THE EFFECT OF ANTIBIOTIC TREATMENT ON BACTERIAL LOAD AND CRYOPRESERVATION OF BOVINE SEMEN

CHAMPAK BHAKAT, V.S. RAINA AND P.K. NAGPAUL

National Dairy Research Institute
Karnal - 132 001

ABSTRACT

The semen samples were collected from twenty-four bovine bulls and extended with egg yolk citrate and treated with various combinations of antibiotics at different dose level. The different combinations were Streptomycin (@ 1000 mg/ml extender) + Benzyl Penicillin (@ 1000 IU/ml extender), Streptomycin + Benzyl Penicillin + Gentamicin (@ 1000 mg/ml extender), Streptomycin + Benzyl Penicillin + Ampicillin (@ 1000 mg/ml extender). The effect of different combinations of antibiotics on individual motility of spermatozoa (68.24 ± 1.05) non-eosinophilic spermatozoa (74.69 ± 0.63), sperm abnormalities (2137 ± 0.48), acrosomal integrity (94.23 ± 0.29) was non-significant. However, total bacterial count (TBC) was significantly different ($P < 0.01$). The effect of freezing to ultra low temperature had a significant ($P < 0.01$) influence on individual motility of spermatozoa (42.31 ± 1.34), non-eosinophilic count (54.67 ± 0.85), sperm abnormalities (26.87 ± 0.55), acrosomal integrity (88.54 ± 0.37) and on TBC. TBC was reduced from a level of 239.81 c.f.u/ml to 132.04 cfu/ml after deep freezing. 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 deep freezing purpose. It was concluded that the most efficient antibiotic combination for checking bacterial load was Streptomycin + Benzyl Penicillin + Gentamicin (TBC = 117.78 cfu/ml) followed by Streptomycin + Benzyl Penicillin + Ampicillin (TBC = 183.61 cfu/ml) and Streptomycin + Benzyl Penicillin (TBC = 256.39 cfu/ml) in the semen samples before and after deep freezing without affecting the quality and preservability of bovine semen. Though Gentamicin treatment combination resulted in marked reduction of colonies as compared to Ampicillin combination treatment, but the count was under permissible level. Ampicillin being cheaper can, therefore, also be recommended for controlling the microbial load of frozen semen.

Artificial insemination (AI) through preserved semen is an indispensable tool for the rapid improvement in the milk production. However, preservation technique employed will also preserve most of micro organisms which will be subsequently deposited in the female reproductive tract along with the semen. The bacteriocidal agent may either be not fully effective or may have detrimental effect on the preservability of spermatozoa. The present investigation was, therefore, undertaken to study the effect of various combinations of antibiotics on bacterial load and cryopreservation of bovine semen.

MATERIALS AND METHODS

Twenty four disease free cattle bulls (3-5 years of age) belonging to Artificial Breeding Complex (ABC), NDRI, Karnal, were used as semen donor. Semen was collected by Artificial Vagina (AV) technique under aseptic conditions. Split ejaculate was extended with Egg Yolk Citrate (EYC) extender containing various combinations of antibiotics, viz., Streptomycin and Benzyl Penicillin (Na-Salt) (SP), Streptomycin and Benzyl Penicillin and Gentamicin sulphate (SPG), Streptomycin and Benzyl Penicillin and Ampicillin (SPA mp)

combination. The level of antibiotics were kept as 1000 mg/ml of extender for Streptomycin, 1000 IU/ml of extender for Benzyl Penicillin, 1000 $\mu\text{g/ml}$ of extender for Gentamicin, 1000 mg/ml of extender for Ampicillin.

The individual motility, eosinophilic spermatozoa, morphological abnormality, acrosomal integrity and Standard Plate Count (SPC)³ were carried out before (BDF) and after deep freezing (ADF) at ultralow temperature (-196°C). All these processes were carried out in contamination free air zone by using spirit lamp. AV was sterilized in AV sterilizer (steam sterilization) for 40 minutes. Glassware were sterilized in hot air oven at 110°C overnight. Surface sterilization was done by exposure to UV rays. Lab was fumigated at weekly interval. The data were statistically analysed⁽⁴⁾ for the interpretation of results.

RESULTS AND DISCUSSION

The data on semen quality of antibiotic treated and frozen samples are given in Table 1. The effect of different combinations of antibiotic treatment on individual motility of spermatozoa, eosinophilic spermatozoa, abnormal spermatozoa and acrosomal integrity revealed that there was no significant difference between different combinations of antibiotic treatment on semen

Table 1. Mean value of semen quality during freezing process

	BDF	ADF	Overall Average	BDF	ADF	Overall Average
Individual motility (%)				Non-eosinophilic spermatozoa (%)		
SP	67.78	41.67	54.72	74.61	54.89	64.75
	± 1.93	± 1.84	± 2.55	± 0.98	± 1.40	± 1.85
SPG	67.78	43.06	55.42	75.11	54.56	64.83
	± 1.68	± 2.12	± 2.46	± 0.91	± 1.47	± 1.92
SPA mp	69.17	42.22	55.69	74.33	54.56	64.44
	± 1.85	± 2.05	± 2.64	± 1.28	± 1.53	± 1.93
Overall Average	68.24 ^a	42.31 ^b		74.69 ^a	54.67 ^b	
	± 1.05	± 1.34		± 0.63	± 0.85	
Sperm abnormality (%)				Acrosomal integrity (%)		
SP	21.44	26.67	24.06	94.94	89.28	92.11
	± 0.67	± 0.99	± 0.74	± 0.49	± 0.53	± 0.60
SPG	20.94	27.33	24.14	93.83	88.67	91.25
	± 0.85	± 1.01	± 0.85	± 0.39	± 0.55	± 0.55
SPAmp	21.72	26.61	24.17	93.85	87.67	90.75
	± 0.91	± 0.82	± 0.74	± 0.55	± 0.75	± 0.69
Overall average	21.37 ^a	26.87 ^b		94.23 ^a	88.54 ^b	
	± 0.48	± 0.55		± 0.29	± 0.37	

Table 2. Mean value of SPC (cfu/ml) of semen during freezing process

	BDF	ADF	Overall average
SP	334.44	178.33	256.39 ^a
SPG	148.33	87.22	117.78 ^b
SPAmp	236.67	130.56	183.61 ^c
Overall Average	239.81 ^a	132.04 ^b	

Mean with similar superscripts (a, b, c) do not differ significantly from each other ($P < 0.01$).

quality in fresh as well as post thaw samples. A significant ($P < 0.01$) drop in motility was observed because of the process of cryopreservation. The non-significant effect of antibiotic on sperm motility is consistent with the observations of various worker⁴ who also concluded that the combination of penicillin, streptomycin, and either oxytetracycline or Kanamycin did not affect the post thaw motility of spermatozoa but some other worker⁵ has reported the Kanamycin (250 µg/ml) and oxytetracycline (100 µg/ml) reduced the motility of sperm by 3 per cent before freezing and by 15 per cent after freezing. The live spermatozoa count registered a significant ($P < 0.01$) decrease as a result of deep freezing. Number of live spermatozoa decreased after deep freezing was found in the normal range. The post thaw motility obtained from this study was in conformity with Chinnaiya et al.,². The freezing process significantly ($P < 0.01$) increased the abnormal

cell counts in semen samples. However, an almost identical increment was found for all treatments of antibiotic combination used in this investigation. A significant ($P < 0.01$) effect of cryopreservation on intact sperm acrosome observed in the present study is in conformity with other reports^{2,6}.

The data of mean value of TBC (cfu/ml) of semen samples before and after deep freezing (Table 2) when subjected to statistical analysis revealed highly significant ($P < 0.01$) variation in fresh as well as in post thawed samples. The maximum reduction of TBC was observed in Streptomycin + Benzyl Penicillin + Gentamycin combination followed by Streptomycin + Benzyl Penicillin + Ampicillin and Streptomycin + Benzyl Penicillin combinations before and after cryopreservation. A 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.¹, who also found that gentamicin could control the bacterial growth in semen samples without affecting the semen quality after equilibration, after different interval of preservation and cryopreservation. Wayda⁹ also recommended use of gentamicin in combination with penicillin for preparation of diluent for cryopreservation.

Thus, from the present study, it can be concluded that Streptomycin + Benzyl Penicillin + Gentamicin was the most efficient antibiotic combination for checking bacterial load followed by Streptomycin + Benzyl Penicillin in the semen

samples before and after deep freezing without affecting the quality and preservability of bovine semen. Though Gentamicin treatment combination resulted in marked reduction of colonies as compared to Ampicillin combination treatment, but the count was under permissible level. Ampicillin being cheaper can, therefore, also be recommended for controlling the bacterial load of semen without affecting its livability in the process of cryopreservation.)

ACKNOWLEDGEMENTS

The authors wish to express their sincere thanks to Dr. O.S. Tomer, Director, NDRI; Dr. V.K. Batish, Senior Scientist, Deptt. of Microbiology, NDRI and Dr. A.K. Gupta, Scientist, ABC, NDRI, Karnal for providing necessary facilities for the present work.

REFERENCES

1. Ahmed, K.; Borgohain, B.N.; Deca, B.C. and Dutta, G. 1989. Effect of strepto penicillin and gentamicin on bacterial load and quality of frozen semen. *Indian Vety. J.* 66(9): 886
2. Chinnaiya, G.P; Sharma, P.A. and Ganguli, N.C. 1978. The freezability of buffalo semen in different extenders. *India J. Dairy Sci.*, 32(1): 93
3. Cruickshank, R.; Duguid, J.P.; Marmain, B.P. and Swain, R.H.A. 1975. Medical Microbiology. The practice of Medical Microbiology, 12th edn., Vol.2. Churchill, Livingstone, Edinburgh.
4. Gangadhar, K.S.; Rao, A.R. and Subbaiah, G. 1986. Effect of antibiotics on bacterial load in frozen semen of buffalo bulls. *Indian Vety. J.*, 63(6): 489
5. Gangadhar, K.S. and Rao, A.R. 1986. Influence of direct addition of streptomycin to neat semen on the bacterial load of frozen semen. *Indian Vety. J.* 63(8): 686
6. Nehring, H. 1988. Evaluation of acrosome integrity during semen storage. Beurteilung der Funktionstichtigkeit des Akrosoms bei der Konservierung Von spermicin Medizin, 37(1): 109
7. Salisbury, G.W.; Faller, M.K. and Willet, E.L. 1941. Preservation of bovine spermatozoa in yolk citrate diluent and field results from it's use. *Dairy Sci.* 24: 905
8. Snedecor, W. George and Cochran, G. William 1981. Statistical methods. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India.
9. Wayda, E. 1991. Antibiotic resistance of bacterial strains isolated from bull semen. *Medycyna Weterynaryjna*, 47(5): 214