

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 $22.20 \pm 2.62\%$, while the acrosomal defect rate was $12.30 \pm 2.55\%$. 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 insemination

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 mhorh*, *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 (carbofuchsin, 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: $77.6 \pm 4.17\%$ for *Gazelle cuvieri*, $59.5 \pm 6.81\%$ for *Gazelle dama* and $80.6 \pm 7.21\%$ for *Gazelle dorcas*. The abnormality head were: $5.0 \pm 0.99\%$ for *G. cuvieri*, $10.5 \pm 3.89\%$ for *G. dama mhorh* and $2.5 \pm 1.29\%$ for *G. dorcas*. While, the abnormalities of midpiece were: $1.5 \pm 0.37\%$ for *G. cuvieri*, $3.0 \pm 0.88\%$ for *G. dama* and $1.0 \pm 0.33\%$ 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 species 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 $22.20 \pm 2.62\%$, while the acrosomal defect rates was $12.30 \pm 2.55\%$. The most observed acrosomal defect types were swollen, missed and dissolved acrosome. The total other morphological defect (head, midpiece and tail) rates average was $10.20 \pm 3.1\%$. 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	$22.20 \pm 2.62\%$
Mean±SD (Standard Deviation)		



Fig. 1: Electro-ejaculator, UD 42100572

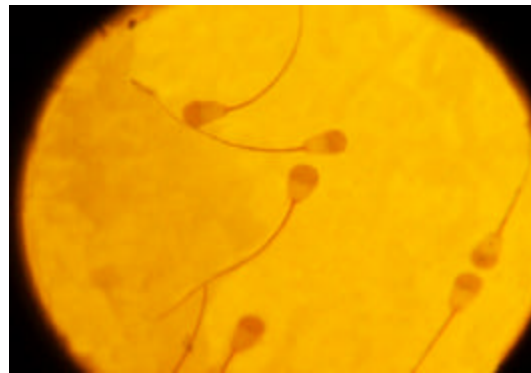


Fig. 2: Smear of Sand Gazelle semen under light microscope showing normal spermatozoa after staining with Spermac stain x400

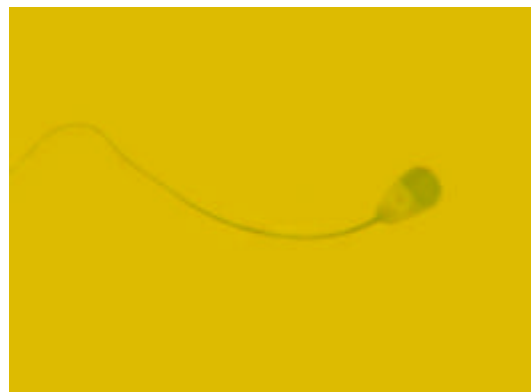


Fig. 3: Smear of Sand Gazelle semen under light microscope showing normal head of spermatozoa after staining with Spermac stain x1000

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

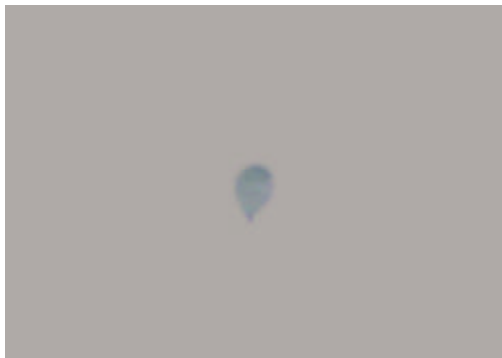


Fig. 6: Smear of Sand Gazelle semen under light microscope showing the swollen acrosome after staining with Spermac stain x1000

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)

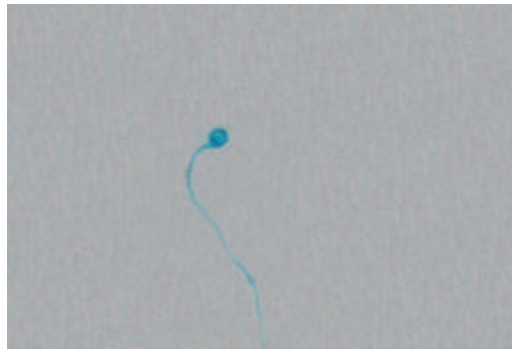


Fig. 7: Smear of Sand Gazelle semen under light microscope showing missed acrosome after staining with Spermac stain x400



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

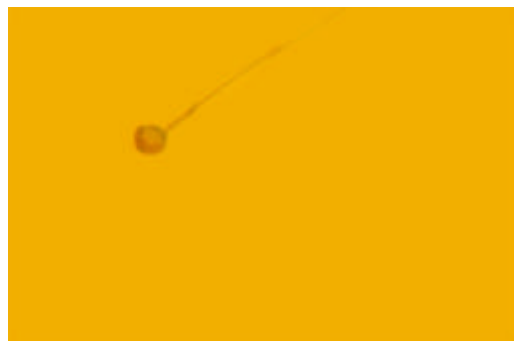


Fig. 9: Smear of Sand Gazelle semen under light microscope showing the head abnormalities: Microcephalic head after staining with Spermac stain x400

- **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)

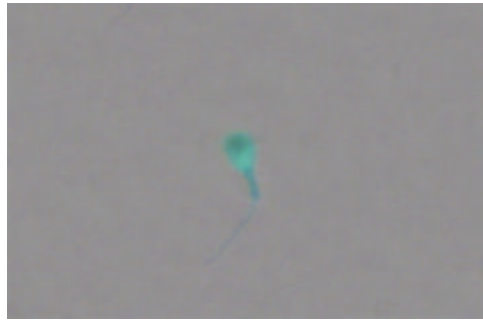


Fig. 10: Smear of Sand Gazelle semen under light microscope showing the mid-piece thickness after staining with Spermac stain x1000



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 $12.30 \pm 2.55\%$ acrosome defects, $10.20 \pm 3.1\%$ other defects and $22.20 \pm 2.62\%$ total morphological defects. The acrosome defect rate was higher than the mean $11.1 \pm 2.23\%$, $12.1 \pm 3.72\%$ for Dama gazelle (*Gazella dama*) and Dorcas gazelle (*Gazella dorcas*) but less than $17.2 \pm 5.77\%$ for Cuvier gazelle (*Gazella cuvieri*) (Cassinello *et al.*, 1998). The data showed that the damaged or modified apical ridge (DAR) was less than $13.8 \pm 4.06\%$ and $14.0 \pm 6.97\%$, for abnormal principal/terminal piece for *Gazelle dama* and *G. dorcas*, respectively, but higher than $8.4 \pm 1.33\%$ 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 $12.30 \pm 2.55\%$, in contrast, that value was less than the value of $17.9 \pm 12.4\%$ which reported by Abaigar *et al.* (2001). Furthermore, the mean of total morphological defect level was $22.20 \pm 2.62\%$ and this similar to the value $22.8 \pm 1.16\%$ which reported by Roldan *et al.* (1998). Also similar to the $21.3 \pm 6.9\%$ 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 mhorrr*. 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.

REFERENCES

- Abaigar, T., W.V. Holt, R.A.P. Harrison and G. Del-Barrio, 1999. Sperm subpopulations in boar (*Sus scrofa*) and gazelle (*Gazella dama mhorrr*) semen as revealed by pattern analysis of computer assisted motility assessments. Biol. Reprod., 60: 32-41.
- Abaigar, T., M. Cano, A.R. Pickard and W.V. Holt, 2001. Use of computer-assisted sperm motility assessment and multivariate pattern analysis to characterize ejaculate quality in Mohor gazelles (*Gazella dama mhorrr*): Effects of body weight, electroejaculation technique and short-term semen storage. Reproduction, 122: 265-273.
- Al-Eissa, M.S., 2007. Assessment of reproductive efficiency of the Arabian Sand Gazelle males (*Gazella subgutturosa marica*) and semen cryopreservation. Ph.D. Thesis, King Saud University, College of Science, Department of Zoology.

- Al-Eissa, M.S., A.R. Al-Hamidi and S. Kandeal, 2007. Semen cryopreservation using triladyl and tris diluents of the Arabian Sand Gazelle males (*Gazelle Subgutturosa marica*). Arabian Gulf J. Sci. Res., 25: 199-206.
- Al-Eissa, M.S., M.A.E.K. Sandouka, S.H. Alkahtani and A.A. Qurtam, 2009. Reproductive characteristics of the Arabian Sand Gazelles (*Gazelle Subgutturosa marica*). J. Saudi Soc. Agric. Sci., 9: 70-80.
- Axner, E., C. Linde-Forsberg and S. Einarsson, 1999. Morphology and motility of spermatozoa from different regions of the epididymal duct in the domestic cat. Theriogenology, 52: 767-778.
- Baran, A., B.E. Sahin, M. Evecen, K. Demir and I.K. Ileri, 2004. Use of spermac staining technique in the determination of acrosomal defects in cat semen. Turk. J. Vet. Anim. Sci., 28: 519-525.
- Cassinello, J., T. Abaigar, M. Gomendio and E.R.S. Roldan, 1998. Characteristics of the semen in three endangered species of gazelles (*Gazella dama mhorrr*, *G. dorcas neglecta* and *G. cuvieri*). J. Reprod. Fertility, 113: 35-45.
- Chan, P.J., J.U. Corselli, J.D. Jacobson, W.C. Patton and A. King, 1996. Correlation between intact sperm acrosome assessed using the spermace stain and sperm-fertilizing capacity. Arch. Androl., 36: 25-27.
- Comizzoli, P., P. Mermillod and R. Mauget, 2000. Reproductive biotechnologies for endangered mammalian species. Reprod. Nutr. Dev., 40: 493-504.
- Curry, M.R., 2000. Cryopreservation of semen from domestic livestock. Rev. Reprod., 5: 46-52.
- Garde, J.J., A.J. Soler, J. Cassinello, C. Crespo and A.F. Malo *et al.*, 2003. Sperm cryopreservation in three species of endangered gazelles (*Gazella cuvieri*, *G. dama mhorrr* and *G. dorcas neglecta*). Biol. Reprod., 69: 602-611.
- Haque, M.N. and T.R. Smith, 1996. Reintroduction of Arabian sand gazelle (*Gazella subgutturosa marica*) in Saudi Arabia. Biol. Conserv., 76: 203-207.
- Harnal, V.K., R. Spindler, S.L. Monfort, B. Pukazhenth, D.M. Bird and D.E. Wildt, 2001. Sperm capacitation *in vitro* in the eld's deer. Theriogenology, 56: 399-413.
- Harrison, D.L. and P.J.J. Bates, 1991. The Mammals of Arabia. 2nd Edn., Harrison Zoological Museum, England, ISBN: 0951731300, pp: 345.
- Hay, M.A., W.A. King, C.J. Gartley, S.P. Leibo and K.L. Goodrowe, 1996. Evaluation of fertilizing potential of fresh, cooled and frozen-thawed canine spermatozoa. Proceedings of the 3rd International Symposium on Reproduction of Dogs, Cats and Exotic Carnivores, Sept. 1996, Utrecht, pp: 31-31.
- Holt, W.V., H.D. Moore, R.D. North, T.D. Hartman and J.K. Hodges, 1988. Hormonal and behavioural detection of oestrus in blackbuck, *Antelope cervicapra* and successful artificial insemination with fresh and frozen semen. J. Reprod. Fertility, 82: 717-725.
- Holt, W.V., T. Abaigar and H. Jabbour, 1996. Oestrus synchronization, semen preservation and artificial insemination in the Mohor gazelle (*Gazella dama mhorrr*) for the establishment of a genome resource bank programme. Reprod. Fertility Dev., 8: 1215-1222.
- Oetjen, M., 1988. Überprüfung verschiedener akrosomfärbungen zur qualitätsbeurteilung von nativ-und tiefgefriersperma des pferdes. Ph.D Thesis., Tierärztliche Hochschule, Hannover.
- Oettle, E.E. and J.T. Soley, 1985. Infertility in a maltese poodle as a result of a sperm midpiece defect. J. S. Afr. Vet. Assoc., 56: 103-106.
- Oettle, E.E., 1986a. Using a new acrosome stain to evaluate sperm morphology. Vet. Med., 81: 263-266.
- Oettle, E.E., 1986b. Changes in acrosome morphology during cooling and freezing of dog semen. Anim. Reprod. Sci., 12: 145-150.

- Purdy, P.H., 2006. A review on goat sperm cryopreservation. *Small. Rumi. Res.*, 63: 215-225.
- Roldan, E.R.S., J. Cassinello, T. Abaigar and M. Gomendio, 1998. Inbreeding, fluctuating asymmetry and ejaculate quality in an endangered ungulate. *Proc. Biol. Sci.*, 265: 243-248.
- Saragusty, J., H. Gacitua, R. King and A. Arav, 2006. Post-mortem semen characterization and cryopreservation in two different endangered gazelle species (*Gazella gazella* and *Gazella dorcas*) and one subspecies (*Gazella gazella acaiae*). *Theriogenology*, 66: 775-784.
- Schafer, S. and A. Holzman, 2000. The use of transmiration and SpermacTM stain to evaluate epididymal cat spermatozoa. *Anim. Reprod. Sci.*, 59: 201-211.
- Soler, A.J., V. Astore, A. Sestelo, M. Rivolta, L.N. Jacome and J.J. Garde, 2003. Effect of thawing procedure on cryosurvival of deer spermatozoa: Work in progress. *Theriogenology*, 60: 511-520.
- Suwanpugdee, A., K. Kornkeawrat, K. Saikhun, B. Siriaronrat and W. Tipkantha *et al.*, 2009. Semen characteristics and sperm morphology of serow (*Capricornis sumatraensis*). *Theriogenology*, 71: 576-585.
- Thurston, L.M., P.F. Waston and W.V. Holt, 1999. Sources of variation in the morphological characteristics of sperm subpopulations assessed objectively by a novel automated sperm analysis system. *J. Reprod. Fert.*, 117: 271-280.
- Viggiano, J.M., M.B. Herrero, S.P. Martinez and M.F. De-Gimeno, 1996. Analysis of the effect of nitric oxide synthase inhibition on mouse sperm employing a modified staining method for assessment of the acrosome reaction. *J. Androl.*, 17: 692-698.
- Watkins, A.M., P.J. Chan, W.C. Patton, J.D. Jacobson and A. King, 1996. Sperm kinetics and morphology before and after fractionation on discontinuous *Percoll gradient* for sex preselection: Computerized analyses. *Arch. Androl.*, 37: 1-5.