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Effect of Scrotal Insulation on Semen Quality Profiles in Mithun

P. Perumal*

ICAR-National Research Centre on Mithun, Medziphema, Nagaland (797 106), India

Corresponding Author

P. Perumal
e-mail: perumalponraj@gmail.com

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Abstract

Testes are highly sensitive to increasing body or scrotal temperature or thermal insulation of testis which in turn causes degenerative changes causes reduction of testicular weight and size as well as alteration in its consistency, which ultimately leads to deleterious effects on semen production and its quality profiles. The present study was conducted to assess the adverse effects of scrotal insulation on semen quality profiles (SQPs), seminal antioxidant profile (total antioxidant capacity; TAC) and oxidative stress (malondialdehyde; MDA) in mithun bulls. The study was conducted in livestock farm, ICAR-National Research Centre on Mithun, Medziphema, Nagaland, India. Semen ejaculates were collected twice a week from six adult healthy mithun bulls with good body condition score (5–6) from day of scrotal insulation (day 0) to day 42 of post insulation to know the effect of scrotal insulation on SQPs, TAC and MDA of mithun semen. Scrotal insulation was applied for 5 days (day 0: day of insulation). Semen ejaculates were collected & evaluated upto the day 42 of experimental period. Result revealed that scrotal insulation has significant (p<0.05) effect on the SQPs, TAC and MDA upto day 42 of insulation. However, these scrotal insulated animals were recovered gradually in SQPs as well as antioxidant profiles. The adverse effects of scrotal insulation on SQPs, TAC and MDA indicated that the semen collection as well as preservation required to be suspended till day 42 of scrotal insulation to get good quality or normal semen to avoid the conception failure in artificial insemination (AI) centre using such semen in mithun.

Keywords: Scrotal insulation, mithun, semen, antioxidant, oxidative stress

1. Introduction

The testes are highly sensitive to increasing body or scrotal temperature or thermal insulation of testis which inturn causes degenerative changes are reported in the form of reduction in weight and size of testis as well as alteration in consistency of testes (McEntee, 1990), which ultimately have deleterious effect on semen production and its quality profiles. Temperature of testicle should be 2-6 °C less than the body temperature in mammals, 33-34 °C is optimum testicular temperature for higher spermatogenesis in bovine species (Wildeus and Entwistle, 1983; Barth and Bowman, 1994). Higher scrotal as well as testicular temperature inturn increased metabolic rate and increased oxygen demand but coiled and lengthier tiny testicular artery complex is not able to supply of oxygen which inturn to testicular hypoxia leads to generation of lipid peroxide molecules leads to poor semen production and its quality and increased different sperm abnormalities and poor fertility results (Setchell, 1998). Based on the available research reports, exotic breeding bulls (*Bos taurus*) have lower sperm output and higher percentages of total sperm abnormality when exposed to scrotal insulation or summer stress or heat/thermal stress or higher ambient temperature were observed (Brito et al., 2003; Arteaga et al., 2005; Rajoriya et al., 2013). However thermal or scrotal insulation tolerance capacity of indigenous cattle (*Bos indicus*) is considerably greater than European cattle (*Bos taurus*) as indicated by higher SQPs and antioxidant profiles and lower total sperm abnormalities and MDA in former than in letter (Brito et al., 2003; Arteaga et al., 2005; Rajoriya et al., 2013). Breed variation and their capability to adapt and adjust to the sub-tropical or tropical temperature is also another important factor to determine the ambient temperatures that alter the bull reproduction and its fertilizing ability (Rajoriya et al., 2013). It is believed that the mithun is cold loving animal and during hot hours or seasons, they enter and retire into their deep cold forest and mostly the mithun is prevalent in tropical rain forest of North-eastern hill States of India and adjoining parts of neighbouring countries and these areas are most favoured chilled condition. Research has been initiated to study the effect of thermal stress or climatic change on reproduction and production potential of mithun and results revealed mithun has performed better in 16 °C and 50% RH than 37 °C and 85% RH (Khate, 2015). Scrotal insulation is a

suitable method to study the effect of increased temperature on semen production and its quality profiles and alteration of biochemical molecules in semen and sperm. Therefore the present study was proposed with the objectives of to study the effect of scrotal insulation on semen quality profiles and alteration of biochemical components of semen in mithun to implement the mithun breeding programme in semi-intensive system to conserve the precious bovine species.

2. Materials and Methods

2.1. Experimental animals

Six numbers (n=6) of healthy adult mithun bulls of 4–6 yr of age with good body condition (score 5–6) were selected from the herd, mithun breeding farm, ICAR-NRC on Mithun, Medziphema, Nagaland, India and the experimental station is located between 25°54´´30´´ North latitude and 93°44´15´´ East longitude and at an altitude range of 250–300m MSL. The mean annual rainfall was 1688.4 mm. The maximum and minimum temperature during the period of experiment year was 32.99 °C and 9.10 °C, respectively. They were maintained under homogenous feeding, housing, lighting and other managemental practices with daily offered *ad libitum* drinking water. The experimental animals were categorised into two groups and each group consisted of three animals. Group I: control and Group II: complete scrotal insulation. In insulation group, the scrotum is covered with pampers baby diapers that was surrounded by a cloth bag and this was tightening by tape around the neck of the scrotum. The scrotal insulation was done for 5 days in the separate animal shed (day 0 is day of insulation).

2.2. *Semen collection and analysis*

Semen ejaculates were collected by trans-rectal massage method from the mithun (attempted twice a week). Briefly, seminal vesicles were massaged centrally and backwardly for 5 min followed by the gentle milking of ampullae one by one for 3–5 min, which resulted into erection and ejaculation. During collection, the initial transparent secretions were discarded and neat semen drops were collected in a graduated test tube with the help of a funnel. Immediately after collection, the samples were preserved at 37 °C in a water bath and evaluated the routine SQPs such as sperm concentration by spectrophotometer, the percentage of sperm total motility (Nikon, Eclipse 80i; magnification 400X with thermostage maintained at 37 °C), viability by eosin nigrosin staining (Tomar, 1997), total sperm abnormality (Tomar, 1997), acrosomal intactness by Giemsa staining (Watson, 1975), the plasma membrane intactness by hypo-osmotic swelling test (HOST) (Jeyendran et al., 1984) and nuclear intactness by Feulgen's staining technique (Barth and Oko, 1989) were determined as per standard procedure. The activities of intra-cellular enzymes such as AST and ALT were estimated in the seminal plasma according to the method described by Reitman and Frankel (1957). Total antioxidants in seminal plasma were estimated by total antioxidant capacity (TAC) colorimetric assay kit (K274; Bio Vision, Mountain View, CA, USA) and malondialdehyde (MDA; TBARS Estimation Kit for Lipid peroxide, HiMedia) as per the manufacturer's guidelines.

2.3. *Statistical analysis*

The results were analysed statistically and expressed as the mean±S.E.M. Means were analyzed by student t test to determine the significant differences between the two experimental groups on the SQPs, TCA and MDA using the SPSS/PC computer program (version 15.0; SPSS, Chicago, IL). One way analysis of variance (ANOVA) was used to assess the significant differences among the days of post insulation period. Differences with values of $p<0.05$ were considered to be statistically significant after arcsine transformation of percentage data by using SPSS 15.

3. Results and Discussion

The result revealed that there was significant ($p<0.05$) variation between control and treatment groups and among the days of post insulation period in treatment group was observed in SQPs, TCA and MDA. SQPs such as sperm concentration (Figure 1), total motility (Figure 2), vitality (Figure 2), intactness of acrosome (Figure 2), plasma membrane (Figure 2) and nucleus (Figure 2) and biochemical profile such as TAC (Figure 5) were significantly ($p<0.05$) higher whereas total sperm abnormality (Figure 3), leakage of AST (Figure 4) and ALT (Figure 3) and production of MDA (Figure 3) were significantly ($p<0.05$) lower in post-insulation period in control than in treatment group

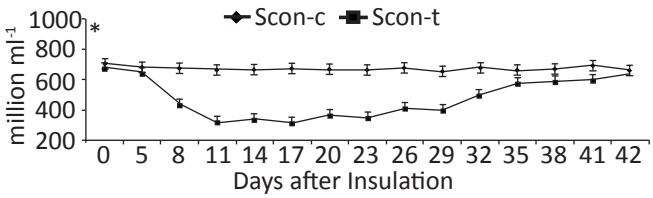

Figure 1: Effect of scrotal insulation on sperm concentration (Scon) in mithun semen (mean±SE), c: control group; t: treatment group. (*indicates $p<0.05$ between control and treatment groups; day 0: day of scrotal insulation)

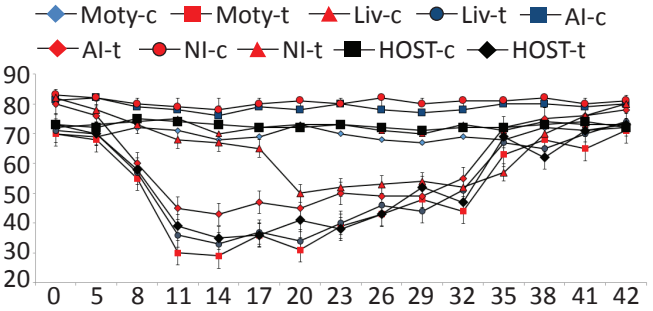

Figure 2: Effect of scrotal insulation on sperm motility (Moty), livability (Liv), acrosomal integrity (AI), nuclear integrity (NI) and hypo osmotic swelling test (HOST) in mithun semen (mean±SE), c: control group; t: treatment group. (*indicates $p<0.05$ between control and treatment groups; day 0: day of scrotal insulation)

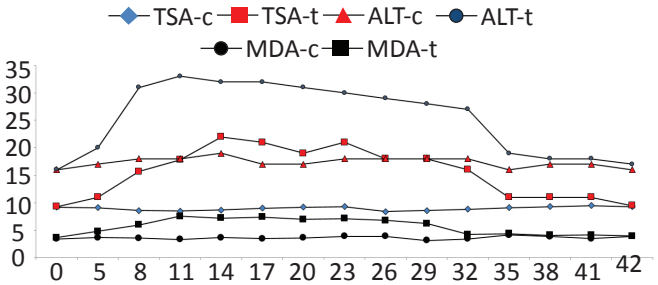

Figure 3: Effect of scrotal insulation on total sperm abnormality (TSA), alanine amino transaminase (ALT) and malondialdehyde (MDA) in mithun semen (mean±SE), c: control group; t: treatment group. (*indicates $p<0.05$ between control and treatment groups; day 0: day of scrotal insulation)

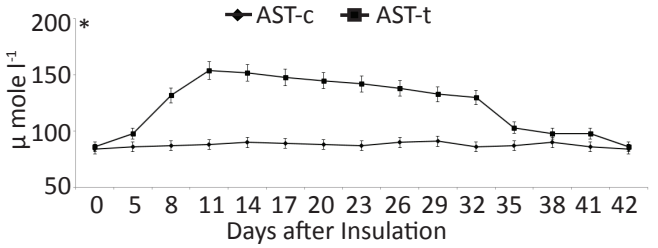

Figure 4: Effect of scrotal insulation on leakage of Aspartate Amino transaminase (AST) in mithun semen (mean±SE), c: control group; t: treatment group. (*indicates $p<0.05$ between control and treatment groups; day 0: day of scrotal insulation)

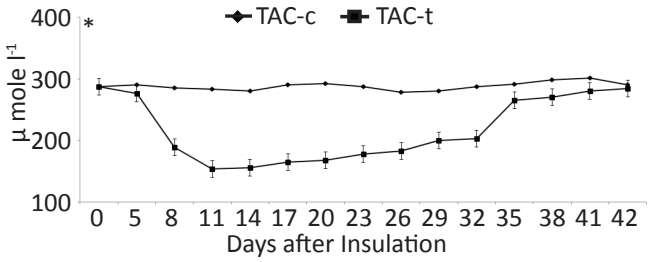

Figure 5: Effect of scrotal insulation on total antioxidant capacity (TAC) in mithun semen (mean±SE), c: control group; t: treatment group. (*indicates $p<0.05$ between control and treatment groups; day 0: day of scrotal insulation)

and pre-insulation than post-insulation period in treatment group. Sperm concentration, motility, viability, intactness of plasma membrane and acrosomal membrane and biochemical profile like TAC were decreased to day 11and were maintained to day 20–24 and then gradually increased to day 35 and finally reached to the level of control group at day 42 whereas intactness of nuclear membrane and nucleus were highly decreased on day 20 and the same were maintained to till day 32 and then gradually increased to day 35 and reached to level of control on day 42. Similarly, leakage of intra-cellular enzymes such as AST and ALT and production of MDA were increased to day 11, maintained to day 32 and then decreased to day 35 and to the level of control at day 42. It was also observed that there was bull to bull variation among the semen quality and biochemical profiles after insulation in the treatment group.

In the present study, the results revealed that scrotal insulation in mithun bulls has affected the SQPs and biochemical profiles of mithun semen and thus it affects the structures and functions of spermatozoa effectively. Thus, the semen from scrotal insulated or heat stressed bulls is not suitable to preserve for artificial insemination. There was no report on effect of scrotal insulation on seminal parameters in mithun and to the best of our knowledge, this is the first report of the effect of scrotal insulation on SQPs and biochemical profiles of semen in mithun.

The scrotal insulation causes thermal stress to the scrotum and testis and affect the Leydig cell function as similar to cryptorchidism or vaccination (Bhakat et al., 2010) as chronic thermal treatment has decreased the testosterone concentration and spermatid population whereas the integrity of sertoli cells has been compromised, which inturn leads to poor quality semen with concomitant drop in total as well as forward progressive motility (Egbounlike et al., 1985). SQPs such as sperm motility, acrosomal intactness and livability were decreased while total sperm abnormalities has been increased significantly ($p<0.05$) after scrotal insulation was in agreement with earlier report in both indigenous as well as crossbred cattle (Brito et al., 2003; Arteaga et al., 2005; Rajoriya et al., 2013). In general, the sperm motility starts and develops during their travel through the epididymis especially cauda epididymis (Moulikrishnan and Ramamohan Rao, 1986). Thermal stress caused by scrotal insulation as expressed by the considerably significant increase in temperature of testes which inturn causes derangement of epididymal functions as well as spermatogenesis in bovine species (Venkatareddy et al., 1991) and which leads to decreased sperm motility as reports of similar picture of testicular hypoplasia as well as testicular degeneration (Arthur et al., 1989). Scrotal insulation caused increased scrotal temperature could cause increase of secondary sperm abnormalities (Brito et al., 2003) as well with rise of sperm tail and mid-piece abnormalities as similar in testicular degeneration and/or partial testicular hypoplasia (Sullivan, 1978). The present finding in the acrosomal intactness is similar to the earlier reports by Brito et al. (2003), who reported decrease the sperm percentage with intact acrosome after scrotal insulation. The acrosome is most sensitive part of the sperm and carried acrosomal enzymes for fertilization, so the adverse effects were more prominent on the acrosomal membrane and its content after scrotal insulation treatment. The acrosomal capsule was either broken or detached inturn causes release of intra cellular enzymes leads to fertilizing ability of spermatozoa has been affected. Similarly, Rathore (1970) has been observed in rams that exposure to artificial hot environment leads to significantly higher acrosomal abnormalities after 9 to10 days of exposure, which indicates the abnormal changes has been occurred during sperm maturation in the epididymis. Plasma membrane intactness was measured by hypo osmotic swelling test has significantly ($p<0.05$) decreased after scrotal insulation than pre-insulation period. There was a decrease of plasma

membrane intactness from day 11–32 of scrotal insulation and return to pre-insulation level after 42days. Similar results were reported by Brito et al. (2003); Vogler et al. (1993) and Barth and Bowman (1994) in bovine species following scrotal insulation. However, it was reported in other investigations that scrotal insulation in indigenous bovine bulls (*B. indicus*) for 4–7 days caused a higher and prolonged reduction of motile as well as normal sperm and which required at least 105–148 days to return to the level of control or normal animals (Fonseca and Chow, 1995). Species breed variation and individual variations within the breed were observed for total sperm motility as well as sperm morphology. HOS test reflects the sperm plasma membrane biochemical integrity, and it is also involved in various process of fertilization such as capacitation and acrosome reaction and finally binding of spermatozoa with the oocyte for fertilization (Argov et al., 2007). Antoine and Pattabiraman (1999) have been observed that HOST positive spermatozoa were reduced in ejaculates collected from buck exposed to scrotal insulation caused rise the temperature of testicle and scrotum. In another study in the breeding bulls, HOST positive spermatozoa were decreased after heat or thermal treatment (Sivaramalingam, 1994). Therefore it can be suggested that scrotal insulation severely affects the plasma membrane integrity by alteration of biochemical structure of the sperm plasma membrane.

In general, the sperm morphology returns to pre-insulation stage within 42 days of the treatment (Kastelic et al., 1996; Wildeus and Entwistle, 1983). But recovery is also depends upon degree and duration of exposure. Prolonged exposure and severe increase of temperature will inturn to increase the interval for from end of the exposure to recovery. It suggests that the reduction in semen quality is associated with higher testicular temperature is finally related to the severity as well the duration of the higher testicular temperature or scrotal insulation (Arteaga et al., 2005). AST and ALT are essential metabolic enzymes and are responsible for metabolic processes which supply energy for viability, motility & velocity and fertilizing ability of spermatozoa and activities of these transaminases in semen are valuable indicators to determine the quality of semen as because they are used to measure the stability of sperm membrane (Perumal et al., 2013). Therefore, it is correlated that high concentration of intra cellular transaminase enzymes in the extra-cellular fluid means there is increasing concentration of abnormal spermatozoa in semen samples due to damage on sperm membrane and leakage of these enzymes from the sperm cells (Gundogan, 2006). Increase concentration of AST and ALT in seminal plasma of scrotal insulated animals due to structural instability of the sperm or its plasma membrane (Buckland, 1971). Significantly higher AST and ALT levels were observed in scrotal insulated animal as because it has destabilises the membrane of acrosome, plasma membrane, mitochondria as well as the flagella of the spermatozoa. Scrotal insulation on TAC and MDA revealed that there was a significant (p<0.05) difference between the scrotal insulated and control groups in the present experiment. Thermal or heat stress is due to scrotal insulation causes increased overflow in blood circulation of the testis which inturn increases the temperature to 42-43 ^{o}C as in local heating (Mieusset et al., 1992) leads to insufficient oxygenation and hypoxic stress to testes results increased formation of ROS leads to arrest of spermatogenic cell cycle and apoptosis (Iida et al., 2002) which leads to depletion of germ cells (Krakowska et al., 2006) and its function (Paul et al., 2009) which ultimately affect the semen production, its quality and fertilizing ability of the spermatozoa.

High concentration of polyunsatured fatty acids present in the mammalian sperm membrane which inturn favour the sperm to susceptible to lipid peroxidation subsequently alters significantly the motility and intactness of sperm membranes as it alters the phase transition and composition of cholesterol and phospholipid of membranes of sperm as well as significant damage to DNA of spermatozoa (Griveau et al., 1995; Perumal et al., 2011a; Perumal et al., 2011b) and finally has significant effect on fertilizing ability of sperm and fertility of breeding bulls. So that it plays critical role in the male reproductive system and the sperm protection system is to be well protected against oxidative cellular injury by reactive oxygen species. Naturally various essential antioxidants available in the mammalian semen and functions against LPO to protect the sperm cells from ROS & LPO and helps to maintain the intactness of the sperm very efficiently (Bucak et al., 2008). It was reported that the free radicals generation rate is temperature as well as time dependent in testis or spermatozoa (Ikeda et al., 1999) and the scrotal insulation leads to heat or thermo stress which inturn deterioration of quality of sperm and also deleterious effect on fertility status of sperm as well as the breeding bulls. Level of the antioxidant is reduced due to thermal or heat stress in the scrotal insulated animals (Ahotupa and Huhtaniemi, 1992) because the antioxidants are produced and released from epididymis (Fouchecourt et al., 2000; Zini et al., 2002) especially from cauda epididymis into the stream of semen as the epididymis is thermo sensitive as well as testosterone dependent as other accessory sex glands (Saeed et al., 1994). Earlier reports suggested that impairment of the endocrinological functions in testes is due to thermo stress may be due to scrotal insulation or any other causes leads to significant reduction in LH receptor numbers (Risbridger et al., 1981) and improper uptake of gonadotropin in the testis (Sharpe, 1983). Moreover, in the thermo stressed or scrotal insulated animals, there was reduction of the functional activity of enzymes responsible for biosynthesis of androgen and also there was decreased androgen-binding protein production (Kerr et al., 1979).

4. Conclusion

Scrotal insulation has severe adverse effects on SQPs, antioxidant profile and favours MDA production in mithun

semen. SQPs and seminal antioxidant were decreased and ROS concentration and sperm abnormalities were increased due to scrotal insulation. Therefore the semen collection and preservation need to be suspended till day 42 of scrotal insulation to get normal or returning to normal SQPs and TAC of semen to avoid the fertilization failure in the artificial insemination centre using such semen in this precious bovine species.

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