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Original article

Maturation of bovine oocytes under low culture temperature decreased glutathione peroxidase activity of both oocytes and blastocysts

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Abstract

It is known that the basic variable in the cellular environment is temperature and low temperature decreases cellular metabolism rate. Also, low cellular metabolic activity reduces oxidative stress, resulting in low ROS production. The aim of this study was therefore to investigate the effect of 36.5°C (low) and 38.5°C (conventional) incubation temperatures during IVM on glutathione peroxidase activity of oocytes and blastocysts following fertilization. Bovine oocytes were matured in medium-199 for 22 hours at either 36.5°C or 38.5°C and they were subjected to in vitro fertilization (IVF). Putative zygotes were then transferred randomly into SOFaa embryo culture media with or without antioxidant (a mixture of GSH and SOD) until development to the blastocyst stage. Glutathione peroxidase enzyme (GSH-Px) activity was lower ($p<0.05$) in oocytes matured at low temperature than those of conventional temperature. Similarly, GSH-Px activity was lower ($p<0.05$) in blastocysts, which were obtained from oocytes matured at low temperature and cultured in antioxidants-supplemented embryo media. The GSH-Px activity of blastocysts, obtained from oocytes matured in low temperature, cultured in antioxidants-free embryo media was similar to blastocysts obtained from oocytes matured in conventional temperature, cultured in antioxidants-supplemented embryo media. The results of the present study show that decreasing the in vitro maturation temperature decreases antioxidant enzyme activity in both oocyte and blastocyst. Additionally, maturation of bovine oocytes at 36.5°C incubation temperature may provide an optimal thermal condition for the enzymatic antioxidant system of both oocytes and blastocyst.

Key words: bovine, temperature, antioxidant activity, oxidative stress, oocytes, blastocysts

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Introduction

For a long time, in vitro embryo production experiments have been carried out in cattle, but this reproductive biotechnology technique is not yet satisfactory to obtain sufficient quantity of good quality transferable embryos (Pfeifer et al. 2008). In vitro production of bovine embryo is affected by many factors (Camargo et al. 2006), but culture conditions of oocyte during in vitro maturation is the most important factor for healthy embryo production (Cetica et al. 2001). Various approaches (Cevik et al. 2011, Kocyigit et al. 2015, Sen and Kuran 2018) have been employed to improve maturation and developmental competence of bovine oocytes following in vitro fertilization (IVF). Improved in vitro maturation (IVM) competence in bovine oocytes has been subjected in many investigations by supplementation such as follicular fluid, co-culture (Moulavi et al. 2006), growth factors or gonadotropic hormones into IVM media (Cevik et al. 2011). However, developmental competence of bovine oocytes not only depends on the composition of the culture medium but also incubation conditions such as gases tension, humidity and temperature (Leese et al. 2008). IVM of bovine oocytes is performed at 38°C to 39°C, as this temperature is close to the rectal temperature in cattle (Shi et al. 1998). However, previous studies reported that the temperature in pre-ovulatory follicles is 1.5 to 2°C cooler than their adjacent stroma in cattle and pig (Grondahl et al. 1996, Hunter et al. 1997, Hunter et al. 2006). The existence of follicular cooling raises the question of whether oocytes develop advantageously at lower temperatures. Reduced temperature may be required for successful oogenesis or oocyte maturation, or for subsequent embryonic or fetal development (Hunter et al. 2006).

Intra cellular ATP production is realized as a result of oxidative metabolism, but reactive oxygen species (ROS), as one undesired by-product, are also produced (Luvoni et al. 1996). The amount of ROS production can vary depending on oxidative metabolism rate and high metabolic activity increases amount of ROS production (Sturmey et al. 2009). High amounts of produced ROS cause cellular damage such as inactivation of cellular enzymes, membrane lipid peroxidation and DNA damages in embryo cells (Halliwell and Gutteridge, 1998). Oxidative modifications of cell components via ROS are one of the most potentially damaging processes for proper cell function (Luvoni et al. 1996). The presence of molecules and cellular damage can trigger cell death in embryo and damage drives an increase in nutrient turnover to fuel repair processes or initiate apoptosis (Leese et al. 2008).

The role of ROS in IVM and its influence on later embryonic development is controversial (Cetica et al. 2001). A physiological level of ROS may be indicative of healthy developing oocytes (Combelles et al. 2009), but the presence of excessive ROS can contribute to the meiotic arrest of the oocyte (Cetica et al. 2001) and embryonic developmental arrest, cell death and apoptosis (Leese et al. 2008). In vitro embryo production systems have developed mechanisms to control ROS levels, including enzymatic (superoxide dismutase, glutathione peroxidase, catalase) and non-enzymatic (α-tocopherol, ascorbic acid, β-carotene, and glutathione) antioxidant agents (Camargo et al. 2006). Superoxide anion and hydroxyl radical activity in embryonic cells is scavenged by endogenous superoxide dismutase to superoxide anion, and endogenous glutathione peroxidase and catalase transform hydrogen peroxide into water and oxygen in vitro (Luvoni et al. 1996, Cetica et al. 2001).

The extent of ROS damage to cell systems is dependent on the balance between their production and removal rates (Halliwell and Gutteridge 1998). Generation of ROS is an inevitable consequence of oxidative reactions, but various antioxidant agents remove radical species before they can cause significant damage in in vitro embryo production systems. However, if metabolic activity of in vitro-produced embryos can be reduced, oxidative metabolism may be decreased, resulting in low production of ROS. Thus, in vitro-produced embryos may not need the addition of antioxidant agents against ROS in culture media. Also, these embryos attempt to scavenge ROS with its antioxidant mechanisms. Probably, establishment of in vitro environmental conditions, which will not increase metabolism of embryo, may eliminate necessity of supplemental antioxidant defense systems against ROS in culture media in vitro. It was hypothesized that IVM at a temperature that mimics the thermal environment of the bovine pre-ovulatory follicles in vivo may provide a better culture condition for optimum metabolic activity and for post-fertilization development of oocytes. The aim of this study was therefore to investigate the effect of low (36.5°C) and conventional (38.5°C) incubation temperatures during IVM on glutathione peroxidase enzyme (GSH-Px) activity of oocytes and embryos cultured in SOFaa media with or without antioxidant.

Materials and Methods

All chemicals and media used in this study were purchased from Sigma-Aldrich, Turkey, except where otherwise indicated.

Collection and in vitro maturation of oocytes

Bovine ovaries were collected from a local slaughterhouse and transported to the laboratory in 0.9% NaCl (w/v) containing 0.1% v/v antibiotic-antimycotic solution (including 10.000 IU penicillin, 10 mg streptomycin and 25 µg amphotericin B in per ml) at 32.8±1.4°C within 3 h after slaughter. Cumulus-oocyte complexes (COCs) were aspirated with an 18 gauge needle fixed to a 10 ml syringe from follicles 2 to 8 mm in diameter. COCs were collected in 3-4 ml of HEPES-buffered medium-199 (Sigma, M7528) containing Earle's salts and supplemented with 1% v/v antibiotic-antimycotic solution, 100 µg/ml L-glutamine and 5% v/v heat-inactivated fetal calf serum (FCS). COCs were assessed morphologically and only oocytes with compact, intact cumulus cells around and homogeneous cytoplasm were selected for maturation.

COCs were then washed three times in HEPES-buffered medium-199 and twice in maturation medium. Maturation medium was prepared as reported by Cevik et al. (2011). Maturation medium was sodium bicarbonate-buffered medium-199 (Sigma, M4530) containing Earle's salts and L-glutamine supplemented with 5.5 µg/ml sodium pyruvate, 1% v/v antibiotic-antimycotic solution, 10% v/v heat-inactivated FCS, 5.0 µg/ml LH, 0.5 µg/ml FSH and 10 ng/ml EGF. COCs were separately placed in 500 µl of maturation medium (approximately 25-35 COCs per well) covered with 300 µl mineral oil in four-well dishes (Nunc, Roskilde, Denmark) and matured for 22 hour with humidified 5% CO_2 in air at 36.5°C (low) or 38.5°C (conventional) incubation temperatures. At the end of maturation period 50 % of COCs were completely denuded of cumulus cells by vortexing and first polar body of oocytes was evaluated. In vitro matured denuded oocytes were washed 3 times in Ca^{2+} and Mg^{2+} free PBS supplemented with 1 mg/ml polyvinyl alcohol and were stored in microtubes (approximately 25 / 10 µl) at -80°C until enzyme activity analysis of GSH-Px.

Spermatozoa preparation and IVF

IVF of matured bovine oocytes was performed as described by Sen (2015). Following maturation culture, the rest of COCs were washed twice in HEPES--buffered medium 199 and then twice in IVF medium. The IVF medium was glucose-free modified TALP supplemented with 25 mM bicarbonate, 22 mM Na-lactate, 1 mM Na-pyruvate, 6 mg/ml fatty acid-free BSA, 10 mg/ml heparin-sodium salt and 0.5 µl/ml antibiotic-antimycotic solution (adjusted pH 7.4 and 280-300 mOsm/kg). COCs were transferred into 48 µl fertilization drops (approximately 15 COCs per drop) covered with mineral oil. Frozen-thawed semen from a single bull was used for the fertilization of oocytes. Percoll density gradient system was used for the separation of the motile fraction of the frozen-thawed semen as described by Sen and Kuran (2018). The sperm concentration was counted in hemocytometer using a phase-contrast microscope at 400× magnification. Sperm was then diluted to 50×10^6/ml spermatozoa with fertilization medium. The sperm motility was visually checked for acceptable motility (at least 80% progressively motile). The oocytes were fertilized with 2 µl diluted semen per fertilization drops for 22 h in a humidified atmosphere of 5% CO_2 in air at 38.5°C.

In vitro culture

Following IVF, the putative zygotes were washed three times in HEPES-buffered medium-199 and they were vortexed 5 min to remove cumulus cells layer. The denuded zygotes were washed twice in HEPES-buffered medium-199 and then twice in synthetic oviduct fluid (SOF) embryo culture media. The SOF embryo culture media was supplemented with 40 µg/ml sodium pyruvate, 8 mg/ml fatty acid-free BSA, 20 µl/ml MEM non-essential amino acids solution (100×), 10 µl/ml BME Amino Acids Solution (50×) and 0.5 µl/ml antibiotic-antimycotic solution on the day of use. The zygotes were placed in 50 µl drops (approximately 15 zygotes per drop) of SOFaa embryo culture media with or without antioxidant (a mixture of 1mM GSH and 1500 IU SOD) under mineral oil and cultured in a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2 in air at 38.5°C. İn vitro fertilization was considered as 0 day. Zygotes cleaved were recorded on day 3 of development. Morula and blastocyst development were evaluated on days 5 and 8, respectively. Embryos developed to blastocyst were washed 3 times in Ca^{2+} and Mg^{2+} free PBS supplemented with 1 mg/ml polyvinyl alcohol and were stored in microtubes (approximately 5 blastocyst/10 µl) at -80°C until enzyme activity analysis of GSH-Px.

Determination of GSH-Px Activity

The freezing-thawing oocytes and blastocyst were sonicated at 50W for 2 minutes for enzymatic extracts. They were then centrifuged at 4°C and 10,000 g for 20 min. Supernatants were used to determine enzymatic activity. GSH-Px activity of oocytes and blastocyst was determined spectrophotometrically using a commercial sensitive kit (GSH-Px Assay, Northwest Life Science Specialties, LLC, and Vancouver, WA USA) as suggested by the manufacturer, which was based on the method of Paglia and Valentine (1967) by coupling the oxidation of glutathione and nicotine adenosine dinucleotide phosphate (NADPH) using glutathione reductase.

Table 1. Developmental competence of bovine embryos obtained from oocytes maturated at low (36.5°C) or conventional (38.5°C) incubation temperatures.

IVM condition (°C)	Antioxidants*	Developmental competence of bovine embryos (%)			
		Putative zygotes	Cleavage	Morula	Blastocyst
36.5	–	116	69.4 ± 2.02	38.7 ± 2.60	21.6 ± 3.47[b]
	+	83	71.3 ± 2.06	40.2 ± 5.49	30.1 ± 4.68[a]
38.5	–	115	70.7 ± 2.46	39.6 ± 2.83	21.7 ± 1.89[b]
	+	85	72.0 ± 2.45	41.7 ± 2.67	29.4 ± 3.66[a]

[a,b] – Different letters in the same row indicate significant difference (P<0.05).
* a mixture of 1mM glutathione and 1500 IU superoxide dismutase, IVM – in vitro maturation.

In this protocol, GSH-Px catalyzes the reduction of hydrogen peroxide (H_2O_2), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase (GR) and B-nicotinamide adenine dinucleotide phosphate (NADPH) forming NADP+ (resulting in decreased absorbance at 340 nm) and recycling the GSH. Because GSH-Px is limiting, the decrease in absorbance at 340 nm is directly proportional to the GSH-Px activity. One unit of enzyme activity was defined as 1 mmol of NADPH oxidized/min at room temperature. Specific activities were expressed as mmol NADPH oxidized/min/mg protein or units/mg protein. The analysis for each sample was duplicated.

Statistical Analysis

Effect of temperature during IVM and antioxidants in embryo culture on developmental competence of embryos was analyzed after arcsine-transformation using the General Linear Model (GLM) of the SPSS package program. Significant differences between GSH-Px activity in oocytes and blastocysts were tested using Duncan's test. The results are presented as untransformed mean ± SE values for developmental competence of embryos, and statistical significance was determined at the level of 0.05.

Results

Developmental competence of bovine embryos obtained from oocytes matured at low or conventional incubation temperatures and cultured in SOFaa with or without antioxidants following fertilization are presented in Table 1. There were no significant differences between embryos obtained from oocytes matured at different incubation temperatures and cultured in embryo culture media with or without antioxidants in terms of cleavage and morulae rates. Also, percentage of these embryos developed to blastocyst stage was similar in both oocytes maturation culture temperatures, but supplementation of antioxidants into embryo culture media increased ($p<0.05$) percentage of embryos developed to blastocyst stage in both oocytes maturation culture temperatures.

The GSH-Px activity of in vitro matured bovine oocytes, cultured at low or conventional incubation temperatures, and its blastocysts, obtained following IVF are present in Figs. 1 and 2, respectively. Bovine oocytes matured at low incubation temperature had significantly lower GSH-Px activity than those of oocytes matured at conventional incubation temperature ($p<0.05$). GSH-Px activity of blastocysts obtained from oocytes matured at low incubation temperature was significantly lower ($p<0.05$) than those of oocytes matured at conventional incubation temperature. Blastocysts from oocytes matured conventional incubation temperature and cultured in antioxidants-free embryo media have shown higher GSH-Px activity ($p<0.05$). Additionally, supplementation of antioxidants into embryo culture media decreased ($p<0.05$) GSH-Px activity in blastocysts obtained from oocytes matured at both incubation temperatures. However, antioxidants were more effective in reducing GSH-Px activity in blastocysts from oocytes matured at low incubation temperature compared to blastocysts from oocytes matured at conventional incubation temperature ($p<0.05$). Interestingly, GSH-Px activity of blastocysts obtained from oocytes matured at low temperature and cultured in antioxidants-free embryo media were similar with blastocysts obtained from oocytes matured at conventional temperature and cultured in antioxidants-supplemented embryo media.

Discussion

The present study demonstrates that application of low (36.5°C) incubation temperature, which is representative to the in vivo pre-ovulatory follicle temperature (Grondahl et al. 1996), during in vitro maturation

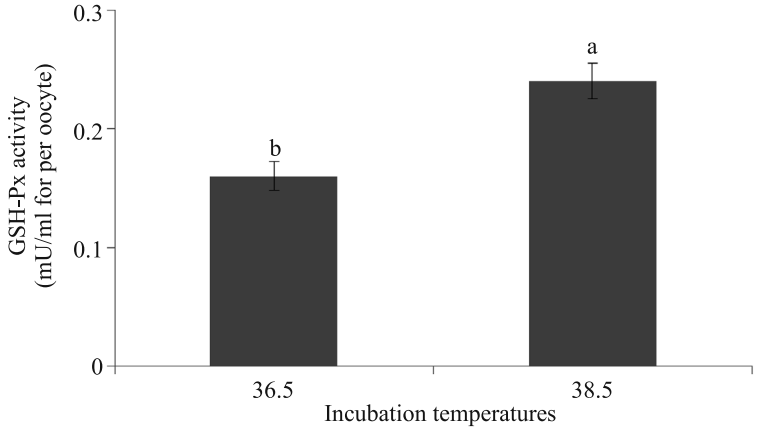

Fig. 1. Glutathione peroxidase (GSH-Px) activity of in vitro matured bovine oocytes at low (36.5°C) or conventional (38.5°C) incubation temperatures. The error bars represents the standard error of means and bars with different letters are significantly different at $p<0.05$.

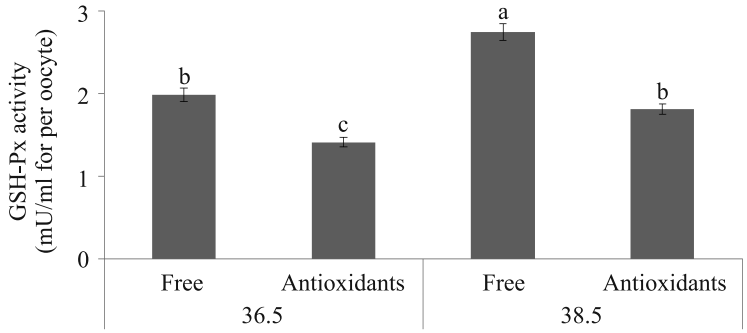

Fig. 2. Glutathione peroxidase enzyme (GSH-Px) activity of blastocysts obtained from in vitro matured bovine oocytes at low (36.5°C) or conventional (38.5°C) temperatures and cultured with antioxidants or antioxidants-free embryo culture media after fertilization. The error bars represents the standard error of means and bars with different letters are significantly different at $p<0.05$.

of bovine oocytes decreased GSH-Px activity oocytes and its blastocysts following fertilization in vitro. Moreover, the culture of bovine oocytes in low incubation temperature during in vitro maturation may eliminate need to antioxidant supplementation in embryo culture media following IVF due to similar GSH-Px activity of its blastocysts obtained from conventional incubation temperature and antioxidant-doped embryo culture media.

The environmental conditions of in vitro production of bovine embryos are basic factors determining developmental competence and viability of embryo (Camargo et al. 2006). Commercial in vitro maturation of bovine oocytes is maintained at 38°C to 39°C culture temperatures, which is close to the core body temperature in cattle (Shi et al. 1998). Previous studies reported that pre-ovulatory follicles are approximately 1.5 to 2.0°C cooler than their adjacent stroma in pigs (Hunter et al. 2006) and cattle (Grondahl et al. 1996). Hunter et al. (1997) suggested that one approach to the contribution of temperature in in vivo maturation of the oocyte would be to undertake in vitro studies at temperatures lower than those conventionally used. Therefore, in the present study we chose oocyte maturation culture temperature of 36.5°C as it was temperature recorded in pre-ovulatory follicles in cattle (Grondahl et al. 1996). Previous studies showed that low (36.5°C or 37.0°C) or conventional (38.5°C or 39.5°C) incubation temperature during IVM had similar effect on embryonic development until development to the blastocyst stage in different farm animal species such as cattle (Shi et al. 1998, Sen and Kuran 2018), buffalo (Ravindranatha et al. 2003) and pig (Ye et al. 2007). In the present study, embryos from oocytes matured at either the pre-ovulatory follicle (low; 36.5°C) or conventional incubation temperatures (body temperature of cattle; 38.5°C) showed a similar development rate until the blastocyst stage. However, antioxidant supplementation (a mixture of GSH and SOD) increased ratio of embryos developed to blastocyst, which was obtained from oocytes matured at both incubation temperatures. These results show that different maturation culture temperatures did not affect the development of embryos to the blastocyst stage after fertilization, but the supplementation of antioxidants to the embryo culture increased the rate of embryos reaching the blastocyst stage. Similarly, Luvoni et al. (1996), Uysal et al. (2003) and Sen and Kuran (2018) reported that GSH

and SOD supplementation improve embryo development rate until the blastocyst stage.

The increase or decrease in the rate of a biological or chemical system in response to a 10°C change in temperature, which is defined as the Q10 temperature coefficient, is between 2- and 3-fold. Therefore, incubation temperature applied to oocyte maturation, fertilization, and embryo in vitro cultures may affect oocyte and embryo metabolism rate. In this context, McEvoy et al. (2000) suggested that commercial in vitro maturation technology is using an incubation temperature, which doesn't mimic the thermal condition of pre-ovulatory follicles in vivo. This is why conventional incubation temperature applied during in vitro oocytes maturation has been set to reflect the core body temperature. However, it is known that the temperature of the maturing oocyte's follicular environment is 2-3°C lower than the stroma of the ovary (Grondahl et al. 1996, Hunter et al. 1997, Hunter et al. 2006). There is no data on the direct effects of conventional culture temperature (core body temperature) on oocyte and embryo metabolic activity and antioxidant mechanisms of in vitro bovine embryo production systems. However, in the present study, maturation of oocytes at low (36.5°C) incubation temperature decreased GSH-Px activity, contrarily conventional (38.5°C) incubation temperature increased GSH-Px activity. These results may indicated that low incubation temperature (38.5°C), which represents pre-ovulatory follicles temperature, decrease oxidative metabolism due to low metabolic activity of bovine oocytes. GSH-Px converts ROS such as superoxide anion and hydroxyl radical into water and molecular form of oxygen (Luvoni et al. 1996, Cetica et al. 2001). Especially, in mammalian cells it plays a critical role to protect them from oxidative stress (Misra and Fridovich 1972). The antioxidant defense system of the cell may be affected due to changes in GSH-Px activity as well as have an impact on the cell's metabolic activity (Gudmundsdóttir et al. 2008). Moreover, low activity of GSH-Px can increase the amount of reduced gluthatione and allow a rise in ROS concentrations and oxidative stress (Yanar et al. 2011). It is known that the enzymatic antioxidant GSH-Px is starting to be transcribed from the early embryonic stage to blastocyst stage (Lee et al. 2001). GSH-Px mRNA was shown to be expressed highly from blastocyst stage (Harvey et al. 1995). Unfortunately, observations regarding the production level of ROS, metabolic activity level, and gene expression level of GSH-Px were examined in neither oocytes nor blastocysts in the present study, but it can be expected that data on GSH-Px activity would correspond to a given situation, because of the low GSH-Px activity in oocytes, which were matured in low incubation temperature, and blastocyst derived from it, indicating that ROS production would have decreased. Moreover, low incubation temperature during maturation culture may decrease metabolic activity of oocytes and in subsequent development stage following fertilization and low metabolic activity of oocytes or embryos develop to blastocyst stage may lead to drop in ROS production and it may cause decrease in GSH-Px activity in both the maturation and embryonic development process. Hence, oocyte and early embryo function at a lower temperature could encourage the expression of a low metabolism than core body temperature in in vitro (Leese et al. 2008).

In conclusion, bovine oocytes have completed maturation process when cultured at 36.5°C and they have shown similar degree of developmental competence to the blastocyst stage following IVF compared to counterparts cultured at conventional (38.5°C) temperature. Moreover, bovine oocytes matured at low incubation temperature exhibited low glutathione peroxidase enzyme activity. Similarly, blastocysts obtained from oocytes matured at low incubation temperature had low glutathione peroxidase enzyme activity. These results may suggest that application of pre-ovulatory follicle temperature during in vitro oocyte maturation may provide better thermal conditions for the enzymatic antioxidant system of both oocytes and blastocyst after fertilization.

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References

Camargo LS, Viana JH, Sa WF, Ferreira AM, Ramos AA, Vale Filho VR (**2006**) Factors influencing in vitro embryo production. Anim Reprod 3: 19-28.

Cetica PD, Pintos LN, Dalvit GC, Beconi MT (**2001**) Antioxidant enzyme activity and oxidative stress in bovine oocyte in vitro maturation. IUBMB Life 51: 57-64.

Cevik M, Sen U, Kocyigit A, Soydan E, Kuran M (**2011**) Effects of serum, gonadotropins, epidermal growth factor and estradiol 17-beta on cumulus expansion and nuclear maturation of bovine oocytes. Kafkas Univ Vet Fak Derg 17: 1009-1014.

Combelles CM, Gupta S, Agarwal A (**2009**) Could oxidative stress influence the in-vitro maturation of oocytes? Reprod Biomed Online 18: 864-880.

Grondahl C, Greve T, Schmidt M, Hunter RH (**1996**) Bovine pre-ovulatory follicles are cooler than ovarian stroma and deep rectal temperature. Theriogenology 45: 289.

Gudmundsdóttir KB, Kristinsson J, Sigurdarson S, Eiríksson T, Jóhannesson T (**2008**) Glutathione peroxidase (GPX)

activity in blood of ewes on farms in different scrapie categories in Iceland. Acta Vet Scand 50: 23.

Halliwell B, Gutteridge JM (**1988**) Free radicals and antioxidant protection: mechanisms and significance in toxicology and disease. Hum Toxicol 7: 7-13.

Harvey MB, Arcellana-Panlilio MY, Zhang X, Schultz GA, Watson AJ (**1995**) Expression of genes encoding antioxidant enzymes in preimplantation mouse and cow embryos and primary bovine oviduct cultures employed for embryo coculture. Biol Reprod 53: 532-540.

Hunter RH, Einer-Jensen N, Greve T (**2006**) Presence and significance of temperature gradients among different ovarian tissues. Microsc Res Tech 69: 501-507.

Hunter RH, Grondahl C, Greve T, Schmidt M (**1997**) Graafian follicles are cooler than neighbouring ovarian tissues and deep rectal temperatures. Hum Reprod 12: 95-100.

Kocyigit A, Cevik M, Sen U, Kuran M (**2015**) The effect of macromolecule and growth factors combinations on in vitro development of bovine embryos. Turk J Vet Anim Sci 39: 308-313.

Lee JH, Park JH, Choi KM, Im KS, Jin DI (**2001**) Improvement of in vitro development of bovine embryos in a medium containing selenium. Asian-Aust J Anim Sci 4: 170-173.

Leese HJ, Baumann CG, Brison DR, McEvoy TG, Sturmey RG (**2008**) Metabolism of the viable mammalian embryo: quietness revisited. Mol Hum Reprod 14: 667-672.

Luvoni GC, Keskintepe L, Brackett BG (**1996**) Improvement in bovine embryo production in vitro by glutathione-containing culture media. Mol Reprod Develop 43: 437-443.

McEvoy TG, Sinclair KD, Young LE, Wilmut I, Robinson JJ (**2000**) Large offspring syndrome and other consequences of ruminant embryo culture in vitro: relevance to blastocyst culture in human ART. Hum Fertil 3: 238-246.

Misra HP, Fridovich I (**1972**) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247: 3170-3175.

Moulavi F, Hosseini SM, Ashtiani SK, Shahverdi A, Nasr-Esfahani MH (**2006**) Can Vero cell co-culture improve in-vitro maturation of bovine oocytes? Reprod BioMed Online 13: 404-411.

Paglia DE, Valentine WN **(1967)** Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70: 158-169.

Pfeifer LF, Schneider A, Correa MN (**2008**) Factors that affect the in vitro production of bovine embryos: A review. Rev Colom Cienc Pecua 21: 109-120.

Ravindranatha BM, Nandi S, Raghu HM, Reddy SM (**2003**) In vitro maturation and fertilization of buffalo oocytes: effects of storage of ovaries, IVM temperatures, storage of processed sperm and fertilization media. Reprod Domest Anim 38: 21-26.

Sen U (**2015**) Effects of sperm from different bulls on developmental competence, blastosist quality and cell number of bovine embryos in vitro. Kafkas Univ Vet Fak Derg 21: 339-344.

Sen U, Kuran, M (**2018**) Low incubation temperature successfully supports the in vitro bovine oocyte maturation and subsequent development of embryos. Asian-Aust J Anim Sci 31: 827-834.

Shi DS, Avery B, Greve T (**1998**) Effects of temperature gradients on in vitro maturation of bovine oocytes. Theriogenology 50: 667-674.

Sturmey RG, Hawkhead JA, Barker EA, Leese HJ (**2009**) DNA damage and metabolic activity in the preimplantation embryo. Hum Reprod 24: 81-91.

Uysal O, Akçay E, Tekin N (**2003**) Effects of glutathione and superoxide dismutase on in vitro maturation, fertilization and culture of bovine oocytes. Indian Vet J 80: 758-762.

Yanar K, Aydın S, Cakatay U, Mengi M, Buyukpınarbaşılı N, Atukeren P, Sitar ME, Sönmez A, Uslu E (**2011**) Protein and DNA oxidation in different anatomic regions of rat brain in a mimetic ageing model. Basic Clin Pharmacol Toxicol 109: 423-433.

Ye J, Coleman J, Hunter MG, Craigon J, Campbell KH, Luck MR (**2007**) Physiological temperature variants and culture media modify meiotic progression and developmental potential of pig oocytes in vitro. Reproduction 133: 877-886.