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The application of *in vitro* cattle embryo production system to study the influence of elevated temperature on oocyte maturation, fertilization and early embryonic development

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Abstract

Higher air temperature in summer causes a significant reduction in fertility in cattle. Increase in female body temperature during the period of reproduction by only 2°C, also known as hyperthermia, leads to disturbances in the functioning of the female reproductive system, oocytes maturation, fertilization and embryos development. Particularly sensitive to high temperatures are embryos in the first and second day after fertilization (thermosensitive), but just at third till fifth day after fertilization their resistance to thermal stress significantly increases. Morula-stage and blastocyst-stage bovine embryos are insensitive to elevated temperatures (thermoresistant). Most probably this is due to the increasing number of cells within the embryo and the capacity to activate defense mechanisms based on the synthesis of various factors providing resistance to high temperatures. These factors include heat shock protein 70 (HSP70), antioxidants such as glutathione, and IGF-1. One of the responses of the embryo to elevated temperature is the induction of apoptosis, which is associated with the activation of embryonic genome. Owing to the apoptosis, cells damaged by high temperature may be eliminated from the embryo, which increases their chance of survival. Precise examination of the mechanisms responsible for the development of thermotolerance of preimplantation bovine embryos will enable their protection from the consequences of elevated temperature. The aim of this review is to summarise experiments in which *in vitro* embryo production system was used to estimate the influence of elevated temperature on cattle fertility.

Key words: cattle, elevated temperature, embryos, oocytes, sperm, in vitro, HSP70, GSH, IGF-1, apoptosis

Introduction

Higher temperature has a negative impact on the reproduction of animals. This is important especially for domestic animals like cattle, sheep, goats or horses, which are more exposed to sun radiance and elevated temperature as compared to laboratory animals. Due to their physiology, cattle are especially sensitive to higher temperature (Hansen and Arechiga, 1999; García-Isperito et al., 2007; Roth et al., 2000). The optimal air temperature for cattle is approximately 20°C when the physiological body temperature ranges between 38.5 and 39°C. Elevation of the air temperature above 27°C causes elevation of body temperature to 40-42°C, which is defined as hyperthermia (García-Isperito et al., 2007). Hyperthermia in cattle leads to adverse changes in the functioning of their reproductive organs, course of reproductive cycle and pregnancy (Hansen and Arechiga, 1999).

The influence of elevated air temperature on bovine fertility *in vivo*

In vivo experiments indicate that the dysfunctions caused by elevated air temperature result in a considerable decrease in the number of pregnant females, from 50% in the winter to less than 20% in the summer (Roth et al., 2000). Elevated air temperature in the summer causes disturbances in the secretion of reproductive hormones, such as estrogens and progesterone, which leads to irregularities in follicle development (Wolfenson et al., 1995). Elevated air temperature also affects the duration and intensity of estrus (Zeron et al., 2001) in cows, and decreases blood flow in the uterus (Rivera and Hansen, 2001).

Elevation in the air temperature affects the quality of oocytes. These oocytes isolated from ovaries by ovum pick up (OPU) in the summer are of lower quality than

oocytes isolated in the winter and are less capable of undergoing fertilization and embryonic development (Rocha et al., 1998).

Early bovine embryos as well as oocytes are susceptible to elevated air temperature. Until the second day after fertilization, embryos are especially sensitive to thermal stress. Resistance of the embryos to elevated temperature increases significantly between the third and fifth day post fertilization (Ealy et al., 1993). Thus, bovine preimplantation embryos *in vivo*, like other farm animal embryos, become more resistant to thermal stress as the development proceeds (Putney et al., 1988; Dutt, 1963; Tompkins et al., 1967; Wolfenson and Blüm 1988). There are reports indicating that a higher air temperature in the summer disrupts oocyte maturation, fertilization and embryonic development. Molecular mechanisms underlying these processes could not be determined by *in vivo* experiments. Accordingly, for many years *in vitro* research have been conducted.

Experimental system for studying the influence of elevated temperature on oocyte maturation, fertilization and early development of bovine embryos

The *in vitro* production of the embryos seems to be a good system for studying the mechanisms associated with the influence of elevated temperature on animal reproduction. *In vitro* models of oocyte maturation, fertilization and development of bovine embryos (Duszewska et al., 2010) allow for the examination of the processes related to thermosensitivity and thermoresistance of gametes and embryos (García-Isperito et al., 2007; Zeron et al., 2001). In most *in vitro* experiments, stress was induced by increasing temperature of culture to 40.5-41 °C and the control temperature ranged between 38.5 and 39 °C.

In vitro oocyte maturation

In the *in vitro* experiments on thermal stress, both immature and mature oocytes, are used. Oocytes can be isolated in winters as well as in summers, from stimulated or nonstimulated cows. They can be isolated from live females by OPU method or from ovaries of slaughtered cows. In most *in vitro* experiments, immature oocytes arrested at the first prophase of meiotic division are used. Maturation of the oocytes, defined as the transition to the second metaphase of meiotic division, takes place during a 24-hour *in vitro* culture (Duszewska et al., 2010).

Maturation of mammalian oocytes is a complex process and comprises nuclear maturation and cytoplasmic maturation (Duszewska et al., 2010). Some of the authors claim that elevated temperature mostly disrupts nuclear maturation of the oocyte (Roth et al., 2005), while the others believe that it affects mainly cytoplasmic maturation (Dorado et al., 2001).

The entire oocyte maturation process depends on correct functioning of the cell cytoskeleton compound of microtubules and microfilaments (Gallicano, 2001; Shin and Kim, 2003; Duszewska et al., 2010). Microtubules and microfilaments are highly susceptible to elevated temperature (Coss and Linnemans, 1996). In somatic cells, in M phase of the cell cycle, elevated temperature leads to a disruption of mitotic spindle and in consequence to improper chromosome segregation to descendant cells, polyploidy and failure of cytokinesis (Roth and Hansen, 2005).

Roth and Hansen (Roth and Hansen, 2005) demonstrated that the changes in karyokinesis are the main causes of oocyte maturation disruption after thermal stress. The majority of oocytes exposed to elevated temperature (41 °C) were arrested at the first meiotic metaphase. It is possible that thermal stress has activated the meiotic spindle checkpoint, which is the mechanism responsible for correct chromosome segregation during anaphase (Brunet et al., 2003).

Activation of meiotic spindle checkpoint takes place when incorrect arrangement of chromosomes in metaphase plate or incorrect attachment of chromosome to the spindle is recognized, and consequently leads to anaphase delay (Zhou et al., 2002; Cleveland et al., 2003). Roth and Hansen (Roth and Hansen, 2005) also discovered meiotic spindle deformation, decrease in the amount of actin connected to the membrane, and simultaneous increase in the amount of actin dispersed in the cytoplasm.

Contrary to the previous authors, Dorado and Edwards (Dorado et al., 2001) found that elevated temperature mostly affected cytoplasmic maturation of the oocyte. They noticed that the percentage of oocytes that completed nuclear maturation was independent of the culture temperature. Moreover, the percentage of oocytes with disrupted chromatin was similar for oocytes cultured at elevated temperature (41 °C) and those cultured at the control temperature (38.5 °C).

In the experiments described by Roth and Hansen (Roth and Hansen, 2005), majority of the oocytes arrested by thermal stress underwent apoptosis due to inhibition of meiosis. Adverse changes in the cytoskeleton as a result of thermal stress possibly triggered apoptotic cascade in the oocyte. The adverse relation would be also possible, when actin microtubules and microfilaments were damaged as a result of apoptosis activation. These experiments indicate that apoptosis of oocytes cultured at elevated temperature is the main cause of the decrease in fertilization rate (Roth and Hansen, 2005).

A very important issue in context of thermal stress during oocyte maturation is the role of cumulus cells. Many *in vitro* experiments revealed that cumulus cells are necessary for correct oocyte growth and maturation. Loss of cumulus cells leads to a limited protein synthesis in the oocyte (Chian and Girard, 1995). Most probably, cumulus cells are also very important for the resistance of the oocyte to elevated temperature, because they partially limit the negative impact of thermal stress on protein synthesis in the oocyte (Chian and Girard, 1995). Cumulus cells are in permanent contact with the oocyte by gap junctions (Edwards and Hansen, 1997). Possibly through these gap junctions, cumulus cells supply to the oocyte some of the regulatory agents which can activate the mechanisms of thermoresistance within the oocyte. Itis also possible that cumulus cells deliver to the oocyte some molecules, for example glutathione (GSH), which can directly improve the resistance of the oocyte to elevated temperature.

After fertilization, zygotes lose cumulus cells in a natural manner. This can be the reason of significant thermosensitivity of zygotes and two-cell embryos which are not yet able to synthesize resistance agents such as GSH (Edwards and Hansen, 1997).

In vitro fertilization

In vitro fertilization process follows the same steps as *in vivo* (Duszewska et al., 2010). The first step of *in vitro* fertilization is spermatozoa capacitation, which enables spermatozoa penetration through the zona pellucida of the oocyte. Subsequently, the sperm fuses with the membrane of the oocyte and penetrates into the ooplasm. Sperm penetration is a factor stimulating the oocyte to meiosis termination (oocyte activation), which is manifested by second polar body extrusion and formation of female pronucleus (Yanagimachi, 1994). At the same time, nucleus of the sperm transforms into male pronucleus. Thereafter, DNA in every pronucleus replicates, followed by a fusion of pronuclei and formation of the nucleus of the zygote. The envelopes of both pronuclei disperse and chromosomes arrange into the metaphase plate, and subsequently first mitotic division of the zygote occurs (Gilbert, 2000).

Results of *in vitro* experiments held by Rivera and Hansen indicate that fertilization at elevated temperature (41°C) leads to a decrease in the number of fertilized oocytes and to disrupted development of the embryos. Elevated temperature also disturbs the fusion of the gametes and the course of first mitotic division of the zygote, mainly due to cytoskeleton disruption (Rivera and Hansen, 2001). Thus elevated temperature during *in vitro* fertilization limits not only the capacity of the zygote to divide but also the capacity of embryos to subsequent development (Rivera and Hansen, 2001).

In vitro culture of embryos

The last stage of *in vitro* production of bovine embryos is the culturing of embryos for 168 h, from the zygote until the blastocyst stage (Duszewska et al., 2010). After 168 h, embryos at various stages of development are recovered – from early morula through late morula and early blastocyst to late or even hatching blastocyst (Van Som and De Kruif, 1996). The very early stage of development of bovine embryos is similar to that of human embryos, and therefore cattle have been considered as a model species to study embryogenesis (Niemann and Wrenżycki, 2000). Bovine embryos cultured *in vitro* develop slower than embryos *in vivo*, because in the former the cell cycle between eight-cell and sixteen-cell stage embryos is longer (Barnes and Eyestone, 1990; Grisard et al., 1994).

In cattle, activation of the embryonic genome occurs at the eight-cell stage (Eystone and First, 1986). The maternal-embryonic control transition is associated with ultrastructural changes in the nucleus and nucleoli and with the protein synthesis based on expression of embryonic genes. However, the expression of some embryonic genes occurs much earlier in development, at the two-cell stage (Niemann and Wrenżycki, 2000). In the majority of mammals, one of the earliest genes expressed is heat shock protein 70 gene (*hsp 70*) (Edwards and Hansen, 1996).

Two-cell and four-cell stage bovine embryos are more sensitive to thermal stress than oocytes, morulas and blastocysts (Rivera et al., 2004). The mechanism of development disruption of two-cell and four-cell embryos by elevated temperature is unclear. One of hypotheses assumes that the main cause of disturbances of embryonic development following thermal stress is the increased production of free radicals (Flanagan et al., 1998). The culture of two-cell embryos at elevated temperature (41°C) and at two different oxygen concentrations (source of free radicals) did not confirm this hypothesis (Rivera and Hansen, 2001). Therefore, lower oxygen content does not decrease the negative influence of elevated temperature on embryo development.

Results of recent studies indicate that in early stages of cattle embryonic development mitochondria are more active than in the later stages (Tarazona et al., 2006). The presence of active mitochondria might enhance free radical production in two-cell and four-cell embryos as a consequence of thermal stress and make them highly thermosensitive (Hansen, 2007). It is also possible that inhibition of embryonic development by elevated temperature is caused by such changes inside the blastomeres that are impossible to overcome by embryo genome activation at the eight-cell stage (Rivera et al., 2004).

Contrary to two-cell and four-cell stage embryos, the development of morulas and early blastocysts is not disrupted by elevated temperature (41°C) and most of them form normal blastocysts on the seventh day of *in vitro* culture (Ealy et al., 1995; Edwards and Hansen, 1997). However, it does not mean that morulas and early blastocysts are fully resistant to elevated temperatures, since elevation of the *in vitro* culture temperature to 42°C resulted in the inhibition of the development of morulas and blastocysts (Ealy et al., 1995).

Interestingly, the embryo resistance to elevated temperature *in vitro* is sex related. At the moment of fertilization, the sex of the embryo is established, and cascades of reactions leading to the fixation of sex-related features are triggered (Kochhar et al., 2001). *In vitro* experiments conducted on a number of species, including cattle, revealed that male embryos develop faster than female embryos (Yadav et al., 1993) and male blastocysts are composed of more cells than female (Xu et al., 1992). Most probably a faster development of male embryos is caused by earlier expression of genes situated on Y chromosome (Edwards et al., 2001). Burgoyne (Burgoyne, 1993) revealed a gene on Y chromosome, whose expression leads to accelerated preimplantation development.

Male and female embryos also differ with respect to resistance to elevated temperature. Unpublished results of *in vitro* experiments conducted by Kawarsky and King showed that elevated temperature (42°C) caused increased mortality of male embryos in comparison with female embryos. Thus female embryos are more thermoresistant. The mechanism underlying the difference in resistance to elevated temperature between male and female embryos is unclear. It is possible that higher thermoresistance of female embryos is related to the presence of two active X chromosomes until the blastocyst stage (De la Fuente et al., 1993). Thus during the preimplantation development female embryos contain a double dose of transcripts of genes localised on X chromosomes (De la Fuente et al., 1993). Products of those genes participate in basic metabolism of the cell and are responsible for neutralisation of free radicals (Flanagan et al., 1998; Sakatani et al., 2004).

Oocyte and embryo defence against the elevated temperature *in vitro*

There are several mechanisms in bovine oocytes as well as in embryos, which protect them from elevated temperature. These mechanisms function both in the cytoplasm and the nucleus. It seems that the important element of the thermoresistance of bovine gametes and embryos are HSP 70 proteins.

Heat shock proteins

Heat shock proteins are present in many cell types, including gametes and embryos, and are responsible for limiting the negative impact of elevated temperature on cell functioning (Hendrey and Kola, 1991; Lindquist, 1993). They belong to the protective protein family called molecular chaperones. These proteins can recognize other proteins with incorrect folding and force them to adopt proper conformation. They can also assist newly synthesized proteins during their folding. Chaperones can also select dysfunctional proteins and control their discharge to the site of their degradation. These proteins also participate in transporting other proteins through biological membranes. Under physiological conditions, heat shock proteins are constantly present in all cells of an organism, and when the cell is stressed the level of their synthesis increases (Kawarsky and King, 2001; Chan-

dolia et al., 1999). Within the heat shock protein family, five classes/families – HSP 100, HSP 90, 70, HSP 60, HSP 25/27 (Krawczyk and Lisowska, 2000) can be distinguished. The best known family of heat shock protein is the HSP 70 family. These proteins are responsible for induction of thermoresistance at the early stage of embryonic development in mammals, including cattle. Within this family there is the constitutively synthesized form (HSP 70) and a form induced by elevated temperature (HSP 68) (Chandolia et al., 1999). Of all the heat shock proteins only HSP 70 is not synthesized in cells under physiological conditions, or its synthesis occurs at a minimum level.

In bovine oocytes, during meiotic maturation, HSP 70s are connected mostly with microtubules of meiotic spindle, which indicates their importance for stabilising the spindle. Despite temperature elevation, the level of synthesis in the oocyte of both forms – constitutive and inducible – remains unaltered (Chandolia et al., 1999). It might be the consequence of the transcriptional activity of mature oocyte, where mRNA synthesis is suspended and the metabolism of the cell is based on mRNA accumulated within the ooplasm (Edwards and Hansen, 1997).

In two-cell bovine embryos at elevated temperature the level of HSP 70 synthesis increases. Chandolia et al. (Chandolia et al., 1999) revealed that in two-cell embryos following thermal stress, the amount of HSP 70 mRNA increased within the cytoplasm. Simultaneously, the addition of transcription inhibitors such as DRB (5,6-dichloro-1β-D-ribofuranosylbenzimidazole) or actinomycin D to the culture lead to the inhibition of the increase in the amount of mRNA, and subsequent inhibition of embryonic development.

Edwards et al. (Edwards and Hansen, 1997) noticed that in two-cell embryos, contrary to four-cell and older embryos, the level of inducible HSP 68 synthesis is not regulated at the transcriptional level. The addition of transcription inhibitor (alfa-amanitin) to the culture stopped HSP 68 synthesis in four-cell embryos while in two-cell embryos the level of HSP 68 synthesis was unaltered. It is possible that two-cell embryos, after thermal stress, do not increase the level of HSP 68 synthesis because even under physiological conditions the transcription of this gene is at the maximum level (Kawarsky and King, 2001).

Kawarsky and King (Kawarsky and King, 2001) suggested that since the synthesis of HSP 68 in two-cell embryos is not regulated by modifications in transcription, it is possible that it relies on increased stability of mRNA accumulated in the cytoplasm or increased efficiency of translation.

The results of *in vitro* experiments indicate that from the eight-cell to the blastocyst stage, the intensity of HSP 68 synthesis increases after exposition to elevated temperature. This increase is entirely based on *de novo* transcription, as proven by the increase in HSP 68 mRNA amount within the blastomeres (Kawarsky and King, 2001).

Under physiological conditions in eight-cell embryos, heat shock proteins are localised mostly within the cytoplasm of blastomeres and such localisation indicates their role as protective proteins and anti-apoptotic agents. During thermal stress HSP 68 aggregates and migrates from the cytoplasm to the nucleus, in particular, where it is responsible for protecting and fixing nucleoli which are very sensitive to elevated temperature (Pelcham, 1984; Welch and Feramisco, 1984; Thayer and Mirkes, 1997).

Within a single eight-cell stage embryo some of the blastomeres contain large amounts of HSP 70 while the others do not contain this protein at all. This may reflect transcriptional competence of these blastomeres or their viability, or indicate a population of blastomeres that have already gained thermotolerance (Kawarsky and King, 2001).

HSP 70 is synthesized in bovine embryos at every stage of preimplantation development, but the increase in its amount as a consequence of elevated temperature does not occur until the blastocyst stage. Thus, the resistance of blastocysts to thermal stress may be in part connected with their capacity to increase the synthesis of HSP 70 (Muller et al., 1985; Hahnel et al., 1986; Edwards and Hansen, 1997) The influence of elevated temperature on advanced blastocysts is a very interesting phenomenon, since an increase in temperature in the culture of more advanced blastocysts to 42°C results in increased protein synthesis (Edwards and Hansen, 1997). Most probably, high temperature accelerates the metabolism of blastomeres in embryos, which already became thermotolerant.

Glutathione

In the majority of cells examined so far, the agents determining the resistance to elevated temperature,

apart from heat shock proteins, are several antioxidants, for example glutathione (GSH) (Griffith and Meister, 1979; Loven, 1988; Aréchiga et al.,1995). Glutathione is the most important intracellular antioxidant. It is synthesized in oocytes, and after fertilization its production stops and is not resumed until the blastocyst stage. GSH is responsible for decondensation of sperm nucleus after its penetration into the oocyte and for pronuclei formation (Yoshida et al., 1993). However, the principal role of glutathione is neutralisation of free radicals (Loven, 1988), which are produced in cells at elevated temperature. GSH acts as a substrate for such enzymes like glutathione peroxidase, catalase and enables the transformation of free radicals into H_2O (Bray and Taylor, 1993).

Edwards and Hansen (Edwards and Hansen, 1997) used the specific inhibitor of GSH synthesis – BSO (DL-buthionine-[S,R]-sulfoximine) (Griffith and Meister, 1979) to examine the role of glutathione in oocyte resistance to elevated temperature, and showed that BSO presence during IVM carried out at elevated temperature decreased the rate of resulting blastocysts. The result was confirmed by the experiment based on the use of cysteamine, which causes an increase in the amount of GSH within the cell (de Matos et al., 1995). Cysteamine added to the *in vitro* culture of oocytes at elevated temperature caused increase in the number of oocytes forming blastocysts after fertilization and culture.

In the experiments conducted by Ealy et al. (Ealy et al., 1995) the culture of two-cell embryos at elevated temperature with the addition of glutathione did not improve the viability and the quality of development of the embryos. GSH is insoluble in the cell membrane thus its transport through the membrane is hampered (Meister, 1983). To address this issue Ealy et al. applied monoethyl ester of glutathione (glutathione analogue soluble in cell membrane (Anderson and Meister, 1989) that passes from environment inside the cell), but this modification did not limit the disadvantageous impact of elevated temperature on two-cell embryos development (Ealy et al., 1995).

A couple of years earlier Ealy et al. (Ealy et al., 1992) obtained different results of the experiments with the use of GSH in the culture of morula-stage embryos at elevated temperature. In this case, an addition of glutathione to the embryo culture caused partial limitation of the disadvantageous influence of the temperature on the viability of morulas and their development to the blastocyst stage. The authors suggested that the discrepancy in thermoprotective competence of GSH in respect to two-cell embryos and morula-stage embryos was due to the requirement of cooperation with other protective agents that are active only at later stages of development. It is also possible that for two-cell embryos free radicals are less harmful than for later-stage embryos.

The lack of significance of free radicals for two-cell embryos exposed to elevated temperature was confirmed by Rivera et al. (Rivera et al., 2004). They noticed that the glutathione content within blastomeres of embryos cultured at elevated temperature (41 °C) did not differ from the content of GSH within control embryos, which suggests that free radicals are not the main source of the damage of two-cell embryos after thermal stress (Rivera et al., 2004).

Insulin-like Growth Factor (IGF-1)

The results of *in vitro* experiments conducted by Jousan and Hansen (Jousan and Hansen, 2004) indicate that Insulin-like Growth Factor-1 (IGF-1) protects preimplantation bovine embryos from the consequences of elevated temperature, such as a decrease in cell number or increase in the number of apoptotic cells within the embryo. An addition of IGF-1 to the culture medium promoted embryo development by increasing the total cell number and reducing the extent of apoptosis. IGF-1 is anti-apoptotic agent and cell proliferation stimulator, which *in vivo* is secreted by oviduct (Makarevich and Sitorkin, 1997) and uterus (Robinson et al., 2000) as well as by the embryo (Lonergan et al., 2000).

Activation of apoptosis is the reaction of the embryos to thermal stress, and to a certain degree, it protects them from elevated temperature by eliminating the damaged cells. Results of Jousan and Hansen's (Jousan and Hansen, 2004) experiments did not reveal if the promoting effect of IGF-1 is based on its role as an anti-apoptotic agent or rather on some other mechanisms that is independent of its anti-apoptotic activity.

Therefore the same authors conducted another experiment and proposed that IGF-1 activity was independent of its anti-apoptotic role (Jousan and Hansen, 2007). Thus the results of this experiment confirmed the thermoprotective properties of IGF-1 and, on the other hand, showed that IGF-1 protects preimplantation embryos from the consequences of elevated temperature independently of its anti-apoptotic role. Firstly, the ad-

dition of LY294002, an agent which blocks anti-apoptotic action of IGF-1, to the *in vitro* culture of embryos at an elevated temperature did not inhibit thermoprotective role of IGF-1. Secondly, DEVD-fmk, another anti-apoptotic agent, added to the embryo culture did not have the same advantageous influence on the embryo development after thermal stress as IGF-1 (Paula-Lopes and Hansen, 2002). Surprisingly, simultaneous addition of two anti-apoptotic agents – IGF-1 and DEVD-fmk – to the embryo culture blocked the thermoprotective role of IGF-1 (Jousan and Hansen, 2007).

Jousan and Hansen suggest that another possible role of IGF-1 is supporting the glucose uptake, which has been previously observed in mice blastocysts (Pantaleon and Kaye, 1996). Such a mechanism might enhance the development of embryos damaged by elevated temperature (Jousan and Hansen, 2007).

Apoptosis as an answer of embryos to elevated temperature in vitro

A particular kind of reaction of bovine embryos cultured *in vitro* to elevated temperature is the induction of apoptosis. Apoptosis is a form of cell death, which acts as a specific mechanism of quality control (Jacobson et al., 1997; Meier et al., 2000; Beere and Green, 2001), to eliminate cells damaged by elevated temperature. Paula-Lopes and Hansen (Paula-Lopes and Hansen, 2002) demonstrated that the capacity of the preimplantation bovine embryos to activate apoptosis after thermal stress is developmentally regulated. In two-cell and four-cell stage embryos, elevated temperature does not activate apoptotic traits, and the first apoptotic cells do not appear until the stage of 8 to 16-cell embryos, and not earlier than 4 days post fertilization (Matwee et al., 2000; Paula-Lopes and Hansen, 2002). This may indicate that the ability to activate apoptotic mechanisms in preimplantation bovine embryos depends on the number of mitotic divisions as well as the time elapsed from the moment of fertilization. The negative impact of elevated temperature in two-cell and four-cell stage bovine embryos is significantly stronger than in morula-stage embryos (Aréchiga et al.,1995, Edwards and Hansen, 1997). This suggests that more resistant to elevated temperature are those embryos that have passed through the activation of embryonic genome, because during this time embryos are already able to induce programmed cell death and it is possible to eliminate damaged cells from the embryo. Therefore, thermotolerance is most probably correlated with apoptosis (Paula-Lopes and Hansen, 2002).

Summary

In vivo experiments indicate that elevated temperature disrupts oocyte maturation, fertilization and embryo development. Oocytes and early embryos are more thermosensitive than morulas and blastocysts. *In vitro* models of oocyte maturation, fertilization and the development of bovine embryos allow for the examination of mechanisms related to thermosensitivity and thermoresistance of oocytes, sperm and preimplantation embryos. Mechanisms protecting bovine oocytes and embryos from elevated temperature function at the molecular and biochemical level in the cytoplasm and the nucleus, and are based on mRNA, proteins (HSP70, IFG-1), antioxidants (GSH) and apoptosis.

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