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Influence of season in Kerala on the yield and quality of bovine oocytes obtained by ultrasound-guided transvaginal oocyte recovery#

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Abstract

The present study was carried out to assess the influence of three seasons in Kerala on the yield and quality of oocytes from crossbred (CB) dairy cows (n=27), subjected to ultrasound-guided transvaginal oocyte recovery (TVOR). Meteorological data including, ambient temperature (AbT) and relative humidity (RH) was recorded and Temperature Humidity Index (THI) was calculated. In each season, selected animals (n=9) were subjected to TVOR; data regarding the number of follicles available for puncture (>2mm), number of aspirated follicles, number of oocytes retrieved and oocyte recovery rate were recorded. The retrieved oocytes were subjected to morphological evaluation. Significantly higher (P<0.05) AbT and THI was recorded during summer (28.92±0.64°C and 80.61±0.76) when compared to rainy (26.95±0.17°C and 78.68±0.21) and post-monsoon (27.29±0.22°C and 77.75 ± 0.57) seasons. However, due to the availability of rainfall throughout the year, RH did not differ significantly between seasons. The mean number of follicles imaged (available for puncture) by ultrasonography per session was significantly higher (P<0.01) during the rainy season (11.19±0.74) compared to post-monsoon (8.08±0.47) and summer (5.28±0.38). Total mean number of follicles aspirated and oocytes retrieved per session was highest during rainy

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season (7.86±0.49 and 3.17±0.28) and least during summer (4.36±0.29 and 1.81±0.20). However, the oocyte recovery rate and the yield of culture-grade oocytes did not differ significantly between seasons. It was observed that the THI during all the seasons was above the thermo-comfortable level suggested for dairy cows. Ovarian activity of CB dairy cows was compromised during the summer season in Kerala, as evidenced by the lowest number of follicles aspirated and oocytes retrieved per session.

Keywords: *Season, crossbred cows, ambient temperature, temperature humidity index, transvaginal oocyte recovery*

The Indian economy relies considerably on the livestock sector. As per the 2019 census, there was an increase in the crossbred cattle population in India, compared to the 2012 census (Livestock Census, 2019). In spite of the increasing trend in the cattle population, the reproductive outcome of these animals is considerably compromised. In the scenario of global warming and simultaneous rising demand for more production, heat stress has become a major challenge for livestock producers in tropical and subtropical countries resulting in hefty financial losses that affect nearly 60 per cent of the world cattle population. Because of the prevailing tropical monsoon climate, India is not an exception to these ill effects. The ill effects of heat stress are not limited to the hotter months; the carryover effects of this phenomenon to the subsequent cooler months results in long-term effects on reproduction (Bernabucci *et al*., 2014).

India's weather is divided into three distinct seasons: winter (October to February), summer (March to June), and wet (July to September) respectively (ICAR, 1977). Somanathan (1980) analysed the climate data of Kerala over a period of three years between 1974 and 1978 and divided the climate of Mannuthy (Thrissur, Kerala) into two seasons: rainy season (May to November), and dry season (December to April) respectively. Nishant (2009) identified that the summer in Kerala lasts from February to May in Thrissur district of Kerala. Rainy (June–September), post–monsoon (October–January), and summer (February-May) are the three seasons that dominate the climate of Thrissur, Kerala (Biya,2011). The THI-based zonation of India by NICRA (2012) shows the high THI periods and describes Kerala as a high-stress zone.

Elevation of maternal body temperature negatively affects several aspects related to reproduction, either directly through the effects on oocyte quality and its developmental competence or indirectly by limiting nutrient availability. Oocytes in the ovarian pool are highly susceptible to heat stress; changes in follicular activity may diminish the developmental competence of the enclosed oocyte (Wolfenson and Roth, 2019).

Although there have been reports of seasonal impact on follicular turnover, oocyte recovery rate and yield of culture-grade oocytes in B *taurus* and B *indicus* cattle, the *in vivo* and *in vitro* studies in crossbred cattle of the tropics are lacking. Hence, the present research work was carried out to assess the influence of season on the yield and quality of oocytes in crossbred dairy cows retrieved by TVOR during different seasons prevailing in Kerala.

Materials and methods

The study was carried out at the University Livestock Farm and Fodder Research and Development Scheme (ULF & FRDS), Mannuthy during the period from June 2021 to May 2022. Apparently healthy postpartum dairy cows (n=27) with a history of normal calving and maintained under identical management conditions were selected for the study. The study was carried out during three different seasons prevailing in Kerala *viz.,* rainy (June to September), post-monsoon (October to January) and summer (February to May), classified as per Biya (2011). In order to nullify the carryover effect of the previous season, the study was initiated 60 days after the onset of each season (Roth, 2017). The selected animals in each season (n=9) were subjected to TVOR twice weekly for two consecutive weeks (4 sessions of TVOR for each animal).

Meteorological data collection

Ambient temperature (°C) and RH (%)

of the Mannuthy campus area, where the farm is located collected from the Centre for Animal Adaptation to Environment and Climate Change Studies (CAADECCS), Mannuthy. In order to assess the stress level, THI was calculated (LPHSI, 1990) by incorporating *AbT* (dry bulb temperature) (°C/°F) and RH (%).

$$THI = T_{(db)} - \{(0.55-0.55RH)(T_{(db)}-58)\}$$

Where,

$T_{(db)}$ = Dry bulb temperature (°F)

RH = Relative humidity (%)

Donor preparation and TVOR

The animals were prepared for TVOR and restrained in a trevis to limit movement. For ease of handling during the procedure, 4 mL of 2 per cent lignocaine hydrochloride (lidocaine) was given as epidural anaesthesia just prior to TVOR. The transducer was inserted into the vagina after proper lubrication, cleaning the vulva and perineal region and emptying the rectum. The ovaries were manipulated per rectum and positioned in front of the probe, in order to obtain a clear image on the ultrasonographic monitor. Aspiration of the follicles was performed with the aid of an ultrasound machine (Honda HS-2100, Honda Electronics Co., Ltd, Japan) with a transvaginal convex transducer (5MHz) equipped with a needle (COVA Needle; Kitazato, Fuji, Schizuaka, Japan) and its guidance system. Overall, 36 sessions of TVOR were performed on nine selected animals (4 sessions/ per animal) in each season.

Grading of retrieved oocytes

The retrieved oocytes were subjected to quality evaluation and classified as good, fair, poor and denuded, based on the gross morphological appearance of the cumulus oophorous complexes (COCs), number of cumulus cells surrounding the oocytes and granularity of oocyte cytoplasm (Abdoon and Kandil, 2001). Compact COCs with an unexpanded cumulus mass having more than five layers of cumulus cells and evenly granular homogenous ooplasm were graded as excellent quality oocytes (Grade A) and COCs with 3-5 layers of compact cumulus cells with evenly granular homogenous ooplasm were graded as good quality (Grade B). Both A and B-grade oocytes were considered culture-grade oocytes.

Statistical analysis of the data

The data generated were analyzed statistically (Snedecor and Cochran,1994) by using SPSS V 24.0. A factorial design of the experiment was conducted and statistical analysis was done with one-way ANOVA followed by Duncan Multiple Range Test (DMRT).

Results and discussion

Climatological parameters

Micro-climatic variables in the experiment area were recorded during the study period (June 2021 to May 2022). The variables recorded were minimum temperature (°C), maximum temperature (°C) and RH (%). During each season, THI was calculated.

Ambient temperature

Among the three different seasons, summer (February- May) exhibited significantly higher ($P< 0.05$) AbT (28.92±0.64°C) compared to rainy (26.95±0.17°C) and post-monsoon (27.29±0.22°C) seasons. Minimum, maximum and average AbT during each season were recorded and the data are depicted in Table 1. The minimum and maximum (Mean±SE) AbT recorded did not differ significantly between the seasons. A similar study on seasonality in Kerala was done by Alanteena (2016), who reported that Palakkad is the mid-land area of Kerala with maximum AbT. The maximum AbT in Palakkad during their study period went up to 40°C during the summer months. The microclimatic variables during different seasons were studied by Revathy *et al*. (2021) also and reported that the summer experienced a significantly higher mean maximum AbT (36.29±0.21°C) than post-monsoon (34.58±0.13°C) and rainy (30.40±0.26°C) season, but the recorded temperature was higher when compared to the mean maximum (Mean±SE) AbT recorded in this study (Table 1). This could be due to the change in time and location of the study.

Table 1. Season-wise ambient temperature (Mean ± SE) at Mannuthy area, as recorded in CAADECCS, Mannuthy

Season	Ambient temperature (°C)			Relative Humidity (%)	THI
	Minimum (Mean±SE)	Maximum (Mean±SE)	Average (Mean±SE)		
Rainy	24.45±0.31	29.33±0.94	26.95±0.17^{b}	85.52±0.87	78.68±0.21^{b}
Post Monsoon	25.23±0.18	28.79±0.39	27.29±0.22^{b}	73.93±6.72	77.75±0.57^{b}
Summer	26.38±1.02	30.61±0.41	28.92±0.64^{a}	76.51±3.94	80.61±0.76^{a}
P-value	0.147^{ns}	0.167^{ns}	0.015*	0.218^{ns}	0.016*

**Significant at 5% level; ns non-significant*

Means having different letters as superscripts differ significantly between rows

Relative humidity (RH)

The average RH (%) during each season were recorded and data is depicted in Table 1. Among the three different seasons, a higher RH (Mean±SE) was observed during the rainy season (85.52±0.87), but did not differ significantly from post-monsoon (73.93±6.72) and summer (76.51±3.94). Contrary to this, Revathy *et al.* (2021) reported significantly higher RH during the rainy season in Kerala. But the RH per cent reported by the authors during the rainy season (81.56±1.36) is comparable with the RH recorded in the present study during the rainy season (85.52±0.87). The availability of rainfall throughout the year during the study period could be the reason for the non-significant difference in RH among the three seasons.

Temperature Humidity Index

The seasonal comparison revealed that THI during summer (80.61±0.76) was significantly higher compared to rainy (78.68±0.21) and post-monsoon (77.75±0.57) seasons (Table 1). This can be corroborated with the findings of Prasad (2014), who recorded the highest THI and period of highest thermal discomfort for cattle in Kerala from 19-Feb to May 27 (summer season). He also reported that the comfortable THI zone of Kerala crossbred dairy cows is below 75.52. In the present study, THI values recorded in all the seasons were above the comfortable THI level described by him, which can be attributed to high temperatures recorded during the summer season and heavy rainfall obtained during rainy and post-monsoon seasons in the study period.

Influence of season on yield and quality of oocytes

In the present study, a total of 108 TVOR sessions were carried out in 27 animals, with 36 sessions of TVOR during each season. Data on the number of follicles aspirated and the number of oocytes recovered during different seasons are presented in Tables 2 and 3.

Number of follicles available for puncture

In each session, while performing TRUS and subsequent TVOR, follicles (>2mm) present in both ovaries were recorded (Fig 1). The number of follicles imaged per session (Mean±SE) by trans-rectal ultrasonography (TRUS) in both ovaries during different seasons is shown in Table 2.

A highly significant difference (P<0.01) between seasons was recorded in the total number of follicles in the right ovary that could be sonographically imaged. The number of follicles imaged was 6.56±0.47, 4.94±0.58 and 2.67±0.43, respectively during the rainy, post-monsoon and summer seasons. The mean number of follicles imaged in the left ovary during the rainy season (4.63±0.36) was significantly higher (P<0.05) than in post-monsoon (3.14±0.49) and summer (2.61±0.56) seasons. But there existed no significant difference between post-monsoon and summer

Table 2. Number of follicles (Mean±SE) imaged by trans-rectal ultrasonography in crossbred dairy cattle during different seasons prevailing in Kerala

Variables	Number of follicles imaged			F-value (P-value)
	Rainy	Post monsoon	Summer	
Right ovary (n=36)	6.56±0.47[a]	4.94±0.58[b]	2.67±0.43[c]	15.467** (< 0.001)
Left ovary (n=36)	4.63±0.36[a]	3.14±0.49[b]	2.61±0.56[b]	4.317* (0.025)
Total	11.19±0.74[a]	8.08±0.47[b]	5.28±0.38[c]	28.95**

*Significant at 5% level ** Significant at 1% level

• a-b: means with different superscripts differ significantly between columns

Table3. Number of follicles aspirated and number of oocytes retrieved during trans-vaginal ultrasound guided ovum recovery from crossbred dairy cattle during different seasons prevailing in Kerala

Season	Number of follicles aspirated per session			No. of oocytes retrieved (Mean±SE)
	Right ovary (Mean±SE)	Left ovary (Mean±SE)	Total follicle (Mean±SE)	
Rainy	4.69±0.36[a]	3.17±0.24[a]	7.86±0.49[a]	3.17±0.28[a]
Post-Monsoon	4.03±0.30[a]	2.72±0.26[a]	6.78±0.43[ab]	2.64±0.22[a]
Summer	2.44±0.24[b]	1.92±0.27[b]	5.33±0.33[b]	1.81±0.20[b]
F-value	14.420**	5.906**	2.974*	8.52**

** Significant at 5% level; ** Significant at 1% level.*

ab: Means with different superscripts differ significantly between rows

seasons in the total number of follicles imaged by TRUS.

The total number of follicles imaged by TRUS (>2mm) per animal per session during rainy, post-monsoon and summer seasons were 11.19 ± 0.74, 8.08 ± 0.47 and 5.28 ± 0.38, respectively. There existed a highly significant difference between seasons (P<0.01) with the highest number of follicles imaged during the rainy season, followed by post-monsoon and least during summer.

Heat stress seems to have a negative impact on the effectiveness of follicular development including selection and dominance as well as the quality of ovarian follicles (Badinga *et al*., 1993). In bovine, the progression of follicular growth is lengthy and takes around 180 days; thus, thermal stress that occurs during the early stages of follicular growth (i.e., primordial follicles) might further affect follicular function at later stages of growth (Campbell, 2018). Studies have specified that early antral follicle, approximately 0.5–1 mm in diameter, are highly sensitive to heat stress (Roth *et al.,*2000), further expressed in reduced steroid production in medium-sized and preovulatory follicles (Roth *et al*., 2001).

Number of follicles aspirated during different seasons

Significantly lower (P<0.05) number of follicles were aspirated during summer (4.36±0.29) than rainy (7.86±0.49) and post-monsoon (6.78±0.43) seasons (Table 3).

The number of follicles aspirated from the right ovary was significantly lower (p<0.01) during summer (2.44±0.24), compared to the other two seasons (4.69±0.36 during the rainy season and 4.06±0.30 during post-monsoon). Similarly, the number of follicles aspirated from the left ovary was significantly lower (p<0.01) during summer (1.92±0.27), compared to the other two seasons (3.17±0.24 and 2.72±0.26 during rainy and post-monsoon seasons, respectively). All these findings clearly indicate that heat stress significantly affects ovarian follicular growth and development.

Manik *et al*. (2003) and Abhilash (2017) reported the number of follicles aspirated from crossbred cows as 6.80±0.70 and 5.15±0.10, respectively, which is comparable

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Table 4. Oocyte recovery rate and yield of culture grade oocytes during trans-vaginal ultrasound guided ovum recovery from crossbred dairy cattle during different seasons prevailing in Kerala

Seasons of TVOR (n=36)	Oocyte recovery rate			Culture grade oocytes	
	No of follicles aspirated	No of oocytes recovered	Rate of recovery (%)	Number	Per cent
Rainy	284	114	40.14	56	49.12
Post monsoon	244	95	38.93	48	50.53
Summer	154	65	42.21	31	47.69
Total (108)	682	274	40.18	135	49.27
χ^2 Value = 0.421^{ns}; P-value = 0.810				χ^2 Value = 1.059^{ns}; P-value = 0.589	

to the present report of 6.78±0.43 during post-monsoon season. The yield of greater number of follicles from exotic breeds was reported by Garcia and Salaheddine (1998) (12.40±0.61) and by Goodhand *et al*. (1999) (14.70±2.27).

Number of oocytes retrieved during different seasons

The mean number of oocytes retrieved per session was significantly lower (p< 0.01) during summer (1.81±0.20) when compared to rainy (3.17±0.28) and post-monsoon (2.64±0.22) seasons (Table 3). Reduced oocyte yield during summer can be related to the reduced number of follicles available for puncture during the season. This is in agreement with the findings of Zeron *et al*. (2001) who reported a higher number of oocyte recovery per ovary during the winter compared to summer season (7.50 vs 5.00). Alanteena *et al*. (2016) reported an oocyte yield of (3.13±0.08) from CB cows of Kerala, which is in agreement with the oocyte yield recorded during the rainy season.

Oocyte recovery rate

A total of 108 TVOR sessions were carried out from 27 crossbred dairy cattle; 36 sessions of TVOR during each season. During rainy, post-monsoon and summer seasons, 284, 244 and 154 follicles, respectively were aspirated (a total of 682 follicles). A total of 274 oocytes were recovered from the total of 682 follicles, yielding an oocyte recovery rate of 40.18 per cent. Data regarding oocyte recovery are presented in Table 4. Oocyte recovery rates during rainy, post-monsoon and summer seasons were 40.14, 38.93 and 42.21, respectively. No significant difference in the oocyte recovery rate was observed between the seasons. Contrary to our finding a higher oocyte recovery rate of 59 and 61.74 per cent was reported by Manik *et al.* (2003) and Abhilash (2017), respectively in Indian CB dairy cows. Though the total number of follicles >2mm imaged and aspirated was higher in rainy than in other seasons, any such influence on the oocyte recovery rates between the seasons was not evident in this study. This could be attributed to the fact that the determinant of oocyte recovery rate in OPU is the influence of the environment and timing of the procedure as reported by Kim *et al*. (2018).

Yield of culture-grade oocytes

All the retrieved oocytes were subjected to grading by morphological evaluation based on the number of cumulus cell layers surrounding the oocyte and the granularity of the cytoplasm. Based on the morphological grading, they were categorized as grade A (n=45), grade B (n=96), grade C (n=77) and grade D (n=56). Grade A and B oocytes were together considered culture-grade oocytes (Fig 2).

The proportion of culture-grade oocytes retrieved during rainy, post-monsoon and summer seasons was 49.12 (n=56) 50.53 (n=48) and 47.69 per cent (n=37), respectively (Table 4). There existed no significant difference in the yield of culture-grade oocytes between seasons. This is in agreement with the findings of Kim *et al.* (2018) who recorded no significant difference in the yield of culture-grade oocytes between autumn and summer seasons (51.10±5.00 vs 46.20±6.30 %). Contrary to

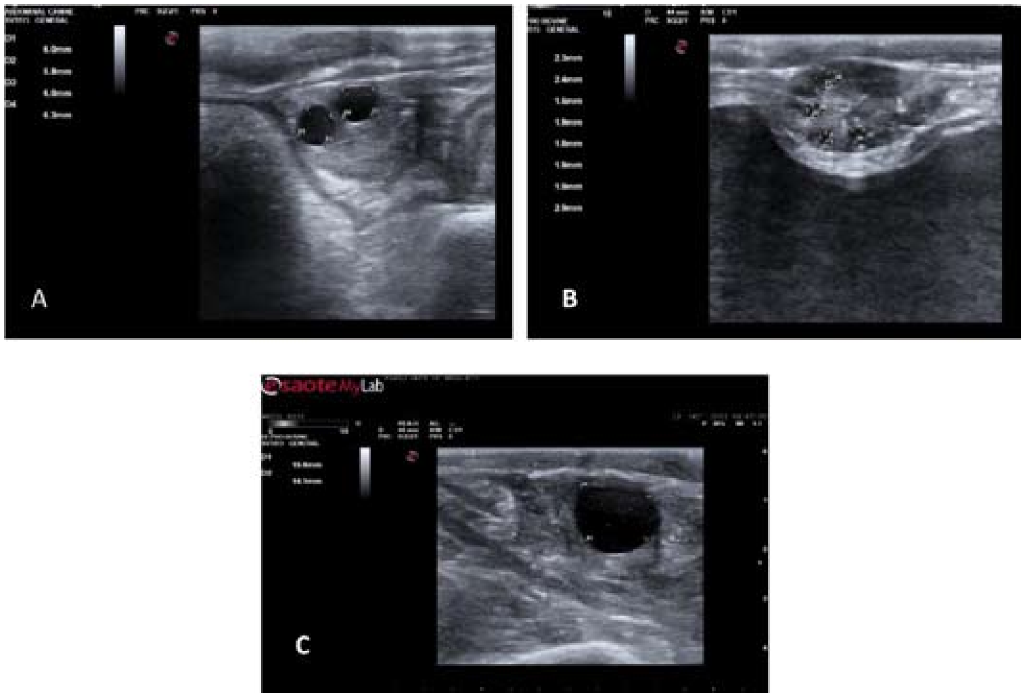

Fig 1. Sonographic imaging of ovarian follicles prior to transvaginal oocyte recovery (TVOR) (A) Ovary with small sized follicles (3-5mm diameter), (B) Ovary with medium sized follicles (5-9mm diameter) and (C) Ovary with large sized follicle (>9mm diameter).

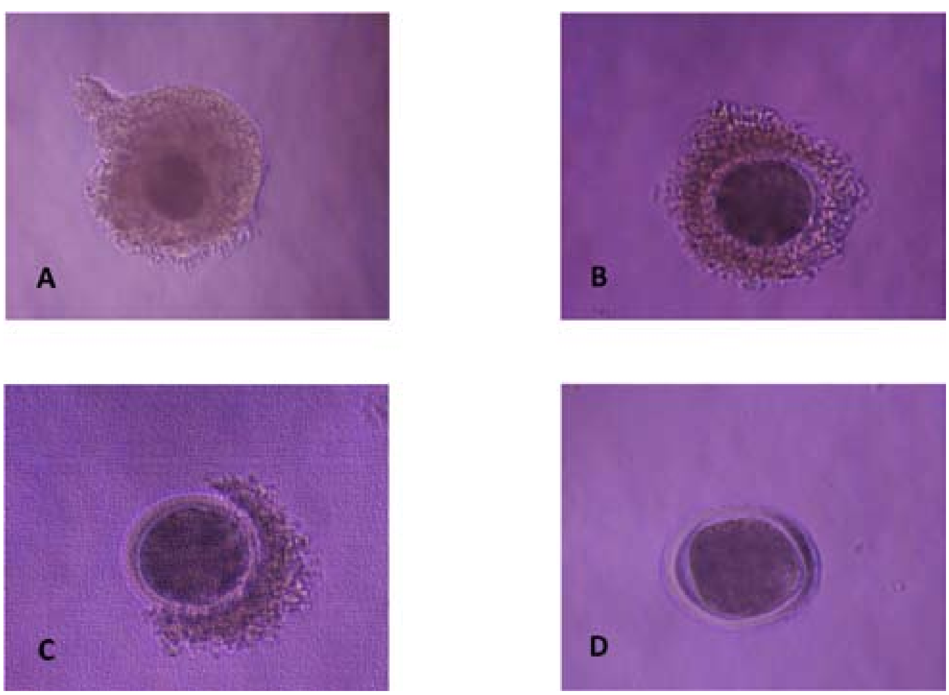

Fig 2. Grading of oocytes based on morphology, as visualized under inverted microscope (20X): (A): Grade A oocyte - compact COCs with unexpanded cumulus mass with more than five layers of cumulus cells. (B): Grade B oocyte - three to five layers of compact cumulus cells, with evenly granular homogenous ooplasm. (C): Grade C oocyte – partially denuded oocyte with scattered cumulus cells. (D): Grade D oocyte - denuded oocyte with irregular shrunken ooplasm.

these findings Chrenek *et al*. (2015) reported a significantly higher (P<0.05) yield of good-quality oocytes during summer when compared to the spring season.

The non-significant difference in the yield of culture-grade oocytes among the three seasons in the present study could be due to the superior adaptation of the CB cattle in tropical and subtropical environments. Studies show that the impacts of heat stress are more distinct in *Bos taurus* cattle. Heat stress increases the disparity in membrane fatty acid profiles, which reduces the oocyte's developmental competence. It also alters the transcriptional stages of genes involved in folliculogenesis, oogenesis, and embryo development, as well as disrupts processes that take place in the nucleus and cytoplasm, such as the translocation of the cortical granule to the oolemma and cytoskeleton reorganization, which may eventually result in cell death (Gendelman and Roth, 2012). Whereas such changes are less distinct in *Bos indicus* cattle, due to their superior adaption to tropical and subtropical environments, typically outclass *Bos taurus* cows in terms of reproduction (Camargo *et al.*, 2007; Silva *et al.*, 2013) during extreme weather conditions.

Conclusion

From the present finding of high THI irrespective of the seasons, above the thermo-comfortable level specified for dairy cows, it could be inferred that CB cattle in Kerala are always under some degree of stress, due to the climatic conditions prevailing in Kerala. The significantly lower mean number of follicles available for aspiration and oocytes retrieved per session during TVOR points to a compromised gonadal function during the summer season. However, such a compromise on the oocyte recovery rate and yield of culture grade oocyte was not observed in this study.

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Conflict of interest

The authors declare that they have no conflict of interest.

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