



# Basic Reproductive Parameters of Kathiawari Horses Reared Under Arid Zone Region

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## ABSTRACT

The current study was undertaken with an aim to record and analyse the data related to basic reproductive parameters such as length of estrous cycle, estrus duration, pre-ovulatory follicle size at ovulation, day of ovulation, length of gestation and days of occurrence of foal heat in respect of Kathiawari mares for a period of thirteen consecutive breeding seasons (2010-2023). The mares were monitored closely for their estrus signs, 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 Kathiawari stallions. This is the first time ever report that live foals have been obtained using frozen semen *via* artificial insemination in Kathiawari horses. The data on reproductive indices of the mares of Kathiawari breed in India are scanty and there are no available reports on reproductive characteristics of Kathiawari breed until today in the literature. The results of the present findings are of much useful and may serve as a ready reckoner for the reproductive traits of Kathiawari breed reared under arid region. The differences in the reproductive parameters observed in relation to other breeds of Indian horses may be attributed to breed differences and other environmental factors.

**Key words:** Arid zone, Estrous cycle, Gestational length, Kathiawari horses, Foal heat.

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## INTRODUCTION

India has a complex amalgamation of equids in the form of three horse breeds (Marwari, Kathiawari and Kachchisindhi) and four endangering pony breeds (Bhutia, Spiti, Manipuri and Zanskari) besides indigenous donkeys and

wild asses (Gupta *et al.*, 2014). All these breeds have evolved and adapted to different geographical, harsh climatic and ecological conditions (Talluri *et al.*, 2016 and Talluri *et al.*, 2020). Kathiawari breed of Indian horse is known for elegance and endurance. Their native tract is Kathiawar (Saurashtra region) region of Gujarat from where the name

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of breed was originated. Breed characteristics of these horses include shorter back with concave profile, stout neck, short legs and squared quarters (Singh *et al.*, 2002; Ravi *et al.*, 2013). These breed are also famous for their speed, stamina, endurance capability of fast moving, etc. (Zala, 2010). Indiscriminate breeding with exotic or non-descript horses and development in mechanization led to drastic decrease in the population of indigenous equine breeds. It necessitates immediate measures to be taken for conservation and propagation of Kathiawari breed of horses (Ravi *et al.*, 2013). Hence, with an intention to conserve and propagate the germplasm of the Kathiawari 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 the year 2010.

Developing a ready reckoner of the reproductive parameters like estrus length, follicular size at ovulation and gestation length of mares are of immense importance as reproductive cycle of the mare is subject to the greatest variability among all the domestic animals (Talluri *et al.*, 2016) and will be easier for developing breeding plans. Some mares appear to be truly polyestrous and they can produce offspring at any time of the year. 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). However, majority of the mare populations are seasonally polyestrous and many of them show behavioral estrus without accompanying ovulation (Hafez, 1993). 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 (Morel *et al.*, 2002).

The differences are existing in reproductive parameters of individual mare within the same breed as well as between the mares of different breeds. 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 some 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 Kathiawari 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 Kathiawari mares for a period of thirteen consecutive breeding sea-

sons (2010-2023) at arid region and a comparison in the reproductive parameters were made with other indigenous breeds of horses for which already parameters were recorded and reported earlier.

## MATERIALS AND METHODS

The Kathiawari mares present at the Equine Production Campus, Bikaner, Rajasthan were used and data were recorded for every breeding season. A total of 187 estrous cycles in a period of thirteen years were collected and analyzed. The mares aged between 5 to 15 years old were included in the study. All the mares were housed under uniform managemental conditions and the animals had the access to feed and water at *libitum*. There was no source of artificial light provided at night.

For recording the estrus behavior through teasing, an adult fertile 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 of 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). 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 5 MHz linear-array, real-time B mode transducer (CHISON, China and Exago, IMV, France). During the study period all mares were clinically healthy. When pre-ovulatory follicle diameter obtained 35 mm, the follicle was considered as a dominant follicle. Then, cross-sectional follicular area (FA) was taken as a product of the two largest perpendicular follicular diameters. 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 42 mm in diameter approximately, and tear shape, 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 48h 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.

**Determination of length of gestation:** The length of gestation period was calculated as the interval from ovulation until foaling. 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.

**Artificial insemination of the mares:** Frozen semen was thawed by immersing the straws in a water bath at 37°C for 30 sec, and 4 to 5ml of semen was artificially inseminated in the estrus mare after detecting the suitable period of near ovulation. Mares were inseminated until ovulation is confirmed. Pregnancy diagnosis and various embryonic developmental changes were recorded using ultrasound machine from day 12 onwards. Mares that were not conceived were rebred again.

**Statistical analysis:** All the results were expressed in Mean  $\pm$  SE. Statistical analysis was performed using Statistical Package for Social Science (SPSS® Version 20.0 for Windows®, SPSS Inc., Chicago, USA). The means were compared using Analysis of Variance, Duncan's multiple range test and presented as mean  $\pm$  standard error (SE) at the significance level of  $P < 0.01$  or  $P < 0.05$ .

## RESULTS AND DISCUSSION

Summary of various reproductive indices of Kathiawari mares were recorded, analyzed statistically and presented in the Table 1.

**Table 1.** Reproductive parameters of Kathiawari mares.

SNo.	Reproductive Parameters	Mean	Range
1	Estrous cycle duration (days) (n = 75)	20.47 $\pm$ 2.87	18 – 24
2	Length of estrus period (days) (n = 75)	8.79 $\pm$ 1.92	6 – 9
3	Size of the follicle at ovulation (mm) (n=158)	42.47 $\pm$ 2.67	37.2 – 48.9
4	Gestation period (days) (n = 15)	326.47 $\pm$ 7.93	315–339
5	Foal heat (days) (n = 15)	8.27 $\pm$ 4.09	6 – 14
6	Size of the ovulatory follicle in foal heat (mm)	38.27 $\pm$ 1.21	37-42
7	Foal Birth Weight (Kgs)	25.43 $\pm$ 2.49	24-36

**Estrus duration and estrus characteristics:** The day when mares which start exhibiting the estrus signs on presenting a stallion were taken as estrus day one and were also

examined through ultrasonography for the same. The signs of estrus signs included a stance of micturition (squatting) and a swelling and winking of the vulvar lips, a prolonged rhythmic exposure of clitoris. In the present study, an average duration of estrus was 8.74  $\pm$  1.92 days with the range of 6-9 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, Zanskari and Manipuri Mares (Arangasamy *et al.*, 2008; Talluri *et al.*, 2016; Talluri *et al.*, 2020) 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, 1 day 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).

In the present study, mares exhibited an estrous cycle throughout the year (polyestrous) with more than 80% of mares exhibiting estrus during mid of March through November. The main part of the breeding season reported to occur 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).

**Duration of estrous cycle:** The estrous cycle length was recorded to be 20.47  $\pm$  2.87 (range 18-24) days in Kathiawari horses. 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 have reported an average length of 21 days in exotic horses and 14 to 26 days in Marwari and Zanskari mares. Observations for the same parameters in Zanskari breed of mares revealed a mean of 18.58  $\pm$  0.56 (range 15-26) days in the earlier study (Talluri *et al.*, 2016) whereas it was 19.85  $\pm$  0.44 days (18-26 days) for Manipuri mares (Talluri *et al.*, 2020). Various factors such as 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). Duration or length of estrus of mares may vary among individuals of same breed and different breeds and also among the estrous cycles of the same mare.

**Follicular dynamics:** Both the ovaries of the Kathiawari mares were diagnosed every day from the day of showing prominent visual signs of estrus using ultrasound



machine with transrectal linear array probe to know the status of follicular growth and to determine ovulatory size of the dominant follicle. There was increase about 0.24 to 0.51 cm in size of the follicle every day during estrus phase. The size of the ovulatory dominant follicle was observed from 37.2 to 48.9 mm with an average size of  $42.47 \pm 2.67$  mm (Fig.1, A- D).

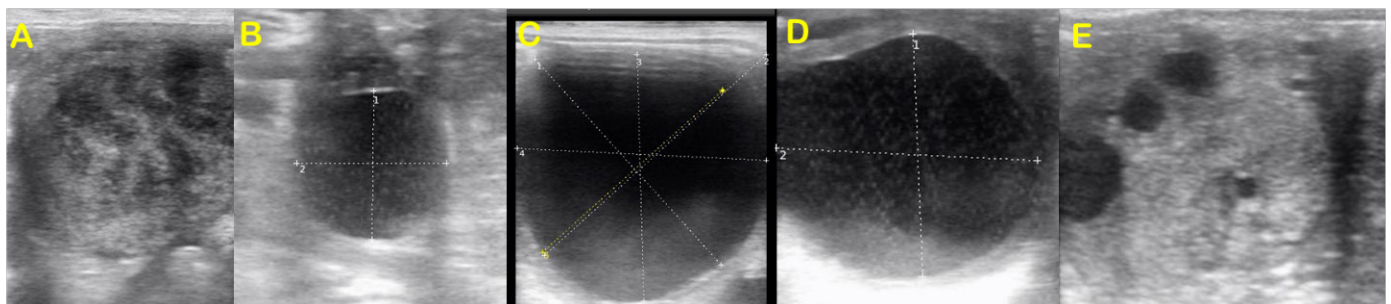
To achieve pregnancy and get foals from Kathiawari mares, we, for the first time collected and cryopreserved successfully Kathiawari stallion semen and artificially inseminated (AI) with the frozen thawed semen in estrus mares which later became pregnant with a fertility index of 1.62 to 3.97 (*data not shown*). The inseminated mares were conceived and the embryonic developmental stages were also recorded (Fig. 2 A-D) and viable foals have been obtained for the first time with AI from the cryopreserved semen of stallions from Kathiawari breed (Fig.3.).

**Gestation Length:** The average gestation length in Kathiawari mares was  $326.47 \pm 7.93$  days. The length of gestation was reported as 328 days with a ranging 301 to 368 days in Marwari mares (Arangasamy *et al.*, 2008), and in Zanskari mares it was  $326.11 \pm 3.23$  days and ranged from 314 to 342 days (Talluri *et al.*, 2016). The average gestation length in Manipuri mares was recorded to be  $338.47 \pm 5.21$

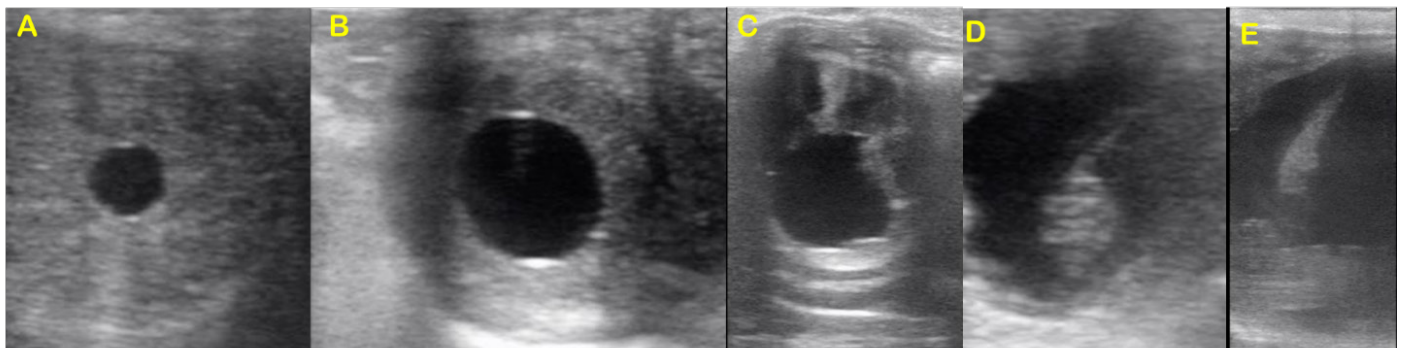


**Fig. 3.** Kathiawari mare with foal at foot. For the first time foals were produced through frozen semen AI in this breed at ICAR-NRCE.

(Talluri *et al.*, 2020). The present findings of the gestation length are also in normal range and corroborated with the



**Fig.1.** Uterine and follicular dynamics observed during estrus in Kathiawari mares A. Typical cart wheel pattern observation of uterine folds during estrus stage in mares B. follicle on day 3 C. Pre-ovulatory dominant follicle D. typical tear shape of follicle just before ovulation E. Corpus luteum formation after ovulation.



**Fig.2.** Embryonic developmental stages in Kathiawari mares identified through ultrasonography. A. 12<sup>th</sup> day Pregnancy B. 17<sup>th</sup> day pregnancy C. 20<sup>th</sup> day pregnancy D. 28<sup>th</sup> day pregnancy E. 46<sup>th</sup> day pregnancy in Kathiawari mare.

reports of Arangasamy *et al.* (2008) in Marwari mares, Talluri *et al.* (2016 & 2020) in Zanskari and Manipuri mares and Davis Morel (2005) in Thoroughbred mares. 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 Morel, 2002). 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 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). 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:** In the current study, we observed neither the twin pregnancies nor any dystocia during foalings. Mares are unusual among mammals in showing her first estrus very soon after foaling often within 4-10 days. In the present study, an average duration of foal heat was  $8.27 \pm 4.09$  days (6-14 days) which is correlating with the reports of Arangasamy *et al.* (2008) and Talluri *et al.* (2016 & 2020) for Marwari mares, Zanskari and Manipuri mares. Foal heat is fertile and it is advised to inseminate the mares during foal heat to minimize the duration between foalings (Talluri *et al.*, 2021). Postpartum estrus usually occurs 5 to 15 days after foaling and some mares, however, may show estrus as late as 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 found to be  $25.43 \pm 2.49$  kg (24-36 kg). This observation was lesser to the earlier reports of Davies Morel (2003) who reported as 32 kg in thoroughbred mares. The observations were corroborating with the earlier reports observed for Zanskari and Manipuri mares (Talluri *et al.*, 2016).

The current study reports the reproductive parameters of indigenous horses of Kathiawari breed. 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. However, it needs to be taken into consideration the fact that the population of Kathiawari horse breed 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.

## CONCLUSIONS

The current study gives a reference about some of the basic reproductive indices of Kathiawari breed of horses. Data on reproductive parameters would assist 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 precisely, taking care of a due mare and a foal properly, and better planning for the subsequent breeding season. The present study findings may be useful as a ready reckoner for the indigenous breeds of horses reared under arid region.

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## CONFLICT OF INTEREST

None.

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