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## Study on fresh semen characteristics in Poitou donkeys

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### Abstract

The aim of this study was to evaluate the semen Characteristics of Poitou donkey. 32 ejaculates from four Poitou donkeys 8 ejaculates from each was collected between month September to November 2016, using Colorado style of AV and Each fresh semen sample was evaluated for total semen volume, gel in semen, gel free volume, progressive sperm motility, sperm concentration, live sperm count and Abnormal sperm percentage were subjected to gross and microscopic evaluation. Mean ( $\pm$ SD) of total semen volume, Gel-free volume, Gel volume and pH were  $58.00 \pm 4.45$  ml,  $44.43 \pm 3.92$  ml,  $13.09 \pm 1.40$  ml and  $7.74 \pm 0.04$  respectively. Progressive sperm motility, sperm concentration, live sperm percentage and abnormal sperm percentage were  $88.46 \pm 0.35\%$ ,  $282.12 \pm 4.81$  million/ml,  $91.84 \pm 0.32\%$  and  $8.75 \pm 0.26\%$  respectively. There was no significant individual difference in most semen parameters. The study thus revealed that semen can be successfully collected and evaluated as part of a breeding soundness examination of Poitou donkey.

**Keywords:** Poitou donkey, semen, sperm, Colorado AV

### 1. Introduction

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% [1-2]. Artificial insemination (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 [3-4]. Another study [5] 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 [6-7]. Pregnancy rate is affected by factors such as the freezing technique, extenders used, time of insemination, number of spermatozoa used for the AI [6, 8]. Study [9] in donkey semen showed that pregnancy with fresh or chilled semen is similar for Jennies and mares. Previously 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 [10]. Some studies support the addition of homologous seminal plasma during re-suspension of frozen semen to improve fertility [11-12]. The study was conducted to evaluation of the fresh seminal characteristics of Poitou donkey and to know the fertility and conception rate for production of good quality Mules.

### 2. Materials and Methods

#### Animals

A total of 4 Poitou jacks previously selected for semen collection at Indian Council of Agricultural Research-National Research Centre on Equines (NRCE), Bikaner were used. The jacks were aged between 6 to 8 years with scored good body condition. The jacks were mainly used to produce donkey crosses and mules. Donkeys were housed in individual boxes attached with closed paddock for ample exercise. Individual houses were provided with watering as

well as feeding troughs. The concentrate ration 2 Kg fed to donkey comprised of oat/barley (40%), gram (30%), wheat bran (27%) with mineral mixture and common salt (3%). A total 7 Kg of fodder including dry fodder (Sewan grass, oat, bajra, sorghum, and ground nut) and green fodder (Lucerne, sorghum and bajra/millet) in ratio of (1:3) was fed to donkeys. Poitou jacks were identified as Brand no. M-28, M-29, M-30 and M-31

### Collection and Evaluation of Semen

Semen was collected twice a week after the sufficiently stimulated Poitou donkeys using Colorado style artificial vagina and jenny in estrus as dummy in the morning hours before feeding. Twice a week for a total of 32 collections were carried out. Immediately after collection, the color and the total volume of the each ejaculate were recorded. Semen was then filtered and the gel-fraction removed and placed in water bath at 37 °C. Semen was then filtered and the gel-fraction removed and placed in water bath at 37 °C. Aliquot of 5-micro lit semen was removed from the gel-free fraction for each of the following microscopic evaluation: Progressive sperm motility, sperm concentration, live sperm percentage and abnormal sperm percentage. pH was determined using strip. Sperm concentration ( $10^6/\text{ml}$ ) was measured using Neubauer hemocytometer. Progressive sperm motility was evaluated using Computer Assisted Semen Analyzer (CASA) and a minimum seven fields and 500 spermatozoa were measured for each sample. Live sperm percent and percent abnormal sperm were determined by using eosin-nigrosin stained smears of semen sample under light microscope at 100X oil immersion 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.

### Statistical Analysis

Data were collected, arranged, summarized and statistical analysis performed. Analysis included mean values, standard error and analysis of variance (ANOVA) using F-test.

### 3. Results

A total of 32-semen collection procedures were carried out. Summary of fresh semen parameters are given below in (Table.1)

**Table 1:** Fresh semen characteristics in Poitou donkey (n=32 collections)

Parameter	Mean ( $\pm$ SD)	Range
Total semen volume (ml)	58.0 $\pm$ 4.45 ml	39-79
Gel volume (ml)	13.09 $\pm$ 1.40	7-16
Gel-free volume (ml)	44.43 $\pm$ 3.92	27-63
Ph	7.74 $\pm$ 0.04	7.6-7.8
Semen concentration ( $10^6/\text{ml}$ )	282.12 $\pm$ 4.81	261-313
Progressive sperm motility (%)	88.46 $\pm$ 0.35	86-90
Sperm viability (%)	91.84 $\pm$ 0.32	91-93
Morphologically abnormal sperm (%)	8.75 $\pm$ 0.26	8-10



**Fig.** Semen collection from a Poitou donkey using artificial vagina (Colorado style)

Semen was collected successfully from all males on all selected days. Visually observed, Semen color was creamy white. There was no significant difference in semen parameters among individual Poitou jacks except of semen. The mean total semen volume recorded for Poitou donkeys were in the range of 39.00 $\pm$ 6.24 to 79.12 $\pm$ 10.21 ml with an overall mean of 58.0 $\pm$ 4.45 ml. The mean gel free semen volume recorded for Poitou donkeys ranged between 27.75 $\pm$ 4.14 to 63.12 $\pm$ 10.08 ml with an overall mean of 44.43 $\pm$ 3.92 ml. The mean gel volume in semen recorded for Poitou donkeys were in the range of 7.5 $\pm$ 1.92 to 16 $\pm$ 3.55 ml with an overall mean of 13.09 $\pm$ 1.40 ml. The mean of semen pH recorded for Poitou donkeys ranged between 7.66 $\pm$ 0.06 to 7.82 $\pm$ 0.06 with an overall mean of 7.74 $\pm$ 0.04. The value of mean sperm motility recorded for Poitou donkeys were varied from 86.25 $\pm$ 0.72 to 90.25 $\pm$ 0.49% with an overall mean of 88.46 $\pm$ 0.35%. The mean sperm concentration recorded for Poitou donkeys varied from 261.75 $\pm$ 8.96 to 312.5 $\pm$ 6.85 million/ml with an overall mean of 282.12 $\pm$ 4.81 million/ml. The mean value of live sperms count recorded for Poitou donkeys varied from 89.87 $\pm$ 0.44 to 93.25 $\pm$ 0.49 % with an overall mean of 91.84 $\pm$ 0.32%. The mean value of abnormal sperms counted for Poitou donkeys' ranges between 8.25 $\pm$ 0.36 to 9.37 $\pm$ 0.37% with an overall mean of 8.75 $\pm$ 0.26%

### 4. Discussion

In present study the color of Poitou donkey was graded as creamy white while the color of semen milky-white to creamy was made for exotic (Poitou) jack semen by [13-16]. The total semen volume (ml), gel volume (ml), and gel-free volume (ml) of jack semen were 58.00 $\pm$ 4.45, 13.09 $\pm$ 1.40, and 44.43 $\pm$ 3.92 respectively. The variations between the donkeys among group with respect to the three characteristics total semen volume, gel volume and gel-free volume were found Non-significant difference. It was similar to the reports of [13-15] in exotic (Poitou) jacks. The overall average pH of Poitou jack semen was observed 7.74 $\pm$ 0.04 Non-significant difference was found among donkeys for seminal pH. Our findings were in support with [13, 15] who had reported that exotic (Poitou) jack semen was neutral or slightly alkaline with pH ranging between 6.98 and 7.40 in different seasons. The average sperm concentration of Poitou jack semen was 282.12 $\pm$ 4.81 million/ml. The variations between the jacks were differing non-significantly. The average sperm concentration ( $10^6/\text{ml}$ ) of Indian jack semen was lower than the Poitou jacks, maintained under similar condition [13] Progressive motility of the spermatozoa in the semen was found 88.46 $\pm$ 0.35%. The variation between the donkeys with respect to Progressive motility was significantly differ ( $P<0.01$ ). In present study the progressive motility of spermatozoa were fond comparatively higher than to the report of [13] in exotic (Poitou) donkeys. The mean value of live sperms count recorded for Poitou donkeys overall mean of 91.84 $\pm$ 0.32%. Non-significant difference was found among donkeys for live sperm count. Our finding indicated that live sperm (%) of Poitou donkey was higher ( $P<0.01$ ) than Indian jacks while, live sperm (%) in Indian jacks was higher ( $P<0.01$ ) than the live sperm concentration in exotic (Poitou) jack semen, reported by [13]. The proportion of abnormal sperms was 8.75 $\pm$ 0.26%. Our finding supported the report of [16] with respect to the percentage of abnormal sperms in exotic (Poitou) donkeys.

## 5. Conclusion

Evaluation of fresh semen characteristic as part of the breeding soundness. Evaluation can give a more objective assessment of Poitou jacks breeding ability. Outcomes of semen analysis in our study were generally good with acceptable level of fertility for Poitou jacks. Present study would be helpful in developing artificial insemination (AI) and semen cryopreservation protocol for genetic improvement of Poitou jacks and production of good quality mules.

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