EFFECT OF DIFFERENT ANTIBIOTIC COMBINATIONS IN EXTENDER ON BACTERIAL LOAD AND SEMINAL CHARACTERISTICS OF MURRAH BULLS

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ABASTRACT

In order to evaluate the effect of different antibiotics in extender a study was conducted on 36 ejaculates from six Murrah buffalo bulls maintained at Artificial Breeding Research Centre (ABRC), National Dairy Research Institute (NDRI), Karnal. Each ejaculate wassplit in to three parts and the each part was diluted in Tris egg yolk dilutor with separate combination of antibiotics [Conventionally used Benzyl penicillin (1000 IU/ml) + Streptomycin (1000 ug/ml) (PS), Benzyl penicillin (500 IU/ml) + Streptomycin (1000 ug/ml) + Gentamicin (500 ug/ml) (PSG) and Benzyl penicillin (500 IU/ml) + Streptomycin (1000 ug/ml) + Amikacin (1000 ug/ml) (PSA)]. The microbial load and sperm quality was evaluated in refrigerated (0, 24 and 48 h) and cryopreserved (at -196^{o}C) semen. The results depicted that individual sperm motility, sperm abnormalities, percent intact acrosome, HOST was significantly higher and lowest bacterial load was observed in semen fortified with benzyl penicillin, streptomycin and amikacin combination at refrigerated temperature, but the differences in bacterial load between different treatment combinations were not significant. The results revealed that benzyl penicillin, streptomycin and amikacin combination had higher efficacy in maintaining the individual sperm motility, percent intact acrosome and HOST, but non-eosinophilic counts and sperm abnormalities were higher in semen treated with amikacin combination. No significant difference in bacterial load was observed in different antibiotic combination treatments in cryopreserved semen, but lowest values of bacterial load were observed in semen treated with benzyl penicillin, streptomycin and amikacin combination. Overall the results suggested that fortification of semen extender with benzyl penicillin, streptomycin and amikacin combination would have positive effect on preservability of semen.

Keywords: antibiotic, bacterial load, buffalo semen, preservability, semen quality

INTRODUCTION

AI using frozen semen technology is an important biotechnological tool for the rapid improvement in milk production and germplasm

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improvement. The success of AI technique is associated with effective prolongation of fertile life of spermatozoa obtained from high pedigree bulls under in vitro storage condition. However, there in inherent problem of quality semen production in buffalo due to poor libido, seasonality, low epididymal sperm reserve, low sperm harvest, freezability, contamination of micro-organisms and other foreign particles. Further, unhygienic surroundings, repeated entry of penis into AV, improper handling of semen and inflammatory condition of reproductive system adversely influence the microbial quality of semen. The AI in buffalo is not as popular as in cattle because there is low fertility rate with frozen semen (Shukla and Misra, 2007). Even under best conditions, some microbial contamination can occur as the microbes have ample access to contaminate the semen during collection, processing and preservation stages. Further, the extender used for extension of semen is rich in nutrients most suitable for the growth of microorganisms. Bacteria present in the ejaculates can affect fertilization directly (Morrell, 2006).

Addition of antibiotics in freezing diluents may affect the viability or fertility of cryopreserved bovine spermatozoa by controlling the bacterial load (Morrell, 2006). As it has been proven that bacteria in buffalo semen are resistant to single antibiotics like penicillin (Ahmed *et al*., 2001). So recently combination of antibiotics for preservation of semen has been advocated (Akhter *et al*., 2008). Though it is known that antibiotics reduce bacterial load in any subject including semen but scanty information is available on the use of different combinations of antibiotics with specific dose on the preservability of buffalo bull semen. Therefore, the present study was conducted to compare the efficacy of alternate combinations of antibiotics vis-à-vis the antibiotics conventionally used in semen extender for preservation of semen of Murrah buffalo bulls.

MATERIALS AND METHODS

Thirty six ejaculates (initial motility >70%) of six Murrah buffalo bulls maintained at Artificial Breeding Research Centre of National Dairy Research Institute (NDRI) Karnal, were collected twice a week from each bull using sterilized artificial vagina during spring season. Each ejaculate wassplited in to three parts and the each part was diluted in Tris egg yolk dilutor with separate combination of antibiotics. The combinations were Benzyl penicillin (1000 IU/ml) + Streptomycin (1000 ug/ml) (PS) as control as well Benzyl penicillin (500 IU/ml) + Streptomycin (1000 ug/ml) + Gentamicin (500 ug/ml) (PSG) and Benzyl penicillin (500 IU/ml) + Streptomycin (1000 ug/ml) + Amikacin (1000 ug/ml) (PSA) antibiotic combinations. Thereafter, each of three parts was further splitted into two equal parts. Out of these one part of each was placed in refrigerator at 5^{o}C and other part was subjected to freezing for further analysis. Semen was analysed for motility, non-eosinophilic counts, acrosome integrity and plasma membrane integrity by hypo osmotic swelling test. The microbial load and semen quality was evaluated in different hours of refrigeration (0, 24 and 48 h) and frozen (at -196^{o}C) thawed semen. The bacterial count /ml of the sample were estimated by multiplying the dilution factor with the mean number of colonies in media plates (B*acterial load /ml = Bacterial colonies counted on plate X Dilution factor*). The percent data on semen characteristics were subjected to arcsine transformation and microbial count was subjected to log transformation for analysis (Snedecor and

Cochran, 1994). Data was analyzed by two-way ANOVA with interaction and least squares analysis technique(Harvey, 1975).

RESULTS AND DISCUSSION

Least squares means of individual sperm motility, percent non-eosinophilic counts, HOS reacted spermatozoa and total plate counts in different treatment groups at 0, 24 and 48 h of preservation at refrigerated temperature as well as pre freeze and post thawed semen samples in different treatment groups are presented in Table 1 and 2.

Preservation of semen at 5°C

The overall least squares means of individual sperm motility (%) estimates in semen preserved at 5°C for 0, 24 and 48 h are presented in Table 1. At 0 h of preservation, individual motility was significantly higher in PSA group. However, at 24 and 48 h of preservation no significant difference was observed in individual motility in different groups.

The of average non-eosinophilic sperm (%) estimates in semen preserved at 5°C for 0, 24 and 48 h are presented in Table 1. After 24 h of preservation the non-eosinophilic sperm percent were significantly higher in PSA compared to PS group; however, there was no significant difference among other treatment combinations. At 0 and 48 h of preservation there was no significant difference in non-eosinophilic sperm percent among different antibiotic combinations. The total sperm abnormalities at 0, 24 and 48 h of preservation are presented in Table 1. After 0, 24 and 48 h of preservation, the total sperm abnormalities were significantly lower in PSA group as compared to PS and PSG.The percent values of intact acrosome at 0, 24 and 48 h of preservation are presented in Table 1. At 0 and 48 h of preservation there was no significant difference in the percent intact acrosome values. After 24 h of preservation, intact acrosome values were significantly higher in PSA than PSG group. It is clear from the findings that the combination of antibiotics used has no damaging effect on sperm survival. Results of present study are in agreement with those of Bhakat and Raina (2001). The liveability of the spermatozoa was sustained till 48 h of storage with a decrease in motility. The acrosomal damage due to preservation at refrigerated temperature observed in the present study was in conformity with the findings of various workers (Bhakat and Raina, 2001) who also reported increased acrosomal damage with the process of semen preservation at 4°C to 7°C temp. The average HOST (%) estimates in semen preserved at 5°C. After 0 and 24 h of preservation, the HOST percent values were significantly better in PSA than PS and PSG. After 48 h of preservation, there was no significant difference in HOST values in different antibiotic combinations treatment groups. The decrease in quality with increase in storage hours are in consonance with the finding of Azawi and Ismaeel (2012) in ram semen.

The average microbial load (10 X) in semen preserved at 5°C after 0, 24 and 48 h are presented in Table 1. After 0, 24 and 48 h of preservation, no significant difference was observed among all antibiotic combinations. However, the values of microbial load were lower in PSA followed by PSG and PS. The reduction in bacterial count in semen samples following addition of different combinations of antibiotics are in agreement with the studies of Yániz *et al*. (2010) and Azawi and Ismaeel (2012).

Table 1. Mean ± SE for semen characteristics (%) and bacterial load in refrigerated semen (up to 48 h) preserved with different antibiotic combinations in extenders.

Time	Quality Tests	Antibiotics		
		PS	PSG	PSA
0 h	IM	73.51^{a}±0.01	74.52^{a}±0.01	76.54b±0.01
	N-eosin	83.41±0.01	83.70±0.01	84.69±0.02
	SPA	8.87^{a} ±0.00	8.48^{a}±0.01	5.80^{b}±0.00
	IA	89.38±0.01	87.630.01	90.02±0.02
	HOST	54.18^{a}±0.01	55.48^{a}±0.01	60.44^{b}±0.01
	TPC	173.08±1.17	149.12±1.12	127.32±1.10
24 h	IM	55.06±0.01	56.90±0.01	59.70±0.01
	N-eosin	63.76^{a}±0.01	66.33^{ab}±0.01	68.16^{b}±0.01
	SPA	8.50^{a}±0.43	8.67^{a}±1.43	7.17^{b}±0.79
	IA	74.84^{ab}±0.01	72.24^{b}±0.02	76.66^{a}±0.01
	HOST	48.60^{a}±1.68	49.97^{a}±3.71	52.52^{b}±2.48
	TPC	37.43±0.02	38.30±0.02	40.01±0.02
48 h	IM	46.71^{a}±0.02	47.99^{b}±0.01	49.58^{b}±0.01
	N-eosin	46.71^{a}±0.02	47.99^{b}±0.01	49. 58^{b}±0.01
	SPA	13.16^{a}±0.00	12.15^{a}±0.00	10.20 ^{b}±0.00
	IA	57.54±0.02	55.65±0.01	56.18±0.03
	HOST	31.55±0.02	32.39±0.02	34.27±0.02
	TPC	100.45±1.16	94.18±1.12	72.79±1.09

PS = Penicillin + Streptomycin; PSG = Penicillin + Streptomycin + Gentamicin; PSA = Penicillin + Streptomycin + Amikacin IM: Individual sperm motility (%); N-eosin: Non-eosinophilic count (%); SPA: Total sperm abnormalities (%); HOST: Hypo-osmotic swelling reactivity (%);

IA: Intact acrosome (%); TPC: Total plate count (X10) (cfu/ ml).

Values with different superscripts within a column differ significantly.

Preservation of semen at -196°C

Individual motility values in pre freeze and post thaw semen are presented in Table 1 and Table 2. In pre freeze semen the percent motility values were significantly higher in PSA than PS and PSG. Similarly, in post thawed semen percent individual motility was significantly better in PSA than PS and PSG groups. The present findings are in agreement with those of Ronald and Prabhakar (2001); Ahmed and Mohan (2002) who reported that the sperm motility treated with Amikacin and Norfloxacin before and after freezing was significantly higher than control.

The percent values of non-eosinophilic sperm in pre freeze and post thaw semen are presented in Table 1 and Table 2. In pre freeze semen no significant difference was observed for live spermatozoa values in different treatment groups, but in post haw semen, percent non-eosinophilic sperm values were significantly higher in PSA and PSG than PS. However, no significant difference was observed between PSA and PSG groups. The percent total abnormality values in pre freeze and post thaw semen are presented in Table 1 and Table 2. In pre freeze semen, the percent total abnormalities were significantly lower in PSA than PS and PSG. In post thaw semen, the percent total abnormalities were significantly higher in PS as compared to PSG and PSA. The difference between PSA and PSG was not significant.

The percent intact acrosomes in pre freeze and post thaw semen are presented in Table 1 and Table 2. In both pre freeze and post thawed semen, no significant difference was observed in percent intact acrosome values of different treatment groups. However, in both pre freeze and post thawed semen PSA group had highest intact acrosome values. The acrosomal damage due to cryopreservation observed in the present study is in conformity with various workers (Nehring and Stähr, 2001) who reported increased acrosomal damage with the process of semen freezing. HOST percent values in pre freeze and post thaw semen are presented in Table 1 and Table 2. In pre freeze

Table 2. Mean ± SE for semen characteristics (%) and bacterial load in post freeze semen with different antibiotic combinations in extender.

Quality Tests	Antibiotics		
	PS	PSG	PSA
IM	46.28^{a}±0.01	49.60 ^{b}±0.01	50.93 ^{b}±0.01
N-eosin	54.92^{a}±0.01	57.79^{b}±0.01	58.13^{b}±0.00
SPA	19.16^{a}±0.43	14.63^{b}±1.43	14.54^{b}±0.79
IA	61.35^{ab}±0.01	63.30^{b}±0.02	65.28^{a}±0.01
HOST	38.20^{a}±0.01	42.83 ^{b}±0.01	42.84^{b}±0.01
TPC	115.57±1.18	97.95±1.18	76.43±1.18

PS = Penicillin + Streptomycin; PSG = Penicillin + Streptomycin + Gentamicin; PSA = Penicillin + Streptomycin + AmikacinIM: Individual sperm motility (%); N-eosin: Non-eosinophilic count (%); SPA: Total sperm abnormalities (%); HOST: Hypo-osmotic swelling reactivity (%);

IA: Intact acrosome (%); TPC: Total plate count (X10) (cfu/ ml).

Values with different superscripts within a column differ significantly.

semen, HOST percent values were significantly better in PSA than PSG and PS. In post thaw semen, the HOST percent values were significantly better in PSA and PSG than PS. However, no significant difference in HOST percent values was observed between PSG and PSA groups.

The microbial load values (X 10) in pre freeze and post thaw semen are presented in Table 1 and Table 2. In pre freeze and post thaw semen, no significant differences were observed among different antibiotic combinations. However, values of microbial load were lowest in PSA group followed by PSG and PS. Results of present study are in accordance with the earlier studies of Ahmed and Mohan (2002), who reported better efficacy of Amikacin and penicillin in reducing the bacterial counts and improving the motility of semen. Studies are also akin with the report of Ronald and Prabhakar (2001) who revealed that Amikacin, Chloramphenicol and Gentamicin were effective against isolated organisms. Antibiotic addition helps in reduction of microbial population, otherwise there was interference with motility (Diemer *et al*., 1996) due adherence of microorganism with spermatozoa and impair sperm quality and fertilizing capacity (Griveau *et al*., 1995; Thibier and Guerin, 2000; Morrell, 2006) due to production of reactive oxygen species and toxins (Fraczek *et al*., 2007) as well as induce acrosome reaction (El-Mulla *et al*., 1996).

CONCLUSION

It can be concluded that there is scope of substitution of conventionally used penicillin and streptomycin antibiotics with a combination of benzyl penicillin, streptomycin and amikacin antibiotics in extenders, which have positive effect on preservability of semen. Antibiotic addition is essential to prevent proliferation of microorganisms in ejaculated spermatozoa.

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