Review Article

Physiological mechanisms through which heat stress compromises reproduction in pigs†

Running Head: Reproductive consequences of heat stress in pigs

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Abbreviations: HSP, Heat-shock protein; LPS, lipopolysaccharide.

Quote: *[U]nderstanding the physiological implications and mechanisms employed in individual organs, or orchestrated through a whole animal response, is critical to establishing the core causes of heat induced losses in animal agriculture efficiency.*

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Abstract

Seasonal variations in environmental temperatures impose added stress on domestic species bred for economically important production traits. These heat-mediated stressors vary on a seasonal, daily, or spatial scale, and negatively impact behavior and reduce feed intake and growth rate, which inevitably lead to reduced herd productivity. The seasonal infertility observed in domestic swine is primarily characterized by depressed reproductive performance, which manifests as delayed puberty onset, reduced farrowing rates, and extended weaning-to-estrus intervals. Understanding the effects of heat stress at the organismal, cellular, and molecular level is a prerequisite to identifying mitigation strategies that should reduce the economic burden of compromised reproduction. In this review, we discuss the effect of heat stress on an animal's ability to maintain homeostasis in multiple systems via several hypothalamic-pituitary-end organ axes. Additionally, we discuss our understanding of epigenetic programming and how hyperthermia experienced in utero influences industry-relevant postnatal phenotypes. Further, we highlight the recent recognized mechanisms by which distant tissues and organs may molecularly communicate via extracellular vesicles, a potentially novel mechanism contributing to the heat-stress response.

Key Words: heat stress, reproduction, ovary

1. Heat stress compromises animal production efficiency

Successful mammalian reproduction requires optimally employing a combination of homeostatic and homeorhetic mechanisms that facilitate nutrient partitioning to produce healthy gametes, and ultimately the successful production and nourishment of healthy offspring (Bauman and Currie, 1980). While these physiological mechanisms are critical to survival under a variety of conditions, the capacity to maintain an organism's reproductive performance at a level consistent with its genetic potential is markedly compromised by environmental stresses, particularly thermal stress.

Assessing the economic costs of heat stress on animal agriculture is difficult. Heat stress costs the global swine industry millions of dollars annually due to poor sow reproductive performance alone, suppressed growth performance, and altered carcass composition (St-Pierre et al. 2003; Pollmann, 2010). While the economic severity of heat stress on animal agriculture varies depending on multiple factors, there is little doubt that most pig producing regions in the world experience production drags during (and following) the warm summer months. The negative effects of heat stress on pork production efficiency will likely escalate in the future as the combination of changing climate and the intensification of pork production in warm regions is occurring concomitant with genetic-selection emphasis on lean growth and reproductive performance creating a scenario that increases the susceptibility to thermal stress. Ultimately, all such factors will hinder efficient animal protein production and our ability to effectively meet the demands of a growing global population.

The numerous documented responses to heat stress can directly or indirectly influence reproduction in pigs (Figure 1). This review focuses on specific responses to heat stress known to occur in some tissues, and describes potential implications for other cells and tissues in the pig that may alter reproduction efficiency.

2. Seasonal Infertility and Heat Stress

The drop in reproductive competence due to seasonal infertility has been characterized in the United States of America (Hurtgen and Leman 1981), Finland (Peltoniemi et al. 1999), Germany (Wegner et al. 2016), and Thailand (Tummaruk et al. 2004). The manifestations of seasonal infertility include delayed puberty onset, decreased signs of estrus after weaning, and a reduction in the proportion of sows conceiving and maintaining pregnancy (Bertoldo et al. 2009; Hurtgen and Leman 1981; Love 1978; Omtvedt et al. 1971; Peltoniemi et al. 1999; Tompkins et al. 1967). Although photoperiod likely plays a role in seasonal infertility (Love et al. 1993), elevated temperatures during that time contribute directly or at least cumulatively to the negative effects on reproductive competence (Auvigne et al. 2010; Boma and Bilkei 2006; Prunier et al. 1994).

In mammals, metabolic heat, generated through processes such as muscle contractions, biochemical reactions, and digestion, can be offset by the body's ability to dissipate heat through convection or conduction (Mount 1978). This heat dissipation mechanism only works when the ambient temperature is lower than the body's thermal regulatory zone. When the ambient temperature rises above this level, mammals utilize evaporative cooling mechanisms, such as sweating and respiration (Mount 1978). Swine are especially susceptible to heat stress as the sweat glands they have are essentially non-functional, an increased metabolic rate due to genetic selection for efficient lean tissue accretion, and a substantial layer of subcutaneous adipose tissue

(Baumgard and Rhoads 2013; Brown-Brandl et al. 2001; O'Hea and Leveille 1968; Patience et al. 2005).

When ambient temperatures are above the level that evaporative cooling is effective, an animal regulates physiological and metabolomic changes, such as redistribution of blood flow from the body core to the periphery and reducing feed intake, in an effort to reduce heat retention and production (Hansen 2009). While successful at reducing the internal euthermic temperature, such changes can be detrimental to other physiological processes (Hansen 2009; Sakurada and Hales 1998). Indeed, physiological changes to reduce core temperature can cause disturbances in metabolism of water and protein, energy and mineral balance, enzymatic reactions, hormonal secretions, and blood metabolites (Marai et al. 2006).

Acclimation to heat stress involves changes in hormone abundance – i.e. epinephrine, leptin, prolactin, glucocorticoids, thyroid hormones, and somatotropins – that potentially dysregulate physiological processes (Bernabucci et al. 2010). For example, heat stress was shown to increase insulin circulation in cows (O'Brien et al. 2010; Wheelock et al. 2010), and pigs (Pearce et al. 2013; Sanz Fernandez et al. 2015). This increase in insulin could be due to an activated cellular stress response (Li et al. 2006), as heat is toxic to cells (Gao et al. 2013). Heat stress is associated with increased circulating pro-inflammatory cytokines, which lead to inflammation (Leon 2007; Leon et al. 2006), and has been shown to injure vascular endothelium (Lugo-Amador et al. 2004).

3. Whole-animal response to heat stress

A consistently observed initial response to a thermal load across species is the immediate reduction in nutrient intake, presumably a strategy to reduce metabolic heat (Baumgard and Rhoads, 2013). Corroborating this, the negative effects of heat stress in pigs has become increasingly apparent in recent years, suggesting that genetic selection for reproductive and lean tissue growth (heat-producing traits) contribute to their increasing sensitivity to heat (Renaudeau *et al.* 2011). The negative impact of heat stress on animal productivity has primarily been attributed to suppressed feed intake although recent experimental results challenge this dogma. When animals are provided equivalent nutrient intake, physiological responses still vary between heat-stress and thermal neutral environments (Baumgard and Rhoads, 2013; Pearce *et al.* 2013a), suggesting that heat stress exerts direct and indirect effects on physiological processes that affect animal health and productivity. However, quantifying the consequences of heat stress on animal productivity in relation to reductions in feed intake is difficult as weight loss is different between heat-stressed animals and pair-fed counterparts in thermal neutral conditions (Prunier *et al.* 1997; Pearce *et al.* 2013a; Sanz Fernandez *et al.* 2015a). While heat stress-induced reductions in feed intake compromise productivity in growing animals, heat stress is also likely altering tissue synthesis priorities through other direct and/or indirect mechanisms.

3.1 Heat stress compromises intestinal integrity

Heat stress compromises gastrointestinal health and its effectiveness as a biological barrier. In an attempt to dissipate heat and to reduce core temperatures, blood flow is diverted to the periphery, resulting in intestinal hypoxia, which contributes effectiveness of the intestinal barrier through compromised tight junctions (Hall *et al.* 1999; Lambert *et al.* 2002; Pearce *et al.* 2013b). As a

consequence, heat stress enables the passage of lipopolysaccharide (LPS) into circulation (Hall *et al.* 2001; Pearce *et al.* 2013b), which in turn may impact reproductive ability through numerous mechanisms (Ross et al., 2015). Circulating LPS may also be a contributing factor to the increased insulin secretion associated with heat stress in multiple species (Baumgard et al., 2016).

3.2 Endocrine-mediated responses to stress influence reproduction

Pigs are subject to multiple events of acute and chronic stress throughout their productive lifetime including weaning, mixing, and increased temperatures. In particular, prolonged chronic stressors inhibit normal reproductive function (Turner 2002) via specific endocrine-related pathways that control homeostasis and homeorhesis. Sows that successfully achieve estrus after the stress associated with weaning exhibit lower plasma concentrations of cortisol and beta-endorphin and higher concentrations of Luteinizing hormone at the time of weaning, compared to those who remained anestrous after weaning (Tsuma *et al.* 1995). With respect to heat stress, pigs chronically exposed to increased ambient temperatures have increased plasma cortisol levels (Hao *et al.* 2014) while serum testosterone in boars is decreased after 5 days of heat stress (Wettemann *et al.*1979), underscoring the sensitivity of the hypothalamic pituitary axes. Additionally, when stress is simulated through repeated injections of synthetic Adrenocorticotropic hormone during the window of estrus, reproductive performance is inhibited (Brandt 2006), resulting in increased loss of oocytes and embryos, shortened standing estrus length, and altered progesterone, Luteinizing hormone, Inhibin alpha, and estradiol production.

While the mechanisms through which stress alters endocrine signaling are not completely clear, evidence suggests that the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes are particularly sensitive to stresses, including heat stress. When stress is perceived, the hypothalamic-pituitary-adrenal axis is activated, resulting in increased glucocorticoid levels. Glucocorticoid production is critical in the "fight-or-flight" response, and in reallocating biological resources towards resuming homeostasis. This response ultimately suppresses reproductive function (Einarsson *et al.* 2008) by imposing negative feedback on the hypothalamus by impeding Gonadotropin-releasing hormone production, which affects the downstream release and action of gonadotropins. For example, dairy cows subjected to heat stress exhibit decreased Luteinizing hormone and estradiol levels, which are believed to contribute to seasonal dips in fertility (Madan & Johnson 1973). Additionally, stress-induced glucocorticoids regulate kisspeptin neurons in the hypothalamus, which potentially contributes to infertility in mice (Wang *et al*, 2011). Such inhibition of gonadotropins has significant downstream effects, impacting steroidogenesis and inhibiting meiotic development of the pig oocyte (Yang 1999).

3.3 Metabolic response and insulin dysregulation during heat stress

Basal levels of plasma non-esterified fatty acid are typically suppressed in mammals (Sanders *et al.* 2009; Shwartz *et al.* 2009; Pearce *et al.* 2013a) in response to heat stress; particularly in comparison to thermo-neutral control animals pair-fed to mimic heat-stressed-induced reductions in feed intake (Rhoads *et al.* 2009; Sanz Fernandez *et al.* 2015a). The presumed dampening of the lipolytic capacity of adipose tissue in the face of reduced nutrient intake is perplexing considering the similar energetic and catabolic states are normally associated with increased non-esterified fatty acid levels (Baumgard and Rhoads, 2013). The heat-induced blunted adipose

mobilization is a likely reason why pigs harvested late in the summer and early fall can be fatter than bioenergetically predicted (Johnson et al., 2015a).

The altered insulin dynamic is another intriguing hallmark associated with heat stress that can influence reproduction. Basal and stimulated insulin concentrations increase in a variety of animal models in response to heat-stress (Wheelock *et al.* 2010; O'Brien *et al.* 2010; Pearce *et al.* 2013a; Sanz Fernandez *et al.* 2015a). This phenomenon is especially apparent when compared to thermo-neutral animals on a similar plane of nutrition (Baumgard and Rhoads, 2013). The increase in insulin, during an intensely catabolic condition induced by heat stress is paradoxical, for which the purpose may be involved with respect to insulin's role in activating the molecular and cellular stress response (Li *et al.* 2006). Using the hyperinsulinemic-euglycemic clamp technique, it has been demonstrated that increased whole-body insulin sensitivity occurs in growing calves (Rhoads *et al.* 2009) and pigs (Sanz Fernandez *et al.* 2015b) during heat stress. Although not entirely clear as to which tissue(s) or cell types are responsible for the induced increase in glucose disposal, recent evidence suggests that the immune system (activated by intestinally derived LPS) is likely a large contributor to the overall improved insulin sensitivity observed during heat stress (Sanz Fernandez et al., 2015b). While elevated circulating insulin during heat stress is presumably a necessary metabolic response to ensure survival, it can also negatively affect intracellular signaling pathways essential for successful reproductive function as outlined below.

4. Female reproductive fecundity is compromised during heat stress

In North America, annual seasonal infertility and the accompanying reproductive efficiency nadir parallels matings in late summer. While seasonal infertility is related to multiple environmental factors including photoperiod, the compromised reproductive ability is closely associated with periods of elevated ambient temperatures. Indeed, elevated temperature above the thermo-neutral zone compromises farrowing rates and is thought to delay puberty onset (Bertoldo *et al.* 2009). Thus, heat stress is likely a causative factor contributing to seasonal infertility (Love, 1978; Prunier *et al.* 1994), and probably negatively impacts reproduction through numerous mechanisms, including compromised early embryo development (Tompkins *et al.* 1967; Omtvedt *et al.* 1971).

Heat stress tolerance and the effect of temperature on fertility appear to be genetically influenced. As an example, selection of sows for improved farrowing rate resulted in an increased susceptibility to heat stress realized via reduced litter size and number of pigs born (Bloemhof *et al.* 2008). The current understanding of how exactly heat stress compromises gilt and sow fertility is not sufficient although likely involves alterations in the production of gametes and embryos altering developmental ability. As example, the developmental competence of in vitro fertilization-derived and parthenogenetically activated pig embryos is compromised as a result of heat stress (Isom *et al.* 2007b; Bertoldo *et al.* 2010; Pennarossa *et al.* 2012). Heat-shock protein (HSP), so named because of their increase in abundance in response to heat stress (Tissiéres *et al.* 1974), are present in the ovary (Driancourt *et al.* 1999; Guzeloglu *et al.* 2001; Maniwa *et al.* 2005; Narayansingh *et al.* 2004; Pennarossa *et al*., 2012; Salvetti *et al.* 2009), indicating that a hyperthermia response can impact developing oocytes. While the direct effects of heat stress on gamete function are consequential and compromise reproduction, this outcome likely represents a compounded, downstream effect of heat stress on somatic tissues and

organs throughout the animal – which affect the gonads through altered endocrine signaling.

5. Molecular regulation of the heat stress response

Extracellular vesicles are vehicles of intercellular signaling. These nanometer-sized, membrane-bound containers shuttle molecular messages (DNA, micro RNAs, mRNAs, and proteins) to and from different cells (Valadi et al., 2007; Yáñez-Mó et al., 2015). Since their first defined role in immunological processes (Raposo et al., 1996), many types of extracellular vesicles were identified – including exosomes, microvesicles, and apoptotic bodies – that have been classified on the basis of size and biogenesis (Colombo et al., 2014; Yáñez-Mó et al., 2015). Exosomes, (30-100 nm in diameter) are derived from an intracellular source, a multi-vesicular body that fuses with the plasma membrane to release its vesicular contents (Colombo et al., 2014). Microvesicles (up to 1 μm in diameter) are generated directly from the plasma membrane (Colombo et al., 2014). Other classifications of extracellular vesicles are based on protein markers, such as tetraspanins CD9 and CD63 on exosomes – although recent evidence revealed that traditional exosome markers are also found in other extracellular vesicles (Kowal et al., 2016). Different subtypes can also be found within the same extracellular vesicle class sizes, exemplifying the degree of molecular heterogeneity and complexity within a certain population (Kowal et al., 2016). All extracellular vesicles, regardless of the specific variety, are released into the extracellular milieu, and can be taken up by cells in a paracrine or endocrine fashion (Yáñez-Mó et al., 2015). Importantly, in vitro and in vivo experiments demonstrated cellular uptake of exogenous extracellular vesicles, with functional consequences in the targeted cell that are mediated by the molecular cargo within the vesicles (Tian et al., 2014; Ruiz-Gonzalez et al., 2015).

5.1 Molecular response of extracellular vesicles during heat stress

The molecular content of extracellular vesicles changes in response to heat stress, mainly involving an increased packaging of heat shock proteins. Exposing B cells to a three-hour heat challenge in vitro increased packaging of HSP27, -70, -90, and Heat shock cognate 70, but not HSP60 or GP96 (Clayton et al., 2005). Jurkat cells also demonstrated increased packaging of HSP70 and -90 in response to in vitro heat stress (Clayton et al., 2005). The intensity of the heat stress may also proportionally affect HSP concentration in extracellular vesicles, as HSP70 content increased from a mild temperature to a more intense temperature in human peripheral blood mononuclear cells (Lancaster and Febbraio, 2005). Non-immune related cells also demonstrated increased HSP packaging: HSP60, -70, -90, and HSC70 protein content increased in 3LL (Lewis lung carcinoma) cells after exposure to heat stress (Chen et al., 2011). Collectively, these studies demonstrate that one response to hyperthermic stimulation shared among different cell types is the packaging of more heat-sensitive molecular cargo. Such elevation in extracellular vesicle-loaded HSP content could represent one mechanism by which immune cells become activated (De Maio, 2011). This cellular response may represent an adaptive strategy under heat stress that allows tissues throughout the body to communicate regarding the perceived presence and severity of the stress itself.

5.2 Extracellular vesicles as mediators of endocrine signaling

The export and delivery of molecular information via circulating extracellular vesicles creates a novel concept of ‘organ cross-talk’ via non-classical endocrine mechanism, whereby different cell types and tissues can communicate to each other during particular physiological states

(Zierath and Wallberg-Henriksson, 2015). For example, extracellular vesicles may influence reproduction by affecting important biological roles within the follicle via their micro RNA and small RNA cargo (Di Pietro 2016; Navakanitworakul et al., 2016). We hypothesize that extracellular vesicles of major metabolic organs may help coordinate the heat-induced alterations in carcass composition and metabolism observed in pigs under heat stress. For instance, under certain stimuli, adipocyte-derived extracellular vesicles can deliver lipogenic mRNAs (in the form of lipid droplet structural proteins, triacylglyceride-synthesizing enzymes, and adipokines) to recipient adipocytes, which respond by increasing triacylglyceride esterification (Muller et al., 2011). Whether or not this extracellular vesicle-mediated transfer of lipogenic or anti-lipolytic (Muller *et al.*, 2010) information contributes to the increased carcass adiposity in conjunction with increased circulating insulin under heat stress is of interest. Adipose-derived extracellular vesicles can also influence insulin action directly on hepatocytes (Kranendonk *et al.*, 2014) or indirectly in muscle cells, via activation of macrophages in vitro (Deng et al., 2009), which may contribute to the changes in insulin sensitivity of pigs under heat stress (Sanz Fernandez *et al.*, 2015b). Thus, extracellular vesicles represent a novel form of intra-organismal communication that may help explain some of the unique body composition and reproductive phenotypes observed during heat stress.

5.3 Elevated insulin alters ovarian function

As described above (Section 3.3), insulin secretion increases in response to heat stress in numerous species, and this hyperinsulinemia may impact the ability of the ovary to function. Insulin could affect oocyte development through the Phosphatidylinsoitol-3 kinase (PI3K) pathway (Kasuga, 1996). Indeed, reproductive issues that cause reduced fertility are concomitant with increased insulin levels in specific physiological conditions, such as obesity or polycystic ovary syndrome. The potential connection between oocyte viability and elevated insulin seems plausible as Insulin receptor gene expression is elevated in ovaries of gilts during heat stress (Nteeba *et al.* 2015), suggesting an increased ovarian sensitivity to insulin during heat stress. In addition, expression of genes that encode steroidogenic enzymes are altered in the ovary of pigs exposed to heat stress (Nteeba *et al.* 2015). Follicular fluid composition changes can differ as a result of environmental conditions (Gosden *et al.* 1988; Fortune, 1994), implying that the oocyte microenvironment can also be affected due to heat stress. Therefore, our working hypothesis is that reproduction can be compromised through heat stress-induced hyperinsulinemia that subsequently compromises the production of female gametes that are capable of producing healthy offspring.

Development and maturation of the oocyte represents a vulnerable stage of female reproduction that is affected by heat stress. Studying early reproductive effects in vivo is logistically arduous, which explains why studies investigating the effects of heat stress during oocyte maturation and embryo development in pigs are primarily conducted using in vitro systems. Maturation of the oocyte in the laboratory mimics the developmental processes that would occur in vivo during the follicular phase in a female until ovulation occurs, when behavioral estrus is approximately 55-60% complete (Soede and Kemp, 1997). Our group also applied heat stress during in vitro oocyte maturation to explore its influence on subsequent developmental capability. Cumulus-oocyte-complexes heat stressed during the first half of in vitro maturation exhibited a reduced capacity to reach metaphase II (Wright and Ross, unpublished data). Furthermore, oocytes that did reach metaphase II following a heat-stress challenge during their maturation demonstrated

impaired embryonic development following in vitro fertilization compared to those matured in thermo-neutral conditions. While heat stress can directly impair oocyte quality, usually via incomplete meiosis or poor early development following fertilization, additional biological responses to heat stress Heat stress applied during in vitro oocyte maturation and development prior to activation of the zygotic genome (at the 4-cell stage in pigs) clearly established the sensitivity of oocytes and early embryos to heat (Isom *et al.* 2007a, Tseng *et al.* 2006). The increased sensitivity of early-stage embryos to heat stress therefore identified a potential source of the phenotypic observations during seasonal infertility: the reduced ability of early-stage embryos to mount a heat-shock response (Sakatani *et al.* 2012).

5.4 Ovarian function is negatively impacted by exposure to LPS

In a number of species, LPS exposure in the ovary negatively affects ovarian function. In rodents, LPS reduces primordial follicle number mediated through Toll-like receptor 4 (TLR4) signaling, given that *Tlr4*-null mice were impervious to the atretic effects of LPS (Bromfield and Sheldon 2013). This LPS effect on the ovarian follicular reserve was also observed in bovine ovarian cortical strips exposed to LPS in culture, which had fewer primordial follicles (Bromfield and Sheldon 2013). This exposure paradigm is consistent with the detection of LPS in follicular fluid (Herath *et al.* 2007) – an observation that also demonstrates that LPS can reach the ovary under physiological conditions that increase systemic, circulating levels of it.

Circulating LPS can also impact the hypothalamic-pituitary-ovary axis: LPS suppresses Luteinizing hormone release, but increases Prolactin and cortisol concentrations in non-cycling ewes (Herman *et al.* 2010). Indeed, expression of genes encoding Luteinizing hormone (*LHB*) and Luteinizing hormone receptor (*LHR*) were reduced, whereas expression of genes encoding FSH and FSH receptor (*FSH* and *FSHR*, respectively) and Prolactin and Prolactin receptor (*PRL* and *PRL* receptor) were increased by LPS (Herman *et al.* 2010). Taken together, LPS can impact ovarian function as well as alter the female endocrine milieu.

5.5 Molecular response of the ovary to heat stress

Little is known about the specific in vivo response of ovarian cells to heat stress. Heat stress can reduce the size of dominant follicles in the first third (Badinga et al. 1993) or in the second half (Wilson et al. 1998a; Wilson et al. 1998b) of the estrus cycle in cows. In goats, heat stress delays follicular recruitment and lowers blood estradiol concentration (Ozawa et al. 2005). Heat stress increased the susceptibility of granulosa cells in culture to apoptosis as well as decreased estrogen and prostaglandin secretion by granulosa cells in culture (Luo *et al.* 2016; Shimizu *et al.* 2005). By far the most-studied molecular response to thermal stress among different, cultured cell types involves the HSPs, which assist in the non-covalent assembly or disassembly of protein structures (Ellis 1997; Lindquist 1986). HSP70 may partly regulate hormone synthesis within the ovary (Sirotkin 2010), while HSP90 can act as an inhibitor of ovarian aromatase activity and estrogen production (Driancourt *et al.* 1999). Heat stress increases HSP70 in porcine granulosa cell culture as well as decreases cell proliferation (Sirotkin 2010). Heat stress also increases the mRNA abundance of an array of HSP family genes in cultured, bovine granulosa cells (Li *et al.* 2016).

5.6 Heat stress-induced autophagy signaling in the ovary

Autophagy is a mechanism through which somatic cells reutilize cellular components. The primary forms of autophagy include chaperone-mediated autophagy, microautophagy, and macroautophagy. Macroautophagy is the most-characterized process, as it plays a large role in homeostasis and stress response (Klionsky 2005). During autophagy, cytoplasm is sequestered via the formation of the autophagosome. After the autophagosome has formed around its target components, the vesicle itself fuses with the lysosome, which causes the degradation of the autophagosome contents (Klionsky and Emr 2000). The process of autophagy consists of induction, autophagosome formation, autophagosome-lysosome fusion, and ultimately degradation (Pyo *et al.* 2012). This step-wise process is regulated largely at the post-translational level, and involves the formation of large protein complexes (Mizushima 2010; Mizushima *et al.* 2011).

Autophagy, whether basal levels or stress-induced, has been observed in the oocyte and embryo, and an inadequate abundance of autophagy-related gene expression appears to negatively influence embryonic development (Cecconi *et al.* 2008; Fimia *et al.* 2007; Qu *et al.* 2007; Zeng *et al.* 2006). Embryonic induction of autophagy may also occur in response to external stressors (Adastra *et al.* 2011; Xu *et al.* 2011). For example, knock-out of Autophagy-related gene 5 (*Atg5*) in mice results in embryos that do not progress beyond the 4-cell embryo stage (Tsukamoto *et al.* 2008). Furthermore, autophagy signaling is altered in the pig ovary as a result of heat stress applied during the follicular phase of estrous cycle. Specifically, heat stress elevated the abundance of markers of autophagy induction, Beclin 1 and LC3-II (Hale et al., 2017). Beclin 1 is responsible in autophagosome formation and has the capacity to respond to numerous points of regulation (Funderburk *et al.* 2010; Liang *et al.* 1998; Pyo *et al.* 2012; Wurmser *et al.* 1999) whereas LC3-II is a core constituent facilitating autophagosome membrane expansion.

Autophagy and apoptosis share upstream regulators that are important for cell fate decisions (Mukhopadhyay et al; 2014). One manner in which the regulation of both autophagy and apoptosis is regulated is through BCL2, which can interact with Beclin 1 or BAX to inhibit either autophagy or apoptosis, respectively. Initially, low-level phosphorylation of BCL2 is thought to decrease interactions with Beclin 1, enabling activation and induction of autophagy. BCL2 can be hyper-phosphorylated, which then decreases its interaction with BAX and promotes induction of apoptosis. Therefore, BCL2 phosphorylation likely represents a mechanism that cells use to distinguish responses to different levels of stress.

6. Developmental consequences of heat stress

Epigenetics refers to the regulation of a cellular phenotype by chromatin modifications that affect gene expression. The most frequent epigenetic changes involve histone modification and DNA methylation, albeit other modifications exist. A minor change to the epigenetic code can have long-term phenotypic effects throughout life (and in some cases, across generations) as these epigenetic modifications can influence cell-specific functions later in life in response to specific environmental cues or physiological states.

6.1 Developmental imprinting is subject to epigenetic mechanisms

Methylation of DNA is a relatively firm alteration either inherited or acquired during life though regulated tightly during development (Rivera and Ross, 2013). Following fertilization, the demethylated single cell zygote begins development and is subject to cell specific genetic reorganization and reprogramming facilitating cellular differentiation (Reik, 2007; Kelly and Trasler, 2004). Examples of *in utero* conditions, including maternal nutrition, resulting in permanent physiological consequences are widespread (Roseboom *et al.* 2006; Tobi *et al.* 2014; Boddicker et al., 2016).

The postnatal effects of prenatal environment are still being explored in agriculturally important animals. Experimental approaches utilizing intrauterine growth retardation in pigs results in compromised lifetime performance, altered skeletal muscle and carcass quality (Bee, 2004; Foxcroft *et al.* 2006; Cerisuelo *et al.* 2009; Foxcroft *et al.* 2009). Programming of the epigenetic code and resulting changes in the endocrine milieu in mammalian offspring as a result intrauterine growth retardation is thought to be analogous to that observed in animals exposed to prenatal heat stress (Ross et al., 2015). For example, epigenetic conditioning as a result of heat stress exposure in some species has conferred thermal tolerance later in life, as demonstrated in chickens (Yahav and McMurtry, 2001), may be the result of histone code modifications facilitating an epigenetic molecular memory (Kisliouk *et al.* 2010).

6.2 Heat stress during gestation in pigs impacts offspring

The hypothesis that heat stress exposure *in utero* alters postnatal pig performance was tested and these studies demonstrate that exposure to heat stress altered back-fat depth and elevated circulating insulin in offspring later in life (Boddicker *et al.* 2014). This study was replicated and offspring growth performance of pigs from dams subjected to heat stress during gestation was examined. Remarkably, during the lipid accretion growth phase (60 to 90 kg), *in utero* heat stress exposure resulted in a more efficient accretion of adipose tissue compared to piglets from dams gestated in thermal neutral conditions (Johnson *et al.* 2015c). However, this observation was not observed in piglets from heat-stressed dams during the growth phase favoring lean tissue accretion (30 to 60 kg) suggesting that the heat-induced epigenetic imprint established during gestation only manifests at specific stages of production later in life (Johnson *et al.* 2015b).

In utero exposure to heat stress also appears to impact the thermoregulation of piglets later in life (Johnson *et al.* 2013). Pigs developed during *in utero* heat stress experienced elevated body temperature (~0.3˚C), regardless of postnatal environmental conditions (Johnson *et al.* 2013; Johnson et al., 2015d). These differences in body temperature, while small, accumulate over a lifetime, resulting in significant effects on maintenance costs and feed efficiency – assuming the increase in body temperature is due to endogenous heat production (Johnson *et al.* 2015a). A better understanding of the mechanisms through which body composition and body temperature regulation can be altered as a result of gestational heat stress exposure was achieved by employing RNA-sequencing on several tissues to identify alterations in mRNA expression patterns that may be causative for these phenotypes associated with gestational heat exposure (Boddicker *et al.* 2015).

Conclusion

As is the trend in agriculturally important species, genetic selection aimed at improving growth and fecundity may have unintentionally increased heat susceptibility, which has inevitably led to reproductive inefficiency during the warm summer months. Thus, it is pertinent that we understand the mechanisms through which heat stress affects productivity and how critical milestones in management practices can be modulated (Bloemhof et al., 2013) to mitigate the deleterious effects brought on by exposure to climate extremes. However, with the growing tendency of pork production moving to warmer climates, there is also an urgent need to select pig lines for heat tolerance in addition to methods to alleviate the incidence of heat stress. Differences in heat stress tolerance as measured by the differences in reproductive performance are an indication of genetic differences in sow lines (Bloemhof et.al. 2014). Although both reproductive performance and heat tolerance have low heritability, and the inheritance of thermoregulatory traits is poorly described in pigs, there seems to be enough genetic variation between breeds and/or lines to potentially achieve an improvement through genetic selection or crossbreeding or both (Gourdine et al., 2006; Bergsma and Hermesch, 2012). In addition, understanding the physiological implications and mechanisms employed in individual organs, or orchestrated through a whole animal response, is critical to establishing the core causes of heat induced losses in animal agriculture efficiency so that mitigation and genetic selection strategies can be fully effective.

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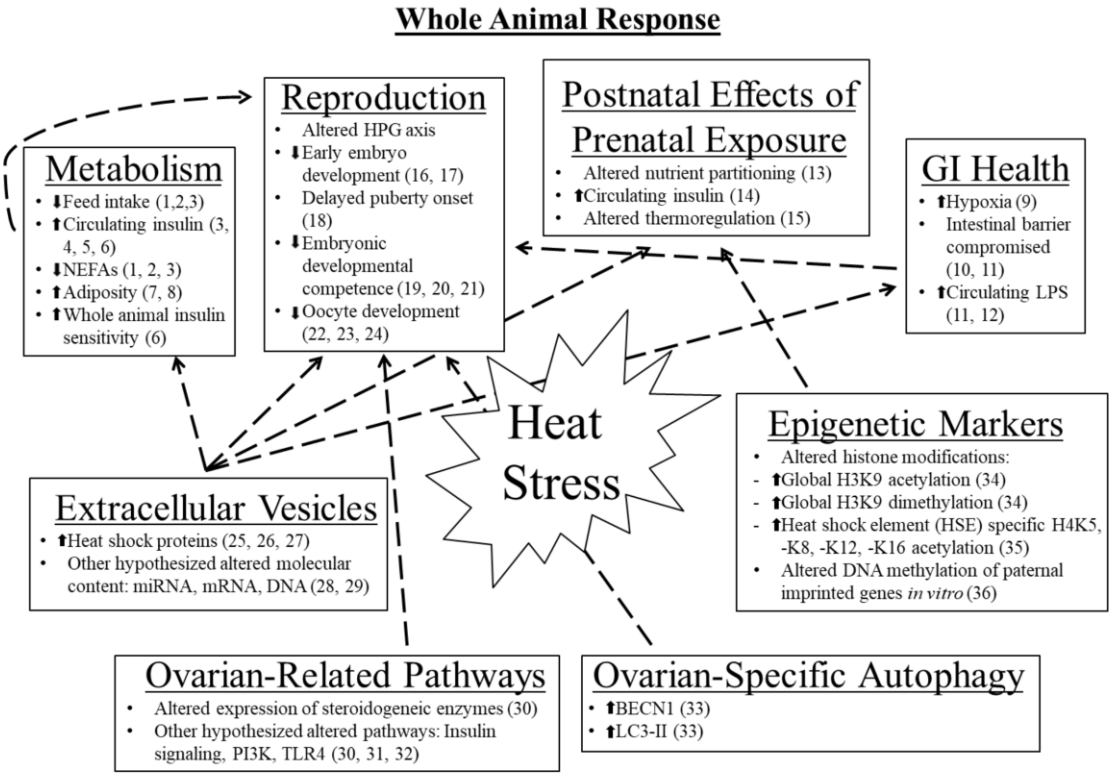
Figure Legend

Figure 1. The effects of heat stress at the whole-animal and the molecular and cellular levels. Although heat stress has direct impacts on swine reproduction, the heat-stress response is complex and systematically involves many non-reproductive related organs, which in turn may influence reproductive processes. Each effect is listed with its associated citation. Dashed arrows indicate potential crosstalk of one component impacting the processes of another.



1	Sanders et al. 2009	19	Isom et al. 2007b
2	Shwartz et al. 2009	20	Bertoldo et al. 2010
3	Pearce et al. 2013a	21	Pennarossa et al. 2012
4	Wheelock et al. 2010	22	Wright, Ross, et al., unpublished results
5	O'brien et al. 2010	23	Tseng et al. 2006
6	Sanz Fernandez et al. 2015	24	Isom et al. 2007a
7	Baumgard and Rhoads 2013	25	Clayton et al. 2005
8	Johnson et al. 2015a	26	Lancaster and Febbraio 2005
9	Hall et al. 1999	27	Chen et al. 2011
10	Lambert et al. 2002	28	Valadi et al. 2007
11	Pearce et al. 2013b	29	Yáñez-Mó et al. 2015
12	Lambert et al. 2002	30	Nteeba et al. 2015
13	Johnson et al. 2015c	31	Kasuga, 1996
14	Boddicker et al. 2014c	32	Bromfield and Sheldon 2013
15	Johnson et al. 2013	33	Hale et al. 2017
16	Tompkins et al. 1967	34	Kisliouk et al. 2009
17	Omtvedt et al. 1971	35	Tetievsky and Horowitz 2010
18	Bertoldo et al. 2009	36	Zhu et al. 2008

Figure 1