

**EFFECT OF PENICILLIN –CIPROFLOXACIN
COMBINATION ON SEMEN CHARACTERISTIC AND
BACTERIAL CONTAMINANTS OF DILUTED BULL SEMEN**

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Summary

Effect of penicillin- ciprofloxacin combinations on some semen characteristics and bacterial contaminants of diluted bull semen was determined and compared with those of penicillin- streptomycin combinations. Sperm motility, percentage of dead and abnormal spermatozoa and survival index did not differ significantly for penicillin (1000 I.U) and ciprofloxacin (125, 250, 500, 1000 µg) combinations per milliliter of diluted bull semen from those of penicillin (1000 I.U) and streptomycin (500 µg) combination / ml. Each penicillin-ciprofloxacin combination effectively controlled bacterial growth. However penicillin-streptomycin combination did not effectively control the growth. Accordingly, the combination of penicillin- ciprofloxacin seems to be a satisfactory substituent for penicillin- streptomycin combination for controlling bacterial contaminants in diluted bull semen without impairing semen characteristics.

تأثير مزيج البنسلين و السبروفلوكساسين على خصائص السائل المنوي و الملوثات

البكتيرية في السائل المنوي المخفف للثيران

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الخلاصة

تم دراسة تأثير مزيج البنسلين و السبروفلوكساسين على بعض خصائص السائل المنوي و الملوثات البكتيرية في السائل المنوي المخفف للثيران وقد تم مقارنة تأثيراتها مع مزيج البنسلين و الستربتومايسين. لم تظهر فروقات معنوية في حركة النطف و النسبة المئوية للنطف الميتة و المشوهة و معامل الحيوية عند مزج البنسلين (1000 وحدة دولية) مع السبروفلوكساسين (125, 250, 500, 1000 مايكروغرام) لكل مليلتر من السائل المنوي المخفف للثيران عند مقارنتها مع مزيج البنسلين (1000 وحدة دولية) و الستربتومايسين (500 مايكروغرام) / مل، بينما ظهر ان كل مزيج من البنسلين و السبروفلوكساسين كان أكثر كفاءة من مزيج البنسلين و الستربتومايسين في السيطرة على النمو البكتيري. و على ضوء ما تقدم يمكن اعتبار مزيج البنسلين و السبروفلوكساسين بديلا مناسباً لمزيج البنسلين و الستربتومايسين للسيطرة على الملوثات البكتيرية في السائل المنوي المخفف للثيران بدون احداث تأثيرات سلبية على خصائص السائل المنوي.

Introduction

Antimicrobial agents have been added to diluted bull semen to control semen – borne microorganisms and improve fertility of low- fertility bulls⁽³⁾. Penicillin- streptomycin combination is commonly used by the artificial inseminating centers in Iraq and in most countries⁽¹⁸⁾. However, due to the restricted use of streptomycin⁽²⁰⁾ a search for a suitable replacement for streptomycin is required. Different combinations of antibacterial agents are tested to ensure the control of various pathogenic micro- organisms that contaminate bull semen⁽¹³⁾. Ciprofloxacin is a fluoroquinolone derivative with a wide spectrum antibacterial activity including gram- negative and gram- positive bacteria as well as some chlamydia and mycoplasma⁽¹²⁾ via the inhibition of the bacterial DNA gyrase⁽⁴⁾. Combinations of ciprofloxacin with other antibacterial agents are indicated to expand the spectrum of activity or to exploit bacterial synergy against drug resistant organism⁽⁵⁾. Despite the differences in the antibacterial activity and the differences in spermatozoal tolerance to these agents⁽¹⁹⁾, the concentrations of the antibacterial agents added to semen should have no deleterious effect on semen quality. Our objective was to determine the effects of penicillin – ciprofloxacin combinations in comparison to penicillin – streptomycin combinations on spermatozoa livability and bacterial contaminants of diluted bull semen since the effect of ciprofloxacin against mycoplasmas in bull semen has been improved previously⁽¹⁰⁾.

Materials and Methods

Twenty-one ejaculates of semen were collected using artificial vagina from three bulls maintained at the Artificial Inseminating Center, Baghdad. The diluent was prepared freshly from egg yolk and sodium citrate (1:20). Stock antibacterial solutions were prepared by dissolving each of penicillin, streptomycin and ciprofloxacin in the diluent and placing them in test tubes held at 37°C in water bath until semen was added.

The treatments included 1000 I.U penicillin plus 500 µg streptomycin per milliliter of diluent⁽²⁾ and penicillin 1000 I.U plus one of the four concentration of ciprofloxacin 125, 250, 500 and 1000 µg per milliliter of diluent. The antibacterial combinations were added to the diluent before dilution of semen. Freshly collected semen was diluted at a ratio of one part semen to four parts diluent.

Percentage of sperm motility, dead and abnormal spermatozoa were estimated⁽¹⁶⁾ and the survival index was also estimated⁽⁹⁾.

Analysis of variance⁽¹⁷⁾ was used to analyze the data, between both antibacterial combinations and within each treatment concentration of penicillin – ciprofloxacin combination. Effectiveness of both antibacterial combinations for controlling bacterial contaminants of semen were tested by inoculating Trypticase Soy Agar and MacConkey agar with each type of treatment. Thereafter, cultures were incubated under aerobic and anaerobic conditions at 37°C and examined after 24 – 48 hours.

Results

Combination of penicillin 1000 I.U plus ciprofloxacin at 125, 250 and 500 µg /ml of diluted semen appeared to be harmless to semen characteristics tested as they did not differ statistically from those of penicillin – streptomycin combination effects (Table 1).

Ciprofloxacin at 1000 µg and penicillin 1000 I.U / ml of diluted semen appeared to produce deleterious effect on semen quality by increasing the percentage of dead and abnormal spermatozoa and reduced the survival index in comparison to the other concentrations of ciprofloxacin 125, 250 and 500 µg plus penicillin 1000 I.U / ml diluted semen and to those of penicillin – streptomycin combination (Table 1).

Table 1: Effect of antibacterial combinations on semen characteristics of bulls

Semen characteristics	Penicillin 1000 I.U + Streptomycin 500 µg / ml	Penicillin 1000 I.U + Ciprofloxacin µg / ml			
		125	250	500	1000
Individual Motility %	65.91 ± 3.88 ^a	65.68 ± 4.07 ^a	65.64 ± 4.24 ^a	64.32 ± 4.34 ^a	63.64 ± 4.81 ^a
Survival Index	67.93 ± 2.80 ^a	68.71 ± 2.26 ^a	67.89 ± 3.95 ^a	66.79 ± 3.28 ^a	50.77 ± 4.22 ^b
Dead Spermatozoa %	18.03 ± 1.39 ^b	17.68 ± 1.66 ^b	18.18 ± 1.52 ^b	18.35 ± 1.41 ^b	26.93 ± 3.82 ^a
Abnormal Spermatozoa %	16.37 ± 2.82 ^b	15.36 ± 3.19 ^b	17.03 ± 3.90 ^b	17.22 ± 4.00 ^b	24.64 ± 3.10 ^a

Means + S.D with different superscripts are statistically different (P< 0.05).

Results of microbiological examination demonstrated the isolation of *Bacillus* spp. From the semen of the three bulls and *Corynebacterium* spp. In addition to *Bacillus* spp. from the semen of one of them (Table 2).

The effect of the different concentrations of penicillin – ciprofloxacin combination revealed effective control of bacterial growth whereas the combination of penicillin – streptomycin failed to control the growth effectively (Table 2).

Table 2: Effect of antibacterial combinations on bacterial contaminants of bulls semen

Animal No.	Untreated semen	Penicillin 1000 I.U + Streptomycin 500 µg / ml	Penicillin 1000 I.U + Ciprofloxacin µg / ml			
			125	250	500	1000
923	+ *	+	-	-	-	-
802	+ *⊗	+	-	-	-	-
838	+ *	+	-	-	-	-

+ = Growth, - = No growth, * *Bacillus* spp., ⊗ *Corynebacterium* spp.

Discussion

For effective control of bacterial contaminants in diluted bull semen usually combinations of antimicrobial agents are recommended instead of using a single agent. Since the use of ciprofloxacin in controlling bacterial contaminants of bull semen has not been reported, preliminary trials were made with 125, 250, 500, and 1000 µg ciprofloxacin per milliliter of diluted semen combined with 1000 I.U penicillin.

Results of testing twenty one ejaculates of bull semen indicated that combination of 1000 I.U penicillin and ciprofloxacin at either 125, 250 and 500 µg/ml diluent was not toxic to important characteristics of bull semen and they were similar to those effects produced by the combination of penicillin and streptomycin. Lower concentrations of ciprofloxacin and penicillin than those we tested should be studied to establish an effective combination since at the lowest concentration 125 µg of ciprofloxacin effective bacterial control was achieved without any toxic effect on the studied semen characteristics. However, combination of 1000 µg ciprofloxacin and 1000 I.U penicillin was detrimental to sperm, this might be due to interaction between this high concentration of ciprofloxacin and the sperm since 1000 I.U of penicillin combined with lower concentrations of ciprofloxacin produced no deleterious effect. The interaction might be due to change in pH and osmolarity as these parameters are essential determinants to spermatozoa or might be due to direct action of ciprofloxacin on spermatozoa. This undesired effect of ciprofloxacin should be considered during therapy with this drug since large dosage of ciprofloxacin (up to 15 µg / kg) is recommended in severe conditions⁽⁷⁾ and from pharmacokinetic study, it has been shown that ciprofloxacin attain high concentration in prostate (3-4 times than those in plasma)⁽¹¹⁾.

Bacillus spp. and *Corynebacterium* spp. are usually accused to be one of the natural bacterial contaminants of bull semen^(8,14). In 1989, Sadiq⁽¹⁵⁾ examined 300 seminal samples from bulls in the Artificial Inseminating Center, Baghdad, *Bacillus* spp. constituted higher percentage (27.3 %) and *Corynebacterium* spp. constituted (18. 2 %) among the other bacterial contaminants isolated.

The *Bacillus* spp. that was isolated from the semen of the three bulls used in this study and *Corynebacterium* spp. isolated from only one of the seminal samples indicates that *Bacillus* spp. still highly contaminates semen of the bulls in the center and *Corynebacterium* spp. comes next to it.

The combination of ciprofloxacin and penicillin at all treatment levels of ciprofloxacin were effective in controlling the growth of *Bacillus* spp. and *Corynebacterium* spp. present in all the semen samples. The failure of penicillin – streptomycin combination in controlling bacterial contamination of semen could be due to the resistance of those micro – organisms⁽¹⁾ as this combination has been used for a long time in the center for the elimination of bacterial contaminants of semen.

Since it is unlikely that a bull shedding pathogenic organisms could be treated systematically, than it requires the choice of antibacterial agents in the semen⁽⁶⁾ most likely to be effective in eliminating the organisms without altering the fertilizing capacity of the spermatozoa.

According to the results obtained in this study and in a previous one⁽¹⁰⁾ it seems that effective control of microbial contaminants of bull semen could be achieved by utilizing ciprofloxacin.

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