Indian Journal of Animal Sciences **87** (5): 587–589, May 2017/Short communication

Quantitative and qualitative assessment of seminal parameters of Manipuri breed stallions reared in arid zone of Rajasthan

YOGESH SONI[1], THIRUMALA RAO TALLURI[2], S K RAVI[3], ASHOK KUMAR CHAUDHARY[4], J S MEHTA[5], R A LEGHA[6], YASH PAL[7], A K GUPTA[8] and B N TRIPATHI[9]

ICAR-National Research Centre on Equines, Bikaner, Rajasthan 334 001 India

Received: 19 May 2016; Accepted: 20 October 2016

Key words: Acrosomal integrity, DNA intactness, HOST, Manipuri, Seminal characteristics, Stallion semen.

Manipuri ponies are known for their sturdiness, stamina, speed on hilly and difficult terrains, high disease resistance, surefootedness, and used mainly for riding and as pack animals in cold hilly regions of India (Gupta *et al*. 2014). The population of purebred animals of this breed has reduced considerably low, which has brought this breed the category of threatened equine breed in the country. This necessitates immediate measures to be taken for conservation and propagation of Manipuri breed of horses. Keeping this in view, recently, this breed has been brought to Equine Production Campus, ICAR-NRC on Equines, Bikaner, Rajasthan for efficient breeding and their *ex-situ* conservation and propagation.

Three apparently healthy Manipuri Stallions maintained at the ICAR- National Research Centre on Equines, Equine Production Campus, Bikaner were utilized for the current study.

The semen from stallions were collected (6 collections from each stallion, a total of 18 collections), twice per week using artificial vagina (Colorado model) equipped with a disposable liner as per Talluri *et al*. (2012). Semen collection, evaluation and processing for freezing were done as previously described (Talluri *et al*. 2012). Immediately after semen collection, seminal parameters like appearance, volume, colour, consistency, pH were recorded by visual observation. The other semen parameters that were evaluated were total and progressive sperm motility, sperm concentration, liveability, Hypo-osmotic swelling test (HOST) to determine plasma membrane integrity, acrosome integrity and DNA intactness to study the chromatin stability of the spermatozoa.

The sperm morphology and live/dead status was determined according to Bloom's method (eosin/nigrosine) (Talluri *et al*. 2012). Evaluation of the plasma membrane/ functional integrity of sperm membrane were determined by HOS test as described by Talluri *et al*. (2012). The number of spermatozoa (%) with tail coiling (HOS +ve) was recorded for each sample. The experiment was repeated five times to obtain a consistent result.

For the detection of acrosome integrity of the sperms, Giemsa method as described by Watson (1975) was followed. For detecting the DNA intactness of spermatozoa the method described by Talluri *et al.* (2012) was adopted. The percentage of spermatozoa with single-stranded DNA was calculated from the ratio of spermatozoa with red, orange, or yellow fluorescence to all spermatozoa counted per sample.

Semen with progressive motility of more than 60% was processed for cryopreservation. Collected semen was cryopreserved using conventional method of freezing and plunged in liquid nitrogen and stored in liquid nitrogen (–196°C) as per Talluri *et al.* (2016). Each frozen thawed semen sample was further evaluated for determining live and dead %, post thaw motility, HOS test, acrosome integrity and DNA intactness as described earlier in case of fresh semen analysis. The data were analysed as per the SPSS17.

Quantitative and qualitative seminal parameters of fresh and frozen thawed semen from Manipuri breed stallions were evaluated and presented in Table 1. In general, Manipuri stallion semen was observed to be milky white to creamy in colour, however creamy appearance was very frequently observed in this study and similar with the earlier observation recorded in donkeys (Arangasamy *et al*. 2009), Poitou donkey and Marwari (Pal and Legha 2009), Kathiawari (Ravi *et al*. 2013), and in Zanskari (Talluri *et al*. 2016) stallions. Consistency of the semen was observed as thick to thin in the current study is also in accordance with the earlier reports in Poitou donkeys and Marwari Stallions (Arangasamy *et al*. 2009).

Total semen volume ranged from 10 ml to 40 ml/ ejaculate. Gel volume and gel free semen volume ranged

Present address: [1]Teaching Assistant (dr.ysoni89@gmail.com), [4]Assitant Professor (vbg611ashok@gmail.com), [5]Professor and Head (drjsmehta12@gmail.com), CVAS, RAJUVAS, Bikaner. [2,3]Scientist (raotalluri98@gmail.com, skravivet@gmail.com), Equine Production Campus. [6]Prinicipal Scientist and Incharge EPC (ralegha@gmail.com). [7,8]Principal Scientist (yashpal1888@gmail.com, akguptanrce@hotmail.com), [9]Director (bntripathi1@yahoo.co.in), ICAR-NRCE, Hisar.

Table 1. Seminal characteristics of fresh and frozen thawed semen in Manipuri breed stallions

Fresh seminal characteristics of Manipuri stallions	
Total volume (ml)	54.53±7.09
Gel volume (ml)	19.60±5.6
Gel free volume (ml)	34.93±4.29
pH	7.29±.03
Concentration (million(10^6) /ml)	262.33±15.89
Total motility %	80.20±2.82
Progressive motility %	75.00±2.84
Live and dead %	77.56±.99
HOST+ve sperm %	56.80±5.37
Acrosome integrity %	91.86±.37
DNA Intactness %	$95.8±0.73^{A}$

Means with different superscripts differ significantly (P≤0.05), letters in lower case denote significance among stallions within breed. (n=3)

Frozen thawed seminal characteristics Manipuri Stallions	
Post thaw motility %	39.72^{b}±4.80
Live and dead %	53.44^{d}±1.01
HOST+ve sperm %	44.05^{d}±1.40
Acrosome integrity %	87.00^{c}±0.75
DNA intactness %	82.33^{a}±1.12

Means with different superscripts differ significantly (P≤0.05), letters in lower case denote significance among stallions within breed. (n=3)

from 2 to 30 ml, and 8 ml to 70 ml, respectively. Great variations were observed between ejaculates of stallions and from stallion to stallion. The observed values for Manipuri were found to be correlating with previous reports on semen collected from Marwari (Pal and Legha 2009), Kathiawari (Ravi *et al*. 2013) and Zanskari (Talluri *et al*. 2016) stallions. The pH of the semen was slightly alkaline and observed mean pH was 7.29±.03 with a range from 7.0 to 7.6. The average spermatozoa concentration observed to be ranging from 262.33±15.89/ml/ejaculate in this study (Table 1). The observed values for Manipuri were found to be correlating with previous reports on semen collected from Zanskari (Talluri *et al*. 2016) stallions whereas, the values were found to be on lower side when compared to the values reported for Marwari (Pal *et al*. 2011) and Kathiawari (Ravi *et al*. 2013) breeds of stallions.

Mean progressive motility of Manipuri stallion's spermatozoa was ranged from 45 to 85%, this is similar with the values of Marwari stallions as reported by Arangasamy *et al.* (2009) and Pal and Legha (2009). The observed post-thaw motility 39.72±4.80 in the present study is also in the range of the earlier reports of Arangasamy *et al*. (2009) in Marwari stallions. The liveability of spermatozoa in fresh semen sample ranged from 65.62 to 82.28 and that of frozen semen 34.29 to 57.61 (Fig. 1). The present values are in correlation with the previous findings (Arangasamy *et al*. 2009), who observed the same values

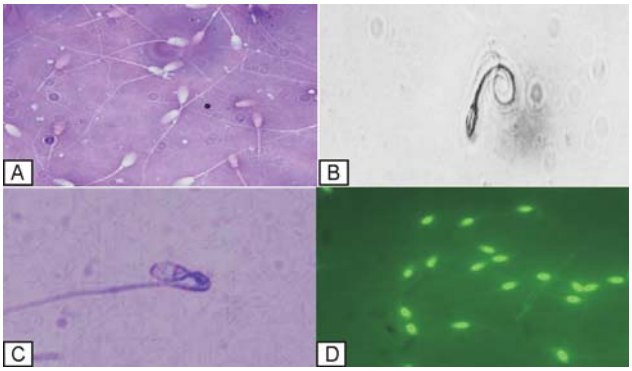

Fig. 1. Seminal parameters of Manipuri stallion. (A). Live and dead spermatozoa (B). HOS positive sperm. (C). Acrosome integrity of spermatozoa. (D) DNA intactness of Manipuri stallion spermatozoa.

in Marwari stallions and also in Kathiawari stallions (Ravi *et al*. 2013).

HOS test was conducted and the values ranged from 55.96 to 65.19% and 38.97 to 52.31%, respectively, in fresh and post-thaw semen samples respectively (Fig. 1). These values were lesser than the values reported by Arangasamy *et al*. (2009) in Marwari stallions. The acrosome integrity of the Manipuri stallions was ranged from 85.45 to 94.87% and 80.42 to 89.87% in fresh and frozen thawed semen from Manipuri stallions respectively (Fig. 1). DNA intactness of the fresh and frozen thawed semen varied from 90 to 96% and 85 to 91% respectively (Fig. 1), which is in well correlation to that of reported in Zanskari stallion (Talluri *et al*. 2012).

SUMMARY

This is the first report about the seminal parameters of Manipuri stallions. The evaluation of quantitative and quality seminal parameters of fresh and frozen thawed semen of Manipuri breed stallion were studied. The current study revealed that the semen from Manipuri breed stallion can be cryopreserved successfully at arid zone of Rajasthan, which is outside its natural habitat. The seminal characteristics observed in the current study are correlating with that of other indigenous breeds of horses. This enables the *ex- situ* conservation of this breed as its present strength is alarming.

ACKNOWLEDGEMENT

The authors are highly thankful the Dean, College of Veterinary and Animal Science, RAJUVAS, Bikaner, Director and In charge, Equine Production Campus, National Research Centre on Equine, Bikaner, India for providing the necessary facilities and infrastructure to conduct the present study.

REFERENCES

Arangasamy A, Bansal R S and Singh J. 2009. Quality assessment of frozen Poitou jack semen by HOS test and conception rate. *Indian Veterinary Journal* **86**(11): 1138.

Gupta A K, Chauhan M, Bhardwaj A, Gupta Neelam, Gupta S C,

Pal Y, Tandon S N and Vijh R K. 2014. Comparative genetic diversity analysis among six Indian breeds and English Thoroughbred horses. *Livestock Science* **163**: 1–11.

Pal Y and Legha R A. 2009. Seminal characteristics of Marwari stallions. *Indian Veterinary Journal* **86**: 918–20.

Ravi S K, Legha R A, Pal Y and Sharma R C. 2013. Characteristics and freezability of Kathiawari horse semen. *Indian Journal of Animal Sciences* **83**(11): 1146–48.

Thirumala Rao Talluri, Arangasamy A, Ravi S K, Yash Pal, Gupta A K and Singh R K. 2012. Characteristics of fresh and frozen semen of Zanskari stallions. *Indian Veterinary Journal* **89**(07): 62–65.

Thirumala Rao Talluri, Ravi S K, Singh J, Legha R A, Yash Pal, Gupta A K, Singh R K and Tripathi B N. 2016. Some reproductive parameters of Zanskari mares reared in an organized farm under tropical climate. *Indian Journal of Animal Sciences* **86**(2): 163–67.

Watson P F 1975. Use of Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. *Veterinary Record* **97**: 12–15.