RESEARCH ARTICLE

Effect of Streptomycin-Penicillin and Gentamicin-Tylosin-Lincomycin-Spectinomycin on the Quality and Bacterial Load of Frozen-Thawed Semen of Surti Buffalo Bull

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Abstract

The present study evaluated the effect of two different antibiotic combinations SP (streptomycin, penicillin) and GTLS (gentamicin, tylosin, lincomycin and spectinomycin), as antibiotic regimens in TCFY (Tris-citric acid-fructose-yolk) semen extender on Surti buffalo bull semen. A total of 12 ejaculates from 6 bulls were collected. After evaluation of the dilution rate, the fresh semen was divided into three parts. Each part of the semen was extended upto 80 million spermatozoa/mL. One control group (part 1) was maintained with no antibiotics, while antibiotic combination SP and GTLS was added into part 2 and part 3 at the concentration of streptomycin 1000 µg/mL, penicillin 1000 IU/mL (Treatment-1), and gentamicin 500 µg/mL, tylosin 100 µg/mL, lincomycin 300 µg/mL and spectinomycin 600 µg/mL (Treatment-2). Pre-freeze and post-thaw microbial load (CFU/mL) and sperm abnormalities were found to be significantly ($p<0.05$) lower, whereas individual progressive sperm motility, live sperm percentage and HOS responsive spermatozoa were significantly ($p<0.05$) higher in semen samples treated with antibiotic combinations SP and GTLS in comparison to control. Further, the GTLS had a significantly ($p<0.05$) lower bacterial load at the pre-freeze and post-thaw stage as compared to SP. However, the observations on sperm parameters with SP and GTLS were statistically similar. It was concluded that SP and GTLS in semen extender show significant improvement in pre-freeze and post-thawed semen quality, statistically both at par as compared to control, and more efficiently reduced the bacterial load compared to SP and non-added control suggesting its preference for use in bovine frozen semen extender.

Key words: Antibiotic combinations, Bacterial load, Buffalo semen, Cryopreservation, Semen Quality

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Introduction

Artificial insemination has proved to be a valuable tool for increasing milk production and reducing the spread of venereal diseases but at the same time, it has also posed a threat of sudden, mass-scale distribution of pathogens from infected bulls, if inducted by mistake. Bacteria can decrease the fertility of the bull by causing disease of the reproductive tract or by affecting spermatozoa to prevent fertilization (Givens and Marley, 2008). Therefore, superior class infection-free semen production from sires along with its wider spread for breed improvement is one of the key objectives of semen stations in India and throughout the world (Meena *et al.*, 2015). Although bacteria can contaminate semen at any step of semen production but most quota of bacterial contamination occur at the time of collection (Singh, 2018). Among all the sources of bacterial contamination, the preputial cavity was the main source (Bhakat and Raina, 2001), and though the majority of bacteria in prepuce are non-pathogenic but it can become the habitat of pathogenic bacteria at times (Aurich and Spergser, 2007).

The use of antibiotics in semen extenders to check the growth of several organisms originating from bulls or contamination during semen processing provided a major contribution to the development of AI over the last 50 years. Streptomycin-Penicillin (SP) is the traditional combination added to buffalo semen extender. However, some of the microorganisms, which were previously sensitive to SP, may have become resistant to these antibiotics (Andrabi *et al.,* 2001; Hasan *et al.,* 2001). An alternative combination of antibiotics comprising gentamicin, tylosin, lincomycin, and spectinomycin (GTLS) added to the extender was tested for bull semen for the first time by Shin *et al.* (1988) and was

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proved to be more efficient in eliminating bacteria. Moreover, GTLS was not detrimental to post-thaw semen quality (Shin *et al.,* 1988; Hasan *et al.,* 2001) or fertility in bovine (Andrabi *et al.,* 2001). The effect of the relatively new antibiotic combination (GTLS) in frozen-thawed semen has been assessed in cattle (Kommisrud *et al.,* 1996; Bousseau *et al.,* 1998), but sparsely studied in buffalo (Andrabi *et al.,* 2001). Hence, the present study was designed to assess the comparative effect of traditional antibiotic combination (SP) and relatively modern combination of antibiotics (GTLS) in semen extender in terms of the bacterial load and semen quality parameters in pre-freeze and post-thaw semen of Surti buffalo bull.

Materials and Methods

The study was conducted on six adult healthy Surti buffalo bulls of the age group between 5.5 and 7.0 years. The bulls were reared at Network Project on Buffalo Improvement at Livestock Research Station, Vallabhnagar, Udaipur (India), in the campus of Veterinary College Navania, Vallabhnagar, Rajasthan. Semen samples were collected from each bull twice a week in the morning hours by the artificial vagina method. A total of twelve ejaculates (fresh semen samples) were collected from six Surti buffalo bulls. After evaluation of the dilution rate, the fresh semen was divided into three parts, part 1, 2, and 3. Each part of the semen was extended up to 80 million spermatozoa/ mL. Antibiotic combination SP (streptomycin 1000 µg/mL, penicillin 1000 IU/mL) and GTLS (gentamicin 500 µg/mL, tylosin 100 µg/mL, lincomycin 300 µg/mL and spectinomycin 600 µg/mL) was added into part 2 and part 3 as treatment 1 and 2, respectively. Part 1 of the extended semen was considered as control. These samples were then processed for cryopreservation and thawed thereafter. At pre-freezing and post-thawing, semen samples were evaluated for bacterial load (CFU/ mL), progressive sperm motility (%), live spermatozoa (%), abnormal sperm (%) and HOST-positive spermatozoa (%). The data were statistically analyzed using one way ANOVA and means were compared at $p<0.05$ as per the standard statistical procedures described by Snedecor and Cochran (1994) by using SPSS 20.0.0 version

Results and Discussion

Bacterial Load (CFU/mL)

At both pre-freeze (on equilibration) and post-thaw stage, the treatment T2 (GTLS) showed significantly lowest bacterial load followed by T1 (SP) and both had significantly lower bacterial load than in untreated control group (Table 1). Similar to the present study, Andrabi *et al.* (2016) reported significantly reduced total bacterial count in post-thawed water buffalo bull semen as compared to control (log 2.78 CFU/mL) group after addition of antibiotic combination SP (log 0.88 CFU/mL) and GTLS (log 0.00 CFU/mL) in Tris-citric acid extender. Akhter *et al.* (2008) reported significantly ($p<0.05$) lower total aerobic bacterial count in semen samples treated with GTLS compared to SP and control stored at 5°C. Hasan *et al.* (2001) also reported significantly ($p<0.05$) lower total aerobic bacterial load in the GTLS treated group compared to SP treated and control groups at the equilibration stage (log 1.30 Vs. 1.95 and 2.5 CFU/mL) and post-thaw stage (log 0.00 Vs. 1.60 and 3.69 CFU/mL) in Sahiwal bull semen, and almost same were the observations for buffalo semen. The reduction in bacterial load in equilibrated semen samples of control compared to that of fresh was attributed to the dilution factor and that of GTLS compared to SP was most likely due to gentamicin and lincomycin-spectinomycin, being a broad spectrum and more effective against Gram-positive and negative bacteria (Shin *et al.*, 1988; Hasan *et al.*, 2001). The reduction in bacterial load in our study may be due to the addition of these antibiotics, diluting effect of extender, and hygienic practices followed during the processing of semen.

Sperm Progressive Motility (%)

The mean percent progressive sperm motility was significantly ($p<0.05$) higher in T1 and T2 extenders as compared to the control, and the effect of both the antibiotics combinations (SP, GTLS) was statistically similar on sperm motility both

Table 1: Effect of antibiotic combinations SP (T1) and GTLS (T2) on seminal and microbial parameters of Surti buffalo bull semen at pre-freeze and post-thaw stage

Quality parameters	Stage of freezing	Control	T-1, SP	T-2, GTLS
Bacterial load (CFU/mL)	Pre-freeze	3116.67 ± 257.86^{c}	1350 ± 104.08^{b}	19.17 ± 7.33^{a}
	Post-thaw	2083.34 ± 169.15^{c}	841.67 ± 115.77^{b}	0.84 ± 0.41^{a}
Sperm progressive motility (%)	Pre-freeze	67.00 ± 0.44^{a}	70.09 ± 0.40^{b}	69.42 ± 0.38^{b}
	Post-thaw	56.17 ± 0.42^{a}	59.42 ± 0.38^{b}	58.75 ± 0.45^{b}
Live spermatozoa (%)	Pre-freeze	77.09 ± 0.39^{a}	80.17 ± 0.41^{b}	79.59 ± 0.33^{b}
	Post-thaw	69.00 ± 0.39^{a}	72.50 ± 0.44^{b}	71.67 ± 0.45^{b}
Sperm abnormality (%)	Pre-freeze	11.67 ± 0.35^{b}	9.34 ± 0.33^{a}	9.71 ± 0.42^{a}
	Post-thaw	14.75 ± 0.29^{b}	12.17 ± 0.34^{a}	12.67 ± 0.53^{a}
HOS response (%)	Pre-freeze	62.17 ± 0.42^{a}	65.50 ± 0.52^{b}	64.67 ± 0.54^{b}
	Post-thaw	55.17 ± 0.41^{a}	58.50 ± 0.44^{b}	57.75 ± 0.45^{b}

Means with different superscripts within a row differ significantly ($p<0.05$).

at pre-freeze and post-thaw stages (Table 1). Similar to the present observations, Akhter *et al.* (2008) also did not find significant difference ($p>0.05$) in motility of spermatozoa in Nili-Ravi buffalo bull due to SP or GTLS combination in extender until the third day of storage at 5°C. Our findings concurred with Andrabi *et al.* (2016), who reported higher percentage of post-thaw linear motility in extender containing SP and GTLS compared to the control group in water buffalo bull semen, and similar were the observations of Hasan *et al.* (2001) on buffalo bull sperm in SP, GTLS and control groups. Furthermore, gentamicin was found deleterious to equine spermatozoa when used at a dose level of 2000 µg/mL (Jasko *et al.,* 1993), while in another study no decrease in motility was recorded for equine (Price *et al.,* 2008) and ovine (Moustacas *et al.,* 2010) semen stored in extenders containing 0.25 mg/mL gentamicin. It is suggested that bacteria in semen can cause depletion of energy by competing with sperm, which may lead to a reduction in motility.

Live Spermatozoa (%)

In this study, there was significant ($p<0.05$) improvement in live sperm percentage in T1 and T2 during pre-freeze equilibration of semen compared to control in Surti buffalo bulls' semen (Table 1). Akhter *et al.* (2008) reported that there was no difference ($p>0.05$) in the longevity of spermatozoa in Nili-Ravi buffalo bull due to the extender containing SP or GTLS combination until the third day of storage at 5°C. However, on the fifth day of storage, sperm viability was better ($p<0.05$) in the extender containing SP compared with that in GTLS and control. Our results concurred with Ali *et al.* (1994) who reported an increase in the longevity of buffalo bull spermatozoa with SP compared to gentamicin added-extender at refrigerated temperature.

In the present study, a significant ($p<0.05$) improvement in the mean live sperm count (%) was observed in antibiotic combinations SP and GTLS in comparison to control group (Table 1). Akhter *et al.* (2013) reported higher ($p<0.05$) sperm viability of buffalo bull spermatozoa in an extender containing antibiotic combination SP compared with control at 0 h post-thaw semen samples. Similarly, Ishaq *et al.* (2019) reported that the percentage of viable sperm was higher ($p<0.05$) in extender containing SP as compared to control in bulls of Sahiwal breed in post-thaw semen samples.

Sperm Abnormalities (%)

In this study, as compared to the control (11.67±0.35 %), there was a significant ($p<0.05$) reduction in sperm abnormalities in T1 (9.34±0.33 %) and T2 (9.71±0.42 %) during pre-freeze equilibration of semen in Surti buffalo bulls (Table 1). Akhter *et al.* (2008) reported that sperm abnormalities (head, midpiece, and tail) did not differ due to antibiotics in extender during 5 days of storage at 5°C. The reduction in sperm abnormalities in T1 and T2 groups compared to the control group in our study may be due to the lower bacterial load reported in these two groups compared to the control group.

Similarly, a significant ($p<0.05$) reduction in the abnormal sperm percentage was observed in T1 (12.17±0.34 %) and T2 (12.67±0.53 %) group in comparison to the control (14.75±0.29 %) group in post-thaw Surti buffalo bull semen samples (Table 1). Our results are in agreement with Hasan *et al.* (2001).

HOST (%)

In this study, the HOS response during pre-freeze and post thawed stage in T1 and T2 groups was significantly ($p<0.05$) higher than control (62.17 ±0.42) group (Table 1). Akhter *et al.* (2008) reported that there was no difference in plasma membrane integrity of Nili-Ravi buffalo bull spermatozoa in an extender containing SP or GTLS combination until the third day of storage at 5°C, whereas PMI on the fifth day of storage was significantly better with SP than with GTLS or Control. Our findings corroborated with Andrabi *et al.* (2016), who assessed the efficacy of antibiotics on the percentage of intact plasma membranes of water buffalo bull semen samples. The plasma membrane integrity percentage with SP and GTLS was higher than the control group of the post-thaw semen samples and found that the effect of antibiotics on post-thaw plasma membrane integrity of buffalo bull spermatozoa was not significant. Similar results were observed by Hasan *et al.* (2001) for the effect of antibiotics on the percentage of intact plasma membranes of Sahiwal cow bull semen samples. The percentages of post-thaw plasma membrane integrity were higher in the extender containing antibiotic SP (74.0±5.9) and GTLS (66.5±2.5) than control (61.3±5.9) semen samples.

Conclusions

It was observed that at pre-freeze and post-thaw stage of cryopreservation of buffalo semen, both Streptomycin-Penicillin (SP) and Gentamicin-Tylosin-Lincomycin-Spectinomycin (GTLS) antibiotics combinations in Tris extender significantly improved sperm quality parameters, both at par, however, the bacterial load was reduced significantly with GTLS combination as compared to SP and control. Therefore, it can be concluded that GTLS improves the bacteriological quality significantly over the SP and Control regimens, while not affecting the semen quality of Surti buffalo bull.

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