

## Physiological consequences of heat stress in pigs

J. W. Ross<sup>A,C</sup>, B. J. Hale<sup>A</sup>, N. K. Gabler<sup>A</sup>, R. P. Rhoads<sup>B</sup>, A. F. Keating<sup>A</sup> and L. H. Baumgard<sup>A</sup>

<sup>A</sup>Department of Animal Science, Iowa State University, Ames, IA 50046, USA.

<sup>B</sup>Department of Animal and Poultry Sciences, Virginia Tech University, Blacksburg, VA 24061, USA.

<sup>C</sup>Corresponding author. Email: [jwross@iastate.edu](mailto:jwross@iastate.edu)

**Abstract.** Heat stress negatively influences the global pork industry and undermines genetic, nutritional, management and pharmaceutical advances in management, feed and reproductive efficiency. Specifically, heat stress-induced economic losses result from poor sow performance, reduced and inconsistent growth, decreased carcass quality, mortality, morbidity, and processing issues caused by less rigid adipose tissue (also known as flimsy fat). When environmental conditions exceed the pig's thermal neutral zone, nutrients are diverted from product synthesis (meat, fetus, milk) to body temperature maintenance thereby compromising efficiency. Unfortunately, genetic selection for both increased litter size and leaner phenotypes decreases pigs' tolerance to heat, as enhanced fetal development and protein accretion results in increased basal heat production. Additionally, research has demonstrated that *in utero* heat stress negatively and permanently alters post-natal body temperature and body composition and both variables represent an underappreciated consequence of heat stress. Advances in management (i.e. cooling systems) have partially alleviated the negative impacts of heat stress, but productivity continues to decline during the warm summer months. The detrimental effects of heat stress on animal welfare and production will likely become more of an issue in regions most affected by continued predictions for climate change, with some models forecasting extreme summer conditions in key animal-producing areas of the globe. Therefore, heat stress is likely one of the primary factors limiting profitable animal protein production and will certainly continue to compromise food security (especially in emerging countries) and regionalise pork production in developed countries. Thus, there is an urgent need to have a better understanding of how heat stress reduces animal productivity. Defining the biology of how heat stress jeopardises animal performance is critical in developing approaches (genetic, managerial, nutritional and pharmaceutical) to ameliorate current production issues and improve animal wellbeing and performance.

**Additional keywords:** epigenetics, metabolism, reproduction, swine.

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### Introduction

Abiotic stress and heat stress, in particular, severely impairs efficiency at every stage of the production cycle. Pigs are particularly sensitive to heat stress because they lack functional sweat glands and despite decades of intense genetic selection, still have a thick layer of subcutaneous adipose tissue that acts as an effective insulation layer. The swine industry prioritises production efficiency and as a result, has achieved rapid improvements in the lean growth of market pigs and reproductive efficiency over the past several decades. The bulk of heat stress-induced financial burden occurs through reduced and inconsistent growth and poor sow reproductive performance but is also realised through increased mortality and morbidity, and decreased carcass value. Consequently, heat stress is one of the largest economic barriers to the USA pork industry (St-Pierre *et al.* 2003). Although currently a large impediment, heat stress will likely become more of a production hurdle in the future if traditional production traits continue to be genetically emphasised, as selection for improved lean tissue accretion rates and reproductive capacity (piglets born and weaned) are both accompanied with increased basal heat production (Brown-Brandl *et al.* 2004). This reduced thermal

tolerance may be partly mediated by altered body composition (i.e. increased lean tissue), as synthesising and maintaining skeletal muscle generates metabolic heat (Brown-Brandl *et al.* 2001). Thus, genetic selection for economically important production traits will likely further decrease tolerance to heat stress (Nienaber and Hahn 2007; Baumgard and Rhoads 2013). The focus of this review is to evaluate our current understanding of the physiological basis for compromised growth and reproduction in pigs as a result of heat stress. Deepening our understanding of the physiological consequences of heat stress in pigs is essential to developing strategies to mitigate the deleterious effects of current heat stress and future climate change on the global swine industry.

### Heat-stress effects on swine production

Accurately determining the heat-induced economic loss is difficult, but a recent estimate suggests poor sow performance alone (not including reduced offspring growth, carcass quality) costs the USA swine industry \$450 million annually (Pollmann 2010). Even if optimal heat-stress abatement strategies were implemented by all pig producers at all stages of production, heat stress is estimated to cost the USA swine industry millions

annually (St-Pierre *et al.* 2003). The combination of climate change forecasts, increased pork production in tropical and subtropical regions of the globe, and improved genetic capacity for lean tissue accretion and fecundity, all point to increasingly negative impacts of heat stress on pork production efficiency and quality in the future.

#### *Heat stress impacts feed intake*

Reduced nutrient intake during a thermal load is a highly conserved response across species and presumably represents an attempt to decrease metabolic heat production (Baumgard and Rhoads 2013). Additionally, a meta-analysis of publications (1970–2009) revealed the effect of heat stress on feed intake and growth in pigs to be more pronounced in recent years, supporting the posit that genetic selection for growth and carcass traits increases pig thermal sensitivity (Renaudeau *et al.* 2011). Traditionally, the detrimental effects of heat stress on production have been solely attributed to inadequate feed intake. However, recent findings from this laboratory challenge this dogma as it has been repeatedly demonstrated that, given the same plane of nutrition, production responses differ between thermal neutral and heat-stress environments in both cattle and growing pigs (Baumgard and Rhoads 2013; Pearce *et al.* 2013a). This led to the hypothesis that heat stress has both direct and indirect (via reduced feed intake) effects on animal productivity.

Identifying how much of the decreased productivity is caused by heat-induced reductions in feed intake is difficult. This is primarily because the composition of tissue accretion is not taken into consideration when measuring gross changes in bodyweight gain. For example, heat-stressed sows (Prunier *et al.* 1997) do not lose as much bodyweight and body condition as do their pair-fed thermal neutral counterparts; this holds true also for growing pigs (Pearce *et al.* 2013a; Sanz Fernandez *et al.* 2015). Therefore, reduced feed intake may appear to explain a majority of the decreased performance in growing animals, but the direct effects of heat may be altering the hierarchy of tissue synthesis.

#### *Heat stress impacts carcass composition*

In addition to the aforementioned reduced productive measures, heat stress also alters carcass composition (more fat and less lean). It is well known that pigs reared in heat-stress conditions have reduced muscle mass and increased adipose tissue (Close *et al.* 1971; Verstegen *et al.* 1978; Heath 1983; Bridges *et al.* 1998; Collin *et al.* 2001). Although there are some inconsistencies in the literature regarding the effects of thermal stress on carcass composition (Nienaber *et al.* 1987; Le Bellego *et al.* 2002), these differences are explained by dissimilar environmental conditions and experimental animal size as heat stress has little effect on carcass tissue mass in young pigs but markedly increases adipose tissue accretion and reduces carcass nitrogen content in heavier pigs (Christon 1988). This phenomenon is not unique to pigs, as heat stress also alters carcass composition similarly in rodents (Schmidt and Widdowson 1967; Katsumata *et al.* 1990) and growing poultry (Geraert *et al.* 1996; Yunianto *et al.* 1997). This metabolic shift in heat-stressed animals is energetically interesting as animals in thermal neutral conditions consuming a restricted diet will deposit protein at the expense of lipid accretion (i.e. the carcass lipid to protein ratio decreases

meaning the carcass becomes leaner), and the quantity of lipid deposited per unit of energy consumed decreases (Le Dividich *et al.* 1980; Van Milgen and Noblet 2003; Oresanya *et al.* 2008). Hence, the reduced feed intake to body composition relationship is exactly opposite in pigs reared in heat-stress conditions and is independent of the plane of nutrition (Baumgard and Rhoads 2013). Therefore, it is clear that heat stress alters the hierarchy of normal nutrient partitioning and this unusual metabolism is not conducive to profitable pig production.

#### **Metabolic consequences of heat stress in pigs**

The aforementioned production data suggest that heat stress alters metabolism differently than would be expected based upon calculated whole-body energy balance. Surprisingly, we and others have demonstrated that basal plasma non-esterified fatty acid levels are typically reduced in heat-stressed rodents (Sanders *et al.* 2009), pigs (Pearce *et al.* 2013a), and cattle (Shwartz *et al.* 2009) despite marked reductions in feed intake, and especially when compared with pair-fed thermal neutral controls (Rhoads *et al.* 2009; Sanz Fernandez *et al.* 2015). Furthermore, this laboratory has recently reported that both heat-stressed cows and pigs have a decreased non-esterified fatty acid response to an epinephrine challenge compared with pair-fed thermal neutral counterparts (Baumgard *et al.* 2011; Sanz Fernandez *et al.* 2015). The blunted lipolytic capacity of adipose tissue is especially unusual as heat-stressed animals are severely nutrient restricted, which is an energetic state typically associated with elevated circulating non-esterified fatty acid levels (Bauman *et al.* 1988).

#### *Heat stress alters insulin circulation*

Despite hallmarks traditionally associated with hypoinsulinemia such as (1) marked reductions in feed intake, (2) calculated negative energy balance and (3) rapid bodyweight loss, it has been demonstrated that basal insulin concentrations gradually increase in lactating heat-stressed cows (Wheelock *et al.* 2010), growing calves (O'Brien *et al.* 2010) and pigs (Pearce *et al.* 2013a; Sanz Fernandez *et al.* 2015). The increase in insulin, a potent anabolic hormone, during heat stress, an intensely catabolic condition, is seemingly a biological paradox. The reason for this counter-intuitive physiological occurrence is not clear although may involve insulin's role in the activation of cellular stress responses (Li *et al.* 2006). Regardless, increased plasma insulin concentrations in these experiments agree with data from other heat-stressed ruminant reports (Itoh *et al.* 1998), a malignant hyperthermic pig model (Hall *et al.* 1980), and a heat-stressed rodent model (Torlinska *et al.* 1987). In addition, and in response to a glucose tolerance test, heat-stressed cows and calves have an increased insulin response compared with pair-fed thermal neutral controls, whereas glucose disposal is quicker or remains unchanged (O'Brien *et al.* 2010; Wheelock *et al.* 2010). It is also demonstrated, using the hyperinsulinemic-euglycemic clamp technique, that insulin sensitivity is improved in growing heat-stressed calves (Rhoads *et al.* 2009) and growing pigs (Sanz Fernandez *et al.* 2015), as heat-stressed animals required more glucose to maintain euglycemia. Similarly, growing pigs appear to be more insulin sensitive based on the insulin to glucose response to a glucose tolerance test (Sanz Fernandez *et al.*

2015). Whole-body glucose utilisation appears to increase during heat stress; however, the contribution of the different tissues to this net effect remains unknown. The immune system is a potential glucose utiliser that, as described below, might be stimulated due to the deleterious effects of heat stress on intestinal health. Once activated, immune cells become obligate glucose utilisers, and this altered hierarchy of fuel requirements may trigger a whole body shift in nutrient partitioning in order to spare glucose for the immune system (Baumgard and Rhoads 2013). In this scenario, adipose and muscle become refractory to insulin whereas activated immune cells become insulin sensitive, and the immune system's glucose utilisation may exceed that of systemic tissue.

#### *Heat stress compromises intestinal health*

Mechanisms responsible for altered nutrient partitioning during heat stress are not clear, however, heat-stress effects on gastrointestinal health and function might mediate them. The small intestine is one of the first tissues upregulating HSP during a thermal load (Flanagan *et al.* 1995). 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 oxygen 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, heat stress increases the passage of luminal content [e.g. lipopolysaccharide (LPS) from Gram-negative bacteria] into the portal and systemic blood (Hall *et al.* 2001; Pearce *et al.* 2013b). Increasing evidence suggests that LPS directly or indirectly increases pancreatic insulin secretion as infusing LPS into the mammary gland increased (~2-fold) circulating insulin in lactating cows (Waldron *et al.* 2006). In addition, intravenously infusing LPS into growing pigs and calves demonstrated a >10-fold increase in circulating insulin (Rhoads *et al.* 2009; Stoakes *et al.* 2015).

#### **Reproductive consequences of heat stress in pigs**

In North America, the effects of heat stress on swine reproduction are also quite apparent with pregnancy detection rates on Day 28 of gestation reaching their lowest levels in August into October and reduced farrowing rates in November and December (based on inseminations conducted in June–September). Although this repeated observation, which is referred to as 'seasonal infertility', could arguably be related to factors such as photoperiod, seasonal infertility in pigs is associated with periods of excessive heat, and heat stress has been repeatedly demonstrated to negatively impact reproductive efficiency, particularly due to lost embryonic development (Tompkins *et al.* 1967; Omtvedt *et al.* 1971).

#### *Heat stress compromises boar fertility*

Although the effects of heat stress on female pig reproduction are notable, boars are also negatively affected by heat stress, which are explained primarily by poor semen production and quality. In comparison to boars in thermal neutral environments, exposing boars to heat stress for 90 days (34.5 and 31.0°C for 8 and 16 h/day, respectively), reduced sperm motility and

increased sperm abnormalities within 2 weeks from heat-stress initiation (Wettemann *et al.* 1976). Furthermore, utilisation of semen from heat-stressed boars reduced the number of fetuses on Day 30 post-insemination (Wettemann *et al.* 1976). Similarly, boars exposed to heat stress had increased presence of abnormal sperm within 2–3 weeks following induction of heat-stress exposure (Cameron and Blackshaw 1980). Therefore, the boar is obviously an important part of a successful summer reproductive management plan, and efforts to minimise the negative effects of heat stress on male fecundity are imperative.

#### *Heat stress compromises female fertility*

Heat stress decreases fertility in sows and gilts that is typically manifested during seasonal infertility (Love 1978; Prunier *et al.* 1994). Increased ambient temperature lowers farrowing rates and is postulated to delay the onset of puberty (Bertoldo *et al.* 2009). Tolerance to heat stress and heat stress-induced exacerbation of infertility appears to occur differentially in different genetic lines. For example, Bloemhof and colleagues (2008) demonstrated that sows selected for increased farrowing rate were more sensitive to heat stress (i.e. reduced litter size and total number born) and that farrowing rate per first insemination was compromised compared with those lines not selected for increased farrowing rate (Bloemhof *et al.* 2008). How exactly heat impacts female fertility is not well understood, although it most likely involves compromised production of gametes and embryos capable of development. Heat stress has been associated with reduced developmental competence, and induction of apoptosis in *in vitro* fertilised and parthenogenetically activated pig embryos (Isom *et al.* 2007b; Bertoldo *et al.* 2010; Pennarossa *et al.* 2012). Although the heat shock protein (HSP) machinery is constitutively expressed in the somatic cells of the ovary, there is only a change in abundance in the oocyte in response to whole-ovary heat stress (Pennarossa *et al.* 2012), suggesting that regulation in response to hyperthermia may occur within the porcine oocyte.

#### *Heat stress during oocyte and embryo development impairs reproductive success*

An inability to maintain a healthy body temperature has significant implications for the production of gametes capable of yielding developmentally competent embryos. Ewes exposed to heat stress before oestrus or after insemination have suppressed reproductive ability, including reduced ability to demonstrate behavioural oestrus despite ovulation and suppressed pregnancy rates (Sawyer 1979a, 1979b; Sawyer *et al.* 1979). Interestingly, it appears a sizeable portion of seasonal infertility could be explained by the thermal sensitivity of early stage embryos, as early stage bovine embryos are unable to mount an effective and sufficient heat shock response (Sakatani *et al.* 2012). Consequently, early stage embryos are incredibly sensitive to subtle increases in body temperature. *In vitro* oocyte maturation mimics the *in vivo* oocyte development that occurs during late proestrus and during the first part of behavioural oestrus before ovulation, concomitant with the first service, as ovulation occurs at ~55–60% of the way through the behavioural oestrus (Soede and Kemp 1997). We have developed an *in vitro* oocyte maturation model to investigate effects of heat stress on oocyte

development. Oocytes subjected to heat stress during *in vitro* maturation have an impaired ability to survive beyond the 4-cell stage of development, despite being fertilised and cultured in thermal neutral conditions (E. C. Wright and J. W. Ross, unpubl. data).

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 a 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 before ovulation and complete oocyte maturation, as pigs typically ovulate in the mid to latter half of oestrus (Soede and Kemp 1997). The severity of negative effects of heat stress during pregnancy in pigs appears to depend on the stage of gestation. Omtvedt *et al.* (1971) demonstrated this by exposing pregnant gilts to heat stress for 8 days during different stages of gestation. Heat stress (37.8°C for 17 h and 32.2°C for 7 h) beginning either on Day 0 or Day 8 of gestation compared with thermal neutral conditions (constant 23.3°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 whereas heat stress during late gestation (Days 102–110) resulted in a significantly increased number of dead piglets born and a four-piglet reduction in the number born alive (Omtvedt *et al.* 1971). However, a more moderate cyclic heat stress, on bred gilts beginning on Day 3 and extended to either Days 24 or 30 of gestation, did not impact embryo survival (Liao and Veum 1994). Thus, there are specific reproductive stages that are sensitive to heat stress and identifying those phases and mechanisms involved are of both academic and practical interest.

Due to the difficulty for such studies *in vivo*, characterisation 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. Culture of pig embryos at 42°C for 9 h following porcine *in vitro* fertilisation significantly reduced blastocyst formation rate (Isom *et al.* 2007a), and heat shock of 41.5°C following *in vitro* maturation also reduced oocyte development (Tseng *et al.* 2006). The impact of *in vitro* heat stress during oocyte maturation and its impact on subsequent developmental competency have also been demonstrated. Oocytes exposed to heat stress (41°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 whereas heat stress during only the second half (21 h) of *in vitro* maturation did not impact maturation rate (E. C. Wright and J. W. Ross, unpubl. data). Metaphase II arrested oocytes following heat stress during *in vitro* maturation demonstrated impaired developmental competency compared with oocytes matured at 39°C, as measured by their ability to develop to the blastocyst stage following *in vitro* fertilisation and culture in thermal neutral conditions. This model has subsequently been used to demonstrate differences in gene expression in developing 4- to 8-cell embryos as a result of

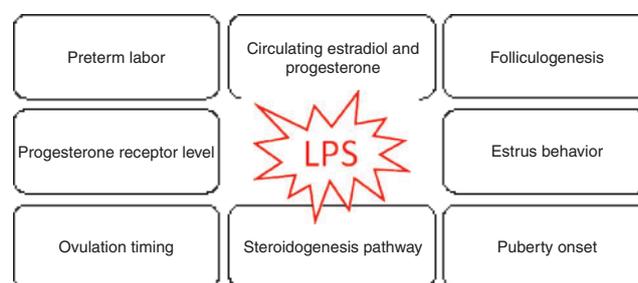
heat-stress conditions during *in vitro* maturation (E. C. Wright and J. W. Ross, unpubl. data).

#### *LPS-induced signalling influence on ovarian function*

As already mentioned, intestinal integrity is compromised by heat stress and is associated with increased circulating endotoxin. Elevated endotoxin may be a mechanism through which heat stress compromises ovarian function. From a reproductive perspective, LPS-induced poor fecundity is a phenomena reported throughout the literature (as summarised in Fig. 1). Interestingly, follicular fluid that surrounds and nourishes the maturing oocyte contains LPS levels reflective of the systemic circulation. Thus, LPS appears to reach the ovary via the systemic circulation having the ability to directly interact with the oocyte (Herath *et al.* 2007). In cattle, LPS contact with ovarian cortical explants was associated with reductions in the number of primordial follicles simultaneous with increased atresia of the ovarian reserve (Bromfield and Sheldon 2013). In rodents, LPS exposure *in vivo* also reduced primordial follicle number, potentially mediated via Toll-like receptor 4 signalling as Toll-like receptor 4 null mice were resistant to LPS-mediated primordial follicle depletion (Bromfield and Sheldon 2013). In addition to compromising the follicular pool, LPS alters anterior pituitary hormone secretion. In anestrus ewes, LPS infusion suppressed luteinising hormone release while having a stimulatory effect on prolactin and cortisol levels. Furthermore, mRNA for luteinising hormone and luteinising hormone receptor was suppressed by LPS infusion, although follicle stimulating hormone (FSH) and FSH receptor as well as prolactin and prolactin receptor genes were elevated (Herman *et al.* 2010).

#### *Elevated insulin alters ovarian function*

Elevated insulin secretion has repeatedly been observed in response to heat stress and hyperinsulinemia likely alters ovarian function. One mechanism is through insulin's ability to activate phosphatidylinositol-3 kinase pathways (Kasuga 1996), which in turn can regulate oocyte recruitment and activation. During specific physiological states such as polycystic ovary syndrome and obesity, where insulin levels are elevated, associated reproductive problems including reduced fecundity and increased pregnancy loss also exist. Nteeba *et al.* (2015) demonstrated increased expression of



**Fig. 1.** Summary of reproductive areas that can be impacted by lipopolysaccharide (LPS) exposure. Based upon a wide variety of literature sources from different species, LPS has been demonstrated to induced pre-term labour, affect gene expression, impact ovarian sex steroid hormone production, and influence puberty onset. Many of these phenotypic consequences of ovarian LPS exposures are observed in heat-stressed sows and gilts.

the gene encoding the insulin receptor in ovaries of gilts exposed to heat stress, indicating ovarian sensitivity to increased insulin during heat-stress conditions. Additionally, heat stress increased genes encoding the ovarian steroidogenic enzymes, suggesting the potential for altered oestradiol synthesis during heat-stress conditions. Furthermore, the composition of follicular fluid, essential for providing an important microenvironment for maintaining oocyte competency, can also be altered by environmental conditions (Gosden *et al.* 1988; Fortune 1994). It is our postulate that elevated insulin signalling during heat stress in female pigs is associated with insulin-induced altered signalling in the ovary that compromises the production of viable oocyte capable of fertilisation and full-term development.

#### *Autophagy-induced signalling in the ovary*

Autophagy is emerging as an additional mechanism through which heat stress alters cellular function. Autophagy is the process by which somatic cells recycle energy through the reutilisation of cellular components, and is activated in somatic cells by a variety of stressors. There are three major types of autophagy: chaperone-mediated autophagy, microautophagy, and macroautophagy. Macroautophagy accounts for the largest amount of energy reacquisition of the three different types (Klionsky 2005). Autophagy is the sequestration of cytoplasm into a double-membraned cytosolic vesicle, the autophagosome, which fuses with a lysosome to form an autolysosome for degradation by lysosomal hydrolases (Klionsky and Emr 2000). The steps of autophagy can be broken down into induction, autophagosome formation, autophagosome-lysosome fusion, and degradation (Pyo *et al.* 2012). These processes are marked by the formation of large protein complexes, and much of the regulation occurs at the post-translational level (Mizushima 2010; Mizushima *et al.* 2011). Both basal and stress-induced autophagy have been observed in the embryo and oocyte. Deficiencies in autophagy-related genes negatively affect both early- and late-stage embryonic development (Zeng *et al.* 2006; Fimia *et al.* 2007; Qu *et al.* 2007; Cecconi *et al.* 2008). Embryos also respond to external stressors by the induction of autophagy (Adastra *et al.* 2011; Xu *et al.* 2011). In the oocyte, autophagy-related gene 5 knockout mice fail to develop past the 4-cell embryo stage (Tsukamoto *et al.* 2008). Furthermore, microtubule-associated protein 1 light chain 3 (LC3)-II, an autophagy marker, is detectable during initial culture of pig oocytes (Lee *et al.* 2014), and the autophagy protein, Beclin1 (BECN1), has been observed in the mouse oocyte (De Felici *et al.* 2008).

It has been well documented that regulation between autophagy and apoptosis is highly coordinated (Mukhopadhyay *et al.* 2014). In this way, B-cell lymphoma 2 (BCL2) is known to interact with both BECN1 and Bax, and inhibit the activation of both proteins. Initial phosphorylation of BCL2 decreases its interaction with BECN1, therefore activating BECN1 and inducing autophagy (Pattinre *et al.* 2005). Though the mechanism is not well understood, it is hypothesised that BCL2 can be hyper-phosphorylated, causing it to no longer interact with Bax and induce apoptosis (Oltval *et al.* 1993; Ling *et al.* 1998). This has led investigators to hypothesise a model where different levels of stress affect the level of phosphorylation of BCL2 and act as a regulator between autophagy and apoptosis in response to an

increasing gradient of environmental stress (Levine *et al.* 2008; Mukhopadhyay *et al.* 2014).

To investigate the effects of heat stress-induced autophagy signalling in the pig ovary, synchronised follicular development in a group of gilts occurred using Matrix (DPT Laboratories, San Antonio, TX, USA), for which the active component, altrenogest, functions as a progesterone receptor agonist. For 5 days following Matrix withdrawal, during follicular development, gilts were subjected to cyclical heat stress or thermal neutral conditions. After 124 h of cyclical heat stress and the emergence of the dominant follicle pool, gilts were sacrificed and whole ovaries collected. Using whole-ovary extract, it was determined that cyclical heat stress increased the abundance of BECN1 and cleavage of LC3-I to LC3-II (B. J. Hale and J. W. Ross, unpubl. data), key markers of autophagy induction. The protein BECN1 has a known role in autophagosome formation and its ability to react to a wide variety of regulators (Liang *et al.* 1998; Wurmser *et al.* 1999; Funderburk *et al.* 2010; Pyo *et al.* 2012) whereas LC3-II is a key component of the conjugative system necessary for the expansion of the autophagosome membrane. Interestingly, it has also been shown that the increase of BECN1 abundance correlates with an increase in phosphorylation of BCL2 at the threonine 56 (B. J. Hale and J. W. Ross, unpubl. data).

#### **Epigenetics of heat stress**

Epigenetics is translated to mean 'above the genome', and is the study of DNA modifications outside of base-pair sequence information that are capable of impacting gene expression. Multiple modifications, such as DNA methylation, histone regulation, chromatin state, and miRNA expression, to name a few, all have the ability to impact the epigenetic code. Importantly, environmental influences occurring both pre-natally and post-natally can influence cell specific imprinting of the epigenetic profile and result in altered responses to environmental conditions or stimuli.

#### *Epigenetic mechanisms*

DNA modification via methylation is a stable DNA modification that can be either inherited or acquired throughout an animal's lifetime, although is very dynamically regulated during early development (Rivera and Ross 2013). Methylation of DNA is achieved through the enzymatic actions of DNA methyltransferases capable of transferring a methyl group from the methyl donor, S-adenosyl methionine, commonly to the cytosine of CpG dinucleotides located upstream of gene promoters. Hyper-methylation of these CpG islands in promoter regions of genes will most often result in suppression of gene expression through recruitment of DNA-binding proteins capable of interfering with transcription factor function. Alternatively, methylation of DNA in non-CpG can result in more variable regulation patterns. *De novo* or maintenance methylation is accomplished via two classes of DNA methyltransferases (Klose and Bird 2006). Copying existing methylation patterns during replication and development is referred to as maintenance methylation whereas *de novo* methylation introduces new methylation patterns. Induction of *de novo* methylation in response to environmental stress is one potential mechanism for observations of an imprinted response to stress.

### *Developmental imprinting*

Following fertilisation, both parental genomes experience global demethylation producing a single cell totipotent zygote. After several rounds of holoblastic cleavages, the embryonic cells undergo dynamic reorganisation and genetically identical cells begin differentiation leading them towards specific cell lineages (Reik 2007). The mechanism controlling the molecular programming of these cells is not well understood but is thought to be largely attributed to differences in DNA methylation of CpG islands as well as alterations in histone methylation and acetylation patterns (Kelly and Trasler 2004). Continued differentiation and programming of cells during pre-natal development is largely the result of continued modifications of DNA methylation patterns. During cellular differentiation, some epigenetic modifications can be changed whereas others persist, such is the basis of numerous disease statuses as the result of abnormal epigenetic imprints. Functionally, epigenetic modifications can improve the plasticity of the genome to improve responses to specific environmental conditions or can permanently impair an individual's ability to cope or respond to particular environmental conditions. Classical examples of gestational imprinting have been observed in multiple species. One example is the lifelong consequences of humans exposed to the Dutch famine *in utero* (Roseboom *et al.* 2006). Individuals exposed to the famine *in utero* demonstrate lifelong consequences as a result of the intrauterine environment created by the famine with exposure during the first trimester having significant impairments with respect to metabolic syndrome (Roseboom *et al.* 2006). More recently, the metabolic alterations in response to the famine incurred during gestation have been demonstrated to be in part due to altered DNA methylation profiles (Tobi *et al.* 2014).

### *Epigenetic programming due to maternal stress*

In addition to decreased fertility, pre-natal stress can also result in intrauterine growth retardation (IUGR) that may result in further losses as piglets born during periods of maternal stress have diminished performance in post-natal life. In agricultural species, the post-natal effects of pre-natal stresses are still being characterised. The majority of research on epigenetic regulation in response to maternal stressors has been conducted in pigs and sheep. Although there is little direct evidence suggesting that heat stress *in utero* confers an epigenetically mediated response to heat in later life, there is ample evidence of stress-induced epigenetic changes in these species.

A variety of intrauterine events can have extensive and permanent effects on post-natal pig performance (Foxcroft *et al.* 2009). Experimental models, such as IUGR, indicate growth/development and lifetime piglet performance is impaired and is associated with alterations in skeletal muscle phenotype (Bee 2004; Foxcroft *et al.* 2006, 2009; Cerisuelo *et al.* 2009). In pigs, IUGR has lasting effects on growth potential and carcass quality (Foxcroft *et al.* 2006, 2009). In addition to IUGR, maternal diet has been demonstrated to impact on post-natal performance of piglets. Supplementing gestating sow diets with omega 3 fatty acids improved glucose uptake in offspring (Gabler *et al.* 2009). Moreover, maternal stress may also result in an imprint on immune response. Exposure to maternal restraint stress, for example, caused

a greater inflammatory response to endotoxin challenge in offspring (Collier *et al.* 2011).

Similar to IUGR, metabolic imprinting describes a lasting epigenetic imprint in response to the pre-natal metabolic environment (Waterland and Garza 1999). This imprint is characterised by changes in organ and tissue structure, cell number, and differentiation. Csaba *et al.* (1984) demonstrated the phenomenon of metabolic imprinting by injecting parental rats with insulin, which in turn altered the insulin-binding response in the offspring in a sex-dependent manner. Metabolic and hormonal imprinting has since been demonstrated through the study of obesity-prone offspring born from diabetic mothers in rodents and humans (Poston 2011).

Epigenetic programming and hormonal changes in IUGR animals are somewhat similar to that observed in animals exposed to pre-natal heat stress. This change is likely a result of alterations in metabolism, uterine blood flow, and reproduction caused by heat stress. The effects of maternal heat stress may alter a variety of physiological parameters in offspring later in life. For example, in mice and guinea-pigs, pre-natal exposure to heat stress resulted in reduced post-natal weight gain and smaller brain weights that lasted into maturation (Jonson *et al.* 1976; Shiota and Kayamura 1989). The cause of suboptimal post-natal performance in mice exposed to pre-natal heat stress has been suggested to be due to interference with establishment of the hypothalamic-pituitary-axis. In mouse embryos, a gene-specific DNA methylation imprint of heat was observed following heat stress (Zhu *et al.* 2008).

### *Epigenetic programming in response to heat stress*

In some species, epigenetic conditioning following exposure to heat results in thermal tolerance to heat stress later in life. This has been demonstrated in chickens where heat stress at 3 days of age resulted in protection against acute heat stress-related mortality during adulthood (Yahav and McMurtry 2001), and is thought to be mediated by modifications to the histone code thereby enabling an epigenetic memory (Kisliouk *et al.* 2010). Chicks acutely heat stressed 3 days post-hatching and again 1 week later had increased H3K9 acetylation and H3K9 dimethylation. Chronic heat stress has also been reported to alter histone modifications in rodents resulting in elevated expression of HSP-70 and increased HSP-90 protein in response to heat acclimation and re-acclimation (Tetievsky and Horowitz 2010). Additionally, mice fibroblasts exposed to a conditioning heat load had improved survival in response to what would normally be a lethal heat load (Luft *et al.* 2001).

How pre-natal heat stress exposure impacts post-natal performance is poorly understood but is likely a result of epigenetic programming. Epigenetic programming is significantly influenced by differences in DNA methylation of CpG islands (Klose and Bird 2006), that when negatively impacted during development can have lasting implications on gene expression (Bernal and Jirtle 2010) and is thought to occur in pigs having a potentially significant impact on lifetime production (Foxcroft *et al.* 2009). These epigenetic modifications in chromatin structure, (which can last short periods or lifelong) influence the condensation of the DNA reducing the recruitment and ability of DNA-binding proteins, such as RNA polymerases, to interact with and transcribe

genes from the genome. Intrauterine modifications via DNA methylation can occur in temporal- and tissue-specific manners (Schneider *et al.* 2010). Because the biological underpinning of the pig heat-stress response involves the coordinated interactions between adipose, hepatic and muscle tissues, an objective is to understand which molecular responses in these tissues are associated with pre-natal epigenetic programming through DNA methylation.

#### *Gestational heat stress in pigs impacts the offspring*

Boddicker *et al.* (2014) tested the hypothesis that *in utero*, heat-stress exposure alters future piglet performance through some measure of epigenetic imprinting. Interestingly, gilts exposed to heat stress during the first half of gestation produced piglets that tended to have greater back-fat depth at 12 weeks of age and had increased circulating insulin at 19 weeks of age. This study was replicated and examined the growth performance of pigs produced from dams exposed to heat-stress conditions for the entire length of gestation. Interestingly, during the lipid accretion phase of growth (60–90 kg), exposure to heat stress *in utero* resulted in a propensity to accrete adipose tissue more efficiently than piglets gestated in dams in thermal neutral conditions (Johnson *et al.* 2015c), although this was not the case with piglets during the lean tissue accretion phase (30–60 kg) (Johnson *et al.* 2015b). In addition to gestational heat stress repartitioning nutrient priorities during offspring growth and development, it also appears that *in utero* exposure to heat stress impacts thermoregulatory capabilities of offspring (Johnson *et al.* 2013). In particular, post-natal pigs exposed to heat stress *in utero* have an increased body temperature (~0.3°C) during both thermal neutral and heat-stress conditions (Johnson *et al.* 2013; J. S. Johnson and L. H. Baumgard unpubl. data). This small difference in body temperature could have large implications on feed efficiency if this due to increased basal heat production, as this thermal energy would have been derived from feed energy (Johnson *et al.* 2015a). To investigate the mechanisms by which these phenotypes (body composition and body temperature) occur, Boddicker *et al.* (2015) utilised RNA sequencing on adipose tissue, liver and *M. longissimus dorsi*, and demonstrated differential expression of hundreds of mRNA transcripts as a result of exposure to heat-stress conditions *in utero*.

#### Conclusions

Genetic selection for rapid, lean tissue accretion and fecundity in the swine industry is associated with an increased susceptibility to heat-induced suppression of production efficiency. These production losses primarily occur through compromised production during the finishing phase and through seasonally compromised losses in reproductive efficiency. Epigenetic imprinting during *in utero* exposure to heat stress is another mechanism through which heat stress compromises swine production. Offspring from gilts exposed to heat stress during gestation had increased insulin levels, fatter carcasses, and had increased body temperature during post-natal life. These altered phenotypes represent an underappreciated consequence to heat stress, and combined with the well documented effects of heat

stress on growth and reproduction, create a massive impediment to efficient global pork production.

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