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# Improvement of sexual behavior and semen quality by therapeutic approach and zinc supplementation on Karan Fries

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ABSTRACT

Sub-fertility is one of the major concerns in crossbred bulls as 42.98% bulls are getting culled due to sub-fertility problems therefore, a study was planned on 18 Karan Fries bulls producing poor quality semen or not mounted. They were randomly grouped into 3 treatment groups of 6 animals each and 2 normal bulls were kept as control. Sexual behavior and semen quality were studied during pre-therapy (1 month), therapy (90 days) and post-therapy (90 days) period. Data were analyzed using ANOVA. During therapy period, first group treated with 10 g of herbal product orally, second group with 0.0082 mg of GnRH intramuscularly at 10 days interval and third group with 80 ppm of zinc for 90 days for each group. The results depicted that herbal and GnRH treatment significantly reduced the reaction time, whereas herbal, GnRH treatment and zinc supplementation significantly improved the libido score and reduced per cent of non-mounts during the therapy and post-therapy period. The volume of semen and level of testosterone improved in all treatments groups. Herbal, GnRH treatment and zinc supplementation increased the average number of good and medium quality ejaculates/month/bull and maximum efficiency achieved during the treatment period. There was increase in percent live sperm and decrease in abnormality in herbal treated and zinc supplemented group. Therefore, it can be concluded that GnRH therapy, herbal treatment and zinc supplementation will be effective to overcome the sub-fertility problem in crossbred bulls.

**Key words**: Crossbred bull, GnRH therapy, Herbal treatment, Semen quality, Sexual behavior, Zinc supplementation

In India contribution of 20.81% crossbred cows for improvement of milk production cannot be ignored, but major constrains were witnessed during use of crossbred bulls. Poor semen quality, freezability and poor libido are the major reasons of culling in crossbred bulls (Khatun *et al*. 2013). To overcome such problems and to maximize the efficiency of breeding bulls researcher have administered hormones (Gauthaman *et al*. 2003, Sieme *et al.* 2004 and Ramchander *et al*. 2004), fed zinc (Osman *et al.* 2000, Kendall *et al*. 2000) and given Ayurvedic treatment (Brown 2000, Giuliano and Allard 2001). Defective secretion of reproductive hormones leads to lack of libido, poor semen quality and poor semen freezability in adult animals. Treatment of bulls with Ayurvedic product and hormone act through hypothalamic-gonadal-axis to improve the libido and semen production performance was attempted so far by different researchers (Severiano *et al*. 2007, Baskaran and Dubey 2004). Besides, genetical and climatic factors, one of the major contributors of sub-fertility problem in crossbred bulls is mineral deficiency especially zinc (Zn) as it plays an important role in improving male fertility as zinc is a major component of various enzymes especially 200 metallo-enzymes, which regulate several cellular metabolic activities. Researchers (Kendall *et al*. 2000, Osman *et al*. 2000) reported that higher concentration of zinc has positive impact on semen quality, may be due to presence of high zinc concentrations in male accessory sex glands, seminal plasma and spermatozoa. Therefore, the study was planned to overcome sub-fertility problem in crossbred bull by therapeutic intervention using hormone, Ayurvedic treatment and zinc supplementation.

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## MATERIALS AND METHODS

The study was carried out at Artificial Breeding Research centre, ICAR-National Dairy Research Institute, Karnal. Bulls were kept in loose housing system and provide 1 h exercise in the bull exerciser, 1 day before semen collection to maximize the libido of bulls and to ensure quality semen production (Singh 2014). Karan Fries bulls (18) that are not fulfilling the criteria of possessing more than 60% progressive motility, not exceeding more than 20% total abnormality and 4% primary abnormalities with post-thaw motility of 40% for more than 3 months were selected. Karan Fries bulls (18) were randomly grouped into 3 treatments of 6 animals each. Experimental period was

categorized as pre-therapy, therapy and post-therapy. During therapy period following regimen was followed

| Treatment groups | No. of animals | Period of observation | Treatments |
|---|---|---|---|
| Control ($T_1$) | 2 | 7 months | Normal fertile bulls (control) |
| Herbal ($T_2$) | 6 | 7 months | 5 g speman powder + 5 g Tentex Forte daily for 90 days (orally)# |
| GnRH ($T_3$) | 6 | 7 months | 0.0084 mg of Buserelin acetate (Synthetic GnRH)* for 90 days at 7 days interval (Intra muscularly after semen collection) |
| Zinc ($T_4$) | 6 | 7 months | 80 PPM extra zinc feeding daily for 90 days (In feed)# |

* Gynarich Intramuscular injection immediately after the end of semen and blood collection at weekly intervals; #, inorganic zinc (zinc sulphate) and Ayurvedic powder mix were given along with concentrate mixture for 90 days.

Immediately after semen collection, semen was evaluated for volume, sperm concentration (hemocytometer), mass activity, eosin-nigrosin staining and HOST. Sexual behavior parameters like reaction time, libido score and per cent non-mount activity were evaluated as per Anzar *et al*. (1993).

*Statistical analysis:* The per cent of semen parameter and other different parameters were calculated by proportion using descriptive statistics. The semen production records were subjected to statistical analysis using ANOVA. Group comparison, percentage data arcsine transformations were also done for data analysis (Snedecor and Cochran 1994).

## RESULTS AND DISCUSSION

Improvement of semen quality and sexual behaviour after zinc supplementation, Ayurvedic and GnRH treatment are presented in Tables 1, 2.

*Sexual behavior:* In $T_1$ group, post-therapy period average reaction time (38.57 sec) was significantly higher ($P<0.01$) as compared to the pre-therapy and therapy period, may be due to summer stress during post-therapy period. Herbal and GnRH treatment significantly ($P<0.01$) reduced the reaction time during therapy (29.96 and 41.10 sec) and post-therapy period (30.90 and 33.89 sec) as compared to the pre-therapy period (44.41 and 62.10 sec). In similar line Hadi (1970) reported the use of Tentex Forte in Nagpuri Murrah buffaloes and Sahiwal bulls, Baskaran and Dubey (2004) reported that use of Buserelin acetate in Holstein Friesian and Jersey bulls reduced reaction time. The decrease in reaction time in case of zinc supplementation is in consonance with finding reported by Osman *et al*. (2000) in buffalo bulls may be due to increased level of circulatory testosterone, which is also evident after GnRH treatment (Jimenez *et al*. 2007).

Herbal treatment, GnRH injection and zinc supplementation significantly ($P<0.01$) improved the libido score from pre-therapy period to therapy period and it reached highest during post-therapy period. This might be due to the increased testosterone level mediated through hormonal-gonadal-axis, resulted in decreased number of mounts before ejaculation (Sieme *et al*. 2004). The improvement of reaction time and libido score by use of herbal product may be due to androgen activity of the herbs as Giuliano and Allard (2001) reported that presence of protodioscin (Brown 2000) and dopamine in the herbal product improved the mounting activity and erection. Non mount frequency (3.13%) encountered while semen collection in control animals during post-therapy period, but it was not found during pre-therapy and therapy period, may be due to better environmental condition as compared to post-therapy period.

In T2, T3 and T4 group, the non-mount frequency was reduced. Reduction of non-mount frequency using herbal treatment may be due to presence of sexually enhancing chemicals protodioscin and dopamine in *T. terrestris* and Mucuna plants, which stimulated the mounting activity followed by better erection (Gauthaman *et al*. 2003). Whereas, in GnRH treatment and zinc supplementation group circulating testosterone level increased from 1.58 ng during pre-therapy period to 2.79 ng during therapy period. In similar line Osman *et al.* (2000) reported reduction of testosterone production in case of zinc deficiency.

Table 1. Mean± SE values of sexual behavior during different therapy periods

| Treatments | Therapy period | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pre | | | During | | | Post | | |
| | Reaction time (sec) | Libido score | % Non mount activity | Reaction time (sec) | Libido score | % Non mount activity | Reaction time (sec) | Libido score | % Non mount activity |
| Control (T1) | $22.97_{b}$ ±4.62 | $8.67_{a}$ ±0.33 | 0 | $26.69_{b}$ ±1.64 | $9.18_{a}$ ±0.38 | 0 | $38.57_{a}$ ±5.87 | $7.70_{b}$ ±1.16 | 3.13 |
| Herbal (T2) | $44.41_{a}$ ±1.42 | $4.33_{b}$ ±0.50 | 16.67 | $29.96_{b}$ ±1. 90 | $6.60_{a}$ ±0.20 | 0.81 | $30.99_{b}$ ±6.36 | $6.47_{a}$ ±0.97 | 0 |
| GnRH (T3) | $62.10_{a}$ ±19.75 | $3.33_{b}$ ±0.76 | 33.33 | $41.10_{ab}$ ±6.34 | $5.21_{a}$ ±0.44 | 8.11 | $33.89_{b}$ ±10.21 | $6.14_{a}$ ±1.64 | 2.33 |
| Zinc (T4) | 32.63±8.97 | $2.33_{b}$ ±0.71 | 46.15 | 27.67±2.81 | $4.50_{a}$ ±0.45 | 7.77 | 33.13±12.43 | $4.87_{a}$ ±2.74 | 7.23 |

Mean bearing different superscript within row differ significantly (*$P<0.05$).

*Seminal characteristics:* The semen quality recorded for different treatment groups in 3 different therapy periods are presented in Table 2. In control group volume, mass activity, individual motility, sperm concentration, acrosome integrity and HOST were nonsignificant (P>0.05) during 3 treatment periods whereas, the per cent live sperm count was significantly reduced during therapy period and post-therapy period as compared to pre-therapy period. In herbal treatment group highest individual motility, concentration, live per cent sperms, acrosome integrity and HOST% was recorded during therapy period. The decline in the values during post-therapy period for above parameters may be due to withdrawal of herbal treatment. The improvement in semen quality may be due to improved spermatogenesis functions of testes and accessory sex gland like prostate and seminal vesicles along with improvement of libido as reported by Pardanani et al. (1976) after treatment with speman powder in oligospermic men.

In GnRH treatment group all semen quality parameters improved. The results are in agreement with Sieme *et al.* (2004) and Ramchander *et al*. (2004) also reported significant improvement in semen volume achieved by GnRH treatment. The improvement of semen quality may be due to stimulatory effect of GnRH leads to release of FSH and LH from anterior pituitary. LH act on leydig cells to synthesize testosterone, which inturn improves the functions of all sex glands. There is also a school of thought that GnRH treatment restores seminiferous tubule function either through FSH and LH or FSH, and testosterone through synthesis of androgen binding protein and secretion of tubular fluid. In case of sperm concentration contradictory finding was reported by Bhaskaran and Dubey (2005) may be due to the variation in GnRH dose.

All seminal parameters were increased during zinc supplementation except concentration (Table 2). The experiment result was in compliance with the earlier findings of Kendall *et al*. (2000). The increase in semen volume after zinc supplementation may be due to improvement of secretory function of accessory sex glands as Zn stimulates spermatogenesis, primary and secondary sex organ's growth and development and function of prostate gland in various species (Kumar *et al.* 2014). Zinc supplementation increased the mass activity and percent motile spermatozoa during supplementation which was in correspondence with the findings of Osman *et al*. (2000) and Kumar *et al.* (2006). Zinc is an important element for tail morphology and sperm motility, and it is involved in the catabolism of lipids in the sperm mid-piece that is the principal source of energy required for the movement of spermatozoa. Zinc supplementation significantly improved (P<0.01) mean percent of live sperm count during and post-therapy periods as compared to the pre-therapy period. The results are in consonance with Kumar *et al.* (2014). The improvement in livability and membrane integrity is due reduction of oxidative stress through production of antioxidative enzymes, superoxide dismutase and

Table 2 Mean ± SE values of semen quality during different therapy periods in KF bulls

| Treatment | Therapy period | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-therapy | | | | | | During therapy | | | | | | Post-therapy | | | | | |
| | Volume | Mass activity | Individual motility | Conc. | Live | Acrosome integrity | HOST | Volume | Mass activity | Individual motility | Conc. | Live | Acrosome integrity | HOST | Volume | Mass activity | Individual motility | Conc. | Live | Acrosome integrity | HOST |
| Control | 4.00 | 2.44 | 48.75 | 925.00 | 88.00 a | 85.88 | 47.25 | 3.78 | 2.36 | 48.33 | 791.03 | 82.54 b | 84.10 | 46.13 | 3.65 | 2.55 | 49.68 | 864.52 | 83.07 b | 84.29 | 48.35 |
| (T1) | ±0.37 | ±0.11 | ±2.80 | ±44.32 | ±0.96 | ±1.20 | ±1.10 | ±0.22 | ±0.13 | ±2.45 | ±43.54 | ±0.92 | ±0.84 | ±1.56 | ±0.21 | ±0.11 | ±2.65 | ±32.971 | ±0.71 | ±0.84 | ±1.40 |
| Herbal | 4.23 | 1.87 | 35.33 | 626.67 | 67.91 b | 79.60 a b | 37.93 | 4.66 | 1.79 | 37.38 | 666.80 | 79.40 a | 82.26 a | 40.19 | 5.06 | 1.58 | 31.75 | 589.58 | 76.43 a | 78.71 b | 35.88 |
| (T2) | ±0.38 | ±0.21 | ±4.15 | ±58.73 | ±1.88 | ±1.42 | ±2.07 | ±0.17 | ±0.08 | ±1.67 | ±25.31 | ±0.84 | ±0.57 | ±0.96 | ±0.18 | ±0.10 | ±1.97 | ±29.921 | ±0.92 | ±0.63 | ±1.03 |
| GnRH | 3.45 $_{b}$ | 1.55 | 33.00 | 630.00 | 71.58 | 79.70 | 36.20 | 4.60 $_{a}$ | 1.75 | 36.23 | 657.97 | 78.87 | 80.06 | 39.04 | 4.88 $_{a}$ | 1.80 | 36.38 | 667.72 | 76.91 | 79.81 | 39.01 |
| (T3) | ±0.52 | ±0.20 | ±4.36 | ±65.49 | ±3.71 | ±1.59 | ±2.32 | ±0.21 | ±0.11 | ±2.14 | ±32.46 | ±1.44 | ±0.79 | ±1.29 | ±0.20 | ±0.11 | ±2.24 | ±33.012 | ±1.04 | ±0.77 | ±1.29 |
| Zinc | 3.93 | 1.71 $_{a}$ | 35.00 $_{a}$ | 685.71 a | 59.41 c | 79.14 a | 37.00 b | 4.39 | 1.84 $_{a}$ | 37.79 $_{a}$ | 682.63 a | 81.73 b | 80.75 a | 40.13 a | 4.88 | 1.36 $_{b}$ | 28.20 $_{b}$ | 549.33 b | 75.21 a | 76.61 b | 33.89 b |
| (T4) | ±0.70 | ±0.24 | ±4.76 | ±64.29 | ±2.227 | ±1.63 | ±2.42 | ±0.164 | ±0.11 | ±2.07 | ±31.88 | ±1.06 | ±0.77 | ±1.24 | ±0.19 | ±0.11 | ±2.269 | ±33.71 | ±1.23 | ±0.77 | ±1.26 |

Mean bearing different superscript within row differ significantly (*P<0.05).

glutathione peroxidase as zinc act as co-factor for the production of antioxidative enzymes.

Zinc supplementation did not show significant improvement (P>0.05) in sperm concentration, but maintained the pre-therapy sperm concentration without further decline during therapy period. However, withdrawal of zinc supplementation significantly reduced the sperm concentration in post-therapy period might be due to disruption of spermatogenesis process.

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