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Semen characteristics and fertility assessment of *Sennar* jackass *(Equus asinus)* in Ethiopia

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The aim of this study was to evaluate the semen characteristics and fertility of *Sennar* jacks. Semen was collected between January and March using Missouri AV model and was subjected to gross and microscopic evaluation. Fertility was evaluated from pregnancy rate after AI was carried out with fresh semen on 12 Abyssinian jennies, 5 *Sennar* jennies and 17 local mares. Mean (±SD) total and gel-free volume; and spermatozoa concentration were 61.1±12.6 ml, 50.3±12.3 ml, and 257± 8.1 x 10^6/ml, respectively. Total and progressive sperm motility, sperm viability and abnormal sperm percentage were 84.2±2.1; 67.4 ± 6.1%, 89%, and 10.9±2.9, respectively. There was no significant individual difference in most semen parameters. Pregnancy rate was 40% (2/5) in *Sennar* jennie, 58.3% (7/12) in Abyssinian jennies and 64.7% (11/17) in mares. The study thus revealed that semen can be successfully collected and evaluated as part of a breeding soundness examination of *Sennar* jacks during cross breeding using AI. Further study on cryopreservation of semen and improving pregnancy rate after extending semen in optimized donkey semen extender is necessary in the future.

Key words: AI, fertility, *Sennar* jacks, semen, Ethiopia.

INTRODUCTION

With an estimated 6.2 million heads (CSA, 2011), donkeys are known to play a great role particularly in rural areas of Ethiopia, where they are used on a daily basis to carry out numerous tasks in the house and agricultural fields (Alemu et al., 1997). Classifications of Ethiopian donkeys based on size and coat colour, includes four major types namely *Abyssinian, Jimma, Ogaden,* and *Sennar* Donkeys (Fesseha, 1991). The *Sennar* donkey is by far the largest and the only one reputed for producing good mules. The natural habitat of the *Sennar* donkeys is the Northwestern lowland of Ethiopia. Donkey crossing with selected descendants of the Kessella Nubian asses is a very common practice, while mule production from Sudan *Sennar* donkeys is more common in the highlands around Ethio-Sudan border. Mating, in almost all cases, except for the crossing, is uncontrolled and hence usually associated with year-round foaling.

Due to their distinct phenotypic features, *Sennar* donkeys are expensive and are not easily available in all

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areas of the country. Moreover, anecdotal information suggests that the work performance of *Sennar* donkeys is nearly twice that of Abyssinian types. Regardless, there is little published information on their genetic potential, selection, and reproductive management. Particularly, no work has been initiated to consider the use of AI, a technology which is relatively well developed in horses (Squires, 2005). At natural mating, the average fertile jack ass ejaculates 3.3-18 billion spermatozoa directly into the body of the uterus. Fewer than 100 spermatozoa pass through the uterotubal junction to reach the site of fertilization to give a per cycle conception rates of 60 – 70% (Allen et al., 2001; Hagstrom, 2004). AI in donkeys can improve the reproductive performance; however, expanded use of frozen semen is dependent on proper laboratory assessment of sperm quality as an essential procedure of the AI technology. Mares bred with frozen semen are often examined 4-6 times/day and inseminated immediately before or within 6 h post-ovulation because of lower survivability of spermatozoa in the reproductive tract (Squires et al., 2003; Contri et al., 2012). Another study (Samper, 2005) has shown that deep insemination into the horn ipsilateral to the ovary with the pre-ovulatory follicle results in 80% of the sperm remaining in that oviduct with-higher conception. On the other hand, with the apparent differences from horses, the efficient application of AI in donkeys requires an understanding of peculiar semen characteristics (Rota et al, 2012; Qeusada et al., 2012). This study was aimed at assessing *Sennar* donkey semen characteristics and evaluation of fertility of fresh ejaculate through AI.

MATERIALS AND METHODS

Animals

A total of 5 *Sennar* jacks previously selected for breeding at *Sennar* Donkey Multiplication Centre (Wekin, North Ethiopia) were used. The jacks were aged between 6 and 8 years, and had an average BCS of 7 (on 1-9 scale, Pearson and Quasat, 2000) body weight ranging 243 to 280 kg. The jacks were mainly used to produce donkey crosses and mules. Study jacks were allowed to graze in the field freely and were supplemented with ample amount of hay and concentrate. Water was provided *ad libitum*. All jacks were dewormed against common parasites, and vaccinated against African Horse Sickness and Anthrax before introduction to the stable.

Collection and evaluation of semen

The jacks were allowed to individually interact with jennies well into oestrus for 30 min. Semen was collected after the jacks were sufficiently stimulated using a Missouri model equine AV (Agatech, Manhattan, USA) twice a week for a total of 25 collections between January and March. Immediately after collection, the color and the total volume of the each ejaculate was recorded. Semen was then filtered and the gel-fraction removed and placed in water bath at 37°C. Aliquot of 5 μl semen was removed from the gel-free fraction for each of the following microscopic evaluation: total motility, progressive motility and sperm viability, abnormal sperm percent, pH was determined using strip. Sperm concentration (10^6/ml) was measured using Neubauer hemocytometer. Total and progressive motility were evaluated by phase contrast microscopy at ×100 and ×200 magnifications. The differential staining was made after taking a 10 μl aliquot of the semen sample mixed at 1:1 ratio of with 3% sodium citrate buffer and 1% eosin and made into thin smear. The slide was quickly dried on a pre-warmed plate before evaluation. Live percent and percent abnormal sperm were determined under light microscope (oil immersion, x1000) after counting 200 spermatozoa. White (unstained) sperm was classified as live and those that showed pink or red coloration were classified as dead. Morphological defects were classified into head, mid-piece or tail defects.

Fertility assessment

Fresh semen was extended in 1:1 volume by volume ratio of semen and a modified equine semen extender (prepared from 100 ml skimmed milk, 2.5 ml egg yolk, 4 g of glucose, 150,000 IU crystalline penicillin and 150,000 μg streptomycin) (Davies-Morel, 1999). Sperm longevity was evaluated at 15 min intervals for the first one hour, followed by final evaluation after one hour. The semen was then evaluated again after 24 h of chilling in Equitainer-I Tube Style (Agtech, Inc., Manhattan, U.S.A.) at +6°C. Spermatozoa fertilizing ability was afterwards determined by inseminating 17 jennies (5 *Sennar* jennets, 12 Abyssinian jennets) and 17 mares that were induced for estrus prior to AI. Both the jennies and mares used for AI were selected based on their previous history of breeding and each received 1ml prostaglandin (Clorprostenol, Pharmacia and Upjohn Company, USA) for induction of estrus. Jennies and mares were followed for manifestation of estrus signs. Semen used for insemination was collected the day of AI, prepared to make up $100x10^6$/ml (4 ml of semen; $400x10^6$ sperm per insemination) Insemination was carried out after ovulation (but as close to time of ovulation as possible) which was determined by ultrasound. A catheter was inserted per vaginum into the uterus and semen was deposited using a plunger free syringe. Small amount of air was pushed into the catheter to gently drive out the remaining semen. Pregnancy was diagnosed using ultrasonography (Mindray, Hong Kong) after 30 days post insemination. Fertility assessment was performed from pregnancy rate per insemination.

Statistical analysis

All collected data was stored in Microsoft Excel data sheet. The statistical analysis was performed using SPSS for Windows (Version-15) and STATISTICA for Windows (version 6, Statsoft, USA). The data was summarized using descriptive statistics. Comparison between jacks in fresh semen parameters was done using One Way ANOVA. Correlation between variables was computed using Pearson correlation (r). Differences were considered significant when $P< 0.05$.

RESULTS

A total of 25 semen collection procedures were carried out. Summary of fresh semen parameters are given in Table 1. The most prominent pre-coital sexual displays observed during teasing included nibbling and/or sniffing of the vulva, head, neck and back of the knee, flank, perineum and tail; Olfactory investigation of voided urine, flehman response, mounting with and without erection (Figure 1) and naso-nasal contact. Once a jack gets

Table 1. Fresh semen characteristics in *Sennar* jacks (n=25 collections).

Parameter	Mean (±SD)	Range
Total semen volume (ml)	61.1 ± 12.6	25 - 100
Gel-free volume (ml)	50.3 ± 12.3	15 - 85
pH	7.4 ± 0.1	6.5 – 7.7
Semen concentration (10^6/ml)	257 ± 8.1	65 – 387
Total sperm motility (%)	84.2± 2.1	78 - 92
Progressive sperm motility (%)	67.4 ±6.1	60 - 75
Sperm viability (%)	89.1 ± 2.3	82 – 94
Morphologically normal sperm (%)	89.0 ± 2.9	80 - 94

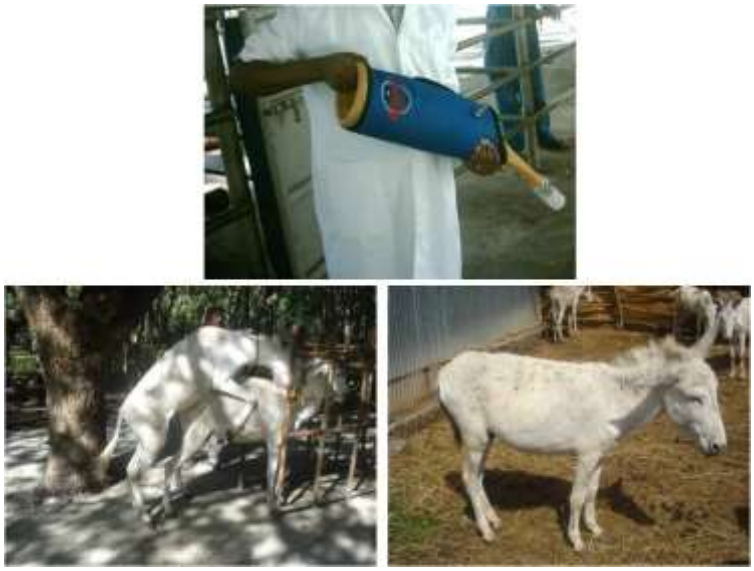

Figure 1. Semen collection in *Sennar* donkeys. Top: Missouri model equine AV used to collect *Sennar* jacks (Bottle cover removed), Left: False mount without erection during teasing, Right: Jack after semen collection displaying refractory isolation from estrous jennies.

erection, then it took on average less than two minutes to reach full erection and eventually being collected.Semen was collected successfully from all males on all occasions.Jacks entered into refractory period and often isolated themselves from the jenny in estrus after ejaculation.

Semen color was creamy white (91.5%), or milky (85%). There was no significant difference in semen parameters among individual jacks except for pH of semen. Live percent was successfully estimated in 1% eosin stain. Sperm abnormality was also fairly identifiable in the same slide prepared for determining sperm viability. The most common sperm defect was bent tail (40.9± 2.9%; $p<0.05$) as compared to head (17.6±01%) and other sperm defects. Sperm longevity declined with time and total motility was reduced to <60% at 2 h post ejaculation at 37°C water bath temperature. However, after 24 h of chilling, total motility remained at 36.2±4.3% while progressive motility was 24 ±7.4%.

Pregnancy rate was 40% (2/5) in *Sennar* jennies, 58.3% (7/12) in Abyssinian jennies and 64.7% (11/17) in mares. Pregnancy confirmation at early stage using ultrasonography was fairly easier in all cases (Figure 2).

DISCUSSION

Although donkey population in Ethiopia is high population of *Sennar* donkeys is very low. Sparsely located within the country, they are mostly used in the production of best mule using hand mating. Their semen characteristics have never been assessed. Artificial insemination with cooled transported semen would allow the development of appropriate breeding plans and a

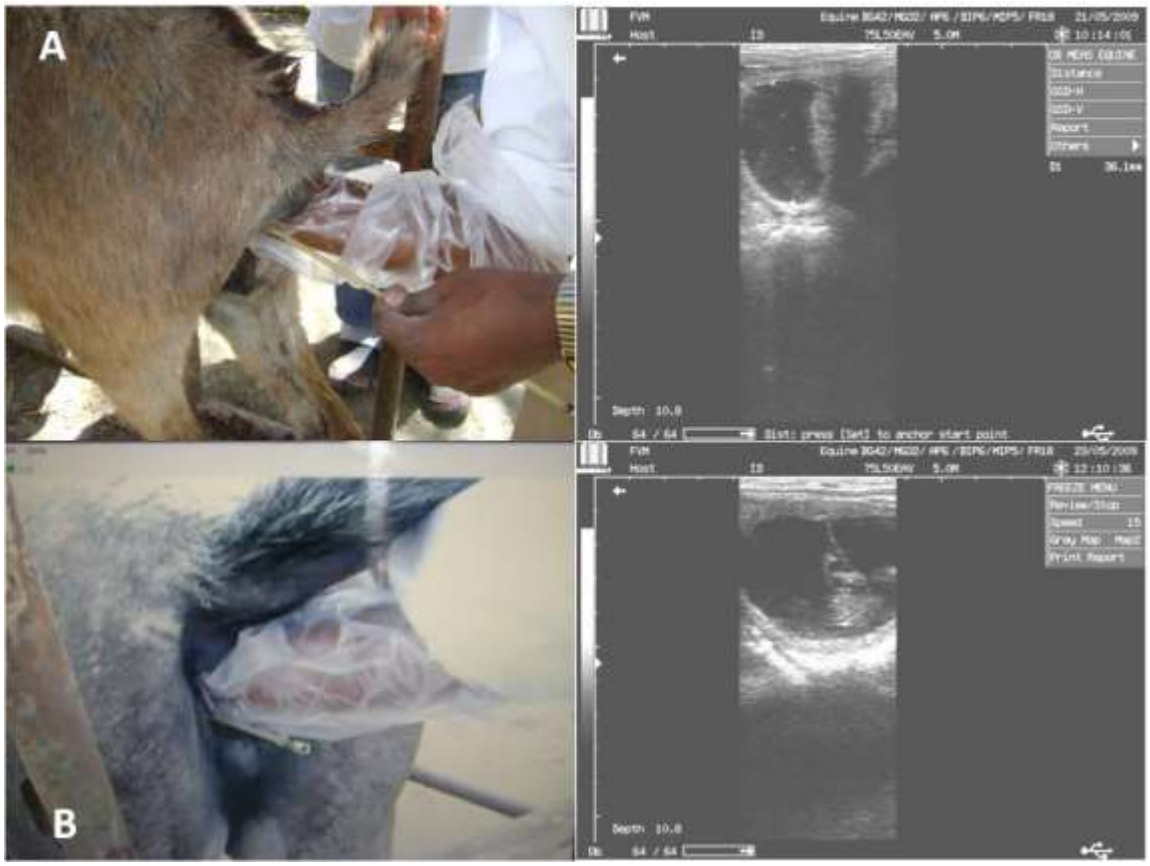

Figure 2. AI in Abyssinian donkey and 30-day embryo of the same jenny, AI in mare and 35-day embryo of the same mare.

better gene distribution, reducing the risks of excessive inbreeding in small populations (Canisso et al., 2011). Semen characteristics are very good showing comparatively better sperm motility; however this quickly deteriorates through time and particularly after chilling. Total volume and concentration are lower than Amiata breeds of donkeys (Rota et al., 2008), and are higher than Abyssinian donkeys (Lemma and Deressa, 2009) but comparable to report by Purdy (2005). Apart from semen characteristics, an important difference from stallion might be the continued reluctance of jacks to mount jennets during semen collection. Tail abnormality is much higher than previous reports for other donkeys (Henry et al*.,* 1991). Pregnancy rate is affected by factors such as the freezing technique, extenders used, time of insemination, number of spermatozoa used for the AI (Rota et al., 2012; Saragusty, 2015). Previous study (Vidament et al., 2009) in donkey semen showed that pregnancy with fresh or chilled semen is similar for jennies and mares. Previous studies confirm freezing donkey semen with addition of glycerol can reduce pregnancy dramatically. An improved technique of freezing large volumes of semen as in directional freezing has been found to improve quality of frozen semen and fertility (Arav and Saragusty, 2013). Some studies support the addition of homologous seminal plasma during re-suspension of frozen semen to improve fertility (Okazaki et al., 2012; Sabatiniet al., 2014). If seminal plasma has influenced the pregnancy rate in this study has to yet be verified. Pregnancy rate however is still much better than the 40% previously reported by Oliveira et al*.* (2006).

Conclusion

Evaluation of semen characteristic as part of the breeding soundness evaluation can give a more objective assessment of *Sennar* jacks breeding ability. Outcomes of semen analysis in the present study are generally good with acceptable level of fertility both in jennies and mares. The application of multiparametric evaluation could further improve quality of semen that can be used for AI. A notable setback observed in this study is the reluctance of the jacks to mount even on jennies well into estrous. AI both in mares and jennies also require meticulous ultrasonic evaluation of ovulatory follicle to match the time of ovulation with the time of insemination to get good pregnancy results.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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