The Bioscan 8(1): 181-183, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFECT OF AMBIENT TEMPERATURE ON MEMBRANE INTEGRITY OF SPERMATOZOA IN DIFFERENT BREEDS OF BULLS

S. R. MISHRA*, A. K. KUNDU AND A. P. K. MAHAPATRA
Division of Physiology and Climatology, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh – 243 122
[1]Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar - 751 003, Odisha, INDIA
E-mail:smruti.mishra1983@gmail.com

KEYWORDS
Spermatozoa
Bull
Membrane integrity
Live sperm
HOS test
Acrosome integrity

Received on :
10.12.2012

Accepted on :
16.01.2013

***Corresponding author**

ABSTRACT

The study was conducted to assess the effect of ambient temperature on membrane integrity of fresh and post thaw sperm in 4 different breeds of bulls like CB (crossbred), Red sindhi, Haryana and Jersey. Semen was collected during different temperatures throughout the year *i.e.* 10ºC-18ºC (lower range temperature), 25ºC-35ºC (Medium range temperature) and more than 35ºC (Higher range temperature). Membrane integrity of spermatozoa was assessed by live sperm percent count test, acrosome integrity test (AI test) and hypoosmotic swelling test (HOS test). The live sperm (%), intact acrosome (%) and HOS positive sperm (%) in all the four breeds were decreased significantly ($p < 0.01$) with increase in temperature. During lower and medium temperature range, no significant difference was observed in live sperm (%) where as significant difference ($P < 0.01$) was observed in acrosome integrity (%) and HOS positive sperm (%). In higher temperature range, significant difference ($p < 0.01$)

INTRODUCTION

Bull's fertility is highly important as it involves the fertilization of oocyte to produce a good, viable and genetically potential conceptus. Artificial insemination is the most widely used technique followed throughout the world, to increase numbers of genetically potential breeding bulls, offering many advantage over natural service. High quality semen is crucial for successful artificial insemination. Though the technique is being used to increase bull fertility status still the numbers of efficient breeding bulls are reduced day by day. Male related embryonic death owe to reduced semen quality due to heat stress (Setchell *et al*., 1988; Colas, 1983). Heat stress affects the body growth, the biological functions and the productive and reproductive characteristics of sheep (Sevi *et al*., 2002). Season is one of the major factors influencing the reproductive performance of breeding bulls and it exerts its effect through macro and micro climatic factors like temperature, humidity, rainfall, photo-period (Mandal *et al*., 2000). In case of bulls the increased evidence of sperm abnormality and reduced sperm output has been reported during warmer seasons of the year (Igboeli *et al*., 1971; Parkinson, 1987). In the light of above facts, it is very essential to evaluate the semen quality before artificial insemination. Semen quality study can be best assessed by the funtional integrity of sperm membrane in cattle (Correa *et al*., 1994). So the present study was undertaken with the objective to find out the status of membrane integrity of fresh and post thaw sperm in bulls during different range of temperature

MATERIALS AND METHODS

Four different breeds of bull *i.e.* Crossbred (CB), Red sindhi, Haryana, Jersey bulls within age group of 4 to 6 years were studied during the period from December'10 to August'11 at Frozen Semen Bank, Cuttack, Odisha. A total of 360 ejaculates at the rate of five from one bull (six numbers of bulls) from each breed in three different temperature periods were collected for assessment. The whole study duration was divided into three temperature periods, as low temperature range *i.e.* from 10ºC to 18ºC (Dec-Feb), medium temperature range from 25ºC to 35ºC (Mar-Apr) and high temperature range *i.e.* more than 35ºC (May-Aug). Eosin-Nigrosin dye test (sperm livability % test), acrosome integrity test and Hypo-osmotic swelling test (HOS test) were conducted to find out the status of membrane integrity of spermatozoa.

In Eosin-Nigrosin dye test minimum 200 spermatozoa were counted and identified as live or dead under the oil immersion objective (45X) basing upon the staining character Saxena (2000). In acrosomal integrity test 200 spermatozoa per slide were examined under high power phase contrast-microscope and the integrity of the acrosome was studied as per the method previously described by Watson (1975). Hypo-osmotic swelling test was conducted following the technique used by Revell and Mrode (1994). Statistical analysis was done as by

Table 1: Similar superscript shows no significant difference,Dissimilar superscript shows highly significant difference ($p < 0.01$), LIVE %- Live sperm percent, A-I%- Acrosome integrity percent, HOST%- hypo-osmotic swelling test percent. T -1(Temperature between 10ºC-18ºC)**

	Parameter	Crossbred	Red Sindhi	Haryana	Jersey
T-1	LIVE%	88.73 ± 0.47^{a}	88.90 ± 0.47^{a}	88.70 ± 0.59^{a}	89.53 ± 0.59^{a}
	A-I%	82.20 ± 0.47^{a}	80.16 ± 0.47^{b}	81.00 ± 0.69^{a}	81.73 ± 0.69^{a}
	HOST%	83.90 ± 0.51^{a}	81.16 ± 0.51^{b}	83.53 ± 0.66^{a}	84.36 ± 0.66^{a}

Table 2: Similar superscript shows no significant difference; Dissimilar superscript shows highly significant difference ($p < 0.01$); LIVE %- Live sperm percent, A-I%- Acrosome integrity percent, HOST%- hypo-osmotic swelling test percent. T-2(Temperature between 25ºC-35ºC)**

	Parameter	Crossbred	Red Sindhi	Haryana	Jersey
T-2	LIVE%	83.83 ± 0.45^{a}	83.70 ± 0.45^{a}	82.87 ± 0.38^{a}	84.50 ± 0.38^{a}
	A-I%	77.57 ± 0.50^{a}	75.00 ± 0.46^{b}	75.50 ± 0.42^{b}	75.80 ± 0.42^{b}
	HOST%	76.46 ± 0.50^{a}	73.66 ± 0.50^{b}	72.73 ± 0.41^{b}	74.00 ± 0.41^{b}

Table 3: Similar superscript shows no significant difference; Dissimilar superscript shows highly significant difference ($p < 0.01$); LIVE %- Live sperm percent, A-I%- Acrosome integrity percent, HOST%- hypo-osmotic swelling test percent. T-3(Temperature more than 35ºC)**

	Parameter	Crossbred	Red Sindhi	Haryana	Jersey
T-3	LIVE%	78.23 ± 0.37^{a}	78.60 ± 0.37^{a}	77.20 ± 0.41^{a}	77.70 ± 0.50^{a}
	A-I%	72.66 ± 0.43^{a}	69.36 ± 0.43^{b}	70.53 ± 0.34^{b}	69.47 ± 0.34^{b}
	HOST%	72.20 ± 0.35^{a}	69.36 ± 0.35^{b}	69.80 ± 0.32^{b}	68.70 ± 0.31^{b}

Revell and Mrode (1994). Statistical analysis was done as per the Snedecor and Cochrone (1994).

RESULTS AND DISCUSSION

The comparison of membrane integrity in low range ambient temperature is illustrated in Table 1. No significant difference was observed between all studied breeds with respect to live sperm percent. In AI test and HOST, significant difference ($P < 0.01$) was observed only between CB bulls and Red sindhi bulls.

In medium temperature range, the comparison of membrane integrity between different breeds was evaluated (Table-2). In live sperm percent, no significant difference was observed with in breeds but significant difference ($p < 0.01$) was observed when CB bulls were compared with Red sindhi bulls, Haryana bulls and Jersey bulls with respect to AI test as well as HOST.

Table 3 reperesents the comparison of membrane integrity between different breeds at upper range ambient temperature. The finding was similar to the observation at low and medium range temperature with respect to live sperm percent. Significant statistical difference ($p < 0.01$) was obtained between CB bulls, Red sindhi bulls, Haryana bulls and jersey bulls in AI test as well as HOST.

The live sperm count %, A. I % and HOST % was decreased significantly with increase in ambient temperature. Our study revealed that there was a significant decrease ($p < 0.01$) in live sperm %, acrosome integrity % and and HOS test positive sperm %, with increase in ambient temperature corroborating with the study undertaken by (McNitt and First, 1970); (Heitman and Cockrell, 1984); (Larsson and Einarsson, 1984); (Colenbrander and Kemp, 1990). This may be due to the disturbed spermatogenesis caused by high temperature. The current breed wise findings obtained is comparable with various earlier studies like tomar *et al*. (1966) in Haryana bulls, dixit *et al*. (1984) and mandal *et al*. (2000) in murrah buffalo bulls as there was decrease in live sperm % in high temperature. Regarding the signifiacant decrease in acrosome integrity during high temperature, the present study is corroborated with the study undertaken by De las Heras *et al*. (1996). This study is in accordance with the result given by mandal *et al*. (2000) as the functional integrity of sperm membrane in murrah buffalo is highest in low temperature and lowest during high temperature. Apart from the above similarly in observation, our result is contradicting the findings of Nie *et al*. (2001) and Neild *et al*. (1999) as they found lowest HOS test positive sperm % during winter season and low temperature range in stallion. This deviation in our result may be due to climatic variation of countries or due to the fact of stallion sperm getting deteriorated in winter seasons, as they are long day breeder.

The membrane integrity status of fresh spermatozoa from all the studied bulls was found to be best during low ambient temperature and it was found to decrease significantly with increase in ambient temperature.

REFERENCES

Setchell, B. R., D'Occhio, M. J., Hall, M. Laurie, M. S., Tucker, M. and Zupp, J. L. 1988. Is embryonic mortality increased in normal female rats mated to subfertile males. *J. Reprod. Fern.* **82:** 567-574.

Colas, G. 1983. Factors affecting the quality of ram semen. *In: Haresign W, ed. Sheep Production. Stoneham. Massachusetts: Butterworths.* pp. 285 - 291.

Sevi, A., Rotunno, T., Di Caterina, R. and Muscio, A. 2002. Fatty acid composition of ewe milk as affected by solar radiation and high ambient temperature. *J. Dairy Res.* **69:** 181-194.

Mandal, D. K., Nagpaul, P. K. and Gupta, A. K. 2000. Seasonal variation in seminal attributes and sexual behaviour of Murrah buffalo bulls. *Indian J. Dairy Sci.* **53:** 278-283.

Igboeli, G. and Rakha. 1971. Seasonal changes in ejaculate characteristics of Angoni (short horn Zebu) bulls.National council for scientific research, Chilanga, Zambia. *J. Anim. Sci.* **33:** 651-654.

Parkinson, T. 1987. Seasonal variations in semen quality of bulls: correlations with environmental temperature. *Vet. Rec.* **120:** 479-482.

Correa, J. R. and Zavos, P. M. 1994. The hypoosmotic swelling test: its employment as an assay to evaluate the functional integrity of the frozen-thawed bovine sperm membrane. *Theriogenology.* **42:** 351–60.

Saxena, M. S. 2000. Veterinary Andrology and artificial insemination, 1st Edn., CBS publishers and distributors, New Delhi, India.

Watson, P. F. 1975. Use of Giemsa stain to detect change in acrosome of frozen ram spermasatozoa. *Vet. Rec*. **97:** 12-15.

Revel, S. G. and Mrode, R. A. 1994. An Osmotic Resistance test for bovine semen. *J. of Anim. Repro. Sci.* **1(36):** 77-86.

Snedecor, G. W. and Cochran, W. C. 1994. Statistical methods, 8th Edn. *Iawa state University*.

McNitt, J. L. and First, N. L. 1970. Effects of 72-hour heat stress on semen quality in boars. *Int. J. Biometeor*. **14:** 373.

Heitman, H. and Cockrell, J. R. 1984. Cycling ambient temperature effect on boar semen. *Anim. Prod*. **38:** 129–132.

Larsson, K. and Einarsson, S. 1984. Seminal changes in boars after heat stress. *Acta. Vet. Scand*. **25:** 57–66.

Colenbrander, B. and Kemp, B. 1990. Factors influencing semen quality in pigs. *J. Reprod. Fertil*. **40(Suppl.):** 105-115.

Tomar, N. S., Misra, B. S. and Johari, C. B 1966. Seasonal variations in reaction time and semen production and prediction of some semen attributes on initial motility of spermatozoa in Hariana and Murrah bulls. *Indian. J. Dairy. Sci*. **19:** 87-93.

Dixit, N. K., Agarwal, S. P., Agarwal, V. K. and Dwaraknath, P. K. 1984. Seasonal variations in serum levels of thyroid hormones and their relation with seminal quality and libido in buffalo bulls. *Theriogenology*. **24:** 293-303.

De las Heras, M. A., Valcarcel, A., Furnus, C., Perez, L., Moses, D. and Baldassarre, H. 1996. Changes in sperm-bound amidase activity suggest subtle damage to ram sperm acrosomes by freezing/thawing, not detected by light microscopy. *Anim. Reprod. Sci*. **45:** 81–89.

Nie, G. J. and Wenzel, J. G. W. 2001. Adaptation of the hypoosmotic swelling test to assess functional integrity of stallion spermatozoal plasma membranes. *Theriogenology*. **55:** 1005–1018.

Neild, D., Chaves, G., Flores, M., Mora, N., Beconi, M. and Aguero, A. 1999. Hypoosmotic test in equine spermatozoa. *Theriogenology*. **51:** 721–727.