Indian Journal of Animal Sciences **87** (4): 456–460, April 2017/Article

Effect of vitamin E supplementation on semen and blood profile of vaccinated crossbred bulls*

T K S RAO[1], T K MOHANTY[2] and M BHAKAT[3]

ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

Received: 13 September 2016; Accepted: 4 October 2016

ABSTRACT

Vaccination is one of the major stress factors that affects the semen productivity of the breeding bulls who showed consistent higher body temperature for several days. Therefore, a study was planned to assess the ameliorative strategy of vaccination stress by Vitamin E supplementation on twelve Holstein-Friesian crossbred bulls (432 ejaculates) maintained at Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal. The bulls were vaccinated for FMD @ 3 ml by SC injections. In treatment group (6 bulls), Vitamin E was supplemented @ 4000 I.U./bull/day, ten weeks before and nine weeks after vaccination and another group without Vitamin E supplementation served as control. Vitamin E supplementation resulted in significantly reduced sperm abnormalities and improved sperm concentration, total motile spermatozoa (10^6), total live spermatozoa per ml (10^6), total sperm (10^6), live sperm/ml and total dose harvested in treated group as compared to control. Individual motility (%), mass motility, NEC (%), HOST (%) and IA (%) were higher in the treatment group, but it was not significant. Better blood biochemical profile of bull was evident in Vitamin E supplemented group though it was not significant statistically. It can be concluded that Vitamin E supplementation in vaccinated bulls was effective in ameliorating vaccination stress to some extent.

Key words: Blood biochemical profile, Crossbred bull, Vitamin E, Semen quality, Vaccination.

Quality germplasm production from disease free breeding bulls is the mandatory requirement for the dissemination of better germplasm. In India, vaccination of breeding bull against communicable disease is an essential component of standard management practice in the bull station. However, more emphasis is still required to be laid on vaccination of exotic and crossbred bulls, as they are more prone to Foot and Mouth diseases (FMD), Hemorrhagic Septicemia (HS), Black Quarter (BQ) and other diseases (Bhakat *et al.* 2011a). Available report on the effect of vaccination on semen quality are conflicting in nature; while some studies reported no significant effect of vaccination on semen quality (Mangurkar *et al.* 2000), others found increased incidence of sperm abnormalities (Pankaj *et al.* 2007). Semen quality affected by vaccination (Bhakat *et al.* 2008, 2010, 2011a, 2015) as a consequence of stress caused by a rise in the body as well as testicular temperature during the post-vaccination period. With the increase in testicular temperature, there may be a decrease in the proportion of progressively motile and live spermatozoa, and an increase in the incidence of morphologically abnormal spermatozoa, especially those with defective heads and recovery is dependent upon the nature and duration of the thermal insult. The increased body temperature may not only affect spermatogenesis (Pankaj *et al.* 2007), but also the maturation of sperm. All stages of spermatogenesis are susceptible, the severity of damage being related to the degree and duration of the increased temperature.

Scanty information is available regarding ameliorative measures of vaccination stress through antioxidant supplementation and immune-modulation. For optimum sperm production, preproduction nurturing (before ejaculation) like minimum exposure to high temperature, pro-inflammatory factors, xenobiotics and use of nutraceutical like folic acid, Zn, Vitamin E and Vitamin C and postproduction nurturing (after ejaculation) which involves sperm processing, *in-vitro* treatment with pentoxifylline, PFA, antioxidant etc. are advocated (Bhakat *et al.* 2011b). The preproduction nurturing is supposed to be more effective in improvement of semen quality. Supplementation of Vitamin E as antioxidant provides three level of protection like prevention, interception, and repair of sperm, especially from generated free radicals. The present investigation was therefore undertaken to find out the effect of Vitamin E supplementation on amelioration of vaccination stress in crossbred bulls.

Present address: [1]Assistant Professor (tksrao.vet@gmail.com), Vanbandhu College of Veterinary Science and Animal Husbandry, NAU, Navsari. [2]Principal Scientist (mohanty.tushar@gmail.com), [3]Senior Scientist (bhakat.mukesh@gmail.com).

*Part of PhD. thesis of the first author.

MATERIALS AND METHODS

The experiment was carried out on twelve elite Karan Fries (Holstein Friesian cross) bulls stationed at Artificial Breeding Research Centre of ICAR-National Dairy Research Institute, Karnal during monsoon and post-monsoon season (July to November 2008) to observe the effect of vitamin E supplementation on vaccination stress alleviation. The bulls were thoroughly observed before selection for similar physical traits. The bulls were divided into two groups (Control and vitamin E supplemented) with six animals in each group. FMD (concentrated tetravalent) vaccine was administered @ 3.0 ml by S/C injection route which contained FMD virus types O, A, C and Asia1 strains.

Vitamin E feeding was started two and half month prior to vaccination and continued two-month post-vaccination to treatment group only @ 4000 IU/bull/day (Velasquez-Pereira *et al.* 1998). A total of 240 and 192 ejaculates (KF bulls) were collected and evaluated before (10 weeks) and after vaccination (9 weeks) for two months each at weekly interval. Semen was evaluated for volume, pH, mass activity, individual motility, osmolality, sperm concentration using Haemocytometer (Neubauer's chamber) method, eosinophilic/non-eosinophilic sperm count, abnormality of spermatozoa, hypo-osmotic swelling test (HOST), intact acrosome (IA) as per standard semen quality evaluation. Reaction time (Kilgour *et al.* 1984) and harvested semen dose were recorded.

Data were analyzed by two-way ANOVA with interaction and significant differences between means were compared by DMRT (Duncan's Multiple Range Test). Prior to analysis of proportionality (percent non-eosinophilic count, HOST, motility etc.), data were transformed using the arcsin transformation [asin(sqrt(percent/100))] with adjustment to allow for zero values.

RESULTS AND DISCUSSION

The result of Vitamin E supplementation on semen quality of crossbred bulls before and after vaccination is presented in Table 1.

Reaction time: The reaction time during pre-vaccination stage was lower as compared to the post-vaccination period for both treatment and control group (Table 1). The increase in reaction time after vaccination might affect the libido of the bulls. On the other hand, less reaction time in treated group showed a positive effect of Vitamin E supplementation in bulls.

Rectal temperature (°F): Overall rectal temperature during post-vaccination stage was lower as compared to the pre-vaccination stage in both treatment and control group; moreover, it was lower in treatment as compared to control group (Table 1). Rectal temperature increases just after vaccination, especially for first two week, might be due to the sensitivity of testicular epithelium with afebrile condition affecting testis by impairing the pampiniform plexus resulting into testicular degeneration.

Mass activity (0–5 Scale): In Vitamin E supplemented group, mass motility during pre-vaccination was better as

Table 1. Mean±SE of semen quality parameters and reaction time during pre and post vaccination in crossbred bulls

Parameter	Group	Pre-Vaccination	Post-Vaccination
		Mean±SE	Mean±SE
Reaction time (Sec)	Control	74.78±3.78	90.6±7.43
	Treatment	55.13±1.77	70.41±2.55
Mass motility (0-5 scale)	Control	2.64±0.14	2.89±0.19
	Treatment	3.04±0.15	2.81±0.18
Volume (ml)	Control	4.32±0.15	4.16±0.17
	Treatment	5.08±0.15	4.44±0.19
Rectal temp (°F)	Control	101.34±0.05	100.9±0.1
	Treatment	101.05±0.05	100.83±0.12
Concentration (million/ml)	Control	884.21±51.73	1073.12±76.89
	Treatment	1121.66**± 66.71	1230.31**± 80.7
IM (%)	Control	54.77±0.08	59.24±0.14
	Treatment	62.9±0.1	59.02±0.12
NEC (%)	Control	60.17±0.19	64.65±0.15
	Treatment	68.85±0.1	64.1±0.13
Total abnormality (%)	Control	12.44±0.01	12.79±0.02
	Treatment	10.26**±0.01	10.69**±0.02
HOST (%)	Control	44.47±0.09	49.19±0.16
	Treatment	53.78±0.1	49.26±0.16
IA (%)	Control	64.05±0.09	68.19±0.16
	Treatment	73.51±0.11	67.91±0.41
R- Value (Resistance to NaCl in ml)	Control	4837.5±30.75	4410.78±5.96
	Treatment	4875±97.37	6043.75±24.95
Resazurin II (Sec)	Control	4.62±0.37	4.97±0.10
	Treatment	4.25±0.43	4.90±0.23
Total sperm (Million)	Control	4088.87 ±354.93	4541.97 ±392.02
	Treatment	5549.01** ±359.38	5484.68** ±440.87
Total motile sperm (Million)	Control	2589.66 ±285.73	3111.31 ±333.5
	Treatment	3787.75** ±302.08	3662.60** ±366.72
Live sperm (Million)	Control	2791.66 ±304.18	3318.93 ±350.51
	Treatment	4071.55** ±317.9	3914.25* ±390.27
Live sperm/ml	Control	588.79±50.44	775.16±72.83
	Treatment	837.12** ±64.88	863.15** ±76.02
Dose harvested	Control	129.48±14.28	155.56±16.67
	Treatment	189.38**±15.1	183.13*±18.33

Means bearing *, ** within same column differ significantly (* $P<0.05$, ** $P<0.01$).

compared to control. Likewise, post-vaccination mass motility was almost similar and the effect of supplementation was not significant (Table 1). Supplementation of vitamin E increased mass motility however, post-vaccination decrease was very clear cut might be due to adverse effect of vaccination on developing sperm in testes which were affected more as compared to epididymal sperm. A similar finding was also reported by Hafez (2000) and Cameron and Blackshaw (1980) in sheep.

Volume of semen (ml): Semen volume during pre-vaccination was higher as compared to post-vaccination for both treatment and control group. Additionally, trend showed an increase in semen volume in treatment group over control (Table 1). More semen volume in treated group showed a positive effect of vitamin E on nourishing accessory sex gland.

Individual motility (*IM*)*:* The mean individual motility was higher in supplemented group during pre-vaccination as compared to control. Furthermore, during post-vaccination individual motility decreased as compared to pre-vaccination in both group but the decrease was prominent in control group (Table 1). Decrease in individual motility post-vaccination may be due to vaccination stress because sperm that is developing in testes and fully mature sperm (ready to release) at the time of vaccination may show the damage, whereas epididymal sperm is unaffected, therefore the adverse effect was observed either immediately after vaccination or 3–5 week later as observed by Hafez (2000) and Cameron and Blackshaw (1980). A similar trend was also observed for NEC, HOST and IA (%).

Vaccination stress was observed in all stages, however, it was prominent at first and fourth-week post vaccination; later animal recovered well and overall effect during the post-vaccination period was negligible. In our finding, individual motility was numerically greater in vitamin E supplemented group but this was not statistically significant. A similar finding was also reported in male Japanese quail (Biswas *et al.* 2007).

Non-eosinophilic count (NEC): Mean non-eosinophilic count percent was higher in supplemented group during pre-vaccination as compared to control. Moreover during post-vaccination, it was almost similar in both groups. A similar trend was observed in HOST and IA (Table 1). The present finding was similar to the finding of Pankaj *et al*. (2007) who reported vaccination stress up to two months. Moreover, Murugavel *et al*. (1997) also reported a decrease in the live sperm count after vaccination which is due to decreased sperm concentration in the ejaculates.

Antoine and Pattabiraman (1999) and Bhakat *et al.* (2015) reported a decrease in HOST positive spermatozoa due to increase in testicular temperature. This suggests vaccination affects the HOST percentage by affecting sperm plasma membrane.

The present finding was similar to the earlier report of Gowda (1993) who reported a decrease in the intact acrosome percentage after vaccination. The acrosome was either detached or broken which subsequently caused the release of enzymes and affected the fertilizing capacity of spermatozoa.

Sperm concentration (million/ml): Perusal of result reflects higher sperm concentration post-vaccination (1230.31±80.70 and 1073.12±76.89) as compared to pre-vaccination (1121.66±66.71 and 884.21±51.73) (Table 1). Total motile spermatozoa ($\times10^6$), total live spermatozoa per ml ($\times10^6$), total sperm ($\times10^6$), live sperm/ml and total dose harvested were significantly ($P<0.01$) higher in supplemented group. These parameters decreased in KF bulls notably at first and fourth-week post-vaccination but overall it was not decreased. The decrease was observed in treatment group rather than control. Post-vaccination increase in sperm concentration as observed in this study was also reported by Pankaj *et al.* (2007) in buffalo and KF bulls. However, Narsimhan *et al.* (1970) reported no change in sperm concentration after RP (Rinder Pest) vaccination.

Sperm abnormalities: Sperm abnormalities were significantly lower in supplemented group. Trend show higher sperm abnormalities in control group as compared to the vitamin E supplemented group (Table 1). Higher sperm abnormalities were observed during post-vaccination and were very specific in control group as compared to the vitamin E supplemented group. Post vaccination increase in abnormalities was also reported by Cameron and Blackshaw (1980). Vitamin E supplementation to bulls can overcome adverse effect of sperm profile like increased sperm abnormalities, decreased sperm production and depressed sexual behavior produced by gossypol feeding (Velasquez-Pereira *et al.* 1998). Biswas *et al.* (2007) also reported significant ($P<0.05$) effect of vitamin E supplementation on reducing sperm abnormalities although semen characteristics didn't differ significantly in male Japanese quail. Moreover, Rekkas *et al.* (2000) found no positive effects of feeding increased dosage of vitamin E to mice with respect to abnormalities.

Since the sperm cell undergoes maturation changes during their passage through the epididymis, the high incidences of sperm tail and midpiece abnormalities are probably due to epididymal dysfunction. Sperm morphology usually returns to pre-treatment values approximately within six weeks of thermal insult (Katelic *et al.* 1996). However, prolonged increase in testicular temperature will add to the gap for recovery.

FMD vaccination adversely affects the semen quality (Singh *et al.* 2004) of exotic bulls. The febrile reaction is due to antigen and adjuvant. Adjuvant exerts nonspecific lymphocyte proliferation (Singh 2009), increasing the immune response to weak antigens. It also mediates through increasing cellular infiltration, inflammation and promoting APCs by regulating co-stimulatory signals or major histocompatibility complex (MHC) expression; improving antigen presentation; or inducing cytokine release for indirect effects (Srivastava *et al.* 2006).

Vitamin E like selenium is recognized as being an antioxidant and over the years there have been innumerable reports advocating its use in the treatment of sub-fertile men. However, the results have been ambiguous and vitamin E therapy has not become established as a dependable method of treating male sub-fertility in general and reduced sperm motility in particular. Supplementation of vitamin E in much higher doses was proved more effective as the high antioxidant intake was associated with high-grade semen (Eskenazi *et al.* 2005). Vitamin E can treat ROS (reactive oxygen species) associated male infertility.

The dietary antioxidant may confer protective properties through a novel mechanism unrelated to their conventional free radical scavenging abilities like reducing degenerative and inflammatory changes. Up-regulation of antioxidant defense, xenobiotic metabolism, or DNA-repair genes may all limit cellular damage and hence promote maintenance of cell integrity. Eskanazi *et al.* (2005) on the basis of food frequency questionnaire and semen profiling of healthy person reported higher sperm concentration and motility associated with higher antioxidant intake. Supplementation of vitamin E increase sperm output and concentrations and improved qualities of fresh and frozen semen in ram (Zhu *et al.* 2009).

Vaccination against FMD followed by two weeks of sexual rest was practiced to cope with post-vaccination stress. However, vitamin E had shown its effect a little in reducing vaccination stress, there is a need to explore more about these issues to minimize the stressful post-vaccination conditions to the bulls.

R-value: This test shows the ability of the spermatozoa to withstand 1% sodium chloride solution. Higher values suggest good quality sperms. At the pre-vaccination stage, R-value in supplemented group was significantly ($P<0.05$) higher as compared to control at post vaccination stage, the value was higher in supplemented group but it was not significant (Table 1).

Resazurin test: It is time taken by spermatozoa to reduce Resazurin, more the time taken to reduce, lesser the quality. Overall result showed lower Resazurin reduction time in supplemented group suggesting that Vitamin E supplementation had a good effect on improvement of semen quality in vaccinated bulls (Table 1).

Effect of vitamin E supplementation on blood profiles: Blood plasma concentration of minerals P (mg/dl), Ca (mg/dl), Mn, Fe and Cu were higher in vitamin E supplemented group as compared to control but it was not significant (Table 2) except Zn.

Zinc (ppm) pre-vaccination value was less in the treatment group as compared to control and the post-vaccination value was more in the treatment group. Iron level was higher in the treatment group as compared to the control in both pre and post vaccination stages, however, it was not significant. A similar trend was observed with P, Ca, copper (ppm) and manganese (ppm) level also.

Higher serum Zn value post vaccination in the treatment group might be due to mitigation of stress in supplemented group. Zinc is an essential element, which is also present in hormone insulin and enzymes carbonic anhydrase and lactic dehydrogenase. Hidiroglou (1979) observed that zinc deficiency severely affects the final stage of sperm maturation as it decreases gonadotropin and androgen output. Sperm motility improved (62.3 vs. 52.8%) by supplementation of zinc (Rowe *et al.* 2014). Zinc content in semen may thus be a useful indicator of the possible cause of reproductive dysfunction (Croxford *et al.* 2011).

Table 2. Mean±SE of blood biochemical profile during pre and post vaccination in crossbred animals

Parameter	Groups	Pre-vaccination Mean±SE	Post vaccination Mean±SE
Zn (ppm)	Control	1.47±0.13	1.28*±0.22
	Treatment	0.98±0.07	1.53*±0.24
Cu (ppm)	Control	0.97±0.09	0.83±0.06
	Treatment	1.52±0.14	0.92±0.06
Fe (ppm)	Control	0.71±0.08	0.67±0.08
	Treatment	0.84±0.13	0.77±0.21
Ca (mg/dl)	Control	6.68±0.39	6.09±0.64
	Treatment	6.74±0.43	6.27±0.69
P (mg/dl)	Control	3.37±0.19	3.03±0.32
	Treatment	3.62±0.2	3.25±0.33
Glucose (mg/dl)	Control	59.21±0.93	58.31±1.45
	Treatment	54.51±2.1	61.24±0.81
BUN (mg/dl)	Control	15.55±0.66	15.81±0.56
	Treatment	15.77±0.81	15.08±0.54
Total Ig (mg/ml)	Control	22.18±0.68	22.37±0.43
	Treatment	22.92±0.44	23±0.42
NEFA (µM/ml)	Control	237.53±17.85	215.4±19.12
	Treatment	232.87±18.59	194.1±14.21

The iron content was found positively correlated with calcium content as a high concentration of both the element negatively affect physical parameters. Singh and Gangwar (1977) reported similar finding in semen instead of serum. As copper is an important constituent of enzyme tyrosinase, Uricase and also act as a catalyst in the oxidation of sulph-hydral group. So it may have a synergistic effect on motility of spermatozoa.

Metabolic profile: In the present study, plasma glucose and total immunoglobulin was higher but BUN (blood urea nitrogen) and NEFA was lower in supplemented group, although it was not significant (Table 2). The lower blood level of BUN and NEFA suggest higher energy level of treated bull and it helps in better protection from vaccination stress. Very few studies are there to compare our finding for metabolic profile for vitamin E supplementation in bulls.

Impact of vaccination is evident as semen quality, blood profile and metabolic profile in both vitamin E supplemented and control group, but it was also evident that vitamin E had beneficial role in ameliorating vaccination stress as there was little improvement in semen quality, reaction time, blood profile and metabolic profile in supplemented group in positive direction. This may be due to using a single regimen of 4000 IU, which may not be sufficient. Therefore, further study is warranted by increasing the dose rate of vitamin E supplementation to improve semen production performance and amelioration of vaccination stress. Further studies on *in vitro* and *in vivo* fertility will give insight on actual time the semen is restored to normal fertility status after vaccination.

ACKNOWLEDGEMENT

Author is highly thankful to Director, ICAR-NDRI, Karnal for providing necessary facilities for research work.

REFERENCES

Antoine D and Pattabiraman S R. 1999. Spermatozoa response to hypo-osmotic swelling and freezability tests after testicular degeneration in bucks. *Indian Journal of Animal Reproduction* **20**(1): 54–56.

Bhakat M, Mohanty T K, RainaV S, Gupta A K and Brahma B. 2010. Effect of FMD vaccination on semen quality parameters in Karan Fries and Murrah buffalo bulls. *Tropical Animal Health Production* **42**(7): 1363–66.

Bhakat M, Mohanty T K, Raina V S, Gupta A K and Brahma B. 2011a. Effect of TRIOVAC vaccination on semen quality parameters in Karan Fries and Murrah buffalo bulls. *Indian Veterinary Journal* **88**(3): 33–34.

Bhakat M, Mohanty T K, Raina V S, Gupta A K, Pankaj P K, Mahapatra R K and Sarkar M. 2011b. Study on suitable semen additives incorporation into the extender stored at refrigerated temperature. *Asian Australian Journal of Animal Science* **24**(10): 1348–57.

Bhakat M, Mohanty T K, Gupta A K and Abdullah M. 2014. Effect of season on semen quality of crossbred (Karan Fries) bulls. *Advances in Animal and Veterinary Sciences* **2**(11): 632–37.

Bhakat M, Mohanty T K, Gupta A K, Chakravarty A K, Singh P and Abdullah M. 2015. Effect of HS and BQ vaccination on semen quality parameters of Murrah buffalo bulls. *Journal of Infection and Molecular Biology* **3**(1): 24–27.

Bhakat M, Mohanty T K, Raina V S, Gupta A K, Mondal G and Khan H M. 2008. Effect of FMD vaccination on various semen characteristics of Sahiwal bulls. *Pakistan Journal of Agriculture Science* **45**(2): 327–32.

Biswas A, Mohan J, Sastry K V H and Tyagi J S. 2007. Effect of dietary vitamin E on the cloacal gland, foam and semen characteristics of male Japanese quail. *Theriogenology* **67**(2): 259–63.

Cameron R D A and Blackshaw A W. 1980. The effect of elevated ambient temperature on spermatogenesis in the boar. *Journal of Reproduction Fertility* **59**: 173.

Croxford T P, McCormick N H and Kelleher S L. 2011. Moderate zinc deficiency reduces testicular Zip 6 and Zip 10 abundance and impairs spermatogenesis in mice. *Journal of Nutrition* **141**(3): 359–65.

Eskenazi B, Kidds S A, MarksA R, Sloter E, Block G and Wyrobek A J. 2005. Antioxidant intake is associated with semen quality in healthy men. *Human Reproduction* **20**(4): 1006–12.

Gowda B K. 1993. 'Effect of foot and mouth disease vaccination on pre and post freezing semen parameters.' MVSc Thesis, University of Agricultural Science, Banglore, India.

Hafez E S E. 2000. Reproduction in Farm Animals. 7thed. Lippincott Williams and Wilkins, A wolterskluwer co. Philadelphia, Baltimore, NewYork.

Hidiroglou M. 1979. Trace element deficiencies and fertility in ruminants: A Review. *Journal of Dairy Science* **62**(8): 1195–1206.

Kilgour R J, Barwick S A and Fowler D G. 1984. Comparison of the mating behavior of sexually active and sexually inactive rams. Proceedings of 10th International Congress on Animals Reproduction and AI. pp.10–14. Urbanchampaign.

Kstelic J P, Cook R B, Coulter G H and Saacke R G. 1996. Insulating the scrotal neck affect semen quality and scrotal/testicular temperatures in the bull. *Theriogenology* **45**: 935–42.

Mangurkar B R, Phadnis Y P and Bhosrekar M R. 2000. Effect of foot and mouth disease vaccination on semen characteristics of exotic and cross-bred bulls. *Indian Journal of Animal Reproduction* **21**(2): 135–37.

Murugavel K, Veerapandian C and Subramanian A. 1997. Post-thaw motility of buffalo semen following FMD vaccination. *Indian Veterinary Medicine Journal* **26**: 241–42.

Narsimhan K S, Krishna A R and Qqayam S A. 1970. Effect of Rinderpest vaccination on spermatogenesis in bulls. *Indian Veterinary Journal* **47**(1): 19–22.

Pankaj P K, Raina V S, Roy B, Mohanty T K and Mishra A. 2007. Studies on effect of vaccination on seminal characteristics of riverine buffaloes (*Bubalus bubalis*). *Buffalo Bulletin* **26**(3): 91–103.

Rekkas C, Kokolis N, Belibasaki S, Tsantarliotous M and Smokovitis A. 2000. Effect of α-tocopherol on plasma testosterone and plasminogen activation activity or inhibition in raw spermatozoa. *Theriogenology* **53**: 751–60.

Rowe M P, Powell J G, Kegley E B, Lester T D and Rorie R W. 2014. Effect of supplemental trace-mineral source on bull semen quality. *Professional Animal Scientist* **30**(1): 68–73.

Singh C and Gangwar P C. 1977. Zinc iron and citric acid in buffalo bull semen. *Indian Journalof Animal Sciences* **57**: 250–56.

Singh R, Verma H K, Kumar S and Matharoo J S. 2004. Effect of foot and mouth disease vaccination on the reproductive capacity of the buffalo bulls. *Indian Journal of Animal Reproduction* **25**(1): 45–47.

Srivastava A K, Singh R K and Yadav M P. 2006. Animal Biotechnology. 1st edn. Oxford and IBH Publ. Co., New Delhi.

Velasquez-Pereira J, Chenoweth P J, McDowellL R, Risco C A, Staple C A, Prichard D, Martin F G, Cahoun M C, Williams S N and Wilkinson N S. 1998. Reproductive effect of feeding Gossypol and vitamin E to bulls. *Journal of Dairy Science* **76**: 2894–2904.

Zhu H, Luo H L, Meng H and Zhang G J. 2009. Effect of vitamin E supplementation on development of reproductive organs in Boer goat. *Animal Reproduction Science* **113**: 93–101.