Animal Review
2015 Vol. 2, No. 4, pp. 87-98
ISSN(e): 2409-6490
ISSN(p): 2412-3382
DOI: 10.18488/journal.ar/2015.2.4/101.4.87.98

IN VITRO DIFFERENTIAL EFFECT OF NERVE GROWTH FACTOR ON FUNCTIONAL PARAMETERS OF MURRAH BUFFALO SPERMATOZOA IN LOW AND HIGH FERTILE GROUPS

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ABSTRACT

Aim of the present study was to examine the influence of in vitro supplementation of Nerve growth factor (NGF) on functional parameters of Murrah buffalo (Bubalusbubalis) spermatozoa from fresh semen, like, motility, plasmalemma integrity, acrosomal integrity, ATP concentration. Fresh semen samples (n=6) were washed in Tris buffer and divided into two equal parts (control and NGF groups). Only in the NGF group, NGF was added to a final concentration of 50 and 100 ng/ml. The samples were incubated at 37° C for different time intervals in TCM 199 medium supplemented with BSA and the effects were observed at 0, 30, 60 and 120 min of incubation. The experiment was performed in low (LF) and high fertile(HF) groups based on previous three years conception rate and taking cutoff value , below and above this value were categorized as LF and HF group. The mean concentration of the buffalo seminal plasma (n=12) NGF was 67.7±3.25ng/ml and 65.5± 2.76 in LF and HF groups respectively. The concentration of NGF in blood plasma was 83.5±7.82 and 68.6±3.82 in HF and LF groups respectively. The concentration of blood plasma NGF being higher (P<0.05) in HF group. With either dose of NGF in vitro significant effect on the total motility (P<0.05), progressive forward motility (P<0.05) was observed. It could be maintained in HF group till 120 min but in LF group it was restricted to 60 min when compared with their respective control. The functional membrane integrity did not differ significantly between groups (control and NGF treated) in both LF and HF groups with either concentration of NGF. The plasma lemma integrity was significantly less (P<0.05) at 120 min of incubation when compared with the initial value at 0 min of incubation. The percentage of acrosomal intact spermatozoa decreased continuously over a period of time in both the groups. As compared to 0 min of incubation, the significant (P<0.05) loss of acrosome was observed at 60 and 120 min of incubation in LF and HF control groups

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and NGF supplementation could maintain acrosome integrity in HF group for 60 min where as in LF group it could be maintained significantly only till 30 min of incubation when compared with the initial values of respective groups. Viability of spermatozoa was not significantly different when compared between groups with their respective control, However when compared at different time intervals, the viability of sperms in HF and LF supplemented groups was significantly different from initial values only at 120 min of incubation whereas in respective control groups the loss in viability was significant from 60 min itself. With respect to ATP concentration of spermatozoa in different groups it was observed that 100ng dose could increase the concentration of ATP in HF group significantly (P<0.05) at 60 min of incubation only with NGF in vitro when compared with its respective control. In conclusion, all the parameters decreased significantly at 120 min of incubation when compared with their respective initial values for all the groups. In HF group supplementation of NGF@50ng/ml could maintain functional parameters of spermatozoa for a greater duration of time when compared with LF or control group.

Keywords: Spermatozoa, Nerve growth factor, Murrah buffalo, Fertility, Time of incubation.

Received: 30 August 2015/ **Revised:** 30 September 2015/ **Accepted:** 5 October 2015/ **Published:** 10 October 2015

1. INTRODUCTION

Although various neurotrophins have been detected in mammalian testis [1] only NGF seems to have a potential role in male reproduction [2]. Report of Server and Shooter [3] demonstrated the source of Nerve growth factor (NGF) as sub mandibular gland of adult mouse but later presence and purification of NGF was done from seminal plasma of bovine species and was partially characterized.

The biological unit of NGF was observed to be more per ml of semen in bovine, when compared with the activity of NGF of other ruminants and human [4]. In buffaloes no work has been carried out, hence the present study was undertaken for estimating the concentration of blood and seminal plasma NGF in low and high fertile group and study *in vitro* effect of NGF on buffalo spermatozoa functional parameters. Studies on NGF/TRKA system indicated it's role in the physiology of male reproduction [5-7]. Exogenous NGF as cry oprotectant improved sperm viability and motility, increased intracellular NO concentration, and decreased apoptosis content in normal human spermatozoa [8] NGF, belongs to a family of neurotrophins, whose role is in maintaining development of neurons. Their role is not only restricted to nervous system, but they also have role in regulating reproductive system.

It has been well studied in humans [9-11]. Extensive work by Li, et al. [12] immunolocalized the receptor on ejaculated bovine spermatozoa. The study also reported that exogenous supplementation of NGF to bovine sperm samples increased leptin secretion, sperm viability when concentration of NGF was increased from 20-120 ug/L. In humans, it's differential level has been reported and lower level has been related with respect to inferior quality of the semen [2]. In the present study, a comprehensive study has been done to estimate the blood and semen plasma level of NGF, localize, NGF receptors in the spermatozoa of high and low fertile

Murrah buffalo bull. *In vitro* effect of NGF @ 50ng and 100ng on spermatozoa functional parameters was also evaluated.

2. MATERIAL AND METHODS

Enzyme immunoassay kit (EIA) for estimation of NGF in blood and seminal plasma was purchased from WKEA MED. supplies Corporation, China. For estimation of ATP in spermatozoa samples, kit was purchased from Abnova Co., Germany. The primary antibody used against NGF receptor was purchased from Biorbyt Ltd., UK. The FITC conjugated secondary antibody was purchased from Chromus Co., Bengaluru, INDIA. For analyzing the PCR products for the NGF receptor and for capturing the image of gel with products , Alpha Digidoc, documentation system was used.

2.1. Grouping of Murrah Buffalo Bulls

A total of 15 Murrah buffalo bulls from Artificial Breeding Research Center, NDRI were selected for the present study. They were maintained under routine management conditions and were fed ad libitum. Data for total number of services for each bull was collected from the records available at the center. Number of services, for these bulls ranged from 46 to 85. Baseline for number of services was considered as 46. The conception rate was calculated based on first 46 no. of services of each bull. The conception rate obtained with semen of the selected bulls was fitted in normal distribution, and they were divided into high, medium and low fertile groups. Average CR was 36.57% and SD value was 7.54. High and low fertile bulls were selected with higher and lower CR than the estimated value. Six bulls for each high (HF) and low fertile (LF) groups were selected. Mass activity of the semen sample was also considered, average being 2.7 ± 0.52. After grouping, average values for different parameters like body weight (Kg), concentration of sperms (millions/ml) , testosterone (ng/ml) and other factors were also estimated/recorded for both the groups (Table A).

Table-A. Comparison of different parameters between two groups of Murrah bulls

Parameters	Low fertile	High fertile
Body weight (Kg)	685±9.0	704±7.2
Concentration of sperms (10^6/ml)	849±12	875±32
Concentration of testosterone (ng/ml)	1.85±0.14	2.09±0.17
Conception rate(%)	28±4.05	44±2.86
Average volume of semen (ml)	2.9±0.54	3.2±0.28

2.2. Collection of Blood

For estimation of testosterone and nerve growth factor (NGF) in blood and seminal plasma samples, blood was collected by jugular vein puncture once in 15d for two months. Immediately after collection, blood samples were centrifuged at 3000 rpm, plasma was separated and stored at -20°C until assay was done.

2.3. Collection of Semen Samples and Processing

For assay of NGF in semen samples, fresh semen sample was collected from Murrah bulls six number, each in HF and LF group using sterile artificial vagina (CMV France). Hygienic conditions were followed during collection of semen. After collection of semen, samples were centrifuged at 5585 g for 10 min at 4°C to separate out the supernatant fraction containing seminal plasma. The seminal plasma samples were also stored at -20°C.Spermatozoa fraction was also separated out, the fraction was washed with Tris buffer (pH 7.2). Then the sperm samples were suspended in TCM 199 medium to a concentration adjusted to 10 million sperms/ml.

2.4. Evaluation of Different Spermatozoa Functions

Sperm motility was estimated at 37°C, by examination of a wet mount using bright-field microscopy (400X). Sperm viability was determined by preparing an eosin-nigrosin smear (37°C) and assessing at least 100 sperm under bright-field microscopy at 1000X; [13].

Acrosome reaction evaluations were carried out using 10µL-semen smears that had been stained with Trypan-Blue/Giemsa (TB) according to Kitiyanant, et al. [14]. In each smear at 200x magnification, 200 spermatozoa were analyzed and two distinct spermatozoal classes were identified: 1) intact 2) damaged.

Sperm plasma membrane integrity was evaluated using supravitalhypoosmotic swelling test. After the incubation of HOS, equal drop of HOS solution and eosin [0.5% (w/v), sodium citrate 2.92%] was placed on a warm slide, mixed for 10 seconds and cover slip was placed before the evaluation for plasma membrane integrity under phase contrast microscope at 400X. A total of one hundred spermatozoa were observed in at least five different fields. Clear heads and tails and swollen tails were considered intact with biochemically active sperm membranes, while pink heads and tails and unswollen tails were considered disrupted, inactive sperm membranes. Hypo-osmotic swelling (HOS) assay was performed as described by Ramu and Jeyendran [15].

2.5. IN VITRO Studies

2.5.1. Supplementation of NGF for *in Vitro* Studies

After separating out seminal plasma from ejaculated semen, the precipitate fraction containing spermatozoa was dispersed in TCM 199 medium and they were adjusted to ten million number of sperms in one ml of the medium. Nerve growth factor was supplemented @ 50 ng and 100 ng/ml for three different time periods of incubation viz. 30, 60 and 120 min for both LF and HF groups. Percentage of motile or viable spermatozoa were estimated and spermatozoa were also evaluated for acrosome integrity, plasmalemma integrity by HOST test and ATP production. Each trial was conducted in triplicate and four trials for each parameter was conducted.

2.5.2. Localization of NGF Receptor by Immunofluorescence

For localizing receptor, spermatozoa were fixed with 100% methanol for 30 min followed by washing with PBS. Subsequently sperms were incubated with mouse monoclonal antibody specific

for human NGF receptor (1: 100 dilution) or with PBS (for control) for 3 h followed by washing with PBS. Specimens were then incubated with FITC-conjugated secondary antibody (anti-rabbit IgG FITC conjugate, 1:100 dilution) for 90 min. at 37°C. Further, samples were treated with 1,4-diazabicycol (2.2.2) octane, covered with a cover slip and sealed. Specimen were examined under a fluorescence microscope at 40 X magnification and as described by Li, et al. [2].

2.5.3. Confirmation of Receptor by PCR

Polymerase chain reaction was also performed for localizing the receptor. DNA was extracted from spermatozoa samples using modified phenol chloroform extraction method [16]. A 100 ng DNA was precipitated out from 40-50 X10^6 spermatozoa. A100 ng of extracted DNA was amplified with the NGF primers (Table-B) by PCR with different componants of PCR mixture as given in (Table-C)as per the time for different events as given under reaction programme for PCR. PCR product obtained was evaluated by electrophoresis on a 1.8% agarose gel [17]. Purity of the DNA was estimated by taking the ratio of the absorbances recorded at 260 nm and 280 nm. The amount of DNA (ng/ml) present was calculated as O.D. 1 at 260 nm is equivalent to 50 ng ds DNA/ul).

Table-B. Sequences of forward and reverse primers

Receptor	Primer Selection	Fragment size
NGF r (TrKA)	5'-CTGGGTGAGGGTGCCTTT 3'-CGCTCAGACACCTCCTTCAG	112 bp

Table-C. Concentration of different componants in PCR mixture

Contents	Quantity
Total mixture	50ul
Genomic DNA	2ul
Primer (F)	0.5ul
Primer (R)	0.5ul
Master mix	25ul
MilliQ water	22ul

Reaction Programme for PCR

Initial denaturation	95°C	2 min.
Denaturation	95°C	30 s
Annealing	59°C	30s
Extension	72°C	45 s.

The step was continued for 35 cycles and final extension was carried out at 72°C for 4 min. PCR was carried out in thermocycler (QB96, Quanta Biotech., UK).

2.6. Statistical Analyses

All analyses were done using software package. Data from different experiments are presented a Mean±SEM. Significance of the parameters was evaluated by using three way ANOVA considering concentration of supplemented NGF *in vitro* ,Incubation time and group factors.

3. RESULTS

Blood plasma concentration of NGF was observed to be higher (P<0.05) in HF group when compared with LF group. The concentration of NGF in seminal plasma when compared between groups, the difference was not significant. The Mean±SEM concentration of testosterone was also not significantly different between the groups (Table 1).The intra and interassay CV percentage as analyzed for hormone estimations was always <10%.

Table-1. Concentration of NGF in blood and seminal plasma and testosterone in blood plasma of low and high fertile groups

Parameter	LF	HF
Blood plasma NGF(ng/ml)	68.60^{A}±3.75	83.5^{B}±7.92
Blood plasma Testosterone(ng/ml)	1.85±0.14	2.09±0.17
Seminal plasma NGF (ng/ml)	67.58±3.39	65.00±2.76

Values are expressed as Mean±SE. Values with capital superscripts differ significantly (P<0.05) within a row.

3.1. *In Vitro* Supplementation of NGF on Percentage of Motile Sperms

When 10 million spermatozoa were treated with different concentrations of 50 or 100 ng NGF, change in the percentage of motile spermatozoa was not significant in LF or HF group when compared with their respective control, when time factor was kept constant. Although at 60 min, it was observed that supplementation with either dose could maintain significantly greater percentage of motile sperms. When the motility parameter was estimated for different time intervals of incubation, with either dose of NGF, it was observed that there was decrease in the percentage of motile sperms with increase in the time of incubation but the decrease was significant (P<0.05) only at 120 min of incubation in HF group, where as in LF control group it was significant as early as at 60 min and was further significant also at 120 min (P<0.05), but such further significant decrease could not be observed at 120 min, in HF control group samples (Table 2) .

Table-2. Effect of *in vitro* supplementation of NGF (ng/ml) on percentage of motile sperms

Time of Incubation	LF group			HF group		
	Dose of NGF supplemented			Dose of NGF supplemented		
	0	50	100	0	50	100
0	77.17^{a}±0.99	77.17^{a}±0.99	77.17^{a}±0.99	88.17^{a}±0.94	88.17^{a}±0.94	88.17^{a}±0.94
30	71.00±0.90	75.13±1.03	74.08±0.98	81.92±1.03	84.58±1.02	83.67±0.91
60	68.75^{b}±0.95	72.75±1.05	70.83±0.85	77.67^{b}±1.05	82.08±0.98	80.67±0.82
120	65.67^{c}±0.98	69.33^{b}±0.93	69.08^{b}±0.98	74.83^{c}±0.96	78.50^{b}±1.02	78.08^{b}±0.95

Values are expressed as Mean±SE. Values with small superscripts differ significantly (P<0.05) within a column.

3.2. *In Vitro* Supplementation of NGF on Percentage of Viable Spermatozoa

On addition of NGF to HF/LF group spermatozoa samples; there was no significant effect on percentage of viable spermatozoa when time factor was kept constant except at 60 min supplementation increased the viability in HF group when compared with the viability of sperms of respective control group. When concentration of NGF @ 50 or 100ng was kept constant, it was observed that in both LF and HF groups percentage of viable sperms decreased significantly ($P<0.05$) only at 120 min of incubation, but without supplementation in the control group, viability could be maintained only till 30 min without significant change but further from 60 min the decrease was significant ($P<0.05$) from initial value (Table 3).

Table-3. Effect of *in vitro* supplementation of NGF (ng/ml) on percentage of viable sperms

Time of incubation	LF			HF		
	Dose of NGF supplemented			Dose of NGF supplemented		
	0	50	100	0	50	100
0	$54.50^{a}\pm1.08$	$54.58^{a}\pm1.05$	$54.58^{a}\pm1.02$	$78.50^{a}\pm0.99$	$78.33^{a}\pm1.02$	$78.33^{a}\pm1.06$
30	46.92 ± 1.05	48.92 ± 0.99	47.25 ± 0.95	72.8 ± 0.85	75.56 ± 0.85	75.28 ± 0.88
60	$44.83^{b}\pm0.99$	$43.67^{b}\pm0.85$	$44.75^{b}\pm0.90$	$68.58^{bA}\pm0.88$	$74.42^{B}\pm0.87$	$75.56^{B}\pm0.87$
120	$30.83^{c}\pm0.85$	$37.75^{c}\pm0.88$	$34.17^{c}\pm0.85$	$65.58^{b}\pm1.05$	$69.87^{b}\pm0.95$	$68.45^{b}\pm0.79$

Values are expressed as Mean±SE. Values with capital superscripts differ significantly ($P<0.05$) within a row. Values with small superscripts differ significantly ($P<0.05$) within a column.

3.3. *In Vitro* Supplementation of NGF on Acrosome Integrity

When time factor was kept constant, within a group the acrosome integrity of spermatozoa of supplemented groups was not significantly different from the acrosome integrity of control group. On supplementation of either dose of NGF to spermatozoa of LF/HF group, the decrease in acrosome integrity observed was not significant, till 60 min of incubation from the initial value, but decreased significantly ($P<0.05$) at 120 min. In the LF control group samples, the values were significantly less ($P<0.05$) at 60 min of incubation and could be maintained only till 30 min. Whereas in HF control group acrosome integrity of spermatozoa could be maintained till 60min but at 120 min it decreased significantly ($P<0.05$)(Table 4).

Table-4. Effect of *in vitro* supplementation of NGF (ng/ml) on percentage of sperms with acrosome integrity

Time of Incubation	LF group			HF group		
	Dose of NGF supplemented			Dose of NGF supplemented		
	0	50	100	0	50	100
0	$80.8^{a}\pm1.07$	$79.83^{a}\pm1.05$	$80.8^{a}\pm0.99$	$89.58^{a}\pm1.23$	$89.89^{a}\pm1.12$	$88.99^{a}\pm1.20$
30	77.92 ± 1.05	77.17 ± 1.02	77.25 ± 1.00	85.58 ± 0.98	86.83 ± 1.20	87.79 ± 1.08
60	$72.33^{b}\pm1.02$	75.25 ± 0.98	74.56 ± 0.96	84.42 ± 0.88	86.64 ± 0.95	87.32 ± 1.09
120	$69.08^{c}\pm0.99$	$71.92^{b}\pm0.97$	$72.33^{b}\pm0.95$	$80.25^{b}\pm0.95$	$79.59^{b}\pm0.98$	$80.23^{b}\pm0.99$

Values are expressed as Mean±SE. Values with small superscripts differ significantly ($P<0.05$) within a column.

3.4. *In Vitro* Supplementation of NGF on Plasmalemma Integrity

In control group without supplementation of NGF, the integrity of plasmalemma as assessed by HOST test decreased with increase in the time of incubation, the decrease was significant

(P<0.05) at 120 min of incubation. Similar was the trend observed in LF group on supplementation with either 50 or 100 ng dose of NGF.

Keeping the time factor constant, the plasmalemma integrity of spermatozoa was not significantly different from control when any time period of incubation was considered. In HF group supplementation of NGF did not alter the integrity of plasmalemma of spermatozoa significantly at different fixed time intervals when compared with the respective control. Similar were the results when compared with the values at initial time period of incubation and also at different time intervals keeping dose factor constant (Table 5).

Table-5. Effect of *in vitro* supplementation of NGF (ng/ml) on percentage of sperms with plasmalemma integrity

Time of Incubation	LF			HF		
	Dose of NGF supplemented			Dose of NGF supplemented		
	0	50	100	0	50	100
0	58.08^{a}±1.02	58.45^{a}±1.00	58.54^{a}±1.03	65.25±1.02	65.86±0.99	68.83±0.95
30	56.23±0.99	56.89±1.02	56.89±0.98	64.23±1.01	65.23±1.00	67.45±0.98
60	56.45±0.96	56.23±0.99	56.45±0.92	63.32±0.98	64.89±0.97	67.23±0.96
120	46.98^{b}±0.85	50.23^{b}±0.75	49.87^{b}±1.02	60.52±1.03	64.42±1.02	65.01±0.88

Values are expressed as Mean±SE. Values with small superscripts differ significantly (P<0.05) within a column.

2.5. *In Vitro* Supplementation of NGF on Concentration of Spermatozoa ATP

The concentration of ATP in spermatozoa decreased significantly, as the time of incubation increased irrespective of whether the group was supplemented or not supplemented. When the time factor was kept constant, only at 60 min of incubation, supplementation with 100 ng dose of NGF in HF group could increase the concentration of spermatozoa ATP, which was significantly(P<0.05) different from control, where as supplementation could not increase the concentration of ATP significantly at 30 or 120 min of incubation (Table 6).

Table-6. Effect of *in vitro* supplementation of NGF (ng/ml) on concentration of ATP in spermatozoa lysates

Time of Incubation	LF			HF		
	Dose of NGF supplemented			Dose of NGF supplemented		
	0	50	100	0	50	100
0	6.417±0.02	6.421±0.02	6.454±0.03	6.425±0.03	6.423±0.02	6.419±0.02
30	6.361±0.02	6.328±0.02	6.388±0.02	6.342±0.02	6.352±0.03	6.360±0.03
60	6.309±0.01	6.305±0.02	6.308±0.03	6.325^{A}±0.03	6.320±0.02	6.358^{B}±0.02
120	6.208±0.02	6.222±0.01	6.231±0.02	6.210±0.02	6.221±0.02	6.225±0.01

Values are expressed as Mean±SE. Lysate of $10x10^{6}$ sperms was used. Values with different capital superscripts differ significantly (P<0.05) within a row.

When all the parameters were compared between low and high fertile groups, keeping time factor constant at a time , it was observed that all the parameters were greater for HF group when compared with LF group, although were not significantly different, except at 60 min for motility, viability and concentration of spermatozoa ATP. When different parameters were

compared for different time periods of incubation, in both high and low fertile group, all the parameters decreased significantly with increase in the time period of incubation, except for one parameter namely plasmalemma integrity which was not significantly different at any time period of incubation .In HF group supplementation of spermatozoa with NGF could maintain motility, viability and acrosome integrity for greater period of time i.e. 60 min whereas in LF supplemented groups it could be maintained till 30 min only .

By immunoflourescence technique, using fluorescent antibody, it was observed that fluorescence was intense at the acrosomal and post acrosomal regions, irrespective of high or low fertile group. Fluorescence could be observed in both the groups (Fig 1). The test was qualitative. When the extracted spermatozoa DNA was subjected to PCR, and run on 1.8 % agarose gel a 112 bp product was obtained for both LF and HF groups confirming the presence of receptor (Fig 2).

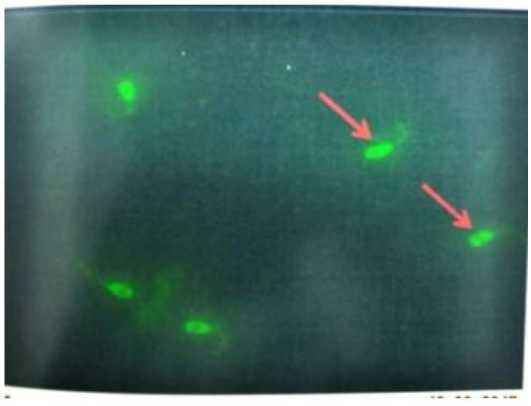

Fig-1. Immunolocalization of NGF receptor in Murrah buffalo bull spermatozoa

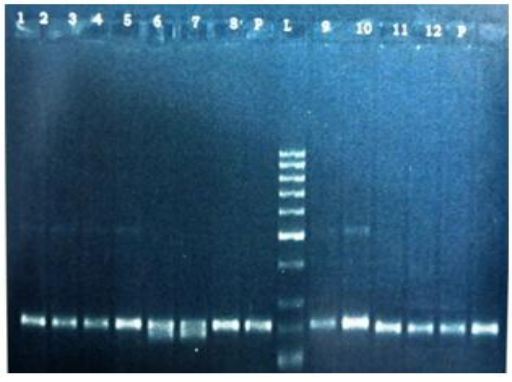

Fig-2. PCR product of NGF receptor (112 bp)
L- 50 bp ladder LF (1- 8),HF (9-12)
P- positive marker for NGF receptor

3. DISCUSSION

There is evidence that a neurotropin like NGF has important role in non neuronal cells in reproductive system [18]. In the present study, higher concentration of blood plasma NGF of HF group indicates positive relation between plasma concentration of NGF and spermatozoa functional parameters and fertility of bulls. In the sperm lysate, NGF could not be detected by EIA. Reports of Li, et al. [19]; Li, et al. [2] have suggested that NGF is present in spermatozoa, but the methodology for estimation was by Western blotting and PCR. In the present study it was observed that all the parameters related to spermatozoa, were higher in the HF when compared with LF group. At the same time, concentration of blood plasma NGF was higher in HF group. It suggests regulatory role of circulatory NGF on buffalo sperm functional parameters. The physiological concentration of NGF (50/100 ng/ml) was more closely related in augmenting sperm physiological functions *in vitro* in the present study. Li, et al. [2] reported the same in bovines and humans. With immunofluorescence technique it was observed that TrKA receptor is present on spermatozoa and PCR studies confirmed the presence of NGF receptor on Murrah bull spermatozoa. Using an immunofluorescent technique, NGF-immunoreactivity was localized to the sperm head and tail, whereas that of TrkA was detected in the acrosomal cap,

nucleus, and tail regions [2, 20].This is the first report in Murrah bull confirming presence of NGF receptor in spermatozoa. *In vitro* studies regarding effect of NGF on human sperm motility by CASA showed that sperm motility were enhanced in a time and dose dependant manner [21]. Study by Saeednia, et al. [8] reported that addition of NGF to medium containing post thawed sperms can enhance motility and viability of human sperms. Hence from the present study, it can be suggested that supplementation of NGF *in vitro* might have enhanced buffalo spermatozoa viability and motility in both LF and HF groups till one hour of incubation when compared with the non supplemented group. This study was carried out for short duration of time; technology has to be improvised for conducting experiments for longer durations. Report of Perrard, et al. [22] suggested that NGF is produced by germ cells and acts on sertoti cells. In the present study, however, by EIA technique, we could not measure NGF in sperm lysates. May be other techniques can demonstrate the presence/secretion of NGF by Murrah bull spermatozoa. It's known that TRKA binds NGF and its presence could be detected in buffalo bull spermatozoa, suggesting role of circulatory/seminal plasma NGF in regulating spermatozoa function. This was confirmed by in vitro studies with supplementation of NGF on different sperm functions. With respect to spermatozoa integrity and viability on NGF supplementation *in vitro*, it was observed that NGF could maintain viability/reduced permeability of membrane and plasmalemma integrity of spermatozoa for a greater duration of time *in vitro* when compared with control within 120 min of study. Although the extent of its effect in augmenting functions or reducing the damage to the membrane was different when compared between LF and HF group . Hence its effect was more prominent in maintaining spermatozoa functional parameters of HF group when compared with the functional parameters of LF group. The mechanism by which NGF affects the viability and motility parameters is not understood and study is required in this area. This study also suggests that enhancement of the sperm functions by supplementation of NGF *in vitro* may be applicable in assisted reproductive technologies yielding better results with Murrah bull spermatozoa.

Funding: This study received no specific financial support.

Competing Interests: The authors declare that they have no competing interests.

Contributors/Acknowledgement: Both authors contributed equally to the conception and design of the study.

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