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Some reproductive parameters of Zanskari mares reared in an organized farm under tropical climate

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ABSTRACT

The present study was undertaken to record the data on duration of estrus, estrus cycle length, size of the follicle at ovulation, period of gestation and estrus after foaling (foal heat) in respect of Zanskari mares for 4 consecutive breeding seasons (2010–2014). The mares were closely monitored with transrectal ultrasonography for their follicle size variation and to determine size of the follicle at ovulation. The mares were inseminated with frozen thawed semen from Zanskari stallions. This is the first time ever that Zanskari stallion semen was cryopreserved successfully and viable foals were produced by using frozen semen via artificial insemination. The reproductive parameters of the mares of Zanskari breed in India are poorly investigated and thus no reports are available on reproductive characteristics of Zanskari breed till today in the literature. Our results can be useful as a ready reference for some of the reproductive characteristics and traits of Zanskari breed as well as indigenous horses reared under tropical region. The reproductive parameters analysed in the present study are fitting within the characteristics of the other Indian horse breeds reared under this region and the differences may be caused by breed effect and other environmental factors.

Key words: Cryopreservation, Estrus, Estrus cycle, Foal heat, Follicular size, Gestation length, Ovulation, Semen, Ultrasonography, Zanskari mare

India is enriched with different breeds of horses that have evolved and adapted to different geographical, harsh climatic and ecological conditions. Zanskari horses, found in Leh and Laddakh region of Jammu and Kashmir of India (Behl *et al*. 2006). Since, their height at wither is between 125 and 127 cm, hence this breed was clubbed under the category of pony breeds (Gupta *et al*. 2010).

This breed is considered as the hardy amongst all the reported pony breeds of India and have their exceptional ability to survive and perform at mountain terrains and under the harsh climatic conditions of their high altitude habitat (between 3 to 5 km) even at the extremes of altitudes and temperatures. Horses are of medium size, well built and 120 to 140 cm high. Only a few hundred horses presently exist in the Zanskar and other valleys of Laddakh (Gupta *et al*. 2010). Large scale breeding with non-descript ponies has endangered this breed. The population of purebred animals of Zanskari has come down considerably below 10,000, which brought this breed under threatened breed category. Hence, with an intention to multiply and conserve the germplasm of the Zanskari breed and to obtain true to breed population and to curtail non-descript breeding, these mares were brought to Equine Production Campus, National Research Centre on Equines, Bikaner, Rajasthan in 2010.

Horses are seasonal breeders with extensive sexual activity in spring, summer, and autumn. Mares can be classified into 3 groups based on their breeding seasons. They are (A) the defined seasonal breeders (group A): the estrous cycle of most wild breeds coincides with the longest days of the year and foaling during a restricted season; (B) transitory seasonal breeders (group B): some breeds manifest estrous cycles throughout the year, but ovulation only occurs during the breeding season; (C) year round breeders (group C): some domestic mares show estrous cycle accompanied by ovulations throughout the year (Hafez 1993).

The determination of the length of estrus, 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 (Bos and van der Mey 1980). 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, taking care of a due mare and a foal properly, and better planning for the subsequent breeding season (Davies

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Morel *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 was 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 characteristic 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 Zanskari mares after acclimatisation to the tropical climate. Hence, documentation of major reproductive characteristics was initiated for future reference and to set the standards for various reproductive parameters for this breed. Therefore, the present study was undertaken to record different reproductive traits in respect of Zanskari mares for 4 consecutive breeding seasons (2010–2014) at National Research Centre on Equines, Equine Production Campus, Bikaner and also a comparison in the reproductive parameters were made with other Indian breed Marwari, for which already parameters were reported earlier.

MATERIALS AND METHODS

Zanskari mares (10 observations), 4 to 8 year old, present at the Equine Production Campus, Bikaner, Rajasthan were managed under uniform management and housing conditions. All the animals were fed uniformly and no source of artificial light and no special feed were provided for all the animals. Mares were provided with water and feed *ad lib.* at all the time.

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) and cessation of dominant follicular activity. For recording the estrus behavior, a fertile stallion of Zanskari was used. Daily the stallion was brought to the premises of all the mares to record the signs of estrus activities. Length of the estrous cycle was recorded as the time interval in days from the beginning of estrus (day 0) to the beginning of subsequent estrus (Hafez 1993), and 42 observations were made for this parameter. Duration of estrus activity were monitored closely by the transrectal ultrasonography at regular intervals. All the Mares were monitored with ultrasound machine from the day of onset of estrus symptoms shown with proximity of a Zanskari stallion and repeated daily using a linear-array scanner equipped with a 5.0 MHz, B-mode, transrectal transducer. Mares were inseminated when ultrasonographic prominent features for ovulation were observed in the pre-ovulatory follicle (i.e. follicles with 34 to 40 mm in diameter approximately, and spherical shape of the follicle and consistency (hard/soft) 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 34 to 40 mm in size) was last observed and the first observation of when it was not present; 55 observations were recorded. The length of gestation period is calculated as the interval from ovulation and fertile mating to foaling or parturition. Gestation period was calculated in 10 numbers of observations as the date of successful breeding to the date of foaling. Foal heat is an important reproductive parameter of horses as it comes within short period after foaling and is measured as the time interval between foaling to the occurrence of first visual signs of estrus, and this foal heat is fertile in mares; 10 observations were made.

The semen of Zanskari stallions was collected by artificial vagina method using estrus mare as dummy. The stallion semen having progressive motility more than 60 % was processed for cryopreservation using lactose-glucose-EDTA extender. Gel free semen was mixed with glucose EDTA primary extender (Cochran *et al.* 1984) in the ratio of 1:1 and centrifuged. The supernatant was discarded and sperm pellet was dissolved so as to get 100–200 million spermatozoa / ml with a modified secondary extender (Cochran *et al*. 1984). The diluted semen was kept in the semen cooling cabinet at 4ºC for 2 h as equilibration period. The equilibrated semen was filled in 0.5 ml straws and it was slowly cooled to –100º C using Bio-med planner. Thereafter, the straws were plunged and stored in liquid nitrogen (–196°C) till use. Frozen semen was thawed by immersing the straws in a water bath at 37°C for 1 min, and 4 to 5 ml of semen was artificially inseminated in the estrus mare after detecting the suitable period of near ovulation. The data were analysed as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The results on reproductive characteristics of Zanskari mares are presented in Table 1.

Estrus characteristics: Estrus Zanskari mares showed typical signs of estrus characteristics like mares responding to the stallion's proximity and showing a stance of urination (squatting) and a swelling and winking of the vulvar lips

Table 1. Reproductive characteristics in Zanskari mares

Reproductive parameter	Mean	Range
Length of the estrous cycle (days)(42)	17.58±0.56	15–26
Duration of estrus (days)(55)	5.76±1.02	3–8
Size of the follicle at ovulation (mm) (55)	36.24±3.72	32.8–40.5
Gestation period(10)	326.11±3.23	314–342
Foal heat(10)	16.09±3.84	6–48
Foal birth weight	22.88±0.88	19–28

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

(arrow), a prolonged rhythmic exposure of clitoris (Fig.1A, B) and the pinkish turning of vulval lips. The non estrus mares 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 5.76±1.02 days with the range of 3–9 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 mares (Arangasamy *et al*. 2008) 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 factors like follicles have to migrate to the ovulation fossa prior to 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), whereas in the southern hemisphere, it is from November to January (Osborne 1966). In present study, we observed that the Zanskari mares though not conceived during all estrous periods, they exhibited estus symptoms all round the year (polyestrous) with more than 80% of mares exhibiting estrus during March through September.

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, Bikaner, revealed a mean of 17.58±0.56 (range 15–26) days of estrous cycle in the current study. Our findings are lesser than the earlier reports of Roberts (1971) and Hafez (1993) reported for exotic horses and in Marwari horses (Arangasamy *et al*. 2010). They reported an average length of 21 days in exotic horses and 14 to 26 days in Marwari mares. The present finding is also having wider range in the estrous cycle compared to exotic horses as per Hafez (1993) who reported to be 19–23 days but correlating with that of the Marwari mares that were reported earlier by Arangasamy *et al*. (2010). 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 Zanskari mares were checked 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 0.5 to 0.8 mm in size of the follicle every day during estrus phase. The size of the ovulatory dominant follicle ranged from 32.8 to 40.5 mm with an average size of 36.24±3.72 (Fig. 1C). During non-breeding season also, the mares showed false estrus and multiple unovulatory follicles found during examination.

For making Zanskari mares pregnant, Zanskari stallion semen was collected and cryopreserved successfully for the

Fig. 1. A-C. A,B. Prominent estrus symtroms while teasing with a fertile Zanskari stallion. C. An ovulatory dominant follicle up on transrectal ultrasonography.

Fig. 2. A-C. The foals born out of artificial insemination by using frozen thawed stallion semen for the first time.

first time, and artificial insemination with frozen thawed semen was done in estrus mares. The pregnancy record revealed a fertility index of 1.5 to 3 (data not shown). The inseminated mares were conceived and viable foals were obtained for the first time with AI from the cryopreserved semen of stallions from this breed (Fig.2 A-C).

Gestation length: The mean gestation period in horses varies more when compared to other farm domestic animals like cows, sheep or pigs (Bos and van der Mey 1980), may be due to the fact that their long gestation period is influenced by physiological factors that do not influence the length of gestation of other livestock species like, embryonic diapause (Lofstedt 1992, Winter *et al*. 2007). The average gestation length in Zanskari mares in the current study was recorded to 326.11±3.23 days and ranged from 314 to 342 days. 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 320 and 335 days in pony breeds (Davies Morel 1993). Hafez (1993) and Davis Morel (2005) reported similar results 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). The present findings about the gestation length are also in normal range and correlating with the reports of Arangasamy *et al*. (2008) in Mawari mares and Davis Morel (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).

Various studies on gestation duration in relation to foal gender emphasized prolonged gestation in colts compared to fillies (Bos and Vander Mey 1980, Davies Morel *et al*. 2002, Perez *et al*. 2003, Taveira and Mota 2007). The present study findings are also corroborative with these observations (data not shown). The reason is considered unknown, however it has been often attributed to diverse endocrine functions in male and female fetuses whose interaction with the endocrine control of the parts is different (Jainudeen and Hafez 2000).

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 16.09±3.84 days (ranging from 6–48 days). This finding is longer than the earlier observation made in exotic horses (Davis Morel *et al.* 2002), but correlating with the reports of Arangasamy *et al*. (2008) for Marwari mares. Postpartum estrus usually occurs 5 to15 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 Zanskari foals born was 22.88±0.88 with a range of 19 to 28 kg. This observation was little lower than that of the earlier reports of Davies Morel (2003) who reported as 32 kg in thoroughbred mares. The current study gives a reference about some of the reproductive characteristics of our indigenous horses of Zanskari breed. This information would be of value for horse owners or farm practitioners.

The present study findings may be useful as a baseline data for the indigenous breeds of horses reared under tropical climate. However, it needs to be taken into consideration the fact that the Zanskari population in India is small and declining day by day due to indiscriminate breeding, 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, especially for twin reduction, the reproductive performance could be enhanced.

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