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In vitro Fertilisation Capacity of Frozen Crossbred Bull Semen Cryopreserved During Different Seasons in Kerala

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ABSTRACT

Background: Assessment of semen quality of bulls in frozen semen stations is of paramount importance as they are used for inseminating large number of cattle. The present study was conducted to assess the *in vitro* fertilisation capacity of crossbred bull semen cryopreserved during different seasons in Kerala as reports of such an evaluation are scarce.

Methods: Semen samples from six crossbred bulls of same exotic inheritance, cryopreserved during rainy, post monsoon and summer seasons were procured from KLDB, Dhoni. The collected samples were evaluated for their *in vitro* fertilisation potential.

Result: Hot dry summer season in Kerala adversely affects the fertilisation capacity of spermatozoa. Rainy season was observed to be the most favourable season for good quality semen production and post monsoon season was intermediate between summer and rainy season. It can be concluded from the present investigation that semen cryopreserved during summer season have lower fertilisation and cleavage rate than rainy and post monsoon season. This might be due to the harmful effect of significantly higher average maximum temperature and lower relative humidity occurred in the area during summer season on spermatogenesis.

Key words: Cleavage rate, Fertilisation rate, *In vitro* fertilisation, Relative humidity, Season, Semen, Temperature.

INTRODUCTION

Semen production and quality shows significant seasonal fluctuations (Anderson, 1945). Season affects all the semen traits of young bulls and most traits of mature bulls (Mathevon *et al*. 1998). Ambient temperature of 40°C and relative humidity of 35- 45 per cent for a short period of 12 h could reduce the semen quality. Reports suggest that *Bos taurus* bulls are more susceptible to heat stress when compared to *Bos indicus* bulls and crossbred bulls will be affected less compared to *Bos taurus* animals (Kastelic, 2013).

All the external body forces that alter the homeostasis can be considered as stress (Stott, 1981). Due to stress, the animal is unable to cope up with its environment and so it fails to achieve its genetic potential (Dobson and Smith, 2000). At temperatures above 30°C, production potential of the animals will be severely compromised (Cheeke, 1986). In temperate climate it is very difficult to differentiate between seasons. According to Biya (2011), seasons in Kerala were divided based on temperature humidity index as rainy (June-September), post monsoon (October-January) and summer (February-May).

Bovine *in vitro* fertilisation (IVF) promises a better way to assess functional performance of gametes in both sexes. The *in vitro* matured bovine oocytes can be used to assess the semen quality in a meaningful way. The technique involves *in vitro* maturation of oocytes (IVM), IVF and *in vitro* culture of fertilised oocytes. Semen quality can be assessed by performing the penetration tests or by assessing the IVF rate.

Cryopreserved semen produced by Kerala Livestock Development Board (KLDB) is used extensively for the cattle breeding programme in Kerala. Assessment of fertility rate of bulls during different season by assessing conception rate following artificial insemination is time consuming. *In vitro* fertilisation is the best available option to assess the multiple sperm parameters having connection with fertility in a short time. Many studies were carried out to assess the viability and acrosome integrity of spermatozoa. However, no *in vitro* fertilisation studies were carried out in crossbred bulls to assess the influence of season on fertilisation capacity of spermatozoa. The present research work was designed to assess the *in vitro* fertilisation capacity of frozen crossbred bull semen cryopreserved during different seasons of Kerala.

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MATERIALS AND METHODS

The present study was conducted in Dhoni region of Palakkad District in Kerala with a temperature range of 29-36°C and relative humidity of 59-79% (Alanteena, 2016). Semen cryopreserved from six crossbred bulls of same exotic inheritance was selected from the bull station of Kerala Livestock Development Board (KLDB), Dhoni. Micro- climatic variables in the area were collected from nearest weather station (Integrated Rural Technology Centre, Mundur) during the study period. The variables collected were maximum temperature, minimum temperature and relative humidity from June 2017 to May 2018. From the collected data, Temperature Humidity Index (THI) in the area during the research period was calculated (Sabes-Alsina *et al*. 2019). Frozen semen straws of the selected bulls were collected from mid to end of each season to nullify the carry over effect of previous season and those straws having post-thaw motility of more than 50 per cent were included in the study. Semen quality analysis was carried out after thawing the straws in a water bath at 37°C for 30-45 seconds.

Bovine slaughter ovaries were collected during summer months of 2019 from Corporation slaughter house, Kuriachira, Thrissur and were transported to the laboratory in normal saline fortified with gentamycin at 37±2°C within 2 h of slaughter. After washing, ovaries were kept at 37°C till the oocyte recovery. Aspiration technique was used to collect oocytes from the visible surface follicles of 2-8 mm diameter. The aspirated oocytes were subjected to *in vitro* maturation and the matured oocytes were randomly divided into three groups as group I, II and III.

Group I, II and III oocytes were allowed to fertilize with the semen cryopreserved during rainy, post monsoon and summer season, respectively in the Central Instrumentation Laboratory of College of Veterinary and Animal Sciences, Mannuthy. Semen processing and *in vitro* capacitation of semen sample was done by swim up method as described by Parrish *et al*. (1986). After 18 h of co-incubation with sperms in modified Tyrode's medium the presumed zygotes were transferred into modified SOF medium for culture. After 48 h of culture, oocytes were examined for fertilisation changes (Plate A, B, C and D). The changes observed were cleavage, second polar body extrusion and penetration of sperm into cytoplasm and formation of male and female pro-nuclei. All the uncleaved oocytes without second polar body extrusion and sperm penetration into the cytoplasm were stained with aceto-orcein stain and examined for male and female pro-nuclei (Clark *et al*. 2005).

Seasonal influence of frozen crossbred bull semen on *in vitro* fertilisation capacity was evaluated by subjecting the collected data to statistical analysis using SPSS version 24.0 software. Repeated measures ANOVA were used to compare the means. Means of micro- climatic variables were compared using students one way ANOVA.

RESULTS AND DISCUSSION

The micro climatic variables in the Palakkad area during the period from June 2017 to May 2018 are summarized in Table 1. Maximum temperature observed in the present investigation was significantly higher during summer season (36.29±0.21°C) than post monsoon (34.58±0.13°C) and rainy (30.40±0.26°C) season. Relative humidity was significantly higher during rainy season (81.56±1.36%) than post monsoon (61.10±1.37%) and summer (53.32±1.46%) season. However, no significant difference was noticed for minimum temperature and THI during the study period.

For *in vitro* fertilisation studies, a total of 184 slaughter ovaries were subjected to follicular aspiration and grade A and B oocytes were selected for the study. The selected oocytes were subjected to *in vitro* maturation and matured oocytes were randomly allotted to three treatment groups and were inseminated with the semen cryopreserved during three different seasons. The number of matured oocytes allotted to rainy, post monsoon and summer season were 381, 280 and 334 respectively.

Effect of season on *in vitro* fertilisation capacity of spermatozoa is summarized in Table 2 and Fig 1. There was a significantly higher cleavage rate obtained in group I ($p \leq 0.05$) than group III (50.45±0.93 vs 47.02±0.22) and no significant difference was observed between group I

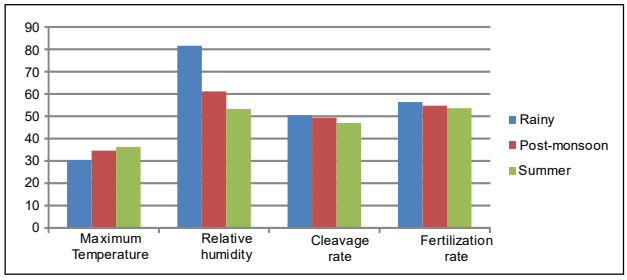

Fig 1: Effect of micro climatic variables on cleavage rate and fertilisation rate.

Table 1: Micro climatic variables (Mean±S.E) in the Palakkad area during the period from June 2017 to May 2018.

Season	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	THI
Rainy	30.40±0.26^{a}	24.32±0.09	81.56±1.36^{a}	78.37±0.22
Post-monsoon	34.58±0.13^{b}	23.98±0.15	61.10±1.37^{b}	78.64±0.28
Summer	36.29±0.21^{c}	24.43±0.17	53.32±1.46^{c}	78.98±0.24
p-value	0.000**	0.060^{NS}	0.000**	0.212

For each parameter means with different superscripts differ significantly between groups.

**Significance at 1% level, NS- Non significant.

and II (50.45±0.93 vs 49.32±0.66). Cleavage rate observed in group II was intermediate between group I and III and it was significantly higher ($p \leq 0.05$) than group III.

The *in vitro* fertilisation rate of oocytes fertilized with semen cryopreserved during rainy season (Group I), was found to be statistically higher ($p \leq 0.05$) than group III containing oocytes fertilized with semen cryopreserved during summer season (56.36±0.50% vs 53.65±0.42%). However, no significant difference was observed between group I and II (56.36±0.50% vs 54.74±0.75%) as well as between II and III (54.74±0.75% vs 53.65±0.42%). In the present study, frozen crossbred bull semen produced during summer season was found to be having significantly lower *in vitro* fertilisation rate and cleavage rate than rainy season.

The ability of spermatozoa to move through the female reproductive tract and the capacity to cause fertilisation and later embryonic development will change as the semen quality changes. At present there is no dependable method that mimics the most important and complicated interactions of spermatozoa with female reproductive tract during the passage of spermatozoa to the place of fertilisation. *In vitro* fertilisation can be used as a tool to evaluate the fertilisation capacity of spermatozoa. Micro-climatic variables in the Palakkad area were collected during the research period. From the collected observations of maximum temperature, minimum temperature and relative humidity, THI was calculated for the study period. There was no statistical difference noticed among seasons for minimum temperature and THI. During rainy season, relative humidity was significantly higher (81.56±1.36%) and maximum temperature was significantly lower (30.40±0.26°C) than post monsoon and summer seasons. Significantly higher maximum temperature (36.29±0.21°C) and lower relative humidity (53.32±1.46%) was observed during summer season in the area.

In the current study, *in vitro* fertilisation rate was analysed by considering parameters like cleavage rate, formation of male and female pro nuclei, second polar body extrusion and penetration of sperm into cytoplasm. Summer showed a statistically lower ($p \leq 0.05$) fertilisation rate (53.65±0.42 vs 56.36±0.50) than rainy season. However, the difference was not statistically significant between post monsoon and summer seasons. Alanteena (2016) reported that Palakkad is a mid- land area of Kerala having maximum temperature which ranged from 29-36°C and relative humidity ranged from 59-79 per cent. The maximum temperature in the area during the study period went up to 40°C during summer months. The climates during summer months were hot and dry in the area.

Sabes-Alsina *et al*. (2019) observed no significant variation for the *in vitro* fertilisation rate of cryopreserved semen produced during different seasons. However, the authors opined that there was a significant variation among individual bulls on the *in vitro* fertilisation rate. The maximum temperature found during the summer months in the research area was 40°C and the corresponding relative humidity recorded was 32 per cent. So the semen produced after 45 to 60 days after this period may be affected by the severe heat stress during March. Kastelic (2013) reported that an ambient temperature of 40°C and relative humidity of 35-45 per cent for a short period of 12 h could reduce the semen quality significantly. *Bos taurus* and crossbred bulls are more susceptible to the effect of heat stress than the *Bos indicus* bulls.

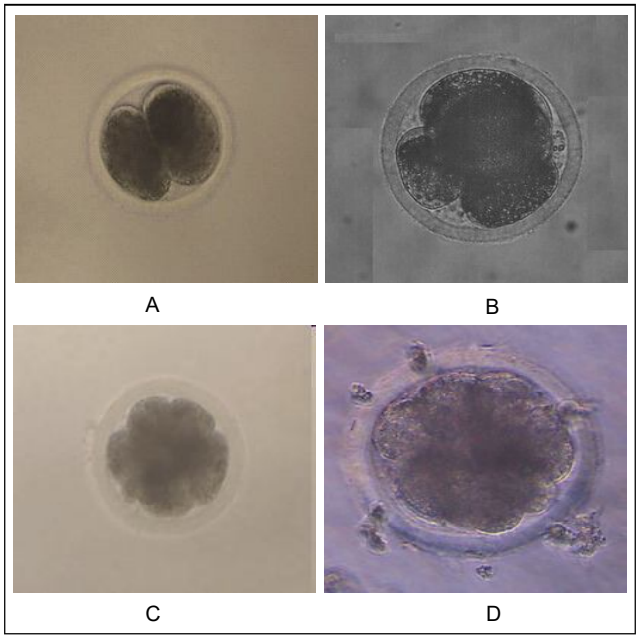

Plate embryo development visualised under inverted microscope (20X).
A- Two cell stage embryo.
B- Four cell stage embryo.
C- Eight cell stage embryo.
D- Morula.

Table 2: Effect of season on *in vitro* fertilisation capacity of spermatozoa from semen of crossbred HF bulls cryopreserved during different seasons.

Season	No. of oocytes				Cleavage rate	Fertilisation rate
	Kept for fertilisation	Penetrated by sperm (%)	With second polar body (%)	With male and female pronuclei (%)		
Rainy	381	14 (3.67)	3 (0.79)	5 (1.31)	50.45±0.93^{a}	56.36±0.50^{a}
Post monsoon	280	10 (3.57)	2 (0.71)	3 (1.07)	49.32±0.67^{a}	54.74±0.75^{ab}
Summer	334	11 (3.29)	6 (1.80)	5 (1.50)	47.02±0.23^{b}	53.65±0.42^{b}

Means with different superscripts differ significantly between groups ($p \leq 0.05$).

The maximum average temperature (36.29±0.21°C) in the area was observed during summer months and was significantly higher than the other two seasons. A significantly lower average relative humidity ($p \leq 0.01$) was observed during summer month (53.32±1.46%). In the research area, the average THI observed during all the three seasons was between 78 and 79 and there was no significant difference was observed between seasons. The THI values ranging from 75-80 were considered to be the alert zone for the livestock (Hahn *et al*. 2009).

Significantly lower fertility rate was observed during summer than rainy season and marginally lower fertility compared to post monsoon season might be due to the significantly higher average maximum temperature and lower relative humidity occurred in the area during summer season. Reduced fertility observed during hot and dry climate might be due to significantly higher numbers of abnormal sperms, DNA damage of spermatozoa caused by oxidative stress, reduced efficiency of bulls to maintain testicular temperature during summer months and also lower expression of Heat Shock Protein-70 during summer months (Kastelic and Thundathil, 2008).

CONCLUSION

It can be concluded from the present investigation that hot dry summer season in Kerala adversely affected the fertilisation capacity of spermatozoa. Rainy season in Kerala was the most favourable season for good quality semen production and post monsoon season is the intermediate between summer and rainy season. Study also suggests that mitigation strategies should be followed to reduce temperature and humidity inside the bull shed to reduce heat stress.

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