

ANIMAL

Heat waves impair cytoplasmic maturation of oocytes and preimplantation development in Korean native cattle (Hanwoo)

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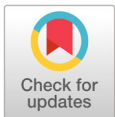
Abstract

There has been widespread warming and a general increase in summer temperatures over the Korean peninsula (0.5°C/ 10 years from 2001 to 2010). South Korea is transforming into a subtropical region, and the productivity of livestock is affected by the climatic changes. In this study, we investigated whether the summer heat waves affect the developmental competency of Korean native cattle (Hanwoo), a taurine type of cattle with a small portion of *indicine* varieties. We collected oocytes during the summer (heat stress, HS) and autumn (non-HS condition) and examined the developmental competencies including *in vitro* maturation and preimplantation embryo development. No significant differences were observed between the HS and non-HS oocytes in nuclear maturation (extrusion of the polar body); however, the cleavage and blastocyst rates were significantly lower in the HS group than those in the non-HS group. The lower developmental competence of the HS oocytes compared to the non-HS is, in part, due to insufficient cytoplasmic maturation because of a higher production of Reactive oxygen species (ROS) levels as well as peri/cortical distributed mitochondria in the HS oocytes after *in vitro* maturation. Next, we examined the ROS and mitochondria distribution and found a significant increase in the levels of ROS in the HS oocytes and a polarized distribution (pericortical cytoplasm) of mitochondria in the HS oocytes. In summary, impaired cytoplasmic maturation of oocytes from exposure to HS affects the preimplantation embryo development by dysfunction of mitochondria. To improve reproductive performance, embryo transfer using cryopreserved embryos/oocytes is recommended in the hot summer season of South Korea.

Keywords: *Bos Taurus*, Hanwoo, heat stress, Korean native cattle, oocyte maturation

Introduction

Recent trends in climate change observed on the Korean peninsula showed a rate of change of 0.41°C / 10 years from 1981 to 2010 and 0.5°C / 10 years from 2001 to 2010, and scenarios using the regional climate model for the future climate forecast predicted the temperature increased by 4°C and the precipitation



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increased by 17% for all regions of the Korean peninsula (Chung et al., 2004; Lee, 2015). According to the report, South Korea is transforming into a subtropical region, and the climatic changes can impact on productivity of livestock (Rojas-Downing et al., 2017). Moreover, the occurrences of heat waves defined as extreme weather conditions such as daily maximum temperature over 33°C has increased in the 1990s and 2000s, and the hot events have been more intense in summer from June to August over southern provinces in South Korea (Shim et al., 2017).

Interestingly, our analysis of reproductive performance in Korean native cattle (Hanwoo) demonstrated that calving interval was increased 4.3% and pregnancy rate was decreased about up-to 2.8% year-on-year due to subfertility in summer season (Cho et al., 2016; Choi and Cho, 2016). In this study, we investigated association of heat waves in summer with developmental competency of oocyte in Korean native cattle, Hanwoo.

Material and Methods

All other chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise indicated.

Statement of Animal Rights

All practices and procedures for this experiment were reviewed and approved by the Animal Ethics Committee of the Chungnam National University (CNU-00599).

Ovary collection and *in vitro* production of embryos

Ovaries were collected from a local slaughter house within 7 days after heat waves during summer, and at November, respectively. *In vitro* maturation, parthenogenetic activation, and embryo culture procedures have been described elsewhere (Kelly et al., 2010). The maturation and embryo development rates were assessed by extrusion of the first polar body and morphology such as blastocoel.

Reactive oxygen species (ROS) levels and mitochondrial distribution

The ROS levels were examined to determine whether heat stress induced by heat waves affect cytoplasmic competency of oocytes. ROS levels of oocytes were measured with Image-iT Live Green Reactive Oxygen Species Detection kit (Invitrogen, Eugene, OR, USA). To investigate mitochondrial distribution, matured oocytes were incubated with 100mM MitoTracker Red TM (Invitrogen) for 30 min and examined using a confocal laser-scanning microscope (Zeiss LSM 710 META; Jena, Germany). The fluorescent intensities of ROS level and the distribution of fluorescence signal intensities along a line drawn across MII oocytes were analysed using Image J (1.48v) software (<https://imagej.nih.gov/ij/index.html>).

Results and Discussion

There were no significant differences in nuclear maturation assessed by polar body extrusion between HS (heat stress) and Non-HS groups. The cleavage and blastocyst development was examined on day 2 and 7 after oocyte activation. 2-cell cleavage and blastocyst development were significantly lower in the HS groups, respectively (Table 1).

We examined ROS and mitochondria distribution because mitochondria are essential for oocyte maturation and subsequent embryo development (Wang et al., 2009). We found a significant increase in the levels of ROS in the HS oocytes after IVM, compared to those in non-HS oocytes (Table 2). Mitochondria in non-HS oocyte were homogeneously distributed in the cytoplasm, however mitochondria were polarized in the HS oocyte, particularly in the pericortical cytoplasm (Fig. 1).

Oocytes from summer season showed lower cleavage and blastocyst rates although nuclear maturation rates were not different from oocytes obtained in winter season. We therefore postulated that insufficient cytoplasmic maturation affect further developmental competency. Our findings that oocytes exposed to summer heat waves increased the production of ROS and had polarized peripheral distribution of mitochondria, rather than homogeneous cytoplasmic distribution, indicate that the lower developmental competency of bovine oocytes collected under heat waves is attributed to insufficient cytoplasmic maturation such as increased generation of ROS and dysfunction of mitochondria (Roth et al., 2008; Roth, 2015; Paes et al., 2016). Moreover, taurine type of Korea native cattle (Hanwoo) has more genetic diversities including genetic features of African taurine and *indicine* cattle breeds (less than 10%) (Lee et al., 2014; Makina et al., 2014; Sharma et al., 2016).

Conclusion

We supposed that Hanwoo might be less resistant to increased temperature and humidity as seen in *Bos taurus* cattle, compared to *Bos indicus* (Paula-Lopes et al., 2013; Silva et al., 2013). Taken

Table 1. Comparison of HS and Non-HS oocytes to maturation and blastocyst rates.

Condition	No. of oocytes	No. of MII oocytes (%)	No. of cleaved 2-cell zygote (%)	No. of blastocyst (%)
HS	175	149 (85.14)	100 (67.11) ^a	19 (12.75) ^a
Non-HS	163	147 (90.18)	125 (85.03) ^b	42 (28.57) ^b

HS, Heat stressed by heat waves; Non-HS, Non-heat stress.

a - b: Superscript significant differences between Heat waves and winter conditions.

Table 2. Level of ROS production (mean \pm SEM) in matured oocytes.

Condition	ROS (fluorescence unit)
HS	37 \pm 6.23 ^a
Non-HS	25 \pm 8.23 ^b

HS, Heat stressed by heat waves; Non-HS, Non-heat stress.

a - b: Superscript significant differences between Heat waves and winter conditions.

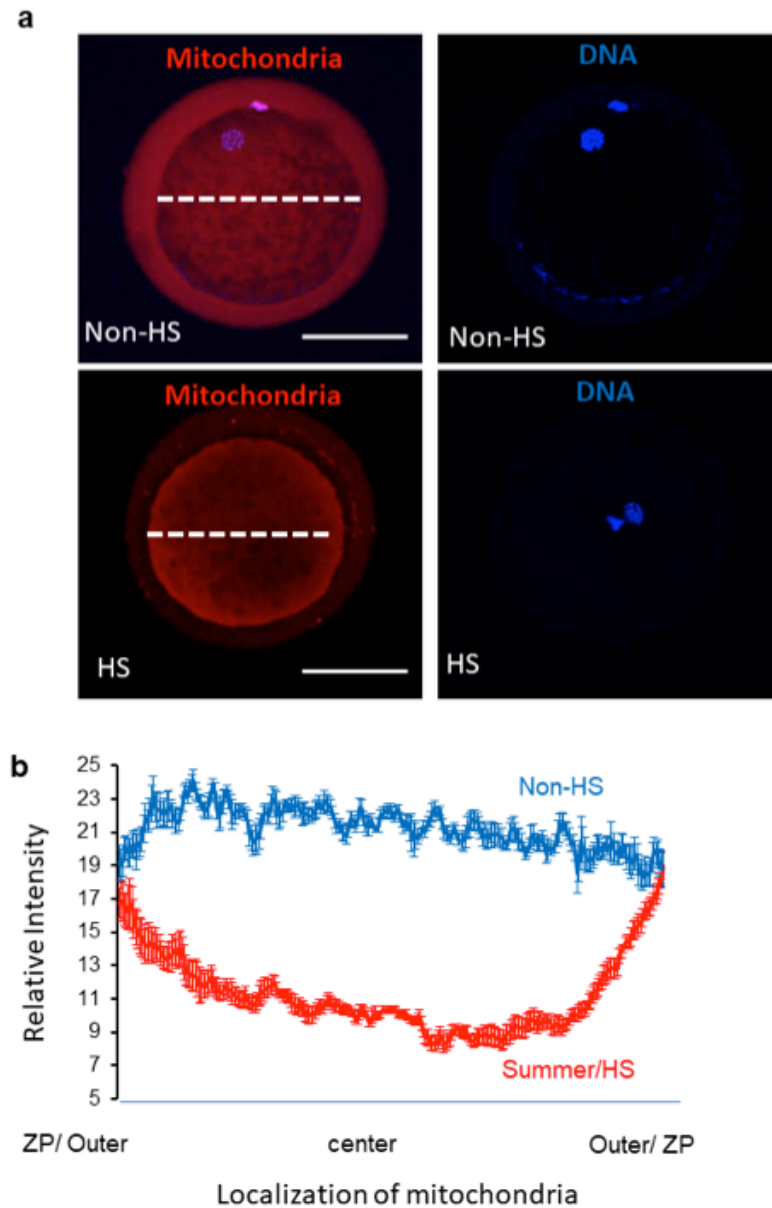


Fig. 1. Intercellular distribution of mitochondria in matured oocytes. a: Mitochondria are stained red (MitoTracker Red) Non-HS (Non-heat stressed condition; oocytes collected and matured in winter), HS (Heat stressed condition; oocytes collected following exposure of heat waves). Scale bar 50 μ m. b: Intensities of stained mitochondria (along a line drawn across MII oocytes; white dot line in Fig 1. a) are measured using image J. ZP (zona pellucida).

together, our findings suggest that embryo transfer using cryopreserved embryos/oocytes obtained during non-heat stress season can improve pregnancy rates in the summer because HS adversely affects cytoplasmic maturation and subsequent embryonic development.

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