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effect of addition of prostaglandin pgf2α and oxytocin in vitro to diluted and cooled semen of friesian bull

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ABSTRACT

The study was carried out to investigate the effects of PGF2α and oxytocin added to diluted and cooled bull semen. Two Frisian bulls aged about Three years were used in this study presented in A.I center, Abu-Graib, Baghdad during a period from Jan.,18 to March,18 ,2017. Semen samples were collected weekly with artificial vagina using a cow as a teaser. Semen parameters were taken for the fresh pooled semen which includes volume (7.62±0.31 ml), Color (creamy), conc. (1022.26±151.38×10^6) per cubic mm, Mass activity (35±1.77)%, Individual motility (45.87±4.96)%, Dead (35±1.29)%, a live sperm (65±1.29)%, primary abnormalities (0.87±0.17)% and the secondary abnormalities (6.17±0.53)%. Semen was diluted with 1:10 fold with Tris-based extender according to its concentration. Diluted semen were divided into three parts (T1) added to it (37.5) µg/ml of PGF2α, the second part (T2) added to it 5 I.U/ml of oxytocin and the third part (T3) serve as a control. The PGF2α treated group showed a significant difference (P≤0.05) as compared with oxytocin or control groups. Also cooled diluted semen at 4°c were showed the PGF2α treated group a significant difference (P≤0.05) as compared with other groups. It was concluded from this study that addition of PGF2α with 37.5 µg to diluted or cooled semen are beneficial for bull ejaculate.

Keyword: Semen, Friesian bull, prostaglandin PGF2α and oxytocin.

تأثير اضافة البروستكلاندين والاوكسيتوسين مختبرياً الى السائل المنوي المخفف والمبرد للثيران الفريزيان

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الخلاصة

اجريت الدراسة لمعرفة تأثير اضافة هرموني البروستاكلاندين PGF2α والاوكسيتوسين الى السائل المنوي المخفف والمبرد لثيران الفريزيان. اجريت الدراسة على ثورين من سلالة الفريزيان بعمر ثلاث سنوات تواجدت في مركز التلقيح الصناعي – ابوغريب – بغداد للفتره من ١٨ كانون الثاني الى ١٨ آذار ٢٠١٧. تم جمع السائل المنوي لمره واحدة اسبوعيا من الثيران بواسطة المهبل الصناعي باستخدام بقرة كدمية. تم تقييم السائل المنوي بعد مزجه لكلا الثورين، بعد الجمع مباشرةً من حيث الحجم (7.62±0.31) اللون (حليبي) التركيز (10^6×151.38±1022.26) بالملليتر المكعب الواحد الحركة الجماعية (35±1.77)% الحركة الفردية (٤٥.٨٧±٤.٩٦)% والحيامن الميتة (35±1.29)% والحيامن الحية (65±1.29)% والتشوهات الاولية (0.87±0.17)% والتشوهات الثانوية (6.17±0.53)%. خفف السائل المنوي بنسبة ١٠:١ باستخدام المخفف Tris-based، أستخدم خليط السائل المنوي طبقاً لتركيزه وتم تقسيم السائل المنوي الى ثلاثة اقسام: القسم الاول (T1) اضيف له (37.5µg/ml) PGF2α، القسم الثاني (T2) اضيف له 5 وحده دوليه من هرمون الاوكسيتوسين، اما القسم الثالث (T3) اعتبر كمجموعه سيطرة. اظهرت النتائج وجود فرق معنوي (P≤0.05) في المجموعه المعاملة بـ PGF2α عند المقارنة مع مجموعتي الاوكسيتوسين والسيطرة في صفات السائل المنوي المخفف. كما وأظهرت النتائج وجود فرق معنوي (P≤0.05) في المجموعه المعاملة بـPGF2α عند المقارنة مع مجموعتي الاوكسيتوسين والسيطرة في صفات السائل المنوي المخفف والمبرد عند درجة حرارة ٤°م. وقد استنتج من الدراسة ان اضافه البروستاكلاندين وبجرعة ٣٧.٥µg/ml الى السائل المنوي المخفف او المخفف والمبرد يحسن صفات السائل المنوي لثيران الفريزيان.

INTRUDUCTION

Artificial insemination (A.I.) has many advantages to offer dairy farmer. The major advantage is the genetic gain which probably together with disease control, was one of the main reason to the development A.I., technique enable superior genes to be spread widely amongst the cattle population (Ball & peters, 2004). In developing countries limitations of technology and communications have restricted the development of A.I. services A.I. plays an important role in the development of dairy industry, however, follow up of cows that have been inseminated and assessing success of A.I. by regular pregnancy diagnosis is a problem especially with rectal palpation (FAO/ IAEA, 2007). These limitations lead to long calving intervals and economic losses to the farmers. A.I. allow single animals to have multiple progeny and leads to significant increase in the intensity of selection and proportional increases in genetic improvement of production (FAO/ IAEA, 2007; jamal & lemmam, 2015). Good extender should provide energy for metabolic activities with in sperm cell; maintain osmotic pressure and PH of the medium (Anzar et.al., 2003). Extender also protect semen from microbial growth. Different semen extenders should provide adequate nutrition in the form of fructose sugar to sperm cell during storage (Rehman et. Al., 2013). Moreover, liquid extended semen produces a higher conception rate with a relatively less number of sperm cell (Anzar et.al., 2003). Citric acid are very common used buffers in various types of diluents used for ruminants semen. Tris containing egg yolk glycerol extender was developed in 1963 and become most popular for both fresh and frozen semen (Rehman et. Al., 2013). The widespread use of dairy bull semen in A.I. requires that the semen production be as efficient as possible. Several techniques were used to increase the number of fertile spermatozoa in the ejaculate to inseminate cows of a pedigree bull (Ball & petters, 2014; Youngquist & threifal, 2007). It has been found by many worker that injection of $PGF_2\alpha$ and oxytocin to the bull, ram, rabbits and stallion prior to semen collection will improve ejaculate quality by increasing the total sperm number in the ejaculate (Hafs, 1974; Ibrahim, 1988; Mckonnen et. Al., 1989; El_Badry et. Al., 2013; Amoregouda, 2014) . The addition of high amount of $PGF_2\alpha$ to the semen of the bull associated with poor fertility. It was found that supplementation of semen with $PGF_2\alpha$ and oxytocin increases the rate of sperm motility and have a beneficial effect on ejaculate in bull, ram, and human (Grunberger et. al., 1981; Karahan et. al., 2006; El_Badry et.al., 2013). Therefore, the present study was conducted to investigate the effect of addition of $PGF_2\alpha$ and oxytocin to bull semen after dilution and cooling.

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MATERIALS AND METHODS

The study was carried out on two Friesian bulls, aged about 3 years, presented in Artificial Insemination Center Abu Graib, Baghdad, during the period from January 18 to March 18, 2017. The bulls were fed concentrated meal supplemented with lucerne hay and fresh drinking water was provided. Semen samples were collected with an artificial vagina using cow as a teaser and the semen were taken from each bull once a week over a course of 8 weeks (eight samples per bull) semen samples were put in a water bath with 35c°. Pooled semen of both bulls were evaluated. One milliliter of semen was removed from each sample for the determination of spermatological characteristics. Semen volume was measured by direct reading of graduated marks of collecting tubes (0.5-15ml) also the color has been taken visually a according to Salisbury et. al. (1978). For determination of mass activity, a non-cover slipped drop of fresh non-diluted semen was placed warm slide (37c°) and placed under a light microscope with hated stage at 100 magnification. Swirl can be observed in samples which have adequate numbers of motile spermatozoa. The ranking of this estimate are as follows according to Chenoweth (2002). Rapid swirling, very good (VG); slower swirling, good, (G); generalized oscillation, fair (F); sporadic oscillation, poor (P). Immediately after collection samples were evaluated for mass activity and individual motility using pre-warmed stage of phase contrast microscope (one drop from fresh semen plus one drop of sod. Citrate) and check percent of individual motility. two smears of semen stained with eosin-nigrosin, were prepared (Blom, 1950), and used to determine the percentage of live-dead and morphologically abnormal spermatozoa (primary and secondary) (Bielanski et.ai., 1982) sperm concentration measured with hemocytometer (Salisbury et. al., 1978). Semen samples were diluted 1:10 fold with a Tris-based extender according to the concentrated (Tris= 24.4 gm; ctric acid= 13.4 gm; fructose= 10gm: glycerin= 64 ml; egg yolk= 192 ml; distilled water up to one liter) according to Eidan (2016). Diluted semen were taken and divided into 3 parts (each parts 2 ml). The first part (group T1) added to it 37.5 µg $PGF_2\alpha$ per ml of semen (Veteglan, d.cloprostenol, caler, barcilona, spian). The second part (T2 group) added to it 5 I.U. of oxytocin per ml of semen (Hoga mauw 900-B-2370 Arendok-Belgium). The third part (T3 group) not added to it anything and serve as a control. Another fresh semen samples were taken Then diluted semen cooled gradually by addition a piece of ice till it reaches 4c° within 2 hrs. After cooling semen divided into 3 parts and adding to it T1, T2 as in diluted semen. Also semen parameters were measured after cooling. Statistical analysis were done using Tukey's -w- procedures and chi-square test according to Trrie (1980).

RESULTS AND DISCUSSION

Semen characteristics of fresh bull semen are shown in Table 1. There is no significant differences in semen parameters were determined between bulls and different ejaculates of the same bull ($P \leq 0.05$) . The results indicate that the bulls of low fertility. This might be due to breed and /or age of the bulls that affect their fertility (Salisbury et. al., 1978).

Table -1- parameter of fresh semen

Volume Ml	color	Conc.*10^6 ml	Mass Activity %	Individual Motility %	Dead %	Alive %	Abnormalities	
							Primary %	Secondary %
7.62±0.31 Range (5-11)	creamy	1022.26 ± 151.38	35±1.77	45.87±4.96	35±1.29	65±1.29	0.87±0.17	6.17±053

***Values=M ± SE**

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Table -2- showed the semen parameters after dilution with Tris-based extender with addition of 37.5µg of $PGF_2\alpha$ and 5 I.U. of oxytocin to each of the diluted semen. There was a significant difference ($P\leq0.05$) in Individual motility%, Dead%, alive% and secondary abnormalities% between $PGF_2\alpha$ treated group as compared with oxytocin or control groups. Similar observations has been found in bull, stallion, Ram and buck (Karahan et. al., 2006; El_Badry et. al., 2013; Barwary et. al., 2013).

Table -2- Semen parameter after dilution and addition of $PGF_2\alpha$ and oxytocin

Treatment	Individual motility %	Dead %	Alive %	Abnormalities	
				Primary %	Secondary%
T1/$PGF_2\alpha$	51.11 ±3.51^a	35.34±4.19^a	64.56±1.19^a	1.32±0.14^a	6.08±0.76^a
T2/Oxytocin	38.31±3.1^b	47.18±4.94^b	52.81±4.94^b	1.25±0.29^a	9.06±0.29^b
T3/control	42.1± 2.34^b	48.0±1.47^b	52.06±4.53^b	1.0±0.75^a	10.56±1.94^b

***Values=M ± SE**

***Different superscript showed significant difference (P≤O.O5)**

It has been found that repeated Injection of $PGF_2\alpha$ to the ram resulted in significant increase in semen volume , sperm cell conc. and total number of spermatozoa per ejaculate (El_Badry et. al., 2013). Similar observations have been made in bulls (Haynes et. al., 1975; Masoumi et. al., 2011; Titiroonguang et. al.,2011) .The results disagreed with Berndtson et. al. (1979) who found no effect of $PGF_2\alpha$ treatment on various semen characteristics in bulls. In bulls and rams , the addition of $PGF_2\alpha$ to the semen increased the conception rate (Gustafsson et. al., 1975) and increased the fertility in rams by more than 15% (Dimov and Georgiev, 1977). It has been found that injection of Holstein Friesian bulls with oxytocin leads to significant difference in treated bulls (Shankar et. al., 1985).In contrast Alkass et. al. (1987) found that injection of oxytocin to the Ram had no significant effect on semen volume, sperm conc. or number of spermatozoa per ejaculate. Tras and kustritz (2004) observed that the use of oxytocin does not appears to be a valuable treatment to increase the number of spermatozoa in male dog.

Table-3 showed the effects of addition of $PGF_2\alpha$ and oxytocin after cooling of diluted semen. There was a significant difference($P\leq0.05$) between different treatments in individual motility ,dead and alive%. The treatment with $PGF_2\alpha$ give the best results as compared with oxytocin and control groups.

Table -3- Semen parameters after cooling with addition of $PGF_2\alpha$ and oxytocin.

Treatment	Individual motility %	Dead %	Alive %	Abnormalities	
				Primary%	Secondary%
T1/$PGF_2\alpha$	45±2.8^a	42.21±1.64^a	57.8±1.51^a	0.87±0.14^a	9.18± 0.51^a
T2/Oxytocin	36.67±2.74^b	61±4.47^b	38.68±4.63^b	1.91±0.12^a	12.45±0.82^a
T3/control	26.15±8.16^c	70.3±2.4^c	29.68±2.11^c	1.0±0.24^a	17.75±1.94^b

***Values=M ± SE**

***Different small letters showed significant difference (p≤0.05).**

There was no significant difference in primary abnormalities between different groups of treatments while there was a significant difference ($P\leq0.05$) between $PGF_2\alpha$ and oxytocin group as compared with control group similar observation have been made by Karahan et. al. (2006) on cooled semen . The effect induced by the addition of $PGF_2\alpha$ in our study was consistent with the

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findings reported by other investigators (Karahan et. al., 2006; Cebisen and Akcay, 2015). The increase in sperm motility might be explained by the direct effect of prostaglandins on spermatozoa possibly acting on contractile elements of sperm (tail myofibrils) and stimulating its kinetic activity. It has been found by many investigators that the addition of oxytocin to cooled semen have a little effect on semen parameters as compared with $PGF_2\alpha$ (Bozkart et. al., 2007). similar observation have been made by Berndston and Igboeli (1988) in bull and Bozkart et. al. (2007) in rams.

COONCLUSION AND RECOMENDATION

It was concluded from this study that addition of $PGF_2\alpha$ (37.5µg/ml) of diluted semen are beneficial of bull ejaculate. However more research was required by the use of different doses of $PGF_2\alpha$ and oxytocin. Also we can added vitamin such as (vit. A, C and E).

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