Indian Journal of Animal Sciences **87** (7): 840–843, July 2017/Short communication

Levamisole as an immunomodulator to ameliorate vaccination stress in crossbred bulls

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Received: 26 November 2016; Accepted: 16 January 2017

Key words: Blood profile, Cattle, Levamisole, Semen quality, Trio-vac vaccination

Bulls kept at artificial breeding centre should be healthy with adoption of strict hygienic semen collection and freezing protocol standards to improve the conception rate through artificial insemination. Emphasis has required to be on vaccination, as exotic and crossbred bulls are more prone to diseases (Bhakat *et al.* 2011). Semen stations are losing semen productivity due to mandatory requirement of vaccination, which is a foremost stress factor causing decreased sperm quality (Bhakat *et al.* 2010, Bhakat *et al.* 2011) as a result of post-vaccination rise in body and testis temperature. Information regarding ameliorative measures of vaccination stress through immunomodulation is scanty. Levamisole is immuno-modulator to potentiate the immunity. It belongs to the class of synthetic imidazothiazole derivative. Levamisole refurbishes cell mediated immune response by potentiating the rate of T-lymphocyte differentiation and proliferation of macrophages (Temizel *et al.* 2012) and stimulating phagocytosis by monocytes. The present investigation was therefore undertaken to find out the effect of Levamisole treatment on amelioration of vaccination stress on semen production performance in cross bred bulls.

Karan Fries (HF cross) bulls (8) (weighing 450–580 kg) were selected randomly from Artificial Breeding Research Centre, ICAR-NDRI, Karnal. The bulls were divided into 2 groups (control and levamisole injected), 4 in each. The bulls were maintained under identical and optimal conditions of feeding and management during the course of experiment. Semen was collected once a week from the bulls using sterilized artificial vagina (IMV model-005417) over a male dummy. The seminal attributes were compared between the 2 groups. A total of 144 ejaculates (8 animals × 18 ejaculates) were collected during the trial. Trio-vaccine (FMD, HS, BQ oil adjuvant) was administered @ 3 ml by deep intra muscular route. Levamisole was injected 1 week prior to vaccination @ 2.5 mg/kg body wt to the treatment group only. Semen ejaculates were evaluated during pre (1 week) and post-vaccination period (8 weeks).

Data were analyzed by 2-way ANOVA with interaction and significant difference between means were compared. Prior to analysis of proportionality data of sperm parameters were transformed using arc-sin transformation [asin(sqrt(percent/100))] with adjustment to allow for zero values.

Collection and analysis of semen was done weekly, 1 week before and 8 weeks after vaccination and the results are presented in Tables 1 and 2.

Reaction time (*sec*)*:* Mean reaction time during pre vaccination stage was slightly lower in treatment group as compared to the control group; during post vaccination reaction time was significantly lower in treated group (Table 1).

Reaction time increased during 1 week after vaccination but later animal recovered and showed less reaction time in post-vaccination period in treated group. Increase in reaction time was also reported by Pankaj *et al.* (2007) in vaccinated bulls.

Rectal temperature (*°F*)*:* There was no significant change in rectal temperature pre- and post- vaccination in both control and treatment group bulls. Rectal temperature was higher in first week post vaccination as compared to the pre-vaccination but during second week onwards it became normal.

A significant ($P<0.05$) rise in rectal temperature during post vaccination period, especially first week which reached normal during second week was also reported by Pankaj *et al.* (2007). Testicular or germinal epithelium of testis is affected by the febrile reaction, which impairs heat exchange mechanism in pampiniform plexus. In the present study vaccination has not impacted much in treatment group may be due to less increase in rectal temperature.

Semen quality

Volume (*ml*)*:* During pre-vaccination stage, the semen volume in treatment group was lower but during post vaccination semen volume was higher in treatment group as compared to control (Table 1). The present report of higher semen volume during post-vaccination is in line with

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Table 1. Mean ± SE of reaction time and semen characteristics of levamisole treated group during pre- and post-vaccination in Karan Fries bulls

Parameters	Groups	Pre-vaccination Mean±SE	Post-vaccination Mean ± SE
Mass motility (0-5)	Control	3.91±0.29	3.2±0.08
	Treatment	3.75±0.25	3.52±0.1
Reaction time (Second)	Control	105±10.4	84.69±7.83
	Treatment	98.75±14.77	65.41**±4.3
Volume (ml)	Control	5.87±0.65	4.9±0.24
	Treatment	5.25±0.47	5.77±0.3
Rectal temp (°F)	Control	100.7±0.4	100.79±0.21
	Treatment	101.07±0.32	100.97±0.16
Concentration (million/ml)	Control	907.5±69.92	911.25±35.63
	Treatment	1157.00*±66.87	1129.25**±45.23
Testicular temp (°F)	Control	76.59±1.89	81.18±0.76
	Treatment	74.43±1.67	81.11±0.89
Individual motility (%)	Control	79.68±0.43	66.15±0.1
	Treatment	78.03±0.33	71.95±0.05
NEC (live cells %)	Control	85.26±0.52	72.94±0.35
	Treatment	83.81±0.41	78.67±0.25
Total abnormality (%)	Control	8.67±0.03	10.1±0.02
	Treatment	7.42±0.03	7.33*±0.01
HOST (%)	Control	83.86±0.32	69.15±0.05
	Treatment	80.84±0.33	74.65±0.06
Intact acrosome (IA) (%)	Control	91.84±0.68	78.72±0.79
	Treatment	91.03±0.43	84.2±0.1
R-Value (ml)	Control	4837.5 ±30.37	6043.75±24.95
	Treatment	4875 ±97.37	4410.78±5.96
Osmolality (mOsmol)	Control	258±3.62	240.34±2.35
	Treatment	243.50±2.72	240.69±2.89
Total sperm	Control	5265±587.34	4513.75±220.3
	Treatment	6155±430.67	6582.97**±430.2
Total motile sperm	Control	4197.5±663.05	3120.4±186.4
	Treatment	4865.5±543.69	4832.60**±270.35
Live sperm	Control	4471.77±686.57	3409.73±400.21
	Treatment	5207.75±593.64	5264.46**±410.23
Live sperm/ml	Control	775.82±101.25	680.76±35.23
	Treatment	967.12±95.82	906.48**±25.96
Semen dose harvested	Control	209.87±33.15	156.02±14.89
	Treatment	243.27±42.18	241.63**±20.53

Means bearing *, ** within same column differ significantly (* P<0.05, **P<0.01) for each parameter separately.

reports by Singh *et al.* (2004).

Mass activity (0–5)*:* Pre vaccination stage, mass activity was higher in control group. At post vaccination stage mass motility was significantly (P<0.05) higher in Levamisole injected group most of the time (Table 1).

Decrease in mass motility in control as compared to treatment group showed clear cut stress in bulls due to vaccination. Decrease in mass motility as a result of FMD vaccination was also reported by Bhakat *et al.* (2010).

Osmolality (*mOsmol/kg*)*:* Osmolality in treated group was lower as compared to the control during pre vaccination stage, slight difference was observed at post vaccination stage (Table 1). Osmolality was lowered post vaccination as compared to pre vaccination. Osmolality of semen also decreases after vaccination was in agreement Pankaj *et al*. (2007) prominently in KF bulls.

Individual motility (*IM*)*:* The mean individual motility was lower in treated group during pre-vaccination, during post-vaccination individual motility was higher in treated group as compared to control group (Table 1).

Sperm cells develop the capacity for motility during passage through the epididymis. Epididymal dysfunction following vaccination could be the possible cause for decline in motility and increase in sperm abnormalities.

Non-eosinophilic count (*NEC*): Mean non-eosinophilic count was lower in treated group during pre-vaccination as compared to control, during post-vaccination non-eosinophilic count was higher in treated group (Table 1).

The decrease in non-eosinophilic count in post vaccination samples especially in control group of present study was due to the decrease in the live sperm count, which was again due to decreased sperm concentration in the ejaculates. FMD vaccination has produced adverse effects on the live spermatozoa, resulting into increased dead spermatozoa (Bhakat *et al*. 2015). The result of this study revealed increase in motility and NEC in levamisole treated group, however Bozkurt *et al.* (2004) reported the adverse effect of levamisole. Author reported significant (P<0.05) decrease in semen volume, sperm motility, concentration and total sperm number during the period, it may be due to variation in dose of levamisole, since they used the levamisole @ 7.5 mg/kg body weight, the said dose is actually treatment dose, but the dose used in present experiment was only immune-stimulant dose (@ 2.5mg/kg body wt), which perhaps was not enough to express negative effect in experimental bulls.

Sperm Abnormalities: Tail abnormality was lower in levamisole treated group as compared to control in both pre- and post-vaccination (Table 1).

Sperm abnormality within control group increased after vaccination, which reflect the post vaccination stress. As the tail abnormality constituted the major portion of total abnormality, therefore trend was similar in tail and total abnormalities. Similar to present finding, increased incidence in sperm abnormalities following vaccination were observed by Pankaj *et al.* (2007) and Bhakat *et al.* (2011). The primary and secondary abnormalities of spermatozoa noticed by them were attributed to the effect of temperature on spermatocytes and epididymal spermatozoa, respectively.

Hypo osmotic swelling test (*HOST*)*:* The mean HOST% was lower in treated group during pre-vaccination however, during post-vaccination HOST was higher in treated group (Table 1). HOST positive cells decrease after vaccination especially in control group. Antoine and Pattabiraman (1999) reported decrease in HOST positive spermatozoa following scrotal insulation in buck and heat treatment due to increased testicular temperature. Thus the vaccination has similar effect as scrotal insulation.

Intact acrosome (*IA*)*/ acrosomal integrity:* The mean acrosomal integrity was slightly lower in levamisole treated group during pre-vaccination, and during post-vaccination acrosomal integrity was higher in treated group as compared to control (Table 1).

Similar finding was also reported by Gowda (1993). The acrosome was either detached or broken which releases the enzymes affecting fertilizing capacity of spermatozoa. Abnormal acrosomal development could be due to testicular degeneration, which is temporarily caused by vaccination stress.

R- value: This test showed the ability of the spermatozoa to withstand 1% sodium chloride solution. Higher values suggest good quality sperms. At pre-vaccination stage R-value in treated group was higher, at post vaccination stage also the value was higher in treated group as compared to control although it was not significant (Table 1).

Sperm concentration (*million/ml*)*:* Sperm concentration was significantly (P<0.05) higher at pre-vaccination stage in levamisole treated bulls and it was also significantly (P<0.01) higher in treated group at post-vaccination stage (Table 1). Total motile spermatozoa ($\times 10^6$), total live spermatozoa/ml ($\times 10^6$), total sperm ($\times 10^6$), live sperm/ml and total dose harvested was all significantly (P<0.01) higher in levamisole treated group. Similar to present finding Pankaj *et al.* (2007) reported an increase in sperm concentration after vaccination while no change in sperm concentration after viral vaccination was also observed. In the present finding, total motile spermatozoa ($\times 10^6$) decreased, similar post-vaccination decline was also reported by Bhakat *et al.* (2015).

Vaccination is one of the major stress factors that affect the semen quality (Pankaj *et al.* 2007, Bhakat *et al*. 2011). Earlier Singh *et al.* (2004) studied the effect of FMD vaccination on semen quality of exotic and crossbred bulls, but our study was different from said investigation as we have used Raksha triovac (FMD, HS, BQ) vaccine. Rise in temperature causing degenerative change in semen quality may be due to antigen and adjuvant used. Immunogenicity of the vaccine is responsible for febrile reaction and degenerative changes in testes. As a result of rise in testicular temperature, metabolism increases hence the testis turns hypoxic. Therefore, the testes became extremely susceptible to temperature increases. The testis usually operates on the brink of hypoxia.

Post-vaccination increase in body temperature causes defect in epididymal tube which further induces testicular degeneration. Epididymal dysfunction is solely responsible for sperm abnormalities. Rise in abnormalities in this study showed impaired maturation process of spermatozoa due to vaccination. Effect of temperature on the fully formed epididymal spermatozoa could give rise to secondary abnormalities. Heating affects both sertoli and Leydig cell functions; however germ cells are the most sensitive. During early stage spermatocytes are killed by heat, whereas mature spermatozoa showed structural abnormalities.

The result of this study revealed increase in quality of semen in levamisole treated group. Although Bozkurt *et al.* (2004) reported decrease in semen quality due to levamisole treatment however significant increase in the hyaluronidase activity was reported in same experiment, which has significant positive effect on fertilizing capability of sperm even at dose rate of 7.5 mg/kg body wt, as hyaluronidase activity could be an index of fertilization ability (Tanyildizi and Bozkurt 2003) in rams.

Vaccination against foot and mouth disease followed with 2 weeks sexual rest was done to cope with post vaccination stress. However, levamisole had shown its positive effect in reducing vaccination stress, there is need to explore more about these issues to minimize the stressful conditions to the bulls.

Effect of levamisole treatment on blood profile: Blood plasma concentration of minerals Fe, Cu and Zn was higher in levamisole treated group as compared to control but it was not significant except Zn. During pre-vaccination Zn (ppm) concentration was lower in treatment group and post-vaccination zinc concentration was significantly (P<0.05) higher in treatment group as compared to control in KF bulls (Table 2).

Zinc is an essential element, which is also present in hormone insulin and enzymes carbonic anhydrase and lactic dehydrogenase. Zinc deficiency severely affects the final stage of sperm maturation as it decreases gonadotrophins and androgen output. Sperm motility improved by zinc supplementation (Rowe *et al.* 2014). Zinc content in the semen may thus be useful indicator of possible cause of reproductive dysfunctions. The iron content was found positively correlated with calcium content as high concentration of both the element have negative effect on physical parameters.

Effect of levamisole treatment on metabolic profile: During post vaccination total immunoglobulin was

Table 2. Blood profile and metabolic profile of levamisole treated vs control bulls during pre and post vaccination in Karan Fries bulls

Parameters	Groups	Pre-vaccination Mean±SE	Post-vaccination Mean±SE
Fe (ppm)	Control	0.56±0.1	0.56±0.06
	Treatment	0.85±0.09	0.64±0.06
Cu (ppm)	Control	0.66±0.14	0.62±0.07
	Treatment	0.67±0.08	0.65±0.08
Zn (ppm)	Control	1.44±0.24	1.44*±0.12
	Treatment	1.26±0.23	1.92*±0.42
Total Ig (mg/ml)	Control	22.02±0.9	24.57*±0.56
	Treatment	23.83±0.79	25.76*±0.49
Glucose (mg/dL)	Control	61.31±3.12	56.86±1.35
	Treatment	58.9±1.33	58.43±1.52
BUN (mg/dL)	Control	12.12±0.39	12.24±0.59
	Treatment	10.17±0.67	11.46±0.85
NEFA (uM/L)	Control	220±27.98	222.19±11.25
	Treatment	233.25±34.23	222.10± 15.34

Means bearing *, within same column differ significantly (* P<0.05) for each parameters separately.

significantly higher in treatment group. There was increase in blood glucose level and decrease in NEFA and BUN in levamisole treated group of bull after vaccination but it was not significant (Table 2). Result revealed that treated bulls are in positive energy balance with respect to metabolic profiles this might be due to additional immunity developed in levamisole group. Unfortunately no literature was accessed to compare our finding.

SUMMARY

Present study was planned on 8 crossbred bulls (144 ejaculates) to study effect of levamisole on vaccination stress. All the bulls were vaccinated with Trio-vac vaccine as per routine schedule of the farm. In treatment group (4 bulls) levamisole was injected 1 week prior to vaccination, whereas in control normal saline was injected. Semen ejaculates were evaluated during pre-vaccination (1 week) and post-vaccination (8 week) period. Results revealed significantly lesser reaction time, sperm abnormalities and higher sperm concentration (million/ml), total motile spermatozoa ($\times 10^6$), total live spermatozoa/ml ($\times 10^6$), total sperm ($\times 10^6$) and total dose harvested in treated group. Individual motility, mass motility, NEC, HOST and IA (%) was higher in levamisole treated group, but it was not statistically significant. Vaccination stress was evident in both group, however the stress was prominent in control. Level of serum Zn and immunoglobulin improved significantly in treated bulls. Levamisole treatment in vaccinated bulls has positive effect to some extent in amelioration of stress.

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