EFFECT OF VARIOUS ANTIBIOTICS ON CONTROL OF BACTERIAL GROWTH IN THE PROCESSED BUFFALO SEMEN

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(Manuscript received 7March 1994)

Abstract

Studies have been conducted on the value of amikacin, gentamicin, cephapirin and doxycyline when added singly or in double combination to the processed buffalo semen for control of bacterial growth.

The antibiotics were added to each ml. of primary diluted semen. They showed a significant reduction in total bacterial colony count after dilution and when left about 15 minutes at 37 °C and after thawing. The combined antibiotics were effective than single addition of each antibiotic .

The combination of Cephapirin (100 µg/ml) with Gentamicin (750µg/ml) was more effective for control of bacterial contamination than Penicillin and Streptomycin mixture.

The total bacterial count (T.B.C.) was higher in milk than in tris diluent on addition of doxycycline, while lower in milk than tris on addition of other antibiotecs.

INTRODUCTION

Bacterial contamination of semen and its associated effects on fertility had led to the general use of penicillin and streptomycin in semen. These regimen have been applied for many years in artificial insemination programmes to prevent propagation of specific microorganisms in diluted semen. The long term use of penecillin and streptomycin resulted in resistance in some strains (Shin *et al.*

1988).

Several broad-spectrum antibiotics are now available and effective against different microorganisms found in bull semen . Amikacin, Gentamycin, Cephapirine and Doxycycline are effective antibiotics specially to resistant bacteria (Hassan 1985) and did not affect the motility or the fertility of thawed semen.

The elimination of semen bacterial contaminants regimen should remain as one of the important hazards to the microbiologists for an effective artificial insemination policy. Combination of antibiotics have been recommended to control bacterial contaminants in frozen semen. Other factors influence the efficiency of antibiotics added to semen and/or semen extender to control contamination include temperature and duration of incubation of the treated samples (Shisong *et al.* 1990 and Eaglsone *et al.* 1992).

The present study was done to throw a beam of light on the value of Amikacin, Gentamycin, Cephapirine and Doxycycline,and the most effective concentration of any of them individually or in combination to control the bacterial propagation in the processed buffalo semen.

MATERIALS AND METHODS

Semen samples were provided from five mature buffalo bulls (8 years old) raised under identical conditions of management and nutrition in the experimental farm of Animal Reproduction Research Institute. These animals were free from venereal diseases, Brucella and T.B. Semen samples were collected by a sterile artificial vagina. Only semen with good motility (over than 60%) were pooled and used for freezing. Semen samples were incubated in water bath adjusted at 37 ^{o}C till primary diluted with non-glycerolated part of each dilutent separately (partially skimmed buffalo milk with 20% egg yolk).

First trial was made by adding Amikacin, Gentamycin, Cephapirine and Doxycycline singly to primary diluted semen as follows:

1. Amikacin sulphate (A1 = 250μg/ml; A2 =500 μg/ml and A3 = 750μg/ml).
2. Gentamycin sulphate (G1 =250μg/ml; G2 =500 μg/ml and G3 = 750μg/ml).
3. Cephapirin sodium (Ce1=250μg/ml; Ce2 =500 μg/ml and Ce3 = 750μg/ml).
4. Doxycyclin hydrochloride (D) = 100 μg/ml .

Second trial was made by adding antibiotics in a combined form of Cephapirin sodium and Amikacin sulphate or Cephapirin sodium and Gentamycin sulphate. In this experiment, the following system was applied:

1. Cephapirin sodium (Ce) + Amikacin sulphate (A).

A1 + Ce1 ; A1 + Ce2 ; A1 + Ce3

A2 + Ce1 ; A2 + Ce2 ; A2 + Ce3

A3 + Ce1 ; A3 + Ce2 ; A3 + Ce3

2. Cephapirin sodium (Ce) + Gentamycin sulphate (G):

G1 + Ce1 ; G1 + Ce2 ; G1 + Ce3

G2 + Ce1 ; G2 + Ce2 ; G2 + Ce3

G3 + Ce1 ; G3 + Ce2 ; G3 + Ce3

In all experiments we have added positive control (diluted semen containing 1000 I.U./ml. Penicillin G sodium and 1000 µg/ml . Streptomycin sulphate), and negative control (diluted semen without antibiotics).

We have applied the : Mini-tub "system for freezing".

The technique was previously reported in detail by El-Sheltawi (1989), Abdel Aziz (1988), Ziada (1989), Abdel Malak (1989) and El Sheltawi *et al.* (1991). The primary diluted semen was gradually cooled from 37 °C to 5 °C within one hour. At 5 °C, the glycerolated part of the extender added gradually and carefully was incubated for two hours at 5 °C. The diluted semen was then aspirated to medium sized straws. After sealing, straws were arranged in special metal racks placed horizontally on freezing grill 5cm above surface of liquid nitrogen for ten minutes, then, immersed completely in liquid nitrogen. After one week stabilization period in liquid nitrogen, straws were thawed in water bath adjusted at 38 °C for 30 seconds

The bacteriological examination of processed semen was carried out after dilution, equilibrium and thawing for determination of the total bacterial colony count according to Diliello (1979).

RESULTS

Effect of single addition of antibiotics:

TAble 1 shows the values of total bacterial count (T.B.C.) during buffalo se-

men processing with different antibiotics concentrations.The reduction in T.B.C. increased by increasing antibiotics concentrations in case of Gentamycin (G). T.B.C. was significantly higher in tris than milk diluent ($P< 0.05$), while in case of Doxycycline (D) T.B.C. was higher in milk than tris diluent significantly ($P< 0.05$). The highest T.B.C. was recorded for Penicillin-Streptomycin and D which were significantly higher than other antibiotics. From the results obtained, there was no significant effect of freezing steps on T.B.C.

Effect of combined antibiotics on the T.B.C.:

The values of T.B.C. of processed buffalo semen treated with combined antibiotics were reported on Table 2. The results did not reveal any significant effect for diluents used or steps of freezing on T.B.C. The reduction in T.B.C. was significantly higher ($P< 0.01$) in combination of Cephapirin with Amikacin or Cephapirin with Gentamycin than Penicillin-Streptomycin. The effect on the T.B.C. was recorded for Ce3 + A3 and Ce3 + G3.

DISCUSSION

The addition of antibiotics to processed semen for elimination of bacteria has become a routine procedure to guard infection. Chemotherapeutic control of microorganisms in semen should receive continous attention, as many organisms survive freezing and may become resistant to commonly used antibiotics. This study elucidate that, Pencillin and Streptomycin mixture had the lowest effect against bacterial contaminant in processed semen. Meanwhile, the highest reduction of T.B.C. was recorded under the effect of Cephapirin Amikacin, and Gentamycin. The results were in accordance with those of Hassan (1985) and Shin et al. (1988). In the current study, it has been noted that Gentamycin was more effective when used in milk than in tris diluent. This may be due to pH of milk diluent (7.2) which was more suitable for Gentamycin activity than to that of tris diluent (6.8) (Kucers and Bennette 1979). In case of Doxycycline, it was more effective in tris than in milk diluent. The chelation of calcium and magnesium ions in milk diluent by Doxycycline is almost responsible for this reduction in the antibacterial activity of Doxycycline (Booth and McDonaled,1988).

The combination of Cephapirin with Amikacin or Cephapirin with Gentamycin-

Table 1. Effect (mean ± S.E.) of single addition of antibiotics on T.B.C.
(X 105 bacteria / ml).

Antibiotic	buffalo milk diluent			tris-yolk diluent		
	After dilution	After equ-ilibrarion	After thawing	After dilution	After equ-ilibrarion	After thawing
Control	a 4.1±0.31	a 4.7±0.32	a 4.44±0.32	a 4.16±0.30	a 4.7±0.31	a 4.46±0.3
P/S	b 2.58±0.2	b 2.46±0.19	b 2.21±0.19	b 2.55±0.9	b 2.44±0.18	b 2.36±0.18
$Ce_1 + A_1$	c 0.47±0.03	c 0.45±0.03	c 0.43±0.03	c 0.48±0.03	c 0.46±0.04	c 0.45±0.04
$Ce_1 + A_2$	c 0.45±0.03	c 0.42±0.25	c 0.40±0.26	c 0.46±0.03	c 0.44±0.29	c 0.42±0.27
$Ce_1 + A_3$	c 0.42±0.27	c 0.39±0.27	c 0.39±0.25	c 0.42±0.024	c 0.38±0.23	c 0.37±0.
$Ce_2 + A_1$	c 0.4±0.22	c 0.38±0.23	c 0.36±0.22	c 0.40±0.22	c 0.38±0.26	c 0.37±0.26
$Ce_2 + A_2$	d 0.24±0.11	d 0.22±0.01	d 0.21±0.01	d 0.24±0.13	d 0.22±0.14	d 0.21±0.14
$Ce_2 + A_3$	d 0.22±0.14	d 0.20±0.12	d 0.19±0.01	d 0.22±0.01	d 0.21±0.01	d 0.20±0.01
$Ce_3 + A_1$	e 0.17±0.13	c 0.14±0.12	e 0.13±0.17	e 0.17±0.01	c 0.15±0.01	e 0.14±0.01
$Ce_3 + A_2$	e 0.14±0.01	e 0.012±002	e 0.11±0.01	e 0.15±0.01	e 0.13±0.01	e 0.12±0.01
$Ce_3 + A_3$	f 0.22±0.027	f 0.019±0.02	f 0.16±0.02	f 0.021±0.02	f 0.18±0.03	f 0.015±0.002
$Ce_1 + G_1$	c 0.47±0.03	c 0.45±0.27	c 0.43±0.02	c 0.47±0.28	c 0.45±0.23	c 0.44±0.28
$Ce_1 + G_2$	c 0.45±0.21	c 0.43±0.21	c 0.41±0.21	c 0.42±0.22	c 0.42±0.22	c 0.40±0.026
$Ce_1 + G_3$	c 0.41±0.20	c 0.39±0.22	c 0.37±0.22	c 0.43±0.26	c 0.39±0.23	c 0.37±0.24
$Ce_2 + G_1$	c 0.39±0.25	c 0.37±0.25	c 0.35±0.27	c 0.36±0.18	c 0.36±0.02	c 0.35±0.02
$Ce_2 + G_2$	d 0.29±0.28	d 0.27±0.02	d 0.25±0.17	d 0.28±0.02	d 0.26±0.02	d 0.25±0.17
$Ce_2 + G_3$	d 0.25±0.18	d 0.22±0.17	d 0.21±0.17	d 0.23±0.16	d 0.26±0.02	d 0.20±0.018
$Ce_3 + G_1$	e 0.17±0.11	e 0.15±0.01	c 0.14±0.08	e 0.17±0.12	c 0.15±0.13	c 0.14±0.01
$Ce_3 + G_2$	e 0.14±0.01	e 0.12±0.01	e 0.11±0.08	e 0.12±0.01	e 0.12±0.01	e 0.11±0.01
$Ce_3 + G_3$	f 0.023±0.026	f 0.018±.02	f 0.16±0.02	f 0.22±0.002	f 0.108±0.02	f 0.016±0.002

Within columns : The differences between a and b,c,d,e,f are significant at ($P<0.01$), the differences b and c,d,e,f are significant at ($P<0.01$) the differences between f and c,d,e are signifcant at ($P<0.01$) and the differences between c,d,e, are significant at ($P<0.05$).

Table 2. Effect (mean ± S.E.) of antibiotic combination on T.B.C. (X 10^5 bacteria / ml).

Antibiotic	buffalomilk diluent			tris-yolk diluent		
	After dilution	After equ-ilibrarion	After thawing	After dilution	After equ-ilibrarion	After thawing
	a A	a A	a A	a A	a A	a A
Control	3.69±0.23 b A	4.33±0.25 b A	4.09±0.24 b A	3.8±0.26 b A	4.34±0.28 b A	4.18±0.3 b A
P/S	2.38±0.08 d A	2.32±0.2 d A	2.25±0.18 d A	2.39±0.11 d A	2.35±0.1 d A	2.27±0.1 d A
A_1	1.46±0.07 e A	1.35±0.06 e A	1.29±0.06 e A	1.46±0.07 e A	1.37±0.06 e A	1.28±0.07 e A
A_2	1.23±0.03 c A	1.12±0.03 c A	1.04±0.04 c A	1.23±0.04 c A	1.13±0.05 c A	1.06±0.05 c A
A_3	1.01±0.06 d A	0.90±0.05 d A	0.83±0.06 d A	0.98±0.04 d A	0.90±0.04 d A	0.86±0.03 d A
Ce_1	1.45±0.09 e A	1.36±0.08 e A	1.29±0.06 c A	1.44±0.06 e A	1.34±0.06 e A	1.29±0.06 e A
Ce_2	1.22±0.04 c A	1.15±0.04 c A	1.1±0.04 c A	1.25±0.05 c A	1.13±0.04 c A	1.08±0.05 c A
Ce_3	0.99±0.09 d A	0.90±0.06 d A	0.88±0.04 d A	1.04±0.05 f B	0.96±0.06 f B	0.9±0.09 f B
G_1	1.45±0.04 e A	1.38±0.04 e A	1.34±0.05 e A	1.84±0.04 d B	1.74±0.05 d B	1.66±0.05 d B
G_2	1.29±0.04 A	1.22±0.04 c A	1.15±0.07 c A	1.61±0.06 c B	1.49±0.05 c B	1.43±0.04 c B
G_3	1.04±0.04 b A	0.95±0.04 b A	0.88±0.03 b A	1.27±0.03 b B	1.16±0.03 b B	1.07±0.03 bA
D	2.64±0.14	2.7±0.09	2.59±0.09	2.12±0.18	2.03±0.14	1.93±1.1

* Within columns : The differences between a and b,c,d,e,f are significant at (P<0.01), the differences between b and c,d,e,f are significant at (P<0.01) and the differences between c,d,e,f are significant at (P<0.05).

* Withon rows : The diffences between A and B are significant at (P<0.05)

* Control : Contain no antibiotics

* P/S : 1000 IU penicillin - 1000 Ug. streptomycin / ml

* A_1 A_2 A_3 : amikacin sulphate (250 - 500 - 750 Ug/ml)

* G_1 G_2 G_3 : gentamycin sulphate (250 - 500 - 750 Ug/ml)

* Ce_1 Ce_2 Ce_3 : cephepirin sodium (250 - 500 - 750 Ug/ml)

* D : doxycycline hydrochloride (100 Ug/ml)

greatly reduced T.B.C. more than single addition of each antibiotic, and that was in accordance with Fass (1980).

In the present study, the antibiotics, whether added singly or in combination, showed highest reduction in T.B.C. only after dilution and when left about 15 minutes at 37 °C.

The activity of antibiotics was reduced at equilibrium period. This observation was due to cooling and addition of glycerol which has deleteriously limiting effect upon the antibiotic activity (Sullivan *et al.* 1981).After thawing, T.B.C. showed a non-significant decrease from that noted after equilibrium. That may be due to the presence of glycerol which acts as Cryoprotective material for sperm cells as well as bacterial cells (Salisbury *et al.* 1978).

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تأثير بعض المضادات الحيوية علي نمو البكتيريا المتواجدة في السائل المنوى المجمد للجاموس

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تم دراسة إستعمال نوع أو نوعين مجتمعين من المضادات الحيوية لتقييم تأثيرها على نمو البكتيريا المتواجدة فى السائل المنوى الجاموسى أثناء عملية التجميد.

عند إضافة اميكاسين او جينتاميس او سيفابرين او دوكس سيلين لكل ملى لتر من السائل المنوى فى ثلاث تخفيفات مختلفة (٢٥٠، ٥٠٠، ٧٥٠ ميكروجرام/مليلتر) حدث إختزال واضح فى العد البكتيرى بعد التخفيف وبعد ١٥ دقيقة عند درجة ٣٧م، وايضا بعد تسيح السائل المنوى المجمد.

وجد ان عمل مجموعات من سيفابرين (١٠٠٠ ميكروجرام/ملى لتر) مع جينتا ميسين(٧٥٠ ميكروجرام لكل ملى لتر) او سيفابرين (١٠٠٠ ميكروجرام/ملى لتر) مع إميكاسين (٧٥٠ ميكروجرام/ملى لتر) . أدى الى نقص واضح للملوثات البكتيرية فى السائل المنوى فيها عند استعمال البنسلين والاستريبتوميسين مجتمعين.