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MOLECULAR RESPONSES OF HEAT STRESS DURING EARLY EMBRYONIC DEVELOPMENT AND ALLEVIATION STRATEGIES

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SUMMARY

It is well-known that exposure of female farm animals to heat elevation negatively affects all biological activities required to establish pregnancy. The reduction in pregnancy rate during the hot season can approach 50% in dairy cows and 60% in dairy buffaloes. Indeed, earlier investigations done in buffalo indicated seasonal fluctuations of oocyte quality and most studies confirmed a significant reduction in morphological oocytes quality when recovered during hot season. In vivo studies have reported a clear reduction in meiotic maturation of oocytes exposed to heat stress due to decreased blood flow to reproductive tract, low progesterone level and increased glucocorticoids profile. Buffalo oocytes recovered during summer had a lower percentage of nuclear maturation in vitro (42.9%) compared to those collected in winter (85%). Heat shock at 42 °C for 6 hours during in vitro maturation of camel oocytes significantly decreased the polar body extrusion rate (20.7%) compared to control group (32.3%). At the cellular level, decreased nuclear maturation during heat stress was coupled with reduction in mitochondrial activity, protein biosynthesis and impairment of cellular microstructure system organizing nuclear progression to accomplish maturation such as spindle formation, microfilament and microtubule distribution. Heat-induced alterations in cytoplasmic as well as nuclear maturation were coupled with down-regulation of oocyte transcripts such as GDF9, POU5F1, and C-MOS and genes involved in metabolism (GLUT1) and antioxidant defense (SOD2) that regulate maturation process and preimplantation development. These cellular and molecular defects led to compromising early cleavage and blastocyst formation. Blastocyst rate was lower (42%) for camel oocytes exposed to heat shock compared with control counterparts (48%). The rate of developed buffalo blastocyst was lower during summer (13%) than winter (28.3%) which explained by high rate of chromosome abnormalities and alteration of genes regulating key molecular processes that compromise viability of embryos. Providing females farm animals with optimum management system including housing is the first strategy to reduce environmental heat load. Changing composition of animal feeds is a second possible way to alleviate heat stress. Genetic selection for heat tolerance is long term breeding goal with continuous global warming that ensure good productive as well as reproductive performance.

Keywords: Global warming, farm animals, reproduction, preimplantation development, genes.

INTRODUCTION

Large attention is paid internationally to the global warming, especially in tropical and subtropical regions. Climate change represents a great threat for human and animals' life (Pasqui and Di Giuseppe, 2019). In this context, scientists searched for solutions to overcome or alleviate the negative impacts of climatic change. Rather than strengthening stress resistance, the cattle industry has spent the last quarter-century focusing on increasing output, altering the environment, and enhancing nutritional management. Domestic animals' productivity was greatly boosted as a result of this strategy, but their susceptibility to high temperature was increased (Bernabucci, 2019). One of the ways to cope with the results of the climatic change is to enhance the productivity and reproductivity of animals, which are already adapted to extreme environmental conditions. The vulnerability of farm animals to drastic effects of heat stress varies according to biological factors (species, genetic background, life stage and coat characteristics), environmental factors (duration, humidity, and wind), management or production system and nutritional status (Das *et al*., 2016).

Heat stress may be defined as an elevation of environmental temperature that caused an increase in the animal body temperature above its normal set-point (Hansen 2009). Indeed, heat elevation or increasing temperature-humidity index (THI) has caused retardation of animal growth (Baumgard *et al*., 2012), meat production (Archana *et al*., 2018), milk yield (Das *et al*., 2016), and reproductive performance (Rhoads *et al*., 2009). Noteworthy, the upper critical ambient temperature of lactating dairy cattle is approximately 25–28°C, which assumed relative to 78.2 THI (Dikmen and Hansen, 2009). In addition, when the measured THI increases beyond the upper critical value, the conception rate declined sharply to reach 20 to 30% (Collier *et al*., 2017; Wolfenson *et al*., 2019). The pregnancy rate after artificial insemination in dairy cattle has dramatically reduced during summer season (De Rensis *et al*., 2017). An estimation of pregnancy rate in 20,606 cows managed under seven farms was done (Lozano *et al*., 2005) at different THI levels and indicated that it was recorded to be 39.4% (THI<72.0), 38.5% (72.0

to 73.9), 36.9% (74.0 to 75.9), 32.5% (76.0 to 77.9), and 31.6% (>78.0).

When dairy cows are exposed to heat stress subsequently the body temperature increases which is considered detrimental impacts to the reproductive capabilities of dairy cows (Sakatani 2017). Indeed, hot summer season is associated with elevation in vaginal temperature, which might cause irregular ovarian activity and silent heat incidence (Barkawi, 1981), disturbing uterine environment (Sakatani 2017), oviduct environment (Kobayashi *et al*., 2013), reducing oocyte competence (Abdoon, 2014). Additionally, elevated ambient temperatures also negatively increased incidence of embryo loss prior to attachment which resulted in low conception rate (Ealy *et al*., 1993; García-Ispierto *et al*., 2006; Sakatani 2017). So, according to global warming changes, the animal productivity is susceptible to be reduced leading to economical loss especially in dairy farms especially those raising temperate breeds such as Holstein because its limited ability to adapt to heat stress compared with tropical cattle. Therefore, adopting nutritional, management and breeding strategies are the possible future solutions to mitigate global warming (Sejian *et al*., 2018).

The cumulus-oocyte complexes could be negatively influenced by heat stress depending on the duration of exposure (Roth, 2017; Islam *et al*., 2018). The exposure of oocytes to heat shock during maturation under *in vitro* condition caused an increase in percentage of apoptotic oocytes (Camargo *et al*., 2019). Recently, it was demonstrated that camel oocytes are more sensitive to heat stress when exposed to short acute heat shock, and subsequently this was coupled with decreased the diameter of the cytoplasm and reduction in nuclear maturation rate as well as increased incidence of chromosomal abnormalities (Islam *et al*., 2018). Therefore, the decline of pregnancy rate of female farm animals during the periods of heat stress may be due to the decline in quality of oocytes and changing the environment of reproductive tract to be unfavorable for the developing embryos.

Effect of heat stress on reproductive performance:

Heat stress has negative effects on the reproductive efficiency of dairy cows. Female sexual behavior and fertility were negatively affected by heat stress, so the reproductive efficiency of buffalo is decreased during hot months because of silent estrus (Singh *et al*., 2013). It has been reported that heat stress increased ovarian inactivity which manifested by anestrous (Wolfenson *et al*., 2000; Oseni *et al*., 2003). It was observed that majority of heat signs were more incidence throughout the hot season in Indian (Zakari 1981) and Baladi cows (Barkawi *et al*. 2001), while cows that displayed standing behavior reached 50% in hot months (Folman *et al*. 1979). Noticeably, the elevation of ambient temperature during summer season reduced follicular growth which reflected in reduction of circulating estradiol (Wolfenson *et al*. 1995) and subsequently, this led to silent heat or reduction in symptoms of estrus behavior (Abilay *et al*., 1975; Gwazdauskas *et al*., 1981; Thatcher and Collier 1986). The level of estradiol hormone was also reduced in the follicular fluid of preovulatory follicles (Ozawa *et al.*, 2005). The reduction of steroid secretion was caused by the abnormal expression of their synthetic genes in ovaries under heat stress (Argov *et al*. 2005).

It was reported that there is a reduction in signs of estrus in Egyptian cows during summer season (Damarany, 2017). Noteworthy, increased of high temperature humidity index (THI) has negatively influenced the incidence of cow's heat symptoms (Peralta *et al*., 2005). The reduction in estradiol biosynthesis is the mechanism by which heat stress deteriorates ovarian follicle development and impaired expression of estrus (Badinga *et al*. 1993; Wolfenson *et al*. 1988a, b). This sharp reduction in circulating estradiol hormone has resulted in decreasing expression of cow's estrus signs, ovulation, and the development of corpus luteum (Wolfenson *et al*., 1988a, b). Moreover, low level of estradiol caused reduced intensity and duration of estrus as well as increased occurrence of silent ovulation. Heat elevation during the hot months of the year decreased growth of corpus luteum and delayed its regression (Wilson *et al*. 1998; Wolfenson *et al*. 1988a, b).The mechanism by which hyperthermia induces infertility depends on disturbing the surge center of GnRH in the hypothalamus, and thus, reducing release of the circulating levels of gonadotropin hormones (LH and FSH) from anterior pituitary (Howell *et al*., 1994; Wolfenson *et al*., 2000). Using binding assay, it was observed that gonadotropin receptors (FSH-R and LH-R) was reduced in granulosa cells recovered from rats exposed to heat stress (Shimizu *et al*., 2005). Administration of flunixin meglumine (FM) to dairy cows inseminated during heat stressed has resulted in maintaining of corpus luteum (Kaveh *et al*. 2011). Recently, administration of Egyptian local cows with FM or aspirin at 14 and day 15 post-mating increased pregnancy rate of heat stressed animals (60% and 40%) compared to untreated group (30%).

Early embryonic development in cows relies on progesterone secretion by the corpus luteum. The decline in pregnancy rate in dairy cattle was attributed to low circulating progesterone concentrations or a delay in the rise of progesterone concentrations post-ovulation (Mann and Lamming 1999; Khodaei-Motlagh *et al*., 2011; Masoumi *et al*., 2017) Researchers have suggested that prolonged lifespan of the corpus luteum by administration of agents that suppress release of prostaglandin $F_{2\alpha}$ to improve pregnancy in cattle (Elli *et al*. 2001; Geary *et al*. 2010). Wilson *et al*., (1998) reported that dairy heifers exposed to heat stress led to delay in corpus luteum regression. Kaveh *et al*. (2011) reported that treatment of heat stressed dairy cows with FM after-insemination between days 2-5 or 10-13 led to maintaining of corpus luteum. Higher concentration

of circulating progesterone immediately after conception has been linked with an advancement of conceptus elongation and increased interferon tau (IFN-τ) production which subsequently improved cattle pregnancy rate (Inskeep 2004; Stronge *et al*., 2005; McNeill *et al*., 2006). Clemente *et al*. (2009) suggested that progesterone concentrations -induced fluctuated in the uterine environment are answerable for the advancement in embryo elongation in cattle.

Moreover, an increase by 0.5^{o}C in uterine temperature during hot days lead to a decrease in fertilization rate (Schuller *et al.,* 2014).). In addition, high THI will cause an increase in rectal temperature from 38.5^{o} C to 40.5^{o} C during 72 hour after insemination, which decreased pregnancy rate to 50% in cows (Schuller *et al.,* 2014) and 59% in Murrah buffalos (Dash, 2013). Heat stress during 0-3 days or 0-7 days of pregnancy commence increased the risk of embryonic losses (Das *et al*., 2015) because the blood flow in the heat-stressed animals decreased in the deep vessels to increase the flow to the superficial vessels affecting the blood circulation and female reproductive system causing an impairment of its function (Hufana-Duran & Duran, 2020).

Assessment of dairy cattle THI in addition to measurement of animal stress parameters such as rectal temperature and respiration rate are considered useful tools to predict the exact heat load on animals (Herbut *et al*., 2018; Liu *et al*., 2019). Sammad *et al*. (2020) have summarized the strategy that could be applied to mitigate heat stress in dairy cattle by (1) Proper management which includes sufficient shading, sprinklers and monitoring of animal health and hygiene (2) feeding management which include supplementation of minerals, additions of rumen buffer, balanced energy and protein content. Indeed, traditional breeding for genetic selection of dairy cattle can be used for selecting tolerant animals for heat stress however it requires several generations to obtain good results due to its slow rates to get tangible genetic gain as a result of long generation intervals. Garner *et al*. (2016) have suggested that application of genomic selection of dairy cattle for functional traits including immunity and heat tolerance is a key step towards selecting animals that could cope with future global warming.

Effect of heat stress on preimplantation development:

The climate change has raised tangible concerns about global warming which represents a significant threat to the feasibility and sustainability of livestock farming worldwide particularly in regions located in tropical and subtropical zones. Egypt is located in subtropical area where heat stress is intense during summer months, which usually accompanied with high ambient humidity that make the situation worse and represent a constraint to farm animal welfare, productivity and reproductive performance (Marai and Habeeb, 2010). In this regard, cumulus-oocytes complexes are highly affected when the female is exposed to environmental heat stress during follicular development, ovulation and *in vivo* events of oocyte maturation (Al-Katanani *et al*., 2002; Gendleman and Roth, 2012; Sadeesh *et al*., 2016).

Buffalo cumulus-oocyte complexes that were collected during hot season had a high percentage of arrested oocytes in metaphase I stage of nuclear progression after *invitro* maturation (Abdoon *et al*., 2014). Interestingly, bovine oocytes that have been exposed to increased temperatures at 40.0°C and 41.0°C recorded lower rates of nuclear and cytoplasmic maturation that was linked with reduced *in vitro* embryo development (Maya-Soriano *et al*., 2013). Similarly, El-Sayed *et al*. (2018) have indicated that exposure of buffalo COCs to heat shock reduced the percentage of *in vitro* matured oocytes at temperature of 39.5°C and 40.5°C compared with that of non-treated control group (38.5°C). Subsequently, the percentage of buffalo embryos that arrested at early stages of cleavage (2- or 4-cell) or at late stages of cleavage (8-cell, 16-cell and blastocyst) was significantly higher in heat-shocked groups than the counterparts developed in control group.

In support to previous observation, experimental exposure of COCs to heat shock during the maturation has revealed reduced cleavage rate as well as blastocyst development (Edwards and Hansen 1997). In addition, *in vivo* experiments have reported reduced pregnancy rate by 25% for each 1°C elevation in body temperature which is due to the negative impact of heat stress on preimplantation development of embryos (Nabenishi *et al*., 2011). Therefore, it could be implicated from our results and of other studies that exposure of animals or the preimplantation developmental stages to heat stress has a negative impact on their ability to continue normal growth and development.

Ashour *et al*., (2020) have demonstrated that exposure of COCs to heat stress at 41°C and 42°Cfor the first 6 hours significantly decreased the ratio of good PB (31.44±0.92%; 31.63±0.47%) and bad (20.73±0.49%; 20.30±0.52%) compared to the control good and bad oocytes (32.31±0.54%; 30.98±1.36%). Indeed, buffalo cumulus-oocyte complexes that were collected during hot season had a high percentage of arrested oocytes in metaphase I stage after *in vitro* maturation (Abdoon *et al*., 2014). Interestingly, bovine oocytes that have been exposed to heat shock at 40.0°C and 41.0°C recorded lower rates of nuclear and cytoplasmic maturation (Maya-Soriano *et al*., 2013). Similarly, El-Sayed *et al*. (2018) have indicated that exposure of buffalo COCs to heat shock reduced the percentage of *in vitro* matured oocytes at temperature of 39.5°C and 40.5°C compared with that of non-treated control group

(38.5°C). Bovine oocytes exposed to heat stress at 40.0°C and 41.0°C reduced embryo development rate (Maya-Soriano *et al*., 2013). Saadeldin *et al*. (2018) demonstrated that camel oocytes exposed to short acute heat shock at 45°C for 2, showed reduction in maturation rate, linked with decreased ooplasmic diameter and increased percentage of chromosomal abnormalities. Ovine oocytes that *in vitro* matured after exposure to heat stress at 41°Cfor the first 12 h have remained at the germinal vesicle breakdown (GVBD) stage, and they showed an aberrant chromatin configuration (Gharibzadeh *et al*., 2015). Immunofluorescence of heat-shocked oocytes indicated an uneven distribution pattern of cortical granules beneath plasma membrane (Gharibzadeh *et al*., 2015). Heat shock reduced nuclear maturation by inducing alterations in the configuration of chromatin microtubules, and microfilaments (Ju *et al*., 2005). Heat elevation (40–41°C) during *in vitro* maturation of bovine oocytes may cause DNA damage and enhance induction of apoptosis (Roth and Hansen, 2004).

Ashour *et al*. (2020) have reported that the blastocyst rate was significantly lower for good quality oocytes exposed to heat stress at 41°C (6 ± 0%; 2 ± 0.2%1), compared with control groups (15 ± 0.22%; 9 ± 0.22%). This reduction in blastocyst rate was more noticeable when oocytes exposed to heat stress at 42°C (0, 3 ± 0.22%;0%). In support to previous observation, experimental exposure of COCs to heat shock during maturation has revealed reduced cleavage rate as well as blastocyst development (Edwards and Hansen 1997). In addition, *in vivo* experiments have reported reduced pregnancy rate by 25% for each °C elevation in body temperature, which is due to the negative impact of heat stress on preimplantation development of embryos (Nabenishi *et al*., 2011). Recently, heat shock at 40°C for 24 h during IVM of bovine COCs had no effect on PB extrusion rate however; it reduced the rate of embryo cleavage and blastocyst development (Pöhland *et al*., 2020).

It was reported (Li *et al*., 2016) that addition of resveratrol (2.0 μmol/L) to maturation medium relieved the adverse effects of heat stress on porcine oocytes by increasing the proportion that reached nuclear maturation which may be attributed to its antioxidant capability in alleviation of oxidative stress (Liu *et al*., 2015).In addition, resveratrol has a direct protecting and enhancing effect on the nuclear maturation of porcine oocytes during heat stress, by inducing GSH synthesis and preventing accumulation of intracellular ROS (Liu *et al*., 2015). At the molecular level, resveratrol increased the expression of SIRT1gene, which linked with improving mitochondrial function under oxidative stress (Ou *et al*., 2014). Indeed, several antioxidants have supplemented to preimplantation culture media in order to alleviate the adverse effects of heat stress including dithiothreitol (de Castro e Paula and Hansen, 2008) and anthocyanin (Sakatani *et al*., 2007), while other researchers demonstrated little effect of some chemical agents such as vitamin E (Paula-Lopes *et al*., 2003) and glutathione (Ealy *et al*., 1995).

Molecular responses of preimplantation development to heat stress

Maya-Soriano *et al*. (2013) indicated that heat stress induces a series of cellular alterations affecting nuclear and cytoplasmic maturation. Also, heat stress compromise oocyte cytoskeleton, impair mitochondrial activity and induce cell apoptosis (Roth and Hansen, 2004 and Paula-Lopes *et al*., 2012). Impairment of mitochondrial activity under heat stress leads to a reduction of ATP availability at the oocytes level and failure of embryonic development (Roth, 2015). Apoptosis is considered one of the causes of impaired developmental competence of mammalian COCs. In addition, cytoskeletal changes and apoptosis were observed in oocytes after heat stress during the first 12 h of maturation, which might trigger the impairment of further embryo development (Roth and Hansen, 2005). It was demonstrated that heat elevation had a negative impact on maturation of porcine oocyte due to increasing ROS and reducing GSH content (Nabenishi *et al*. 2012). Similarly, Ozawa *et al*., (2002) have observed reduced developmental ability of mouse embryos when zygotes were exposed to heat shock that led to an increase in the level of intracellular ROS causing oxidative stress on preimplantation embryos. The induction of oxidative stress caused by heat stress in the oviduct of mice was possibly involved in heat stress-induced early embryonic death (Ozawa *et al*., 2005).

Heat stress, either short-term or long-term, triggers the expression of HSP (Hassan *et al*., 2019). HSPs promote survival by suppressing apoptosis. Pöhland *et al*. (2020) have observed that the highest expression of HSP70 gene was in cumulus cells of Zebu cattle COCs *in vitro* matured at 40°C while the expression of HSP90 gene was the highest in cumulus cells and their corresponding oocytes matured at 37°C. This data provide evidence that there is a differential regulation of heat shock genes (HSP70 and HSP90) in COCs in response to heat and cold stress (Pöhland *et al*., 2020). Exposure to heat stress enhanced elevation of ROS accumulation, thereby inducing cellular oxidative stress (Kim *et al*., 2005).

Down regulation of maternal transcripts GDF9, C-MOS and POU5F1 was reported in bovine oocytes during hot season (Gendelman, and Roth, 2012). Similarly; reduction in the relative abundance of OCT4,(IGF2R), GDF9 (MnSOD) and GLUT1 was observed in buffalo oocytes recovered during the hot season (Sadeesh *et al*., 2016).

CONCLUSION

In conclusion, global warming represents a real threat for livestock farming. It has tangible negative effect on animal productivity and their reproductive efficiency. Apoptosis inducing oxidative stress is the

one main molecular mechanism that negatively compromised oocytes and embryo viability under heat stress conditions. Monitoring farm environmental factors such as ambient temperature, humidity, THI, wind is a prerequisite for providing suitable mitigation strategy. The management practices through selecting housing type, bedding, and sprinklers are essential tools for alleviating seasonal heat stress. In addition, nutritional management by balancing mixed ration that has suitable composition of energy and protein is also key element for successful mitigation of heat stress. Finally, long-term breeding strategy for selecting animals that cope well with scenario of climate change is a prerequisite for sustainable dairy cattle farming. Application of new technologies such as genomic selection and reproductive biotechnologies will help in speeding up the process of animal selection that fitted for climate change scenario.

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الإستجابات على مستوى البيولوجيا الجزيئية للإجهاد الحرارى خلال مراحل التطور المبكرة للأجنة وإستراتيجيات تخفيفها

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من المعروف جيدا إن تعرض إناث الحيوانات المزرعية لإرتفاع الحرارة يؤثر بالسلب على كل الأنشطة الحيوية المطلوبة لإحداث الحمل. ان الانخفاض فى معدل الحمل فى الموسم الحار قد يصل إلى نسبة ٥٠% فى الأبقار الحلابة و إلى نسبة ٦٠% فى الجاموس الحلاب.الدراسات المبكرة التى أُجريت على الجاموس بينت تغيرات موسمية فى جودة البويضات، و معظم الدراسات أكدت وجود انخفاض ملحوظ فى جودة البويضات عند تقييمها مورفولوجياخلال الموسم الحار.بالاضافه الى ذلك اثبتت الابحاث التى أجريت على الاناث ان هناك انخفاض واضح فى معدل الانضاج الميوزى للبويضات عند تعرض هذه الاناث للإجهاد الحرارى كنتيجة لتناقص تدفق الدم للمسار للجهاز التناسلى و إنخفاض مستوى هرومون البروجيستيرون وزيادة نشاط هرمونات الغده الكظريه. فى أحدى هذه الدراسات وجد ان بويضات الجاموس المستخرجة خلال الصيف كانت ذات معدل منخفض فى إنضاجها النووى معمليا بنسبة تصل الى ٤٢.٩% مقارنة بنظيرتها المستخرجة فى الشتاء بنسبة تصل الى ٨٥%. كما إن تعرض بويضات الجمال لصدمة حرارية معمليا على درجة حرارة ٤٢ مئوية لمدة ٦ ساعات خلال عملية الإنضاج المعملى أدت بشكل الى انخفاض معدل تحرر الجسم القطبى بنسبة ٢٠.٧% مقارنة بالمجموعة الغير معامله بنسبة ٣٢.٣%. على المستوى الخلوى، انخفاض الإنضاج النووى خلال فترة الإجهاد الحرارى متلازما مع تضاؤل فى نشاط الميتوكوندريا و التخليق الحيوى للبروتين و إضعاف البنية الهيكليه الدقيقه للبويضات. بالاضافه الى اختلال تكوين الخيوط المغزلية واختلال توزيع كلا من الأُنيبيبات الدقيقه المنظمه للإنضاج النووى. إن التغيرات فى الإنضاج السيتوبلازمى و النووى يكون تلازما مع حدوث تغيرات على مستوى البيولوجيا الجزيئيه مثلما يحدث فى أختلال التعبير الجينى المسؤوله عن العمليات الحيويه بالبويضه (GDF9 وPOU5F1 و C-MOS وGLUT1 وSOD2) التى تنظم عمليات الإنضاج و التطور فى مراحل التطور الجنينى ما قبل الإنغراس. و هذه العيوب / الإختلالات الخلوية و الجزيئية تؤدى للتأثير المباشر على الإنقسامات المبكرة و تكوين الجنين الكامل التطور

(بلاستوسيست). لذا يلاحظ ان معدل الوصول لمرحلة الأجنة كاملة التطور (بلاستوسيست) كان منخفضا بنسبة ٤٢% لبويضات الجمال التى عُرضت لصدمة حرارية مقارنة بنظيراتها الغير معامله بنسبة ٤٨%. و كان معدل الأجنة كاملة التطور (بلاستوسيست) فى الجاموس منخفضا خلال الصيف بنسبة ١٣% عنه فى الشتاء بنسبة ٢٨.٣% ، والذي عزى الى زياده معدل التشوهات الصبغية و تغييرات فى الجينات المنظمة لعمليات جزيئية رئيسية تمس بحيوية الأجنة. من ذلك يتبين أن تزويد إناث الحيوانات المزرعية بنظام إدارة أمثل، شاملا التسكين فى الحظائر المناسبه، هو الإستراتيجية الأولى لتقليل الحمل الجرارى البيئى. كما ان تغيير التركيبة العلفية للحيوانات هو طريقة ثانية محتملة لتخفيف الإجهاد الحرارى. و فى النهايه يتضح أن الإنتخاب الوراثى لتحمل الحرارى هو هدف طويل المدى للتربية، و ذلك لضمان إنتاج جيد علاوة على الأداء التناسلى وذلك مع إستمرارية ظاهره الإحتباس الحرارى.