Effects of heat stress on the immune system, metabolism and nutrient partitioning: implications on reproductive success

Efeitos do estresse térmico no sistema imunológico, metabolismo e partição de nutrientes: implicações sobre o sucesso reprodutor

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Abstract

Heat stress (HS) is a global problem which jeopardizes animal welfare, profitability, and global food security. Indirect effects of HS such as reduced feed intake contribute to, but do not fully explain, decreased productivity. Heat stressed animals initiate metabolic changes that do not reflect their plane of nutrition. This indicates that HS directly effects metabolism and productivity independent of reduced feed intake. In a variety of species, environmental hyperthermia compromises the intestinal barrier function resulting in increased permeability to luminal content including bacteria and bacterial components. Presumably, heat stress causes leaky gut in ruminants as well. The leakage of luminal content into the portal and ultimately the systemic circulation elicits an inflammatory response that may facilitate the detrimental effects of HS on animal agriculture. Identifying flexible management strategies (i.e. nutritional supplementation) to immediately decrease HS susceptibility without negatively influencing production traits would be of great value to global animal agriculture. Infertility and subfertility in production animals represent important economic, health and welfare issues. Non-successful breeding and embryonic mortality are major limitations to reproductive efficiency. Additionally, with increased requirements for efficient production of animal protein to feed a growing world economy, compromised fertility represents a global food security concern. This proceedings paper will focus chiefly on ruminant and swine reproduction for which the majority of knowledge has been acquired. The primary objective of this paper is to review an environmental stress (hyperthermia) and a physiological condition (bacterial infection) that both impair fecundity and fertility in domestic animal species. The secondary objective is to provide a brief summary of potential mitigation strategies to improve animal reproductive efficiency in the face of such stressors.

Keywords: heat stress, insulin, reproduction.

Resumo

O estresse térmico (HS) é um problema global que põe em risco o bem estar animal, rentabilidade e segurança alimentar global. Efeitos indiretos de HS como redução de ingestão alimentar contribuem para a queda em produtividade, sem, portanto, explicar por completo. Animais com estresse térmico iniciam mudanças metabólicas que não refletem seu plano de nutrição. Isso indica que HS afeta diretamente o metabolismo e produtividade independente da ingestão reduzida de alimentos. Em diversas espécies a hipertermia ambiental compromete a função da barreira intestinal resultando em uma permeabilidade aumentada ao conteúdo do lúmen, incluindo bactéria e componentes bacterianos. O estresse térmico presumidamente também causa vazamento no intestino de ruminantes. O vazamento de conteúdo do lúmen no portal e finalmente na circulação sistêmica gera uma resposta inflamatória que pode facilitar os efeitos prejudiciais do HS na agricultura animal. A identificação de estratégias de gerenciamento flexíveis (i.e. suplementação nutricional) para imediatamente diminuir a susceptibilidade ao HS sem influenciar de forma negativa os traços de produção seria de grande valor para a agricultura animal global.Infertilidade e subfertilidade em animais de produção representam questões econômicas, de saúde e bem estar importantes. A reprodução sem sucesso e mortalidade de embriões são grandes limitações para a eficiência reprodutiva. Adicionalmente, com requisitos crescentes para a produção eficiente de proteína animal para alimentar uma crescente economia mundial, a fertilidade comprometida representa uma preocupação global de segurança alimentar. Este estudo de procedimentos focará principalmente na reprodução de ruminantes e suínos para os quais a maior parte do conhecimento foi adquirido. O objetivo principal do estudo é revisar um estresse ambiental (hipertermia) e condição fisiológica (infecção bacteriana) que impedem a fecundação e fertilidade em espécies de animais domésticos. O objetivo secundário é fornecer um breve resumo de estratégias potenciais de mitigação para melhorar a eficiência de reprodução animal em face a tais estressores.

***Palavras-chave**: estresse térmico, insulina, reprodução.*

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Overview of heat stress

Economic impact

Heat stress negatively impacts a variety of dairy production parameters including milk yield, milk quality and composition, rumen health, growth and reproduction, and is a significant financial burden (~$900 million/year for dairy, and >$500 million/year in beef and swine in the U.S. alone; St. Pierre et al., 2003; Pollman, 2010). When the ambient temperature and other environmental conditions create a situation that is either below or above the respective threshold values, efficiency is compromised because nutrients are diverted to maintain euthermia as preserving a safe body temperature becomes the highest priority, and product synthesis (milk, meat, etc.) is deemphasized. Advances in management (i.e. cooling systems; Burgos et al., 2007) and nutritional strategies (West, 1999) have partially alleviated the negative impacts of HS on cattle, but productivity continues to decline during the summer. The detrimental effects of HS on animal welfare and production will likely become more of an issue in the future if the earth's climate continues to warm as predicted (Intergovernmental Panel on Climate Change - IPCC, 2007) and some models forecast extreme summer conditions in most U.S. animal producing areas (Luber and McGeehin, 2008). A 2006 California heat wave purportedly resulted in the death of more than 30,000 dairy cows (California Department of Food and Agriculture - CDFA, 2006) and a recent heat wave in Iowa killed at least 4,000 head of beef cattle (Drovers Cattle Network, 2011). Furthermore, almost 50% of Canadian summer days are environmentally stressful to dairy cows (Ominski et al., 2002). This illustrates that most geographical locales, including temperate and northern climates, are susceptible to extreme and lethal heat. Thus, for a variety of aforementioned reasons, there is an urgent need to have a better understanding of how HS alters nutrient utilization and ultimately reduces animal productivity. Defining the biology of how HS jeopardizes animal performance is critical in developing approaches (genetic, managerial, nutritional and pharmaceutical) to ameliorate current production issues and improve animal well-being and performance. This would help secure the global agricultural economy by ensuring a constant supply of animal products for human consumption.

Direct and indirect effects of heat stress

Reduced feed intake during HS is a highly conserved response among species and presumably represents an attempt to decrease metabolic heat production (Baumgard and Rhoads, 2012). It has traditionally been assumed that inadequate feed intake caused by the thermal load was responsible for decreased milk production (Beede and Collier, 1986; West, 1999). However, our recent results challenge this dogma as we have demonstrated disparate slopes in feed intake and milk yield responses to a cyclical heat load pattern (Shwartz et al., 2009). To test this, we employed the use of a thermoneutral pair-fed group in our experiments which allowed us to evaluate thermal stress while eliminating the confounding effects of dissimilar nutrient intake. Our experiments demonstrate that reduced feed intake only explains approximately 35-50% of the decreased milk yield during environmental-induced hyperthermia (Rhoads et al., 2009a; Wheelock et al., 2010; Baumgard et al., 2011). This indicates that HS directly effects nutrient partitioning beyond that expected by reduced feed intake.

An appreciation of the physiological and metabolic adjustments to thermoneutral negative energy balance (NEBAL; i.e. underfeeding or during the transition period) is prerequisite to understanding metabolic adaptations occurring with HS. Early lactation dairy cattle enter a unique physiological state during which they are unable to consume enough nutrients to meet maintenance and milk production costs and typically enter NEBAL (Baumgard and Rhoads, 2013). Negative energy balance is associated with a variety of metabolic changes that are implemented to support the dominant physiological condition of lactation (Bauman and Currie, 1980). Marked alterations in both carbohydrate and lipid metabolism ensure partitioning of dietary and tissue derived nutrients towards the mammary gland, and not surprisingly many of these changes are mediated by endogenous somatotropin which naturally increases during periods of NEBAL. One classic response is a reduction in circulating insulin coupled with a reduction in systemic insulin sensitivity. The reduction in insulin action activates adipose lipolysis, leading to the mobilization of non-esterified fatty acids (NEFA; Bauman and Currie, 1980). Increased circulating NEFA are typical in transitioning cows and represent (along with NEFA derived ketones) a significant source of energy (and precursors for milk fat synthesis) for cows in NEBAL. Postabsorptive carbohydrate metabolism is also altered by reduced insulin action during NEBAL resulting in reduced glucose uptake by systemic tissues (i.e. muscle and adipose). Reduced nutrient uptake coupled with the net release of nutrients (i.e. amino acids and NEFA) by systemic tissues are key homeorhetic (an acclimated response *vs*. an acute/homeostatic response) mechanisms implemented by cows in NEBAL to support lactation. The thermoneutral cow in NEBAL is metabolically flexible, and can depend upon alternative fuels (NEFA and ketones) to spare glucose. Glucose can then be utilized by the mammary gland to copiously produce milk (Bauman and Currie, 1980).

Well-fed ruminants primarily oxidize acetate (a rumen produced VFA) as a principal energy source. During NEBAL, cattle increase their energy dependency on NEFA. However, despite the fact that heat stressed

cows have marked reductions in feed intake and are losing considerable amounts of body weight, they do not mobilize adipose tissue (Rhoads et al., 2009a; Wheelock et al., 2010). Therefore, it appears that heat stressed cattle experience altered post-absorptive metabolism compared to thermoneutral counterparts, even though they are in a similar negative energetic state (Moore et al., 2005; Rhoads et al., 2013). The unusual lack of NEFA response in heat stressed cows is probably in part explained by increased circulating insulin levels (O'Brien et al., 2010; Wheelock et al., 2010), as insulin is a potent anti-lipolytic hormone. Increased circulating insulin during HS is unusual as malnourished animals are in a catabolic state and experience decreased insulin levels. We have recently demonstrated that heat stressed growing pigs undergo similar metabolic adaptations (Pearce et al., 2013a), suggesting that this is a well conserved response vital for the acclimation to HS. Increased insulin action may also explain why heat stressed animals have greater rates of glucose disposal (Wheelock et al., 2010). Therefore, during HS, preventing or blocking adipose mobilization/breakdown and increasing glucose "burning" is presumably a strategy to minimize metabolic heat production (Baumgard and Rhoads, 2013). The enhanced extra-mammary glucose utilization during HS creates a nutrient trafficking problem with regards to milk yield. The mammary gland requires glucose to synthesize milk lactose which is the primary osmoregulator determining overall milk volume. Therefore, the mammary gland may not receive adequate amounts of glucose resulting in reduced mammary lactose and subsequent milk production. This may be a primary mechanism accounting for additional reductions in milk yield beyond the portion explained by decreased feed intake.

Leaky gut: responsible for the direct effects of heat stress?

Mechanisms responsible for altered nutrient partitioning during HS are not clear, however, they might be mediated by HS effects on gastrointestinal health and function (Fig. 1). The small intestine is one of the first tissues up-regulating heat shock proteins during a thermal load (Flanagan et al., 1995), demonstrating a higher sensitive to heat damage (Kregel, 2002). During heat stress, blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat (Lambert et al., 2002), leading to intestinal hypoxia (Hall et al., 1999). Enterocytes are particularly sensitive to hypoxia and nutrient restriction (Rollwagen et al., 2006), resulting in ATP depletion and increased oxidative and nitrosative stress (Hall et al., 2001). This contributes to tight junction dysfunction, and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013b). As a result, HS increases the passage of luminal content as lipopolysaccharide (LPS) into the portal and systemic blood (Hall et al., 2001; Pearce et al., 2013b). Further, endotoxemia is common among heat stroke patients (Leon, 2007) and it is thought to play a central role in heat stroke pathophysiology, as survival increases when intestinal bacterial load is reduced (Bynum et al., 1979) or when plasma LPS is neutralized (Gathiram et al., 1987). It is remarkable how animals suffering from heat stroke or severe endotoxemia share many physiological and metabolic similarities such as an increase in circulating insulin (Lim et al., 2007). Infusing LPS into the mammary gland increased (~2 fold) circulating insulin in lactating cows (Waldron et al., 2006). In addition, we intravenously infused LPS into growing calves and pigs and demonstrated >10 fold increase in circulating insulin (Rhoads et al., 2009b; Stoakes and Baumgard, 2015, Iowa State University, Ames, IA, USA; unpublished). Again, the increase in insulin in both models is energetically difficult to explain as feed intake was severely depressed in both experiments.

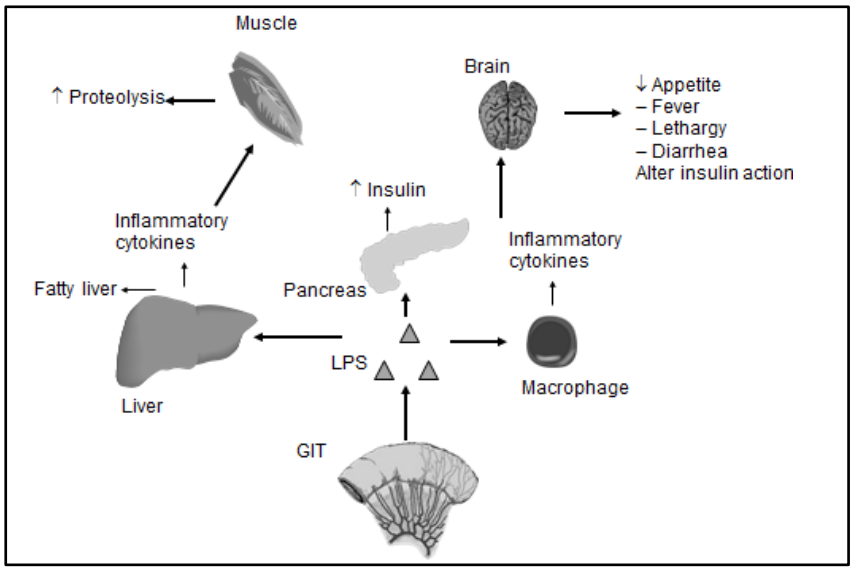

Figure 1. Etiology of heat stress induced leaky gut. Adapted from Baumgard et al., 2012.

Intestinal integrity & Stea to hepatitis

Interestingly, a variety of diseases associated with increased intestinal permeability such as heat stress and stroke, Crohn's disease, inflammatory bowel disease, Celiac disease, and alcoholism are often associated with increased plasma LPS concentrations and an inflammatory acute phase response (Draper et al., 1983; Bouchama et al., 1993; Parlesak et al., 2000; Ludvigsson et al., 2007; Pearce, et al. 2013b). There is increasing evidence that translocation of gut microbiota contributes to hepatic inflammation (Bieghs and Trautwein, 2013) which might impair liver function leading to fat accumulation and ultimately steatohepatitis (Solga and Diehl, 2003; Dumas et al., 2006; Farhadi et al., 2008; Ilan, 2012). The association between leaky gut and fatty liver is of particular interest in the ruminant animal who is already an inefficient exporter of hepatic lipids. There is reason to believe that similar breakdown of gut integrity may be responsible for hepatic disorders (e.g. fatty liver and ketosis; Fig. 2) frequently observed in the transition dairy cow. A transitioning dairy cow undergoes a post-calving diet shift from a mainly forage based to a high concentrate ration. This has the potential to induce rumen acidosis which can compromise the gastrointestinal tract barrier (Khafipour et al., 2009). In addition, calving is a physically stressful event and cytokines released from the damaged reproductive tract may have an impact on the liver's ability to export lipids. Preliminary data has shown an increase in plasma lipopolysaccharide binding protein (LPSBP), an acute phase protein which binds LPS to stimulate an immune response, in cows that required treatment for clinical ketosis compared to healthy transition cows (Nayeri et al., 2012). Nevertheless, the effects of the transition period on the intestinal barrier function and its role in the development of fatty liver and ketosis among other transition disorders remain unknown and require further investigation.

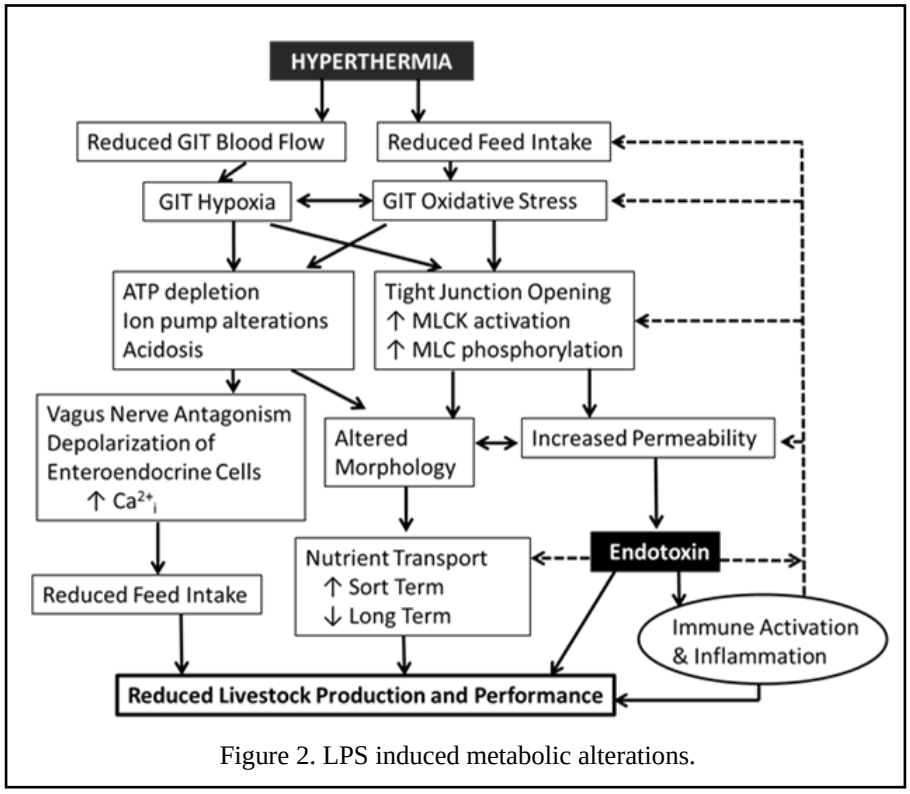

Figure 2. LPS induced metabolic alterations.

Overview of mammalian reproductive physiology

The ovary is the site of female gamete production and maturation as well as steroid hormone synthesis. At birth, the ovary is endowed with a finite number of oocytes housed in follicular structure. The pre-antral follicle is comprised of the oocyte and granulosa cells. Following antrum formation, another layer of cells, theca, are recruited to surround the granulosa cells. Within the antrum, follicular fluid provides nourishment to the developing oocyte (Hirshfield, 1991). The oocyte is arrested in the prophase I stage of meiosis, and will not resume and complete meiosis I and II until ovulation and fertilization, respectively. The vast majority of oocytes are lost to a process known as atresia, and only approximately 1% of oocytes which initially endow the ovary will ovulate. The female sex steroid hormones, 17β-estradiol (E2) and progesterone (P4) are produced by pre-ovulatory, dominant follicles and the corpus luteum (CL), respectively. Briefly, an upper E2 threshold is required to induce a surge of luteinizing hormone (LH) from the anterior pituitary in order to induce ovulation. In addition, LH regulates ovarian steroidogenesis and the process of luteinization by which CL formation occurs.

The CL produces P4, a hormone needed for implantation and pregnancy maintenance. In addition to inducing the LH surge, E2 is required for appropriate display of secondary female sex characteristics and is the dominant hormone involved in the demonstration of behavioral estrus. Should pregnancy occur, E2 synthesis and release from the developing porcine conceptus prevents the luteolytic (CL degradation) action of PGF2α, and the CL's, and thus the pregnancy, are maintained. Both E2 and P4 have specific ovarian receptors - the estrogen receptors α (ERα) and β (ERβ) and the progesterone receptor isoforms A and B (PRA and PRB). The process of folliculogenesis which comprises oocyte development and maturation within the follicular structure, and steroidogenesis for hormone production are essential for efficient reproduction. Any stressor that negatively affects either process will compromise fertility and fecundity.

Reproductive impacts of heat stress

Hyperthermia in swine

The swine industry suffers considerably due to impaired reproductive performance during periods of seasonal infertility, particularly during late summer and early autumn months (Pollmann, 2010). The impact is particularly visible in the U.S. with day 28 pregnancy rates reaching a nadir in August into October and subsequently reduced farrowing in November and December. This phenomenon is not limited to specific regions and occurs internationally (Auvigne et al., 2010; Pollmann, 2010). Several components can contribute to seasonal infertility, such as photoperiod and environmental conditions (i.e. temperature) and deciphering the precise contribution of each on swine reproductive performance is difficult. Despite that, heat stress has been repeatedly demonstrated to negatively impact reproductive efficiency in pigs by affecting gamete development, pregnancy establishment, maintenance of gestation, and lactation performance.

Folliculogenesis

The impact of heat stress during oocyte maturation and early embryonic development is evidenced in that sows exposed to hyperthermia for 5 days following breeding have significantly reduced number of viable embryos after day 27 of gestation, with control pigs possessing an average of 11.0 (68.8% survival) viable embryos and heat stressed sows containing only 6.8 (39.1% survival) viable embryos (Tompkins et al., 1967). In this study, heat stress was administered following breeding, which generally occurs prior to ovulation and complete oocyte maturation, as pigs typically ovulate in the mid to latter half of estrus (Soede et al., 1992).

Due to the difficulty for such studies *in vivo*, characterization of heat stress effects during oocyte growth and maturation and early embryonic development in pigs has been demonstrated using *in vitro* oocyte maturation and embryo culture systems. Some evidence of *in vitro* heat stress models during the transition between germinal vesicle breakdown and the 4-cell stage of development demonstrates the susceptibility of this stage to heat stress. A nine hour culture of pig embryos at 42^{o}C following porcine *in vitro* fertilization reduced blastocyst formation rate from 20.6 to 8.8% (Isom et al., 2007) and heat shock of 41.5^{o}C for 4 h following *in vitro* maturation also reduced oocyte development (Tseng et al., 2006).

We have also demonstrated the impact of *in vitro* heat stress during oocyte maturation and its impact on subsequent developmental competency. Oocytes exposed to heat stress (41^{o}C) for the first half (21 h) or the duration of (42-44 h) of *in vitro* maturation demonstrated impaired ability to reach metaphase II arrest while heat stress during only the second half (21 h) of *in vitro* maturation did not impact maturation rate (Wright and Ross, 2015, Iowa State University, Ames, IA, USA; unpublished data). Metaphase II arrested oocytes following heat stress during *in vitro* maturation demonstrated impaired developmental competency compared to oocytes matured at 39^{o}C, as measured by their ability to develop to the blastocyst stage following *in vitro* fertilization and culture in thermal neutral conditions. We have subsequently used this model to demonstrate differences in gene expression in developing 4- to 8-cell embryos as a result of heat stress conditions during *in vitro* maturation (Wright and Ross, 2015, Iowa State University, Ames, IA, USA; unpublished data).

Gestational impacts

The effect of heat stress during pregnancy in pigs is variable as different stages of gestation can be variably affected. This is demonstrated by a study conducted by Omtvedt et al. (1971) in which exposed pregnant gilts to heat stress for 8 days during different stages of gestation. Heat stress (37.8^{o}C for 17 h and 32.2^{o}C for 7 h) beginning either on day 0 or day 8 of gestation compared to thermal neutral conditions (constant 23.3^{o}C) reduced the number of viable embryos by day 30 of gestation. Interestingly, the same heat stress conditions administered on days 53-61 did not affect farrowing performance while heat stress during late gestation (days 102-110) resulted in a significantly increased number of dead piglets born and a 4 piglet reduction in the number of piglets born alive (Omtvedt et al., 1971). However, a more moderate, cyclic heat

stress, on bred gilts beginning on day 3 and extended to either day 24 or 30 of gestation did not impact embryo survival (Liao and Veum, 1994).

Lactation

Heat stress during lactation can also have a profound impact on production. Temperatures exceeding the evaporative critical temperature during lactation resulted in reduced feed intake and lowered milk production (Black et al., 1993). Elevated core body temperature results in the redirection of blood flow from the mammary gland towards the skin in an effort to facilitate heat dissipation. In response, lactation and piglet growth (during lactation) are reduced (McGlone et al., 1988; Black et al., 1993; Johnston et al., 1999). In addition to reduced performance, heat stress during lactation can also reduce the number of sows returning to estrus within 15 days post weaning (Johnston et al., 1999).

Semen quality

While the effects of heat stress on pig reproduction is notable, it is difficult to distinguish the consequences resulting from heat stress between the male or female. While it is clear that reproductive parameters in gilts and sows are affected by heat stress, exposure of boars to heat stress can also be detrimental to swine reproduction through impacts on semen quality. Boars subjected for heat stress for 90 days (34.5^{o}C and 31.0^{o}C for 8 and 16 h per day, respectively) demonstrated reduced motility and increased percentage of abnormal sperm within 2 weeks from the initiation of heat stress compared to thermal neutral boars (constant 23^{o}C; Wettemann et al., 1976). Utilization of semen from heat stress boars resulted in reduced number of embryos on day 30 post-insemination compared to thermal neutral boars (Wettemann et al., 1976). Similar results were demonstrated by Cameron and Blackshaw in boars exposed to heat stress demonstrated a significant increase in abnormal sperm in 2-3 weeks following initiation of heat stress (Cameron and Blackshaw, 1980).

Thermal stress effects on ruminant reproduction

The physiological effects of heat stress on productivity can be financially devastating for the animal production systems. During periods of heat stress, dry matter intake (DMI) decreases and maintenance requirements increase as livestock attempt to dissipate excess heat load (West, 1999). In addition, changes in blood flow and the production of various hormones ultimately result in decreased reproductive performance. During summer months, conception rates can decline by 20-30% (Rensis and Scaramuzzi, 2003). This observed reduction in fertility, is attributed to several factors, including a reduction in estrus detection ability, early embryonic death, inhibition of follicular dominance, and reduced ovarian steroidogenic output (Putney et al., 1988; Wolfenson et al., 2000; Rensis and Scaramuzzi, 2003). Thus, heat stress has a wide range of reproductive effects beginning with the developing follicle and continuing through early embryonic development. The biological mechanisms that mediate these effects, however, are not completely understood.

Inevitably, the decrease in DMI that occurs during periods of heat stress is accompanied by changes in circulating concentrations of several metabolic hormones. In turn, these metabolic adaptations alter the production and secretion of the hormones controlling the reproductive cycle (Wolfenson et al., 2000). Such consequences are far-reaching and may involve detrimental effects on ovarian follicular development, oocyte competence, early embryonic development and the maternal recognition of pregnancy.

During heat stress, the development of the dominant ovarian follicle is attenuated and circulating concentrations of E2 are lower. In addition, the luteal phase of heat-stressed cattle is extended and follicular wave dynamics are altered (Wilson et al., 1998). These changes in ovarian function appear to be the result of decreased LH pulse amplitude (Gilad et al., 1993). As a reminder, LH is directly involved in the processes of follicular growth, E2 production, ovulation and P4 production. These changes in LH pulsatility may simply be a consequence of lower feed consumption during heat stress (nearly a 35% decrease compared to thermal-neutral controls (Rhoads et al., 2009a). Decreased feed intake is associated with changes in circulating insulin, leptin and ghrelin, which have all been shown to affect LH pulsatility in several species (Szymanski et al., 2007).

Effects on the oocyte and embryo

Preovulatory oocytes can be damaged directly by heat stress, and indirectly by prolonged estrous cycles. These longer estrous cycles presumably result in the ovulation of an aged oocyte that has reduced potential for developmental competence. Oocytes contained within antral follicles appear to be the most susceptible to the damaging effects of heat stress. As a result, conception rates remain depressed extending into the fall as the oocytes that were damaged during the summer heat stress are cleared from the ovary via ovulation or degradation.

Reproductive impacts of infection

Lipopolysaccharide (LPS) is a marker of bacterial infection and is elevated in animals suffering from mastitis, as well as from leaky gut in the transition period. Additionally and interestingly, LPS is increased in hyperthermic animals. From a reproductive perspective, the LPS-induced poor fecundity phenomena is reported throughout the literature. Interestingly, follicular fluid that surrounds and nourishes the maturing oocyte contains LPS levels reflective of the systemic circulation. Thus, LPS is reaching the ovary via the systemic circulation and directly interacts with the oocyte proportionately as extra-ovarian tissues (Herath et al., 2007).

Folliculogenesis

Bovine ovarian cortical explants exposed to LPS had reduced numbers of primordial follicles, concomitant with increased atresia of the ovarian reserve (Bromfield and Sheldon, 2013). Similarly, mice exposed to LPS *in vivo* had reduced primordial follicle number which was described as a TLR4-mediated effect, since *Tlr4*$^{-/-}$ mice were refractory to LPS-mediated primordial follicle depletion (Bromfield and Sheldon, 2013).

Steroidogenesis

LPS alters the level of anterior pituitary hormones, through direct or indirect mechanisms. Using anestrous ewes as a model, LPS infusion decreased LH but stimulated systemic prolactin (PRL) and cortisol levels. Additionally, mRNA abundance of genes encoding LH (LHβ) and the LH receptor (LHR) were reduced by approximately 60% in both cases (Herman et al., 2010). Interestingly, the FSH and FSH receptor as well as PRL and PRL receptor genes were increased by LPS infusion (Herman et al., 2010).

LPS exposure did not impact cell number or androstenedione production from cultured theca cells from either small, medium or large ovarian follicles, but did reduce the amount of E2 produced from cultured granulosa cells isolated from all three follicular sizes (Williams et al., 2008). In an *in vitro* system where ovarian cortical explants were cultured with LPS and provided with FSH or androstenedione, E2 and P4 conversion was reduced; potentially due to the observed decreased expression of *Cyp19a* mRNA and protein, an enzyme critical for production of E2 (Price et al., 2013). Cultured granulosa cells had increased expression of TLR4, likely in response to mediating LPS signaling, and negative impacts of LPS on E2 production were demonstrated (Herath et al., 2007). While no overall impact of LPS on E2 was observed *in vivo*, a temporal decrease in bovine P4 concentrations and lower ovulation rates resulted from LPS treatment (Williams et al., 2008). In agreement with reduced E2 level, when LPS was infused into the uterine lumen, the pre-ovulatory LH surge was attenuated and may be the result of an insufficient stimulation from E2 driving the LH surge (Peter et al., 1989). Furthermore, LPS-treated females had delays in the time to the LH surge (Fergani et al., 2012).

In a regularly cycling animal, in the absence of fertilization and pregnancy, endometrial synthesis and release of PGF2α causes CL regression. LPS itself also causes CL regression by inducing the production of PGF2α (Moore et al., 1991; Hockett et al., 2000). LPS administration causes delayed ovulation, and lengthens the time to CL formation and sufficient P4 production (Suzuki et al., 2001; Lavon et al., 2011). Additionally, the size of CL are reduced by LPS (Herzog et al., 2012), perhaps due to activation of pro-apoptotic pathways (Herzog et al., 2012). Interestingly, a temporal pattern of LPS on circulating P4 has been demonstrated, whereby P4 is initially increased and then declines in LPS-treated, relative to their control females (Herzog et al., 2012).

Estrus behavior

Not surprisingly both heat stress (Doney et al., 1973; Sejian et al., 2010) and LPS (Battaglia et al., 2000) impact female estrus behavior and frequency. As in the case of the LH surge, a threshold of E2 is needed to induce behavioral display of estrus, however the amount required for the latter is thought to be at a lower level (Saifullizam et al., 2010). LPS-induced reductions in E_2 production may explain the observed impacts on behavior estrus display since E2 is required for this female phenotypic response.

Pre-term labor

P_4 is essential for pregnancy maintenance, and LPS reduces the PR in uteri of pregnant mice, thus impacting pregnancy maintenance (Agrawal et al., 2013). The effect of LPS on the ability of P4 to sustain gestation could cause spontaneous abortion, a phenotypic event frequently also associated with hyperthermia. Infection from gram negative bacteria or their outer wall components (LPS) triggers pre-term labor in many species (Koga and Mor, 2010), and, as a testament to the efficacy of LPS at inducing preterm labor, intraperitoneal LPS injection is an established experimental model for inducing pre-term labor (Deb et al., 2004; Agrawal et al., 2013). In addition, infertility can be the result of gynecological infections in both humans and production animals (Williams et al., 2008; Price et al., 2013).

Potential mitigation strategies

As might be expected, a major effort has gone into designing housing facilities for production animals that provides shade, misting and fans to cool animals, and these efforts have greatly ameliorated the occurrence of hyperthermia induced reproductive calamities in production animals. In addition, it is recognized that heat stressed animals do not consume the same amount of feed as their thermal neutral counterparts, however, "off feed" only accounts for approximately 50% of the heat stress-induced alterations to lactation (Baumgard, 2013), thus likely is also not the sole contributor to negative consequences of heat stress of reproduction. Thus, greater research remains to be done to bridge our knowledge gaps in terms of how to overcome the negative impacts of hyperthermia on reproduction.

Heat-stressed induced changes in ovarian dynamics ultimately result in unique challenges for reproductive management and potentially translating to the production of a substandard oocyte. Many producers now rely on timed artificial insemination programs during periods of heat stress because estrus detection ability is reduced as a result of reduced behavioral demonstrations of estrus. Indeed mounting activity declines by nearly half and is likely the result of lower circulating E_2 concentrations. Extended luteal phases during periods of heat stress also make it more difficult to predict when individual animals will come into estrus. Timed artificial insemination alleviates these challenges by allowing the producer to control the time of ovulation.

One management technique has shown promise for overcoming the oocyte-specific problems associated with heat stress. Conception rates by transferring fresh *in vitro*-produced embryos into heat stressed cattle (Stewart et al., 2011). Used as a management practice, this allows the producer to completely bypass the challenges associated with substandard oocyte quality. Currently, however, the advantage is only evident with the use of fresh embryos. Using frozen embryos yields conception rates that are similar to those resulting from timed artificial insemination during heat stress. This presents a logistical challenge since few producers have access to an economical source of fresh *in vitro*-produced embryos. The source of the oocytes is also a concern if the offspring are needed as replacement animals: collecting oocytes from genetically superior females (housed in a cool environment) is more costly, while the least expensive alternative is indiscriminately collecting oocytes from ovaries at the slaughterhouse. Depending on the geographical region, animals sent to the slaughterhouse vary widely in breed and genetics, and therefore would not be desirable as replacement animals. Future advances in *in vitro* embryo production and freezing will make this technique a more viable alternative for use during periods of heat stress.

There are a number of other avenues for exploration, however, including improving intestinal integrity to prevent "toxic" compounds from reaching the reproductive tract. Heat stressed animals are hyperinsulinemic (Baumgard, 2013), a biological paradox since they are consuming less feed. Systemic hyperinsulinemia could be reduced via pharmaceutical insulin-sensitizing agents, which could lessen blood insulin levels. In addition, compromised PR level and function could perhaps be overcome through supplementing with P_4, a strategy routinely used in humans at risk for preterm spontaneous abortion. These potential avenues for mitigation of infertility that results from exogenous exposures remain reliant on generation of science-based understanding of the problem.

Note: Portions of this review have been previously published in the 2014 South West Nutrition and Management Conference in Tempe, AZ, USA and the 2014 Ensminger School in Lima Peru: Advancing Animal Agriculture.

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