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TRIAL TO CONTROL *CHLAMYDIA PSITTACI* IN PROCESSED BUFFALO-SEMEN

(With 5 Tables)

By

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محاولة ضبط الكلاميديا سيتيسى فى منى الجاموس المحضر

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يعتبر ميكروب الكلاميديا سيتيسى من الميكروبات التى تنتقل عن طريق السائل المنوى فمن المعروف أن المعاملة التى يتم بها تجميد وحفظ السائل المنوى لاستخدامه فى التلقيح الاصطناعى هى معامله مناسبة لحفظه. وبذلك يكون التلقيح الطبيعى أو الاصطناعى باستخدام سائل منوى يحتوى على الميكروب طريقه سريعة لانتشاره. ولمحاولة التخلص من ميكروب الكلاميديا سيتيسى فى السائل المنوى الجاموسى المعدى معمليا تم استخدام بعض المضادات الحيوية (لينكومايسين - اوكسىتتراسيكلين - كلورامفينيكول - مركب البنسلين والاستريبتومايسين) بتركيزات مختلفة. وقد وجد أن كل من الاوكسىتتراسيكلين والكلورامفينيكول بالتركيزات المختلفة لها تأثير جيد للتخلص من الميكروب بينما كل من لينكومايسين ومركب البنسلين والاستريبتومايسين ليس له تأثير للتخلص من الميكروب. ومن جهة أخرى فان كل من الاوكسىتتراسيكلين والكلورامفينيكول بالتركيزات المختلفة لها تأثير سام على الحيوانات المنوية المختبرة. ونظرا لما تسببه الإصابة بميكروب الكلاميديا سيتيسى من خسائر اقتصادية نتيجة إصابة الجهاز التناسلى وحدوث الإجهاض فانه ينصح بفحص الطلائق والسائل المنوى قبل استخدامه فى التلقيح للتأكد من عدم وجود الميكروب حيث انه لا يمكن التخلص من هذا الميكروب بإضافة مضادات حيوية مناسبة.

SUMMARY

Lincomycin, oxytetracycline, chloramphinicol and the standard combination of penicillin G sodium streptomycin sulphate were evaluated for the control of *Chlamydia psittaci* in processed buffalo-semen. The chloramphinicol and oxytetracycline in different concentrations were effective, while lincomycin and the standard combination of penicillin and

streptomycin were not effective. On the other hand, the chloramphinicol and tetracycline were very toxic to sperm and its using as antibacterial agent in semen diluents should be contraindicated. In conclusion, *Chlamydia psittaci* could not be controlled in diluted semen samples by adding chemotheraputic drugs as the effective drugs on *Chlamydia psittaci* are very harmful to sperm picture. So, it is recommend that the semen samples must free from *Chlamydia psittaci* before using them in artificial insemination due to its hazard effect on animal reproduction.

Key Words: *Chlamydia, Psittaci, Processed, Buffalo-Semen*

INTRODUCTION

Currently, buffalo has special attention in Egypt as the main animal for meat and milk production. Bacterial diseases of the genital system comprised one of the factors associated with depressed reproductive performance. While the incidence of the major diseases of reproduction has been reduced in some countries, the improvement has been matched by a growing importance of other bacteria as a cause of reproductive disorders (Bartlett, 1981). The cryopreservation of semen increased the national and international distribution of semen and the possibility of spreading diseases among cattle populations. At present, artificial insemination (AI) is used for cattle and buffaloes in Egypt. For AI, extended semen may be placed directly into the uterus and, as a result, is not exposed to the bactericidal effects of vaginal and cervical secretions at estrous (Bartlett et al., 1976).

Some microorganisms, including *Chlamydia psittaci*, may be present in bull semen and transmitted to females by natural or artificial breeding causing genital diseases. The source of *Chlamydia psittaci* in semen may be due to general systemic or local infection and *Chlamydia psittaci* shedding through genital tract (Storz, 1971). Regardless of the source of *Chlamydia psittaci*, the conditions of storage and handling of bovine semen are ideal for the preservation of Chlamydia pathogens and their spread of infection among cattle.

Chlamydia psittaci was excreted in semen intermittently for the next three weeks after infection (Shewen, 1986). Infertility, retained placenta, repeat breeding abortion, stillbirth or the delivery of weak calves and milk production drops were observed in cow herds naturally

bred by bulls excreting chlamydiae in their semen (Storz, 1971). A seminal vesiculitis syndrome was observed in bulls after chlamydial infection (Shewen, 1986). Storz et al. (1968) reported that semen, which collected from bulls infected with *Chlamydia psittaci,* characterized by pyospermia and a high percentage of morphologically abnormal spermatozoa.

Unlike antibiotic treatment of semen to reduce or prevent the spread of some bacterial diseases, use of antimicrobial agents have not been studied to control *Chlamydia psittaci* in render semen. The objective of our present study was to determine the effect of some antibiotics in different concentrations for controlling *Chlamydia psittaci* contaminants in extended buffalo-bull semen. Parallel studies on the effects of the same antibiotics on semen quality were also performed.

MATERIAL and METHODS

***Chlamydia psittaci*:**

Chlamydia psittaci strain which isolated from aborting cow (Amin, 1993) was used. The proper concentration (1.8×10^7 $TCID_{50}$) of *Chlamydia psittaci* was prepared by culturing the isolate in one day old Baby Hamster Kidney (BHK) cell monolayer (Youder et al., 1986). The cells were maintained in Iscov's medium (GIBCO) supplemented with 10% fetal calf serum (FCS), 50 µg/ml gentamicin, and 25 Units of nystatin /ml. Chlamydial suspension was inoculated into a monolayer and then incubated at 37°C for 60 minutes. After which Iscov's medium containing 10% FCS, 50 µg/ml gentamicin, 25 units of nystatin /ml, and 1 µg/ml cycloheximide was added and reincubated at 37°C and 5 % CO_2. Seven days after inoculation, the cell debris was removed by centrifugation the tissue culture fluid at 500 xg for 10 minutes. The supernatant was stored at -70°C after the titer of *Chlamydia psittaci* was determined (Youder et al., 1986).

Antibiotics:

Four antibiotics in different concentrations were used in semen diluents (Table1)

Table 1: Antibiotics used in semen diluents

Antibiotic	Concentration	Antibiotic	Concentration
Lincomycin[1]	100 µg/ml	Oxytetracycline	170 µg/ml
Lincomycin	150 µg/ml	Chloramphinicol[3]	10 µg/ml
Lincomycin	200 µg/ml	Chloramphinicol	20 µg/ml
Lincomycin	250 µg/ml	Chloramphinicol	40 µg/ml
Lincomycin	300 µg/ml	Chloramphinicol	50 µg/ml
Oxytetracycline[2]	10 µg/ml	Chloramphinicol	75 µg/ml
Oxytetracycline	20 µg/ml	Chloramphinicol	100 µg/ml
Oxytetracycline	30 µg/ml	Chloramphinicol	125 µg/ml
Oxytetracycline	40 µg/ml	Chloramphinicol	170 µg/ml
Oxytetracycline	50 µg/ml	Chloramphinicol	200 µg/ml
Oxytetracycline	100 µg/ml	Chloramphinicol	260 µg/ml
Oxytetracycline	130 µg/ml	Chloramphinicol	300 µg/ml
Oxytetracycline	150 µg/ml	Penicillin G[4] + Streptomycin[5]	1000 IU/ml + 1 mg / ml

1 = Lincomycin (Upjohn). **2** = Oxytetracycline (Pfizer). **3** = Chloramphinicol (CID).
4 = Penicillin G sodium (CID). **5** = Streptomycin sulphate (CID).

Experiment 1:

Semen was collected twice weekly from 4 healthy buffalo-bulls, in Animal Reproduction Research Institute farm, by a sterile artificial vagina. Good semen samples (above average) were pooled and primary diluted with non glycolated portion of tris-fractose-yolk extender at 37°C (Steinbach and Foote, 1967). Pooled diluted semen samples were then contaminated artificially by *Chlamydia psittaci* isolate in a final concentration of $1.8x10^7$ $TCLD_{50}$/ml. Infected diluted semen was classified into 26 parts for treatment with different antibiotics as mentioned in Table (1). Whereas an aliquot was remained as a control. Semen samples representing all treatments were cooled to 5°C over 45 minutes. Glycerolated portion of tris diluent which preliminary cooled at 5°C was then added to the treated diluted samples and stored at 5°C for 3 days. Individual motility and percentage of sperms with intact acrosomes were determined (Ziada, 1994) from all treated and control samples during the first, second and third day. The presence of *Chlamydia psittaci* was evaluated in all treated and control samples.

Experiment 2:

Methods for semen collection, contamination and treatment with antibiotics were the same as experiment 1.The samples were then equilibrated at 5°C for 2 hours. Each sample was packaged in 0.5 me French straws, frozen on static nitrogen vapor at -160°C for 10 minutes

and plunged in liquid nitrogen for storage. Thawing was performed one week after freezing at 37°C for 30 seconds. Thawed samples were evaluated for sperm individual motility (%), viability index (Ziada, 1994) and percentage of intact acrosome (%). The presence of *Chlamydia psittaci* was determined in all treated and control samples as follow:
0.5 ml of each semen sample was diluted with the addition of sufficient sterile complete sucrose phosphate glutamate solution (CSPG) (Spencer and Johnson, 1983). The isolation of *Chlamydia psittaci* in cell culture was performed in BHK cell culture. BHK was cultured at 37°C in Iscov's medium supplemented with 10 % FBS, 500 ug/ml gentamicin, and 25 units of nystatin /ml. The medium was then buffered by the addition of 3.024 g/L sodium bicarbonate. Cell culture monolayers were prepared by seeding one ml of cell suspension on 12 mm diameter glass coverslips in 12x24 mm flat-bottomed shell vials. The cultures were incubated overnight at 37°C. Each freshly prepared semen samples was added to five shell vial cell cultures after removing the media. The shell vials were centrifuged at 2100 rpm for one hour. Medium 199 was supplemented with 10% FBS, 500 µg/ml gentamicin, and 25 units of nystatin /ml, and 2 µg/ml cycloheximide was added to the cultures after removing the excess of inoculum. The shell vials were incubated as before for three to five days. The monolayers were examined by the DIF test (Ross and Borman, 1963). The effect of different concentrations of used antibiotics on *Chlamydia psittaci* was classified into 4 categories viz., highly effective, moderate effective, partial effective and not effective according to the titer of reisolated *Chlamydia psitttaci*.

Statistical analysis:

Data were subjected to standard analysis of variance using computerized Co-state program.

RESULTS

The results of effect of different antibiotics on individual sperm motility (%) and on spermatozoa with intact acrosome (%) at 5 °C are shown in Tables 2 and 3, respectively. The results of effect of different antibiotics on post-thaw motility, viability and intact acrosomal percentages of spermatozoa are shown in Table 4. The results of effect of

different antibiotics on *Chlamydia psittaci* in processed semen are shown in Table 5.

Infected diluted semen samples with *Chlamydia psittaci* did not vary significantly in percentages of motile spermatozoa and intact acrosomes (68.33 ± 7.64 and 82.33 ± 7.02, respectively) from those of the control-diluted samples (71.67 ± 2.89 and 88.00 ± 2.65, respectively) along the three days of evaluation (Tables 2 and 3).

The post dilution sperm motility was 63.33 ± 2.89% and acrosomal integrity was 85.67 ± 3.05%. While post-freezing and thawing sperm motility was 40.00 ± 10.00; viability index was 45.83 ± 3.82 and its intact acrosomal percentage was 79.67 ± 4.51 as shown in Tables 2, 3 and 4.

Streptomycin sulphate could not affect *Chlamydia psittaci* (Table 5). Multiplication of reticulate bodies was observed in the presence of Penicillin G sodium in stained smears of chlamydia-infected BHK cells and cell lysis was seen, but a small dense-centered elementary bodies were not observed.

Lincomycin in different concentrations (100, 150, 200, 250 and 300 µg/ml) could maintain, by different degrees, the post-dilution sperm motility and acrosomal integrity (Tables 2 and 3) and post-thawing sperm motility, viability and acrosomal integrity (Table 4). The same concentrations of lincospectin used in the present study couldn't inhibit *Chlamydia psittaci* (Table 5).

Oxytetracycline in different concentrations (10, 20, 30, 40, 50, 100, 130, 150 and 170 µg/ml) and chloramphinicol in different concentrations (10, 20, 40, 50, 75, 100, 125, 170, 200, 260 and 300 µg/ml) were found to be effective on *Chlamydia psittaci* replication by different degrees (Table 5). On the other hand, the same chemotheraputics (oxytetracycline and chloramphinicol) badly effected the locomotion system in diluted and frozen-thawed spermatozoa translated in their post-dilution sperm motility and post thawing sperm motility and viability as shown in Tables 2 and 4. Meanwhile, the bad affect of the mentioned chemotheraputics was not very bad on the integrity of the sperm acrosomes post-dilution and thawing (Table 3).

Table 2: Effect of different antibiotics on post-dilution sperm motility (%) of buffalo-spermatozoa at 5°C (Mean ± SD)

Type	Average of Motility percent		
	First day	Second day	Third day
Net semen	73.33 ±2.89^{ab}	-	-
primary diluted semen	71.67±2.89^{abc}	45.00±5.00	43.33 ±2.89
infected diluted semen (IDS)	68.33±7.64^{abc}	55.00±5.00	45.00 ±5.00
IDS + Lincomycin (100 µg/ml)	76.67 ±5.77^{a}	43.33± 2.89	25.00 ±8.66
IDS + Lincomycin (150 µg/ml)	70.00±5.00^{abc}	41.67±10.41	18.33 ±2.89
IDS + Lincomycin (200 µg/ml)	68.33±7.64^{abc}	40.00 ±10.00	16.67 ±2.89
IDS + Lincomycin (250 µg/ml)	66.67±2.89^{abc}	38.33 ±7.64	15.00 ±5.00
IDS + Lincomycin (300 µg/ml)	65.00±5.00^{abc}	33.33 ±5.77	8.83 ±2.87
IDS+Oxytetracycline (10 µg/ml)	10.00 ±5.00^{ghi}	3.33 ±2.89	0
IDS+Oxytetracycline (20 µg/ml)	6.67 ±2.89^{hi}	1.67 ±2.89	0
IDS+Oxytetracycline (30 µg/ml)	3.33 ±2.89^{i}	0	0
IDS+Oxytetracycline (40 µg/ml)	3.33 ±2.89^{i}	0	0
IDS+Oxytetracycline (50 µg/ml)	3.33 ±2.89^{i}	0	0
IDS+Oxytetracycline (100 µg/ml)	1.67 ±2.89^{i}	0	0
IDS+Oxytetracycline (130 µg/ml)	1.67 ±2.89^{i}	0	0
IDS+Oxytetracycline (150 µg/ml)	1.67 ±2.89^{i}	0	0
IDS+Oxytetracycline (170 µg/ml)	1.67 ±2.89^{i}	0	0
IDS+Chloramphinicol (10 µg/ml)	43.33 ±10.4^{d}	23.33±15.28	0
IDS+Chloramphinicol (20 µg/ml)	33.33 ±20.2^{de}	16.67 ±2.89	0
IDS+Chloramphinicol (40 µg/ml)	28.33 ±2.89^{ef}	11.67 ±2.89	0
IDS+Chloramphinicol (50 µg/ml)	28.33 ±7.64^{ef}	11.67 ±2.89	0
IDS+Chloramphinicol (75 µg/ml)	20.00 ±8.66^{fg}	10.00 ±5.00	0
IDS+Chloramphinicol (100 µg/ml)	18.33 ±7.64^{fgh}	6.67 ±2.89	
IDS + Chloramphinicol 125 µg/ml)	13.33 ±2.89^{ghi}	6.67 ±2.89	
IDS+Chloramphinicol (170 µg/ml)	11.67 ±2.89^{ghi}	6.67 ±7.64	0
IDS+Chloramphinicol (200 µg/ml)	11.67 ±7.64^{ghi}	3.33 ±2.89	0
IDS+Chloramphinicol (260 µg/ml)	10.00 ±5.00^{ghi}	3.33 ±2.89	0
IDS+Chloramphinicol (300 µg/ml)	8.33 ±2.89^{ghi}	3.33 ±5.77	0
IDS + Penicillin G (1000 I U/ml) + Streptomycin sulphate (1 mg/ml)	63.33 ±2.89^{bc}	51.67 ±2.21	36.67±11.55

Superscript small letters within the same column are significantly different ($P<0.05$)
n = 5 samples for each treatment

Table (3): Effect of different antibiotics on intact acrosome percentage of buffalo-spermatozoa at 5°C (Mean ± SD)

Type	Intact acrosome percentage		
	First day	Second day	Third day
Net semen	89.67 ±3.51^{a}	-	-
Primary diluted semen	88.00 ±2.65^{ab}	82.33 ±3.05	66.00 ±7.93
Infected diluted semen (IDS)	82.33±7.02^{abc}	81.00 ±6.24	66.67 ±7.63
IDS + Lincomycin (100 µg/ml)	78.00±9.85^{bcd}	76.00 ±7.93	76.33 ±5.13
IDS + Lincomycin (150 µg/ml)	78.00±7.21^{bcd}	75.67 ±9.81	73.00 ±9.85
IDS + Lincomycin (200 µg/ml)	68.00±8.72^{def}	66.00 ±7.93	64.67 ±8.08
IDS + Lincomycin (250 µg/ml)	67.0±10.44^{def}	65.00 ±9.54	64.00 ±6.24
IDS + Lincomycin (300 µg/ml)	65.67 ±6.03^{ef}	64.33 ±7.50	63.67 ±7.23
IDS+Oxytetracycline (10 µg/ml)	60.00 ±1.00^{fg}	54.00 ±4.58	52.00 ±3.00
IDS+Oxytetracycline (20 µg/ml)	51.33±2.09^{ghij}	48.33 ±1.53	48.00 ±2.65
IDS+Oxytetracycline (30 µg/ml)	46.00 ±3.61^{ijk}	43.67 ±4.62	43.00 ±5.66
IDS+Oxytetracycline (40 µg/ml)	44.67±4.51^{ijkl}	43.00 ±4.3	42.33 ±2.52
IDS+Oxytetracycline (50 µg/ml)	42.33±4.93^{ijkl}	42.33 ±4.16	41.67 ±3.78
IDS+Oxytetracycline (100 µg/ml)	38.00 ±2.65^{kl}	39.00 ±4.36	37.33 ±2.52
IDS+Oxytetracycline (130 µg/ml)	37.33 ±2.52^{kl}	37.66 ±2.52	36.67 ±3.78
IDS+Oxytetracycline (150 µg/ml)	36.67 ±4.04^{kl}	34.67 ±4.72	33.67 ±4.73
IDS+Oxytetracycline (170 µg/ml)	33.00 ±5.19^{l}	33.00 ±4.36	23.33 ±4.04
IDS+Chloramphinicol (10 µg/ml)	58.00±7.00^{fgh}	55.33 ±7.57	54.67 ±4.51
IDS+Chloramphinicol (20 µg/ml)	54.00±5.30^{ghi}	55.33 ±3.51	53.67 ±6.35
IDS+Chloramphinicol (40 µg/ml)	53.67±5.51^{ghi}	54.00 ±4.35	52.67 ±4.62
IDS+Chloramphinicol (50 µg/ml)	52.00±4.36^{ghij}	51.66 ±4.61	48.67 ±6.35
IDS+Chloramphinicol (75 µg/ml)	47.67±7.23^{hijk}	46.33 ±6.11	48.33 ±7.37
IDS+Chloramphinicol (100 µg/ml)	47.00±8.18^{hijk}	45.00 ±8.14	45.67 ±5.51
IDS + Chloramphinicol 125 µg/ml)	44.33±7.57^{ijkl}	44.00 ±5.29	44.00 ±5.56
IDS+Chloramphinicol (170 µg/ml)	44.33±4.04^{ijkl}	42.33 ±4.93	40.00 ±8.89
IDS+Chloramphinicol (200 µg/ml)	43.66±4.16^{ijkl}	41.33 ±4.51	41.00 ±5.00
IDS+Chloramphinicol (260 µg/ml)	42.67±4.16^{ijkl}	41.00 ±3.61	40.00 ±5.00
IDS+Chloramphinicol (300 µg/ml)	40.33 ±4.16^{kl}	40.33 ±4.04	37.67 ±4.62
IDS + Penicillin G (1000 I U/ml) + Streptomycin sulphate (1 mg/ml)	85.67±3.05^{abc}	78.33 ±2.88	69 ±5.29

Superscript small letters within the same column are significantly different ($P<0.05$)
n = 5 samples for each treatment

Table 4: Effect of different antibiotics on Post-thaw motility, viability and intact acrosome percentage of buffalo-spermatozoa (Mean ± SD)

Type	P.T.M	P.T.V.I	P.T I.A P
Primary diluted semen	46.67±7.64^{a}	73.33±10.41^{a}	80.33±4.62^{a}
Infected diluted semen (IDS)	25.00± 5.00b^{c}	35.00±2.5^{c}	70.00±10.0^{ab}
IDS + Lincomycin (100 µg/ml)	23.33±7.64^{bc}	30.00±2.5^{cd}	67.00±7.00^{bc}
IDS + Lincomycin (200 µg/ml)	18.33±2.89^{bcd}	27.5±7.5^{cde}	66.33±9.45^{bc}
IDS + Lincomycin (300 µg/ml)	15.00±5.00^{cde}	15.83±7.64^{fg}	61.3±6.02^{bcd}
IDS+Oxytetracycline (10 µg/ml)	10.00±5.00 ^{def}	11.67±1.44^{g}	51.33±3.51^{de}
IDS+Oxytetracycline (20 µg/ml)	8.33±2.89^{def}	10.83±1.44^{g}	47.67±3.51^{e}
IDS+Oxytetracycline (50 µg/ml)	1.67±2.89^{b}	0.83±1.44^{h}	45.67±7.37^{e}
IDS+Chloramphinicol (10 µg/ml)	26.67±2.89^{b}	30.83±2.88^{cd}	51.00±2.65^{de}
IDS+Chloramphinicol (50 µg/ml)	15.00±5.00^{cde}	20.83±2.89^{ef}	46.00±3.61^{e}
IDS + Penicillin G (1000 IU/ml) + Streptomycin (1 mg/ml)	40.00± 10.00^{a}	45.83±3.820^{b}	79.67±4.51^{a}

P.T.M. = Post-thaw sperm motility. P.T.V.I. = Post-thaw sperm viability index.
P.T.I.A.P. = Post-thaw sperm with intact acrosomal percentage.
Superscript small letters within the same column are significantly different ($P<0.05$)
n = 5 samples for each treatment

Table 5: Effect of different antibiotics on *Chlamydia psittaci* in processed semen

Antibiotic (concentration)	Effect on *Chlamydia psittaci*
IDS + Lincomycin (100 µg/ml)	N.E.
IDS + Lincomycin (150 µg/ml)	N.E.
IDS + Lincomycin (200 µg/ml)	N.E
IDS + Lincomycin (250 µg/ml)	N.E.
IDS + Lincomycin (300 µg/ml)	N.E.
IDS+Oxytetracycline (10 µg/ml)	+++
IDS+Oxytetracycline (20 µg/ml)	+++
IDS+Oxytetracycline (30 µg/ml)	+++
IDS+Oxytetracycline (40 µg/ml)	+++
IDS+Oxytetracycline (50 µg/ml)	+++
IDS+Oxytetracycline (100 µg/ml)	+++
IDS+Oxytetracycline (130 µg/ml)	+++
IDS+Oxytetracycline (150 µg/ml)	+++
IDS+Oxytetracycline (170 µg/ml)	+++
IDS+Chloramphinicol (10 ug/ml)	+
IDS+Chloramphinicol (20 µg/ml)	+
IDS+Chloramphinicol (40 µg/ml)	++
IDS+Chloramphinicol (50 µg/ml)	++
IDS+Chloramphinicol (75 µg/ml)	++
IDS+Chloramphinicol (100 µg/ml)	++
IDS + Chloramphinicol 125 µg/ml)	++
IDS+Chloramphinicol (170 µg/ml)	++
IDS+Chloramphinicol (200 µg/ml)	+++
IDS+Chloramphinicol (260 µg/ml)	+++
IDS+Chloramphinicol (300 µg/ml)	+++
IDS + Penicillin G (1000 IU/ml) + Streptomycin (1 mg/ml)	+ N.E.

IDS = Infected Diluted Semen +++ = Highly effective
++ = Moderately effective + = Partially effective N.E. = Not effective

DISCUSSION

The presence of bacteria in semen used for artificial insemination constitutes a hazard with respect to the transmission of these agents and the contaminant problems which may ensure from their presence in the female reproductive tract. Authors have been confused about the effect of *Chlamydia psittaci* on clinical symptoms of bulls and its semen quality. Storz et al. (1968) and Storz et al. (1976) reported that infected bulls with *Chlamydia psittaci* were suffered from pyospermia and a high percentage of morphologically abnormal spermatozoa. Sobiech et al. (1991) also observed poor semen quality in bulls with serum antibodies to *Chlamydia psittaci*, although clinical signs of infection of the genital organs were not detected. On the other hand, Bicknell et al. (1986) could isolate *Chlamydia psittaci* from satisfactory quality semen of clinically normal bulls. In Egypt, the incidence of antichlamydial antibodies in male and female buffaloes was ranged from 33.15 % to 42.5 % (Schmatz *et al.*, 1978; Ata, 1982 and Amin et al., 1998).

In the present study, infected diluted semen samples with *Chlamydia psittaci* did not vary significantly in percentages of motile spermatozoa and intact acrosomes than those of the control-diluted samples along the three days of evaluation (Tables 2 and 3). These results indicate that the artificial infection of semen samples with *Chlamydia psittaci* did not affect its quality (motility and intact acrosomal percentage). This is in line with the observations of Bowen et al., 1978 and Bicknell et al., 1986 who found that *Chlamydia psittaci* in semen did not affect the sperm motility and that fertilization failure did not appear to be the major cause of infertility. They hypothesized that the reason for any infertility was probably due to the ability of the *Chlamydia psittaci* organism to infect and multiply in the endometrium and thus altered the environment of the embryo, resulting in early embryonic death.

Streptomycin, vancomycin, restoctin and mycostatine which can inhibit multiplication of a great variety of possible bacterial contaminants, they could not affect Chlamydial replication (Gordon and Quan, 1962 and Jenkin and Hung, 1967).

In the present study, penicillin G sodium (1000 IU/ml) and streptomycin sulphate (1 mg/ml) were added to diluted buffalo-semen. Many authors are in line with our results as they found that penicillin and streptomycin are very safe to be added to diluted and frozen buffalo-

semen as both maintain most of sperm characters (Ziada, 1989 and 1994 and Hassan et al., 1997). Meanwhile, streptomycin sulphate could not affect *Chlamydia psittaci* (Table 5). On the other hand, chlamydial agents are susceptible to penicillin G sodium in varying degrees. Multiplication of reticulate bodies was observed in the presence of Penicillin G sodium in stained smears of chlamydia-infected BHK cells and cell lysis was seen, but a small dense-centered elementary bodies were not observed. These results indicated that penicillin G sodium did not interfere with the reproduction of the large reticulte bodies. Simillar conclusions were reported by Tamura and Manire, (1968) and Moulder, (1969). Greenland (1961) reported that penicillin G sodium apparently had no effect on the rate of multiplication of such chlamydial strain.

On the similar ground, Lincomycin in different concentrations could maintain, by different degrees, the post-dilution sperm motility and acrosomal integrity (Tables 2 and 3) and post-thawing sperm motility, viability and acrosomal integrity (Table 4). By the same manner, Hassan et al. (1997) had found that the use of lincospectin at concentrations of 600-1200 μg/ml had not any detrimental effect upon spermatozoal characters. On the other hand, The same concentrations of lincospectin used in the present study couldn't inhibit *Chlamydia psittaci* (Table 5).

The results indicated that oxytetracycline in different concentrations and chloramphinicol in different concentrations were found to be effective on *Chlamydia psittaci* replication by different degrees (Table 5). These results are in line with the results of Storz (1971) who found that tetracyclines and chloramphenicol are the most effective inhibitors for chlamydial multiplication and they could arrest its replication early in the developmental cycle. On the other hand, the same chemotheraputics (oxytetracycline and chloramphinicol) badly effected the locomotion system in diluted and frozen-thawed spermatozoa translated in their post-dilution sperm motility and post thawing sperm motility and viability as shown in Tables 2 and 4. Meanwhile, the bad affect of the mentioned chemotheraputics was not very bad on the integrity of the sperm acrosomes post-dilution and thawing (Table 3). These results were in line with that of Hassan (1990) who found bad effect of tetracycline on buffalo semen characters. Godman and Gillman (1991) had also cited that the chloramphinicol harm effect may refer to its inhibitory effect on protein synthesis and enzyme activity of cells.

In conclusion, *Chlamydia psittaci* could not be controlled in diluted semen samples by adding chemotheraputic drugs as the effective drugs on *Chlamydia psittaci* are very harmful to sperm picture. So, we recommend that the semen samples must be free from *Chlamydia psittaci* before being used them in artificial insemination due to hazardous effect of *Chlamydia psittaci* on reproductive performance of farm animals.

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