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Influence of $PGF_{2\alpha}$ on semen quality and libido in Holstein bulls

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Abstract: The aim of this study was to determine the effects of Cloprostenol ($PGF_{2\alpha}$ analogue) on semen quality and libido in Holstein bulls. Ten low libido Iranian Holstein bulls were randomly allocated to 2 groups and received Cloprostenol (n = 5) or saline (n = 5) 30 min prior to the semen collection 2 days per week for 2 months. Reaction time was significantly decreased in the treatment group. Duration of ejaculation was significantly increased in the treatment group. Semen volume and sperm concentration were higher in treated bulls when compared 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 the Clopostenol. Plasma testosterone concentrations were increased after Cloprostenol injection. It was concluded that injection of $PGF_{2\alpha}$ improved libido, semen quality, and plasma testosterone concentration in low libido Holstein bulls.

Key words: Libido, Cloprostenol, semen, bull

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 (1). 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 (2). Also, it was shown 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 a boar to a dummy sow (3). In that study, the number of false mounts was decreased by the treatment with $PGF_{2\alpha}$. In commercial situations, treatment with $PGF_{2\alpha}$ has been used to expedite mounting behavior, as well as restore libido in boars displaying decreased sex drive (4). When administered prior to ejaculation, $PGF_{2\alpha}$ markedly increased the number of spermatozoa in the first ejaculates from bulls (5-6), rabbits (5), and boars (7). Thus, it was suggested that $PGF_{2\alpha}$ might be useful when collecting semen from bulls for use in artificial insemination (8). Under these circumstances, the increases in spermatozoal output must be attributed to enhanced movement of sperm

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in the excurrent ducts (9). It was demonstrated that nearly all of the sperm produced by a bull can be harvested if semen is collected at sufficient frequency (10). It was demonstrated that administration of 30 mg $PGF_{2\alpha}$ intramuscularly prior to the collection of semen 2 days per week could not increase the libido of yearling beef bulls (11).

In a study (4), treatment with $PGF_{2\alpha}$ (Enzaprost) restored sexual behavior in older boars exhibiting low sex drive. Administration of $PGF_{2\alpha}$ resulted in increased the level of blood serum testosterone in bulls; the peak and duration of the increased testosterone were proportional to the dose of $PGF_{2\alpha}$ (8). Subsequently, it was concluded that luteinizing hormone (LH) release was the primary stimulus for the increased testosterone secretion in bulls injected with $PGF_{2\alpha}$ (12).

Since there is no report on the effects of $PGF_{2\alpha}$ in low libido Holstein bulls, this study was designed to investigate the effects of Cloprostenol on some reproductive characteristics of such bulls.

Material and methods

Animals and experimental design

This experiment was conducted at the center of Progeny Testing of Dairy Cattle, Karaj, Iran, during November to February 2005. Prior to the major experiment, a preliminary experiment was conducted for selecting of low libido bulls. In the preliminary experiment, 92 bulls were stimulated with 2 teasers and observed 2 days a week for 15 min during 3 weeks, and their reaction times were recorded (13).

We selected 10 bulls which had more reaction time as low libido bulls. Selected bulls (41 to 100 months of ages) were assigned to 2 groups (n = 5) according to similar libido and fixed in its group until the end of the experiment. Bulls were housed in small free stalls, fed at maintenance level (14), and had free access to water. Body weights of bulls were measured at 30-day intervals throughout the study.

Bulls in the first group were treated with saline (2 mL) and served as the control group, whereas bulls in the second group (treatment group) received an intramuscular injection of 250 mg Cloprostenol ($PGF_{2\alpha}$ analogue, Nasr LTD, Iran) 30 min prior to the 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 using an artificial vagina by an experienced semen collector.

Two different bulls were used as teaser animals for semen collection. Teaser bulls were used on different days to minimize bull sexual satiation from a teaser, and 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 the treatment on plasma testosterone concentration. Blood was collected and transferred into a Heparinized glass test tube. The tubes containing blood were immediately placed into a 4 °C cooler until centrifugation. The tubes were centrifuged (1000 × *g* for 15 min) at 4 °C; then 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 coefficient of variation of testosterone assay were 0.14 ng/mL 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 a Photometer (IMV, France). Fresh and post-thaw sperm motilities were 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 of ×200.

Morphological analysis of sperm

Stained semen smears were prepared by mixing 10 µL diluted semen with 40 µL nigrosin-eosin stains for 30 s to evaluate the 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 a 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 damaged acrosome cap, bent tail, coiled tail and presence of cytoplasmic droplets (15).

Libido

Libido was assessed based on the reaction time and duration of ejaculation (ejaculation time) (16). 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 the handler had no role.

Statistical analysis

Semen characteristics, libido, and plasma testosterone concentration were analyzed utilizing the Proc MIXED of SAS (17). 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

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 the experiment.

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

Data collected for semen characteristics over the course of experiment are shown in the Table. There were significant effects of treatment on semen volume and sperm concentration. Semen volume (6.8 ± 0.2 mL) in the treatment group was significantly greater than that in the control group (5.7 ± 0.2 mL). Sperm concentration significantly increased in the treatment group (1146.3 ± 48 million/mL) compared with the control group (969.1 ± 47 million/mL). Other semen characteristics in the treatment and control groups 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%, respectively) were not affected by the treatment. Percentage of live sperm was affected by time in the experiment.

Plasma testosterone concentrations in the treatment group (4.1 ± 0.6 ng/mL) were significantly higher than those in the control group (2.3 ± 0.3 ng/mL). As shown in the Figure, the treatment group

Table 1. Characteristics of semen collected from treated with Cloprostenol and control bulls.

Item	Cloprostenol	SE	Control	SE	P-value
No. of bulls	5	-	5	-	-
Semen Volume (mL)	6.8	0.2	5.7	0.2	0.03
Sperm concentration (million/mL)	1146	48	969.1	47	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	0.4
Morphologically normal sperms (%)	85.5	0.3	85.8	0.3	0.36
Living sperms (%)	62.5	1.0	62.9	0.9	0.66

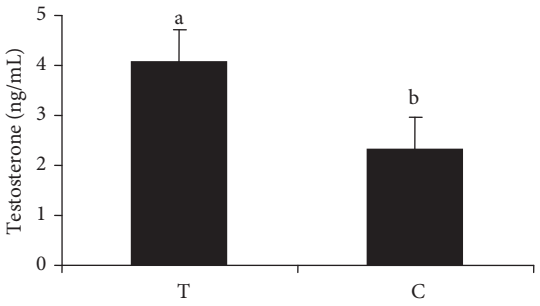

Figure 1. Plasma testosterone concentration of treatment (T) (n = 5) and control (C) (n = 5) groups.

had approximately a 2-fold increase in plasma testosterone concentration.

Discussion

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 on the sexual characteristics of low libido bulls. Results of the present 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 libidos of bulls. Improvement in ejaculate quality following Cloprostenol administration was observed most significantly as increased sperm concentration and semen volume.

In a study (4), treatment with $PGF_{2\alpha}$ (Enzaprost) restored sexual behavior in older boars exhibiting low sex drive. Injection of 30 mg $PGF_{2\alpha}$ (THAM salt) intramuscularly 30 min prior to semen collection to buffalo bulls on a regular semen collection schedule of twice a day, 2 days per week for 6 weeks caused significant reduction in the time to first false mount and the reaction time for the first ejaculations (18). In that study, it was concluded that $PGF_{2\alpha}$ treatment at the dosage and frequency of administration used may be of some value in improving libido in low-libido buffalo bulls. In a commercial boar stud, it was observed that treatment with a $PGF_{2\alpha}$ analog increased the percentage of young boars trained for semen collection after only 1 or 2 exposures to the dummy sow (4).

It was shown that injection of 30 mg $PGF_{2\alpha}$ intramuscularly prior to the collection of semen 2 days per week for 10 weeks had no effect on the libido of Hereford or Angus bulls (11). In that study, bulls were young. Hence, we could conclude that older bulls are more likely to have physical/pathological constraints which confound the assessment of libido. In one study, no influence of $PGF_{2\alpha}$ on the libido of dairy bulls was reported, based on the observations during the collection of semen with an artificial vagina after the injection of $PGF_{2\alpha}$ (6). In the present study, there were dramatic effects of Cloprostenol treatment on the 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 the initial libido, genetics, age or weight of bulls and boars, different $PGF_{2\alpha}$ 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 a dummy cow for semen collection.

Some research has been conducted to determine the effects of prostaglandin treatment on semen characteristics in boars and bulls. When administered prior to ejaculation, $PGF_{2\alpha}$ markedly increased the number of spermatozoa in the first ejaculates from bulls (5,6) and rabbits (5). Thus, it was suggested that $PGF_{2\alpha}$ might be useful when collecting semen from bulls for use in artificial insemination (8).

It was reported that sperm concentration and total number of sperm cells tended to increase after IM treatment of boars with $PGF_{2\alpha}$ (7,16,19). In contrast, it was found that there was no effect of $PGF_{2\alpha}$ treatment on various semen characteristics (20). In a study, it was reported that for boars semen collected at 3-day intervals for 28 days, sperm concentration (by 23%) and total number of sperm cells (by 34%) were increased by IM treatment with 12 mg of $PGF_{2\alpha}$. These studies were all limited by low numbers of experimental boars from which semen was collected (7).

We concluded that Cloprostenol treatment affects some indicators of semen quality, and semen volume

and sperm concentration were significantly greater in the treatment group. 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 (6,10).

It has been established that smooth muscle surrounding the epididymis contracts in response to $PGF_{2\alpha}$ in other species (21). Prostaglandin receptors in the epididymis are most plentiful in the distal segments (22), making these areas more sensitive to the changes in $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$, mature spermatozoa are moved into the deferent duct where they are available for ejaculation. It was clearly shown that $PGF_{2\alpha}$ administration to anesthetized rabbits resulted in the redistribution of spermatozoa from the epididymis to the deferent duct (5,9). In addition to the effects of $PGF_{2\alpha}$ on the smooth muscle of the epididymis, the testicular capsule also contracts in response to $PGF_{2\alpha}$ (13,23,24). Although not specifically investigated in this study, it is likely that the 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 the seminal volume in our study could be an indicator of altered accessory sex gland function and (or) an influence on ejaculation. In the present study, duration of ejaculation was found greater in the treatment group.

We concluded that the percentage of morphologically normal sperm cells and living sperm cells were similar for the treatment and control groups. 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 neither fresh nor frozen spermatozoa (6).

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 $PGF_{2\alpha}$ to bulls caused a surge in the plasma testosterone levels (8,11). The mechanism by which $PGF_{2\alpha}$ influences testosterone secretion is not fully understood and the findings are really equivocal. As suggested, it may be that $PGF_{2\alpha}$ acts in part directly on the testes to enhance the testosterone secretion (8). $PGF_{2\alpha}$ is known to decrease the testicular blood flow in rats (25). That may not have occurred in our experimental bulls because there was no decline in the blood testosterone after $PGF_{2\alpha}$ injection, although testicular blood flow can vary without affecting systemic testosterone concentration. The existence of specific binding sites for $PGF_{2\alpha}$ in Leydig cells has been suggested (26).

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.

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