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Original Research Article

Effect of Ascorbic Acid on Storage Capacity of Murrah Bull Epididymal Spermatozoa at Refrigerator Temperature

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A B S T R A C T

The present investigation was carried out at Deep Frozen Semen Laboratory, Department of Veterinary Gynaecology and Obstetrics, C.V.Sc.&A.H., N.D.U.A.&T., Kumarganj, Faizabad (U.P.) with the aim to examine the effect of ascorbic acid on epididymal spermatozoa of Murrah bull on freezability. A total 56 pair (8 pair of testicle in each groups) of testicle were utilize and semen was collected at 6hrs (G1), 12hrs (G2), 24hrs (G3), 36hrs (G4), 48hrs (G5), 72hrs (G6) and 96hrs (G7) after slaughter. All testicles were stored at refrigerated temperature (4^0C) till semen collection. Basic extender used in this research was Tris-buffer and ascorbic acid. Semen dilution & addition of ascorbic acid to maintain addition of concentration in the dilution in the group T1, T2 & T3 @ 1mM, 2mM & 3Mm respectively along with a control group. Post thawed evaluation of refrigerated semen done after 24 hrs storage at the parameters like % progressive motility, %of live count, % of sperm abnormality and % HOS reactive spermatozoa test. The results showed that, mean (±SE) of percent post thaw motility in Epididymal semen collected indicates a significant ($P<0.05$) improvement in sperm motility was reported in T2 and T3 in all the groups (6, 12, 24, 36, 48, 72 & 96 hrs after slaughter). The mean (±SE) of percent live count in post thaw Epididymal semen collected 6, 12, 24, 36, 48, 72 & 96 hrs after slaughter have a significant ($P<0.05$) improvement in sperm livability in T2 and T3. The results showed that, mean (±SE) of percent post thaw sperm abnormalities in Epididymal semen collected indicates a significant ($P<0.05$) improvement in sperm abnormalities reported in T2 and T3 in all the groups (6, 12, 24, 36, 48, 72 & 96 hrs after slaughter). The mean (±SE) of percent Hypo osmotic swelling test in post thaw Epididymal semen collected after 6, 12, 24, 36, 48, 72 & 96 hrs after slaughter shows a significant ($P<0.05$) improvement in Hyper osmotic swelling test in T2 and T3. It may be concluded that the 6hrs and 12hrs collection of Epididymal semen of Murrah bull critically evaluated from storage point of view and ascorbic acid (@ 2mM) in semen additive appears to provide promising results.

Keywords

Ascorbic Acid, Spermatozoa, Epididymal, Refrigerated Temperature, Murrah Bull

Introduction

Buffaloes are integral part of Indian livestock sector followed by other ruminant species. India possesses 199.1 million cattle and 105.3 million buffaloes and ranks first

in milk production in global world mainly contributed by buffaloes (DAHD, 2010). They served in, prosperity and adversity, peace and war, draught and floods. Buffalo has a favoured position as dairy animal with farmers and dairy owner, in comparison to the cow in India. No other technology in agriculture except hybrid seed and fertilizer use has been so widely adopted at a global scale as Artificial insemination. AI has been intensively used to improve genetic potential of farm animals in developed countries. The high intensity and accuracy of selection arising from AI, can lead to a four-fold increase in the rate of genetic improvement in dairy cattle relative to that from natural mating. Progress in semen collection, dilution and cryopreservation now enables a single bull to be used simultaneously in several countries for up to100000 inseminations a year. This implies that a very small number of top bulls can be used to serve a large buffalo population. Additionally, each bull is able to produce a large number of daughters thus facilitating accurate progeny testing of bulls. Epididymal sperm have been successfully used for AI and in vitro production of embryos (IVP) in several species (Hori *et al.,* 2004; Hori *et al.,* 2005). Motile sperm have been isolated from cool stored epididymis of cattle (Nichi *et al.,* 2007) and African buffalo (Syncerus *caffer*) (Bartels *et al.,* 1999). Ascorbic acid increased the percentage of live, acrosome intact spermatozoa during storage at 5°C. Ascorbic acid does inhibit per oxidation of membrane lipids during storage and thus has protective effects on sperm membranes. The beneficial influences of ascorbic acid can be attributed to the fact that ascorbic acid is a very efficient antioxidant, and a scavenger of oxygen free radicals which are toxic products of many metabolic processes (Dawson *et al.,* 1992). Inhibition of spermatozoa activity by physical (Refrigeration and deep), chemical (reversible chemical inhibition) and flow dialysis technique are the methods by which the semen can preserved *in vitro*, in buffers containing citrate, phosphate, or bicarbonate alone or in combination. In the background of above, it is well concealed that study related with improvement in the freezability of epididymal spermatozoa will be a welcome innovation. Hence the present study was conducted with the objective to find out improvement in the quality of refrigerated epididymal semen of Murrah bull with incorporation of ascorbic acid in dilutor.

Materials and Methods

The present investigation entitled "Effect of Ascorbic Acid on Storage Capacity of Murrah Bull Epididymal Spermatozoa at Refrigerator Temperature" was carried out at Deep Frozen Semen Laboratory, Department of Veterinary Gynaecology and Obstetrics, C.V.Sc.&A.H., N.D.U.A.&.T., Kumarganj, Faizabad (U.P.). The present study was initiated with the aim to assess the effect of ascorbic acid on epididymal spermatozoa of Murrah bull on freezability. A total 56 pair (8 pair of testicle in each groups) of testicle were utilize and semen was collected at 6hrs (G1), 12hrs (G2), 24hrs (G3), 36hrs (G4), 48hrs (G5), 72hrs (G6) and 96hrs (G7) after slaughter. All testicles were stored at refrigerated temperature (4^0C) till semen collection. The testes from each animal were removed immediately after slaughter and transported on ice (4^0C) to a laboratory for further processing. Basic extender used in this research was Tris-buffer and ascorbic acid. The tris buffer was prepared one day before actual use. Semen dilution & addition of ascorbic acid to maintain addition of concentration in the dilution in the group T1, T2 & T3 @ 1mM, 2mM & 3Mm

respectively along with a control group. Post thawed evaluation of refrigerated semen done after 24 hrs storage at the parameters like % progressive motility, %of live count, % of sperm abnormality and % HOS reactive spermatozoa test.

Statistical analysis

Data were presented as mean and standard error of the mean (SEM). Analysis of variance (ANOVA) was used to asses differences among the bulls and treatments. When the F ratio is sign is significant ($P<0.05$).

Results and Discussion

Post Thaw Motility

The mean (±SE) of percent post thaw motility in Epididymal semen collected after 6, 12, 24, 36, 48, 72 & 96 hrs after slaughter in control (56.00±0.65, 51.63±0.75, 48.75±0.75, 43.00±0.80, 36.75±0.60, 23.13±1.32 and 11.75±0.75, respectively), T1 (60.00±0.65, 55.63±0.75, 52.75±0.75, 47.25±0.80, 40.50±0.65, 27.13±1.38 and 14.38±0.82, respectively), T2 (62.88±0.72, 58.13±0.85, 55±0.76, 49.50±0.73, 42.50±0.65, 29.13±1.38 and 16.25±0.81, respectively), and T3 (61.13±0.72, 56.75±0.81, 53.63±0.74, 48.50±0.74, 41.50±0.65, 28.13±1.38 and 15.50±0.84, respectively), is given in the table 1. to 3. and. A significant ($P<0.05$) improvement in sperm motility was reported in T2 and T3 in all the groups (6, 12, 24, 36, 48, 72 & 96 hrs after slaughter). The present finding similar to the observation of Andrabi *et al.,* 2008 in buffalo whereas, Aurich *et al.,* 1997, Ball *et al.,* 2001 did not detect a positive effect after addition of ascorbic acid to cold extended equine spermatozoa similarly, Akthar *et al.,* (2011) also referred no significant improvement in the sperm motility after incorporation of ascorbic acid. Variation in the observation in different studies may be due to variation of concentration of ascorbic acid as an additive in the dilutor. The antioxidant may also have decrease the motility of spermatozoa by reduction of pH value a low pH does induce reversible reduction in the motility of spermatozoa (Acott and Carr 1984) which, is in agreements with our findings that beneficial effect of ascorbic acid on motility may have been marked at higher concentration. As ascorbic acid when added in the semen is known to enhance motility of bull sperm and buffalo sperm under refrigerated temperature for 120 hrs increase post thaw sperm motility and livability however observed no effect on motility after addition of ascorbic acid at the concentration of 25mM. Whereas, various researcher (Foote *et al.,* 2002, Hu *et al.,* 2010) reported that higher concentration of ascorbic acid was detrimental to sperm motility of frozen thawed bull semen confirming the result of the current study.

The results in decrease motility on cold storage of testis in the present study clearly showed that buffalo bull epididymis resulted in the agreement of previous which found in other species that total motility is variable and most affected as a time of storage. (Sankai *et al.,* 2001; Hishinuma *et al.,* 2003; Kaabi *et al.,* 2003 Martinez-pastor *et al.,* 2005). Epididymal environment provides the most ideal condition of sperm survival. However, refrigeration at 5 0C is necessary to diminish energy wastage and extend sperm lifespan.

The percent post thaw motility was significantly ($P<0.05$) and positively correlated with per cent live count and percent HOS in control T1, T2 and T3 in among groups (6hrs, 12hrs, 24hrs, 36hrs, 48hrs, 72hrs and 96hrs).

Table.1 Post Thaw physicomarphological, Epididymal, seminal characteristic and HOS positive sperm (Mean ± S.E.) of Murrah bull in Control group of testis

Group	PD Motility %	PD Live count %	PD HOS %	Sperm Abnormalities %
G1(6hrs)	56.00±0.65a	64.13±0.58a	29.38±0.82a	21.38±0.41a
G2(12hrs)	51.63±0.75b	61.75±0.71ab	28.63±0.56ab	23.25±0.50ab
G3(24hrs)	48.75±0.75bc	58.63±0.67bc	27.50±0.31abc	25.63±0.32bc
G4(36hrs)	43.00±0.80d	52.58±0.71d	28.13±0.70abd	28.25±0.57d
G5(48hrs)	36.75±0.60e	46.63±0.62e	23.38±0.80e	30.25±0.36de
G6(72hrs)	23.13±1.32f	33.88±1.29f	15.00±0.46f	33.63±0.62f
G7(96hrs)	11.75±0.75g	21.50±0.77g	10.13±0.51g	33.63±0.67fg

Mean bearing different superscript (a, b, c, d, e, f, g) in a Column significantly ($p<0.05$) differed; repeatedly for each attribute.

Table.2 Post Thaw physicomarphological, Epididymal, seminal characteristic and HOS positive sperm (Mean ± S.E.) of Murrah bull in T1 group of testis

Group	PD Motility %	PD Live count %	PD HOS %	Sperm Abnormalities %
G1(6hrs)	60.00±0.65a	66.50±0.68a	31.50±0.75a	19.63±0.37a
G2(12hrs)	55.63±0.75b	63.25±0.97ab	30.25±0.45ab	21.13±0.54ab
G3(24hrs)	52.75±0.75bc	60.63±0.67bc	28.63±0.56abc	23.50±0.37c
G4(36hrs)	47.25±0.80d	55.13±0.87d	29.13±0.92abcd	26.88±0.69d
G5(48hrs)	40.50±0.65e	49.25±0.64e	25.38±0.80e	28.25±0.45de
G6(72hrs)	27.13±1.38f	35.50±1.26f	17.75±0.36f	31.63±0.62f
G7(96hrs)	14.38±0.82g	23.00±0.84g	14.13±0.63g	31.38±0.56fg

Mean bearing different superscript (a, b, c, d, e, f, g) in a Column significantly ($p<0.05$) differed; repeatedly for each attribute.

Table.3 Post Thaw physicomarphological, Epididymal, seminal characteristic and HOS positive sperm (Mean ± S.E.) of Murrah bull in T2 group of testis

Group	PD Motility %	PD Live count %	PD HOS %	Sperm Abnormalities %
G1(6hrs)	62.88 ±0.72a	69.13±0.81a	33.00±0.86a	17.50±0.42a
G2(12hrs)	58.13 ±0.85b	64.88±1.04ab	31.63±0.56ab	18.50±0.56ab
G3(24hrs)	55.00 ±0.76bc	62.75±0.72bc	30.63±0.41abc	21.25±0.36c
G4(36hrs)	49.50 ±0.73d	57.13±0.87d	30.88±0.78abcd	23.50±0.75cd
G5(48hrs)	42.50 ±0.65e	51.25±0.61e	27.63±0.65e	26.00±0.42e
G6(72hrs)	29.13 ±1.38	37.00±1.30f	19.88±0.35f	29.38±0.63f
G7(96hrs)	16.25 ±0.81g	24.88±0.81g	16.38±0.88g	29.63±0.49fg

Mean bearing different superscript (a, b, c, d, e, f, g) in a Column significantly ($p<0.05$) differed; repeatedly for each attribute.

Live count

The mean (±SE) of percent live count in post thaw Epididymal semen collected after 6, 12, 24, 36, 48, 72 & 96 hrs after slaughter in control (64.13±0.58, 61.75±0.71, 58.63±0.67, 52.58±0.71, 46.63±0.62, 33.58±1.29 and 21.50±0.77, respectively), T1 (66.50±0.68, 63.25±0.97, 60.63±0.67, 55.13±0.87, 49.25±0.64, 35.50±1.29 and 23.00±0.84, respectively), T2 (69.13±0.81, 64.88±1.04, 62.75±0.72, 57.13±0.87, 51.25±0.61, 37±1.30 and 24.88±0.81, respectively), and T3 (67.13±0.71, 64.13±0.93, 61.63±0.67, 56.13±0.87, 50.25±0.64, 35.25±1.86 and 23.88±0.81, respectively), are given in the table 1. to 3. A significant (P<0.05) improvement in sperm livability was reported in T2 and T3. Cryopreservation of spermatozoa recovered from cauda epididymides, independent of storage time, cause a decrease in post thaw live count percent similar findings were also observed by Martins *et al.,* (2009). Cryopreservation process are known as being damage to the sperm cell and can have an effect of sperm motility and fertilization rate due to compromising the integrity of acrosomal structure (Wakayama and Yanagimachi, 1998). Reports in other species indicate spermatozoa from epididymis are less tolerant to cryopreservation then spermatozoa from an ejaculate (Krzywinski, 1981; Zomborszky *et al.,* 1999). The live count was significantly (P<0.05) and positively correlated with percent HOS reactive sperm and post thaw motility

Hyper osmotic swelling test

The mean (±SE) of percent Hypo osmotic swelling test in post thaw Epididymal semen collected after 6, 12, 24, 36, 48, 72 & 96 hrs after slaughter in control (29.38±0.82, 28.63±0.56, 27.50±0.31, 28.13±0.70, 23.38±0.80, 15±0.46 and 10.13±0.51, respectively), T1 (31.50±0.75, 30.25±0.45, 28.63±0.56, 29.13±0.92, 25.38±0.80, 17+-.75±0.36 and 14.13±0.63, respectively), T2 (33±0.86, 31.63±0.56, 30.63±0.41, 30.88±0.78, 27.63±0.65, 19.88±0.35 and 16.38±0.88, respectively), and T3 (32.13±0.85, 31±0.46, 29.75±0.36, 30.50±0.77, 26.88±0.75, 18.25±0.36 and 15.13±0.78, respectively), are given in table 1. To 3. A significant (P<0.05) improvement in Hyper osmotic swelling test was reported in T2 and T3. Reactive oxygen species molecules at physiological levels are essential for the spermatozoa, and it was observed that higher concentration of Vitamin C may result in impairment of semen quality (Andrabi *et al.,* 2008) and decreases the success of fertility of bull semen *in vitro* (Dalvit *et al.,* 1998). These finding are in agreement with present study that best results of ascorbic acid as an additive is observed at optimal concentration with 2 mM as compared to 3 mM. The HOST was significantly (P<0.05) and positively correlated with percent post thaw motility and livability for control, T1, T2 and T3 treatment group.

Sperm abnormality

The mean (±SE) of percent sperm abnormalities in post thaw Epididymal semen of bull collected after 6, 12, 24, 36, 48, 72 & 96 hrs after slaughter in control (21.38±0.41, 23.25±0.50, 25.63±0.32, 28.25±0.57, 30.25±0.36, 33.693±0.62 and 37.63±0.67, respectively), T1 (19.63±0.37, 21.13±0.54, 23.50±0.37, 26.88±0.69, 28.25±0.45, 31.63±0.62 and 31.38±0.56, respectively), T2 (17.50±0.42, 18.50±0.56, 21.25±0.36, 23.50±0.75, 26±0.42, 29.38±0.63 and 29.63±049, respectively), and T3 (18.63±0.37, 20±.053, 22.75±0.31, 26±0.68, 26.63±0.46, 30.63±0.62 and 30.50±0.50, respectively), are given in the

table 1. to 3. A significant (P<0.05) improvement in sperm abnormalities was reported in T2 and T3 similar findings were also reported by Hu *et al.,* (2010). Whereas Akhter *et al.,* (2011) has observed no significance improvement in sperm quality after incorporation of ascorbic acid as an additive in the dilutor.

Based on present study it can be concluded that the 6hrs and 12hrs collection of Epididymal semen of Murrah bull critically evaluated from storage point of view and ascorbic acid (@ 2mM) in semen additive appears to provide promising results hence; it can be used as supplemented in the dilutor before storage.

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