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EFFECT OF $PGF_2\alpha$ ON SOME SEMEN CHARACTERS OF CROSS-BRED BULLS (BALADY X FRESIAN) DURING STORAGE AT 4°C

(With 6 Tables)

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تأثير اضافة البروستاجلاندين $F_2\alpha$ على بعض خصائص منى الطلائق البقرية الخليط (بلدىXفريزيان) اثناء حفظها عند ٤ °م

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تمت دراسة تأثير البروستاجلاندين $F_2\alpha$ على خصائص السائل المنوى للطلائق البقرية الخليطه (بلدىX فريزيان) و كذلك على الأنزيمات الموجوده فى بلازما السائل المنوى اثناء حفظة علىدرجة حرارة الثلاجة (٤ °م). تم تخفيف السائل المنويبمخفف صفار البيض مع سترات الصوديوم بنسبة (١٠:١) ثم اضيف البروستاجلاندين بنسبة ٠، ٢٠٠،٤٠٠،٦٠٠ ميكروجرام/ ملليتر. تم فحص تلك العينات المحفوظة فى الثلاجة يوميا لمدة أربعة أيام, وقد أظهرت الدراسة أن لهرمون البروستاجلاندين عند تركيز ٢٠٠ ميكروجرام/ ملليتر تأثيرا ايجابيا على الحركة المنفردة للحيامن وكذلك على حيوية الحيامن ونسبة الحيامن المشوهة كما أنه حفظ جدار الخلية مما أدى الى قلة تسريبها لانزيمات الGOT,GPT المختزنة داخلها و عند زيادة تركيز الهرمون الى ٤٠٠،٦٠٠ ميكروجرام/ ملليتر وجد أن له تأثيرا معنويا فى تثبيط حركة الحيامن وكذلك زيادة نسبة الحيامن الميتة والمشوهة .كما ان له تأثير فى زيادة نسبة تشوه الغطاء الطرفى. وكذلك أثبتت الدراسة أن زيادة نسبة الهرمون تؤدى الى التأثير المتلف لجدار خلية الحيوان المنوى مما يؤدى الى خروج الأنزيمات المختزنة داخله الى البلازما المحيطة.

SUMMARY

Incubation of bull spermatozoa with $PGF_2\alpha$ at 4 °C for 4 days was performed to investigate the effect of PG on some sperm cell characters and enzymatic release. Semen from cross-bred bulls (Balady X Fresian) was diluted with egg yolk citrate at a rate of 1:10. Aliquots were

supplemented with PGF_2 α in levels of 0, 200, 400 and 600 ug\ ml and stored at 4°C for 4 days. Daily examination for each treatment was carried during the storage period. The percentage of sperm motility (SM%), alive sperm (AS%) and sperm with intact acrosome (IA%) were decreased significantly with doses higher than 200 ug\ ml. In the same time, abnormal sperm percentage (AbS%) increased in samples which contain 400 and 600 ug\ ml. Continuous increase in transaminases activities in the extra-cellular medium were noticed with the increase level of PGF_2 α and prolongation of storage time. The results suggested that, addition of PGF_2 α with high concentration (more than 200 ug\ ml) to bull spermatozoa during storage at°C adversely affect sperm motility, viability and abnormalities as well as induced membrane damage and impair permeability.

Key Words: $PGF_2\alpha$ - Bull semen- Extension - Invitro

INTRODUCTION

The role of prostaglandins (PG) in reproduction became one of the primary targets of investigations during the last decade. The hormone represents a part of normal constituent of seminal plasma of different species specialy man and ram (Mann and Lutwak Mann, 1981). Cohen et. Al., (1977) cited that prostaglandins increased in case of inflammatory condition of the genital tract and this increase inhibited significantly the sperm motility. Gustafsson et al., (1975); Gamcik et al., (1980) and El-Gaafary, (1987) reported that administration of PGF_2 α, intramuscularly after insemination of ewes, or addition of it to diluted ram semen before insemination improved fertility. Anel et al., (1988a) found that addition of PG led to significant increase in individual sperm motility. In the same time El-Gaafary (1989) showed that supplementation of rabbit semen with PGF_2 α (600 ug\ ml diluted semen) stimulated sperm motility but depressed fertility. This decrease in fertility is explained by the author to be due to decreasing sperm survival in the genital tract, or to loss of ability to fertilize owing to cell damage. Fayed (1996) found that, although the spermatozoal motility was decreased, the activities of transaminases showed a continous increase with increasing level of $PGF_2\alpha$.

Recent studies investigate the effect of $PGF_2\alpha$ on bull spermatozoa in vitro during incubation at 37 °C (Anel et al.,1988; Salwa et al ., 1994 and Fayed, 1996). Information's about the effects of PG on bovine spermatozoa during storage at refrigerator are lacking.

The present investigation was planned to study the effect of adding different levels of $PGF_2\alpha$ to bull semen, extended with egg yolk citrate and stored at 4 °C for 4 days on some characters of sperm. In the same time, the acrosome integrity and leakage of GOT and GPT into the extra-cellular medium were estimated.

MATERIAL and METHODS

Semen used in this study was obtained from three cross-bred bulls (Balady X Fresian), six years age, and maintained under similar nutritional and mangmental conditions in the farm of the Faculty of Veterinary Medicine, Assiut University. Two semen collections were obtained from each bull at morning hours twice weekly using conventional artificial vagina (42-45°C).

Immediately after collection, semen was taken to the laboratory and kept in water bath at 25°C for macroscopic and microscopic examinations. Samples which exhibit active wave motion were pooled together. Pooled samples were divided to four equal parts. Each part was extended by egg yolk citrate extender (1:10). The first part served as control and the $PGF_2\alpha$ (Dinoprost tromethamin, Chinoin, Hungary) was included in the diluent of the second, third and fourth parts at concentration of 200, 400 and 600 ug\ ml extender respectively.

Extended semen was further divided in Epindorf tubes and stored in refrigerator at 4°C. Daily examination for the control and treated samples were performed to evaluate motility, livability and abnormal sperm percentages. In the same time, the percentage of intact acrosome was estimated in a film stained by Giemsa (Barth and Oko, 1989). The rest portions of the extended semen were centrifuged at 3000 rpm for 20 min. and the supernatant fluid was stored at - 20°C till used for the determination of glutamic-oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) colorometrically by test kits supplied from Boehringer Mannheim Dignostica.

The results were analyzed by using least square mean (LSM) test among the groups using PC-stat (1985).

RESULTS

The effects of adding $PGF_2 \alpha$ on bull sperm characters are presented in tables (1-6). Table 1 showed the effect of different doses of $PGF_2 \alpha$ on SM% during refrigeration. Motility significantly improved at 200 ug\ml concentration of $PGF_2 \alpha$ while higher levels (400 and 600 ug\ml) significantly decreased sperm motility. With respect to the duration of storage, the improvement in SM% continued until the second and third days of storage while significant decline in sperm motility started in the fourth day.

The effect of $PGF_2 \alpha$ on AS% is presented in table 2. The first dose of $PGF_2 \alpha$ (200 ug/ml) improved the AS% significantly in comparison with the control sample during the period of storage. However, such improvement decreased with higher concentrations of drug.

The effect of $PGF_2 \alpha$ on secondary AbS% was non significant in the first three days of storage in samples contained 200 and 400 ug\ml. With the highest dose used (600 ug\ml) the significant effect started from the first day of storage. In the same time significant effect appeared in the fourth day of storage with all doses (table 3). Primary abnormalities were very rare in the examined samples.

The percentages of sperm with IA incubated with different doses of $PGF_2 \alpha$ were presented in table 4. Out of this table, the damaged acrosome percentages increased with high doses as well with the prolongation of storage period.

The leakage of GPT and GOT in the extracellular media, increased significantly in samples which contained 600 ug\ ml as with the control samples. In the same time samples which contained 200 and 400 ug\ml showed significant increased in the fourth day of storage (table 5 and 6).

DISCUSSION

The present study was performed to investigate the effect of different levels of $PGF_2 \alpha$ on some characters of bull semen during its storage in refrigerator at $4^{\circ}C$. Out of the study it was observed that increased level of $PGF_2 \alpha$ above 200 ug\ ml has a withdrawal effect on

the different characters of semen especially with extension of storage period up to 4 days. But with 200 ug\ ml of $PGF_2\alpha$ the different characters of semen were improved where the percentages of motility, livability and sperm with intact acrosome increased significantly along the storage period in comparison with control. These results coincided with those obtained for frozen ram semen supplemented with $PGF_2\alpha$ as reported by Anel et al ., (1988 a,b). When the concentration of $PGF_2\alpha$ increased to 400 and 600 µg/ ml the different characters of semen were impaired, (significant decrease in I.M % , A.S.% and I.A.% accompanied with increased Ab.S%). These results are in agreement with those obtained by Memon et al., (1984) in ram semen and Grunberger et al., (1981) in human semen. However, the present results disagree with those of Cohen et al., (1977) who reported significant decrease in sperm motility if $PGF_2\alpha$ are added in concentration 100 time greater than that found in normal human semen. Schlegel et al., (1983) also reported that addition of $PGF_2\alpha$ antisera to semen of rabbit improved sperm motility and fertilization rate. The author returned such improvement to local effect in the female genital tract.

The levels of GOT and GPT in seminal plasma increased with higher concentrations of $PGF_2\alpha$ and as well with the extension of incubation period. These results disagree with those obtained by Daader et al., (1988) who reported that supplementation of ram semen with $PGF_2\alpha$ prevents leakage of GOT and GPT. The present results coincided with those obtained by Salwa et al., (1994) for bull semen incubated at 37°C and Fayed (1996) who examined epididymal bull spermatozoa incubated with $PGF_2\alpha$ in the same temperature and reported that spermatozoal motility was decreased in the same time the activities of transaminases in the extra-cellular medium showed continuous increase with increasing level of $PGF_2\alpha$.

As a general conclusion, the addition of $PGF_2\alpha$ to bull semen in a level above 200 µg/ml adversely affect the sperm characters specially the membrane status which leads to leakage of enzymes in the extracellular medium. Therefore, it is not recommended to add $PGF_2\alpha$ with higher concentrations to bull semen especially in case of preservation at 4°C .

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Table. 1: LSM for motility percentage of semen stored at 4°C for 4 days with different concentrations of $PGF_{2\alpha}$.

Conc.Of $PGF_{2\alpha}$ Ug\ml	Storage Days			
	1st days	2nd days	3rd days	4th days
Control	75.00 ± 5.00^{1}	$68.33 \pm 7.64^{a\,1}$	$56.67 \pm 11.55^{a\,2}$	$40.00 \pm 26.46^{a,c\,3}$
200	85.00 ± 2.89^{1}	$83.33 \pm 2.89^{b\,1,2}$	$73.33 \pm 2.89^{b\,2}$	$58.33 \pm 2.89^{b\,3}$
400	83.33 ± 0.00^{1}	$81.33 \pm 2.89^{b\,1,2}$	$71.67 \pm 2.89^{b\,2}$	$41.67 \pm 2.89^{a\,3}$
600	78.33 ± 2.89^{1}	$73.33 \pm 2.89^{a,b\,1}$	$58.33 \pm 5.78^{a\,2}$	$30.00 \pm 2.89^{c\,3}$

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Table. 2: LSM for alive sperm percentage of semen stored at 4°C for 4 days with different concentrations of $PGF_{2\alpha}$.

Conc.of $PGF_{2\alpha}$ Ug\ml	Storage Days			
	1st days	2nd days	3rd days	4th days
Control	$81.70 \pm 2.68^{a\,1}$	$80.90 \pm 2.68^{a\,1}$	$76.49 \pm 2.68^{a\,2}$	$75.20 \pm 2.68^{a\,2}$
200	$93.45 \pm 2.68^{b\,1}$	$89.39 \pm 2.68^{b\,1,2}$	$80.12 \pm 2.68^{a\,1,2}$	$76.76 \pm 2.68^{a\,2}$
400	$79.44 \pm 2.68^{a,c\,1}$	$77.15 \pm 2.68^{a\,1}$	$68.71 \pm 2.68^{b\,2}$	$63.50 \pm 2.68^{b,c\,2}$
600	$74.26 \pm 2.68^{c\,1}$	$69.95 \pm 2.68^{c\,1}$	$63.48 \pm 2.68^{b\,2}$	$62.56 \pm 2.68^{c\,2}$

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Table. 3: LSM for abnormal sperm percentages of semen stored at 4°C for 4 days with different concentrations of $PGF_{2\alpha}$.

Conc.Of $PGF_{2\alpha}$ Ug\ml	Storage Days			
	1st days	2nd days	3rd days	4th days
Control	$22.64 \pm 2.48^{a\,1}$	$27.83 \pm 2.48^{a\,1,2}$	$28.57 \pm 2.48^{a\,1,2}$	$30.01 \pm 2.48^{a\,2}$
200	$25.31 \pm 2.48^{a,b\,1}$	$26.33 \pm 2.48^{a\,1,2}$	$29.92 \pm 2.48^{a\,1,2}$	$31.46 \pm 2.48^{a\,2}$
400	$29.09 \pm 2.48^{b,c\,1}$	$29.95 \pm 2.48^{a\,1}$	$31.14 \pm 2.48^{a\,1}$	$37.41 \pm 2.48^{b\,2}$
600	32.86 ± 2.48^{c}	38.06 ± 2.48^{b}	38.10 ± 2.48^{b}	37.13 ± 2.48^{b}

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Table. 4: LSM for intact acrosome sperm percentage of semen stored at 4°C for 4 days with different concentrations of $PGF_{2\alpha}$.

Conc.of $PGF_{2\alpha}$ Ug\ml	Storage Days			
	1st days	2nd days	3rd days	4th days
Control	83.84±1.87	81.00±1.87^{a}	79.38±1.87^{a}	79.41±1.87^{a}
200	84.40±1.87^{1}	82.30±1.87$^{a\,1}$	80.08±1.87$^{a\,1,2}$	75.75±1.87$^{a,b\,2}$
400	85.07±1.87^{1}	78.22±1.87$^{a,b\,2}$	72.43±1.87$^{a\,3}$	73.46±1.87$^{b\,4}$
600	79.10±1.87^{1}	75.61±1.87$^{b\,1,2}$	74.24±1.87$^{b\,2}$	74.07±1.87$^{b\,2}$

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Table. 5: LSM for GPT (unit\$10^{6}$sperm) in seminal plasma of semen stored at 4°C for 4 days with different concentrations of $PGF_{2\alpha}$.

Conc.of $PGF_{2\alpha}$ Ug\ml	Storage Days			
	1st days	2nd days	3rd days	4th days
Control	0.00±0.23^{1}	2.36±0.23$^{a\,2}$	2.92±0.23$^{a\,3}$	4.47±0.23$^{a\,4}$
200	0.00±0.23^{1}	0.00±0.23$^{b\,1}$	0.12±0.23$^{b\,1}$	1.39±0.23$^{b\,2}$
400	0.14±0.23^{1}	1.74±0.23$^{c\,2}$	1.93±0.23$^{c\,2}$	1.98±0.23$^{c\,2}$
600	0.05±0.23^{1}	2.08±0.23$^{ac\,2}$	2.32±0.23$^{c\,2}$	4.67±0.23$^{a\,3}$

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Table. 6: LSM for GOT (unit\$10^{6}$ sperm) in seminal plasma of semen stored at 4°C for 4 days with different concentrations of $PGF_{2\alpha}$.

Con. Of $PGF_{2\alpha}$ Ug\ml	Storage Days			
	1st days	2nd days	3rd days	4th days
Control	49.83±1.98^{abl}	57.40±1.98^{a2}	59.45±1.98^{a2}	61.51±1.98^{a2}
200	47.12±1.98^{al}	54.75±1.98^{a2}	57.98±1.98^{a2}	59.25±1.98^{a2}
400	53.50±1.98^{bcl}	62.52±1.98^{b2}	64.72±1.98^{b2}	71.87±1.98^{b3}
600	57.10±1.98^{cl}	64.25±1.98^{b2}	77.00±1.98^{c3}	86.90±1.98^{c4}

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