



**Full Length Research Article**

**QUALITY OF SEMEN COLLECTED BY TRANSRECTAL MASSAGE IN BULLS RESTRAINED IN THE PRESENCE OF A COW**

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**ABSTRACT**

The purpose of this experiment was to determine the effect of PGF and GnRH treatment and exposure of bulls to estrus-cows on semen quality collected by transrectal massage. Three fertile, pure Frisian bulls, 2-3 years old, were followed up weekly for four periods of treatment (control, exposure to estrus-cows, PGF and GnRH). The bulls exposure to a cow in estrus showed high semen volume ( $3.3 \pm 1.3$  ml), percentage of progressive motility of sperm ( $73.3 \pm 12.0$  %;  $P < 0.05$ ) and sperm concentration ( $996.6 \pm 141.6 \times 10^6$ /ml;  $P < 0.05$ ) compared to other treatments. The pH ( $8.2 \pm 0.3$ ;  $P < 0.05$ ) and morphological abnormalities ( $18.1 \pm 4.5$  %) were higher in the control animals compared to other treatments. Live sperm percentages were higher in GnRH treatment ( $72.8 \pm 3.7$ %) than other treatments. However, the difference was not significant. We conclude that transrectal massage was very effective for producing semen and acceptable when the bulls exposure to a estrus-cow was >60 minutes before collection of semen. The lower quality semen after treatment by PGF and GnRH requires further investigation.

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**INTRODUCTION**

Artificial insemination (AI) has very important roles in improving milk and meat production in cattle breeding. The other advantages of AI are genetic selection schemes and long-term storage of genetic materials. However, the AI program depends on selection of superior genetic males, methods of semen collection and freezing. So far, the method of semen collection by using an artificial vagina has been the best method in all AI center when using intact bulls. In bulls suffering with old age, locomotion issues, aggressive behavior or in bulls used for breeding soundness evaluations, the electro ejaculation method is used. Criteria for classification of semen quality in these cases have been established over many decades based on samples obtained by electro ejaculation. However, electro ejaculation is considered by some to be inhumane on the grounds that it is painful (Palmer, 2005). Therefore, transrectal massage of the ampulla may be of some use as an alternative to electro ejaculation in docile bulls and may also be used to decrease the duration of subsequent

electro ejaculation. However, transrectal massage for semen collection is often less consistent in harvesting semen of acceptable quality. The usefulness of this method in breeding soundness evaluations needs to be investigated further. Consequently, the main objective of this study is to improve semen yield by using transrectal massage in bulls which have given PGF or GnRH hormones or subjected to cows in estrus before semen collection.

**MATERIALS AND METHODS**

**Animals:** This study was conducted at the agriculture and veterinary research center, Qassim University. Three mature bulls, age 3 years, were selected. Bulls were kept in open semi shaded pens and each animal was identified by a plastic ear tag. One manual long chute was used next to the pens to facilitate collection of semen and administration of hormones. Bulls were offered regular feed staffs (concentrated feed and alfalfa) at the optimum requirements. Additionally, clean tap water and salt licks were offered as free choice.

**Semen samples:** Four treatments were used for three bulls. Every treatment was done weekly. Bulls received prostaglandin  $F_{2\alpha}$  (500 mcg of cloprostenol; Estrumate),

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gonadotropine releasing hormone (250 ug of GnRH; Gonabred) or were exposed to estrus-cows 2-3 hours before attempting semen collection by transrectal massage. The control is no treatment. Transrectal massage, conducted by holding the thumb and little finger in direct apposition with the ampulla while applying a gentle anterior to caudal motion. Massage was applied to the ampulla for a maximum of 5 min or until a semen sample was obtained and immediately transferred to a 37°C water bath. The semen was investigated immediately for volume (ml), color (cloudy to milky), pH, concentration ( $10^6$ /ml), motility (%), vital stain (%) and morphology (%). Semen volume and color were recorded by reading from graduated tubes and sperm concentration was measured using a haemocytometer. The pH of semen was measured using a pH meter. Subjective sperm motility assessment and viability (eosin-nigrosin) tests were carried out using a light microscope. Percent progressively motile sperm was determined with light microscopy at 400× magnification after placing a cover slip over a 2–4mm drop of semen on a warmed microscope slide. Quantitative data were analyzed by one way ANOVA using (SPSS, version 16).

## RESULTS

The semen quality following treatment in control, exposure to estrus-cows, PGF and GnRH treatment is shown in Table 1. The volume of semen was higher in bulls exposed to estrus-cows ( $3.3 \pm 1.3$ ) compared to other treatments. However, the difference was not significant. The percentage of progressive motility of sperm was significantly higher ( $P < 0.05$ ) in bulls exposed to estrus-cows ( $73.3 \pm 12.0$ ), PGF ( $67.5 \pm 8.5$ ) and GnRH ( $67.5 \pm 6.2$ ) than in the control ( $40.0 \pm 9.3$ ). The pH was significantly higher ( $P < 0.05$ ) in the control ( $8.2 \pm 0.3$ ) than other treatments. Live sperm percentage was higher in the GnRH treatment ( $72.8 \pm 3.7$ ), though not significantly higher, than other treatments. The concentration of sperm was significantly higher ( $P < 0.05$ ) in bulls exposed to estrus-cows ( $996.6 \pm 141.6$ ) than in other treatments. Morphological abnormality was higher in the control ( $18.1 \pm 4.5$ ) than in other treatments. However, the difference was not significant.

stimulated by estrus-cows. It may indicate that sexual stimulation by the presence of a cow induces a greater endogenous oxytocin release (Thackare *et al.*, 2006). Spermatozoa are not able to display their motility within the male tract and hence need to be transported from the testis following spermiation. In the epididymis, spermatozoa undergo maturation and can be stored, for extended periods of time in some species, until their release into the ductus deferens at ejaculation. Oxytocin has been postulated to modulate contractility of the male tract to regulate sperm transport and maturation. Oxytocin hastens the matured sperm transport from epididymis to ampulla and sexual glands secretions. Knight (1974) showed that in the ram, oxytocin has a summative effect on epididymal contractions when administered with adrenaline. Adrenergic blocking drugs inhibited the adrenaline-stimulated contractions but had no effect on Oxytocin-induced contractions (Knight, 1974).

Oxytocin has also been shown in vivo to have a specific action on the ram epididymis and increase sperm transport (Nicholson *et al.*, 1999). Oxytocin (10 and 100  $\mu$ g) significantly increased both fluid output and the number of spermatozoa in the luminal fluid of the cauda epididymis within 10 min of treatment, and the effect was dose dependent (Nicholson *et al.*, 1999). Treatment with an oxytocin antagonist significantly reduced the fluid output and spermatozoal numbers while pre-treatment with an oxytocin antagonist inhibited the stimulatory effects of oxytocin indicating that oxytocin acts on epididymal contractility through its own cognate receptor (Nicholson *et al.*, 1999). This stimulatory effect suggests that oxytocin affects epididymal contractility directly leading to a significant increase in the transport of spermatozoa into the vas deferens and the ejaculate. This may be the reason for the higher semen volume, sperm motility and sperm concentration of samples in bulls exposure to estrus cows compared to those collected from other treatments. PGF, or in this case an analog cloprostenol had some effect on sperm motility, percent live sperm or sperm concentration compared with the control but was low compared to others.

**Table 1. Semen quality following treatment with PGF, GnRH or exposure to estrus-cow in three bulls**

Items	Control	Exposure to estrus-cows	PGF	GnRH
Wave motion	2.7±0.4 <sup>a</sup>	4.0±0.5 <sup>a</sup>	3.5±0.5 <sup>a</sup>	3.0±0.0 <sup>a</sup>
Percentage of progressive motility (%)	40±9.3 <sup>b</sup>	73.3±12.0 <sup>a</sup>	67.5±8.5 <sup>a</sup>	67.5±6.2 <sup>a</sup>
Volume (ml)	2.2±0.1 <sup>a</sup>	3.3±1.3 <sup>a</sup>	2.0±0.3 <sup>a</sup>	1.5±0.3 <sup>a</sup>
pH	8.2±0.3 <sup>a</sup>	7.0±0.0 <sup>b</sup>	7.2±0.2 <sup>b</sup>	6.7±0.2 <sup>b</sup>
live sperm (%)	51.9±5.0 <sup>a</sup>	69.7±5.1 <sup>a</sup>	59.0±6.1 <sup>a</sup>	72.8±3.7 <sup>a</sup>
Concentration ( $10^6$ /ml)	190.8±93.0 <sup>b</sup>	996.6±141.6 <sup>a</sup>	439.0±173.1 <sup>b</sup>	49.8 <sup>b</sup> ±308.7
Normal sperm (%)	81.7±4.5 <sup>a</sup>	87.6±5.2 <sup>a</sup>	85.8±1.6 <sup>a</sup>	91.2±1.5 <sup>a</sup>
Abnormal head of sperm (%)	10.6±4.0 <sup>a</sup>	4.7±1.6 <sup>a</sup>	11.7±2.0 <sup>a</sup>	6.3±1.3 <sup>a</sup>
Abnormal tail of sperm (%)	7.4±1.8 <sup>a</sup>	7.5±5.2 <sup>a</sup>	2.2±0.4 <sup>a</sup>	0.6 <sup>a</sup> ±2.2
Total abnormality (%)	18.1±4.5 <sup>a</sup>	12.2±5.2 <sup>a</sup>	14.0±1.6 <sup>a</sup>	8.5±1.5 <sup>a</sup>

Values with different superscript in the same row differ significantly on  $P < 0.05$ ; ANOVA.

## DISCUSSION

One of the primary requirements for the successful use of AI is a supply of fertile semen. Fertile bulls and their proper management are essential. The correct technique in the collection of semen is necessary so that maximum efficiency can be obtained. Measures, or evaluations of semen and semen processing will be taken up in later exercises (Mader and Price, 1984). The results of this study showed that semen volume and motility of sperm were high in bulls when

These results are in agreement with a previous study in which PGF i.m. was used prior to transrectal massage and electro ejaculation (Palmer *et al.*, 2004). But in another study by Berndtson *et al.*, (1979), PGF had no effect semen parameters. It has been suggested that PGF cannot sustain increases in sperm output beyond that which is achieved with an intensive semen collection schedule (Berndtson *et al.*, 1979), as was the case in the present study. Because it has no effect on spermatogenesis (Berndtson *et al.*, 1979), PGF, or an analog (Cloprostenol), cannot increase sperm output beyond what is

already being produced. It is plausible, that the natural PGF hormone may be more effective than the analog at increasing sperm output. Further research is needed to investigate this possibility. The GnRH test classification was not related to the percentage of progressively motile spermatozoa but was related to the percentage of morphologically normal spermatozoa (Thompson *et al.*, 1992). Therefore, in their study the percentage of morphologically normal spermatozoa was significantly higher ( $P < 0.05$ ) in the bulls with a higher than mean testosterone secretion in response to GnRH injection. However, in this study, the percentage of morphologically normal spermatozoa and live sperm were slightly higher in the bulls in response to GnRH injection. It is possible that since Leydig cells respond consistently to LH, the GnRH response test classification was related, therefore, to the percentage of morphologically normal spermatozoa.

In general, controls (no treatment) have a lower quality semen sample with respect to sperm motility, percent live sperm and concentration of sperm. In addition to having high values in pH and abnormality of sperm morphology. The reasons in this case are: (1) there is no effect of exogenous hormones or sexual stimulation, (2) semen dribbles slowly from the penis resulting in more prolonged exposure to air and ambient temperature on the surface of the semen collection funnel. In addition, due to the frequent lack of penile protrusion with transrectal massage, sperm may be exposed to the environment of the distal preputial cavity (Palmer *et al.*, 2005). It is possible that these are factors that contribute to reduced sperm viability in control samples. It is concluded that semen sample quality can be improved with transrectal massage by sexual stimulation mediated by allowing bulls to observe or being restrained in the presence of a cow in estrus. Therefore, it appears that transrectal massage when bulls are exposed to estrus-cows may be a useful alternative to other methods of semen collection as a means of collecting a semen sample for the purpose of evaluating breeding soundness. Criteria for classification of semen quality in bull breeding soundness evaluations have been established over many decades. Further research is required to evaluate the effectiveness and practicality of transrectal massage as an alternative method of semen collection in range bulls.

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