INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH
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INFLUENCE OF SUMMER HEAT STRESS ON OOCYTE DEVELOPMENTAL COMPETENCE IN JERSEY CROSSBREDS AND NONDESCRIPT INDIGENOUS COWS

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ABSTRACT
The aim of this study was to evaluate effect of summer heat stress on oocyte maturation and fertilization performance in crossbreds and indigenous cows. For this aim, temperature humidity index (THI) was calculated for different seasons as indicator of heat stress. Bovine oocytes from slaughter house were graded based on cumulus compactness and subjected to *in vitro* maturation (IVM) and fertilization (IVF). Crossbred cows showed significantly reduction in good quality oocyte recovery performance (2.74 ± 0.08) in summer season. IVMrates were not significantly different among winter indigenous, winter crossbreds and summer indigenous cows ($p< 0.05$). Cleavage rates in crossbreds during summer time (51.44 ± 2.24) were significantly lower ($p < 0.05$) than winter season. In conclusion, summer heat stress detrimentally affects oocyte maturation and also embryo production competence in crossbreds. Although oocyte developmental competence of summer heat stressed Jersey crossbred cows could be improved in stress free *in vitro* condition.

KEYWORDS: heat stress, bovine, IVM, IVF, THI

INTRODUCTION

Researches in understanding acquisition of bovine oocyte developmental competence and *in vitro* embryo production technology (IVEPT), for last three decades, have been improving the quality transferable embryo production. With the advancement of bovine IVEPT, it can serve as a great tool to investigate the efficiency of crossbreeding scheme in field condition regarding *in vitro* reproductive performance of crossbreds in particular agro-climatic zones. Dairy herds in tropical countries are often composed of crossbred, because they may provide the best characteristics of the two types, namely disease and weather resistance (zebu cattle) and milk production (Jersey). Reproductive performance is dependent on many factors, one of which is oocyte quality to support fertilization and development of the subsequent embryo and there are a variety of environmental factors such as heat stress, nutrition, management practices that have been implicated in affecting it (Hansen *et al.*, 2010). *Bos indicus* cattle are more resistant to severe temperature and humidity than *Bos Taurus*, due to their ability to regulate body temperature during heat stress (Gaughan *et al.*, 1999). In addition, however, there are indications that thermo-tolerant ability extend to the cellular and genomic level (Paula-Lopes *et al.*, 2003).

Assam, one of the North Eastern states of India situated in temperate region with the tropical monsoon rainforest climate and experiences heavy rainfall and high humidity in summer which often causes widespread and destructive flood. During summer, the ambient temperature and relative humidity frequently exceed the critical comfort level of temperature humidity index (THI > 72) (Khan *et al.*, 2013) resulting in elevated body temperature, panting and decrease in milk production, reproduction performance of dairy cattle. Over 9.6 million of the cattle population in Assam are indigenous non-descript (18th Livestock Census, Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries, Govt. of India), reared mostly under low input production system across different villages where quality feed and fodder resources are less available in sufficient quantity and subjected to a higher degree of stress during summer due to high temperatures and humidity. In Assam there has been an increasing interest on Jersey × indigenous nondescript crossbreeding in order to take advantage of heterosis with an aim of higher milk production and better offspring.

The present study was conducted to evaluate influence of summer heat stress on oocyte developmental competence of crossbreds and indigenous cow based on *in vitro* embryo production performance.

MATERIALS & METHODS

Ovaries were collected from local slaughter house from adult, apparently healthy Jersey crossbreds and indigenous nondescript cows of Assam. All media and constituents were obtained from Sigma-Aldrich (Stockholm, Sweden) unless otherwise stated. All media were prepared a day before collection of ovary and sterilized using 0.22µm syringe filter and warmed at 38.5 °C prior to use.

Experimental design

The experiment was conducted to assess good quality oocyte recovery performance and subsequent *in vitro* maturation and fertilization performances of Assam indigenous nondescript and Jersey crossbred cows during winter and summer seasons. The experiment was run in 10

batches for each cattle category (crossbreds / indigenous) in each season. The experiments were conducted during the months of June-August, 2014 in summer and December-February, 2015 in winter. The ambient temperature, relative humidity and rainfall data were collected from online weather database (http://www. worldweatheronline.com) for the months of June'14-August'14 and December'14-February'15. THI was calculated as described by Bohmanova*et al.* (2007):

THI = (1.8×Ta+32) - (0.55- 0.0055× RH)×(1.8×Ta -26)
Where, Ta = ambient temperature in °C
RH = relative humidity

Collection of ovary

Bovine ovaries were collected from local slaughter house and transported in 0.9% NaCl solution (supplemented with 75 µg/mL Gentamicin) in a thermo flask maintaining 38°C and processed in the laboratory within 3 hours following collection. The extraneous tissues were removed with the help of scissors and ovaries were washed three times with physiological saline solution containing antibiotic prior to further processing.

Oocyte recovery and grading

Oocytes were collected by aspiration following slicing technique for maximum recovery into a collection medium (Medium 199, BSA and 75 µg/mL Gentamicin) pre-warmed at 38.5 °C. Aspiration was done from follicles with diameter of 3-8 mm using a 5 ml syringe containing 18 gauge needle. After aspiration of the aspirable follicles, ovaries were placed in a graded glass petridish containing collection medium and sliced into small pieces with the help of a sterilized surgical blade. The petridish containing stromal tissue and collection medium were kept undisturbed for 10-15 minutes. The content and aspiration and slicing media were placed in a squared searching dish and examined under stereozoom microscope to ascertain the presence and grading of cumulus-oocyte complexes (COCs).

COCs were evaluated morphologically and graded (according to cumulus compactness and clarity of ooplasm) into four grades (Satrapa *et al.,* 2011). The COCs with three or more layers of compact cumulus cells and homogeneous ooplasm were classified as grade A. Those with fewer than three intact layers of cumulus cells and homogeneous or slightly nonhomogeneous granulated ooplasm were classified as grade B. The oocytes surrounded only with corona radiata and distinct heterogeneous ooplasm were classified as grade C and denuded oocytes as grade D. Grades A and B were considered as 'good' and combined together for analysis of compact, partially expanded, or expanded, and grades C and D were defined as 'poor'.

***In vitro* maturation (IVM)**

Morphologically good quality COCs (Grade A and B) were selected and washed three times in Medium 199 containing 200 mM L-glutamine solution, 10% FBS, 0.8 M Sodium pyruvate and 50µg/ml Gentamicin and matured in IVM medium containing Medium-199, 200 mM L-glutamine solution, 10% FBS, 0.8 M Sodium pyruvate, 50 µg/ml Gentamicin, 50µM Cysteamine supplemented with p-FSH (5 µg/ml), 10% v/v Follicular fluid and 1 µg/ml 17-β estradiol.

A group of ten oocytes were transferred to 50µl droplet of IVM medium in a 35 mm petridish and covered with prewarmed sterile mineral oil and incubated at 38.5°C in a humidified atmosphere of 5% CO_2 for 24 hours. For confirmation of maturation after 24 hours the oocytes were evaluated for morphological change and *in vitro* maturation performance under stereo zoom microscope. The oocytes with an intact zonapellucida, plasma membrane and homogenous cytoplasm were considered as morphologically normal in the study. The maturation status was observed by examining degree of expansion of cumulus oophorus and extrusion of first polar body. Oocytes were fixed with acetic alcohol (1:3) and stained with 1% aceto-orecin for confirmation of polar body using phase-contrast microscopy (Prentice-Biensch *et al.,* 2012).

***In vitro* fertilization (IVF)**

For *in vitro* fertilization, frozen Jersey semen was prepared for capacitation with swim-up technique using Brackett and Oliphant (B.O.) washing medium (Bracket and Oliphant, 1975). The frozen semen was thawed (30 seconds at 37°C) and purification was done by Percoll gradient centrifugation at 1000×g for 30 minutes. The viable sperm were tested for motility and added to B.O. fertilization medium to make a final concentration of $1x10^6$ sperm/ml. *In vitro* matured oocytes were co-incubated with capacitated sperm in B.O. fertilization medium at 38.5 °C, 5% CO_2 in air and saturated humidity for 20-22 hours.

***In vitro* culture (IVC)**

The expected zygotes were washed in IVC media (*viz.*, mCR2aa containing 5% FBS and supplemented with 2% essential amino acids (v/v), 1% non-essential amino acids (v/v), 1% α- glutamic acid, 0.3% BSA and 0.05 µg/ml gentamicin sulphate) and zygotes were cultured upto four cell stage embryo.

Statistical Analysis

Data were analyzed by ANOVA after arcsine transformation using the SPSS, Version 11.5 for Windows software (SPSS, Inc., Chicago, IL, USA). Ten replicates were conducted for each experiment. Differences with $p < 0.05$ were considered as significant and with $p < 0.01$ as highly significant.

RESULTS

Oocyte quality

Abundance of visible follicle measuring 3-8 mm diameter was significantly lower ($p < 0.05$) in crossbred (3.20 ± 0.30) during summer. In summer, total oocyte per ovary in Jersey crossbreed cows (3.71 ± 0.11) significantly differ ($p < 0.05$) from summer indigenous cows (4.37 ± 0.17), and highly significant difference ($p < 0.01$) was observed compared to winter indigenous (4.46 ± 0.13) and winter crossbred (4.42 ± 0.13) cows. In summer, crossbred cows showed highly significant ($p < 0.01$) reduction in good quality oocyte recovery performance (2.74±0.08) compared to summer indigenous (3.37±0.12), winter indigenous (3.43 ± 0.12) and winter crossbred (3.42 ± 0.11) cows. Numbers of follicles, good quality oocytes and overall oocytes recovered per ovary did not differ significantly ($p < 0.05$) between winter Indigenous, winter crossbreds and summer indigenous cows. Morphologically poor quality oocyte did not show significant differences in all groups (Table 1).

TABLE 1. Oocyte recovery performance of indigenous and crossbred cows during summer and winter seasons

Parameters	Winter Mean ± SEM (No.s)		Summer Mean ± SEM (No.s)	
	Indigenous	Crossbred	Indigenous	Crossbred
Total ovaries	58	54	54	60
3-8mm diameter follicle per ovary	4.28 ± 0.26^{q} (251)	4.27 ± 0.25^{q} (231)	3.74 ± 0.18^{pq} (204)	3.20 ± 0.30^{p} (190)
Grade A oocyte per ovary	1.80 ± 0.14^{ab} (108)	1.87 ± 0.15^{b} (102)	1.69 ± 0.15^{ab} (89)	1.29 ± 0.13^{a} (78)
Grade B oocyte per ovary	1.63 ± 0.09^{NS} (94)	1.55 ± 0.11^{NS} (85)	1.68 ± 0.11^{NS} (91)	1.45 ± 0.15^{NS} (85)
Grade C oocyte per ovary	0.60 ± 0.07^{b} (35)	0.64 ± 0.13^{b} (32)	0.53 ± 0.10^{ab} (28)	0.31 ± 0.07^{a} (18)
Grade D oocyte per ovary	0.43 ± 0.11^{NS} (22)	0.35 ± 0.06^{NS} (20)	0.47 ± 0.10^{NS} (25)	0.66 ± 0.11^{NS} (39)
Good (Grade A, B) oocyte per ovary	3.43 ± 0.12^{q} (202)	3.42 ± 0.11^{q} (187)	3.37 ± 0.12^{q} (180)	2.74 ± 0.08^{p} (163)
Poor (Grade C, D) oocyte per ovary	1.03 ± 0.14^{NS} (57)	0.99 ± 0.12^{NS} (52)	1.00 ± 0.16^{NS} (53)	0.97 ± 0.14^{NS} (57)
Total oocyte per ovary	$4.46 \pm 0.13^{q,b}$ (259)	$4.42 \pm 0.13^{q,b}$ (239)	$4.37 \pm 0.17^{pq,b}$ (233)	$3.71 \pm 0.11^{p,a}$ (220)

Values in the same rows with different superscripts are significantly different at $p < 0.05$ (a, b) and at $p < 0.01$ (p, q), NS: Not Significant at $p = 0.05$

IVM performance

In the case of maturation performance based on COCs expansion in summer crossbred cows (77.79 ± 3.01) differ significantly ($p < 0.05$) from winter indigenous cows (84.56 ± 2.3), winter crossbred cow (86.33 ± 2.30). Similarly, maturation performance based on nuclear maturation rate significantly drops in summer crossbreds (47.50 ± 6.92) compared to winter indigenous (67.50 ± 6.51) and winter crossbred cows (70.00 ± 5.00). Maturation rate did not differ significantly between winter Indigenous, winter crossbreds and summer indigenous cows (Table 2).

TABLE 2: IVM and IVF performance of indigenous and crossbred cows during summer and winter seasons

Parameters	Winter Mean ± SEM (No.s)		Summer Mean ± SEM (No.s)	
	Indigenous	Crossbred	Indigenous	Crossbred
Cumulus expansion	84.56 ± 2.3^{b} (171)	86.33 ± 2.30^{b} (160)	82.69 ± 1.53^{ab} (150)	77.79 ± 3.01^{a} (126)
Nuclear maturation	67.50 ± 6.51^{b} (27/40)	70.00 ± 5.00^{b} (28/40)	62.50 ± 7.68^{ab} (25/40)	47.50 ± 6.92^{a} (19/40)
IVF (Cleavage) rate	58.59 ± 2.09^{b} (78/131)	58.02 ± 1.86^{b} (71/120)	54.41 ± 2.35^{ab} (60/110)	51.44 ± 2.24^{a} (44/86)
4 cell stage	26.26 ± 3.08^{NS} (32/131)	25.23 ± 3.18^{NS} (28/120)	20.15 ± 2.01^{NS} (23/110)	20.00 ± 3.52^{NS} (17/86)

Values in the same rows with different superscripts are significantly different at $p < 0.05$ (a, b) NS: Not Significant at $p = 0.05$

IVF performance

Cleavage rate in summer crossbreds (51.44 ± 2.24) is significantly lower than winter indigenous (58.59 ± 2.09) and winter crossbreds (58.02 ± 1.86). The cleavage rate between winter Indigenous, winter crossbreds and summer indigenous cows showed no significant differences. There were no significant differences recorded in four cell embryo yield between summer and winter irrespective of breed of origin (Table 2).

DISCUSSION

In this study, we made an effort to evaluate the crossbreeding strategy of Jersey × nondescript crossbred cows based on *in vitro* fertilization performance and effect of summer heat stress on oocyte developmental competence. The study reveals that during summer, heat stress can lead to a highly significant reduction of ovarian enclosed oocyte in crossbreds, which ultimately reflects the proportion of recovery rate of good quality oocytes. Due to well-known adaptability, the indigenous nondescripts are able to tolerate heat stress in summer and produce non-significant good quality oocyte almost equivalent to winter season. There were no differences in proportion of normal oocytes related to breed groups.

Reports have been found that high environmental temperature and humidity resulted in a marked decline in oocyte quality from Holstein and crossbred Angus cows (Rocha *et al.*, 1998). Regardless of season, Brahman cow produces higher numbers of oocytes with normal morphology and resulting higher blastocyst rate. Studies

have shown that the season markedly influences oocyte quality of *Bos Taurus* cows, than that of *Bos indicus* or crossbreed cows (Rocha *et al.*, 1998). There are evidences supporting formation of oocytes with reduced competence due to heat stress at the earliest stages of follicular growth (Torres-Junior *et al.*, 2008). A series of in vivo and *in vitro* studies demonstrated that elevated temperature did not affect oocyte cleavage rate in Gir cows (Rutledge *et al.*, 1999 and Payton *et al.*, 2004)

Physiological conditions including age, puberty, COCs morphology, follicular dynamics and oocyte stages differ both *B. indicus* and *B. indicus* crossbred cattle from B. Taurus genotypes. *B. indicus*-derived cattle achieve puberty later as compared with *B. Taurus* cattle (Rekwot *et al.*,2000 and Rodrigues *et al.*, 2002) indicating slower achievement of oocyte developmental competence in *B. Indicus* crossbred. Embryogenic competence of oocytes derived from *B. Indicus* crossbreds largely depends on collection age of the animal (Camargo *et al.*, 2005). COCs can be used as priori to determine visual quality assessment of oocytes and its correlation with blastocyst production. Previous reports showed that zebu cows exhibited smaller ovaries, greater number of small follicles and few oocytes per ovary than *B. taurus* (de Oliveira *et al.*, 1994 and Dominguez, 1995). Summer heat stress along with insufficient nutrition supply may greatly affect oocyte competence as we cannot nullify the influence of nutrition in folliculogenesis (Scaramuzzi *et al.*, 2011).

Germinal vesicle (GV) stage oocytes remain in the antral follicle for 42 days and during this period, animals exposed to summer heat stress may reach body temperatures 40 to 41°C (Rivera and Hansen, 2001). Reports have been found that in Holstein, developmental competence of GV stage oocyte was affected by elevated temperature (Gendelman *et al.*, 2010). So, in summer, blastocyst production was less compared to winter season in Holstein. In contrast Payton *et al.* (2004) reported that effects of heat stress to reduce the development of GV stage COCs were independent of elevated temperature administered in relation to when the resumption of meiosis occurred (*i.e.*, the first or last 6 or 12 hours of culture), but reductions in development have been noted in maturing oocytes, only when heat stress was applied during the first 12 hr of maturation. This disparity of results may be due to inherent differences in GV stage versus maturing COCs due to direct effect of elevated body temperature on antral follicle COCs may reduce fertility in heat-stressed cows. Exposure of crossbred *B. indicus* oocytes to severe heat shock 44°C (0 to 12 hours IVM) and 43°C (45-60 minutes IVM) reduces cleavage and blastocyst rates respectively. Even physiological or moderate heat shock of 40 °C or 41°C for 0-12hours IVM reduced cleavage rate and subsequent blastocyst stage (Paula-Lopes *et al.*, 2008). However, mild heat shock at 40.5°C and 41.5°C for 30 and 60 minutes during IVM did not affect development to the blastocyst stage. These studies indicated that elevated temperature and exposure time of heat shock greatly influences developmental competence of crossbreds oocytes (Paula-Lopes *et al.*, 2013). However, depending upon oocyte thermotolerance capacity heat stress can cause reversible or irreversible cellular damage (Roth and Hansen, 2005), triggering adaptive and/or cellular death responses.

The thermoregulatory ability of Brahman cows reduces maternal hyperthermia compared with Holstein cows and/or oocytes from Brahman cows contain intrinsic mechanisms that allow survival after exposure to elevated temperature (Paula-Lopes *et al.*, 2013). *In vitro* exposure to heat shock at early stages of *B. indicus* embryo can better able to survive as compared to *B. Taurus* embryos. More recently, effects of heat stress on embryonic development in culture were evaluated in Nelore and crossbred (*B. indicus* × *B. taurus*) oocytes and recorded decrease in blastocyst development rates caused by exposure to 41°C at earlier stages of development (Eberhardt *et al.*, 2009).

In our experiment, the reason for the better cleavage rate seen in all cases is most likely that it has been consistently experimented under suitable stress free *in vitro* condition. Although, during summer time, heat stressed crossbred oocytes have significantly lower maturation rate than winter group oocytes. During *in vitro* maturation phase, oocyte competence improves gradually. Despite the fact that only oocytes collected from crossbreds during summer were developmentally compromised upto cleavage stage, although, it revives its competency gradually in normal *in vitro* culture condition. Probably due to embryonic genome activation starts and accelerates on later stage of blastocyst development and embryos become resistant to heat shock as stage of development advances.

CONCLUSION

Summer heat stress detrimentally affects oocyte maturation and also subsequent embryo production competence in crossbreds. In respect to *in vitro* development, the non-significant variation of oocyte developmental competence during summer and winter might be due to gradual elimination of incompetent heat stressed oocytes during the recovery and IVM process. From the experiment it could be concluded that oocyte developmental competence of summer heat stressed Jersey crossbred cows could be improved in stress free *in vitro* condition.

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