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Effect of hormones on semen collection and its quality in mithun (*Bos frontalis*)

KEYWORDS: mithun, oxytocin, PGF2α, sperm functional parameters, massage method

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ABSTRACT *Conservation and propagation of genetic resources of mithun are important to preserve mithun germplasm and livelihood of its rearers. To date, no information is available on effects of oxytocin and PGF2α administration in mithun semen collection and quality. Total of 20 ejaculates were collected through rectal massage method in which these injections were given intra muscularly. These semen samples were analysed for basic seminal parameters. The success rate in semen collection was higher in oxytocin treated animal than other treatments. Similarly the different seminal parameters were showed significant different ($p < 0.05$) between the experimental groups. This study was concluded that the success rate and seminal parameters were superior in oxytocin treated animal.*

INTRODUCTION

Mithun is a unique free – range bovine species available in the North Eastern hill region of India (Simoons 1984). Mithun population is decreased gradually due to lack of suitable breeding bulls, increasing of intensive inbreeding practices and lack of suitable breeding management. In this context, it is necessary to standardize an effective semen preservation protocol for this species to adopt artificial insemination (AI) for breed improvement programme.

Oxytocin may be involved in the movement of sperm in the male duct system, specifically epididymal sperm transport (Whittington et al. 2001). Furthermore, sexual stimulation and ejaculation were associated with a concurrent rise in blood oxytocin concentration in many species (Murphy et al. 1987) and oxytocin treatment prior to semen collection resulted in increased sperm output in rams (Nicholson et al. 1999), buffalos (Ibrahim 1988) and bulls (Berndtson and Igboeli 1988). Therefore, it seems reasonable that since oxytocin is involved in the transit of sperm through the male duct system, it may hasten ejaculation in bulls. PGF2α is known to increase smooth muscle contractility in the male as well as female genital tracts and may be involved in the ejaculation process (Bygdeman 1981). Parenteral PGF2α administration before semen collection resulted in increased sperm output in stallions (Cornwell et al. 1974), dogs (Hess 2002) and buffalo (Ibrahim 1988).

There is apparently no published report on semen collection by transrectal massage with injection of oxytocin or PGF2α in this species. Therefore, the objective of the current study was to determine the feasibility of semen collection from mithun bulls using a transrectal massage in the presence of oxytocin or PGF2α injection without sexual preparation to assess semen characteristics.

MATERIALS AND METHODS

Eight apparently healthy mithun bulls with good body condition (score 5-6) maintained under uniform feeding, housing and managemental conditions, approximately 4 to 6 yr of age were selected for the present study. A total number of 20 ejaculates were collected through rectal massage method from the mithun twice a week from the experimental animals. Immediately after collection, the samples were kept in a water bath at 37°C and evaluated for volume, colour, consistency, mass activity and pH. The treatment groups were divided into three viz. group 1 (control): 5ml 0.9% saline, group 2: Oxytocin 20 IU (Syntocinon, Novartis India limited, Mumbai, India) and group 3: PGF2α 0.5 mg (Cyclix, Intervet international GmbH, Pune, India). These injections were given through intra muscular prior to rectal massage.

During collection, the initial transparent secretions were discarded and neat semen drops were collected in a graduated test tube with the help of a funnel. During semen collection protrusion time, ejaculation time and length of penis exposure were measured. Semen volume was evaluated using the graded collection tube and sperm concentration was determined using a Neubauer hemacytometer. Seminal parameters were analysed by standard procedures. The results were analysed statistically and expressed as the mean ± S.E.M. Means were analyzed by one way analysis of variance, followed by the Tukey's post hoc test to determine significant ($p< 0.05$) differences between the experimental groups using the SPSS/PC computer program (version 15.0; SPSS, Chicago, IL).

RESULTS AND DISCUSSION

The effects of oxytocin and PGF2α on seminal parameters, which were collected from rectal massage method, were presented in Table 1. The percentage of success rate to obtain the semen from the mithun in control, oxytocin and PGF2α treated group were 72.41, 90.56 and 84.48, respectively. Different seminal parameter was showed significant different ($p< 0.05$) between the experimental groups. Results of this study indicated that administration of oxytocin or PGF2α, 10 minutes prior to semen collection by rectal massage in the mithun can improve not only the ejaculate quality, but also the ease of sample collection.

Improvement in ejaculate quality following oxytocin administration was observed significantly ($p< 0.05$) as increased total sperm number, live sperm count, volume and decreased the total sperm abnormality in rectal massage method of semen collection. The increase in sperm number in the ejaculate following oxytocin or PGF2α administration is not due to an increased rate of spermatogenesis. Spermatogenesis is unaffected by collection frequency or short term oxytocin or PGF2α administration (Marshall and Hafs 1976). Rather, oxytocin or PGF2α increases the rate of passage of spermatozoa from the cauda epididymis to

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the deferent duct. The mechanism behind this increases is almost certainly due to redistribution of sperm from the cauda epididymis to the deferent duct during smooth muscle contraction stimulated by oxytocin (DaSilva E Souza et al. 1975) or PGF2α (Hib and Oscar 1978) and is probably involved in sperm transport in the male duct system during emission and ejaculation in several species (Murphy et al. 1987). As oxytocin treatment results is in increased sperm flow through the ducti deferentia (Nicholson et al. 1999), the time required for emission, i.e. the transport of sperm from the epididymis into the pelvic urethra, may be reduced with a consequent reduction in the time required for obtaining a semen sample. The increase in semen volume following oxytocin administration in the current study is consistent with studies that reported increases in ejaculate volume in the ram (Voglmayr 1975), bull (Berndtson and Igboeli 1988) and buffalo (Ibrahim 1988). Oxytocin is also thought to influence the secretion rate of the male accessory glands, which, may account for the increased volume seen in the ejaculate of rams and buffalo following administration (Ibrahim, 1988). However, these reports also demonstrated an increase in sperm numbers in the ejaculate as well. So that the quality of semen was more better than control group. Similarly, Hafs et al. (1974) reported that although PGF2α administration to bulls prior to ejaculation increased sperm number in the ejaculate by 33%, this figure was 30% less than that after sexual preparation in the same bulls.

Serum oxytocin concentrations increase in the male during copulation (Sharma and Hays 1973) and in some species the testicular capsule, seminiferous tubules and epididymis contain contractile tissue sensitive to oxytocin treatment (Ellis et al. 1981). It is evident that in this species oxytocin plays a significant role in regulating physiologic sperm transport.

Lack of serious or significant side effects following oxytocin or PGF2α administration was notable, as the purpose of the experiment was to find a drug that could be used in the clinical setting to improve the mithun ejaculate. There was no side effects would have enhanced the application of oxytocin use in routine assisted reproduction in the mithun but mild irritation on PGF2α administration in mithun bulls.

It was concluded that the percentage of success rate and seminal parameters were significantly higher in oxytocin treated bull than other treated and control group.

Table 1. Mean (±S.E.M.) seminal attributes of semen collected through transrectal massage method with oxytocin and PGF2α treatments

Parameters	Control	Oxytocin	PGF2α
Success rate (%)	72.41	90.56	84.48
Protrusion time (sec)	84.92 ± 4.11^{b}	71.37±2.68 ^{a}	80.16±2.41^{ab}
Ejaculation time (sec)	210.07 ±9.74^{c}	94.32 ±4.01^{a}	111.02±5.81^{b}
Length of penis (inch)	10.32 ±0.12^{a}	11.87 ±0.71^{b}	10.10±0.17^{a}
Volume (ml)	1.39 ±0.59 ^{a}	2.04 ±0.38 ^{b}	1.61±0.56^{a}
Mass activity (1+ to 5+)	2.82 ±0.67^{a}	3.62 ±0.53 ^{b}	2.87±0.60^{a}
Progressive motility (%)	71.12 ±1.83^{a}	77.97 ±1.64^{b}	72.82±1.96^{a}
Live sperm (%)	73.72 ±1.87^{a}	84.56 ±1.58^{b}	74.70±1.17^{a}
Concentration (x10^{6}/ ml)	538.20 ±15.12^{a}	687.32±16.04^{b}	547.00±16.87^{a}
Total sperm abnormality (%)	10.35 ±2.42^{b}	8.21±0.33^{a}	10.17±0.39^{b}
Acrosomal Integrity (%)	78.45 ±1.65^{a}	89.72±1.49^{b}	76.12±1.44^{a}
Plasma membrane integrity (%)	75.82 ± 1.76 ^{a}	85.37±1.46^{b}	76.30±1.05^{a}

Within rows means with different letters (a, b and c) differ significantly ($p < 0.05$) between the treatment groups

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