EFFECT OF PREPUTIAL WASHING AND ANTIBIOTIC TREATMENT ON BACTERIAL LOAD AND PRESERVABILITY OF CHILLED BOVINE SEMEN*

Champak Bhakat **
V.S.Raina ***

The effect of Streptomycin + Benzyl Penicillin (SP), Streptomycin + Benzyl Penicillin + Gentamicin (SPG), Streptomycin + Benzyl Penicillin + Ampicillin (SPAmp) was studied on the preservability of semen collected from 24 cattle bulls. Different combinations of antibiotic treatment had a non-significant effect on individual motility of spermatozoa, non-cosinophilic count, abnormal spermatozoa and acrosomal integrity of sperm cells and a significant ($P < 0.01$) effect on total bacterial count (TBC) for various interval of preservation at refrigerated temperature. The maximum reduction of TBC was observed by SPG combination followed by SP Amp and SP combinations. Preputial washing coupled with SPG was most efficient in reducing bacterial load.

INTRODUCTION

Bacterial contamination of semen may affect spermatozoa directly and compete for substrate in semen extenders, besides infecting the inseminated females. Preputial cavity is the main source of contamination in semen (Reddy *et al.* 1971). Unhygienic surroundings, order of ejaculate, repeated entry of penis into AV, age of bulls, improper handling of semen and inflammatory condition of reproductive system may adversely influence the bacterial load of semen. With the present trend of shipping over greater distances and inseminating animals by the diluted semen

which has undergone longer periods of storage, the microbial count is bound to rise. Special emphasis has been placed on the use of various antibacterial agents including Penicillin, Streptomycin and various sulfonamides for controlling the bacteria which are always found in the semen of bulls with increasing application of A.I. in the field of dairying (Salisbury *et al.* 1941). Penicillin and streptomycin have long been used as semen additives to control the microbial growth. These antimicrobial agents are not fully effective after addition of extenders. The present investigation was, therefore, undertaken to study the effect of preputial washing coupled with antibiotic fortification on bacterial load and preservability of semen.

MATERIALS AND METHODS

Twenty-four disease free bovine bulls (3-5 years of age) belonging to Artificial

* Part of the M.Sc. Thesis submitted to NDRI Deemed University, Karnal 132 001.

** Dairy Cattle Breeding Division, National Dairy Research Institute, Karnal 132 001.

*** Artificial Breeding Complex, National Dairy Research Institute, Karnal 132 001.

Breeding Complex, National Dairy Research Institute, Karnal, were used as semen donor. The bulls were maintained under identical management and fed as per NRC (1989) Standards. Semen was collected by Artificial Vagina (AV) technique (Walton, 1945) taking all aseptic precautions. Preputial washing was given by the antibiotic solution. Procaine Penicillin @ 1000 IU/ml of distilled water was used for this purpose. Hairs around the preputial orifice were clipped when required and cleaned with water. 50 ml antibiotic solution was introduced by the help of catheter and 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. Preputial washing (PW) was carried out in all the twenty-four bulls. A week before preputial washing, ejaculate from the same bull was taken as control.

Each ejaculate was extended by using egg yolk citrate (EYC) extender (Salisbury *et al.* 1941) containing Streptomycin and Benzyl Penicillin (Na salt) (SP), Streptomycin and Benzyl Penicillin and Gentamicin sulphate (SPG), Streptomycin and Benzyl Penicillin and Ampicillin combinations (SP Amp) by split sample technique. For this purpose, each semen sample was split into three aliquots to mix EYC extender which were treated with different combinations of antibiotics at different dose level, viz., streptomycin @ 1000 µg/ml of extender, benzyl penicillin @ 1000 IU/ml of extender, gentamicin @ 1000 µg/ml of extender and ampicillin @ 1000 µg/ml of extender.

Streptomycin and Benzyl Penicillin combination was used as control group and neat semen (NS) used as a benchmark of bacterial count of the samples. The individual motility, eosinophilic spermatozoa (Bloom, 1950; Hancock, 1952) morphological abnormalities (Hancock, 1952), acrosomal integrity (Hancock, 1952) and Standard Plate Count (SPC) (Cruickshank *et al.* 1975) were studied in the semen samples at 0 (just after dilution), 24 and 48 h interval at 4 to 7°C. The analysis was carried out by using standard statistical method (Snedecor and Cochran, 1981).

RESULTS AND DISCUSSION

The effect of different combination of antibiotic treatment on individual motility, non-eosinophilic count, abnormal spermatozoa and acrosomal integrity of spermatozoa (Table 1) revealed that there was no significant difference between three different combinations of antibiotic treatment on semen characteristics for a particular period of preservation. Preputial washing + antibiotic combination treatment had a similar non-significant effect on semen characteristic at various hours of preservation. Ahmed *et al.* (1989) found that there was no difference between Penicillin (800 IU) and Streptomycin (1000 µg/ml) or Gentamicin (500 µg/ml) on motility. The present findings are in agreement with the above observation.

The preservation interval showed significant ($P < 0.01$) drop in motility percentage in antibiotic treatment group as well as in PW + antibiotic treatment group. There was a significant ($P < 0.01$)

Preservation of bovine semen

Table 1
Mean value of semen characteristics of chilled samples at various interval

Combination of Antibiotics	Storage intervals (h)					
	Antibiotic Treatment			PW + Antibiotic Treatment		
	0	24	48	0	24	48
Individual Motility (%)						
SP	67.92 ^a ±1.35	57.21 ^b ±1.59	45.83 ^c ±1.99	65.83 ^a ±1.73	56.46 ^b ±2.01	44.13 ^c ±1.52
SPG	69.46 ^a ±1.25	57.92 ^b ±1.56	45.79 ^c ±1.92	66.67 ^a ±1.81	56.54 ^b ±1.97	44.46 ^c ±1.79
SP Amp	68.00 ^a ±1.36	57.79 ^b ±1.46	46.84 ^c ±2.01	65.50 ^a ±1.72	56.08 ^b ±1.96	44.33 ^c ±1.75
Non-eosinophilic Spermatozoa (%)						
SP	76.92 ^a ±1.12	71.71 ^b ±1.41	64.75 ^c ±1.29	73.79 ^a ±1.00	67.79 ^b ±1.39	61.46 ^c ±1.39
SPG	76.88 ^a ±1.16	71.13 ^b ±1.48	64.92 ^c ±1.38	73.75 ^a ±0.88	67.92 ^b ±1.29	61.63 ^c ±1.29
SP Amp	76.49 ^a ±1.20	70.83 ^b ±1.49	64.46 ^c ±1.38	73.96 ^a ±1.15	67.38 ^b ±1.47	60.92 ^c ±1.22
Sperm Abnormality (%)						
SP	17.33 ^a ±1.06	21.08 ^b ±0.93	25.08 ^c ±0.93	19.79 ^a ±0.84	23.46 ^b ±0.93	26.71 ^c ±0.97
SPG	17.29 ^a ±1.05	21.29 ^b ±0.92	25.13 ^c ±0.86	19.92 ^a ±0.90	23.04 ^b ±1.00	26.33 ^c ±1.10
SP Amp	17.63 ^a ±0.90	21.33 ^b ±0.93	25.79 ^c ±0.87	19.88 ^a ±0.84	23.54 ^b ±1.17	26.00 ^c ±1.09
Acrosomal Integrity (%)						
SP	96.17 ^a ±0.34	93.38 ^b ±0.38	91.25 ^c ±0.47	95.54 ^a ±0.45	92.88 ^b ±0.44	90.33 ^c ±0.67
SPG	96.04 ^a ±0.28	93.46 ^b ±0.33	91.21 ^c ±0.37	95.42 ^a ±0.39	93.04 ^b ±0.37	90.50 ^c ±0.48
SP Amp	95.88 ^a ±0.28	93.08 ^b ±0.37	91.25 ^c ±0.51	95.00 ^a ±0.42	92.25 ^b ±0.45	90.38 ^c ±0.63
Mean with similar superscript (a,b,c) do not differ significantly from each other (P>0.05)						

difference due to preservation with gradual decrease to live count as the storage time passed in case of only antibiotic treatment and also in combination with preputial washing. The abnormal sperm count significantly (P<0.01) increased as

• preservation time passed in chilled semen samples treated with antibiotic combination alone and in combination with preputial washing. The acrosomal integrity registered a significant ($P<0.01$) decrease as a result of preservation time in both case of antibiotic treatment as well as PW + antibiotic treatment. A significant drop in motility of chilled semen stored at refrigerated temperature is consistent with the observation of Duijn *et al.* (1974). The combination of antibiotics used had no damaging effect on sperm survival. On the other hand, some lethal effect of different antibiotic treatment on spermatozoa has been reported by Bonaga *et al.* (1971). The livability of the spermatozoa has been sustained till 48 h of storage with a gradual decrease in motility.

The observed harmless effect of different combinations of antibiotics on sperm morphology is consistent with the observation of Sykes *et al.* (1991). The

combinations of antibiotics did not affect the acrosomes. No interaction between various combination of treatment and preservation at $4-7^{\circ}\text{C}$ was observed. However, the acrosomal damage due to preservation at refrigerated temperature observed in the present study is in conformity with Chinnaiya (1978).

The mean values of total bacterial count of chilled semen sample stored for 0, 24 and 48 h of preservation are given in Table 2. Different combination of antibiotic treatments were highly significant ($P<0.01$) on bacterial count in both groups (antibiotic treatment alone and in combination with preputial washing) at various hours of preservation at refrigerated temperature ($4-7^{\circ}\text{C}$). 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

Table 2
Mean value of SPC (cfu/ml) of chilled semen at various interval

Combination of Antibiotics	Storage intervals (h)					
	Antibiotic Treatment			PW + Antibiotic Treatment		
	0	24	48	0	24	48
NS	32727.08 ^a	19289.58 ^b	14833.33 ^c	2812.46 ^m	1660.42 ⁿ	1365.00 ⁿ
SP	1074.58 ^d	780.83 ^e	432.08 ^h	383.83 ⁿ	242.17 ^r	178.13 ^v
SPG	523.33 ^e	413.33 ⁱ	229.67 ^j	200.63 ^o	145.54 ^s	76.21 ^w
SP Amp	798.75 ^f	472.50 ^k	239.17 ⁱ	285.88 ^p	100.25 ^t	129.29 ^x
Mean with similar superscripts (a,b,c,d) do not differ significantly from each other ($P<0.05$)						

significantly ($P < 0.01$) reduced the TBC in each antibiotic treated semen subsamples at 0 hr of preservation. After 24 hr and 48 h of preservation further lowered TBC in each sub-samples with PW as compared to without PW. The maximum reduction was obtained by PW + SPG treatment followed by PW + SP Amp, PW + SP and PW + NS as compared to samples without PW. The significant ($P < 0.01$) reduction in TBC as a results of preservation of semen samples at low temperature of 4-7°C was also found. The reduction in bacterial counts in semen samples after antibiotic treatment is in accordance with the observations of Nimai Singh *et al.* (1990). Saikia *et al.* (1987) also found that the organisms in bulls semen are highly sensitive to Gentamicin (100%) followed by Ampicillin (83.7%) and least effective drugs were Streptomycin (45.94%) and Penicillin (10.81%). Ahmed *et al.* (1989) found that Gentamicin controls the bacterial growth in the semen sample completely without affecting the semen quality during preservation. However, Streptomycin treatment of semen extended with egg yolk was ineffective in controlling the spread of infection by AI. It could be concluded that Gentamicin is the most effective antibiotic followed by Ampicillin and SP for checking bacterial load of semen.

Flushing the preputial cavity region with antibiotic solution to remove the bacteria and resulting in contamination of semen is recommended. Prasad *et al.* (1985) were able to reduce the bacterial contamination by 61 to 77 per

cent with the help of antibiotic solution.

Thus, it can be concluded that preputial washing coupled with streptomycin + benzyl penicillin + gentamicin combination treatment is more efficient in reducing bacterial load to a great extent in bovine semen at various interval at refrigerated temperature, should be followed by preputial washing coupled with streptomycin + benzyl penicillin + ampicillin combination treatment.

REFERENCES

- Ahmed, K., Dorgohain, B. N., Deca, B. C. and Dutta, G. 1989. Effect of streptomycin and gentamicin on bacterial load and quality of frozen semen. *Indian Vet. J.* 66(9):886-892.
- Bloom, E. 1950. A rapid staining method using eosin-nigresin to distinguish between live and dead spermatozoa. *Nord. Vet. Med.* 2:58-61.
- Bonaga, G., Borghi, G. and Pralo Ram, F. 1971. Toxic and bacteriostatic action of antibiotics and sulphonamides on semen of A.I. bulls. *Zootech. Vety.* 26:139-143.
- Chinnaiya, G. P., Sharma, P. A. and Ganguli, N. C. 1978. The freezability of buffalo semen in different extenders. *Indian J. Dairy Sci.* 32(1):93.
- Cruickshank, R., Duguid, J. P., Marmoin, B. P. and Swain, R. H. A. 1975. Medical Microbiology. The practice of Medical Microbiology. 12th edn., Vol. 2, Churchill, Livingstone, Edinburgh.
- Duijn, C., Von, J. R. and Voorst, C. Van 1974. The effect of ampicillin and doxycycline on spermatozoa *in vitro* compared with the combination of penicillin and streptomycin. Afdeling Biofysive Institute voor Veeteelkundig enderzoek "Schoonoord" Zeist, Netherlands.
- Hancock, J. L. 1952. A method to observe the morphological abnormalities and acrosomal integrity of sperm cells. *J. Exptl. Biol.* 29:445.
- Nimai Singh, Sinha, A. K., Balraj Singh, Singh, L. C., Tiwary, B. K. and Singh, M. P. 1990. Efficiency of specific antibiotic on microbial load in chilled and frozen semen of crossbred bulls. *Indian Vety. J.* 67(6):531-536.

- NRC 1989. Nutrient requirements for dairy cattle. National Research Council, 6th rev. edn. National Academic Sci., Washington, D. C.
- Prasad, J. and Pachauri, A. K. 1985. Significance of intrapreputial wash in collecting low microbial count in raw semen. *Livestock Advisor*. 10(2):9-11.
- Reddy, B. J. C., Krishnamurti, P. S. and Venkataswami, V. 1971. Bacterial flora of prepuce and the effect of intrapreputial treatment. *Indian Vet. J.* 48:722.
- Saikia, G. K., Bora, B. R., Hazarika, A. K. and Ahmed, K. 1987. Bacterial flora of bovine semen and their antibiotic sensitivity pattern. *Indian Vety. Med. J.* 11(7):37-39.
- Salisbury, G. W., Faller, M. K. and Willet, E. L. 1941. Preservation of bovine spermatozoa in yolk citrate diluent and field results from its use. *J. Dairy Sci.* 24:905-910.
- Snedecor, W. G. and Cochran, G. W. 1981. Statistical Methods. Oxford and IBH Publ. Co. Ltd. New Delhi, India.
- Sykes, J. G. and Mixner, J. P. 1991. Toxicity to bull spermatozoa of various salt brands and lots of penicillin, streptomycin, aureomycin and chloromycin. *J. Dairy Sci.* 34:342-346.
- Walton, A. 1945. The technique of artificial insemination. 3rd edn. Holborn - Surgical Instrument Co. Ltd., London.