Original Article

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SEMINAL CHARACTERISTICS IN MURRAH BULLS RECOVERING FROM GOSSYPOL POISONING

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ABSTRACT

The present study was conducted on 5 Murrah buffalo bulls of Central Semen Station, Anjora, Durg. The objective was to analyze the semen properties, semen evaluation after thawing and effect of incubation in frozen thawed semen in Murrah bulls that are in recovery phase of gossypol poisoning. 20 semen ejaculates in total were taken (4 ejaculate from each bull) for the study. Seminal attributes recorded during the recovery period were compared with the data available in Central Semen Station (CSS), Anjora, Durg, Chhattisgarh for the period of poisoning and before poisoning. The average values of fresh semen trait in fresh semen during recovery period *viz*. semen volume (mL), sperm concentration (millions/mL), progressive initial sperm motility (%), live sperm (%), intact acrosome (%), total sperm abnormalities (%), hypo osmotic swelling (HOS %) and sperm penetration distance (SPD-mm) were 2.94±0.70, 1455.03±109.69, 72.50±1.38, 79.60±1.13, 77.93±0.95, 16.45±0.44, 59.00±0.89 and 27.35±0.62, respectively. There was no significant difference between the ejaculate volumes and sperm concentrations of Murrah bulls before the period of gossypol poisoning, during poisoning and during the recovery period. Progressive motility significantly declined ($P<0.01$) during poisoning period. There was no significant difference between the live sperm percentage and per cent normal acrosome before-poisoning and during the recovery phase while the per cent total abnormalities were significantly higher ($P<0.05$) during recovery period as compared to the before toxixity period. After freezing, the mean values of progressive sperm motility (%), total morphological sperm abnormalities (%), intact acrosome (%), HOS reactive (%) and SPD (mm) were 47.00±1.05, 18.70±0.39, 61.35±1.05, 51.55±0.66 and 21.60±0.43, respectively.

Keywords: *Bubalus bubalis*, buffaloes, cryopreservation, gossypol poisoning, seminal characteristics

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INTRODUCTION

Cottonseed and its by-product have been supplemented extensively for years together in ruminant feed to enhance productivity (Zahid *et al*., 2002). The cotton seed gives energy, protein and fibre in the diets of ruminants (Santos, 2008). Gossypol is a yellow pigment present in the cotton plant with highest concentration in seeds and roots. Gossypol is present in variable quantities in whole cottonseed and cottonseed meal (Berardi and Goldblatt, 1980). Total gossypol content in cottonseed meal usually ranges from 0.8% to 1.4%. Cottonseed meal processed by different methods contain free gossypol ranging from 0.05% to 0.30% (Calhoun *et al*., 1991). Non-ruminants are more sensitive to the toxic effects of gossypol than ruminants (Withers and Carruth, 1915; Hale and Lyman, 1957; Kornegay *et al*., 1961; Reiser and Fu, 1962). However, gossypol poisoning has been also found in dairy animals (Lindsey *et al*., 1980; Smalley and Bicknell, 1982) and sheep (Danke *et al*., 1965; Morgan *et al*., 1988). Reproductive effects of gossypol in the ruminants remain a topic of considerable controversy. Marked depression in spermatogenesis caused due to damaged germinal epithelium, reduced sperm motility due to mitochondrial damage to sperm tails and testicular damage have been reported (Randell *et al*., 1992). The pathognomonic lesion for gossypol spermatotoxicity is considered to be the aplasia of the mitochondrial helix (Chenoweth *et al*., 2000). Gossypol toxicosis causes dose and time dependent changes on male bovine reproduction. Therefore, the present investigation was planned to assess the seminal characteristics in fresh and post thaw semen along with its comparative evaluation of pre, during poisoning and the recovery period and effect of freezing on semen in buffalo bulls recovering from gossypol poisoning.

MATERIALS AND METHODS

The study was conducted in 5 Murrah bulls of 3 to 5 years age, maintained at Central Semen Station (CSS) Anjora, Durg (Chhattisgarh) during 2018 to 2019. The commercial concentrate ration fed to bulls at the time of poisoning had free gossypol content 0.034% to 0.070% (i.e. 340 to 700 ppm), which was above the maximum safe level (i.e. 150 ppm for young bulls and 200 ppm for mature bulls, as per NRC). The ration was discontinued after the continuous abnormal semen qualities *viz*., curdling of semen, higher sperm abnormalities and higher dead percentage of spermatozoa. This ration was then replaced with another ration with less than 200 ppm gossypol content along with supplementation of vitamin E.

Semen was collected before poisoning, during poisoning and during recovery from gossypol poisoning for assessment of fresh and frozen seminal characteristics. Semen was collected using artificial vagina (40 cm long and 6.5 cm in diameter) maintained between 42 to 45ºC and prepared as per procedure described by Singh *et al*., 2000. A total of 20 semen ejaculates from 5 bulls (4 ejaculates from each bull) were collected from March to July 2019. The semen was collected by using dummies of the same species after two false months before each collection. Then the semen sample was kept at 37°C in a water bath placed inside the passbox and thereafter, the semen samples were processed after dilution with Tris diluent (Rasbech, 1975). Seminal characteristics and pre freeze in-vitro functional tests were carried out in fresh semen. Seminal volume (Kedia *et al*., 2014), Semen colour was reported from milky creamy to watery (Sonar *et*

al., 2016) whereas the consistency was reported from thick viscus to translucent (Barth, 1997), sperm concentration (Mishra *et al*., 2012), sperm individual progressive motility (Ahmad, 1994), percent live sperm (Campbell *et al*., 1953), percent normal intact acrosome (Watson, 1975), percent total abnormal sperm (Kedia *et al*., 2014), hypo-osmotic swelling test (Jeyendran *et al*., 1984) and cervical mucus penetration test (Prasad *et al*., 1999a) were carried out. The straws were filled and sealed through an integrated system-4 (IS-4, IMV technologies France). Freezing was carried out after equilibration 4°C for 4 h under standard conditions (Graham *et al*., 1985) into the liquid nitrogen (-196°C) and stored. Post thaw progressive motility was assessed 24 h after freezing (Sardar, 2007).

During recovery time the available semen data was compared with the earlier records of semen parameters in the semen station. However, the data were available only for volume, concentration and progressive motility during the poisoning period as all the semen ejaculates during that time were discarded due to poor semen quality. The data was analyzed statistically using standard procedure of ANOVA as per Snedecor and Cochran (1994). Paired t' test was used to compare the data obtained prior to gossypol poisoning with that of the recovery period.

RESULTS AND DISCUSSIONS

Fresh semen characteristics

Semen volume

The average semen volume in Murrah bulls during the recovery period was 2.94±0.70 ml. There was no significant difference between the ejaculate volumes of Murrah bulls before the period of gossypol poisoning, during poisoning and during the recovery period (Table 1) which was in agreement with the findings of Babashani *et al*. (2015) in Yankasa rams fed with diet containing cotton seed. The findings are in contrast with the findings of increased semen ejacutale volume recorded in yearling Holstein bulls fed with cotton seed meal (Jimenez *et al*., 1989) and in male rabbits fed with gossypol (Taha *et al*., 2006).

Colour and consistency

The colour varied from creamy white to watery and consistency varied from thick to translucent in the bulls during the recovery period. Curdling of semen was a major problem reported during the period of poisoning with consistency varying from thin creamy to watery. However, there was not much difference in consistency of semen before the period of poisoning and during recovery period in all bulls. The colour of the semen before poisoning and during recovery was comparable with the findings of Kumar (2008). To the best of our knowledge, no other literature is available regarding sperm colour and consistency in ejaculated semen during gossypol poisoning in farm animals

Sperm concentration

The sperm concentration in Murrah bulls during the recovery period was 1455.03±109.69 million per ml of semen. There was no significant difference in the sperm concentration between all three phases *viz*., before the period of poisoning, during poisoning and during recovery period (Table 1) which was in close agreement with that reported by Cusack and Perry (1995) in bulls fed with whole cotton seed (WCS) for 9 months. Our findings can also be correlated with the findings in goats (Nunes *et al*., 2010) and sheep (Guedes and Soto-Blanco, 2010) in which were fed a diet with 0.5

kg/animal/day cottonseed meal for 120 consecutive days. They reported no deleterious role on semen volume, concentration, progressive motile sperm and sperm morphology. Our findings were in contrast with a reduction in semen concentration in gossypol treated bulls (Velasquez-Pereira *et al*., 1998), male Hamsters (Saksena and Salmonsen, 1982) and rats (De Andrade *et al*., 2006).

Sperm individual progressive motility

The individual motility during the recovery period was 72.50±1.38%. The individual motility of semen in Murrah bulls was significantly reduced ($P<0.01$) during the period of gossypol poisoning as compared to before poisoning and recovery period (Table 1) which was in close agreement with the findings of reduced sperm motility in bulls (Brocas *et al*., 1997), rams (Breitbart *et al*., 1989), male rats (Hadley *et al*. 1981), goats (Zahid *et al*., 2002) and in men, boar, sea urchins, guinea pigs and other experimental animals (Kim *et al*., 1984). In males, the gossypol content reduces sperm motility and their concentration (Guedes and Soto-Blanco, 2010). However, the individual motility assessment is subjective and may slightly vary with person-to-person observation.

Percent live sperm

The average per cent live sperm during the recovery period was 79.6±1.13%. No records for the per cent live sperms were available during the period of gossypol poisoning as all the semen ejaculates were discarded. However, high sperm mortality was reported during that period. High sperm mortality during the period of gossypol poisoning was in close agreement to that reported in bulls (Velasquez-Pereira *et al*., 1998) and rams (Babashani *et al*., 2015). Velasquez-Pereira *et al*. (1998) found more dead sperm in bulls with gossypol content. Babashani *et al*. (2015) observed higher number of dead sperm in rams fed with cotton seed.

Percent normal intact acrosome

The percent normal acrosome was 77.93±0.95 during the recovery period. The percent normal acrosome of Murrah bulls had no significant difference between the period before gossypol poisoning and during recovery period (Table 1). No records for the normal acrosome percentage were available for the period during poisoning. Saacke and White (1972) studied that, assessment of the proportion of sperm with intact acrosome acrosome was associated with fertilizing potential.

Total sperm abnormalities

The head, mid piece and tail abnormal sperm (%) in Murrah buffalo bull during recovery period were 5.28±0.27, 6±0.31 and 5.18±0.32% respectively, and average total abnormalities were 16.45±0.44%. During the recovery period, the percent total abnormalities were significantly higher ($P<0.05$) as compared to the before poisoning period (Table 1). Gossypol may have deleterious effects on certain sperm structural components, particularly the mid piece, without destroying sperm membrane viability (Risco *et al*., 1993; Chenoweth *et al*., 1994). Abnormal morphology of seminal sperm affects the freezing potential of semen (Pangaonkar and Sharma, 1989) and fertility in A.I. bulls (Soderquist *et al*., 1991). Morphology of the spermatozoa is one of the most important aspects in semen evaluation and spermatogenesis through spermatogonial germ cells might be more sensitive to gossypol poisoning in Murrah bulls.

Hypo-osmotic swelling test (HOST)

The sperm with typical tail reactive

swelling (HOS positive) in fresh semen during the recovery period were 59.00±0.89%. In our study, HOS positive sperms in the fresh semen during the recovery period were in comparable with that of the normal values of fresh semen in Murrah (Kumar, 2008), Sahiwal and Red Sindhi bulls (Pathak, 2008), Gir bulls (Sonar *et al*., 2016) and in crossbred bulls (Prasad *et al*., 1999b).

Cervical mucus penetration test (CMPT)

The mean sperm distance covered in neat semen of Murrah bulls during the period of recovery from gossypol poisoning was 27.35±0.62 mm. The sperm penetration distance travelled by freshly ejaculated spermatozoa was comparable to findings in bulls without any gossypol poisoning in Murrah (Kumar, 2008), Gir (Sonar *et al*., 2016), Tharparkar (Kedia *et al*., 2014) and Sahiwal and Red Sindhi (Pathak, 2008) but was lower as compared to SPD of HF and crossbred bulls (Shrivastava and Kumar, 2006) and Jersey bulls (Kumar and Devnathan, 1996). No pertinent literature related to the effect of gossypol poisoning on cervical mucus penetration of bull spermatozoa have been found till date.

Post thaw evaluation of cryopreserved semen

Post thaw motility

The average post thaw motility was 47.00±1.05 (%) in Murrah bulls during the recovery period. There was no significant difference between the post thaw motility before the period of poisoning and that during the recovery period (Table 1). No records for the post thaw motility during the period of poisoning were available as the semen processing was discontinued during that period. The frozen semen samples with more than 30% sperm motility and >65% spermatozoa with normal acrosome, mid piece and tail in combination are regarded as good quality. The reports of Berndtson *et al*. (1976) suggested exposure of semen straws to ambient temperature for one minute can cause significant reduction in post thaw sperm motility. Kumar (2008) reported higher mean post thaw motility in normal Murrah bulls.

Per cent normal intact acrosome

The average post thaw normal acrosome in per cent was 61.35±1.05 (%) during the recovery period. Acrosomal integrity is significantly corresponds with fertilizing ability in post thawed semen (Saacke and White, 1972) and refrigerated semen (Singh *et al*., 1992). The aging or injury to sperm causes the deterioration of acrosomal cap. Abnormal acrosome renders the sperm unfit for fertilization. Thus the characteristics of spermatozoa that influence the integrity of acrosome become equally important. No pertinent literature for the effect of gossypol poisoning on post thaw sperm abnormalities was observed till date.

Percent abnormal sperm

The mean abnormal spermatozoa (%) in post thaw semen was 18.70±0.39 during the period of recovery from gossypol poisoning. There was no role of semen dilution in sperm structural attributes (Saacke, *et al*., 1968; Singh *et al*., 1991), but the sperm morphology was highly affected between equilibration period and 24 h after freezing (Singh *et al*., 1991). A significant increase in total sperm abnormalities was observed after freezing in neat (14.45±1.49) and frozen semen (20.83±3.61) of buffalo bulls (Shetti *et al*., 1981; Hazarika *et al*., 1989; Nath *et al*., 1991). Moreover, about 9% more abnormal sperm was found in frozen thawed semen than fresh semen of Holstein Friesian bulls

Table 1. Semen attributes in Murrah buffalo bulls before gossypol poisoning, during poisoning and during recovery period.

Parameters	Before poisoning	During poisoning	During recovery	Significance
Volume (mL)	2.77±0.19	2.53±0.25	2.94±0.34	P>0.05
Concentration (millions/mL)	1725.43±144.85	1379.52±117.85	1455.03±109.69	P>0.05
Progressive motility (%)	71.50±1.85[a]	37.25±2.09[b]	72.50±1.38[a]	P<0.01
Live sperms (%)	79.30±1.28	-	79.56±1.13	P>0.05
Total sperm abnormalities (%)	15.60±0.72[b]	-	16.45±0.44[a]	P<0.05
Intact acrosome (%)	78.78±0.78	-	77.93±0.95	P>0.05
Post thaw motility (%)	48.25±1.22	-	47.00±1.05	P>0.05

Means bearing different superscripts within a column differed significantly.

P<0.05: Significant, P<0.01: Highly significant, P>0.05: Non significant.

(Luthra and Mariony, 1995). No pertinent literature for the effect of gossypol poisoning on post thaw sperm abnormalities was observed till date.

Post-thaw HOS positive sperm

The average post thaw per cent HOS positive sperm during the recovery period was 51.55±0.66. No records for the post thaw HOST during the pre-poisoning or poisoning period was available with the CSS, Anjora. The average post thaw HOS positive sperm during the period of recovery from gossypol poisoning was in similar with the reports in bulls without gossypol poisoning in Murrah (Kumar, 2008), Sahiwal and Red Sindhi (Pathak, 2008) and Gir (Sonar *et al*., 2016) breeds. The sperm suffer damage that lead to alterations in the plasma membrane and loss in viability during the process of cooling and freezing - thawing (Watson, 2000). Thus, HOST reactivity may be assessed for change in sperm membrane integrity during thawing of semen (Revell and Mrode, 1994). No pertinent literature related to the effect of gossypol poisoning on post thaw HOS tests have been observed till date.

Cervical mucus penetration test (CMPT)

The mean distance covered by spermatozoa was 21.6±0.43 during the recovery period. No records for the post thaw CMPT during the pre-poisoning or poisoning period was available with the CSS, Anjora. The average post thaw sperm penetration distance (SPD) during the recovery period was in corresponds to the findings reported in the bulls of Murrah (Kumar, 2008), Gir (Sonar *et al*., 2016) and Sahiwal and Red Sindhi (Pathak, 2008). Shrivastava and Kumar (2006) found the SPD in fresh semen was more in fresh semen as compared to frozen semen after thawing in HF and crossbred bulls. Prasad *et al.* (1999a) reported SPD in fresh and frozen semen of crossbred bulls was 29.92 to 34.71 mm and 13.75 to 10.83 mm, respectively.

The findings during this investigation, it could be concluded that there was drastic reduction in motility of spermatozoa during gossypol toxicity whereas ejaculate volume and sperm concentration did not differ significantly between before poisoning, during poisoning and during recovery period. In frozen thawed semen, sperm abnormalities were higher during the period of recovery as compared to before poisoning period and there was no significant difference in percent live sperms, intact acrosome and motility between the before poisoning and during recovery period.

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