Review Article

Influence of maternal nutrition and heat stress on bovine oocyte and embryo development

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ABSTRACT

The global population is expected to increase from 7.6 to 9.6 billion people from 2017 to 2050 \cite{1}. In order to meet the nutritional needs of the growing population, there is a critical demand for more efficient livestock production, especially in developing countries \cite{1}. Heat stress (HS) is a major challenge to livestock producers in many countries across the world, especially to those located in desert and tropical climates. As reported by the United States’ Environmental Protection Agency (US EPA) \cite{2}, the average global temperature is expected to increase by 0.3 °C to 4.8 °C by the year 2100 which may have a negative impact on animal production efficiency if effective heat abatement strategies are not implemented. Together, harsh geographical climates and rising global temperatures make heat stress a critical concern for animal production.

The upper critical limit of the thermo-neutral zone for dairy cattle is approximately 25 °C; thus, dairy cows are at risk of HS when exposed to temperatures above 25 °C \cite{3}. In addition to ambient temperature alone as an indicator of HS, the temperature humidity index (THI), which combines temperature and humidity to create an index score, can also be used to estimate the effect of environmental conditions on dairy cattle \cite{4}. As described by Armstrong \cite{5}, THI values can be divided into four categories according to the degree of HS experienced by dairy cows: no HS (≤ 71), mild HS (72–79), moderate HS (80–90), and severe HS (> 90).

1. Introduction

The global human population is expected to increase from 7.6 to 9.6 billion people from 2017 to 2050 \cite{1}. In order to meet the nutritional needs of the growing population, there is a critical demand for more efficient livestock production, especially in developing countries \cite{1}. Heat stress (HS) is a major challenge to livestock producers in many countries across the world, especially to those located in desert and tropical climates. As reported by the United States’ Environmental Protection Agency (US EPA) \cite{2}, the average global temperature is expected to increase by 0.3 °C to 4.8 °C by the year 2100 which may have a negative impact on animal production efficiency if effective heat abatement strategies are not implemented. Together, harsh geographical climates and rising global temperatures make heat stress a critical concern for animal production.

The upper critical limit of the thermo-neutral zone for dairy cattle is approximately 25 °C; thus, dairy cows are at risk of HS when exposed to temperatures above 25 °C \cite{3}. In addition to ambient temperature alone as an indicator of HS, the temperature humidity index (THI), which combines temperature and humidity to create an index score, can also be used to estimate the effect of environmental conditions on dairy cattle \cite{4}. As described by Armstrong \cite{5}, THI values can be divided into four categories according to the degree of HS experienced by dairy cows: no HS (≤ 71), mild HS (72–79), moderate HS (80–90), and severe HS (> 90).

2. Heat stress negatively impact animal production

HS has many negative effects on dairy cow welfare and productivity such as increased rate of respiration, sweating, and peripheral blood flow. HS has also been shown to cause decreased dry matter intake which limits nutrient supply to the mammary gland and results in decreased milk yield and overall feed efficiency \cite{6-8}. Additionally, increased THI results in imbalanced cooling ability of the cow, resulting in heat load that negatively affect DMI and milk production \cite{9,10}.

In addition to altering production, HS also alters metabolic pathways including those involved in acid-base homeostasis. Elevated respiration rate decreases the level of circulating carbon dioxide (CO₂), which disrupts the blood carbonic acid to bicarbonate equilibrium. As a result, urinary bicarbonate excretion increases and blood pH becomes unstable which can lead to a number of metabolic issues for the dairy cow \cite{11}.

3. Heat stress and altered maternal nutrition impair reproduction capacity

In addition to its negative effects on production, HS impairs the reproductive functions of dairy cows \cite{12}. Elevation of maternal body temperature negatively affects several aspects related to reproduction capacity either directly through effects on oocyte quality, success of fertilization, and/or embryo development \cite{13,14} or indirectly by...
limiting nutrient supply to support reproductive function and/or effects on reproductive hormones secretion and level [15–17].

3.1. Impairment of reproductive hormones

The gonadotropin releasing hormone (GnRH) released from hypothalamus that stimulates the release of Gonadotropins; Luteinizing hormone (LH) and Follicle stimulating hormone (FSH); from anterior pituitary gland are main regulators of ovarian functions [12]. Research on the effect of HS on peripheral blood LH is not giving a consistent picture yet, since some studies recorded increase [18], decrease [19,20] and even no effect [21,22] of HS on LH. However, the decreased LH level could be more reasonable, since low LH level could result in decrease estradiol secreted from the dominant follicle, and thus, decrease fertility by poor expression of estrus, poor follicle maturation, and ovarian inactivity [23]. Moreover, steroid production by cultured granulosa and thecal cells was low when cells were obtained from cows exposed to heat stress 20–26 days previously [24]. Implantation failure and early embryonic death resulted from decreased progesterone level during HS condition has been reported in dairy cow [25]. Yet, more research is needed to unravel the mechanism by which heat stress alters the levels of circulating reproductive hormones.

3.2. Altering oocyte quality and embryonic development

During heat seasons, cows showed a high incidence of early embryonic mortality due to several reasons. One of these reasons is the direct effect of elevated temperature on follicular development and oocyte competence [26]. In addition, HS negatively affects the superovulation response of cows and subsequently recovered embryos number, and quality [27]. A recent study on pigs reported that gilts exposed to HS during the follicular phase showed a clear induction of ovarian autophagy, and that HS increases anti-apoptotic signaling in oocytes and early follicles [28]. Ultrasoundography studies revealed that, the size of the first- and second-wave dominant follicles were reduced under HS conditions [29,30]. This affects the development of other follicles and leads to ovulation problems [31]. HS was also correlated with lower steroid concentrations in the follicular fluid obtained from large follicles and reduced granulosa cell viability [32]. Deleterious effect on follicular development and follicular fluid contents directly reflects on the oocyte quality and affects its developmental competence [26,33,34]. On the molecular level, HS seems to alter the maternal RNA stored at the oocyte. This was observed at subsequent developmental stages before embryonic genome activation which can explain the lower quality blastocysts obtained from oocytes collected in hot season than those from oocytes collected in cold season [35]. However, more researches are still needed to elucidate this point. Embryos are highly susceptible to maternal HS during the first early stages of development and this susceptibility reduced as the development proceeds. Exposer of lactating cows to heat stress at day 1 after oestrus (1–2 cell stage embryos), reduced the proportion of embryos that developed to the blastocyst stage at day 8 after oestrus. However, heat stress in later stages had no effect on the proportion of embryos that were blastocysts at day 8 [36].

In vitro studies indicated that exposure of cultured oocytes to physiologically relevant heat shock (41°C) during the first 12 h of maturation decreases their cleavage rate and blastocyst rate by 30 to 65% [37–39]. Recently, it has been reported that HS during oocyte maturation is associated with reduced cytoplasmic events and apoptosis of the cumulus cells and therefore compromise the survival of the oocyte itself [40]. The mechanisms by which elevated temperature affects oocyte and embryo physiology are not completely understood. However, several studies elucidated this effect from different prospective. Based on the gene expression patterns, it has been reported that HS stimulates the apoptosis signaling pathway in oocyte by upregulation of BAX and ITM2B (apoptotic genes) [27] and in early stage embryo by down-regulate genes associated with embryonic survival, such as CDX2, a transcription factor involved in the regulation of embryo implantation and placental development [41]. In another studies, expression of growth/differentiation factor-9 (GDF9) associated with oocyte maturation was downregulated when oocyte was exposed to HS both in vivo [42] and in vitro [43]. Furthermore, the apoptotic pathway of Caspases 2, 3, and 7 were upregulated when bovine oocytes were exposed to HS in in-vitro model leading to mitochondrial damage and nuclear fragmentation [44]. On the other hand, impaired micro-tubulin and microfilaments, which are involved in nuclear and organelles transport have been reported as consequences of oocyte exposure to HS [45,46]. The sensitivity of these cytoskeletal elements to HS affects other cytoplasmic organelles including mitochondria, essential element for oocyte developmental competence. Differences in mitochondrial distribution and shape have been recorded in oocytes isolated in hot season compared to cold season [47]. Mitochondrial functions are also influenced by HS with low membrane potential in association with apoptotic pathway activation [48].

It is also possible that HS directly affects embryonic development via epigenetic regulation, or indirectly through decrease DMI and alter the metabolic status of the animal [49,50]. Dobbs et al. [51] showed that DNA methylation was low during the early stages of embryonic development but increased between the six-to-eight-cell-stage to the blastocyst stage. Additionally, in rat model, low protein diet altered the de novo methylation process in early stage embryo [50]. However, the direct effect of HS on bovine embryo DNA methylation-de-methylation mechanistic is not well established.

3.3. Altering maternal DMI and energy balance

Short term HS has been shown to induce negative energy balance (NEB) [52,53], and even exacerbate the existing NEB by prolonging the period of decreased DMI in highly producing dairy cow [12]. Highly producing dairy cows suffer from NEB which usually occurs after calving due to the disparity between dry matter intake (DMI) and an increase demand for milk production [54]. During HS, cows mobilize adipose reserves which results in elevated non-esterified fatty acids (NEFA) [52,53] that have been shown to decrease oocyte and embryo quality in in vitro studies [54–57].

Blood glucose level was reduced during short term (7 days) [52] and long term (during the hot season) exposure to HS [35]. However, in another in vivo short term (4 days) study [53] blood glucose was not affected by HS. This could be due to the short term exposure to HS which could not predict well the impact of HS on blood glucose level. Even more, elevated blood NEFA concentration is also associated with decreased glucose concentration in ovarian follicular fluid [43,54,58]. This reduction of glucose level can significantly impact oocyte development because glucose serves as the main energy source and provides the necessary elements for oocyte maturation including pyruvate, ATP, and reducing agents such as NADPH and glutathione that neutralize the reactive oxygen species (ROS) [59]. Therefore, HS can have indirect negative effects on oocyte and subsequent embryonic development via this reduction in available glucose [54]. In addition, increased NEFA concentrations affect the expression of genes involved in lipid metabolism in various tissues, such as the mammary gland [60], oocytes [54], and the embryo itself [61]. The mRNA abundance of DNM3A (regulating embryonic DNA methylation), IGFL2R (growth factor), and SLC2A1 (glucose transporter) were up-regulated in blastocysts resulting from NEFA exposed oocytes [61,62] resulting in an imbalance embryonic DNA methylation [41].

3.4. Altering anti-oxidant capacity

During HS, maternal total plasma anti-oxidant capacity decreased [42]. Additionally, ROS such as hydrogen peroxide, superoxide anion, and hydroxyl radical are elevated when both the bovine oocyte [43]
and bovine embryo [63] exposed to HS in vitro, which impairs pre-
implantation embryonic development [64]. Nevertheless, maternal HS
resulted in early embryonic mortality and poor embryo quality in dairy
cow model [13,36]. Additionally, in mice model, maternal HS resulted in
early embryonic mortality and decreased the rate of embryo devel-

opment to morula stage and increase level of embryonic hydrogen

eroxide concentration [65]. This impairment could be explained by
the fact that early stage embryo cannot synthesis glutathione [65,66].
Furthermore, elevated blood NEFA during HS is associated with re-
duced embryonic REDOX (intra-cellular oxidation reduction) [62].

On the other side, when embryo development advances (> 8 cells)
the embryo acquires resistance to HS [63], this could be explained, in
part, by improved oxidative status via a reduction in the level of ROS
and an increase in glutathione anti-oxidant activity, and the de novo
synthesis of embryonic cells glutathione [63,66,67]. On the other part,
before activation of embryonic genome, the embryonic cells are fully
dependent on maternal transcripts formerly stored in their growing
oocytes, thus, impairment of maternal transcripts during HS will be
affecting the performance of embryonic cells until EGA (EGA; em-

bryonic genome activation; embryonic genome becomes active and
massive embryonic gene expression begins to take place) [21,51,52].

4. Nutritional strategies to improve reproduction during HS
conditions

4.1. Dietary cation-anion difference

There is insufficient data on the direct effect of dairy cattle nutrition
on bovine embryo quality and development during HS. However, there are
several nutritional strategies that may be implemented to mitigate the
effect of HS on maternal energy balance and thus, embryo quality
and development. One potential dietary modification that may be used to
minimize the negative effects of HS on dairy cows is the manipulation of
the dietary cation-anion difference (DCAD; mEq/kg of DM) [68,69]. DCAD is an index of the acid-base status of the animal as it is
the relative balance between the principle cations (potassium, K; so-
dium, Na) and the principle anions (chloride, Cl; sulfur, S) in the cow’s
diet [70]. Studies suggest that DCAD can be used as a strategy to im-
prove feed efficiency (3.5% fat-corrected milk per unit DMI) through
altering rumen environment and pH [69,71,72] which could stimulate
DMI. Greater DMI could increase the supply of essential nutrients to

support embryonic growth and development. Reduced DMI is associ-
ated with downregulation of metabolic biomarkers Na⁺/K⁺ ATPase
mRNA and sodium/glucose co-transporter1 (SLC5A1) in ewe oocytes
[73], that might indicate the role of DCAD in modulating the nu-

trigenomic mechanism regulating oocyte quality and hence, embryo
quality however, the exact nutri-epi-genomic role of DCAD on embryo
growth is not yet established.

4.2. Epi-nutrients supplementation

Epi-nutrients are a subset of nutrients such as folate, vitamin B-12, and
choline, which are necessary for epigenetic regulation of the

gene, through DNA methylation and histone modifications [74,75].
Proper supplementation of such nutrients during HS may play a role in
regulating embryonic development; however, the extent to which such
epi-nutrients could regulate embryo epigenome during HS is not yet
investigated. These nutrients play a role in peri-conceptional DNA
methylation which affect embryonic development, subsequent growth,
and health status of the offspring [76]. Most epi-nutrients, such as fo-
late, vitamin B-12, methionine, choline, and betaine, can modulate the
1-carbon metabolism pathways responsible for generating the major
methyl donor, S-adenosylmethionine (SAM), which can directly affect
DNA methylation [77]. Additionally, Folate is involved in de novo
nucleotide synthesis [78].

During early embryonic development, the epi-nutrients choline and
folate are essential for DNA methylation re-programming [79]. The
mRNA of folate–methionine cycle enzymes are expressed in mouse heat
stressed pre-implantation embryos, when incubated in folate containing
medium [78]. In vivo study, [80] folic acid was supplemented in diet
of heat stressed female mice, at the beginning of gestation, and was
successfully reduced the heat stress induced neural tube defects.

Vitamin B-12 is required for the function of methionine synthase, an
enzyme required for the regeneration of methionine from homo-
cysteine, making it an important participant in one-carbon metabolism
[77].

4.3. Long term supplementation of anti-oxidants

As aforementioned HS negatively affects the anti-oxidant scaveng-
ing activity of the cow, oocytes and the resulting embryos, therefore
anti-oxidant supplementation might play a role in relieving the oxida-
tive action of HS. Short-term supplementation of anti-oxidants like vi-
tamin A, E, and C could not scavenge either the maternal or embryonic
ROS developed during HS [81–83]. However, long term effect of these
anti-oxidants on the cow and the embryos is not yet investigated. Ad-
ditionally, long term (90 days) supplementation of β-carotene starting
15 d before calving increased the pregnancy rate by 14 percentage units
[84], on the other side, the effect of β-carotene supplementation for
long term on bovine embryo still needs to be investigated.

Another potent anti-oxidant is the green tea flavonoid compound
epigallocatechinin gallate (EGCG) which was previously investigated in
mouse embryo model [85]. EGCG exerted an anti-oxidant, and anti-
apoptotic action on preimplantation mouse embryos. Further inves-
tigations are needed to investigate if EGCG has similar effects on
bovine embryos during HS.

4.4. Balancing maternal diet for proper protein metabolism

Since HS increased blood urea nitrogen (BUN) and plasma creati-
nine by 71% and 13.2%, respectively in heat stressed lactating dairy
cows [52,86], indicating alteration in protein metabolism and increase
in skeletal muscle protein catabolism [52]. In a recent study by
Kaufman et al. [87], the reduction of rumen degradable protein (RDP)
and rumen un-degradable protein (RUP) levels during hot summer
climate was an efficient strategy to increases the use of amino acids to
limit protein catabolism in heat stressed dairy cow. However, the extent
to which such an approach can improve bovine gamete and em-

bryo quality is not been yet established, therefore, such area of research
of relating amino acid profile balancing to reproduction performance
during heat stress needs to be investigated.

5. Other non-nutritional approaches

Other environmental measure could be taken into consideration to
alleviate the negative HS effect on reproduction is to provide dairy
farms with proper cooling system during hot summer seasons, however,
poor areas and small scale farms might not be able to provide such an
expenses facilities, therefore, having proper nutritional strategy to
alleviate the deteriorating HS effect on animal reproduction and future
production is essential.

6. Future perspectives

More in-depth studies using genomic and metabolomic technologies
are needed to clearly investigate the genomic and epigenetic me-
chanism of action of HS on bovine embryonic development. Most of the
literature discussing the effect of HS and/ or NEB on bovine embryo are
based on in vitro embryo and oocyte maturation models; however, the in
vivo effects are not well understood. Furthermore, the effect of nu-

trigenomic and nutri-epigenetic diets interaction on bovine embryos
and oocytes during HS is a new area that should be considered for
future research.

Another future consideration is proper attention for breeding program, for selection of HS tolerant breeds, instead of only selection for high milk production breeds.

7. Conclusions

The rapidly changing climate, global warming, and the parallel rapidly growing population size, ringing the alarm for critical strategies to manage livestock productivity and reproductibility. As shown in this current review, the effect of HS on maternal nutritional metabolic, and anti-oxidant status is reflected on the oocytes, resulting embryos and the fertility status of the cow. Therefore, long term programming of maternal nutrition is the key factor to mitigate the HS effect on the oocytes and the developing embryos. Additionally, proper nutritional managing of the transition cow to keep the energy balance, could play a positive effect on the oocytes and the resulting embryo, and hence, the future progeny and production.

Competing interests

The authors declare no competing interests.

Author contribution

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