SURVIVAL RATE OF BULL SPERMATOZOA SUPPLEMENTED WITH PROSTAGLANDIN $F_2\alpha$, PROGESTERONE AND ESTROGEN IN CULTURE MEDIA

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ABSTRACT

Three adult Friesian bulls were used in this experiment. Pooled semen samples from bulls were used to study the effect of $PGF_2\alpha$, estrogen and progesterone supplementation on the survival rate of spermatozoa during preservation at 5°C in yolk citrate buffer. Each of these supplements was added at three levels: 100, 200 and 300 mg per ml extender. The levels of 100 and 200 mg $PGF_2\alpha$ per ml were significantly ($p<0.05$) the best in maintaining sperm motility and livability during preservation. Semen supplementation in all levels of progesterone significantly ($p<0.05$) diminished sperm motility and livability. Spermatozoal motility at the control samples were similar to motility in the spermatozoa treated with low level of estrogen, but sperm livability was highest with low level of estrogen (300μg/ml). The high estrogen level markedly diminished survival of spermatozoa during preservation.

INTRODUCTION

Several attempts have been made to improve sperm transport and fertility by injecting animals with hormones. Numerous reports showed that steroid hormones are significant factors in sperm transport (Hawk and Conley, 1973). The significant role of PGS in sperm transport has been demonstrated in rabbits (Mandle,1972) and in sheep (Al-juburi, 1987; Gustafson, 1978). In large animal only a few in vitro studies were carried out to clarify the influence of such hormones on spermatozoa. The present work was conducted to study the influence of $PGF_2\alpha$, estrogen and progesterone supplementation on sperm survival during refrigeration at 5°C.

MATERIALS AND METHODS

Semen used in this study was obtained twice weekly for ten times from each of three Friesian bulls routinely used in AI program in private dairy cattle station in Kut Government, Iraq in cooperation of Animal Production Department, Agriculture Circle, Atomic Energy Organization.

Semen samples from each bull were evaluated, pooled and splitted to ten fractions. Nine fractions were extended with freshly prepared yolk citrate buffer containing three levels (100, 200 and 300 mg per ml extender) of either $PGF_2\alpha$, progesterone or estrogen. One fraction was extended with yolk citrate buffer without any supplementation and served as control. The extender was composed of 2.9% sodium citrate dehydrate, 20% egg yolk, 500 microgram streptomycin and 500 IU penicillin per ml extender, the average dilution rate for all treatment was 1:10 .

Samples were refrigerated at 5°C for four days. Duplicate subsamples were taken daily and examined for sperm motility at 300x magnification on a warm stage to the nearest 10 percent. Daily counts of dead sperm in smears stained with eosin-nigrosin were recorded. The results were analyzed statistically according to Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

Results obtained in this study showed that the extender containing $PGF_2\alpha$ (100 and 200 mg /ml) were the best in maintaining sperm motility during semen preservation at 5°C (Table1). Analysis of variance showed that mean motility percentage of spermatozoa diluted with the low level of $PGF_2\alpha$ was significantly ($p<0.05$) superior to other treatments including the high level of $PGF_2\alpha$, progesterone, control and estrogen. This superiority was observed on the second day of refrigeration till the end of storage time.

Mean sperm motility percentages were inferior ($P<0.05$) when spermatozoa were subjected to all levels of progesterone and 200 and 300 μg/ml estrogen compared to other treatments. In these treatments the decline in motility was noted as early as 24 hours of refrigeration.

The motility and livability of bull spermatozoa declined rapidly after 24 hours of storage in all supplementation, and slightly thereafter.

Results of sperm motility in extenders supplemented with 100 μg/ml of estrogen, at any refrigeration time studied, did not differ ($p<0.05$) from control samples.

Table (2) shows the mean percentages of live spermatozoa over 4-days refrigeration. Analysis of variance involving 300 observations revealed that preserving bull semen in extenders supplemented with $PGF_2\alpha$ at 100 and 200 μg/ml levels resulted in significant ($p<0.05$) increases in the percentages of live spermatozoa more than any other treatments. However, the lowest percentage of live spermatozoa was found with the high dose of progesterone. This was also observed at any tested refrigeration times.

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Table (1): Motility of spermatozoa (%) during storage in egg yolk citrate (EYC) supplemented with $PGF_{2\alpha}$, progesterone and estrogen (means±SE)

Duration of Storage at 5°C (Hours)	EYC	Prostaglandin $F_{2\alpha}$				Progesterone				Estrogen			
		1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	63.0±2.6	77.0±2.3	77.0±2.4	67.0±2.1	74.0±1.3[a]	72.0±2.3	66.0±2.1	60.0±2.1	66.0±1.3[a]	73.0±1.3	68.0±2.2	66.0±1.4	69.0±1.1[a]
24	49.0±2.2	50.0±1.9	53.0±1.2	40.0±2.1	48.0±2.5[b]	31.0±1.1	26.0±1.4	22.0±2.5	26.0±1.5[b]	46.0±2.1	30.0±3.1	27.0±3.2	34.0±1.5[b]
48	37.0±1.8	48.0±1.5	48.0±1.2	34.0±3.1	43.0±1.4[c]	25.0±2.1	19.0±3.3	15.0±1.4	20.0±2.4[c]	39.0±2.8	20.0±4.1	18.0±1.1	26.0±2.1[c]
72	27.0±1.5	40.0±1.5	42.0±1.6	27.0±3.4	36.0±2.1[d]	8.0±4.3	10.0±4.1	5.0±3.2	8.0±3.1[d]	23.0±1.9	14.0±1.1	7.0±2.1	15.0±3.1[d]
96	18.0±0.8	30.0±0.4	31.0±0.4	7.0±3.5	23.0±2.8[e]	1.0±3.1	2.0±3.5	0.5±1.4	2.0±3.8[e]	18.0±2.5	5.0±2.5	1.0±2.2	8.0±3.8[e]
Mean	38.6±1.4[b]	49.1±2.1[a]	50.0±1.1[a]	34.8±1.7[c]		27.4±2.1[d]	24.6±1.3[d]	20.0±2.2[e]		40.0±2.1[b]	27.1±1.2[d]	32.8±2.3[d]	

1= 100 µg per ml extender 2= 200µg per ml extender 3=300 µg per ml extender

a, b, c, d, e = mean values with different superscripts are significantly different (P< 0.05)

Table (2): Percentages of live spermatozoa during storage in egg yolk citrate (EYC) supplemented with PGF2α, progesterone and estrogen (means±SE)

Duration of Storage at 5°C (Hours)	EYC	Prostaglandin $F_{2\alpha}$				Progesterone				Estrogen			
		1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
0	80.0±2.1	88.0±0.8	89.0±0.9	81.0±0.8	86.0±1.3[a]	87.0±1.2	82.0±1.3	80.0±1.1	83.0±1.1[a]	87.0±1.2	83.0±1.3	81.0±1.4	84.0±1.2[a]
24	69.0±2.2	72.0±1.2	73.0±2.1	70.0±1.2	72.0±1.8[b]	60.0±1.3	55.0±1.2	51.0±1.3	55.0±2.1[b]	71.0±1.2	63.0±1.4	60.0±1.3	65.0±1.5[b]
48	64.0±2.1	70.0±2.3	70.0±1.3	66.0±1.4	96.0±2.3[c]	52.0±2.1	40.0±1.3	40.0±1.7	44.0±2.4[c]	68.0±1.3	52.0±1.5	44.0±1.2	55.0±2.1[c]
72	58.0±3.6	60.0±1.2	64.0±1.2	55.0±1.3	60.0±3.2[d]	36.0±2.3	30.0±1.4	15.0±1.8	27.0±3.1[d]	62.0±1.5	33.0±2.4	22.0±2.1	39.0±3.1[d]
96	45.0±3.7	51.0±2.1	53.0±1.1	45.0±1.3	50.0±3.4[e]	17.0±2.1	15.0±2.1	11.0±1.4	14.0±2.8[e]	49.0±0.6	19.0±2.3	8.0±1.9	25.0±2.2[e]
Mean	62.1±2.3[b]	68.6±2.1[a]	70.0±1.3[a]	63.0±0.8[b]		50.2±1.3[e]	44.1±1.2[d]	39.2±1.4[e]		68.0±0.8[a]	51.0±1.2[c]	43.0±0.9[d]	

1= 100 µg per ml extender 2= 200µg per ml extender 3=300 µg per ml extender

a, b, c, d, e = mean values with different superscripts are significantly different (P< 0.05)

The highest ($p<0.05$) mean percentage of live spermatozoa were obtained when semen was subjected to 100, 200 μg PGF2α /ml and 100 μg estrogen/ml.

DISCUSSION

Results presented indicate that supplementation of extenders with 100 and 200 μg PGF2α per ml significantly improved spermatozoal survival during semen storage at 5°C. These findings are in agreement with limited data recently presented by Cohen *et al*. (1977); Daader and EL-Keraby (1982) and الجبورى وآخرون (1995).

Hawk and Cooper (1977) reported that synthetic progestin used for regulation of the estrous cycle, impairs sperm transport and results in a lowered fertility rate. So it is of interest to clarify if progesterone exerts a direct effect on spermatozoa. This study demonstrated that progesterone has a detrimental effect on spermatozoa during refrigeration as indicated by significantly low mean sperm motility and live sperm. Progesterone exerts the detrimental effect upon spermatic cells few hours after the beginning of treatments.

The present work showed that subjecting spermatozoa to low levels of estrogen maintained relatively better sperm motility and liveability during preservation at 5°C. This result as in general agreement with those of Allison and Robinson (1972) who reported that estrogens seem to be necessary for maintenance of sperm populations.

The beneficial effect of low level of PGF2α on sperm motility was explained by Carsten (1972) who found that PGF1 increased the influx of Ca++ into sperm cell leading to metabolic activation of the sperm and consequently increased motility. Mortin et al. (1974) reported that Ca++ ions are involved in the activation of many enzymes necessary for maturation, motility and membrane properties of spermatozoa.

Previous results reported by Cohen et. al. (1977) showed that the addition of high concentration of PGF2α (250μg/ml) on rabbit semen significantly depressed sperm motility.

Further research is needed to establish the most proper level of PGS included in semen extender that gives the highest fertility rates. Furthermore, the economical efficiency for PGS utilization must be also considered.

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صباح عبد الحميد الجبورى و حاتم حمادى ومرتضى الحكيم (1995)، تأثير إضافة البروستاجلاندين $F_{2\alpha}$ على الصفات الطبيعية للسائل المنوى للكباش العواسيه تحت تأثير درجة حرارة الغرفة (20-°18م) أو الثلاجة (5°م). مجلة اتحاد الجامعات العربية للعلوم التطبيقية الجمهورية العربية الليبية العدد الاول من صفحة (161-178).

الملخص العربي

نسبة بقاء الحيوانات المنوية للثيران مضافا اليها البروستاجلاندين $F_{2\alpha}$، البروجسترون أو الاستروجين

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استخدم في هذه التجربة ثلاثة ثيران فريزيان. تم جمع السائل المنوي منهما ثم خلطه لدراسة تاثير إضافة مستويات مختلفة من $PGF_{2\alpha}$ والإستروجين والبروجسترون الى السائل المنوي المخفف (سترات صوديوم مع صفار البيض) على النسبة المئوية لحركة النطف والنسبة المئوية للحيوانات المنوية الحية خلالها الحفظ على درجة حرارة الثلاجة (5°م). و كانت هناك ثلاث مستويات (100-200 و300 مايكروجرام لكل مل مخفف) من البروستاجلاندين أو البروجسترون أوالاستروجين.

استخدام 100 أو 200 مايكروجرام من $PGF_{2\alpha}$ لكل 1 مل مخفف كانت له نتائج عالية معنوية على مستوى ($P<0.05$) و كان الأفضل في ادامة حركة الحيوانات المنوية وحياتها و حتى 96 ساعة حفظ تحت درجة حرارة الثلاجة (5°م) وكانت كل مستويات البروجسترون المستخدم معنوية على مستوى تقليل حركة وحياة الحيوانات المنوية. وأما المستوى المنخفض للاستروجين 100 مايكروجرام لكل ١مل مخفف والكونترول فقط أعطوا نفس النتائج للحيوية، أما حياة الحيوانات المنوية فكانت فيه عالية. المستوى العالي 300 مايكروجرام لكل ١مل مخفف كان غير جيد في بقاء حيوية الحيوانات المنوية خلال الحفظ .