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Cloprostenol Injection Improves Reproductive Characteristics in Low Libido Iranian Holstein Bulls

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Abstract: The objective of this study was to determine whether injections of Cloprostenol (PGF2α analog) in low libido Holstein bulls can improve seminal characteristics and libido. Ten low libido Iranian Holstein bulls were randomly assigned to two groups and received; (1) 250 μg of Cloprostenol (n = 5) or (2) 2 mL of saline (n = 5) 30 min prior to collection of semen 2 days per week for 2 months. Reaction time was significantly decreased in treatment group. Duration of ejaculation was significantly increased in treatment group. Semen volume and sperm concentration were greater in treated bulls in compare with controls. The percentage of morphologically normal sperm cells, percentage of live sperm cells, motile sperm cells and post-thaw motile sperm cells were not affected by treatment. Plasma testosterone concentrations were increased approximately two fold after Cloprostenol injection. Overall, injection of Cloprostenol at this dosage and frequency increased libido, semen volume, sperm concentration and plasma testosterone concentration in low libido Holstein bulls.

Key words: Low libido bulls, Cloprostenol, semen characteristics, testosterone

INTRODUCTION

The widespread use of dairy bull semen in artificial insemination requires that semen production be as efficient as possible. Investigators have shown that low libido is one of the most common problems encountered with bulls and one that frequently leads to culling (Fraser, 1968). Attempts to relate differences in reproductive performance among bulls to differences in hormone concentrations have generally been unsuccessful, which may be related to inaccurate assessment of libido (Post *et al*., 1987). Also, it was concluded that acutely suppressing concentrations of testosterone and estradiol will not abolish sexual behavior in boars, but tends to an increase in the number of unsuccessful mounts of an artificial sow (Estienne *et al*., 2004). In that study, the number of false mounts was decreased by treatment with PGF2α. In commercial situations, treatment with PGF2α has been used to expedite mounting behavior, as well as restore libido in boars displaying decreased sex drive (Szurop *et al*., 1985). When administered prior to ejaculation, PGF2α markedly increased the number of spermatozoa in first ejaculates from bulls (Marshall and Hafs, 1976), rabbits (Hafs *et al*., 1974a), boars (Hashizume and Niwa, 1984) and stallions (Cornwell *et al*., 1974). Thus, it was suggested that PGF2α might be useful when collecting semen from bulls for use in artificial insemination (Haynes *et al*., 1975). Under these circumstances, the increases in spermatozoal output must be attributed to enhanced movement of sperm in the excurrent ducts (Hafs *et al*., 1974b). It was demonstrated that nearly all of the sperm produced by a bull can be harvested if semen is collected at a sufficient frequency (Amann, 1970). It was demonstrated that administration of 30 mg PGF2α intramuscularly prior to the collection of semen 2 days per week couldn't increase libido of yearling beef bulls (Berndtson *et al*., 1979).

In a study by Szurop *et al*. (1985), treatment with PGF2α (Enzaprost) restored sexual behavior in older boars exhibiting low sex drive. Administration of PGF2α resulted in increased blood serum testosterone in bulls; the peak and duration of the increased testosterone were proportional to the dose of PGF2α (Haynes *et al*., 1975). Subsequently, it was concluded that Luteinizing Hormone (LH) release was the primary stimulus for increased testosterone secretion in bulls given PGF2α (Kiser *et al*., 1976).

Since, there is no report on the effects of PGF2α in low libido Holstein bulls; this study was designed to investigate the effects of Cloprostenol on reproductive characteristics of such bulls.

MATERIALS AND METHODS

Animals and experimental design: This experiment was conducted at the center of Progeny Testing of Beef and

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Dairy Cattle, Karaj, Iran during November to February 2005. Prior to major experiment, a pre-experiment was conducted for selecting of low libido bulls. In the pre-experiment, 92 bulls were stimulated with two teasers and observed 2 days a week for 15 min during 3 weeks and their reaction times were recorded (Henney *et al*., 1990).

We selected 10 bulls which had more reaction time as low libido bulls. Selected bulls (41 to 100 months of age) were assigned to two group (n = 5) according to similar libido and fixed in its group until end of experiment. Bulls were housed in small free stalls, fed at maintenance level (NRC, 2001) and had free access to water. Body weights of each bull were measured at 30-day intervals throughout the study.

Bulls in the first group were treated with saline (2 mL) and served as control group, whereas bulls in the second group (treated group) received an intramuscular injection of 250 μg Cloprostenol (PGF2α analog, Nasr LTD, Iran) 30 min prior to collection of the ejaculates on each seminal collection day (2 days per week for 2 months). Two ejaculates from each bull were collected on Saturdays and Tuesdays. Semen was collected in an artificial vagina by experienced semen collector.

Two another bulls were used as stimulous animals (teaser) for semen collection. Different teasers were used on different days to minimize bull sexual satiation from a teaser, to provide uniform stimulus pressure and randomized teaser effects.

Blood collection: At the first and last weeks of the experiment when semen was not collected, 3 blood samples were collected at 20 min interval for 1 h after Cloprostenol or saline injection to determine the effect of treatment on plasma testosterone concentration. Blood was collected and transferred into a Heparinzed glass test tube. The tubes containing blood were immediately placed into a 4°C cooler until centrifugation. The tubes were centrifuged (3000 x g 15 min) at 4°C; plasma was aspirated and placed into 5 mL storage vials which were frozen and kept at -20°C until assay.

Hormone assay: Plasma testosterone concentration was measured with RIA, using validated commercial kits (Spectria, Orion Diagnostica, Finland). Sensitivity and Intra-assay CV of testosterone assay were 0.14 ng mL^{-1} and 5.3%, respectively.

Semen evaluation: The volume of semen was measured with graduated tubes. The total number of spermatozoa per ejaculation was measured by Photometer (IMV, France). Fresh and post-thaw sperm motility was also analyzed by placing a sample on a pre-warmed (37°C) microscopic slide covered with a cover slip and examined under a high power microscope at a magnification x200.

Morphological analysis of sperm: Stained semen smears were prepared by mixing 10 μL diluted semen with 40 μL nigrosin-eosin stains for 30 sec to evaluate sperm morphology and viability. The mixed semen and stain were incubated for 2 to 5 min at 37°C before preparing smears on microscope slides and then leaving them to dry. The nigrosin-eosin-stained slides were evaluated by examining 100 spermatozoa per slide in duplicate slides. Viable spermatozoa were defined as those that did not take up stain. Spermatozoa were examined for the following abnormal morphologies: detached head, abaxial head, malformed head and damage to acrosome cap, bent tail, coiled tail and presence of cytoplasmic droplets (Evans and Maxwell, 1989).

Libido: Libido was assessed based on reaction time and duration of ejaculation (ejaculation time) (Estienne and Harper, 2000). Reaction time was defined as the interval from entering the collection room until the start of first mounting. Ejaculation time was recorded according to how long the semen ejaculation took after entering the collection room. Bulls operated on freely and handler had no role.

Statistical analysis: Semen characteristics, libido and plasma testosterone concentration were analyzed utilizing the Proc MIXED of SAS (1996). Percentile data were transformed by arcsin transformation before analyzing. Significant difference was acknowledged if $p<0.05$. Bulls served as the experimental unit. The statistical model included treatment, time and treatment by time as possible sources of variation.

RESULTS AND DISCUSSION

Mean body weight of the bulls had no change during the experiment. Although the bulls in treatment group were about 24 kg heavier than control bulls at the beginning of experiment.

Reaction time in control group (78±3.0 sec) was significantly greater than that in treated group (51±2.1 sec). Reaction time was not affected by time. Duration of ejaculation was significantly increased in treated group (1.5±0.04 sec) compared with control group (1.1±0.02 sec). Duration of ejaculation was not affected by time. Treated group tended to have lesser reaction time and greater ejaculation time than those in control group. Therefore, the treatment had a positive effect on bull's reaction and ejaculation time.

Table 1: Characteristics of semen collected from treated and control bulls

Items	Cloprostenol	SE	Control	SE	p-value
No. of bulls	5.0	-	5.0	-	-
Semen volume (mL)	6.8	0.2	5.7	0.2	0.03
Sperm concentration (million mL^{-1})	1146.0	48.0	969.1	47.0	0.02
Motile sperm cells (%)	53.9	1.6	51.9	1.6	0.23
Post-thaw motility (%)	36.1	0.8	35.6	0.7	0.40
Morphologically normal sperms (%)	85.5	0.3	85.8	0.3	0.36
Live sperms (%)	62.5	1.0	62.9	0.9	0.66

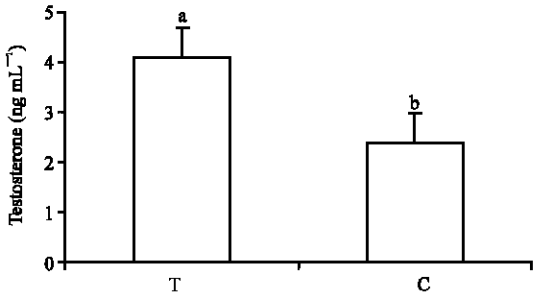

Fig. 1: Plasma testosterone concentration of treated (T) (n = 5) and control (C) (n = 5) group

Data collected for semen characteristics over the course of experiment are shown in Table 1. There were significant effects of treatment on semen volume and sperm concentration. Semen volume (6.8±0.2 mL) in treated group was significantly greater than that in control group (5.7±0.2 mL). Sperm concentration significantly increased in treated group (1146.3±48 million mL^{-1}) compared with control group (969.1±47 million mL^{-1}). Other semen characteristics in treated and control group such as percentage of motile sperm, (53.9 ±1.6 and 51.9±1.6%, respectively), post-thaw motility (36.1±0.6 and 35.6±0.7%, respectively) percentage of morphologically normal sperms (85.5±0.3 and 85.8±0.3%, respectively) and percentage of live sperm (62.5±1.0 and 62.9±0.9%) were not affected by treatment. There was also an effect of treatment by time for percentage of live sperm.

Plasma testosterone concentrations in treated group (4.1±0.6 ng mL^{-1}) were significantly higher than control group (2.3±0.3 ng mL^{-1}). As shown in Fig. 1, treated group had approximately a two fold increase in plasma testosterone concentration.

Experiments investigating the effects of exogenously administered prostaglandins on sexual behavior especially in low libido farm animals have yielded equivocal results. It was our purpose to determine whether Cloprostenol has an effect upon sexual characteristics of low libido bulls. Results of this study indicate that administration of Cloprostenol 30 min prior to semen collection in the bull can improve not only the ejaculate quality, but also the libido of bulls. Improvement in ejaculate quality following Cloprostenol administration was observed most significantly as increased sperm concentration and semen volume.

In a study by Szurop *et al.* (1985), treatment with PGF2α (Enzaprost) restored sexual behavior in older boars exhibiting low sex drive. Injection of 30 mg PGF2α (THAM salt) intramuscularly 30 min prior to semen collection to buffalo bulls on a regular semen collection schedule of twice a day, two days per week for six weeks caused significant reduction in the time to first false mount and the reaction time for the first ejaculations (Narasimha *et al.*, 1986). In that study, it was concluded that PGF2α treatment at the dosage and frequency of administration used may be of some value in improving libido in low-libido buffalo bulls (Narasimha *et al.*, 1986). In a commercial boar study, it was observed that treatment with a PGF2α analog increased the percentage of young boars trained for semen collection after only one or two exposures to the artificial sow (Szurop *et al.*, 1985). In contrast to studies demonstrating positive effects, it was reported that PGF2α treatment did not enhance sexual behavior in boars identified as lacking libido (Wettemann *et al.*, 1992). In that study, im injections of 10 mg PGF2α (Lutalyse) at one minute before exposure to an estrous gilt, or 25 mg PGF2α at 30 min before exposure to an estrous gilt had no effects on genital sniffs, nose to nose contact, nosing the flank, proper mounts or completed matins.

It was shown that injection of 30 mg PGF2α intramuscularly prior to the collection of semen two days per week for 10 weeks had no effect on libido of Herford or Angus bulls (Berndtson *et al.*, 1979). In that study, the bulls which were used were all young. Hence, we suggest that it may be made that older bulls are more likely to have physical/pathological constraints which confound the assessment of libido. In one study, it was reported no influence of PGF2α on the libido of dairy bulls, based on observations during collection of semen with an artificial vagina after the injection of PGF2α (Marshall and Hafs, 1976). In the present study, there were dramatic effects of Cloprostenol treatment on reaction and ejaculation time during the weekly collections. Differences in the effectiveness of prostaglandin therapy to stimulate sexual behavior among studies could be related to initial libido, genetics, age or weight of bulls and boars, different

PGF2α analogues or their doses, or some undetermined management practices. Given the variability in the results, we suggest that the compounds should not be used routinely, but rather judiciously as a potential tool for enhancing libido in certain situations such as stimulating low libido bulls to mount on a teaser or training of young bulls to mount an artificial cow for semen collection.

Some research has been conducted to determine the effects of prostaglandins treatment on semen characteristics in boars and bulls. When administered prior to ejaculation, PGF2α markedly increased the number of spermatozoa in first ejaculates from bulls (Hafs *et al*., 1974a; Marshall and Hafs, 1976), rabbits (Hafs *et al*., 1974b) and stallions (Cornwell *et al*., 1974). Thus, it was suggested that PGF2α might be useful when collecting semen from bulls for use in artificial insemination (Haynes *et al*., 1975).

It was reported that sperm concentration and total number of sperm cells tended to increase after im treatment of boars with PGF2α (Hemsworth *et al*., 1977; Hashizume and Niwa, 1984; Estienne and Harper, 2000). In contrast, it was found no effect of PGF2α treatment on various semen characteristics (Kozink *et al*., 2002). In a study, it was reported that for boars semen collected at 3 day intervals for 28 day, sperm concentration (by 23%) and total number of sperm cells (by 34%) were increased by im treatment with 12 mg of PGF2α. These studies were all limited by low numbers of experimental boars from which semen was collected (Hashizume and Niwa, 1984).

We concluded that Cloprostenol treatment affect some indicators of semen quality. Indeed, semen volume and sperm concentration were significantly greater in treated group. The increase in sperm number in the ejaculate following Cloprostenol administration is not probably due to an increased rate of spermatogenesis. Spermatogenesis is unaffected by collection frequency or short term PGF2α administration (Marshall and Hafs, 1976; Amann, 1970).

It has been established that smooth muscle surrounding the epididymis contracts in response to PGF2α in other species (Hib and Oscar, 1978). Prostaglandin receptors in the epididymis are most plentiful in the distal segments (Bartke and Koerner, 1974) making these areas more sensitive to changes in PGF2α concentration. It seems that endogenous prostaglandins exert more effects on the caudal epididymis than the other segment of epididymis. Caudal epididymis acts as a site of storage for mature spermatozoa. When the caudal epididymis contracts in response to PGF2α, mature spermatozoa are moved into the deferent duct where they are available for ejaculation. It was clearly shown that PGF2α administration to anesthetized rabbits resulted in redistribution of spermatozoa from the epididymis to the deferent duct (Hafs *et al*., 1974a). In addition to the effects that PGF2α has on smooth muscle of the epididymis, the testicular capsule also contracts in response to PGF2α (Free *et al*., 1980; Henney *et al*., 1990; Cosentino and Cockett, 1986). Although not specifically investigated in this study, it is likely that contraction of the testicular capsule in response to Cloprostenol plays a role in increasing the number of spermatozoa available for ejaculation. A significant change in seminal volume in our study could be indicative of altered accessory sex gland function and (or) an influence on ejaculation. Indeed duration of ejaculation in our study was greater in treated group.

We concluded that the percentage of morphologically normal sperm cells and live sperm cells were similar for treated and control group. In agreement with these results, the others reported that administration of PGF2α to bulls prior to ejaculation did not influence the motility of either fresh or frozen spermatozoa (Marshall and Hafs, 1976).

The administration of Cloprostenol 30 min prior to semen collection in our study resulted in an increase in the plasma concentration of testosterone. These observations are consistent with other reports that administration of 30 mg PGF2α to bulls caused a surge in plasma testosterone levels (Haynes *et al*., 1975; Berndtson *et al*., 1979). The mechanism by which PGF2α influences testosterone secretion is not fully understood and the findings are really equivocal. As suggested, it may be that PGF2α acts in part directly on the testes to enhance testosterone secretion (Haynes *et al*., 1975). Prostaglandin F2α is known to decrease testicular blood flow in rats (Einer-Jensen and Soofi, 1974). That may not have occurred in our experimental bulls because there was no decline in blood testosterone after PGF2α injection, although testicular blood flow can vary without affecting systemic testosterone concentration. The existence of specific binding sites for PGF2α in Leydig cells has not yet been investigated, but another study suggests that such sites may be present (Fuchs and Chantharaksri, 1981).

In conclusion, the results suggested that there were positive effects of long-term treatment with Cloprostenol on some indicators of semen quality such as semen volume, sperm concentration and reaction and ejaculation time in bulls. Given the variability in the results, we concluded that the compounds should not be used routinely, but rather judiciously as a potential tool for enhancing libido in certain situations such as stimulating low libido bulls to mount a teaser for semen collection.

REFERENCES

Amann, R.P., 1970. Sperm Production Rates. In: The Testis, Johnson, A.D., W.R. Gomes and N.L. VanDemark (Eds.). Vol. 1, Academic Press.

Bartke, A. and S. Koerner, 1974. Androgenic regulation of the concentration of prostaglandin F in the male reproductive system of rats and mice. Endocrinology, 95: 1739-1743.

Berndtson, W.E., P.J. Chenoweth, G.E. Jr. Seidel, B.W. Pickett and T.T. Olar, 1979. Influence of prostaglandin F2 alpha on spermatogenesis, spermatozoal output, seminal quality, testosterone levels and libido of yearling beef bulls. J. Anim. Sci., 49: 736-742.

Cornwell, J.C., K.L. Koonce and J.L. Kreider, 1974. Effect of prostaglandin F2 alpha on seminal characteristics of the stallion. J. Anim. Sci., 38: 226-230 (Abstr.).

Cosentino, M.J. and A.T. Cockett, 1986. Structure and function of the epididymis. Urol. Res., 14: 229-240.

Einer-Jensen, N. and G. Soofi, 1974. Decreased blood flow through rat testis after intratesticular injection of prostaglandin F2 alpha. Prostaglandins, 7: 377-382.

Estienne, M.J. and A.F. Harper, 2000. PGF2 alpha facilitates the training of sexually active boars for semen collection. Theriogenology, 54: 1087-1092.

Estienne, M.J., A.F. Harper, J.W. Knight, C.R. Barb and G.B. Rampacek, 2004. Sexual behavior after treatment with prostaglandin-F2 alpha in boars with suppressed concentrations of gonadal steroids. Applied Anim. Behav. Sci., 89: 53-57.

Evans, G. and W.M.S. Maxwell, 1989. Salmon's Artificial Insemination of Sheep and Goat. University Press, Sydney, pp: 208.

Fraser, A.F., 1968. Behaviour Disorders in Domestic Animals. In: Abnormal Behaviour in Animals, Fox M.W. (Ed.). Saunders, Philadelphia, USA.

Free, M.J., R.A. Jaffe and D.E. Morford, 1980. Sperm transport through the rete testis in anesthetized rats: Role of the testicular capsule and effect of gonadotropins and prostaglandins. Biol. Reprod., 22: 1073-1078.

Fuchs, A.R. and U. Chantharaksri, 1981. Prostaglandin F2 alpha regulation of LH-Stimulated testosterone production in rat testis. Biol. Reprod., 25: 492-501.

Hafs, H.D., T.M. Louis and J.N. Stellflug, 1974a. Increased sperm numbers in the deferent duct after prostaglandin F2 alpha in rabbits. Proc. Soc. Exp. Biol. Med., 145: 1120-1125.

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

Hashizume, T. and T. Niwa, 1984. Effect of administration of prostaglandin F2 alpha (PGF2 alpha) on the properties of sperm rich fraction of boar semen. Jap. J. Anim. Reprod., 30: 182-185.

Haynes, N.B., H.D. Hafs, R.J. Waters, J.G. Mannas and A. Riley, 1975. Stimulatory effect of prostaglandin F2 alpha on the plasma concentration of testosterone in bulls. J. Endocrinol., 66 (1): 329-338.

Hemsworth, P.H., J. Donnelly, J.K. Findlay and D.B. Galloway, 1977. The effects of prostaglandin F2 alpha on sperm output in boars. Prostaglandins, 13: 933-941.

Henney, S.R., G.J. Killian and D.R. Deaver Libido, 1990. Hormone concentrations in blood plasma and semen characteristics in Holstein bulls. J. Anim. Sci., 68: 2784-2792.

Hib, J. and P. Oscar, 1978. Effects of prostaglandin and indomethacin on rat epididymal responses to norepinipherine and acetylcholine. Arch. Androl., 1: 43-47.

Kiser, T.E., H.D. Hafs and W.D. Oxender, 1976. Increased blood LH and testosterone after administration of PGF2a in bulls. Prostaglandins, 11: 545-553.

Kozink, D.M., M.J. Estienne, A.F. Harper and J.W. Knight, 2002. The effect of lutalyse on the training of sexually inexperienced boars for semen collection. Theriogenology, 58: 1039-1045.

Marshall, C.E. and H.D. Hafs, 1976. Sperm output and quality after PGF2α in bulls. J. Anim. Sci., 43: 296-299 (Abstr.).

Narasimha, A.V., G.B. Haranath and C. Visweswara, 1986. Effects of prostaglandin F2 alpha on libido, seminal quantity and quality of buffalo bulls. Theriogenology, 25: 689-692.

NRC., 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. Edn. Natl. Acad. Press, Washington DC.

Post, T.B., H.P. Christensen and G.W. Seifert, 1987. Reproductive performance and productive traits of beef bulls selected for different levels of testosterone response to GnRH. Theriogenology, 27: 317-328.

SAS., 1996. SAS user's guide: Statistics, version 6.12. Institute Inc., Cary, NC.

Szurop, I., A. Nagy and W. Jochle, 1985. Stimulation of libido in pubertal and mature boars with prostaglandin F2 alpha analogs: Clinical observations. Zuchthyg, 20: 83-86.

Wettemann, R.P., S. Welty and D.K. Bishop, 1992. An attempt to stimulate sexual behavior of boars. Oklahoma Agriculture Experiment Station. Anim. Sci. Rep., pp: 410-412.