Effect of preputial washing and antibiotic treatment on bacterial load and preservability of frozen bovine semen*

CHAMPAK BHAKAT¹ and V S RAINA²

National Dairy Research Institute, Karnal, Haryana 132 001 India

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ABSTRACT

Preputial cavity is a main source of microbial contamination in semen. The semen samples were collected from 24 bovine bulls and extended with egg yolk citrate and treated with various combinations of antibiotics at different dose level. The different combinations were streptomycin + benzyl penicillin, streptomycin + benzyl penicillin + gentamicin, streptomycin + benzyl penicillin + ampicillin. Preputial washing was given by the antibiotic solution containing procaine penicillin. Without preputial washing, ejaculate from the same bull was taken as control. The effect of cryopreservation had a significant ($P < 0.01$) influence on individual motility of spermatozoa, non-eosinophilic count, sperm abnormalities, acrosomal integrity and on total bacterial count. The treatment with different combinations of antibiotic on bacterial count was highly significant ($P < 0.01$). Preputial washing significantly ($P < 0.01$) reduced the total bacterial count. The effect of different combinations of antibiotic treatment on individual motility of spermatozoa, non-eosinophilic count, abnormal spermatozoa and acrosomal integrity of sperm cells was non-significant. The maximum reduction of bacterial count was observed by streptomycin + penicillin + gentamicin combination and followed by other combinations. The preputial washing coupled with streptomycin + penicillin + gentamicin fortification was the most efficient treatment for reducing bacterial load to a greater extent without affecting semen quality and preservability.

Key words: Antibiotic fortification, Bovine semen, Deep freezing, Preputial washing.

The present investigation was undertaken to study the effect of preputial washing coupled with antibiotics fortification on bacterial load and preservability of semen.

MATERIALS AND METHODS

Clinically healthy bovine bulls (24), (3- to 5- year-old,) belonging to artificial insemination section, National Dairy Research Institute, Karnal, were used as semen donor. The bulls were maintained under identical system of management. Semen was collected by artificial vagina (AV) technique taking all aseptic precautions. Preputial washing (PW) was given by the antibiotic solution containing procaine penicillin @ 1000 IU/ml of distilled water. Antibiotic solution (50 ml) was introduced into preputial cavity by the help of catheter and preputial orifice was closed with a rubber band. After introducing solution, the preputial cavity region was massaged with hand. After 10 min interval, band was removed to allow the solution to come out from the cavity. The ejaculate from

the same bull without preputial washing, was taken as control. Each ejaculate was extended by using egg yolk citrate (EYC) extender which were treated with different combination of antibiotics, viz streptomycin and benzyl penicillin (Na salt) (SP) combination, streptomycin and benzyl penicillin and gentamicin sulphate (SPG) combination, streptomycin and benzyl penicillin and ampicillin (SPAmP) combination by split sample technique (Cruick Shank *et al.* 1995). For this purpose, each semen sample was split into 3 aliquots to mix EYC extender, which were treated with 3 different combinations of antibiotics at following dose level viz: streptomycin @ 1000 g/ml of extender, benzyl penicillin @ 1000 IU/ml of extender, Gentamicin @ 1000 g/ml of extender, Ampicillin @ 1000 g/ml of extender. Streptomycin and benzyl penicillin combination was used as control group. The motility, eosinophilic spermatozoa (Bloom 1950, Hancock 1952), morphological abnormalities, acrosomal integrity (Hancock 1952) and standard plate count (SPC) were studied in the semen samples before and after freezing. The analysis was carried out by using standard statistical method (Snedecor and Cochran 1981). For the analysis of SPC, the data were subjected to log transformation. The difference between treatment were analysed with the help of critical difference test.

*Part of the Master Degree Thesis (1994) submitted to NDRI, Deemed University, Karnal, Haryana, 132 001.

Present address: ¹Scientist NRCC, Bikaner Rajasthan 334 001.

²Senior Scientist, Artificial Breeding Complex.

RESULTS AND DISCUSSION

The data on semen characteristics of antibiotic treatment alone and in combination with preputial washing, before and after deep freezing are given in Table 1.

A significant ($P<0.01$) drop in motility was observed because of the process of cryopreservation in antibiotic treatment group as well as in preputial washing + antibiotic treatment group. The live spermatozoa count registered a significant ($P<0.01$) decrease due to deep freezing in antibiotic treatment group and also in combination with preputial washing group. Freezing process, however, significantly ($P<0.01$) in-

creased the abnormal cell counts in semen samples in both treatment groups. There was a significant ($P<0.01$) effect of cryopreservation on intact acrosome in antibiotic treatment case as well as preputial washing + antibiotic treatment. The effect of different combinations of antibiotic treatment on individual motility of spermatozoa, non-eosinophilic count, abnormal spermatozoa and acrosomal integrity of spermatozoa revealed that there was no significant difference between 3 different combinations of antibiotic treatment on semen characteristics in fresh as well as in post-thawed samples. Preputial washing + antibiotic treatment combination had a

Table 1. Mean value of semen characteristics before deep freezing (BDF) and after deep freezing (ADF)

	Antibiotic treatment			Preputial washing + Antibiotic treatment		
	BDF	ADF	Overall average	BDF	ADF	Overall average
<i>Individual motility (%)</i>						
SP	67.78 ^a ±1.93	41.67 ^b ±1.84	54.72 ^c ±2.55	69.72 ^d ±1.64	46.39 ^e ±1.75	58.06 ^f ±2.28
SPG	67.78 ^a ±1.68	43.06 ^b ±2.12	55.42 ^c ±2.46	70.00 ^d ±1.84	46.94 ^e ±1.72	58.47 ^f ±2.30
SPAmp	69.07 ^a ±1.85	42.22 ^b ±2.05	55.69 ^c ±2.64	69.44 ^d ±1.80	46.67 ^e ±1.71	58.06 ^f ±2.27
Overall	68.24 ^a	42.31 ^b		69.72 ^d	46.67 ^e	
Average	±1.05	±1.34		±1.21	±1.51	
<i>Non-eosionophilic spermatozoa (%)</i>						
SP	74.61 ^c ±0.98	54.89 ^d ±1.40	64.75 ^c ±1.85	75.50 ^f ±0.99	54.28 ^g ±1.21	64.89 ^h ±1.93
SPG	75.11 ^c ±0.91	54.56 ^d ±1.47	64.83 ^c ±1.92	75.61 ^f ±0.95	54.39 ^g ±1.23	65.00 ^h ±1.93
SPAmp	74.33 ^c ±1.28	54.56 ^d ±1.53	64.44 ^c ±1.93	74.22 ^f ±1.01	54.72 ^g ±1.14	64.74 ^h ±1.79
Overall	74.69 ^c	54.67 ^d		75.11 ^f	54.46 ^g	
Average	±0.63	±0.85		±0.64	±0.84	
<i>Sperm abnormality (%)</i>						
SP	21.44 ^a ±0.67	26.67 ^b ±0.99	24.06 ^c ±0.74	19.39 ^d ±0.62	24.22 ^e ±1.01	21.8 ^f ±0.72
SPG	20.94 ^a ±0.85	27.33 ^b ±1.01	24.14 ^c ±0.85	19.28 ^d ±0.76	23.44 ^e ±1.01	21.36 ^f ±0.72
SPAmp	21.72 ^a ±0.91	26.61 ^b ±0.82	29.17 ^c ±0.74	19.06 ^d ±0.75	24.67 ^e ±1.18	21.86 ^f ±0.84
Overall	21.37 ^a	26.87 ^b		19.24 ^d	24.11 ^e	
Average	±0.48	±0.55		±0.46	±0.45	
<i>Acrosomal integrity (%)</i>						
SP	94.04 ^c ±0.49	89.28 ^d ±0.53	92.11 ^c ±0.60	94.44 ^f ±0.70	89.22 ^g ±0.73	91.53 ^h ±0.67
SPG	93.83 ^c ±0.39	88.67 ^d ±0.55	91.25 ^c ±0.55	94.83 ^f ±0.47	90.50 ^g ±0.81	92.67 ^h ±0.59
SPAmp	93.83 ^c ±0.55	87.67 ^d ±0.75	90.75 ^c ±0.69	94.78 ^f ±0.43	88.78 ^g ±0.60	91.78 ^h ±0.62
Overall	94.23 ^c	88.54 ^d		94.69 ^f	89.50 ^g	
Average	±0.29	±0.37		±0.30	±0.39	

Mean with similar superscripts do not differ significantly ($P<0.01$) from each other; (SP-streptomycin + benzyle penicillin, SPG - streptomycin + benzyle penicillin + gentamicin, SPAmp - streptomycin + benzyle penicillin + ampicillin).

Table 2. Mean value of total bacterial count (cfu/ml) of semen samples before deep freezing (BDF) and after deep freezing (ADF)

	Antibiotic treatment			Preputial washing+antibiotic treatment		
	BDF	ADF	Overall average	BDF	ADF	Overall average
SP	334.44 ^a	178.33 ^b	256.39 ^c	221.67 ^d	134.44 ^e	178.06 ^f
SPG	148.33 ^g	87.22 ^h	117.78 ⁱ	113.80 ^j	70.56 ^k	92.22 ^l
SPamp	236.67 ^m	130.56 ⁿ	183.61 ^o	147.78 ^p	181.67 ^q	124.72 ^s
Overall average	239.81 ^t	132.04 ^u		161.04 ^v	128.89 ^w	

Mean with similar superscripts do not differ significantly ($P < 0.01$) from each other. (SP, streptomycin+benzyle penicillin, SPG-streptomycin+benzyle penicillin+gentamicin, SPamp-streptomycin+benzyle penicillin+ampicillin).

similar nonsignificant effect on individual motility of spermatozoa, non-eosinophilic count, sperm abnormalities and acrosomal integrity of spermatozoa before and after deep freezing. The non-significant effect of antibiotic on sperm motility as recorded during this investigation is consistent with the observations of Gangadhar *et al.* (1986). He also concluded that the combination of penicillin, streptomycin, chloramphenicol and either oxytetracycline or kanamycin did not affect the post-thaw motility of spermatozoa. They also reported that a combination of chloramphenicol (250 µg/ml), kanamycin (250 µg/ml) and oxytetracycline (100 µg/ml) reduced the motility of sperm by 3% before freezing and by 15% after freezing. The present results revealed that the motility was not affected by the combination of antibiotic used in the study. The combinations seem to be appropriate for semen fortification. Present study, emphasized that the combination of antibiotics used had no damaging effect on sperm survival during the freezing process. Live spermatozoa count was decreased after deep freezing. In general, the post-thaw motility obtained from this study was in conformity with the findings of Chinnaiya *et al.* (1979). In the present study, abnormalities rose with the freezing process. However, an almost identical increment was found for all treatments of antibiotic combination used in this investigation. From this, it was clear that morphology of spermatozoa was not affected by any of the antibiotic treatment combination. The harmless effect of different combinations of antibiotics on sperm morphology is consistent with the observation of Sykes and Mixner (1991). The acrosomal damage due to cryopreservation observed in the present study was in conformity with the report of Chinnaiya *et al.* (1979) and Nehring (1988). Terseron *et al.* (1977) found that spermatozoa were damaged at all the degree of dilution (1:10 and 1:20), cooling, freezing and thawing, thereby, resulting in cumulative acrosomal damage of about 40%. It was clear from the results that the various combinations of antibiotics did not affect the acrosomes and thus revealing no interaction between various combination and freezing process. It further strengthens the previous findings that antibiotic combination have no adverse effect on preservability of bovine semen.

The data of mean value of total bacterial count (TBC) of semen samples before and after deep freezing is presented in

Table 2. Different combinations of antibiotic treatment were highly significant ($P < 0.01$) on bacterial count in both groups viz: Antibiotic treatment fortification and when preputial washing coupled with antibiotic treatment in fresh and post-thawed samples. The maximum reduction of TBC was observed by streptomycin + benzyl penicillin + gentamicin combination followed by streptomycin + benzyl penicillin + ampicillin and streptomycin + benzyl penicillin combinations. Preputial washing significantly ($P < 0.01$) reduced the TBC in each antibiotic treated semen sub-samples in fresh and also in post-thawed states. Before deep freezing, maximum reduction of TBC was observed by PW + SPG treatment followed by PW + SPamp and PW + SP as compared to samples without preputial washing. A similar trend of reduction of bacterial count was found after deep freezing when it compared with samples without preputial washing. The significant ($P < 0.01$) reduction in bacterial counts as a result of preservation of semen samples at ultra low temperature (-196°C) was also found. The reduction in bacterial count after deep freezing could be attributed to injury of some bacterial cells, sensitive to ultra low temperature (-196°C) used for freezing purpose. The reduction in TBC in frozen semen samples after gentamicin treatment, recorded during this investigation was consistent with observation of Ahmed *et al.* (1989) who also found that gentamicin can control the bacterial growth in semen samples without affecting the semen quality after equilibration, after different interval of preservation and cryopreservation. Wayda (1991) suggested that gentamicin in combination with penicillin should be used for preparation of diluent for cryopreservation. Since preputial cavity commonly harbours some bacteria which may contaminate the semen and washing with antibiotic solution could reduce the contamination. Prasad and Pachauri (1985) reported that preputial washing with antibiotic solution could be able to reduce the bacterial contamination in ram semen by 61 to 77%. The maximum reduction of bacterial count was observed by streptomycin + penicillin + gentamicin combination and followed by other combinations. The result of study suggests that preputial washing coupled with streptomycin + benzyl penicillin + gentamicin combination treatment is most efficient to reduce bacterial load to a greater extent in bovine semen, in fresh as well as in post thawed samples without

affecting semen quality and preservability. It is followed by other combinations like preputial washing coupled with streptomycin + penicillin + ampicillin treatment combination.

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