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Freezability and fertility of Marwari stallion semen

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ABSTRACT

Variations in freezability of extended raw semen between individual stallions and rate of conception with cryopreserved semen were studied. Semen was collected through artificial vagina (AV) from 14 Marwari stallions (4 collections each) of 4 organized farms. The semen was filtered through a sterilized gauge for removing gel and volume was recorded. Gel free semen was used for evaluation of certain physical and morphological characteristics like initial motility, progressive motility, pH, colour and consistency of semen and total sperm concentration adopting standard methods. Ejaculates with more than 60% progressively motile sperm were further used for freezing using Bio-med planner. Straws were thawed at 37°C for 1 min in water bath and evaluated for post-thaw motility. Out of 14 stallions studied, the freezability of semen was observed as good, moderate and poor on the basis of post-thaw motility in 6, 5 and 3 stallions, respectively. A total of 98 estrus mares were monitored with ultrasonography from third day of estrus to the time of ovulation and inseminated near to ovulation using the frozen semen. One, two, three and four AI per cycle were performed in 32, 59, 4 and 3 mares depending upon the expected time of ovulation with conception rate as 31, 47, 75 and 100%, respectively. It is concluded that semen of all the stallions do not freeze alike and for optimum conception rate at least 2 to 3 AI per cycle in mares is preferred.

Key words: Conception rate, Fertility, Freezability, Marwari, Semen, Stallion

AI using cryopreserved semen is well in use in all the species including equines in different parts of the world but with limited success. One of the limiting factors in the use of cryopreserved semen in equines is the reduced fertility (Squires *et al.* 2004). In addition to semen quality, many other factors affect the result of AI, including the semen handling and freezing methods, AI dose, timing of AI and management (Colenbrander *et al.* 2003). In our earlier studies semen freezability results were erratic. The present study was initiated to study the variation in semen freezability among the individual stallions as well as to study the fertility rate of the stallions through AI using cryopreserved semen.

MATERIALS AND METHODS

Semen of 14 apparently healthy Marwari stallions (4 collections from each) of normal fertility maintained at 4 organized farms was collected in the morning before feeding and processed. Semen was collected by artificial vagina equipped with a disposable liner as per the standard method. An estrus mare was used as dummy mare for semen collection. The stallions were kept at distance from the mare for visual stimuli to get proper erection before mounting for ejaculation in the AV. Soon after the semen collection, the semen was filtered through sterilized gauze to remove the gel. Gel-free semen was kept at 37°C and used for microscopic and macroscopic analysis. The semen having progressive motility more than 60% was subsequently processed for cryopreservation. Gel-free semen was mixed with modified glucose- ethylenediaminetetraacetic acid (EDTA) primary extender (glucose 0.15 g, sodium citrate dehydrate 2.6 g, disodium EDTA 0.37 g, sodium bicarbonate 0.12 g, streptomycin 0.10 g, benzyl penicillin 0.10 g, made up to 100 ml with double distilled water) as per Cochran *et al.* (1984) in the ratio of 1:1 and centrifuged at 2000 rpm for 4–5 min at 8–10°C. The supernatant was aspirated off and the sperm pellet was dissolved in the modified secondary extender (mixture of 2 solutions i.e. 25 ml from solution 1 + 50 ml from solution 2 with 20 ml egg yolk centrifuged at 3000 rpm at 10°C for 30 min to collect clear supernatant fluid and added glycerol 3% of the total volume). Solution 1 contains glucose EDTA (glucose 6 g, sodium citrate dehydrate 0.37 g, disodium EDTA 0.37 g, sodium bicarbonate 0.12 g, streptomycin 0.08 g, benzyl penicillin 0.08 g, made up to 100 ml with double distilled water). Solution 2 contains lactose 11 g, streptomycin 0.08 g, benzyl penicillin 0.08 g, made up to 100 ml with double distilled water. The diluted semen was kept in semen cooling cabinet at 5°C for 2 h as

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equilibration time. The equilibrated semen was filled and sealed with filling-sealing machine in the poly-vinyl chloride (PVC) straws of 0.5 ml capacity (IMV make) containing 50–60 million motile spermatozoa per straw. These straws were put in the programmable planner biomed for cryo-freezing. The straws in bio-med planner were cooled @ –0.3°C/min from 18°C to 5°C, and frozen @ –10°C/min from +5°C to –15°C @ –19°C/min from –15°C to –100°C. After reaching –100°C, the straws were finally plunged into liquid nitrogen (LN_2) and kept stored in LN_2 till further use for AI. After freezing, 2 straws from each group were thawed at 37°C for 1 min in water bath, immediately wiped off, cut open and emptied into a clean pre-warmed vial (37°C), which was maintained at 37°C and evaluated for post-thaw motility and grading of the stallions. A total of 98 estrus mares free from uterine infections were monitored with ultrasonography from third day of estrus to the time of ovulation and inseminated near to ovulation using the frozen semen. First AI was done when the follicle size reached to 45 mm and thereafter daily till the actual ovulation has taken place during the breeding season (March to September). One, two, three and four AI per cycle was performed in 32, 59, 4 and 3 mares depending upon the expected time of ovulation by the same inseminator. Pregnancy was diagnosed using ultrasound between days 30–45 after insemination. The data were analyzed by standard statistical methods (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The percentage of motile sperms in a stallion's ejaculate is believed to be one of the factors that determine the fertility of the sample. For practical purposes, a stallion having at least 60% of the progressive motile sperm in his ejaculate is considered as a satisfactory potential breeder (Rossdale and Ricketts 1980). Keeping this in view, the ejaculates having more than 60% motility were processed for cryopreservation of semen. Results (Table 1) indicated that mean pre-freezing motility of 14 stallions ranged between 65 and 85% representing all the semen samples was suitable to cryopreserve. Semen processing technique and composition of extender used were similar for all the semen samples. Mean post-thaw motility ranged between 6.25 and 47.5% representing wide variation in the semen freezability of the stallions. Stallions having post-thaw motility <20, 20–40 and >40% were termed as poor, moderate and good freezers, respectively. On the basis of post-thaw motility of the frozen semen, stallions were graded as good (6/14), moderate (5/14) and poor (3/14) freezers (Table 1). It indicated that the semen of stallions do not freeze alike even all other factors affecting freezability are constant. Tischner (1979) also observed that approximately 20% of stallions are good freezers, another 20% are bad freezers, and the majority of stallions (60%), produce 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 cryopreservation. Hence, variation in semen freezability observed during the present study is well supported by Tischner (1979) and Vidament *et al.* (1997). Also, no particular trend was observed between pre-freezing semen motility and freezability of stallion semen during the study.

Table 1. Average value of pre-freezing and post-thaw motility of stallion semen

Stallion No.	Pre-freezing motility	Post-thaw motility	Score of stallion
1	80.0	42.5	Good
2	77.5	45.0	Good
3	82.5	35.0	Moderate
4	85.0	45.0	Good
5	72.5	36.25	Moderate
6	75.0	7.5	Poor
7	77.5	47.5	Good
8	75.0	8.75	Poor
9	80.0	45.0	Good
10	75.0	41.25	Good
11	65.0	30.0	Moderate
12	70.0	33.75	Moderate
13	77.5	6.25	Poor
14	75.0	37.5	Moderate

Table 2. Effect of number of AI on conception rate in mares

No. of mares inseminated	No. of AI per cycle	No. of mares pregnant	Conception rate (%)
32	1	10	31
59	2	28	47
4	3	3	75
3	4	3	100
98	1.77±0.67	44	45

The reproductive performance of mares is expected to be low due to small breeding season during the longer days of the year and plenty of estrous cycle irregularities during the early and late season (Ginther 1974). Coupled with this, because of the longer estrous period and variable time of ovulation, the number of services required per foal born is high (Purohit *et al.* 1999). Effect of number of AI per cycle on conception rate is depicted in Table 2. Out of 98 mares inseminated artificially using frozen semen, 44 were pregnant indicating a conception rate of 45% which is good as far as equines are concerned. Average number of AI per cycle was observed as 1.77. Cristanelli *et al.* (1984) and Gary England (2005) reported conception rate as 56 and 20–55%, respectively, in Thoroughbred horses. In general, pregnancy rates from frozen semen are not as high as those expected from fresh semen in horses and a difference of 5–30% is often quoted (Wockener and Colenbrander 1993). Jhamb *et al.* (2006) reported the pregnancy rate as 61 and 36%, using

fresh semen v/s frozen semen, respectively, in Thoroughbred horses. In a pilot study, Arangasamy (2008) had observed a conception rate of 72% using AI, but the number of mares inseminated was very less. They also reported that ultrasonography assisted pre-ovulatory follicle size confirmation in estrus mare provided suitable time for insemination to get good conception rate with frozen semen. Hence, conception rate of 45% which was achieved in the present study is appreciable due to the fact that the longevity of frozen semen in female reproductive tract is reduced (Watson 2000). Results (Table 2) indicated that to get satisfactory conception rate at least 2 AI per cycle are must in mares. Good conception rate is normally achieved with insemination every 48 h until the oestrus lasts (Watson and Nikolakopoulos 1996). The ideal time of insemination would be the one in which mares ovulates soon before or after insemination, though stallion sperm survive in female reproductive tract for a few days (Troedsson *et al.* 1988). During the present study, conception rate of 75 and 100% was achieved in 7 mares using 3 or 4 AI / cycle, respectively (Table 2) is showing that AI was performed close to ovulation. But it is relatively difficult to inseminate the mare close to the time of ovulation due to non-availability of ultrasound machines and experts to handle the machine in the field as well as mare exhibits estrus for approximately one week.

To study the stallion-wise conception rate, frozen semen of 10 stallions was used for AI (Table 3). An overall conception rate of 45% (44/98) was observed in mares using frozen semen in the present study. Conception rate was observed more than 50% in 5 stallions, between 40 and 49% in 2 stallions and less than 40% in 3 stallions. Number of mares allotted to each stallion for fertility trial was less during the study. Amann (2005) emphasized that fertility studies in horse are notoriously difficult to interpret and sample sizes of statistical relevance are almost impossible to achieve. Also, mares could not be evenly distributed to each stallion due the colour preference of particular stallion by the owner of the mare. Although, all the 3 stallions with less than 40% conception rate were of moderate freezability but no effect of freezability was observed on conception rate during the study. A conception rate of 40 to 100% was obtained using the frozen semen of the stallions with good freezability.

Table 3. Stallion wise per cent conception rate

Stallion No.	No. of mares inseminated	No. of mares pregnant	Conception rate (%)
2	39	16	41
3	5	3	60
4	8	4	50
5	13	4	31
7	8	4	50
9	7	7	100
10	5	2	40
11	4	0	0
12	3	1	33
14	6	3	50
Total	98	44	45

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