Tropical Animal Health and Production (2019) 51:1611–1618
https://doi.org/10.1007/s11250-019-01854-w

REGULAR ARTICLES

Low developmental competence and high tolerance to thermal stress of ovine oocytes in the warm compared with the cold season

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Received: 27 November 2018 / Accepted: 20 February 2019 / Published online: 6 March 2019

Abstract

Heat stress can potentially affect most aspects of reproduction in mammals. To our knowledge, no studies have ever been conducted for evaluating the influences of hot season on the developmental competence of ewe oocytes. In the present study, for the first time, we evaluated the effects of season (winter or summer), in vitro thermal stress, and their interaction on the ewe oocytes harvested from slaughterhouse ovaries. Cumulus-oocyte complexes (COCs) were either incubated at 39 °C for the entire length of IVM period or first incubated at 41 °C for 12 h and then at 39 °C. Evaluated endpoints included the ratios of total aspirated COCs/ovary and good-quality COCs/ovary, the apoptosis (Annexin V staining) and nuclear maturation of oocytes after 24-h IVM, and the developmental competence of oocytes after IVF. Our results showed that the number of aspirated oocytes per ovary was similar in both seasons, but the winter ovaries yielded significantly more oocytes with acceptable morphology in winter than in summer (2.1 ± 0.14 vs. 1.5 ± 0.09, $P < 0.05$). There was a significant interaction between season and thermal stress on the apoptosis, some nuclear maturation parameters, and blastocyst development of oocytes ($P < 0.05$). Although the winter oocytes were more developmentally competent than the summer oocytes, the winter oocytes were more sensitive to the thermal stress than summer oocytes. In conclusion, the developmental competence of ovine oocytes was lower in summer than in winter. However, it seemed that summer oocytes were more resistant to the in vitro thermal stress during IVM period compared with winter oocytes.

Keywords Heat stress · Oocyte · Embryo · Apoptosis · Nuclear maturation

Introduction

High ambient temperatures beyond the animal physiological capability to dissipate excess body heat can lead to hyperthermia and heat stress. In animals, especially in domesticated ones, by increasing the average annual temperature in the process of global warming, heat stress will likely be an important issue during the warm seasons of the year, especially in the tropical and subtropical regions (Root et al. 2003). Different biological functions of animals are impacted by the heat stress, which includes a decrease in feed intake efficiency and utilization; disturbances in water, protein, energy, and mineral balances; enzymatic reactions; hormonal secretions; and blood metabolites (Marai et al. 2007). Heat stress can potentially affect most aspects of reproduction in mammals either directly or indirectly. These include disruptions in the development of male and female gametes, oocyte maturation, early embryonic development, fetal and placental growth, and lactation (Hansen 2009).

The impacts of heat stress have extensively been studied on the various aspects of reproduction in bovine species. In dairy cattle, heat stress has a significant deleterious effect on the fertility of females. In this species, various lines of evidence indicate that heat stress impacts the female fertility by compromising the developmental competence of oocytes (reviewed in Roth 2017). It has been suggested that primordial follicles and enclosed growing oocytes in them are resistant to heat stress (Paes et al. 2016). About pre-antral follicles, it is not clear whether they are sensitive to heat stress or not (Roth 2017). In contrast, experimental data indicate that after antrum formation, follicles become sensitive to heat stress (Roth et al. 2000), and the changes that occur in these follicles due to heat stress may affect the advanced stages of follicular growth.

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Moreover, the effects of these changes extend beyond the hot season (Roth et al. 2004; Zeron et al. 2001). In ovine, to our knowledge, no studies have ever been conducted on the influences of hot season on the developmental competence of oocytes. In this species, the deleterious impacts of high ambient temperatures have been more studied on the animal welfare, production, and physiology than its effects on reproduction (see Marai et al. 2007). However, it is commonly said that various reproductive processes of ewe, such as endocrine function, estrus cycle, ovulation, placental growth, embryonic survival, and fetal growth, can be affected by heat stress (Bell et al. 1989; Casu et al. 1990; Hill and Alliston 1981).

Considering the pivotal role of oocytes in female reproduction, we conducted present study to compensate for the lack of knowledge about the possible seasonal variations in the developmental capacity of sheep oocytes and the effects of thermal stress on them. The data from this study may help us to understand the problems that likely occur in the reproductive processes of animals due to the increasing temperature of the planet. In the present study, the effects of season (cold or warm), in vitro thermal stress, and their interaction on ovine oocytes were evaluated. Evaluated endpoints included (a) the ratios of total aspirated cumulus-oocyte complexes (COCs)/ovary and good-quality COCs/ovary, (b) the apoptosis (Annexin V staining) and nuclear maturation of oocytes after 24-h IVM, and (c) the developmental competence of oocytes after IVF.

Material and methods

All chemicals were obtained from Sigma Chemicals Co. (St. Louis, MO, USA) unless otherwise stated.

Experimental design

This experiment was performed in Shahrekord, Chaharmahal-va-Bakhtiari Province, Iran (32° 19′ N, 50° 51′ E). The maximal and minimal temperatures and relative humidity of the region are presented in Table 1. In the first year of this study, the winter phase was conducted in December 2016 through February 2017, and the summer phase was conducted in June through August 2017. In the second year, the winter phase was conducted in December 2017 through February 2018, and the summer phase was conducted in June through August 2018. COCs were obtained from the ovaries of Lori-Bakhtiari ewes, an Iranian indigenous and wooly sheep breed. COCs were either incubated at 39 °C for the entire length of IVM period (24 h) or first incubated at 41 °C for 12 h and then at 39 °C. In the first experiment and after IVM, the necrosis, apoptosis, and nuclear maturation of oocytes were assessed. In the second experiment, the developmental

Table 1 Meteorological parameters registered in different months during previous 10 years (2008–2017) expressed as mean ± SEM

	January	February	March	April	May	June	July	August	September	October	November	December
Min Hum. (%)	47.3 ± 3.05	41.3 ± 2.47	29.7 ± 1.58	31.5 ± 1.30	24.2 ± 1.01	16.4 ± 1.15	15.7 ± 0.97	17.3 ± 0.84	17.8 ± 1.10	25.1 ± 1.28	41.5 ± 3.21	45.5 ± 3.51
Max Hum. (%)	78.0 ± 2.00	75.7 ± 1.79	64.3 ± 2.80	70.6 ± 1.80	62.3 ± 1.71	49.8 ± 2.27	44.5 ± 1.28	5.4 ± 1.61	49.1 ± 1.72	58.6 ± 1.92	75.7 ± 3.68	78.0 ± 2.80
Min Temp. (°C)	−6.7 ± 1.14	−4.7 ± 0.75	−0.9 ± 0.53	3.1 ± 0.36	6.7 ± 0.49	10.0 ± 0.49	13.7 ± 0.35	11.8 ± 0.57	7.6 ± 0.52	3.4 ± 0.49	−1.0 ± 0.64	−5.1 ± 0.52
Max Temp. (°C)	7.6 ± 1.07	9.7 ± 0.70	15.0 ± 0.57	19.3 ± 0.50	25.1 ± 0.31	31.4 ± 0.40	34.0 ± 0.38	32.6 ± 0.31	29.2 ± 0.25	23.2 ± 0.28	14.3 ± 0.58	10.2 ± 0.78

competence of the thermal-stressed oocytes in comparison to that of the unstressed oocytes was assessed. In each replicate, the number of aspirated ovaries, total recovered COCs, and good-quality COCs were recorded and the ratios of total recovered COCs per ovary and good-quality COCs per ovary were calculated. The same lot of frozen-thawed semen was used in the embryo production experiments in both years. This semen, which had been frozen in the autumn of 2017, was a pool of two consecutive ejaculates of five rams with a proven in vitro fertility.

In vitro embryo production

Ovine ovaries were obtained from a local abattoir and transported to the laboratory in a thermos flask containing normal saline at 25–35 °C. Follicular fluid of follicles with the 2–6 mm diameter was aspirated with 20-G needles using a vacuum pump into the 50-ml conical tubes containing 10 ml aspiration medium (HEPES-buffered medium 199 supplemented with 5% FBS and 100 IU/ml heparin). The vacuum pressure was set on 20 mmHg. All COCs in the aspirated fluid were collected and counted. Then, the cumulus-oocyte complexes with at least three compact layers of cumulus cells and evenly granulated cytoplasm of the oocyte (good-quality COCs) were separated and washed four times in HEPES-buffered medium 199 and one time in IVM medium. Finally, COCs were cultured in the 50-μl droplets of IVM medium (bicarbonate-buffered medium 199 supplemented with 0.33 mM sodium pyruvate, 0.05 IU/ml FSH, and 10% FBS) under mineral oil at 39 °C with 5% CO_2 in humidified atmosphere. After 24-h IVM, the COCs were transferred to the droplets of IVF-TALP and incubated at 39 °C with 5% CO_2 in humidified atmosphere until sperm addition. The spermatozoa were prepared as previously described (Shokrollahi et al. 2014) with some modifications. Briefly, 200 μl of sperm suspension (frozen-thawed spermatozoa) was placed on 1 ml Histoprep® and centrifuged at 300×*g* for 5 min. The pellet of spermatozoa after re-suspension in the 50 μl of Sperm-TALP was added to the fertilization droplets at the final concentration of 1×10^6 motile sperm cells/ml. At 24–26 h post-insemination, presumptive zygotes were denuded from attached spermatozoa and cumulus cells. Finally, five to six morphologically normal zygotes were cultured in the 20-μl droplets of IVC-SOF medium (SOF + amino acids and BSA) in an incubator with 5% CO_2 and 6% O_2 at 39 °C with maximum humidity. Embryonic development was assessed morphologically under a stereomicroscope. On the third day of culture (day 0 = IVF day), cleaved embryos were separated and cultured in IVC-SOF medium supplemented with 10% charcoal-stripped FBS. Blastocyst formation was assessed on the days 6–8 of culture, one time at each day.

Annexin V staining of phosphatidylserine residues and assessment of nuclear maturation

Assessment of apoptosis and nuclear maturation was performed simultaneously on the same oocytes. For detection of phosphatidylserine (PS) externalization on the plasma membrane that occurs in the early stages of apoptosis, Annexin V-FLOUS Staining Kit (Roche Diagnostics, Mannheim, Germany) was employed according to the manufacturer's instructions. For chromatin staining, 5 μg/ml of Hoechst 33342 was added to the binding buffer. During the whole process, reagents were kept at 39 °C.

Denuded oocytes were washed three times with HEPES-buffered medium 199 and one time in binding buffer. The oocytes were transferred to the oil-covered 50-μL droplets of staining solution containing binding buffer, Annexin V/FITC, propidium iodide (PI), and 5 μg/ml of Hoechst 33342, and incubated at 39 °C in an incubator for 15 min. After incubation, oocytes were mounted on glass slides between Vaseline bridges, covered by coverslips, and observed immediately using a fluorescent microscope. Necrotic oocytes due to the diffusion of PI into them stained red and were PI-positive. PI-negative oocytes were classified in two categories: (a) viable healthy oocytes which were Annexin V–negative/PI-negative (AV−/PI−) with little or no green and red fluorescence signal and (b) live apoptotic oocytes which were Annexin V–positive/PI-negative (AV+/PI−) with green fluorescence signal on the plasma membrane.

Nuclear maturation assessment was performed only in PI-negative oocytes. The nuclear status of oocytes was classified as previously described (Shirazi et al. 2010). Briefly, oocytes in which diffuse chromatin could be identified were classified as being in the germinal vesicle (GV) stage. Oocytes possessing slightly condensed or clumped chromatin were classified as being in the germinal vesicle breakdown (GVBD) stage. Oocytes with strongly condensed chromatin that formed an irregular network of individual bivalents (prometaphase), or a metaphase plate but no polar body, were classified as being in metaphase I (MI) stage, and oocytes with either a polar body or two shiny chromatin spots were classified as being in metaphase II (MII) stage of the maturation process. The chromatin was described as fragmented when it had multiple condensed or non-condensed fluorescence foci that could not be described as any stages of nuclear maturation.

Statistical analysis

All proportional data before analysis were subjected to an arcsine transformation. Statistical analysis was performed using IBM-SPSS ver. 20 software package. In this study, factorial ANOVA models were used to determine the statistical differences among the main effects of the season (two levels: winter

and summer), thermal stress (two levels: yes and no), and their interactions (S*TS). In the case of a significant interaction, the interaction was analyzed using a simple main effects analysis. The significant simple main effects were further analyzed by pairwise comparisons using the Bonferroni adjustment for multiple comparisons. If a significant interaction was not detected, the main effects (season and thermal stress levels) were interpreted. The comparison of oocyte yields among two seasons was done by using the independent *t* test. Differences were considered significant at the level of $P < 0.05$.

Results

Since the results of both years of the study followed a similar pattern, the results were combined and analyzed together. As shown in Table 2, the mean number of total recovered COCs per ovary was equal in both seasons. The mean number of good-quality COCs per ovary was significantly lower in summer than in winter ($P < 0.05$).

The statistics of experiment 1 and a summary of two-way ANOVA analysis were presented in Table 3. The main effect of season on the rates of apoptotic and MII-stage oocytes was significant ($P < 0.05$). The main effect of thermal stress and the interaction of season and thermal stress were significant on the rates of apoptotic, MI-stage, and MII-stage oocytes, as well as on the rate of oocytes with fragmented chromatin ($P < 0.05$). Analyzing significant interactions revealed that the incubation of oocytes at 41 °C in the first half of IVM period (thermal stress) had greater influence on the winter oocytes than on the summer oocytes. In this regard, the increases in the rate of apoptotic and MI-stage oocytes and oocytes with fragmented chromatin and also the decrease in the rate of MII-stage oocytes were significantly higher in the winter thermal-stressed oocytes than in their summer counterparts ($P < 0.05$).

The statistics of experiment 2 and a summary of two-way ANOVA analysis were presented in Table 4. The main effect of thermal stress on the cleavage rate was significant as such that thermal-stressed oocytes had lower cleavage rate ($P < 0.05$). The main effect of season only was significant on the day 6 blastocyst rate ($P < 0.05$). The main effect of thermal stress and the interaction of season and thermal stress were significant on the rates of day 6, day 7, day 8, and hatched blastocyst ($P < 0.05$). After analyzing significant interactions, it was revealed that the influence of thermal stress on the winter oocytes was greater than on the summer oocytes. Although the winter oocytes had significantly higher blastocyst and hatched blastocyst rate than the summer oocytes, thermal stress led to a greater decrease in the developmental competence of the winter oocytes than of the summer oocytes ($P < 0.05$).

Discussion

The aim of the present study was evaluating the in vitro developmental potential of ewe oocytes during the warmest and coldest months of the year. Moreover, the tolerance of oocytes to thermal stress was investigated. Our results showed although the number of aspirated COCs per ovary was similar in both seasons, the ovaries yielded more COCs with acceptable morphology in winter than in summer. Moreover, although the status of apoptosis and nuclear maturation was not different between the unstressed oocytes in both seasons, the winter oocytes were more developmentally competent. Nevertheless, it seemed the effect of thermal stress on the winter oocytes was greater than that on the summer oocytes.

In the condition of present study, we observed that ewe ovaries had lower efficiency to yield morphologically normal COCs in summer compared with winter ($P < 0.05$). Total aspirated COCs per ovary were similar in both seasons, and the "lower efficiency" in the summer was due to the higher proportion of low-quality COCs in this season. This phenomenon was observed in both years that the experiment was conducted. Moreover, we tried to provide a uniform condition for performing this experiment. Hence, technical differences were unlikely to be the cause of the observed result. Disqualified COCs in both seasons had a variety of morphological abnormalities in their cytoplasm and surrounding cumulus cells. Although we did not ascertain the exact type and quantity of abnormalities in these COCs and oocytes, it seemed both cumulus and cytoplasmic morphological defects were more prominent in the summer COCs than in the winter COCs. The most probable reason for the increased proportion of low-quality COCs in the summer is the hyperthermia-induced changes in follicles. In cattle, it has been shown that hyperthermia during summer could negatively affect various

Table 2 Aspirated COCs and oocytes from the summer and winter ovaries

Season	Aspirated ovaries	Aspirated oocytes and COCs	Aspirated oocyte/ovary	Acceptable COCs	Acceptable COCs/ovary
Winter	590	1647	2.9 ± 0.14	1204	2.1 ± 0.14[a]
Summer	707	2012	2.8 ± 0.10	1083	1.5 ± 0.09

[a] Significant difference in each column ($P < 0.05$)

COCs cumulus-oocyte complexes

Table 3 Effects of season, in vitro thermal stress, and their interaction on the apoptosis and nuclear maturation parameters of ewe oocytes

			PI+		PI−/AV+		GV		GVBD		MI		MII		Frag.	
		Thermal stress	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
		Winter	2.0	4.2	3.5^{A}	14.9^{a}	4.8	4.5	5.3	6.4	3.6^{A}	13.5^{a}	84.7^{A}	64.2^{a}	1.9^{A}	11.6^{a}
		Summer	2.5	4.5	4.6^{A}	8.2	4.5	1.9	3.6	4.7	4.4	7.3	84.1	79.0	3.4^{A}	7.1
		SEM	1.20		1.20		1.00		2.00		1.40		2.30		0.90	
Main effects	Season	Winter	3.1		9.2*		4.6		5.85		8.6		74.5*		6.6	
		Summer	3.5		6.4		3.2		4.2		5.8		81.5		5.2	
	Thermal stress	No	2.3		4.1$^{\#}$		4.7		4.5		4.0$^{\#}$		84.4$^{\#}$		2.5$^{\#}$	
		Yes	4.4		11.6		3.1		5.6		10.4		71.6		9.4	
Sources of variation																
Season			0.910		*0.029*		0.355		0.530		0.063		*0.004*		0.116	
Thermal stress			0.723		*< 0.001*		0.261		0.660		*< 0.001*		*< 0.001*		*< 0.001*	
S*TS			0.708		*0.003*		0.203		0.381		*0.019*		*0.002*		*0.001*	

Significant values are shown in italics

PI+, necrotic oocytes; *PI−/AV+*, viable apoptotic oocytes; *GV*, germinal vesicle; *GVBD*, germinal vesicle breakdown; *MI*, metaphase I; *MII*, metaphase II; *Frag.*, fragmented chromatin

[A] In each row indicates a significant difference between the thermal-stressed oocytes of each season and their unstressed counterparts in each parameter

[a] In each column indicates a significant difference between the winter oocytes (thermal-stressed or unstressed) and their summer counterparts

*A significant difference between the main effects of season

[#] A significant difference between the main effects of thermal stress

aspects of follicles such as their populations and dynamism (Di Francesco et al. 2011; Trout et al. 1998), growth (Takuma et al. 2010), sensitivity to gonadotropins, and steroidogenesis (de S Torres-Júnior et al. 2008). In buffalo in one study, the mean number of antral follicles and total harvested COCs and oocytes per ovary were lower in hot season than in cold season (Abdoon et al. 2014). On contrary in another study in this species, oocyte recovery per ovary among seasons was not

Table 4 Effects of season, in vitro thermal stress, and their interaction on the developmental competence of ewe oocytes

			Cleavage		D6 B.		D7 B.		D8 B.		H. B.	
		Thermal stress	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
		Winter	85.1	75.4	37.7^{Aa}	17.1	49.0^{Aa}	23.8	50.5^{Aa}	27.1^{a}	31.3^{Aa}	12.5
		Summer	77.9	72.4	24.2	18.8	35.4^{A}	24.1	42.3^{A}	32.8	22.6	17.0
		SEM	3.6		2.1		2.7		2.7		2.8	
Main effects	Season	Winter	80.0		27.4*		36.4		37.1		21.9	
		Summer	75.1		21.5		29.8		37.6		19.8	
	Thermal stress	No	81.5$^{\#}$		31.0$^{\#}$		42.2$^{\#}$		46.4$^{\#}$		27.0$^{\#}$	
		Yes	73.6		17.9		24.0		28.2		14.8	
Sources of variation												
Season			0.192		*0.006*		0.056		0.858		0.473	
Thermal stress			*0.035*		*< 0.001*		*< 0.001*		*< 0.001*		*< 0.001*	
S*TS			0.507		*0.001*		*0.005*		*0.003*		*0.025*	

Significant values are shown in italics

D6 B., the day 6 blastocyst; *D7 B.*, the day 7 blastocyst; *D8 B.*, the day 8 blastocyst; *H. B.*, hatched blastocyst

[A] In each row indicates a significant difference between the thermal-stressed oocytes of each season and their unstressed counterparts in each parameter

[a] In each column indicates a significant difference between the winter oocytes (thermal-stressed or unstressed) and their summer counterparts

*Indicates a significant difference between the main effects of season

[#] Indicates a significant difference between the main effects of thermal stress

different, but the rate of small oocytes was higher during spring and summer than during autumn and winter (Di Francesco et al. 2011). However, the mechanism by which hyperthermia induces destructive changes in growing follicles and reduces the quality of oocyte inside them requires further investigation. Moreover, our observation may have a relationship with the fertility of ewes and needs to be studied further.

Our data indicated that although the potential of summer oocytes to develop to the blastocyst stage was lower than that of winter oocytes, they were more resistant to in vitro thermal stress. In these oocytes, the developmental competence, nuclear maturation, and apoptotic status were not affected by in vitro thermal stress as much as winter oocytes. This phenomenon was previously observed in the bovine oocytes (Maya-Soriano et al. 2013) and might be an adaptation induced by in vivo chronic heat stress. Increased tolerance to stress following applying various types of sublethal stresses to oocytes, spermatozoa, and embryos has previously described (Pribenszky et al. 2010). In this regard, the exposure of bovine embryos to a mild heat shock increased the tolerance of these embryos to the subsequent exposure to more severe heat shocks. This response was attributed to the increased expression of HSP70 due to this mild heat shock (Paula-Lopes and Hansen 2002) and has been observed in other cell types (Beere and Green 2001; Mosser et al. 1997). Besides protecting cells from heat shock, HSP70 can protect cells against several apoptotic stimuli, including DNA damage, UV irradiation, serum withdrawal, and chemotherapeutic agents (Paula-Lopes and Hansen 2002). In this regard, it was observed that relative expression of HSP70 mRNA was upregulated in the buffalo COCs recovered in hot season compared with those recovered in cold season (Abdoon et al. 2014). Collectively, it seemed chronic heat stress during the hot months of summer led to a decrease in the developmental competence of the oocytes enclosed in the growing follicles. Simultaneously, this stress might trigger a heat shock response, which possibly through HSP70 expression increased the tolerance of oocytes to the thermal stress during IVM.

Although the nuclear maturation of summer unstressed oocytes (the entire IVM period at 39 °C) was similar to that of winter oocytes (Table 2), the blastocyst formation and hatchability of blastocysts of summer oocytes were significantly lower than those of their winter counterparts (Table 3, $P < 0.05$). Therefore, the cytoplasmic maturation of summer oocytes might be inferior to that of winter oocytes. The exact mechanism underlying low developmental competence of oocytes during hot season is not clear. However, as mentioned earlier, heat stress can compromise the functions of follicles by affecting early antral stages of them in dairy cattle (Roth et al. 2000; Roth et al. 2001). Moreover, it has been suggested that the exposure of the oocytes pool to heat stress during hot season can impair maternal mRNA storage and/or the mechanism of transcription renewal, which in turn affects embryo gene expression before and after embryonic genome activation (Gendelman and Roth 2012). Studies indicate that seasonal alterations in mitochondrial distribution within the oocytes, proportion of highly polarized mitochondria, and expression of mitochondrion-associated genes are related to the reduced developmental competence of oocytes during summer (Roth 2017). Although the low developmental competence of the oocytes collected in the summer months is a known phenomenon in dairy cattle (Al-Katanani et al. 2002; Zeron et al. 2001), buffalos (Abdoon et al. 2014), and sows (Bertoldo et al. 2010), this issue has not been addressed in sheep before our study. In ovine, it has been shown that high ambient temperatures have negative impacts on the fertility of ewes (Dutt 1964; Kleemann and Walker 2005; Sawyer 1979) and on the quality of in vivo–produced embryos (Naqvi et al. 2004). Perhaps, the negative impacts of heat stress on sheep's reproduction are partly due to the negative effects of this stress on the oocytes inside the growing follicles.

In the present study, the effects of culturing COCs at 41 °C during the first half of the IVM process were investigated. At now, the possibility of the conditions in which the animal's body temperature remains at 41 °C for 12 h is low. However, we provided an extreme condition to investigate the possible consequences of exposing oocytes to this temperature in their final phase of maturation. Regardless of seasonal differences, which discussed earlier, the results showed that the rate of oocytes with the externalized phosphatidylserine residues of the plasma membrane was increased by this treatment. Moreover, both nuclear maturation and developmental competence of oocytes were negatively affected by this treatment. Evidence indicates that apoptosis has a role in the disruption of normal function of bovine oocytes following thermal stress (Roth and Hansen 2004a). In this regard, externalized phosphatidylserine residues of plasma membrane and other apoptotic-related events have been reported in bovine (Kalo and Roth 2011; Roth and Hansen 2004a, b) and porcine (Tseng et al. 2006) oocytes exposed to heat shock during IVM. Alterations in the plasma membrane of heat shock–exposed bovine oocytes have been linked with the reduced developmental competence of these oocytes (Kalo and Roth 2011; Tseng et al. 2006). In the present study, the lower developmental competence of heat-stressed oocytes might partly be related to their altered plasma membrane. Moreover, the more severe thermal-induced decrease in the developmental competence of winter oocytes, in comparison to the summer oocytes, might also be related to the higher rate of apoptosis in these oocytes. Apoptosis might be involved in the thermal-induced nuclear maturation failures, as it has been shown that the effects of heat shock on nuclear maturation can be blocked by the inhibition of apoptosis (Roth and Hansen 2004a, b). Therefore, the lower changes in the nuclear maturation parameters of summer thermal-stressed oocytes likely were related to their lower apoptosis rate.

In conclusion, the developmental competence of ovine oocytes was lower in summer than in winter. However, it seemed that summer oocytes were more resistant to the in vitro thermal stress during IVM period compared with winter oocytes.

Acknowledgements The authors would like to thank the Research Institute of Animal Embryo technology (Shahrekord University, Shahrekord, Iran) for technical and financial supports.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

Abdoon, A.S., Gabler, C., Holder, C., Kandil, O.M. and Einspanier, R., 2014. Seasonal variations in developmental competence and relative abundance of gene transcripts in buffalo (*Bubalus bubalis*) oocytes, Theriogenology, 82, 1055–1067

Al-Katanani, Y.M., Paula-Lopes, F.F. and Hansen, P.J., 2002. Effect of season and exposure to heat stress on oocyte competence in Holstein cows, J Dairy Sci, 85, 390–396

Beere, H.M. and Green, D.R., 2001. Stress management - heat shock protein-70 and the regulation of apoptosis, Trends in cell biology, 11, 6–10

Bell, A.W., McBride, B.W., Slepetis, R., Early, R.J. and Currie, W.B., 1989. Chronic Heat Stress and Prenatal Development in Sheep: I. Conceptus Growth and Maternal Plasma Hormones and Metabolites, Journal of Animal Science, 67, 3289–3299

Bertoldo, M., Holyoake, P.K., Evans, G. and Grupen, C.G., 2010. Oocyte developmental competence is reduced in sows during the seasonal infertility period, Reprod Fertil Dev, 22, 1222–1229

Casu, S., Cappai, P. and Naitana, S., 1990. Effects of high temperatures on reproduction in small ruminants In: B. Ronchi, A. Nardone and J.G. Boyazoglu (eds), International Symposium on Animal Husbandry in Warm Climates, 1990, (EAAP Publication, Viterbo, Italy), 103–111

de S. Torres-Júnior, J.R., de F.A. Pires, M., de Sá, W.F., de M. Ferreira, A., Viana, J.H., Camargo, L.S., Ramos, A.A., Folhadella, I.M., Polisseni, J., de Freitas, C., Clemente, C.A., de Sá Filho, M.F., Paula-Lopes, F.F., Baruselli, P.S., 2008. Effect of maternal heat-stress on follicular growth and oocyte competence in Bos indicus cattle, Theriogenology, 69, 155–166

Di Francesco, S., Boccia, L., Campanile, G., Di Palo, R., Vecchio, D., Neglia, G., Zicarelli, L. and Gasparrini, B., 2011. The effect of season on oocyte quality and developmental competence in Italian Mediterranean buffaloes (*Bubalus bubalis*), Animal Reproduction Science, 123, 48–53

Dutt, R.H., 1964. Detrimental effects of high ambient temperature on fertility and early embryo survival in sheep, Int J Biometeorol, 8, 47–56

Gendelman, M. and Roth, Z., 2012. Seasonal Effect on Germinal Vesicle-Stage Bovine Oocytes Is Further Expressed by Alterations in Transcript Levels in the Developing Embryos Associated with Reduced Developmental Competence, Biology of Reproduction, 86, 1–9

Hansen, P.J., 2009. Effects of heat stress on mammalian reproduction, Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 364, 3341–3350

Hill, T.G. and Alliston, C.W., 1981. Effects of thermal stress on plasma concentrations of luteinizing hormone, progesterone, prolactin and testosterone in the cycling ewe, Theriogenology, 15, 201–209

Kalo, D. and Roth, Z., 2011. Involvement of the sphingolipid ceramide in heat-shock-induced apoptosis of bovine oocytes, Reprod Fertil Dev, 23, 876–888

Kleemann, D.O. and Walker, S.K., 2005. Fertility in South Australian commercial Merino flocks: relationships between reproductive traits and environmental cues, Theriogenology, 63, 2416–2433

Marai, I.F.M., El-Darawany, A.A., Fadiel, A. and Abdel-Hafez, M.A.M., 2007. Physiological traits as affected by heat stress in sheep—A review, Small Ruminant Research, 71, 1–12

Maya-Soriano, M.J., Lopez-Gatius, F., Andreu-Vazquez, C. and Lopez-Bejar, M., 2013. Bovine oocytes show a higher tolerance to heat shock in the warm compared with the cold season of the year, Theriogenology, 79, 299–305

Mosser, D.D., Caron, A.W., Bourget, L., Denis-Larose, C. and Massie, B., 1997. Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis, Molecular and cellular biology, 17, 5317–5327

Naqvi, S.M.K., Maurya, V.P., Gulyani, R., Joshi, A. and Mittal, J.P., 2004. The effect of thermal stress on superovulatory response and embryo production in Bharat Merino ewes, Small Ruminant Research, 55, 57–63

Paes, V.M., Vieira, L.A., Correia, H.H.V., Sa, N.A.R., Moura, A.A.A., Sales, A.D., Rodrigues, A.P.R., Magalhaes-Padilha, D.M., Santos, F.W., Apgar, G.A., Campello, C.C., Camargo, L.S.A. and Figueiredo, J.R., 2016. Effect of heat stress on the survival and development of in vitro cultured bovine preantral follicles and on in vitro maturation of cumulus-oocyte complex, Theriogenology, 86, 994–1003

Paula-Lopes, F.F. and Hansen, P.J., 2002. Heat shock-induced apoptosis in preimplantation bovine embryos is a developmentally regulated phenomenon, Biology of Reproduction, 66, 1169–1177

Pribenszky, C., Vajta, G., Molnar, M., Du, Y., Lin, L., Bolund, L. and Yovich, J., 2010. Stress for stress tolerance? A fundamentally new approach in mammalian embryology, Biology of Reproduction, 83, 690–697

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. and Pounds, J.A., 2003. Fingerprints of global warming on wild animals and plants, Nature, 421, 57–60

Roth, Z., 2017. Effect of Heat Stress on Reproduction in Dairy Cows: Insights into the Cellular and Molecular Responses of the Oocyte, Annu Rev Anim Biosci, 5, 151–170

Roth, Z. and Hansen, P.J., 2004a. Involvement of apoptosis in disruption of developmental competence of bovine oocytes by heat shock during maturation, Biology of Reproduction, 71, 1898–1906

Roth, Z. and Hansen, P.J., 2004b. Sphingosine 1-phosphate protects bovine oocytes from heat shock during maturation, Biology of Reproduction, 71, 2072–2078

Roth, Z., Meidan, R., Braw-Tal, R. and Wolfenson, D., 2000. Immediate and delayed effects of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows, J. Reprod. Fertil., 120, 83–90

Roth, Z., Meidan, R., Shaham-Albalancy, A., Braw-Tal, R. and Wolfenson, D., 2001. Delayed effect of heat stress on steroid production in medium-sized and preovulatory bovine follicles, Reproduction, 121, 745–751

Roth, Z., Bor, A., Braw-Tal, R. and Wolfenson, D., 2004. Carry-over effect of summer thermal stress on characteristics of the preovulatory follicle of lactating cows, Journal of Thermal Biology, 29, 681–685

Sawyer, G., 1979. The influence of radiant heat load on reproduction in the Merino ewe. II. The relative effects of heating before and after insemination, Australian Journal of Agricultural Research, 30, 1143–1149

Shirazi, A., Shams-Esfandabadi, N., Ahmadi, E. and Heidari, B., 2010. Effects of growth hormone on nuclear maturation of ovine oocytes and subsequent embryo development, Reproduction in Domestic Animals, 45, 530–536

Shokrollahi, E., Barati, F. and Gooraninejad, S., 2014. Efficacy of Histoprep gradient for isolating ovine epididymal sperm, Small Ruminant Research, 119, 96–99

Takuma, T., Sakai, S., Ezoe, D., Ichimaru, H., Jinnouchi, T., Kaedei, Y., Nagai, T. and Otoi, T., 2010. Effects of season and reproductive phase on the quality, quantity and developmental competence of oocytes aspirated from Japanese black cows, J. Reprod. Dev., 56, 55–59

Trout, J.P., McDowell, L.R. and Hansen, P.J., 1998. Characteristics of the estrous cycle and antioxidant status of lactating Holstein cows exposed to heat stress, J Dairy Sci, 81, 1244–1250

Tseng, J.K., Tang, P.C. and Ju, J.C., 2006. In vitro thermal stress induces apoptosis and reduces development of porcine parthenotes, Theriogenology, 66, 1073–1082

Zeron, Y., Ocheretny, A., Kedar, O., Borochov, A., Sklan, D. and Arav, A., 2001. Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles, Reproduction, 121, 447–454