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THE EFFECT OF OXYTOCIN, PROSTAGLANDIN $F_{2\alpha}$ OR GNRH INJECTION ON FRESH AND FROZEN-THAWED SEMEN CHARACTERISTICS OF RAMS

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ABSTRACT

The present study aimed to maximize the semen output from rams using oxytocin, prostaglandin $F_{2\alpha}$ (PG $F_{2\alpha}$) or gondatrophin releasing hormone (GnRH) as well as to study the effects of these treatments on quality, freezability and antioxidant activity of ram semen. In the present work, 4 groups (each containing 3 Barki rams) were used as; control (group 1) injected with normal saline intramuscularly (i.m.) 30 min. prior to semen collection, group 2 injected with 10 i.u. oxytocin intravenously (i.v.) 10 min. prior to semen collection, group3 injected with 250 µg of cloprostenol i.m. 30 min. before semen collection and group 4 injected with 50 µg of gonadorelin i.v. 60 minutes before semen collection. Two ejaculates were collected from rams of all groups once weekly for 10 successive weeks. At the 1st, 5th and 10th week semen samples were processesed for cryopreservation and testosterone and antioxidant enzymes were measured in the separated seminal plasma. Results indicated that both PG $F_{2\alpha}$ and oxytocin resulted in significant ($P \leq 0.05$) increase in semen volume and total number of spermatozoa per ejaculates allover the experimental period without altering the quality of the fresh ejaculated or the frozen-thawed semen. Also, PG $F_{2\alpha}$ and oxytocin had no undesirable effect on testosterone level and the values of total antioxidants, superoxide dismutase and glutathione peroxidase in seminal plasma. On the other hand, GnRH elevated the level of testosterone but did not increase the sperm output in rams. Conclusively, repeated injection of $PGF_{2\alpha}$ and oxytocin prior to semen collection was found to be useful when collecting ram semen for use in artificial insemination as they increase the sperm output without altering the quality of fresh or frozen-thawed semen as well as they have no deleterious effects on the antioxidant activities of seminal plasma and testosterone level in rams.

Key words: *Oxytocin, Prostaglandin $F_{2\alpha}$, Rams.*

INTRODUCTION

Nowadays increasing use of assisted reproductive techniques in sheep has uncovered a need for additional aids to help veterinarians for optimizing the ejaculates obtained from rams. Specifically, improving upon the number of spermatozoa obtained during semen collection would benefit most areas of assisted reproduction, including semen cryopreservation and artificial insemination. Oxytocin treatment prior to semen collection resulted in increased sperm output in rams (Nicholson *et al.,* 1999; Bozkurt *et al.,* 2007), buffalos (Ibrahim, 1988) bulls (Berndtson and Igboeli, 1988; Palmer *et al.,* 2004) and rabbits (Fjellstrom *et al.,* 1968). Therefore, it seems reasonable that since oxytocin is involved in the transit of sperm through the male duct system, it may hasten ejaculation in rams. Oxytocin may be involved in the movement of sperm in the male duct system, specifically; epididymal sperm transport (Whittington *et al.,* 2001). Oxytocin receptors have been isolated from the epididymal smooth muscle of several species (Frayne and Nicholson, 1998; Whittington *et al.,* 2001) and in vitro and in vivo studies in rodents have shown that oxytocin increased contractility of the epididymis (Hib, 1977). Furthermore, sexual stimulation and ejaculation were associated with a concurrent rise in blood oxytocin concentration in many species (Murphy *et al.,* 1987).

PG $F_{2\alpha}$ is known to increase smooth muscle contractility in the male and female genital tracts and may be involved in the ejaculation process (Bygdeman, 1981). Parenteral $PGF_{2\alpha}$ administration before semen collection resulted in increased sperm output in stallions (Cornwell *et al.,* 1974; McDonnell, 1992), dogs (Hess, 2001), and buffalo (Ibrahim, 1988). However, the effect of PG $F_{2\alpha}$ treatment on sperm output in the bull is contradictory, with one study reporting increased sperm output after

treatment (Marshall and Hafs, 1976) and a second experiment reporting no treatment effect (Berndtson *et al.,* 1979).

Administration of exogenous GnRH to male dogs induces a dose dependent release of LH immediately following treatment with a resultant increase in testosterone concentration starting 10 minutes after GnRH administration, peak in 50-60 minutes and remains elevated above control values for 4-24 hours (Knol *et al.,* 1993; Hess, 2002). Gabor *et al.* (1995) found that repeated injection of GnRH resulted in higher level of testosterone hormone in bulls.

The present study attempts to evaluate the effects of oxytocin, PGF2α and GnRH injection prior to semen collection on fresh and frozen-thawed semen characteristics of rams.

MATERIALS and METHODS

Animals and treatment:
Twelve mature, clinically healthy Barki rams, aged 1.5-2.5 years were assigned to the study. Rams were kept in the experimental farm of the Animal Reproduction Research Institute (ARRI), and they were divided into four groups (each containing 3 rams); group 1 (control, injected intramuscularly with 2 ml normal saline 30 minutes prior to semen collection), group 2 (injected with 10 i.u. oxytocin i.v. "Oxytocin, Bela-pharm, Germany", ' 10 minutes before semen collection "Knight, 1974"), group 3 (injected i.m. with 250 µg of cloprostenol "Estrumate; Schering Plough Animal Health, Germany" 30 minutes before semen collection "Mekonnen *et al.,* 1989") and group 4 (injected i.v. with 50 µg of the GnRH analogue, gonadorelin diacetate tetrahydrate "Cystorelin; CEVA Sante Animale, France" ' 60 minutes before semen collection "Hess, 2002").

Semen collection and evaluation:
Semen samples were collected from rams of all groups using artificial vagina that was adjusted to a proper condition. Two ejaculates were collected once weekly for 10 successive weeks. Immediately after collection samples were evaluated for mass activity and individual motility using a pre-warmed stage of phase contrast microscope. Two smears of the semen, stained with eosin– nigrosin, were prepared (Blom, 1950) and used to determine the percentage of both live and morphologically abnormal spermatozoa (Bielanski *et al.,* 1982). Sperm concentration was measured with hemocytometer.

Seminal plasma samples:
Seminal plasma samples were obtained at the 1st, 5th and 10th week by centrifugation of portion of semen samples at 3000 rpm for 10 minutes. Supernatant was passed through 1.2 µm nylon syringe filters to obtain sperm-free seminal plasma and stored at -80 °C. Testosterone was measured in seminal plasma samples by ELISA reader in duplicate, at 450 nm wavelength according to Maruyama (1987). The total antioxidant level was measured spectrophotometrically in seminal plasma samples at 37 °C and 421 nm according to Koracevic and Koracevic (2001), the superoxide dismutase level was measured at 560 nm according to Nishikimi *et al.* (1972), while the glutathione peroxidase level was measured at 430 nm according to Paglia and Valentine (1967).

Cryopreservation of ram semen:
Semen samples that collected from all groups at the 1st, 5th and 10th week were diluted at a ratio of 1:19 at 30°C with Tris based extender (Evans and Maxwell, 1986). After that, the extended semen was cooled to 5°C throughout 60 minutes in a cold handling cabinet. The cooled semen was loaded into 0.25 ml French straws (IMV, L'Aigle, France), arranged horizontally on a cooled racks, then lowered into liquid nitrogen vapor inside foam box (Khalifa, 2001). Then the straws were immersed in liquid nitrogen and stored.

Evaluation of cryopreserved semen:
One week after cryopreservation, frozen ram semen samples were thawed in water bath at 37°C for 30 seconds. Individual sperm motility was subjectively assessed post thawing and after 1, 2 and 3 hours of thawing. The post-thawing viability indices were estimated according to Milovanov (1962). The percentage of abnormal acrosome was recorded after thawing in smears stained by fast green FCF (Wells and Awa, 1970).

Statistical analysis:
Two way analysis of variance and Duncan's multiple range tests were done for the data obtained. Such statistical analysis was performed according to Costat computer program copyright (1986).

RESULTS

As shown in table 1, injection of oxytocin and $PGF_{2\alpha}$ resulted in a significantly ($P \leq 0.05$) higher total semen volume of the 1st and 2nd ejaculates (2.84 ± 0.06 and 2.62 ± 0.06 ml, respectively) when compared with the control and GnRH groups (2.17 ± 0.06 and 2.24 ± 0.04 ml, respectively).

Table 1: Effect of injection of oxytocin, $PGF2_{\alpha}$ and GnRH on semen volume in rams (Mean ± S.D.).

Weeks	Semen volume (ml)											
	First ejaculate				Second ejaculate				Total			
	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4
1	1.00 ±0.15	1.53 ±0.09	1.33 ±0.09	1.03 ±0.09	1.03 ±0.18	1.10 ±0.06	1.13 ±0.09	1.17 ±0.03	2.03 ±0.33	2.63 ±0.07	2.47 ±0.18	2.20 ±0.10
2	1.03 ±0.09	1.60 ±0.06	1.27 ±0.03	1.23 ±0.15	1.23 ±0.09	1.33 ±0.12	1.17 ±0.12	1.30 ±0.12	2.27 ±0.19	2.73 ±0.19	2.43 ±0.13	2.53 ±0.12
3	1.23 ±0.14	1.93 ±0.09	1.60 ±0.06	1.27 ±0.17	1.10 ±0.10	1.33 ±0.07	1.23 ±0.12	1.13 ±0.09	2.33 ±0.02	3.27 ±0.33	2.83 ±0.09	2.40 ±0.17
4	0.97 ±0.09	1.47 ±0.14	1.00 ±0.15	1.10 ±0.06	1.07 ±0.13	1.03 ±0.09	1.07 ±0.07	10.03 ±0.09	2.03 ±0.14	2.50 ±0.20	2.07 ±0.22	2.17 ±0.33
5	0.93 ±0.09	1.60 ±0.06	1.30 ±0.06	0.97 ±0.09	1.03 ±0.03	1.03 ±0.12	1.07 ±0.09	1.10 ±0.15	1.97 ±0.06	2.63 ±0.12	2.37 ±0.14	2.07 ±0.14
6	0.93 ±0.03	1.70 ±0.06	1.43 ±0.03	0.90 ±0.17	1.01 ±0.06	1.00 ±0.06	1.17 ±0.09	1.13 ±0.12	2.03 ±0.09	2.70 ±0.06	2.60 ±0.06	2.03 ±0.22
7	1.07 ±0.14	1.67 ±0.12	1.36 ±0.03	1.00 ±0.17	1.23 ±0.14	1.30 ±0.10	1.40 ±0.15	1.23 ±0.17	2.30 ±0.29	2.97 ±0.22	2.77 ±0.17	2.23 ±0.09
8	1.17 ±0.09	1.70 ±0.03	1.50 ±0.09	1.07 ±0.12	1.23 ±0.06	1.27 ±0.09	1.37 ±0.09	1.30 ±0.06	2.40 ±0.15	2.97 ±0.14	2.87 ±0.07	2.13 ±0.12
9	1.07 ±0.09	1.87 ±0.12	1.60 ±0.06	0.97 ±0.07	1.10 ±0.06	1.30 ±0.21	1.33 ±0.21	1.17 ±0.09	2.17 ±0.13	3.17 ±0.18	2.93 ±0.06	2.13 ±0.12
10	1.03 ±0.09	1.70 ±0.12	1.37 ±0.07	1.03 ±0.12	1.13 ±0.09	1.10 ±0.21	1.47 ±0.09	1.20 ±0.06	2.17 ±0.13	2.80 ±1.52	2.83 ±0.13	2.23 ±0.12
Mean	1.04^{c} ±0.03	1.68^{a} ±0.04	1.38^{b} ±0.04	1.06^{c} ±0.03	1.13^{a} ±0.03	1.16^{a} ±0.04	1.24^{a} ±0.04	1.18^{a} ±0.03	2.17^{c} ±0.06	2.84^{a} ±0.06	2.62^{b} ±0.06	2.24^{c} ±0.04

Gp 1: Conrol, Gp2: oxytocin, Gp 3: PGF2α and Gp4: GnRH.

Data regarding the ram sperm concentration were represented in table 2. Injecting rams with $PGF_{2\alpha}$ prior to semen collection resulted in a significant ($P \leq 0.05$) increase in sperm cell concentration when compared to control (2.50 ± 0.04 vs. 2.33 ± 0.05 $x10^9$ sperm/ml, respectively). On the other hand, there were no statistically significant differences ($P \leq 0.05$) between oxytocin and GnRH groups (2.29 ± 0.05 and 2.23 ± 0.06 x 10^9 sperm/ml, respectively) as compared with control group.

Table 2: Effect of injection of oxytocin, $PGF2_{\alpha}$ and GnRH on sperm concentration in rams (Mean ± S.D.).

Weeks	Sperm concentration ($x10^9$/ml)											
	First ejaculate				Second ejaculate				Average			
	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4
1	2.06 ±0.03	2.44 ±0.05	2.34 ±0.09	2.25 ±0.05	2.06 ±0.14	1.97 ±0.05	2.38 ±0.11	2.16 ±0.09	2.12 ±0.09	2.20 ±0.01	2.36 ±0.02	2.21 ±0.04
2	1.94 ±0.02	2.07 ±0.04	2.15 ±0.05	1.91 ±0.02	2.04 ±0.17	1.77 ±0.07	2.42 ±0.06	1.79 ±0.32	1.99 ±0.09	1.92 ±0.02	2.29 ±0.03	1.85 ±0.15
3	2.34 ±0.10	2.45 ±0.06	2.23 ±0.03	2.18 ±0.06	2.35 ±0.20	2.31 ±0.01	2.56 ±0.10	2.51 ±0.21	2.34 ±0.10	2.38 ±0.03	2.40 ±0.03	2.35 ±0.08
4	2.43 ±0.11	2.56 ±0.06	2.43 ±0.06	1.55 ±0.11	2.38 ±0.11	2.15 ±0.09	2.70 ±0.09	1.89 ±0.14	2.41 ±0.11	2.35 ±0.12	2.57 ±0.04	1.92 ±0.12
5	2.83 ±0.06	3.11 ±0.12	2.72 ±0.04	2.54 ±0.06	2.61 ±0.35	2.43 ±0.05	2.96 ±0.07	2.67 ±0.32	2.72 ±0.16	2.77 ±0.09	2.38 0.05	2.61 ±0.13
6	2.19 ±0.10	2.75 ±0.08	2.48 ±0.05	2.35 ±0.02	2.18 ±0.23	2.35 ±0.10	2.68 ±0.27	2.20 ±0.32	2.19 ±0.16	2.55 ±0.09	2.58 ±0.13	2.28 ±0.13
7	2.26 ±0.05	2.34 ±0.05	2.42 ±0.07	2.56 ±0.21	2.39 ±0.23	2.17 ±0.03	2.59 ±0.28	2.68 ±0.30	2.32 ±0.12	2.26 ±0.04	2.51 ±0.15	2.62 ±0.25
8	2.75 ±0.08	2.64 ±0.10	2.47 ±0.05	2.22 ±0.06	2.65 ±1.53	2.16 ±0.10	2.88 ±0.19	2.43 ±0.26	2.70 ±0.06	2.40 ±0.10	2.68 ±0.11	2.33 ±0.11
9	2.15 ±0.03	2.27 ±0.04	2.23 ±0.07	2.05 ±0.12	2.17 ±0.16	1.77 ±0.06	2.51 ±0.24	2.27 ±0.35	2.16 ±0.07	2.02 ±0.05	2.37 ±0.09	2.16 ±0.14
10	2.27 ±0.05	2.22 ±0.05	2.35 ±0.05	1.76 ±0.11	2.33 ±0.05	1.93 ±0.03	2.41 ±0.26	2.14 ±0.05	2.30 ±0.05	2.08 ±0.05	2.38 ±0.14	1.95 ±0.08
Mean	2.33^{b} ±0.05	2.49^{a} ±0.06	2.38^{b} ±0.03	2.18^{c} ±0.05	2.32^{b} ±0.06	2.10^{c} ±0.05	2.61^{a} ±0.06	2.28^{b} ±0.09	2.33^{b} ±0.05	2.29^{b} ±0.05	2.50^{a} ±0.04	2.23^{b} ±0.06

Gp 1: Conrol, Gp2: oxytocin, Gp 3: PGF2α and Gp4: GnRH.

Table 3 illustrates the effect of different treatments on the total number of spermatozoa per ejaculates. Injection of oxytocin and $PGF_{2\alpha}$ significantly ($P \leq 0.05$) increased the total number of spermatozoa per ejaculates as compared with control group (6.59 ± 0.18 and 5.74 ± 0.17 vs. 5.29 ± 0.17 x 10^9, respectively). In contrary, the GnRH group had the lowest total number of spermatozoa per ejaculates (4.86 ± 0.14 x 10^9).

Table 3: Effect of injection of oxytocin, PGF2α and GnRH on total number of spermatozoa per ejaculates in rams (Mean ± S.D.).

Weeks	Total number of spermatozoa ($x10^9$)											
	First ejaculate				Second ejaculate				Total			
	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4
1	2.16 ±0.30	3.75 ±0.27	3.11 ±0.13	2.33 ±0.24	2.38 ±0.42	2.17 ±0.17	2.31 ±0.13	2.64 ±0.16	4.55 ±0.57	5.92 ±0.14	5.42 ±0.40	4.98 ±0.22
2	2.00 ±0.16	3.30 ±0.06	2.72 ±0.10	2.36 ±0.19	2.79 ±0.25	2.03 ±0.29	2.18 ±0.30	2.65 ±0.51	4.79 ±0.15	5.33 ±0.38	5.90 ±0.38	5.02 ±0.216
3	2.85 ±0.21	4.75 ±0.30	3.57 ±0.16	2.78 ±0.44	3.00 ±0.25	3.08 ±0.14	2.37 ±0.19	2.87 ±0.24	5.85 ±0.46	7.83 ±0.14	5.94 ±0.14	5.65 ±0.24
4	2.37 ±0.33	3.76 ±0.42	2.43 ±0.37	2.14 ±0.12	2.71 ±0.59	2.20 ±0.14	1.97 ±0.15	2.23 ±0.17	5.08 ±0.47	5.96 ±0.57	4.39 ±0.65	4.37 ±0.23
5	2.64 ±0.22	4.99 ±0.22	3.53 ±0.14	2.47 ±0.28	3.28 ±0.11	2.51 ±0.17	2.38 ±0.17	3.22 ±0.44	5.91 ±0.03	7.50 ±0.54	5.91 ±0.31	5.69 ±0.24
6	2.05 ±0.16	4.68 ±0.28	3.55 ±0.04	2.11 ±0.23	2.64 ±0.14	2.35 ±0.14	2.57 ±0.27	2.07 ±0.21	4.69 ±0.43	7.03 ±0.33	6.13 ±0.32	4.18 ±0.70
7	2.42 ±0.38	3.89 ±0.20	3.31 ±0.13	2.49 ±0.24	3.26 ±0.31	2.82 ±0.18	2.85 ±0.36	2.62 ±0.37	5.69 ±0.91	6.71 ±0.37	6.16 ±0.81	5.11 ±0.58
8	3.20 ±0.16	4.48 ±0.42	3.70 ±0.11	2.37 ±0.11	3.27 ±0.14	2.75 ±0.32	2.70 ±0.23	2.77 ±0.20	6.47 ±0.29	7.23 ±0.55	6.40 ±0.39	5.14 ±0.27
9	2.30 ±0.21	4.25 ±0.33	3.57 ±0.23	1.97 ±0.07	2.59 ±0.07	2.29 ±0.37	2.62 ±0.07	2.19 ±0.20	4.89 ±0.29	6.54 ±0.45	6.19 ±0.23	4.16 ±0.55
10	2.34 ±0.14	3.76 ±0.17	3.20 ±0.09	1.79 ±0.12	2.63 ±0.20	2.13 ±0.42	2.73 ±0.07	2.58 ±0.16	4.97 ±0.23	5.90 ±0.09	5.93 ±0.11	4.37 ±0.17
Mean	2.43 [c] ±0.09	4.16 [a] ±0.12	3.27 [b] ±0.09	2.28 [c] ±0.08	2.85 [a] ±0.09	2.43 [b] ±0.09	2.47 [b] ±0.07	2.58 [b] ±0.10	5.29 [c] ±0.17	6.59 [a] ±0.18	5.74 [b] ±0.17	4.86 [d] ±0.14

Gp 1: Conrol, Gp2: oxytocin, Gp 3: PGF2α and Gp4: GnRH.

Regarding the total number of motile spermatozoa per ejaculates (table 4), the oxytocin group had the highest one (5.15 ± 0.16 x 10^9) as compared with control, $PGF_{2\alpha}$ and GnRH groups (4.34 ± 0.14, 4.34 ± 0.12 and 4.14 ± 0.13 x 10^9, respectively).

Table 4: Effect of injection of oxytocin, PGF2α and GnRH on mass activity of spermatozoa and total number of motile spermatozoa in rams per ejaculates (Mean ± S.D.).

Weeks	Spermatozoal Mass Activity								Total number of motile spermatozoa (x 10^9)			
	First ejaculate				Second ejaculate							
	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4
1	+++	+++(+)	+++	+++	+++	+++(+)	+++	++++	3.64 ±0.45	4.85 ±0.38	4.33 ±0.25	4.15 ±0.23
2	+++	+++(+)	+++	+++	+++	+++(+)	+++	++++	3.95 ±0.27	4.34 ±0.28	3.96 ±0.28	4.10 ±0.09
3	+++	+++	+++	+++(+)	+++(+)	+++(+)	++(+)	+++(+)	4.79 ±0.42	6.45 ±0.16	4.31 ±0.18	4.75 ±0.21
4	+++(+)	+++	+++(+)	+++(+)	+++(+)	+++	+++	+++	4.28 ±0.42	5.03 ±0.58	3.59 ±0.55	3.68 ±0.17
5	+++(+)	+++	+++	+++	+++	+++	+++	+++	4.83 ±0.2.89	6.08 ±0.55	4.60 ±0.43	4.72 ±0.17
6	+++	+++	+++	++++	+++	+++	+++	++++	3.80 ±0.38	5.56 ±0.37	4.70 ±0.24	3.70 ±0.62
7	+++(+)	+++	++(+)	+++(+)	+++(+)	+++	++(+)	+++(+)	4.61 ±0.80	4.97 ±0.27	4.45 ±0.51	4.47 ±3.33
8	+++	+++	+++	++++	+++	+++	+++	++++	5.33 ±0.25	5.25 ±0.42	4.89 ±0.19	4.50 ±0.32
9	+++(+)	++(+)	++(+)	+++(+)	+++(+)	++(+)	++(+)	+++(+)	4.11 ±0.13	4.68 ±0.33	4.34 ±0.14	3.56 ±0.34
10	+++	+++	+++	+++(+)	+++	+++	+++	+++(+)	4.02 ±0.21	4.32 ±0.32	4.25 ±0.19	3.82 ±0.10
Mean	+++ [b]	+++ [b]	+++ [b]	+++(+) [a]	+++(+) [a]	+++ [b]	+++ [b]	+++(+) [a]	4.34 [b] ±0.14	5.15 [a] ±0.16	4.34 [b] ±0.12	4.14 [b] ±0.13

Gp 1: Conrol, Gp2: oxytocin, Gp 3: PGF2α and Gp4: GnRH.

As demonstrated in tables 4 and 5, injection of GnRH resulted in the highest ($P \leq 0.05$) mass and individual motility of spermatozoa (+++[+] and 8.33 ± 0.61%). On the other hand, injection of oxytocin and $PGF_{2\alpha}$ resulted in a significantly ($P \leq 0.05$) lower spermatozoa individual motility as compared with control group (78.17 ± 1.08 and 76.00 ± 0.85% vs. 81.92 ± 0.57%, respectively).

Table 5: Effect of injection of oxytocin, PGF2α and GnRH on Sperm individual motility in rams (Mean ± S.D.).

Weeks	Sperm individual motility											
	First ejaculate				Second ejaculate				Mean			
	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4
1	78.33 ±1.67	81.67 ±6.01	78.33 ±1.67	81.67 ±1.67	81.67 ±1.67	81.67 ±3.33	81.67 ±1.67	85.00 ±2.89	80.00 ±0.00	81.67 ±4.64	80.00 ±1.44	83.33 ±3.82
2	81.67 ±1.67	81.67 ±3.33	81.67 ±1.67	81.67 ±1.67	83.33 ±1.67	81.67 ±1.67	80.00 ±0.00	81.67 ±1.67	82.50 ±0.00	81.67 ±2.21	80.83 ±0.83	81.67 ±0.83
3	80.00 ±2.89	81.67 ±1.67	73.33 ±1.67	83.33 ±3.33	83.33 ±1.67	83.33 ±3.33	71.67 ±1.67	85.00 ±0.00	81.67 ±2.21	82.50 ±2.50	72.50 ±1.44	84.17 ±0.83
4	80.00 ±2.89	81.67 ±1.67	83.33 ±1.67	83.33 ±1.67	83.33 ±1.67	83.33 ±1.67	81.67 ±2.89	85.00 ±2.89	84.17 ±2.21	84.17 ±1.67	81.67 ±1.67	83.33 ±1.67
5	83.33 ±1.67	81.67 ±1.67	78.33 ±1.67	81.67 ±1.67	80.00 ±2.89	80.00 ±2.89	76.67 ±4.41	85.00 ±2.89	81.67 ±2.21	80.83 ±2.21	77.50 ±2.89	83.33 ±1.67
6	78.33 ±1.67	78.33 ±4.41	76.67 ±1.67	83.33 ±1.67	83.33 ±1.67	80.00 ±2.89	76.67 ±1.67	88.33 ±1.67	80.83 ±1.67	79.17 ±3.63	76.67 ±0.83	83.33 ±0.83
7	81.67 ±4.41	75.00 ±2.89	71.67 ±1.67	86.67 ±1.67	80.00 ±2.89	73.33 ±3.33	73.33 ±1.67	88.33 ±1.67	80.83 ±3.00	74.17 ±3.00	72.50 ±1.44	87.50 ±0.00
8	81.67 ±1.67	73.33 ±1.67	76.67 ±1.67	83.33 ±1.67	83.33 ±1.67	71.67 ±1.67	76.67 ±3.33	86.67 ±1.67	82.50 ±1.44	72.50 ±1.44	76.67 ±2.21	87.50 ±1.44
9	85.00 ±2.89	71.67 ±1.67	68.33 ±1.67	86.67 ±3.33	83.33 ±1.67	71.67 ±1.67	71.67 ±1.67	85.00 ±2.89	84.17 ±2.21	71.67 ±1.67	70.00 ±1.44	85.83 ±3.00
10	80.00 ±2.89	75.00 ±2.89	71.67 ±1.67	86.67 ±1.67	81.67 ±1.67	71.67 ±1.67	71.67 ±1.67	83.33 ±1.67	80.83 ±2.21	73.33 ±2.21	71.67 ±2.21	87.50 ±1.44
Mean	81.50^{b} ±0.80	78.50^{c} ±1.15	75.83^{d} ±0.93	84.83^{a} ±0.74	82.33^{b} ±0.57	77.83^{c} ±1.12	76.17^{c} ±0.92	85.83^{a} ±0.68	81.92^{b} ±0.57	78.17^{c} ±1.08	76.00^{d} ±0.85	85.33^{a} ±0.61

Gp 1: Conrol, Gp2: oxytocin, Gp 3: PGF2α and Gp4: GnRH.

Table 6 illustrates the effect of different treatments on the ram sperm viability. Injection of GnRH prior to semen collection resulted in the highest ($P \leq 0.05$) sperm viability (87.17 ± 0.58%) as compared with control group. On the other hand, injection of oxytocin and $PGF_{2\alpha}$ resulted in a significantly ($P \leq 0.05$) lower sperm viability as compared with control group (80.43 ± 1.04 and 78.30 ± 0.84 vs. 84.08 ± 0.57%, respectively).

Table 6: Effect of injection of oxytocin, PGF2α and GnRH on spermatozoa viability in rams (Mean ± S.D.).

Weeks	Spermatozoal viability (% of live spermatozoa)											
	First ejaculate				Second ejaculate				Mean			
	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4
1	81.33 ±2.19	84.00 ±6.25	81.00 ±1.53	84.00 ±2.08	83.67 ±1.67	83.67 ±3.38	83.33 ±1.85	86.33 ±2.90	82.50 ±0.29	83.83 ±4.81	82.17 ±1.30	85.17 ±2.24
2	84.33 ±1.33	83.67 ±2.85	83.33 ±1.85	85.00 ±1.53	84.33 ±2.19	84.00 ±1.53	82.67 ±0.66	83.00 ±2.00	84.33 ±0.29	83.83 ±1.96	83.00 ±1.26	84.00 ±1.04
3	82.67 ±2.05	84.00 ±1.53	75.67 ±1.45	85.67 ±3.18	85.00 ±1.53	85.00 ±3.51	74.00 ±1.53	87.00 ±0.58	83.83 ±2.05	84.50 ±2.52	74.83 ±1.45	83.66 ±1.59
4	87.33 ±5.04	87.33 ±3.18	83.33 ±1.85	85.00 ±1.53	85.33 ±1.67	85.66 ±1.45	83.33 ±1.85	87.00 ±3.22	86.33 ±2.33	86.50 ±2.02	83.33 ±0.93	86.00 ±2.18
5	84.67 ±1.33	83.33 ±1.85	80.67 ±1.85	82.67 ±1.77	82.33 ±2.60	81.67 ±3.18	81.00 ±4.04	86.33 ±3.12	83.50 ±1.89	82.50 ±2.36	80.83 ±2.60	84.50 ±1.26
6	81.00 ±2.52	80.67 ±3.53	79.33 ±1.85	90.33 ±1.77	82.33 ±2.60	81.67 ±3.18	81.00 ±4.04	86.33 ±3.12	83.50 ±1.89	82.50 ±2.36	80.83 ±2.60	84.50 ±1.26
7	84.67 ±4.34	77.33 ±2.60	74.33 ±1.85	88.33 ±1.33	82.00 ±2.89	76.00 ±2.52	75.33 ±1.20	90.33 ±1.67	83.33 ±3.18	76.67 ±2.52	74.83 ±1.46	89.33 ±1.67
8	84.00 ±1.00	75.67 ±1.85	78.33 ±1.85	89.67 ±1.33	85.67 ±1.85	74.67 ±1.20	79.00 ±3.00	88.67 ±1.20	84.83 ±1.30	75.17 ±1.30	78.67 ±2.21	89.17 ±1.17
9	81.33 ±2.90	77.00 ±2.64	73.67 ±2.40	88.00 ±1.53	84.00 ±1.53	74.00 ±1.15	74.00 ±1.53	90.00 ±1.53	82.67 ±2.05	75.50 ±1.89	73.83 ±1.73	89.00 ±1.32
10	81.33 ±2.90	77.00 ±2.64	73.67 ±2.40	88.00 ±1.53	84.00 ±1.53	74.00 ±1.15	74.00 ±1.53	90.00 ±1.53	82.67 ±2.05	75.50 ±1.89	73.83 ±1.73	89.00 ±1.32
Mean	83.87^{b} ±0.81	80.70^{c} ±1.12	78.03^{d} ±0.90	86.77^{a} ±0.69	84.30^{b} ±0.57	80.17^{c} ±1.06	78.57^{c} ±0.90	87.56^{a} ±0.70	84.08^{b} ±0.57	80.43^{c} ±1.04	78.30^{d} ±0.84	87.17^{a} ±0.58

Gp 1: Conrol, Gp2: oxytocin, Gp 3: PGF2α and Gp4: GnRH.

Data regarding the percentage of morphologically abnormal spermatozoa are presented in table 7. As compared to control (6.35 ± 0.28%), all the treatment groups resulted in a significantly ($P \leq 0.05$) lower percentage of abnormal spermatozoa (5.32 ± 0.24, 5.62 ± 0.31 and 4.52 ± 0.37% for oxytocin, $PGF_{2\alpha}$ and GnRH groups, respectively).

Table 7: Effect of injection of oxytocin, PGF2α and GnRH on sperm abnormalities in rams (Mean ± S.D.).

Weeks	Sperm abnormalities (%)											
	First ejaculate				Second ejaculate				Mean			
	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4	Gp 1	Gp 2	Gp 3	Gp 4
1	7.00 ±0.58	6.33 ±0.33	8.00 ±0.58	7.67 ±0.88	7.67 ±0.33	6.33 ±1.33	7.67 ±2.03	7.00 ±0.58	7.33 ±0.17	6.33 ±0.83	7.83 ±1.17	7.83 ±0.72
2	8.00 ±0.58	5.67 ±1.20	7.00 ±1.15	4.67 ±1.20	6.67 ±1.77	5.67 ±1.67	7.67 ±1.67	5.00 ±0.58	7.33 ±1.17	5.67 ±1.42	7.33 ±0.44	4.83 ±0.88
3	6.67 ±0.88	5.00 ±0.58	6.00 ±0.58	4.67 ±0.88	6.67 ±0.33	5.67 ±0.33	5.33 ±0.33	4.67 ±0.33	6.67 ±0.60	5.33 ±0.33	5.67 ±0.17	4.67 ±0.44
4	6.00 ±0.58	5.67 ±1.20	5.00 ±1.00	3.33 ±0.33	5.67 ±0.33	5.67 ±0.66	5.33 ±0.33	4.00 ±0.58	5.83 ±0.17	5.67 ±0.93	5.17 ±0.93	3.67 ±0.33
5	6.67 ±0.88	6.00 ±0.58	6.00 ±1.15	4.00 ±1.53	6.33 ±1.45	6.33 ±0.66	5.67 ±0.33	3.67 ±0.33	6.50 ±1.15	6.17 ±0.44	5.83 ±0.73	3.83 ±0.88
6	6.67 ±1.20	4.00 ±0.58	4.00 ±0.58	5.33 ±0.66	6.33 ±1.20	4.33 ±0.33	3.33 ±0.33	4.33 ±1.33	6.50 ±1.15	4.17 ±0.17	3.67 ±0.44	4.83 ±0.88
7	4.00 ±0.58	5.00 ±1.15	5.67 ±0.66	4.00 ±1.15	4.67 ±0.66	5.33 ±1.67	5.33 ±1.85	3.67 ±0.66	4.33 ±0.44	5.17 ±1.36	5.50 ±1.26	3.83 ±0.88
8	6.67 ±1.20	5.00 ±0.58	5.33 ±0.88	3.33 ±0.33	6.33 ±1.20	4.67 ±0.66	5.67 ±1.20	2.67 ±0.33	6.50 ±0.87	4.83 ±0.44	5.50 ±1.04	3.00 ±0.29
9	5.00 ±1.15	5.00 ±0.58	4.67 ±0.88	1.53 ±0.33	5.33 ±0.88	4.67 ±0.33	5.00 ±1.00	5.33 ±0.33	5.17 ±0.93	4.83 ±0.33	4.83 ±0.88	4.83 ±0.33
10	7.00 ±1.15	5.00 ±0.58	5.33 ±1.45	4.00 ±0.58	7.67 ±0.33	5.00 ±1.00	4.33 ±0.88	4.67 ±0.66	7.33 ±0.73	5.00 ±0.76	4.83 ±1.17	4.33 ±0.44
Mean	6.37 [a] ±0.31	5.27 [ab] ±0.24	5.70 [bc] ±0.32	4.53 [c] ±0.32	6.33 [a] ±0.31	5.37 [ab] ±0.29	5.53 [ab] ±0.39	4.50 [b] ±0.27	6.35 [a] ±0.28	5.32 [b] ±0.24	5.62 [b] ±0.31	4.52 [c] ±0.37

Gp 1: Conrol, Gp2: oxytocin, Gp 3: PGF2α and Gp4: GnRH.

Table 8 illustrates frozen-thawed semen characteristics of rams of different treatment groups. There were no statistically significant ($P \leq 0.05$) differences between the control and three treatment groups in the percentage of motile spermatozoa either at 0, 1, 2 or 3 hours post-thawing (just after thawing: 52.22 ± 2.06, 52.78 ± 1.09, 48.89 ± 1.38 and 53.89 ± 1.11% for groups 1-4, respectively; after one hour: 36.67 ± 1.07, 37.78 ± 1.88, 35.00 ± 1.18 and 38.33 ± 1.07% for groups 1-4, respectively; after 2 hours: 25.00 ± 1.07, 25.00 ± 1.44, 24.44 ± 1.30 and 25.56 ± 1.76 for groups 1-4, respectively; at 3rd hour post-thawing: 15.00 ± 2.89, 13.33 ± 2.04, 14.44 ± 2.26 and 14.44 ± 2.26% for groups 1-4, respectively). Also, there were no statistically significant ($P \leq 0.05$) differences between the control and three treatment groups either in viability index (102.78 ± 5.26, 102.50 ± 4.81, 98.33 ± 3.93 and 105.28 ± 2.69 for groups 1-4, respectively) or in the percentage of spermatozoa with acrosomal defects (25.44 ± 1.44, 27.56 ± 1.61, 26.56 ± 1.07 and 26.78 ± 1.34% for groups 1-4, respectively).

Table 8: Frozen-thawed semen characteristics of rams injected with oxytocin, PGF2α and GnRH (Mean ± S.D.).

ITEMS	Week	Gp1	Gp2	Gp3	Gp4
Fresh semen motility	**1**	80.00 ± 0.00	81.67 ± 4.64	80.00 ± 1.44	83.33 ± 3.82
	5	81.67 ± 2.21	80.83 ± 2.21	77.50 ± 2.89	83.33 ± 1.67
	10	80.83 ± 2.21	73.33 ± 2.21	71.67 ± 2.21	87.50 ± 1.44
	Mean	80.83 ± 0.93 [ab]	78.61 ± 2.09 [b]	76.39 ± 1.07 [b]	84.72 ± 1.14 [a]
Motility just after thawing	**1**	50.00 ± 2.80	53.33 ± 4.41	46.67 ± 1.67	53.33 ± 1.67
	5	51.67 ± 3.33	53.33 ± 3.33	50.00 ± 2.88	51.67 ± 1.67
	10	55.00 ± 5.06	51.67 ± 1.67	50.00 ± 2.88	56.67 ± 1.67
	Mean	52.22 ± 2.06 [a]	52.78 ± 1.09 [a]	48.89 ± 1.38 [a]	53.89 ± 1.11 [a]
Motility after 1 hr.	**1**	36.67 ± 1.67	38.33 ± 4.41	36.67 ± 1.67	35.00 ± 2.88
	5	35.00 ± 2.88	36.67 ± 4.41	35.00 ± 2.88	36.67 ± 1.67
	10	38.33 ± 4.41	38.33 ± 1.67	33.33 ± 1.67	43.33 ± 1.67
	Mean	36.67 ± 1.07 [a]	37.78 ± 1.88 [a]	35.00 ± 1.18 [a]	38.33 ± 1.07 [a]
Motility after 2 hr.	**1**	26.67 ± 1.67	25.00 ± 2.88	26.67 ± 1.67	21.67 ± 1.67
	5	21.67 ± 3.33	23.33 ± 3.33	25.00 ± 2.88	23.33 ± 1.67
	10	26.67 ± 3.33	26.67 ± 1.67	21.67 ± 1.67	31.67 ± 1.67
	Mean	25.00 ± 1.07 [a]	25.00 ± 1.44 [a]	24.44 ± 1.30 [a]	25.56 ± 1.76 [a]
Motility after 3 hr.	**1**	18.33 ± 1.67	11.67 ± 1.67	16.67 ± 1.67	11.67 ± 1.67
	5	11.67 ± 3.33	11.67 ± 1.67	15.00 ± 2.89	13.33 ± 1.67
	10	15.00 ± 2.89	16.67 ± 1.67	11.67 ± 1.67	18.33 ± 1.67
	Mean	15.00 ± 2.89 [a]	13.33 ± 2.04 [a]	14.44 ± 2.26 [a]	14.44 ± 2.26 [a]
Viability index	**1**	106.67 ± 8.98	101.67 ±10.83	103.33 ± 5.83	95.00 ± 3.82
	5	94.17 ± 18.93	98.33 ± 10.93	100.00 ± 9.46	99.17 ± 5.07
	10	107.50 ±12.50	107.50 ± 4.33	91.67 ± 7.07	121.67 ± 4.17
	Mean	102.78 ± 5.26[a]	102.50 ± 4.81[a]	98.33 ± 3.93 [a]	105.28 ± 2.69[a]
% of acrosomal defects	**1**	25.33 ± 1.45	32.33 ± 2.90	24.67 ± 1.20	29.33 ± 2.19
	5	26.67 ± 4.17	26.33 ± 1.77	28.00 ± 2.64	28.00 ± 2.08
	10	24.33 ± 2.03	24.00 ± 1.00	27.00 ± 1.53	23.00 ± 1.15
	Mean	25.44 ± 1.44 [a]	27.56 ± 1.61 [a]	26.56 ± 1.07 [a]	26.78 ± 1.34 [a]

Gp 1: Conrol, Gp2: oxytocin, Gp 3: PGF2α and Gp4: GnRH.

As shown in table 9, injection of GnRH prior to semen collection resulted in significant increase in testosterone level when compared with control, oxytocin and $PGF_{2\alpha}$ groups (5.16 ± 0.11 vs. 4.34 ± 0.05, 4.37 ± 0.04 and 4.48 ± 0.11, respectively). Concerning the antioxidant activities exerted by ram semen of the different treatments groups (Table 9) represented by total antioxidant, superoxide dismutase and glutathione peroxidase, semen of $PGF_{2\alpha}$ group has a significantly ($P \leq 0.05$) higher antioxidant activities (0.35 ± 0.01, 302.18 ± 5.68 and 18.15 ± 0.92 mM/L, respectively) as compared with control (0.33 ± 0.01, 276.21 ±3.10 and 12.66 ± 0.81 mM/L, respectively), oxytocin group (0.33 ± 0.01, 277.90 ± 3.42 and 13.12 ± 1.11 mM/L, respectively) and GnRH group (0.32 ± 0.00, 224.83 ± 4.52 and 12.66 ± 1.01 mM/L, respectively).

Table 9: The levels of testosterone and antioxidant enzymes in the seminal plasma of rams injected with oxytocin, PGF2α and GnRH (Mean ± S.D.).

ITEMS	Week	Gp1	Gp2	Gp3	Gp4
Testosterone (ng/ml)	**1**	4.36 ± 0.11	4.50 ±0.02	4.54 ± 0.24	5.05 ± 0.09
	5	4.28 ± 0.10	4.32 ± 0.03	4.42 ± 0.22	5.19 ± 0.11
	10	4.38 ± 0.05	4.28 ± 0.05	4.48 ± 0.17	5.25 ± 0.13
	Mean	4.34 ± 0.05 [b]	4.37 ± 0.04 [b]	4.48 ± 0.11 [b]	5.16 ± 0.11 [a]
Total antioxidants (mM/L)	**1**	0.35 ± 0.01	0.33 ± 0.02	0.35 ± 0.01	0.32 ± 0.01
	5	0.32 ± 0.01	0.33 ± 0.01	0.35 ± 0.01	0.32 ± 0.01
	10	0.33 ± 0.01	0.33 ± 0.01	0.36 ± 0.02	0.33 ± 0.01
	Mean	0.33 ± 0.01 [b]	0.33 ± 0.01 [b]	0.35 ± 0.01 [a]	0.32 ± 0.00 [b]
Superoxide dismutase (mM/L)	**1**	280.24 ± 3.55	279.08 ± 7.44	301.59 ± 10.54	228.61 ± 13.89
	5	272.61 ± 6.83	277.37 ± 4.87	301.59 ± 10.54	228.61 ± 13.98
	10	275.77 ± 6.41	277.24 ± 7.75	300.75 ± 11.58	222.55 ± 4.52
	Mean	276.21 ± 3.10 [b]	277.90 ± 3.42 [b]	302.18 ± 5.68 [a]	224.83 ± 4.52 [c]
Glutathione peroxidase (mM/L)	**1**	12.19 ± 2.23	12.99 ± 2.27	18.24 ± 1.10	12.09 ± 1.71
	5	12.75 ± 1.73	13.31 ± 2.56	17.88 ± 1.69	12.24 ± 1.58
	10	13.03 ± 1.01	13.04 ± 1.71	18.34 ± 2.45	12.42 ± 1.58
	Mean	12.66 ± 0.81 [b]	13.12 ± 1.11 [b]	18.15 ± 0.92 [a]	12.66 ± 1.01 [b]

Gp 1: Conrol, Gp2: oxytocin, Gp 3: PGF2α and Gp4: GnRH.

DISCUSSION

In flocks of sheep in which large numbers of spermatozoa must be collected from a few rams over a short period for artificial insemination (A.I.), a substantial increase in the output of spermatozoa per ejaculate per ram would be an advantage (Knight, 1974). The present study aimed to maximize the semen output from certain rams using different regimes as well as to study the effects of these treatments on quality, freezability and antioxidant activity of ram semen.

In the current study, repeated injection of oxytocin resulted in significant increase in semen volume and total number of spermatozoa per ejaculate. Similar results were recorded for oxytocin in rams (Knight and Lindsay, 1970; Knight, 1974; Voglmayr, 1975; Nicholson *et al.,* 1999; Bozkurt *et al.,* 2007) bulls (Sharma and Hays, 1973; Schefels and ElAzab, 1975; Berndtson and Igboeli, 1988; Palmer *et al.,* 2004), buffalo (Ibrahim, 1988), rabbits (Fjellstrom *et al.,* 1968), dogs (Hess, 2002), men (Rezk *et al.,* 2004) and ostriches (Suttiyotin *et al.,* 2012). In contrast, no detectable effect of oxytocin injection was found in rabbits (Agmo, 1975) and oligospermic men (Byrne *et al.,* 2003).

In the present work, oxytocin did not alter sperm concentration, sperm mass activity or abnormal sperm rate in comparison to the control values. Similar findings were observed in rams by Bozkurt *et al.* (2007) and in bulls by Berndtson and Igboeli (1988). Similarly, the addition of oxytocin to bull (Gallagher and Senger, 1989) and stallion semen (Clough *et al.,* 2006) was found to have no effect on sperm motility.

Circulating plasma concentrations of oxytocin increase during sexual arousal and ejaculation in bulls (Sharma and Hays, 1973) and humans (Carmichael *et al.,* 1987). The action of oxytocin is mediated via activation of the oxytocin receptor present in the peritubular smooth muscle cells of the epididymis stimulating contractility in vivo (Hib, 1977) and in vitro (Filippi *et al.,* 2002a). Oxytocin acts directly on smooth muscle cells throughout the entire epididymis and indirectly by stimulating the release of endothelin-1 from epithelial cells of the caput epididymidis (Peri *et al.,* 1997; Filippi *et al.,* 2002b). Endothelin-1 is a potent stimulator of epididymal contraction. Epididymal contractility stimulated by oxytocin may be important for the release of sperm stored in the cauda epididymidis, thereby enhancing

sperm transport within the extragonadal ducts before and/or during ejaculation (Studdard *et al.,* 2002).

In the current work, repeated injection of $PGF_{2\alpha}$ resulted in significant increase in semen volume, sperm cell concentration and total number of spermatozoa per ejaculate. Similar results were recorded for $PGF_{2\alpha}$ in rams (Azawi *et al.,* 2011; Olfati *et al.,* 2013) bulls (Hafs *et al.,* 1974; Haynes *et al.,* 1975; Marshall and Hafs, 1976; Masoumi *et al.,* 2011; Titiroongruang *et al.,* 2011), buffalo (Ibrahim, 1988), stallion (Kreider *et al.,* 1981; McDonnell, 1992), rabbits (Hafs *et al.,* 1974), dogs (Hess, 2002; Kustritz and Hess, 2007) and boars (Hashizume and Niwa, 1984; Estienne and Harper, 2004). In contrast, it was found that there was no effect of $PGF_{2\alpha}$ treatment on various semen characteristics in bulls (Berndtson *et al.,* 1979) and boars (Levis and Reicks, 2005; Kozink, 2002).

In rams, the addition of $PGF_{2\alpha}$ to semen increased the conception rate (Gustafsson *et al.,* 1975) and increased the fertility of rams by more than 15% (Dimov and Georgiev, 1977).

The increase in the sperm number in the ejaculate following Cloprostenol administration is not probably due to an increased rate of spermatogenesis. Spermatogenesis is unaffected by the collection frequency or short-term $PGF_{2\alpha}$ administration (Johnson *et al.,* 1970; Marshall and Hafs, 1976). It has been established that smooth muscle surrounding the epididymis contracts in response to $PGF_{2\alpha}$ in other species (Hib and Oscar, 1978). Cauda epididymidis acts as a site of storage for mature spermatozoa. When the caudal epididymis contracts in response to $PGF_{2\alpha}$, mature spermatozoa are moved into the deferent duct where they are available for ejaculation (Masoumi *et al.,* 2011). In addition to the effects of $PGF_{2\alpha}$ on the smooth muscle of the epididymis, the testicular capsule also contracts in response to $PGF_{2\alpha}$ (Free *et al.,* 1980; Cosentino and Cockett, 1986).

In the present study, although GnRH improved motility and viability and morphologically normal spermatozoa of rams, it has no detectable effect on semen volume, sperm cell concentration and total number of spermatozoa per ejaculate. These results coincided with the findings of Hess (2002) in dogs. In contrary, Gabor *et al.* (1995) observed that repeated injection of GnRH provided valuable results of producing capacity of bull testes. Also, Braun *et al.* (1988) revealed the improvement of semen quality in adult bulls after the administration of GnRH. Garner *et al.* (1990) added that GnRH controls the production of gonadotropins, thereby having an orchestrating effect on the reproductive hormone cascade and spermatogenesis.

In the current investigations, repeated GnRH injection resulted in marked increase in testosterone level as compared to control. These results coincided with that recorded in bulls by Braun *et al.* (1988) and Gabor *et al.* (1995) and in dogs by Knol *et al.* (1993). The data pertaining to the effect of oxytocin on testosterone are controversial, because some researchers (Inaba *et al.,* 1999) have reported that oxytocin treatment reduces the testosterone concentration in goats, whereas others (Nicholson *et al.,* 1987) have reported that this hormone increases the release of testosterone in rats, and some (Nozdrachev *et al.,* 1994; Kumar and Farooq, 1994; Bozkurt *et al.,* 2007 in rat, mice and rams, respectively) have also documented that oxytocin does not alter the concentration of testosterone. In the present study, it was observed that the administration of oxytocin did not affect the levels of seminal plasma testosterone at any time compared to the control group. In the current work, injection of $PGF_{2\alpha}$ had no effects on the level of testosterone. On contrary, plasma testosterone concentrations in bulls were increased after $PGF_{2\alpha}$ injection (Haynes *et al.,* 1975; Kiser *et al.,* 1976; Masoumi *et al.,* 2011).

An unbalanced, excessive production of ROS and decreased level of antioxidant enzymes cause decreased sperm motility and viability, and increased sperm defects by initiating an oxidation chain reaction damaging proteins, lipids and DNA (Sikka, 2004; Aitken and Baker, 2004). Seminal plasma has an antioxidant system that seems to be very relevant to the protection of sperm. The sperm oxidative defence enzymes predominantly include superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Sarlos *et al.,* 2002). In the present study, $PGF_{2\alpha}$ had beneficial effects on the antioxidant activities while repeated injection of oxytocin and GnRH had no deleterious effects on antioxidant activities in ram semen in terms of total antioxidants, superoxide dismutase and glutathione peroxidase levels in seminal plasma. To the best of our knowledge, no previous data are available about the effect of repeated injection of oxytocin, $PGF_{2\alpha}$ and GnRH on the antioxidant activities of semen.

Concerning the post-thaw semen characteristics in the present work, all the treatment groups did not alter motility, viability and acrosomal integrity of the frozen-thawed ram semen. In agreement with these results, the others reported that the administration of $PGF_{2\alpha}$ to bulls prior to ejaculation did not influence the motility of frozen-thawed spermatozoa (Marshall and Hafs, 1976; Masoumi *et al.,* 2011). Berndtson and Igboeli (1988) mentioned that oxytocin did not impair the ability of bull spermatozoa to withstand freezing or thawing.

In conclusion, it was suggested that injection of $PGF_{2\alpha}$ and oxytocin prior to semen collection may be useful when collecting ram semen for use in artificial insemination leading to increase in the semen output and increment in the total number of straws frozen. Moreover, repeated injection of them neither causes depletion of sperm reserves in rams nor alters the quality of fresh and frozen-thawed semen as well as they have no deleterious effects on the antioxidant activities of seminal plasma and testosterone level in rams.

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دراسة تأثير البروستاجلاندين $F_{2\alpha}$ أو الأوكسيتوسين أو الهرمون المحرر للحاثة المنسلية على خصائص قذفة السائل المنوى للكباش وقابلية السائل المنوى للتجميد

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تهدف هذة الدراسة إلى زيادة حجم القذفة وتركيز الحيوانات المنوية المجمعة من الكباش بإستخدام الأوكسيتوسين أو البروستاجلاندين F2α أو الهرمون المحرر للحاثة المنسلية مع دراسة تأثير هذة المعاملات على خصائص السائل المنوى الطازج والمجمد للكباش ودراسة تأثيرهم على مستوى مضادات الأكسدة بالسائل المنوى. تم تقسيم عدد ١٢ كبش برقى إلى ٤ مجموعات متساوية: الأولى ضابطة تم حقنها بالعضل بمحلول ملح فسيولوجى قبل تجميع السائل المنوى ب ٣٠ دقيقة والمجموعة الثانية تم حقنها بالوريد ب ١٠ وحدات دولية من الأوكسيتوسين قبيل التجميع ب ١٠ دقائق والمجموعة الثالثة حقنت بالعضل ب ٢٥٠ ميكروجرام من الكلوبروستينول قبل التجميع ب ٣٠ دقيقة والمجموعة الرابعة حقنت بالوريد ب ٥٠ ميكروجرام من الجونادورولين ٦٠ دقيقة قبل تجميع السائل المنوى. تم تجميع قذفتى سائل منوى من الكباش مرة واحدة أسبوعيا على مدار ١٠ أسابيع وفى الأسبوع الأول والخامس والعاشر تم تجميد السائل المنوى للكباش مع قياس مستويات التستوستيرون وإنزيمات مضادات الأكسدة فى بلازما السائل المنوى. وقد أظهرت النتائج أن حقن البروستاجلاندين F2α والأوكسيتوسين أديا إلى زيادة ملحوظة ($P \leq 0.05$) فى حجم القذفة وفى العدد الكلى للحيوانات المنوية بالقذفة طوال فترة التجربة وبدون حدوث تغيرات ملحوظة فى خصائص السائل المنوى الطازج والمجمد. كما أن البروستاجلاندين F2α والأوكسيتوسين لم يؤثرا سلبا على مستويات التستوستيرون ومضادات الأكسدة الكلية والسوبر أوكسيد ديسميوتاز والجلوتاثيون بيروكسيدازز وفى المقابل، أدى حقن الهرمون المحرر للحاثة المنسلية إلى زيادة مستوى التستوستيرون لكنة لم يؤدى إلى زيادة حجم القذفة وتركيز الحيوانات المنوية بها. نستخلص من هذة الدراسة أن تكرار حقن البروستاجلاندين F2α أو الأوكسيتوسين قبيل تجميع السائل المنوى له فائدة كبيرة عندما يتم التجميع من الكباش للأستخدام فى التلقيح الأصطناعى لأن ذلك يزيد من عدد الحيوانت المنوية المتحصل عليها بدون تغيير ملحوظ فى خصائص السائل المنوى الطازج والمجمد بالإضافة لعدم وجود أثار سلبية على مستوى مضادات الأكسدة والتستوستيرون فى بلازما السائل المنوى.