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Study on the effect of prostaglandin $F_{2\alpha}$ treatment on semen characteristics and enzymatic activates of Awassi rams in breeding and non breeding seasons

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Abstract

The purpose of this research work was to determine the effects of PGF2*α*, given immediately before semen collection, on semen characteristics and libido in Awassi rams during breeding and non breeding season. The experiment was conducted in late summer to early autumn when major breeding activities commence and winter during the non breeding season at Mosul region in northern Iraq at the Animal Research and Practice Farm of the College of The Veterinary Medicine, University of Mosul. Twelve mature Awassi rams were used in this study. Animals were randomly allocated into two equal groups, the first group was administered 7.5 mg IM of PGF2*α* weekly and the second group as a control group received 1 ml of N-saline solution. Semen samples were collected from the Awassi rams 24 h after IM administration. Scrotal circumference (SC) and testicular volume were measured weekly during the study period. Semen ejaculates were evaluated for semen volume, sperm concentration, sperm concentration/ejaculate, mass motility, individual motility, percentage live sperm, sperm abnormalities, and sperm acrosomal defects. Samples of seminal plasma were analyzed for the estimation of alanine amino transferase (ALT), aspartate amino transferase (AST), acid phosphatase (ACP), alkaline phosphatase (ALP) and lactic dehydrogenase (LDH). Results of the present showed that $PGF_{2\alpha}$ treatment to Awassi rams did not improve most semen characteristics in both breeding and non breeding seasons compared with the group. The only improvement of Awassi semen quality observed was in sperm concentration in the breeding season. The testicular volume showed a significant increase ($P<0.05$) in Awassi rams treated with $PGF_{2\alpha}$ in breeding season compared to the control group and $PGF_{2\alpha}$ treated group in the non breeding season. The mean activity of LDH enzyme estimated in the PGF2α treated group and control group showed a significant difference ($P<0.05$) between the two groups in the breeding season and non breeding season (52.34 ± 8.96 and 57.43 ± 19.9 vs. 117.02 ± 5.26 and 131.88 ± 5.01, respectively). Other enzymatic activities including ALT, AST, ACP and ALP showed no significant differences between Awassi rams treated with PGF2α and control groups in both breeding and non breeding seasons. In conclusion, PGF2α treatment of Awassi rams improved sperm concentration and testicular volume.

Keywords: Awassi Ram, Semen, Prostaglandin $F_{2\alpha}$, Enzymes, Seasons

Introduction

Awassi sheep are one of the most economically important skin, milk and meat-breed reared in Iraq. The production potential of livestock can be increased by genetic improvement using one of the modern ways of breed improvement, e.g., artificial insemination (AI). Increasing use of assisted reproductive techniques in the ram has revealed a need for additional aids to optimize the ejaculates obtained using artificial vagina for semen collection. Specifically, improving upon the number of spermatozoa obtained during semen collection in the breeding and non breeding seasons would benefit most areas of assisted reproduction, including semen preservation and artificial insemination. Ram seminal plasma is rich in prostaglandins, and this hormone has been shown to be of importance to sperm transport in ewes (Gustafsson et al., 1977).

Research conducted in bulls and rabbits has identified PGF2α that when administered prior to semen collection improved ejaculate quality by increasing total sperm number in the ejaculate (Hafs et al., 1974; Marshall and Hafs, 1976). Treatment with PGF2α has been used to expedite mounting behavior, as well as restore libido in bulls displaying decreased sex drive (Masoumi et al., 2011). Also, others (Estienne and Harper, 2004) used PGF2*α* to expedite mounting behavior, as well as restore libido in boars displaying decreased sex drive. The mechanism behind the

increase in ejaculate volume and/or concentration in response to PGF2α is not fully understood. It is thought that PGF2α acts directly on the contractile tissues of the testicular capsule and epididymis causing an increased rate of sperm passage from the epididymis to the deferent ducts (Mekonnen et al., 1989). The use of PGF2α prior to collection may optimize the number of sperm in a collection by enhancing sperm movement from the epididymis to the deferent duct where they are available for ejaculation (Hemsworth et al., 1977; Shankar et al., 1984; Estienne and Harper, 2004). The objective of the present experiment was to determine the effects of PGF2*α*, given immediately before semen collection, on semen characteristics and libido in Awassi rams during breeding and non breeding season.

Materials and Methods

The study was carried out at the College of The Veterinary Medicine, University of Mosul. The experiment was conducted in breeding season (August-October 2008) when major breeding activities commence and winter during the non breeding season (December-February 2009) at the Animal Research and Practice Farm of the College of The Veterinary Medicine, University of Mosul. Twelve mature Awassi rams (2-3 years of age) used in this study, and maintained using conventional feeding, housing and lighting conditions. All these rams were in good health. They were maintained in identical nutritional and managerial condition throughout the period of study. The animals were kept in open front barrens, fed individually with concentrated mixture of 1 kg per ram per day, and were given water ad libitum. Rams were randomly allocated into 2 equal groups, first group administered 7.5 mg IM of $PGF_2\alpha$ (Intervet, B.V., Boxmer, Holland) weekly and the second group as a control received 1 ml of N-saline solution. Semen samples were collected from rams 3 h after IM administration. A total number of 288 ejaculates were collected from the rams using an artificial vagina once a week 24 h following administrations starting from August 2008 to February 2009. The scrotal circumference (SC) was taken weekly during the study period. SC was measured with the help of a flexible tape, while the rams were restrained in a sitting position. Scrotal wool was clipped off and the testes were pulled fully into the scrotum before measurement. Testicular volume was estimated by the amount of liquid displaced by immersing the whole scrotal sac of a standing ram into a 2-litre container filled with warm water according to Archimedes law of buoyancy (Piperelis et al., 2008). The sexual behavior expressed as reaction time of the rams was evaluated weekly, on day of semen collection in a pen test with estrous ewes. Ewes were induced into estrus with intravaginal sponges impregnated with medroxy-progesterone acetate (Synncropart 40 mg sheep sponge, Ceva 140 Sante Animale, France) during 6 days and 300 IU of eCG ((Synncropart) IM at sponge withdrawal. Rams were individually located with one estrous ewe in a 5 x 5 m pen and the time of lateral approach, mounts and ejaculation in the artificial vagina were recorded. The intervals to first mounts and ejaculation in the artificial vagina were rerecorded.

The volume of each ejaculate was recorded and sperm concentration was determined using semen diluted with 3% NaCl, the diluted semen was placed on a hemocytometer with the sperm counted in five squares of one chamber. Sperm motility was identified as those sperm cells that demonstrated progressive motility. Sperm motility was scored from 0 to 100% by a qualified and experienced investigator. Semen was placed on a heated glass slide, and scoring was performed at microscopic magnification of x 200. Each sample was evaluated twice. The mean value was used for data analysis. Assessments of abnormal and normal spermatozoa were performed using an eosin–nigrosin staining method. For the percent of spermatozoa with abnormal acrosomes, fast green stain was used (Wells and Awa, 1970).

Samples for the estimation alanine amino transferase (ALT), aspartate amino transferase (AST), acid phosphatase (ACP), alkaline phosphatase (ALP) and lactic dehydrogenase (LDH) were obtained by centrifugation 5 ml of diluted (in a dilution rate 1:20) semen using N-saline solution for volume expansion at 5000 rpm for 15 min using available diagnostic kits (BIOLABO SA, 02160, Maizy, France). Determination of ALT and AST enzymes in diluted cell free semen samples were carried by means of spectrophotometer with photometric determination of the concentration pyruvate and oxaloacetate hydrozone formed with 2,4 dinitrophenylhydrazine as described by Reitman and Frankel (1957). ACP enzyme was determined according to the methods described by Tietz (1999). Determination of ALP enzyme was performed as the methods described by King and King (1954). LDH activity in seminal plasma free cells was determined as the methods described by Henry (1974).

Statistical analysis

Data were expressed as means (±S.E.) and Statistical analyses were performed with the software (Sigma stat, Jandel scientific software V2.0, Richmond, CA, 2004). The differences between means of the same parameter were tested by the analysis of variance (ANOVA) and Duncan's multiple range test.

Results and Discussion

Results of the present showed that $PGF_{2\alpha}$ treatment to Awassi rams did not improve most semen

characteristics in both breeding and non breeding seasons (Table 1) compared with the group. These results were in contrary with the results obtained by previous studies in bulls, boars and rabbits (Marshall and Hafs, 1976; Hafs et al., 1974; Estieranne and Harper, 2004; Masoumi et al., 2011). While agrees with the findings of Hashizume and Niwa (1984), they found no effect or improvement of semen characteristics after $PGF_{2\alpha}$ treatment in bulls. These results revealed that injecting $PGF_{2\alpha}$ to Awassi rams 3 hours prior to semen collection had no effect on most semen characteristics. The only improvement of Awassi semen quality observed was in sperm concentration in the breeding season. These results were in agreement with the results found by Shankar et al. (1984) and Estienne and Harper (2004). The mechanism behind the increase in ejaculate concentration in response to PGF2α could be due to that PGF2α acts directly on the contractile tissues of the testicular capsule and epididymis causing an increased rate of sperm passage from the epididymis to the deferent ducts (Mekonnen et al., 1989). The use of PGF2α prior to collection may optimize the number of sperm in a collection by enhancing sperm movement from the epididymis to the deferent duct where they are available for ejaculation (Narasimha et al., 1986). Free et al. (1980) determined that intravenous administration of PGF2α to rats would increase the flow of fluid from the rete testis 2-3 fold over a period of 20-40 minutes compared to untreated controls. Furthermore, Buhrley and Ellis (1975) demonstrated that spontaneous contraction of the testicular capsule and seminiferous tubules is significantly inhibited by indomethacin, a potent prostaglandin synthesis inhibitor. Bartke and Koerner (1974) found that the prostaglandin composition of the distal epididymis in rats and mice was significantly greater than the proximal portions39. Similarly, the concentration of prostaglandins in the lumen of the ram epididymis is 15-20times greater in the distal regions compared to the proximal regions. The concentration of PGF2α in the cauda epididymis fluid is eight times that of the rete testis fluid in bulls (Voglmayr, 1973). Hafs et al. (1974) used anesthetized rabbits to demonstrate that exogenous PGF2α and found a significant increase in the movement of sperm from the epididymis to the deferent duct. The increase in spermatozoa and fluid flow from the rete testes, epididymis and deferent duct following PGF2α administration does not appear to be the result of an increase in spermatogenesis (Saksena et al., 1978). In addition, those studies that have documented an increase in sperm numbers in the ejaculate following PGF2α administration have shown that the effect is limited to the first of two successive ejaculates (Kreider et al., 1981). Results of this study indicate that PGF2α could be used to enhance sperm numbers in the ejaculate of Awassi ram semen.

The Testicular volume showed a significant increase ($P<0.05$) in Awassi rams treated with $PGF_{2\alpha}$ in breeding season compared to the control group and $PGF_{2\alpha}$ treated group in the non breeding season as shown in Table 1. Reaction time (libido) of Awassi rams treated with $PGF_{2\alpha}$ showed no significant improvement in the breeding and non breeding seasons by expressing shortened reaction time per seconds in collecting semen samples with artificial vagina. Some researchers noted that treated animals had more libidos at the time of semen collection (Shankar et al., 1984; Mekonnen et al., 1989). Results of the present study revealed no effect of PGF2α treatment on ram's libido expressed by reaction time estimation in this study. Libido was assessed using quantifiable observations (as used in this study), such as time to initial false mount and time to ejaculation in buffalo, and time for collection in rams. Initially, the effects on reaction time and collection time were attributed to a surge in testosterone following PGF2α administration. In yearling beef bulls, serum testosterone increased two-fold following PGF2α and remained elevated for over four hours (Berndtson et al., 1979). Prostaglandin F2α treatment caused a release of testosterone in the bull that became apparent in 40-50 minutes and persisted for at least 8 hours. The increase in testosterone following PGF2α treatment was thought to be due to direct stimulation of testicular steroidogenesis. Prostaglandin F2α stimulates cyclic AMP production in the testicle; cyclic AMP then stimulates testosterone synthesis (Reichard et al., 1978).

The mean activity of ALT, AST, ACP, ALP and LDH enzymes in the PGF2α treated and control groups in the breeding and non breeding seasons are summarized in Table 3. The mean activity of LDH enzyme estimated in the PGF2α treated group and control group showed a significant difference ($P<0.05$) between the two groups in the breeding season and non breeding season (52.34 ± 8.96 and 57.43 ± 19.9 vs. 117.02 ± 5.26 and 131.88 ± 5.01, respectively). Other enzymatic activities including ALT, AST, ACP and ALP showed no significant differences between Awassi rams treated with GnRH and control groups in both breeding and non breeding seasons. Pursel et al. (1968) reported that one of the consequences of acrosomal damage is the leakage of enzymes from the sperm. The leakage of five enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP); revealed a positive correlation between enzyme release and sperm cell integrity and acrosomal damage (Azawi et al., 1990a; Chauhan et al., 1994). Enzyme release has generally been recognized as an indicator of cellular injury whereby membranes become inactivated or destroyed resulting in the loss of cellular material (Yousef et al., 1998). The present results revealed no

Table 1: Mean (± SEM) values of semen parameters in breeding and non breeding seasons in $PGF_{2\alpha}$ treated and control groups of Awassi rams.

Groups	Volume (ml)	Sperm concentration $X10^9$ sperm/ml	Sperm concentration $X10^9$ sperm/ ejaculate	PH	Mass motility (%)	Individual motility (%)	Live sperm (%)	Abnormal sperm (%)		Abnormal acrosomes (%)	Methylene blue reduction test
								primary	secondary		
	0.83	1.81[a]	1.75	6.34	94.3	92.7	86.6	1.9	12.9	1.98	2.4
$PGF_{2\alpha}$ in	±	±	±	±	±	±	±	±	±	±	±
breeding season	0.06	0.02	0.19	0.06	3.07	3.33	2.29	0.49	1.27	0.01	0.51
	0.72	1.16[b]	1.52	6.72	87.3	82.5	80.28	2.6	12.21	2.01	2.71
$PGF_{2\alpha}$ in non	±	±	±	±	±	±	±	±	±	±	±
breeding season	0.19	0.11	0.23	0.07	2.11	3.59	2.88	0.26	0.85	0.13	0.45
	1.1	0.89[b]	1.21	6.52	92.8	92.0	85.83	2.83	15.55	1.3	2.61
Control in	±	±	±	±	±	±	±	±	±	±	±
breeding season	0.07	0.11	0.24	0.51	0.83	1.78	2.01	0.39	0.65	0.10	0.44
	0.96	0.88[b]	1.08	6.45	84.16	82.3	81.66	2.79	15.15	1.0	2.72
Control in	±	±	±	±	±	±	±	±	±	±	±
non breeding season	0.09	0.05	0.15	0.177	1.53	1.05	1.05	0.37	1.94	0.01	0.31

Means for the same parameter with different superscripts (a, b) within each column are significantly different ($P<0.05$).

Table 2: Mean (± SEM) values of reproductive traits in breeding and non breeding seasons in $PGF_{2\alpha}$ treated and control groups of Awassi rams

Groups	Reaction time (Seconds)	Scrotal circumference (cm)	Testicular volume (cm^3)
$PGF_{2\alpha}$ in breeding season	13.33±4.52	32.67±0.37	694.2±28.46[a]
$PGF_{2\alpha}$ in non breeding season	16.6±7.14	30.75±0.44	490.1±35.41[b]
Control in breeding season	15.1±2.35	32.1±0.61	475.11±38.98[b]
Control in non breeding season	17.5±2.81	30.1±0.31	462.33±17.28[b]

Table 3: Mean (± SEM) values of seminal plasma enzymes activities in breeding and non breeding seasons in $PGF_{2\alpha}$ treated and control groups of Awassi rams

Groups	Alanine amino transferase (ALT)	Aspartate amino transferase (AST)	Acid phosphatase (ACP)	Alkaline phosphatase (ALP)	Lactic dehydrogenase (LDH)
$PGF_{2\alpha}$ in breeding season	78.87±2.97	174.65±16.0	398.34±10.14	1288.5±224.18	52.34±8.96[a]
$PGF_{2\alpha}$ in non breeding season	109.24±17.49	183.38±19.29	334.48±103.93	2580.8±258.19	57.43±19.9[a]
Control in breeding season	90.89±8.86	167.9±29.71	351.67±42.45	1831.9±138.22	117.02±5.26[b]
Control in non breeding season	114.114±9.42	280.31±49.98	319.19±63.81	2012.2±130.87	131.88±5.01[b]

Means for the same parameter with different superscripts (a, b) within each column are significantly different ($P<0.05$).

significant differences in the activities of AST, ALT, ACP, and ALP in the seminal plasma of PGF2α treated and control groups. Therefore, the stability in the activities of these enzymes coincided with the unaffected semen quality and viability of treated rams with PGF2α and control groups in the breeding and non breeding seasons found in the present study. The significant decrease in the LDH activity in Awassi rams treated with GnRH could be due the significant increase in semen volume and sperm concentration/ejaculate observed in this study. Roussal and Stallcup (1965) and Azawi et al. (1990b) reported that the activity of seminal plasma LDH was negatively correlated with each of ejaculate volume and sperm concentration.

The reproductive activity of the ram appears to be influenced, in certain breeds and regions, by the season of the year (Glover et al., 1990; Karagiannidis et al., 2000) with photoperiod being the key environmental signal timing the reproductive cycle (Lincoln and Short, 1980). The effect of season and/or day length on semen quality has been studied in different breeds of rams (Ibrahim, 1997; Kafi et al., 2004; Deldar Tajangookeh et al., 2007; Makawi et al., 2007). The results of the present study show that Awassi rams have continuous and acceptable spermatogenic activity during all seasons of the year. However, seasonal variations in semen characteristics are observed with no significance. Semen of superior quality was produced in late summer and throughout autumn. Seasonal changes occurred in semen volume, sperm concentration, mass motility, sperm individual motility and percent alive sperm occurred in the Awassi rams without significant. Increase was occurring during late summer to early autumn, which is similar to the findings of Deldar Tajangookeh et al. (2007) and Makawi et al. (2007). During the natural breeding season, (late summer to early autumn) Awassi rams recorded a highest sperm concentration compared to non-breeding season (winter). The increase in sperm concentration of Awassi rams was associated with a marked decrease in photoperiod, which agrees with the findings of Zamiri and Khodaei (2005). High environmental temperatures (average of 25°C), particularly in association with increasing day length during summer months has been demonstrated to result in a reduction in semen quality in Corriedale, and Chios rams (Perez et al., 1997; Karagiannidis et al., 2000). Northern Iraq is regarded as a dry region with a hot summer (40–50°C). Contrary to our expectation, results of the present study show that semen volume, sperm concentration, and semen mass motility of Awassi rams were superior in late summer and autumn compared to that obtained in winter. Similarly, Ibrahim (1997) in a study with Chios crossbred rams reared in the United Arab Emirates

found that semen quality was not reduced during hot months of summer. These findings suggest that the semen quality of Awassi rams is not affected by summer high temperature in northern Iraq. In conclusion, PGF2α treatment of Awassi rams improved sperm concentration and testicular volume.

References

Azawi, O.I., Zand M.M. and Al-Jarrah L.H. 1990a. The correlation between the viability of bovine spermatozoa preserved at ambient temperature and the activity of transferases. *Animal Reproduction Science*, 23: 319 – 323.

Azawi, O.I., Znad M.M. and Al-Jarrah, L.H. 1990b. The effect of preserving bovine spermatozoa at ambient temperature on the release of phosphatases and lactic dehydrogenases enzymes. *Indian Journal of Reproduction*, 11: 1-5.

Bartke, A. and Koerner, S. 1974. Androgenic regulation of the concentration of prostaglandin F in the male reproductive system of rats and mice. *Endocrinology*, 95:1739-1742.

Berndtson, W.E., Chenoweth, P.J. and Seidel G.E. 1979. Influence of prostaglandin F2 alpha on spermatogenesis, spermatozoal output, seminal quality, testosterone levels and libido of yearling beef bulls. *Journal of Animal Science*, 49: 736-743.

Buhrley, L.E. and Ellis, L.C. 1975. Contractility of rat testicular seminiferous tubules in vitro: prostaglandin F_1 alpha and indomethacin-1,2. *Prostaglandins*, 10:151-155.

Chauhan, M.S., Kapila R., Gandhi K.K. and Anand S.R. 1994. Acrosome damage and enzyme leakage of goat spermatozoa during dilution, cooling and freezing. Andrologia 26: 21–26.

Deldar Tajangookeh, A., Zare Shahneh, A., Moradi Shaherbabak, M. and Shakeri, M. 2007. Monthly variation of plasma concentrations of testosterone and thyroid hormones and reproductive characteristics in three breeds of Iranian fat-tailed rams throughout one year. *Pakistan Journal of Biological Science*, 10: 3420-3424.

Estienne, M.J. and Harper, A.F. 2004. Semen characteristics and libido in boars treated repeatedly with PGF2α. *Journal of Animal Science*, 82:1494–1498.

Free, M.J., Jaffe and R.A. and Morford, D.E. 1980. Sperm transport through the rete testis in anesthetized rats: role of the testicular capsule and effect of gonadotropins and prostaglandins. *Biology of Reproduction*, 22:1073-1081.

Glover, T.D., D'Occhio, M.J. and Millar, R.P. 1990. Male life cycle and seasonality. In: Lamming, G.E. (Ed.), Marshall's Physiology of Reproduction, vol. II, Reproduction in the Male, 4th ed. Churchill Livingstone, London, Pp: 213–378.

Gustafsson, B.K., Graham, E.F., Crabo, B.G., Pavelko, M.K. and Wagner, W.C. 1977. Pre-freeze supplementation of ram semen with PGE1 and PGF2 alpha: effects on sperm vitality in vitro and on sperm transport in the ewe. In: Proceedings Annual Meeting Society Study Reproduction, (Abstract).

Hafs, H.D., Louis, T.M. and Waters, R.J. 1974. Increased sperm output of rabbits and bulls treated with prostaglandin F2 alpha. *Prostaglandins*, 8: 417-422.

Hashizume, T. and Niwa, T. 1984. Effect of administration of prostaglandin F2α (PGF2α) on the properties of sperm rich fraction of boar semen. *Japan Journal of Animal Reproduction*, 30: 182–185.

Hemsworth, P.H., Donnelly, J. and Findlay, J.K. 1977. The effects of prostaglandin F2alpha on sperm output in boars. *Prostaglandins*, 13:933-939.

Henry, J.B. 1974. Clinical diagnosis and management by laboratory method. *American Journal of Clinical Science*, 61: 108-115.

Ibrahim, S.A. 1997. Seasonal variations in semen quality of local and crossbred rams raised in the United Arab Emirates. *Animal Reproduction Science*, 49: 161–167.

Kafi, M., Safdarian M. and Hashemi, M. 2004. Seasonal variation in semen characteristics, scrotal circumference and libido of Persian Karakul rams. *Small Ruminant Research* 53: 133–139.

Karagiannidis, A., Varsakeli, S., Alexopoulos, C. and Amarantidid, I. 2000. Seasonal variation in semen characteristics of Chios and Friesian rams in Greece. *Small Ruminant Research*, 37: 125–130.

King, P.R.N. and King, E.J. 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-anti-antipyrine. *Journal of Clinical Pathology*, 7: 322-326.

Kreider, J.L., Ogg, W.L. and Turner, J.W. 1981. Influence of prostaglandin F2 alpha on sperm production and seminal characteristics of the stallion. *Prostaglandins*, 22: 903-908.

Lincoln, G.A. and Short R.V. 1980. Seasonal breeding: nature's contraceptive. *Recent Progress in Hormonal Research*, 36: 1–52.

Makawi, S.A., Elsharif, B.A. and Babiker, E.A. 2007. Effect of season on freezability of semen from two breed types of desert sheep in the Sudan. *Journal of Animal Veterinary Advances*, 6: 846-849.

Marshall, C.E. and Hafs, H.D. 1976. Sperm output and quality after PGF2a in bulls. *Journal of Animal Science*, 43:296.

Masoumi, R., Towhidi, A., Nabizadeh, H., Zhandi, M., and Javaremi, A.N. 2011. Influence of PGF2α on semen quality and libido in Holstein bulls. *Turkish Journal of Veterinary and Animal Science*, 35: 1-6.

Mekonnen, G., Boland, M. and Gordon, I. 1989. The effect of prostaglandin on semen production and libido in the ram. *Irish Veterinary Journal*, Jan/Feb: 56-62.

Narasimha, A.V., Haranath and G.B., Visweswara, C. 1986. Effect of prostaglandin F2 alpha on libido, seminal quantity and quality of buffalo bulls. *Theriogenology*, 25: 689-694.

Perez, G.R., Lopez, A., Castrillejo, A., Bielli, A., Laborde, D., Gastel, T., agle, R., Queirolo, D., Franco, J., Forsberg, M. and Rodriguez-Martinez, H. 1997. Reproductive seasonality of Corriedale rams under extensive rearing conditions. *Acta Veterinary Scandinavia*, 38, 109–117.

Piperelis, S.G., Vafiadis, D., Boscos, C.M., Brozos, C., Kiossis, E. and Alexopoulos, C. 2008. Efficiency Assessment of a Swift Method to Enhance Substandard Viability Ram Ejaculates. *Reproduction in Domestic Animal*, 43: 111–116.

Pursel, V.G., Johnson, I.A. and Gerrits, P.J. 1968. Glutamic oxaloacetic transaminase and lactic dehydrogenase activities of boar spermatozoa and seminal plasma. *Journal of Reproductive Fertility*, 18: 176–177.

Reichard, L.A., Hafs, H.D. and Haynes, N.B. 1978. Sperm output and serum testosterone in rabbits given prostaglandin F2alpha or E2. *Prostaglandins*, 16: 135-138.

Reitman, S. and Frankel, S.A. 1957. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28, 56–63.

Roussal, J.D. and Stallcup, O.T. 1965. Activity of lactic dehydrogenase and its isozymes in bovine semen. *Journal of Dairy Science*, 48, 1506.

Saksena, S.K., Lau, I.F. and Chang, M.C. 1978. Effects of prostaglandin-F2 alpha on some reproductive parameters of fertile male rats. *Prostaglandins*, 1:107-111.

Shankar, U., Benjamin, B.R. and Agarwal, S.K. 1984. Effect of prostaglandin F2α (PGF2α) on reaction time and semen characteristics of buffalo-bulls. *Indian Journal of Animal Science*, 54:38-40.

Tietz, N.W. 1999. Text book of clinical chemistry. 3rd ed., Saunders Co., USA, Pp: 711-715.

Voglmayr, J.K. 1973. Prostaglandin F2 alpha concentration in genital tract secretions of dairy bulls. *Prostaglandins*, 4: 673-678.

Wells, M.E. and Awa, O.A. 1970. New technique for assessing acrosomal characteristics of spermatozoa. *Journal of Dairy Science*, 53: 227-232.

Yousef, M. I. and Zeitoun, M. M. 1998. Bovine ovarian follicular fluids modulate the release of transaminases, acrosome reaction and motility of the rabbit's sperm in vitro. *Alexandria Journal Agricultural Research*, 43: 17–26.

Zamiri, M.J. and Khodaei, H.R. 2005. Seasonal thyroidal activity and reproductive characteristics of Iranian fat-tailed rams. *Animal Reproduction. Science*, 88:245–255.