



Characteristics and freezability of Kathiawari horse semen

S K RAVI¹, R A LEGHA², YASH PAL³ and R C SHARMA⁴

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

ABSTRACT

Semen was collected from 6 Kathiawari horses for its evaluation and freezing. The appearance of fresh semen was milky white to creamy and consistency of gel free semen was variably thin. The volume of total semen, gel free semen and gel in semen were 37.8 ± 3.47 , 25.8 ± 2.13 and 12.0 ± 2.22 ml, respectively. Mass and progressive sperm motility percent in gel free semen was 82.0 ± 1.51 and 77.0 ± 1.51 , respectively. Mean sperm concentration in fresh semen was $173.75 \pm 8.86 \times 10^6 \text{ ml}^{-1}$. Post thaw sperm motility (PTM) % in 17 out of total 21 ejaculates (80%) was recorded up to an acceptable ≥ 30 and mean value 35.62 ± 1.99 . Five Kathiawari horses were moderate to good freezer (PTM, 30 to 45%), whereas one stallion was poor freezer (PTM, 5 to 10%). The study indicated that the semen from Kathiawari stallions can be cryopreserved at farmers' door using vapor freezing technique for encouraging artificial insemination and their conservation.

Key words: Characteristic, Freezability, Freezing, Horse, Kathiawari, Semen

Kathiawari, an important indigenous breed of horses, is native of Saurashtra region of Gujarat. Breed characteristics of these horses include shorter back with concave profile, stout neck, short legs and squared quarters. The face is short, triangular from pole to forehead, small muzzle, big nostrils and curved upright ears at 90° axis that can rotate at 180° , broad forehead and large expressive eyes. Tail is long touching to ground; foot is round and broad. Height of these horses measures between $13^{1/2}$ to $14^{1/2}$ hands. Kathiawari horses are reared for riding, sports and transportation. Indiscriminate breeding with exotic or non-descript horses and development in mechanization led to drastic decrease in the population of indigenous equine breeds. This necessitates immediate measures to be taken for conservation and propagation of Kathiawari breed of horses. The semen of Marwari stallion and Poitou jack was characterized (Pal *et al.* 2009) as well as cryopreserved using vapor freezing technique in Marwari stallion (Pal *et al.* 2011a) and Poitou jack (Legha and Pal 2012). Studies on seminal characteristics and freezability of Kathiawari horse semen has not yet been taken up. Hence, seminal characteristics were recorded to generate baseline information in Kathiawari horses, which is prerequisite for semen cryopreservation and semen was cryopreserved successfully, which is first to be reported in Kathiawari horses.

Present Address: ¹Scientist (skravivet@gmail.com), ^{2,4} Senior Scientist (legha@scientist.com, rcsharmaepc@rediffmail.com), ³Principal Scientist (yashpal1888@gmail.com).

MATERIALS AND METHODS

Semen was collected from 6 Kathiawari horses at Junagadh in which 4 horses (Chanakya, Sarthi, Parijat and Chand) were maintained at Kathiawari Horse Breeding Centre, Junagadh, whereas 2 horses, Manik and Chetak were transported to Junagadh one each from its sub centres at Inaj and Gondal in Gujarat, respectively. Ejaculates (15) were collected over 6 days period including 1 day rest allowed on third day from 3 Kathiawari horses (Chanakya, Sarthi and Parijat). Four ejaculates from 1 horse (Chand) were collected daily over 5 days including 1 day rest allowed on second day which did not mount on first day of schedule. Similarly, 2 ejaculates were obtained 1 each from 2 horses (Manik and Chetak) brought 1 day before last day of semen collection schedule. Artificial vagina method was used for semen collection in the morning hours before feeding during June last and July first week. Semen collection, evaluation and processing for freezing were done according to Pal *et al.* (2011b). Seminal parameters as appearance, volume, colour, consistency, pH, sperm concentration, mass and progressive sperm motility were recorded by visual observation under microscope. Semen was extended up to 150–200 millions sperm/ml, filled into 0.5 ml poly-vinyl chloride (PVC) straws and sealed with polyvinyl alcohol powder (PVA) using manual method. Filled straws were kept for equilibration in refrigerator at 5°C for 2 h; pre-freeze motility was recorded after equilibration and before exposing straws to liquid nitrogen (LN_2) vapor. Straws were kept spreaded over freezing racks, 3cm above the LN_2 level which was up to 3

cm in a tyrofoam box for exposure to LN₂ vapor and then dipped into LN₂ after 12 min. Frozen semen straws were dipped into water bath at 37°C for 1 min to thaw and PTM was recorded. Semen samples having PTM ≥30% were kept stored into separate canisters dipped in LN₂ containers.

RESULTS AND DISCUSSION

Seminal characteristics

Appearance and consistency: Fresh semen of Kathiawari horses was observed milky white to creamy and consistency was variably thin. The appearance of semen was in agreement with observation of Pal *et al.* (2009); however, consistency was reported thick to thin in Marwari stallions. A good semen sample of stallion should appear milky white in colour, though it may range from watery to creamy.

Semen volume: Total semen volume, gel free semen and gel volume in semen were recorded (Table 1). Ricketts (1993) observed that total volume of stallion semen vary between 30 and 250 ml. In present study, average total semen volume, gel free semen and gel in semen was recorded less than reports of Pal *et al.* (2009) in Marwari stallions. This may be due to frequent use of stallions for breeding purpose and breed difference. There is individual variation in semen volume which may be affected by age, season, breed, teasing time, frequency of semen collection and workload (Pickett *et al.* 1988).

pH of semen: The mean value recorded for seminal pH was 7.05±0.04, ranging from 6.7 to 7.4. However, Heckenbichler *et al.* (2011) reported a wider pH range (6.0–7.5) with a mean value of 6.6±0.0 in stallion semen. In present study, semen pH was within this range which was also comparable to report of Pal *et al.* (2009) in Marwari stallions (7.2±0.02) and Poitou donkey stallions (7.17±0.02).

Sperm concentration: Mean sperm concentration in fresh semen was 173.75±8.86 × 10⁶ ml⁻¹ varying from 115 to 275 × 10⁶ ml⁻¹. The values for sperm concentration in horse semen samples were reported to vary widely, ranging from 100 to 200×10⁶ ml⁻¹ (Pickett *et al.* 1988). All values within the range may be considered acceptable and appropriate for use in artificial insemination (AI). In present study, sperm

concentration varied which was not significant. Mean sperm concentration in Kathiawari stallions observed in this study is lower (173.75±8.86 × 10⁶ ml⁻¹ vs 192.0±9.3 × 10⁶ ml⁻¹) compared to Marwari stallions (Pal *et al.* 2009). This could be due to daily collection of semen from the stallions and the reason that all stallions were frequently in use for natural covering to mares. Magistrini *et al.* (1987) reported a decrease in sperm concentration per milliliter for everyday and 3 days collection of semen per week compared to alternating every week.

Mass motility and progressive motility: Mass or initial sperm motility % observed in gel free semen was 82.0±1.51 which is near to value (79.76%) observed in Marwari stallions (Pal *et al.* 2009). Progressive sperm motility % in gel free semen was observed 77.0±1.51 which is near to value (73.33%) observed in Marwari stallions (Pal *et al.* 2009). Progressive sperm motility more than 60% might be considered appropriate for cryopreservation of stallion semen and used in AI program (Davies Morel 1993).

Pre freeze motility and post thaw motility: The pre freeze motility % in extended semen after 2 h of equilibration at 5°C was 69.64±1.18 (range 60 to 75). Observation of pre freeze sperm motility is a check point before further processing of diluted semen for freezing. Pre-freeze motility was slightly lower as reported by Pal *et al.* (2011a) in Marwari stallions. The post-thaw sperm motility % (PTM) was 35.62±1.99 (range 30 to 45). Almost similar trend of PTM was reported by Pal *et al.* (2011a) in Marwari stallions.

Freezability of semen: Freezability of semen from 6 Kathiawari horses was 80% (17 out of 21 ejaculates). Five Kathiawari horses were moderate to good freezers, and had PTM 30 to 45%, whereas 1 stallion was poor freezer, and had PTM 5 to 10%. Pal *et al.* (2011a) reported that semen of all the stallions' do not freeze alike which was also recorded during the present study. Tischner (1979) also observed that approximately 20% of stallions are good freezers, another 20% are bad freezers, and the majority of stallions (60%), produced semen that is affected adversely, but may be freezable using certain techniques. Vidament *et al.* (1997) also observed that 20–40% stallions responded poorly to

Table 1. Seminal characteristics of individual Kathiawari horses

Stallion name	Chanakya	Sarathi	Parijat	Chand	Manik	Chetak
Total volume (ml)	40.6±11.51	43.25±5.96 ^a	24.8±0.80 ^b	43.25±6.49 ^a	35.0	48.0
Gel volume (ml)	17.8±6.39	18.25±5.54 ^a	4.8±1.0 ^b	8.25±1.18	3.0	18.0
Gel free volume (ml)	22.8±5.20	25.0±0.70 ^a	20.0±0.6 ^b	35.0±7.35	32.0	30.0
p ^H	7.12±0.07	7.00±0.11	7.06±0.11	7.1±0.12	6.8	6.9
Sperm conc. (×10 ⁶)	164.0±10.32	157.5±16.89	150.0±11.32	187.5±13.14	250.0	275.0
Mass motility (%)	82.0±3.40	81.25±3.14 ^a	76.0±2.4 ^a	88.75±1.25 ^b	90.0	80.0
Progressive sperm motility (%)	77.0±3.40	76.25±3.14	71.0±2.45 ^a	83.75±1.25 ^b	85.0	75.0
Pre freeze motility (%)	71.25±2.1 ^a	70.0±2.50 ^a	62.5±1.58 ^b	70.0±0.00 ^a	75.0	70.0
PTM (%)	31.25±1.25 ^{ad}	38.33±1.66 ^{bd}	10.0±5.01 ^c	36.66±4.41 ^d	40.0	35.0

Values bearing same superscript or no superscript within a row do not differ significantly (P ≤ 0.05).

cryopreservation. Hence, variation in semen freezability observed during the present study is well supported by Tischner (1979) and Vidament *et al.* (1997).

ACKNOWLEDGEMENT

Authors are thankful to the Director, NRCE, Directorate of Animal Husbandry, Gujarat state for providing the facilities; Dr M L Gujarati, Dr R B Sanwalia, Deputy Directors of A.H. and staffs at Kathiawari Horse Breeding Centre, Junagadh for helping us to carry out this experiment.

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