Indian Journal of Animal Sciences **79** (10): 1028–1029, October 2009

Comparative assessment of seminal characteristics of horse and donkey stallions

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Recieved : 26 September 2008 ; Accepted: 9 May 2009

Key words: Biochemical indices, Donkey, Horse, Stallion, Seminal characteristics, Semen

Physical, morphological as well as biochemical indices of semen of donkey and horse stallions were studied to establish the baseline semen indices for both species as well as the difference in 2 species if any. Semen was collected from 4 Marwari stallions (*Equus cabalus*) and 6 French donkey stallions (*Equus asinus*) maintained at Equine Production Campus, Bikaner in the morning hours before feeding between 7–8 AM with the help of artificial vagina (AV) having temperature of 42–45°C. The stallions were kept standing at a distance for visual stimuli to get proper erection before ejaculation. Total semen volume and gel free semen volume was recorded soon after semen collection. Part of gel free semen was centrifuged and seminal plasma was used for biochemical analysis. Gel free semen kept in water at 37°C in an incubator was used for physical and morphological characteristics like initial motility, progressive motility, pH, colour, consistency of semen and total sperm concentration adopting standard methods (Gupta *et al.* 2003). Seminal plasma was used for biochemical analysis, viz. glutamic oxaloacetic transaminase (GOT), glutamic pyruate transaminase (GPT), lactate dehydogenase (LDH), glucose, cholesterol, total protein and triglycerides using single step reagent kit for each parameter in Auto chemistry analyzer. The data was analyzed by standard statistical methods (Snedecor and Cochran 1989).

Macroscopic and microscopic characteristics

Appearance and consistency: In general, Marwari stallion semen and donkey stallion semen was observed milky white to creamy and off white to creamy in colour, respectively. However creamy appearance was frequently observed in donkey stallions (Gupta *et al.* 2003). As per Kuklin (1983), a good semen sample of stallion should appear milky white in colour, though it may range from watery to creamy. Consistency of the semen was observed as thick to thin in both the species during the study as reported in Poitu donkeys (Gupta *et al.* 2003).

Semen volume: Total semen volume ranged from 30 to 225 ml and 5 to 150 ml ejaculate in horse and donkey stallions, respectively. The average gel volume was 23.21±6.48 and 19.0±3.45 ml in horse and donkey stallions, respectively. The mean gel free semen volume was 53.69±2.64 and 51.30±3.56 ml in horse and donkey stallions, respectively. These values were not significantly different between the 2 species. Dowsett and Pattie (1982) and Ricketts (1993) observed that total volume of semen vary between 30 and 250 ml. On an average, stallions produced 100 ml in total, of which 2–040 ml was gel, leaving 6–080 ml of gel-free semen. In present study the observations regarding semen volume and gel were within normal range (Gupta *et al.* 2003, 2008).

pH of semen: The mean pH was 7.2 ±0.02 and 7.17±0.02 in horse and donkey stallion, respectively. However, Davies Morel (1999) reported a wider pH range (6.2–7.8) in horse stallion semen. Oba *et al.* (1993) advocated pH within this range as the best one. In present study, semen pH was within this range and was not significantly different in the two species.

Spermatozoa concentration: In horse and donkey stallions mean spermatozoa concentration was 192.0 ±9.3 and 305.0±10.1×10^6 ml^{-1} per ejaculate, respectively. Pickett *et al*. (1988) and Ricketts (1993) reported that the values for spermatozoa concentration in horse semen samples vary widely, ranging from 100 to 200×10^6 ml^{-1}. All values within the range may be considered acceptable and appropriate for use with AI. The spermatozoa concentration per ejaculate in donkey stallion was recorded significantly ($P<0.01$) high than the horse stallions.

Initial motility: Spermatozoa were highly motile as more than 70% sperms were motile. Mean initial motility of donkey and horse spermatozoa was observed as 82.60 and 79.76%, respectively. Mean initial motility of donkey spermatozoa was observed significantly ($P<0.05$) higher than Marwari stallion spermatozoa. Gupta *et al*. (2003) also reported initial motility of 76.4 to 86.5% in donkey stallions during different seasons.

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Progressive motility: Mean progressive motility of donkey and horse spermatozoa was observed as 77.80±1.0 and 73.33±0.94%, respectively. Progressive motility was significantly (P<0.01) high in donkey stallions. Davies Morel (1993) and Ricketts (1993) reported that progressive motility more than 60% might be considered appropriate for cryopreservation of stallion semen and used in AI programme.

Biochemical evaluation of seminal plasma

Enzyme: The mean activity of GOT, GPT and LDH was observed as 174.3±6.94, 26.08±6.56 and 1552±114 IU/L in horse stallion semen and 387.7±28.27, 28.2±3.96 and 3913±487 IU/L in donkey stallion semen, respectively. GOT and LDH are located in the spermatozoa head, in the acrosomal region, or in the mid-piece. They can be used to indicate spermatozoon viability as elevated free levels of these enzymes are indicative of damage to the spermatozoon (Graham *et al*. 1978). Activity of GOT and LDH was observed significantly (P<0.01) higher in donkey than horse stallions. Activity of these enzymes was observed with in normal range as reported in exotic donkeys (Gupta *et al.* 2003, Gupta *et al*. 2008). GOT and GPT are important transaminases present in semen which are concerned with oxidative metabolism. The GOT activity is mainly associated with the sperm cell and found in the seminal plasma due to leakage from spermatozoa (Pace and Graham 1970).

Metabolites: Mean glucose (mg/dl), cholesterol (mg/dl), total protein (g/dl) and triglycerides (mg/dl) content observed were 23.36±2.05, 64.72±10.23, 1.25±0.19 and 39.57±8.35 in horse and 24.44±1.81, 74.65±9.70, 3.63±0.50 and 61.72±7.3 in donkey semen, respectively. Levels of total protein content in this study are comparatively lower than that reported for stallion semen (Amann *et al.* 1987). Low levels of seminal proteins may be due to breed difference and nutritional status. Proteins are considered to be responsible for providing a protective coating to the spermatozoa and hence increase their survival time within the female reproductive tract, and this coating might be a prerequisite for capacitation (Samper 1995). Glucose is considered as the major energy source in stallion semen, which is different from bull, and ram in which fructose is the major source (Davis Morel 1999). Total protein and triglycerides were significantly (P<0.01) high in seminal plasma of donkeys as compared to horses.

SUMMARY

The study was undertaken to establish the variation in seminal characteristics *viz*. macroscopic, microscopic and biochemical indices in the horse and donkey stallions if any. Semen was collected from 4 Marwari stallions and 6 French donkey stallions at regular intervals. Fresh semen was subjected to macro-and microscopic examinations, while seminal plasma was used for biochemical estimations. Gel volume, gel free semen volume, total semen volume and pH were not significantly different in both the species. Initial and progressive motility and spermatozoa concentration were observed significantly higher in donkeys than horses. GOT, LDH, total protein and triglycerides were significantly (P<0.01) high in seminal plasma of donkeys as compared to horses. It may be concluded that the variations do exist between some of the microscopic and biochemical indices in donkey and horse semen.

ACKNOWLEDGEMENT

Authors wish to thank the Director for providing the facilities to carry out this experiment.

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