



Reproductive indices of Manipuri horses reared under arid zone region

THIRUMALA RAO TALLURI¹✉, S K RAVI², J SINGH¹ and B N TRIPATHI³

ICAR-National Research Centre on Equines, Post Box No 80, Bikaner, Rajasthan 334 001 India

Received: 18 November 2019; Accepted: 28 February 2020

ABSTARCT

The current study was undertaken with an object to record and analyse the data related to reproductive indices such as estrus cycle length, duration of estrus, size of the follicle at ovulation, day of ovulation, gestation length and estrus after foaling (foal heat) in respect of Manipuri mares. The mares were closely monitored for their estrus exhibition, examined with transrectal ultrasonography for the size of the follicle variation and to determine the size of the follicle at ovulation. The mares were inseminated with the frozen thawed semen collected from Manipuri stallions at appropriate time. This is the first time ever report that Manipuri stallion semen was cryopreserved successfully and live foals have been obtained using frozen semen *via* artificial insemination. The data on reproductive characteristics of the mares of Manipuri breed in India are scanty and there are no available reports on reproductive parameters of Manipuri breed until today in the literature. The results of the present findings are of much useful as a ready reckoner for the reproductive parameters and traits of Manipuri breed as well as indigenous horses reared under arid region. The differences observed in various parameters in relation to other breeds of Indian horses may be caused by breed effect and other environmental factors.

Keywords: Arid zone, Estrus cycle, Gestational length, Manipuri Mares, Stallion

India is enriched with different breeds of horses that have evolved and adapted to different geographical, harsh climatic and ecological conditions. The Manipuri Pony breed of horse is known for its beauty, fastness, intelligence and is believed to be the oldest pony breed in world to be used as 'Polo Pony'. These ponies are known for their sturdiness, stamina, speed on hilly and difficult terrains, high disease resistance, surefooted animals, and used mainly for riding and as pack animals in cold hilly regions. There is high demand for these animals in the north eastern region and rest of areas of the country as one of the most suitable pack pony in region.

Hence, with an intention to conserve and propagate the germplasm of the Manipuri breed and to obtain true to breed population and to curtail non-descript breeding, these horses were brought to Equine Production Campus, ICAR-National Research Centre on Equines, Bikaner, Rajasthan in 2010.

The determination of the estrus length, follicular size at ovulation and gestation length of mares is of immense important as reproductive cycle of the mare is subject to the greatest variability among all the domestic animals (Talluri *et al.* 2016). Data on reproductive parameters would

aid in a precise calculation for breeding or insemination, calculation of expected date of foaling, which would help in better planning and organization of work in stud farms more efficiently, taking care of a due mare and a foal properly, and better planning for the subsequent breeding season (Davies *et al.* 2002) and also in routine equine industry. Some mares appear to be truly polyestrous and they can produce offspring at any time of the year. However, majority of the mare populations are seasonally polyestrous and many of them show behavioral estrus without accompanying ovulation (Hafez 1993). It has been reported that 75–80% of mares showed seasonal or winter anestrus in autumn and winter (Osborne 1966, Ginther *et al.* 1972, Hughes *et al.* 1980).

Breed differences and individual variations in reproductive characteristics do exist in mares. The reproductive parameters of exotic and thoroughbred horses are well studied and records are available for ready references which can be referred as standards. The reproductive characteristics of our indigenous horses are not well studied, documented and reported. However, to our knowledge, no reliable information is available concerning the reproductive parameters of the Manipuri mares either in niche areas or after acclimation to the arid region. Hence, we have initiated documenting major reproductive indices for future reference and to set the standards for various reproductive indices for this breed. Therefore, the present study was undertaken with an object to record different reproductive parameters in respect of

Present address: ¹Equine Production Campus, ICAR-NRC on Equines, Bikaner, Rajasthan. ²ICAR-Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands. ³DDG (Animal Sciences), ICAR, Krishi Bhawan, New Delhi.

✉Corresponding author email: raotalluri79@gmail.com

Manipuri mares at arid region and a comparison in the reproductive parameters were made with respect to the Indian breed horse breeds for which already parameters were reported earlier.

MATERIALS AND METHODS

For the present study, the Manipuri mares (10) present at the Equine Production Campus, Bikaner, Rajasthan were used and data was recorded for every season. Bikaner city is situated in middle of the Thar Desert at an altitude of 238 m above the mean sea level between latitude of 27°11' to 29°3' north and longitude of 71°54' to 74°12' east. The temperature in this arid region register its extreme, rising above 49°C in summer and cooling down to near 4°C during winter. The mares considered for this study included adult mares that are aged between 5 to 15 years old. All the mares were housed under uniform managemental conditions and the animals had the access to feed and water *ad lib*. There was no source of artificial light provided at night.

For recording the estrus behavior, an adult fertile Manipuri stallion with proven fertility was used. Each mare was brought to the vicinity of stallion, to detect the onset of estrus and estrus behavior. The estrus length was considered to be from the first day of onset estrus until the disappearance of the behavioral or physiological signs of estrus (Back *et al.* 1974, Squires 1993) as well as cessation of dominant follicular activity accompanied with corpus luteum formation. Length of the estrous cycle was recorded as the time interval in days from the beginning of estrus (day 0) to the onset of subsequent estrus (Hafez 1993). Observations (68) were made for this parameter. Follicular dynamics of the mares were closely monitored with the help of transrectal ultrasonography at daily intervals during breeding and non-breeding season. Visual observations were followed by daily ultrasonic examination of the mares' reproductive tracts using a linear-array, real-time scanner, equipped with a 5 MHz transducer (Kontron Medical, France and CHISON, China). During the study period, all mares were considered to be clinically healthy. When pre-ovulatory follicle diameter reached 35 mm, the follicle was regarded as a dominant follicle. Then, cross-sectional follicular area (FA) was taken as a product of the two largest perpendicular follicular diameters. In the case of multiple ovulation, the area of all dominant follicles was pooled. The ovulation day was taken as Day 0. In the case of multiple, non-synchronic ovulations, the day of ovulation of the last dominant follicle was taken as Day 0. Mares were inseminated when ultrasonographic prominent features for ovulation were observed in the pre-ovulatory follicle (i.e. follicles with 38 to 44 mm in diameter approximately, and irregular shape of the follicle and soft consistency of the follicle, and/or uterus (i.e. uterine edema/uterine infection), and repeated each 24 to 48 h until the confirmation of ovulation via ultrasonography. The time of ovulation was calculated as the middle of observations when a pre-ovulatory follicle (i.e. anechoic ovarian structure around 38 to 42 mm in size) was last observed and the first

observation of when it was not present. Fifty five Observations were recorded for this parameter in the current study.

Gestational length: The length of gestation period was calculated as the interval from ovulation till foaling. Gestation period was calculated for 11 mares. Foal heat is an important reproductive parameter of horses as it comes within short period immediately after foaling and is measured as the time interval between foaling to the occurrence of first visual signs of estrus. A total of 7 observations were made for the present study and in rest of the mares, foal heat was not observed.

Collection and cryopreservation of Manipuri stallion semen: The semen from Manipuri stallions was collected using Artificial Vagina (AV) (Colorado model) method using estrus Manipuri mare as dummy. The gel portion of the semen was sieved through a sterile gauge and the gel free semen was mixed with lactose-glucose-EDTA primary extender (Yogesh *et al.* 2018) in the ratio of 1:1 and centrifuged at 650 g for 3 min. The supernatant was siphoned off and the sperm rich pellet was dissolved in such a way to get 150–200×10⁶ spermatozoa/ml with a modified secondary extender having Dimethyl formamide (DMF) as the cryoprotectant (5%) (Prashant Kumar *et al.* 2019). The diluted semen was kept in the semen cooling cabinet at 4°C for 2–3 h as equilibration period. The equilibrated semen was filled in to 0.5 ml straws and sealed with the help of an automated filling and sealing machine. The semen was cryopreserved using custom method of freezing using liquid nitrogen vapours. Thereafter, the straws were plunged and stored in liquid nitrogen (–196°C) until use. Frozen semen was thawed by immersing the straws in a water bath at 37°C for 30 sec, and 4 to 5 ml of semen was artificially inseminated in the estrus mare after detecting the suitable period of near ovulation.

Statistical analysis: The data were analysed as per the standard procedure (Snedecor and Cochran 1989) and also by using SPSS 20.0

RESULTS AND DISCUSSION

Summary of various reproductive indices of Manipuri mares were recorded and analysed statistically and are presented in the Table 1.

Table 1. Reproductive characteristics in Manipuri mares

Reproductive parameter	Mean	Range
Length of the estrous cycle (days) (n=68)	19.85±0.44	18–26
Duration of estrus (days) (n=68)	6.47±0.86	5 – 9
Size of the follicle at ovulation (mm) (n=55)	36.24±3.72	36.4–42.7
Gestation period (n=11)	338.49±5.21	309–342
Foal heat (n=7)	12.14±2.61	8–27
Foal birth weight (kg)	20.69±1.78	27–39

Note: Numbers within the parenthesis are actual number of observations/recordings.

Estrus characteristics: The Manipuri mares, which were in estrus showed standard signs of estrus characteristics like a stance of micturition (squatting) and a swelling and winking of the vulvar lips, a prolonged rhythmic exposure of clitoris. The mares which were not in estrus did not show any of the above mentioned symptoms and they also did not respond to the stallion's call and moved away from the stallion. In present study, an average duration of estrus was observed as 6.47 ± 0.86 days with the range of 5–9 days. Daels *et al.* (1991) and Allen (1978) reported the duration of estrus as 5–6 and 3–10 days, respectively in exotic horses and the slight increase in the estrus length was also reported in Marwari and Zanskari Mares (Arangasamy *et al.* 2008, Talluri *et al.* 2016) and are correlating with our present findings. The length of estrus in mares is much longer than that of other domestic species (1–2 days in pigs, 2–3 days in cows). Long duration of the estrus in the mare may be due to the factors like follicles have to migrate to the ovulation fossa prior to their rupture, or the ovaries are less sensitive to exogenous FSH than in other species or may be low level of LH is delaying ovulation (Hafez and Hafez 2000).

The main part of the breeding season occurs during April through June or May through July in the northern hemisphere (Andrews and McKenzie 1941, Hutton and Meacham 1968, Hafez 1993). In the present study, we observed although not necessarily conceived during all estrous periods, mares exhibited an estrus cycle all the year round (polyestrous) with more than 70% of mares exhibiting estrus during mid of February through November.

Length of estrus cycle: Multiple factors including photoperiod, temperature and availability of feed, affect the reproductive cycle of horses. Of these, photoperiod may be one of the most important factors (Burkhardt 1947, Nishikawa 1959), by which an endogenous circannual rhythm is synchronized with seasonal climatic and dietary changes (Bronson and Heideman 1994). Zanskari mares maintained at Equine Production Campus, at arid region, revealed a mean of 18.58 ± 0.56 (range 15–26) days of estrous cycle in the earlier study (Talluri *et al.* 2016) where as it was observed to be 19.85 ± 0.44 (range 18–26 days) for Manipuri mares. Our observed findings are higher than the earlier reports of Roberts (1971) and Hafez (1993) reported for exotic horses and in Marwari and Zanskari mares (Arangasamy *et al.* 2008, Talluri *et al.* 2016). They reported an average length of 21 days in exotic horses and 14 to 26 days in Marwari and Zanskari mares. Duration or length of estrus of mares may vary among individuals of same breed and different breeds and also among the estrous cycle of the same mare.

Follicular dynamics: Both the ovaries of the Manipuri mares were diagnosed every day from the day of showing prominent visual signs of estrus with the aid of transrectal ultrasonography to know the status of follicular growth and to determine ovulatory size of the dominant follicle. There was increase about 0.38 to 0.56 mm in size of the follicle every day during estrus phase. The size of the ovulatory



Fig. 1. Live foals born out of artificial insemination by using frozen thawed stallion semen for the first time.

dominant follicle was observed to be ranging from 36.4 to 42.7 mm with an average size of 36.24 ± 3.72 . During non-breeding season also the mares showed false estrus and multiple anovulatory follicles found during examination.

For Manipuri mares to conceive, for the first time collected and cryopreserved successfully Manipuri stallion semen and artificially inseminated the frozen thawed semen in estrus mares which became pregnant with a fertility index of 1.97 to 3.42 (data not shown). The inseminated mares were conceived and viable foals have been obtained for the first time with AI from the cryopreserved semen of stallions from this breed (Fig. 1).

Gestation length: The mean gestation period in horses varies more when compared to other farm domestic animals like cow, sheep or pig (Bos and van der Mey 1980), may be due to the fact that their long gestation period influenced by physiological factors that do not influence the length of gestation of other livestock species like, embryonic diapause (Lofstedt 1992 and Winter *et al.* 2007). The average gestation length in Manipuri mares was recorded to be 338.47 ± 5.21 . It is assumed that the average length of gestation period in mares' ranges from 330 to 340 days (Bos and Van der Mey 1980) and ranges between 309 and 342 days in pony breeds (Davies 2002). Similar results have also been reported by Hafez (1993) and Davis (2005) for thoroughbred horses. The length of gestation was reported as 328 days with a range of 301 to 368 days in Marwari mares (Arangasamy *et al.* 2008), and in Zanskari mares it was reported to be 326.11 ± 3.23 days and ranged from 314 to 342 days (Talluri *et al.* 2016). The present findings about the gestation length are also in normal range and correlating with the reports of Arangasamy *et al.* (2008) in Marwari mares, Talluri *et al.* (2016) in Zanskari mares and Davis (2005) in thoroughbred mares. Gestation length in the mare is influenced by maternal size, fetal genotype and the stage of the breeding season when conception occurs (Hafez 1993).

Foal heat: Mares are unusual among mammals in showing her first estrus very soon after foaling often within 4–10 days. Foal heat is fertile and it is advised to inseminate the mares during foal heat to minimize the duration between foalings (Ginther 1974). In the present study, an average duration of foal heat was observed as 12.14 ± 2.61 days (ranging from 8 to 27 days). This finding is longer than the earlier observation made in exotic horses (Davis *et al.* 2002), but correlating with the reports of Arangasamy *et al.* (2008)

and Talluri *et al.* (2016) for Marwari mares and Zanskari Mares. Postpartum estrus usually occurs 5 to 15 days after foaling and some mares, however may show estrus as late as up to 45 days (Hafez 1993) and the observation made in the present study are also correlating to these findings.

Foal birth weight: The average fetal birth weight for Manipuri foals born was 20.69 ± 1.78 with a range of 27 to 39 kg. This observation was lesser to the earlier reports of Davies (2003) who reported as 32 kg in thoroughbred mares. The observations were found to be closer that are reported for Zanskari mares (Talluri *et al.* 2016).

The current study gives a reference about some of the reproductive parameters of our indigenous horses of Manipuri breed. Data on reproductive parameters would aid in a precise calculation for breeding or insemination, calculation of expected date of foaling, which would help in better planning and organization of work in stud farms more efficiently, taking care of a due mare and a foal properly, and better planning for the subsequent breeding season. This information would be of value for horse owners or farm practitioners. The present study findings may be useful as a ready reckoner for the indigenous breeds of horses reared under arid region. The data on reproductive characteristics of the mares of Manipuri breed in India are scanty and there are no available reports on reproductive parameters of Manipuri breed until today in the literature. However, it needs to be taken into consideration the fact that the Manipuri population in India is small and declining day by day and consequently this study included a small number of mares. Although indicators point to the breeding strategy heading towards the right direction, by educating the owners and with frequent veterinary supervision, the reproductive performance could be enhanced.

ACKNOWLEDGEMENTS

The authors are highly thankful the Director and In charge, Equine Production Campus, National Research Centre on Equine, Bikaner, Rajasthan for providing the necessary facilities and infrastructure to conduct the present study.

REFERENCES

- Allen W R. 1978. Control of ovulation and oestrus in the mare. Control of ovulation. (Eds) Crighton D P, Haynes H B, Foxcroft G R and Lamming G E. Butterworths, London. pp. 453–468.
- Andrews F N and McKenzie F F. 1941. Estrus, ovulation and related phenomena in the mare. *University of Missouri Agricultural Experiment Station Research Bulletin* **329**: 1–117.
- Arangasamy A, Bansal R S, Sharma R C, Legha R A, Yash P and Tandon S N. 2008. Some reproductive characteristic of Marwari mares. *Indian Journal of Animal Reproduction* **29**: 66–67.
- Bos H and Van der Mey J W. 1980. Length of gestation period of horses and ponies belonging to different breeds. *Livestock Production Science* **7**: 181–87.
- Bronson F H and Heideman P D. 1994. Seasonal regulation of reproduction in mammals. *Physiology of Reproduction*. (Eds) Knobil E and J D Neill. Raven Press, New York. pp. 541–583.
- Burkhardt J. 1947. Anoestrus in the mare and its treatment with estrogen. *Veterinary Record* **59**: 341–42.
- Daels P F, Hughes J P and Stabenfeldt G H. 1991. Reproduction in horses. *Reproduction in Domestic Animals*. (Ed.) Cupps P T. 4th edn. Academic Press, New York.
- Davies Morel M C G, Newcombe J R and Holland S J. 2002. Factors affecting gestation length in the Thoroughbred mare. *Animal Reproduction Science* **74**: 175–85.
- Davies Morel M C G. 2003. Equine reproductive physiology, breeding and stud management, second ed. CABI Publishing International. pp. 169–170.
- Ginther O J. 1974. Occurrence of anestrus, estrus, diestrus and ovulation over a 12-month period in mares. *American Journal of Veterinary Research* **35**: 1173–79.
- Ginther O J, Whitmore H L and Squires E L. 1972. Characteristics of estrus, diestrus, and ovulation in mares and effects of season and nursing. *American Journal of Veterinary Research* **33**: 1935–39.
- Hafez E S E. 1993. *Reproduction in Farm Animals*. 6th edn. Lea & Febiger, USA.
- Hafez E S E and Hafez B. 2000. Horses. *Reproduction in Farm Animals*. (Eds) E.S.E. Hafez, B. Hafez. Lippincott Williams and Wilkins. 7th ed. Philadelphia 192–217.
- Hughes J P, Stabenfeldt G H and Kennedy P C. 1980. The estrous cycle and selected functional and pathologic ovarian abnormalities in the mare. *Veterinary Clinics of North America* **2**: 225–39.
- Hutton C A and Meacham T M. 1968. Reproductive efficiency of fourteen horse farms. *Journal of Animal Science* **27**: 434–38.
- Prashant Kumar, Rabindra Kumar, Jitendra Singh Mehta, Ashok Kumar Chaudhary, Sanjay Kumar Ravi, Sharat Chandra Mehta, Mohd. Matin Ansari, Ram Avtar Legha, Bupendra Nath Tripathi and Thirumala Rao Talluri. 2019. Ameliorative effect of ascorbic acid and glutathione in combating the cryoinjuries during cryopreservation of exotic Jack semen. *Journal of Equine Veterinary Science* **81**: 102796.
- Snedecor G W and Cochran W G. 1989. *Statistical Methods*. 6th Ed. Iowa State University Press, USA.
- Yogesh S, Talluri T R, Ashok K, Ravi S K, Mehta J S and Tripathi B N. 2018. Effects of different concentration and combinations of cryoprotectants on sperm quality, functional integrity in three Indian horse breeds. *Cryobiology*. <https://doi.org/10.1016/j.cryobiol.2018.12.005>.
- SPSS. 2005. SPSS for windows. Brief Guide, version 20.0. SPSS Inc. Chicago, IL.
- 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**: 163–67.
- Winter G H Z, Rubin M I B, De La Corte F D and Silva C A M. 2007. Gestational length and first postpartum ovulation of Criollo Mares on a stud farm in Southern Brazil. *Journal of Equine Veterinary Science* **27**: 531–34.