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Fertility Improvement Following Herbal Treatment in Subfertile Buffalo Bulls

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ABSTRACT

The present experiment was conducted to study the impact of herbal treatment on post thaw semen quality and fertility in subfertile buffalo bulls. Three Murrah subfertile buffalo bulls maintained at bull station, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, India (Latitude/Longitude, 30.55°N, 75.54° E) with the history of poor semen quality (pre-freeze individual motility < 60%, post-thaw individual motility < 40%, Viability < 70% and Abnormality > 20%) were orally supplemented with herbal mixture (*Panax ginseng* roots, Shilajit, *Withania somnifera* roots, *Tribulus terrestris* fruits, *Turnera diffusa* leaves, *Ptychopetalum olacoides* bark of each 400 mg/100 kg body weight and 300 mg/100 kg body weight of *Pausinystalia yohimbe* bark) daily for 60 days of treatment phase. Two semen ejaculates per week per bull were collected during pre-treatment, treatment and post-treatment phases of 60 days each and extended with Tris egg yolk extender. The freezing of extended semen was carried out in a biofreezer (4°C to -15°C @-30°C/min, -15°C to -140°C @-50°C/min) followed by plunging into liquid nitrogen for storage. Post thaw semen quality was assessed in terms of individual motility, viability, total sperm abnormalities. Present study revealed that the oral treatment of subfertile buffalo bulls with *Panax ginseng* roots, Shilajit, *Withania somnifera* roots, *Tribulus terrestris* fruits, *Turnera diffusa* leaves; *Ptychopetalum olacoides* bark each @ 400 mg/100 kg body weight and *Pausinystalia yohimbe* bark @ 300 mg/100 kg body weight for 60 days improved post thaw semen quality and fertility.

Key words: Bull, Semen, Libido, Herbal, Subfertility

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INTRODUCTION

Buffalo bull sperms are more fragile as compared to cattle bull sperm (Andrabi *et al*., 2008; Kumaresan *et al*., 2005). Various factors such as temperature, humidity and nutrition have been ascribed to play a key role in freezability of buffalo bull sperm (Sharma *et al*., 2017; Dahiya *et al*., 2013). Under stress, there is excessive production of reactive oxygen species (ROS) in somatic and spermatogenic cells (Shamsi *et al.,* 2010), thereby creating an imbalance between production and utilization of ROS leading to oxidative stress (Nita *et al*., 2016). Due to the higher contents of polyunsaturated fatty acids (PUFA) and low antioxidant enzymes in buffalo bull sperm, there is more lipid peroxidation leading to poor post thaw recovery in terms of motility, viability, morphology, plasma membrane integrity and acrosomal integrity (Ahmad *et al*., 2014; Bucak *et al*., 2010; Nair *et al*., 2006).

Several studies have been carried out in humans and animals to improve the fresh and post thaw sperm quality by *in-vitro* addition of vitamins, minerals, proteins, amino acids, herbs (Patel *et al*., 2016; Dorostkar *et al*., 2014; Partyka *et al*., 2017; Liu *et al*., 2004) with variable success. However, only few in-vivo studies have been carried out by using oral feeding of herbs in subfertile buffalo bulls. Studies have indicated that herbs and herbal substances containing wide range of bioactive constituents protect semen from ROS induced quality deterioration due to various stressors (Etuk and Muhammad. 2009; Parandin *et al*., 2012). It has been observed that oral supplementation of herbal mixtures improved semen quality and minimized oxidative stress parameters (Shivkumar et al 2018a, Shivkumar et al 2018b, Shivkumar et al 2018c). However, the information pertaining to impact of oral supplementation of herbal mixture on fertility of subfertile buffalo bulls is lacking. Our hypothesis is that oral supplementation of herbal mixture containing *Panax ginseng* roots, Shilajit, *Withania somnifera* root, *Tribulus terrestris* Fruits, *Turnera diffusa* leaves, *Ptychopetalum olacoides* bark and *Pausinystalia yohimbe* bark at the rate of 400 mg/100 kg body weight and 300 mg/100 kg body weight respectively, might improve fertility upon insemination with post thaw semen. In the present study, we studied the effects of oral supplementation of above mentioned herbal mixture to subfertile buffalo bulls on semen quality and fertility following artificial insemination in buffaloes.

MATERIALS AND METHODS

The present study was conducted after the approval of Institutional Animal Ethics Committee with reference number GADVASU/2016/IAEC/35/02 dated 17.07.2016.

Selection of animals

The study was conducted on three (n=3) Murrah buffalo bulls (aged around 5 years and body weight 700-750 kilograms) with the history of poor semen quality (pre-freeze individual motility < 60%, post-thaw individual motility < 40%, Viability: < 70% and Abnormality: > 20%). Buffalo bulls were being maintained loosely in half walled concrete sheds in individual pens (covered area - 12 x 10 ft and uncovered area - 25 x 10 ft) at bull station, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, India (Latitude/Longitude, 30.55°N, 75.54° E). All the animals were being fed according to standard feeding schedule along with *ad libitum* green fodder. The bulls were being given an exercise for half an hour on alternate days.

Oral supplementation of herbs

This experiment was having three treatment phases viz. pre-treatment, treatment and post-treatment phase of 60 days each. During the treatment phase, buffalo bulls were orally supplemented daily with herbal mixture containing *Panax ginseng* roots, Shilajit, *Withania somnifera* roots, *Tribulus terrestris* fruits, *Turnera diffusa* leaves, *Ptychopetalum olacoides* bark each of 400 mg/ 100 kg body weight and *Pausinystalia yohimbe* bark @ 300 mg/100 kg body weight of bulls. Herbs were procured from the Indian Drugs and Botanical Herbs Company, New Delhi, India.

Semen collection and freezing

Two ejaculates per week per bull were collected using artificial vagina method. Ejaculates were evaluated for mass motility, individual motility and sperm concentration. The ejaculates having mass motility +++, individual motility more than 80% and sperm concentration 500 million/ml was further processed for cryopreservation. The semen was extended with freshly prepared Tris egg yolk Fructose extender keeping sperm concentration of 80 million/ml (35°C). The extended semen was filled, sealed and printed in French mini straws (0.25 ml) using integrated filling,

sealing and printing machine (IS4, IMV Technologies, France). The packaged straws were racked and kept for equilibration at 4°C for 4 hours in a horizontal cold handling cabinet (Model E22301CP-2000, IMV Technologies). Equilibrated semen straws were frozen in a programmable bio-freezer (Mini Digitcool, ZH 400, IMV Technologies, France) by using following pre-standardized freezing protocol for buffalo bull semen (4°C to -15°C @ 30°C/min; -15°C to -140°C @ 50°C/min. The straws were picked up from freezing chamber and plunged liquid nitrogen (-196°C) and stored therein for further assessment of post-thaw semen quality and fertility.

Post-thaw semen evaluation

Frozen semen straw was thawed at 37°C for 30 seconds and immediately evaluated for semen quality parameters such as individual motility, viability, total sperm abnormalities. Post thaw individual motility was assessed objectively with Computer Assisted Sperm Analysis software (Biovis CASA 2000, version 4.59, Expert Vision Labs Pvt. Limited, India). The sperm concentration of the sample was adjusted to 20 million sperms/ml using Tris buffer. A drop of semen (5 µl) was put on a pre-warmed Biovis Shukratara chamber (Expert Vision Labs Pvt. Limited, India) and individual motility was recorded under 10x of phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan) attached with the warm stage. Sperm viability and abnormalities of post-thaw semen was assessed by Eosin-Nigrosin stain and Rose Bengal stain, respectively (Kumar *et al.,* 2004).

Effects of herbal treatment on fertility of buffalo bulls

Fertility trial was conducted on 102 normal cycling multiparous buffaloes maintained at GADVASU dairy farm and private dairy farms in the peri-urban area of Ludhiana, Punjab. These buffaloes were being maintained under almost similar feeding and managemental conditions. The buffaloes were examined for their body condition scores, physical problems like lameness and genital infections. Animals with physical or genital tract problems were excluded from this study. In this study, all the buffaloes were inseminated by the same person. Out of 102 buffaloes, 21 buffaloes (7 Buffaloes/bull), 49 buffaloes (16 Buffaloes/bull) and 32 buffaloes (10 Buffaloes/bull) were inseminated with frozen semen produced during pre-treatment, treatment and post-treatment phases, respectively. The pregnancy diagnosis was conducted by per-rectal examination followed by ultrasonography at 60-70 days of insemination. Conception rate was calculated.

Statistical Analysis

Normality of data was checked by Shapiro-Wilk Test. Homogeneity of variance was analyzed by Levene's test. Data were analyzed by one way ANOVA followed by Tukey's HSD post hoc test for the comparison of supplementation phases (IBM SPSS Statistics version 22). Statistical significance was considered at $P<0.05$.

RESULTS AND DISCUSSION

Semen quality parameters

Semen quality parameters of subfertile buffalo bulls obtained during pre treatment, treatment and post treatment phases are presented in Table 1. During pre-treatment phase, individual motility and viability were lower as 32.85 ± 2.40% and 65.78 ± 2.13%, respectively. Following herbal treatment, individual motility and viability improved significantly to 41.72 ± 0.33% and 75.47 ± 1.59%, respectively during treatment phase and 40.36 ± 2.40 and 70.90 ± 1.05%, respectively during post treatment phase.

Table 1: Semen quality parameters (Mean ± SE) following herbal treatment of subfertile buffalo bulls during pre-treatment, treatment and post-treatment phases.

Semen quality parameters	Post-thaw semen		
	Pre-treatment	Treatment	Post-treatment
Motility	32.85 ± 2.40[a]	41.72 ± 0.33[b]	40.36 ± 2.40[b]
Viability (%)	65.78 ± 2.13[a]	75.47 ± 1.59[b]	70.90 ± 1.05[b]
Total sperm abnormalities (%)	37.22 ± 2.33[a]	15.63 ± 1.40[b]	20.03 ± 2.02[b]

Values with superscripts a, b differ significantly within a row (P<0.05)

Total sperm abnormalities were very high in pre-treatment phase (37.22 ± 2.33%), but following herbal treatment significantly reduced to 15.63 ± 1.40% and 20.03 ± 2.02% during treatment phase and post-treatment phase, respectively. However, there was no significance difference between treatment and post-treatment phase.

Effects of herbal treatment on fertility of buffalo bulls

Average conception rates from frozen semen produced during pre-treatment, treatment, and post-treatment phases are presented in Table 2. The conception rates obtained following insemination with frozen semen produced during pre-treatment, treatment and post-treatment phases were 19.05%, 44.89% and 46.88 %, respectively. The lower conception rates with frozen semen produced

during pre-treatment phase may be due to lower sperm motility and higher sperm abnormalities

Table 2: Effects of herbal treatment on conception rate of frozen semen produced from subfertile buffalo bulls during pre-treatment, treatment and post-treatment phases.

Treatment phases	No. of animals insemi-nated	No. of animals conceived	Conception rate (%)
Pre-treatment	21	4	19.05[a]
Treatment	49	22	44.89[b]
Post-treatment	32	15	46.88[b]

Values with different superscripts ([a,b,c]) within a column differ significantly (Tukey' HSD, P<0.05)

It has been reported that improvement in semen quality and reduction in lipid peroxidation (MDA) during herbal treatment and post-treatment phases may be due to the antioxidant effects and adaptogenic nature of bioactive components of supplemented herbs. Ginsenosides (Saponins) of *Panax ginseng* (Lee *et al*., 2005; Leung and Wong, 2010; Lee *et al*., 2017), Humic acid, fulvic acid and Dibenzo Alpha Pyrones of Shilajit (Rege *et al*., 2015; Sharma *et al.,* 2003; Gallardo *et al*., 2012), sitoindosides VII-X and withaferin A of *Withania somnifera* (Bhattacharya *et al.,* 1997; Kulkarni *et al*., 2008), *Ptychopetalum olacoides* (Antunes *et al.,* 2001) and yohimbine of *Pausinystalia yohimbe* (Neha *et al.,* 2017) reduced the lipid peroxidation by elevation of free radicals scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels as reported in the rat, mice and rabbits in chronic disease conditions (Bhattacharya *et al*., 1997; Dhuley, 1998). Improvement in semen quality may be due to the improved androgen level in the body due to *Tribulus terrestris*'s active component protodioscin as reported in the primates (Gauthaman *et al.,* 2002; Gauthaman *et al.,* 2003). Administration of *Turnera diffusa* having Apigenin as an active component, pinocembrin, and acacetin in combination significantly suppressed aromatase activity (Zhao *el al.,* 2008; Kumar *et al.,* 2006). Aromatase inhibition increases the levels of FSH (Tsjoen *et al.,* 2005) and testosterone in adult men (Ronde and Jong, 2011). Increased testosterone level will potentially stimulate the sperm production and it is higher in adult buffalo bulls when compared to aged bulls (Javed *et al.,* 2000). Similar results on sperm motility and antioxidant activity of herbal mixture to subfertile buffalo bulls have also been reported by Shivkumar *et al*. (2018a, 2018b and 2018c). It may be concluded that herbal treatment improved the conception rates by minimizing the lipid peroxidation due to higher antioxidant activity in blood and semen.

CONCLUSIONS

Present study revealed that the oral treatment of subfertile buffalo bulls with *Panax ginseng* roots, Shilajit, *Withania somnifera* roots, *Tribulus terrestris* fruits, *Turnera diffusa* leaves; *Ptychopetalum olacoides* bark each @ 400 mg/100 kg body weight and *Pausinystalia yohimbe* bark @ 300 mg/100 kg body weight for 60 days improved post thaw semen quality and fertility.

CONFLICT OF INTEREST

The authors declare no conflict of interest among themselves.

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