investigated by Saragusty *et al.* (2006). In previous study it was found that the head of the Sand gazelle spermatozoon is 10±0.15 μm long and 6.8±0.04 μm wide, the spermatozoon having an average total length of 65-70 μm (Al-Eissa, 2007). The most frequently observed morphological defect types in gazelle semen are classified as follows: (1) Acrosomal abnormalities (knobbed acrosome, swollen acrosome, acrosome with abnormal borders); (2) Head abnormalities (detached head, narrow head, macrocephalic head, undeveloped head); (3) Mid-piece abnormalities (double mid-piece and midpiece defect) and (4) Tail abnormalities (coiled tail, bent tail) (Axner *et al.*, 1999). Total morphological defect rates recorded by researchers in cat semen using various staining (carbolfuchsin, eosin-nigrosin and papanicolaou) and fixation (formol saline fixed solution) techniques are 2-29% (Baran *et al.*, 2004).

The semen of three species of gazelles examined by Garde *et al.* (2003), also others three species of gazelles were described by Cassinello *et al.* (1998) and gave the results for normal spermatozoa as: 77.6±4.17% for *Gazelle cuvieri*, 59.5±6.81% for *Gazelle dama* and 80.6±7.21% for *Gazelle dorcas*. The abnormality head were: 5.0±0.99% for *G. cuvieri*, 10.5±3.89% for *G. dama mohorr* and 2.5±1.29% for *G. dorcas*. While, the abnormalities of midpiece were: 1.5±0.37% for *G. cuvieri*, 3.0±0.88% for *G. dama* and 1.0±0.33% for *G. dorcas*.

Soler *et al.* (2003) used three different staining methods for comparison and assessment of epididymal red deer sperm morphometry by computerized analysis with ISAS, they were Hemacolor (Merck standard kit, Darmstadt, Germany, Cat. No. 11661), Diff-Quik (Baxter DADE AG 3186, Du¨dingen, Switzerland) and Harris' Hematoxylin (Merck, Darmstadt, Germany, Cat. No 9253) were used to stain two smears from each sperm Sample.

Previously Spermac stain has been used for the staining of fresh and extended-semen from the bull, ram, goat, dog, horse, boar, cheetah and man (Chan *et al.*, 1996; Hay *et al.*, 1996; Oetjen, 1988; Oettle and Soley, 1985; Oettle, 1986a,b; Viggiano *et al.*, 1996; Watkins *et al.*, 1996) recently used for gazelle by Al-Eissa (2007). Spermac is metachromatic stain, is mainly used to make various degrees of acrosomal damages visible and additionally, some of the changes that occur during acrosomal reaction can be seen (Schafer and Halzman, 2000).

To the best of our knowledge very few studies have been done on the reproductive physiology of the Arabian sand gazelle (Al-Eissa, 2007; Al-Eissa *et al*., 2007, 2009). There is an urgent need in Saudi Arabia to study how to preserve the genetic material of endangered spices of gazelle and other wild animals.

MATERIALS AND METHODS

All of the experimental procedures were conducted in King Khalid Wildlife Research Center (KKWRC) (25°03'N, 46°45'E), Saudi Wildlife Commission (SWC), Riyadh, Saudi Arabia, 2009.

Ten males of Arabian Sand Gazelles aged 2-4 years (average 15-18 kg live weight each) served as semen donors. The males were kept in enclosure 100×100 m and all were vaccinated and good health well feed mainly on dry alfaalfa, some concentrated pellets and water.

Semen collection: Semen was collected from the males by using an electroejaculation procedure described by Al-Eissa (2007) and Al-Eissa *et al*. (2007, 2009) (Fig. 1). The animals were anaesthetized using Xylazine (8.4±1.5 mg kg^{-1} b.wt.) and ketamine hydrochloride 6.9±1.5 mg kg^{-1} b.wt.). During the electroejaculation procedure, which typically involved <10 stimulations up to maxima of 4.5-7.0 volts, also similar method was described by Holt *et al*. (1988, 1996).

Sperm morphometry: Semen was diluted with 100 μL of 0.9% NaCl after collection. Morphological defects were determined by staining with Spermac (Stain Enterprise, P.O. Box 12421, 0110, Onderstepoort, Republic of South Africa) stain kit. A drop of semen was placed on a glass slide and a thin smear was prepared and air-dried for 3 min.

The slide was then fixed for 5 min and washed with distilled water 5-6 times. Excess water was removed with a piece of filter paper and the slide was placed into stain solution A for 1-2 min before being totally dried. This procedure was repeated for solutions B and C. Finally, the slide was air dried. The smears were stained with Spermac and 200 spermatozoa were evaluated for abnormal acrosome, head, mid-piece and tail forms under a light microscope at x1000 magnification according to the method described by Axner *et al*. (1999). The acrosome, other parameters and total morphological defect mean values were evaluated by the SPPS (Statistical Package for Social Sciences program) version 11 and by using of ANOVA (one-way).

RESULTS

The data obtained from spermatozoa morphology investigation are presented in Table 1, the mean total morphological defect rates in the study was 22.20±2.62%, while the acrosomal defect rates was 12.30±2.55%. The most observed acrosomal defect types were swollen, missed and dissolved acrosome. The total other morphological defect (head, midpiece and tail) rates average was 10.20±3.1%. The most frequently scored defects were head microcephalic, Mid-piece thickness, coiled tail and rarely double tails. In addition, there were considerable reductions in the number of sperm abnormalities.

Table 1: Sperm morphological of arabian sand spermatozoa; morphology rate for fresh semen

Acrosome (%)	Other (head, midpice and tail)	Total abnormality (%)
12.30±2.55	10.20±3.1	22.20±2.62%

Mean±SD (Standard Deviation)

Fig. 1: Electro-ejaculator, UD 42100572

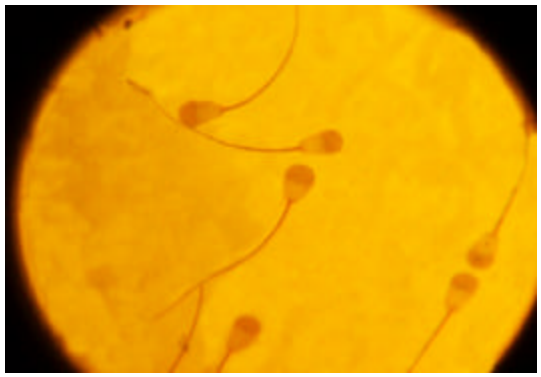

Fig. 2: Smear of Sand Gazelle semen under light microscope showing normal spermatozoa after staining with Spermac stain x400

Fig. 3: Smear of Sand Gazelle semen under light microscope showing normal head of spermatozoa after staining with Spermac stain x1000

The microscope examination illustrated normal spermatozoa and abnormal spermatozoa. Normal spermatozoa morphology were presented in Fig. 2-5 presented the normal shape of spermatozoa that consists of acrosome, mid-piece and tail (Fig. 3) showing the normal head shape of spermatozoa with green color (Fig. 4) showing post-acrosomal region of normal spermatozoa in red color and (Fig. 5) showing the equatorial segment of normal spermatozoa with light green color.

Fig. 4: Smear of Sand Gazelle semen under light microscope showing post-acrosomal region of normal spermatozoa after staining with Spermac stain x1500

Fig. 5: Smear of Sand Gazelle semen under light microscope showing the equatorial segment of normal spermatozoa after staining with Spermac stain x1000

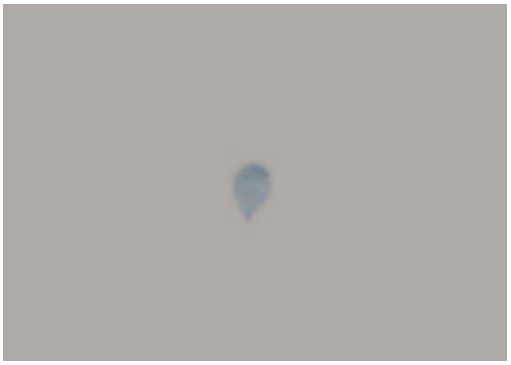

Fig. 6: Smear of Sand Gazelle semen under light microscope showing the swollen acrosome after staining with Spermac stain x1000

The most abnormal spermatozoa morphology observed were acrosomal defects types and the most frequently seen in the other morphological defects (head, mid-piece and tail) and those are:

- **Swollen acrosome:** The acrosomal cap looked swollen and its outer surface was irregular (Fig. 6)

Fig. 7: Smear of Sand Gazelle semen under light microscope showing missed acrosome after staining with Spermac stain x400

Fig. 8: Smear of Sand Gazelle semen under light microscope showing dissolved acrosome after staining with Spermac stain x1000

Fig. 9: Smear of Sand Gazelle semen under light microscope showing the head abnormalities: Microcephalic head after staining with Spermac stain x400

- **Missed acrosome:** Giving the appearance that part of the acrosome was missing or incomplete (Fig. 7)
- **Dissolved acrosome:** The acrosomal cap looked dissolved. The entire surface was characterized by swollen cords and irregularly shaped (Fig. 8)
- **Head micro cephalic:** Microcephalic means 'small head', these spermatozoa has smaller head than normal (Fig. 9)

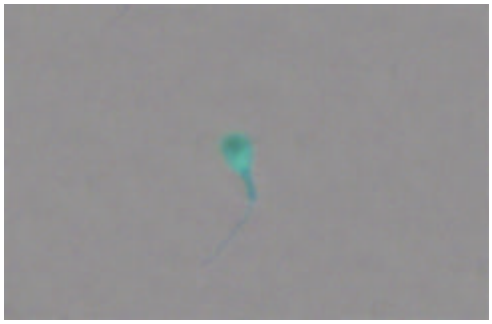

Fig. 10: Smear of Sand Gazelle semen under light microscope showing the mid-piece thickness after staining with Spermac stain x1000

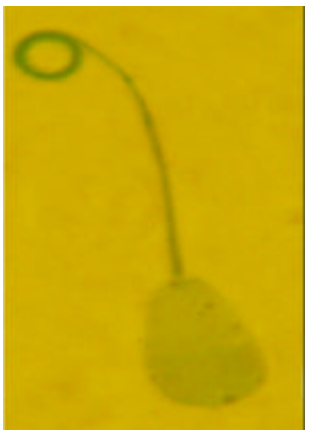

Fig. 11: Smear of Sand Gazelle semen under light microscope showing the coiled tail after staining with Spermac stain x1600

Fig. 12: Smear of Sand Gazelle semen under light microscope showing the double tail after staining with Spermac stain x1000

- **Mid-piece thickness:** The defect was characterized by a local thickening on the midpiece and also more likely to be irregular in shape (Fig. 10)
- **Coiled tail:** The tail was not angularly deviated and eventually assumed an undulated or spiral conformation; it was sometimes in the shape of a ring (Fig. 11)

- **Double tails (rarely):** It appeared to be made up of two flagella which were either completely joined or diverged from different points (Fig. 12).

DISCUSSION

The abnormal spermatozoon rates of fresh semen were 12.30±2.55% acrosome defects, 10.20±3.1% other defects and 22.20±2.62% total morphological defects. The acrosome defect rate was higher than the mean 11.1±2.23%, 12.1±3.72% for Dama gazelle (*Gazella dama)* and Dorcas gazelle (*Gazella dorcas)* but less than 17.2±5.77% for Cuvier gazelle (*Gazella cuvieri*) (Cassinello *et al*., 1998). The data showed that the damaged or modified apical ridge (DAR) was less than 13.8±4.06% and 14.0±6.97%, for abnormal principal/terminal piece for *Gazelle dama* and *G. dorcas*, respectively, but higher than 8.4±1.33% for *G. cuvieri*, this difference can be attributed to the different staining or/and fixative techniques used (Thurston *et al*., 1999; Abaigar *et al*., 1999). However, in the present study revealed that the mean of acrosome defect rate was 12.30±2.55%, in contrast, that value was less than the value of 17.9±12.4% which reported by Abaigar *et al*. (2001). Furthermore, the mean of total morphological defect level was 22.20±2.62% and this similar to the value 22.8±1.16% which reported by Roldan *et al*. (1998). Also similar to the 21.3±6.9% for other wild animal and lower than the 35, 57 and 59% for *Mountain gazelle (Gazella gazelle)*, Dorcas gazelle *(gazella dorcas)* and Acacia gazelle (*G. gazella acaiae)*, respectively (Saragusty *et al*., 2006).

The difference in acrosome and total morphological defect rates among the researchers may be due to the morphological evaluation techniques, the semen collection techniques and the breeds of gazelle used (Cassinello *et al*., 1998), compared the morphology of fresh semen of Dama gazelle (*Gazella dama)*, by using three staining methods included Formaldehyde, Glutaraldehyde and Eosin-nigrosin/Giemsa and observed that the efficacy of Formaldehyde for revealing head, midpiece, principal/terminal piece and defect in tails of *Gazella dama mhorr*. Unfortunately, no study available on using the Spermac for other species of gazelle to compare the defection and morphology of Acrosomal and other pieces of sperm. Several studies reported that the preparations with Spermac stain contained fewer protoplasmic droplet spermatozoa and attributed this to degeneration of the cytoplasmic droplet during staining (Baran *et al*., 2004).

The present study shows that the Spermac staining technique possesses the advantages of being rapid, reliable and practical and can successfully be used in the examination of acrosomal morphology in Gazelle semen, as with that of other species. In the light of our results, this technique is suggested to be beneficial in determining acrosome defects, which are one of the leading defect types in post-thaw semen.

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