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Curcumin Supplementation Ameliorates Heat Stress and Affects Early Embryonic Development

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ABSTRACT

The temperature-humidity index (THI) is used to present the environmental factors causing heat stress. Heat stress effects on the effectiveness of reproduction have been extensively studied and reported. The length of the endocrine state, estrus, follicular growth and development, fertilization, conception rate, uterine function, luteolytic processes, early embryonic development, and fetal growth have all been demonstrated to be altered by heat stress. Heat stress is considered to cause oxygen-derived free radicals, or reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) or superoxide anion, to be produced in oocytes or embryos, hence inducing oxidative stress. Antioxidants, both enzymatic and non-enzymatic, provide vital defense against oxidative damage caused by heat stress. Curcumin has anti-inflammatory, anti-cancer, anti-infectious, and antioxidant properties, etc. Its antioxidant properties scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS), and when supplemented with the culture medium during *in vitro* culture, they protect the embryos from ROS and encourage embryonic growth as well as fetal development. This review aims to summarize the current knowledge on the role of curcumin during heat stress and embryonic development.

Introduction

Heat stress is defined as a gaggle of conditions as a result of over-exposure to or overexertion in excess environmental temperature and the animal is unable to dissipate a sufficient amount of warmth to look out of homeothermy (Mafruchati et al., 2023). The temperature-humidity index (THI) presents the environmental conditions that drive heat stress. Researchers have extensively studied and reported the effects of heat stress on the effectiveness of reproduction. The length of the estrus, fertilization, conception rate, uterine function, endocrine state, follicular growth and development, luteolytic processes, early embryonic development, and fetal growth have all been demonstrated to be altered by heat stress (Dovolou et al., 2023). Heat stress increases the rate of breathing, sweating, and peripheral

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blood flow, among other detrimental impacts on dairy cattle productivity and reproduction. Furthermore, heat stress reduces dry matter intake, limiting the duct gland's ability to receive nutrients and decreasing milk production and feeding efficiency overall (Nzeyimana et al., 2023). Increased THI also causes an unbalanced cooling capacity in the animal, leading to heat stress that alters metabolic pathways, particularly those involved in acid-base homeostasis, and has a deleterious impact on dry matter intake and milk production. Heat stress affects the reproductive systems of animals which causes a detrimental impact on productivity. This can happen directly, by affecting the quality of the eggs, the success of fertilization, and/or the development of the embryo, or indirectly, by reducing the nutrients that are available to support the reproductive process (Khan et al., 2023; Zeng et al., 2023). The primary regulators of ovarian functions are the gonadotropin-releasing hormone (GnRH), which is released from the brain and induces the release of gonadotropins; luteinizing hormone (LH); and follicle-stimulating hormone (FSH) from the anterior pituitary gland (Sesay, 2023). Research on how heat stress affects peripheral blood LH still does not provide a consistent picture because some research found that heat stress causes LH levels to rise while others show that it has no effect at all. On the other hand, a lower LH level may make more sense because it can lead to a decrease in the dominant follicle's production of estradiol, which would impair fertility through poor follicle development, poor ovarian inactivity, and poor regulation of estrus (Chen et al., 2023). Moreover, the production of steroids by cultured granulosa and thecal cells was low when cells were isolated and exposed to heat stress 20–26 days previously (Skliarov et al., 2022). Failure of implantation and early embryonic death resulted from a downregulation of blood progesterone levels during heat stress conditions. Embryos grown *in vitro* under heat-stress conditions exhibited a distinct RNA profile compared to physiologically normal oocytes (Chen et al., 2023). Reportedly, lower embryo growth is another effect of higher temperatures during *in vitro* fertilization (Sakatani, 2017; Arshad et al., 2021).

In addition to *in vitro* culture conditions, heat stress is thought to induce oxidative stress in oocytes by producing oxygen-derived free radicals, or known as reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) or superoxide anion. Heat stress has been linked to low reproductive success in buffalo due to its detrimental effects on blastocyst generation, decreased implantation, and increased embryonic death (Elamaran et al., 2012; Yadav et al., 2013; Kumbhar et al., 2021). Several physiological and reproductive processes, including ovulation, may cause an endogenous increase in ROS levels (Ghanem et al., 2022). Antioxidant supplementation may lessen damage from ROS, reducing oxidative stress and protecting the quantity and quality of oocytes within the ovary (Yang et al., 2021).

Dietary and synthetic antioxidants like superoxide dismutase, β-mercaptoethanol, cysteamine, cystine, and N-acetyl-L-cysteine act as the primary protective agents against oxidative stress caused by free radicals (Abeyta et al., 2023). Whereas, several reports are available on the possible impact of natural antioxidant supplements on *in vitro* maturation of oocytes, *in vitro* fertilization (IVF), and early stages of embryonic development (Sahin et al., 2023).

Curcumin, a hydrophobic polyphenolic chemical, is typically derived from the rhizome of the herb Curcuma longa (Ciuca and Racovita, 2023). Curcumin is widely employed in many biological and medical disciplines because of its anti-oxidant, anti-inflammatory anti-infectious and anti-cancer effects. Its anti-oxidant mechanism is by scavenging properties of reactive oxygen species and reactive nitrogen species (Liu et al., 2023). Curcumin may also decrease the lipid peroxides and increase the activity of antioxidant enzymes i.e. catalase, glutathione peroxidase, glutathione reductase, etc (Mohamed et al., 2023).

Antioxidant capacity of oocytes and embryos during heat stress

When both the bovine oocyte and embryo are exposed to heat stress *in vitro*, the total maternal plasma antioxidant capacity of the animal is reduced, and ROS such as oxide, anion, and free radicals are elevated. This impairs preimplantation embryonic development (Ghanem et al., 2022). Nevertheless, early embryonic death and low-quality embryos were caused by heat-stressed animals. Additionally, this causes the rate at which embryos develop to the morula stage to slow down and the concentration of embryonic peroxide to rise (Sakatani, 2017). The inability of early-stage embryos to synthesize glutathione may account for this deficit. Furthermore, decreased embryonic intracellular oxidation reduction is linked to higher blood NEFA levels under heat stress. Conversely, as the embryo develops (>8 cells), it gains resistance to heat stress. Embryonic cells achieve enhanced oxidative status by reducing ROS levels, increasing glutathione anti-oxidant activity, and undergoing de novo synthesis of glutathione (Menezo et al., 2022). Prior to the activation of the embryonic genome, the developing oocytes exhibit high receptivity to the maternal transcripts previously stored within them. Therefore, heat stress-induced disruption of maternal transcripts impacts the development of embryonic cells until the activation of the embryonic genome (Cuthbert, 2020).

A significant frequency of early embryonic death is exhibited in the animals in the summer. One of the most significant causes is the direct impact of higher temperatures on follicular growth and oocyte competency (Sammad et al., 2020). Ultrasonographic examinations showed that the heat stress made the main first- and second-wave follicles smaller and slowed the growth of other follicles, which made ovulation harder (Wolfenson and Roth, 2019). Heat stress was also associated with reduced granulosa cell viability and lower steroid concentrations in the follicular fluid extracted from big follicles. Oocyte quality and developmental competence are directly impacted by the negative impact on the development of follicles and follicular fluid contents (Huber et al., 2020). Heat stress appears to alter the maternal RNAs that are stored in the oocytes on a molecular level. This may explain the inferior quality of blastocysts acquired from oocytes collected during the hot season as opposed to those obtained from oocytes collected during the cold season, as it was found at later developmental stages prior to embryonic genome activation (Yadav et al., 2013; Ashraf et al., 2014; Roth, 2021). During the early stage of development, embryos are extremely susceptible to maternal heat stress; as development progresses, this susceptibility declines. Embryos exposed to heat stress on day 1 after estrus (1-2 cell stage embryos) had a lower percentage of reaching the blastocyst stage on day 8 after estrus. Nevertheless, heat stress in later phases did not significantly affect the percentage of embryos that reached the blastocyst stage. When oocytes are cultured to physiologically relevant heat shock (41°C) during the first 12 hours of maturation, it results in a 30-65% reduction in cleavage rate and blastocyst production rate. Dias et al (2022) reported that heat stress decreases cytoplasmic events and leads to cumulus cell death during oocyte maturation, compromising the oocyte's survival. The exact processes by which high temperatures impact the physiology of oocytes and embryos remain unclear. Heat stress is reported to stimulate the apoptosis signaling pathway in oocytes by upregulating apoptosis-related genes and in early-stage embryos by downregulating genes related to embryonic survival, such as CDX2, a transcription factor involved in the regulation of embryo implantation and placental development (Siqueira et al., 2020). Exposing oocytes to heat stress in both in vivo and *in vitro* downregulates the expression of growth/differentiation factor-9 (GDF9), which is related to oocyte maturation. Furthermore, exposure of bovine oocytes to high temperatures accelerated the apoptotic pathway of Caspases -2, -3, and -7, resulting in nuclear fragmentation and mitochondrial damage (Ramos-Ibeas et al., 2020).

After fertilization, maternal heat stress lowers the rate of pregnancy, resulting in embryonic death before implantation (Wani et al., 2021). These situations could affect the intrauterine environment of uterine tissue as well as embryos. Collecting beef cow embryos resulted in decreased viability of uterine epithelial cells obtained through uterine flushing, as well as evidence of poor embryo quality. Heat stress impacts the secretion of prostaglandin F2α (PGF2α) in the endometrial tissues of bovine uteruses, both pregnant and non-pregnant. Luteal regression was induced, and embryonic development was altered by a high level of PGF2α-repressed implantation. Summer heat suppresses a luteal function, which suppresses progesterone levels in luteinized granulosa cells, theca cells, and plasma (Abdelnour et al., 2020). Heat stress has a negative effect on placentation and fetal growth, which leads to growth retardation, reduced placental size, and weight in rats and sheep (Cowell et al., 2023). Whereas, in cows, it affects the levels of placental hormones, which compromise placental function and retard fetal development (Ouellet et al., 2020). Maternal heat stress and nutritional status throughout gestation cause significant impacts on fetal development (Rekha et al., 2023).

Curcumin as an antioxidant combating heat stress

Curcumin, a yellow-colored polyphenol compound present in turmeric, has been known for its highly effective antioxidant activity in humans and animals (Memarzia et al., 2021; Ciuca and Racovita, 2023). By improving the antioxidant capacity of embryos and enhancing intracellular levels of ROS scavengers like glutathione (GSH), a number of exogenous antioxidant substances have been frequently utilized to lessen the effects of heat stress (Baskaran et al., 2021). Antioxidants such as ascorbic acid, low taurine, and taurine provide the main exogenous protection to embryos in follicular and oviductal fluids. Enzymes such as glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), and gamma-glutamylcysteine synthetase (GCS) are involved in embryonic intrinsic defense (Labarrere and Kassab, 2022). In the past, researchers have investigated several antioxidants, including melatonin, coenzyme Q10, cysteamine, mercaptoethanol, ascorbic acid, and others, to counteract the effects of heat stress on gametes and embryos *in vitro*. Earlier research has confirmed that antioxidants and ROS in *in vitro* embryo production (IVEP) media can potentially benefit embryonic development (Rakha et al., 2022). Cysteamine is the most frequently employed antioxidant in IVEP protocols, primarily related to the IVM stage (Anand et al., 2008). This molecule promotes the development of the embryo

and the production of glutathione (GSH). This substance is present in both gametes naturally and has a critical role in protecting against the effects of reactive oxygen species (ROS). Currin et al. (2021) successfully incorporated glutathione and cysteine into *in vitro* embryo production. Quercetin, resveratrol, vitamin C, carnitine, and cysteamine are the antioxidants that protect bovine oocytes from ROS damage while they are maturing in a lab (Budani and Tiboni, 2020).

Glutathione-S-transferase (GST), γ-glutamyl cysteine ligase (γ-GCL), and heme oxygenase-1 (HO) are among the cytoprotective enzymes that are activated by curcumin and have the ability to scavenge hydrogen peroxide, peroxyl radicals, superoxide anion, hydroxyl radicals, singlet oxygen, nitric oxide, and peroxynitrite anion (Hunyadi, 2019). Curcumin has been shown to modify transcription factors such as nuclear factor (erythroid-derived 2)-like 2 (Nrf2), activator protein-1 (AP-1), and nuclear factor kappa B (NFκB), which in turn induce innate antioxidant defense systems (Beeraka et al., 2021). Curcumin has anti-inflammatory properties that are similar to those of nonsteroidal and steroidal medicines that can have bad side effects, such as phenylbutazone and indomethacin (Banez and others, 2020). Previous research showed that curcumin modifies the activity of several pro-inflammatory molecules, including xanthine oxidase, cyclo-oxygenase-2 (COX-2), and tumour necrosis factor (TNF-α). Its ability to suppress the induction of COX-2, LOX, iNOS, and the manufacture of cytokines such as interferon and tumour necrosis factor, among others, appears to be the process underlying its anti-inflammatory effect (Guo et al., 2020).

Effect of curcumin on ovarian cells and embryonic development

Curcumin influences the release and receipt of pituitary and ovarian hormones, growth factors, and cytokines, which affect ovarian cell responses to these compounds as well as external environmental variables (Gupta et al., 2020). It may also influence the oxidative processes in the ovaries as well as a variety of intracellular signaling pathways involved in ovarian cell proliferation and apoptosis *in vivo* and *in vitro* (Liang et al., 2023).

Curcumin can lower ROS by blocking methylglyoxal, but it can also disturb metabolic events in early embryonic cells and blastocysts in excessive doses. Curcumin showed harmful effects on embryonic development at higher dosages via interactions with mitochondrial signaling (Saifi et al., 2022). Most studies show that more ROS is being made, which is what causes phospholipid, protein, and nucleic acid peroxidation in oocytes and sperm cells. This makes fertilization and embryonic development less likely (Alahmar, 2019). Accumulation of free radicals and the process of apoptosis accompany reproductive senescence, leading to a decrease in the quantity and quality of ovarian follicles (Szafarowska and Jerzak, 2013). Treatment of mice with curcumin increased ovarian volume and the number of follicles and showed improved oocyte maturation, fertilization, and embryonic development, as well as lower oxidative stress. These embryos showed increased levels of *GDF-9, BMP-15, SIRT-1*, and *SIRT-3* gene expression (Azami et al., 2020). Curcumin improves the folliculogenesis profile by raising growth differentiation factor-9 (GDF-9) and lowering hyaluronate levels in an ectopic endometriosis mouse model (Azami et al. 2020). Because it is phytoestrogenic and an antioxidant, curcumin improves metabolic status, folliculogenesis, and ovarian function in rats that have polycystic ovary syndrome caused by letrozole (Reddy et al., 2018; Alibraheemi et al., 2021).

Curcumin significantly reduced the viability of buffalo granulosa cells (GCs) and increased their levels of mitochondrial activity (Ghanem et al., 2022). However, curcumin-supplemented cultured mouse somatic cells showed a significant rise in ROS as compared to the control. Curcumin supplementation successfully protected these cells from oxidative damage (H_2O_2 challenge) (Alisi et al., 2020). Curcumin regulates numerous signaling pathways, including PI3K/AKT/mTOR, JAK/STAT, ERK/MAPK, Wnt/β-catenin, NF-κB, and p53. At the molecular level, it goes after apoptotic factors like BCL2, caspases, etc., growth factors like VEGF, EGF, etc., proinflammatory cytokines like interleukins, and cell cycle regulatory factors like cyclins, cyclin-dependent kinases (CDKs), etc. Curcumin enhances the activity of antioxidant enzymes, i.e., GPx, CAT, and SOD, that can prevent oxidative stress in a dose-dependent manner and leads to an increase in anti-apoptotic protein expression and a reduction in the expression of pro-apoptotic proteins (Hafez et al., 2022).

There are only a few reports available on curcumin supplementation in embryo culture or during heat stress in livestock. When buffalo zygotes were *in vitro* cultured with and without curcumin supplementation at various concentrations (0, 2.5, 5, 10, and 20 μM), it was found that there was a slight decrease in the cleavage rate from the 0 μM group (control) to the 20 μM group (60.34%, 56.18%, 57.69%, 55.09%, and 45.58%, respectively), but

there was no statistical difference among all groups. However, the blastocyst rate in the 20 µM group (9.82%) was significantly lower than that in the 0 µM to 10 µM groups (30.61%, 26.30%, 26.80%, and 27.48%, respectively). This revealed that exposure of buffalo zygotes to high-dose curcumin (20 µM) during IVC has adverse effects on embryonic development *in vitro* (Shang et al., 2013). Similar findings were also observed in our study, where a lower concentration of curcumin improved the *in vitro* blastocyst production rate in buffalo and ameliorated heat stress in preimplantation embryos (unpublished data).

In pigs, curcumin supplementation at 5, 10, and 20 µM had significantly favorable effects on *in vitro* maturation and fertilization rates. Also, after maturation, there were a lot fewer oocytes with DNA fragmented nuclei, and adding it at a concentration of 10 µM during the *in vitro* maturation of oocytes led to a much higher rate of blastocyst formation compared to a group that wasn't added. Due to decreased oxidative stress, groups exposed to higher doses of curcumin showed better fertilization and embryonic development than those treated with lower concentrations (Namula et al., 2020). When mice were administered with higher doses of curcumin (24 mM), a harmful effect was observed in all the embryonic stages, and a significant reduction in the number of nuclei per blastocyst and an increased proportion of trophoblastic giant cells per outgrowth were reported (Huang et al., 2013). They also reported an adverse effect on mouse embryos between day 3 and day 8 of *in vivo* gestation, or the early post-implantation phases of development. Curcumin treatment of oocytes during *in vitro* maturation led to more embryos breaking down after implantation and a lower birth weight for the fetus (Huang et al., 2013).

Conclusion

Heat stress has negative effects on several aspects associated with reproductive capacity, either directly through an impact on oocyte quality, the success of fertilization, and/or embryonic development. Curcumin is a natural and popular ingredient in many biological and medical disciplines. Supplementation of curcumin in culture media plays an essential role in early embryonic development. This antioxidant can counteract the ROS effect that is generated in both normal culture conditions and situations where heat stress results in a significant increase in ROS levels. The findings of this review will help to develop a curcumin-containing medium that may be used for reducing stress in *in vitro* embryonic development.

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