

## Revisiting summer infertility in the pig: could heat stress-induced sperm DNA damage negatively affect early embryo development?

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**Abstract.** Temperature is a crucial factor in mammalian spermatogenesis. The scrotum, pampiniform plexus, and cremaster and dartos muscles in mammals are specific adaptations to ensure sperm production in a regulated environment 4–6°C below internal body temperature. However, the limited endogenous antioxidant systems inherent in mammalian spermatozoa compounded by the loss of cytosolic repair mechanisms during spermatogenesis, make the DNA in these cells particularly vulnerable to oxidative damage. Boar sperm is likely to be more susceptible to the effects of heat stress and thus oxidative damage due to the relatively high unsaturated fatty acids in the plasma membrane, low antioxidant capacity in boar seminal plasma, and the boar's non-pendulous scrotum. Heat stress has a significant negative impact on reproductive performance in piggeries, which manifests as summer infertility and results in productivity losses that amount to millions of dollars. This problem is particularly prevalent in tropical and subtropical regions where ambient temperatures rise beyond the animal's zone of thermal comfort. Based on preliminary studies in the pig and other species, this article discusses whether heat stress could induce sufficient DNA damage in boar sperm to significantly contribute to the high rates of embryo loss and pregnancy failure observed in the sow during summer infertility. Heat stress-induced damage to sperm DNA can lead to disrupted expression of key developmental genes essential for the differentiation of early cell lineages, such as the trophoblast, and can distort the timely formation of the blastocyst; resulting in a failure of implantation and ultimately pregnancy loss. Confirming such a link would prompt greater emphasis on boar management and strategies to mitigate summer infertility during periods of heat stress.

**Additional keywords:** boar, DNA fragmentation, embryo loss, seasonal infertility, spermatozoa, *Sus scrofa domestica*, temperature.

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### Introduction

Pork production is a major contributor to the agricultural economy, with global production as high as 112 million tons carcass weight equivalent compared with beef and veal at 59.2 million tons carcass weight equivalent and broiler meat at 89.3 million tons ready to cook equivalent, respectively (FAS 2015). A 120-kg pig yields ~91 kg of carcass, providing 371 servings of high quality meat for human consumption (Snelson 2010; National Pork Board 2014). Pigs also contribute many other by-products while providing extensive employment opportunities due to rising production, consumption, and import and export

demands. The demand for food continues to grow as the current population increases exponentially. Average global meat consumption is currently 100 g per person per day, providing at least 16% of the total calories and 34% of the total proteins in the human diet (McMichael *et al.* 2007). Although the latest FAO estimates show a positive trend at reducing global hunger as compared with the previous two decades (FAO, IFAD, WFP 2014), meeting the current and projected demands for food still poses enormous challenges considering that the human population is predicted to rise to 8.9 billion in 2050 (Cohen 2003). The demand for food has been projected to increase

significantly to at least 3050 kcal/person.day in 2050 from an average global food consumption of 2940 kcal/person.day in 2015 (WHO 2003; FAO 2009). Therefore, research efforts should continue to focus on improving the production potential and efficiency of the pig industry.

### Summer infertility: the problem

Seasonal or summer infertility in the pig is a syndrome characterised by an overall reduction in the reproductive performance of the breeding herd that usually occurs in summer when pigs are exposed to a combination of environmental stressors including heat in particular, as well as photoperiod, humidity, genetic background, and management practices among others (Love 1978, 1981; Hennessy and Williamson 1984; Quesnel *et al.* 2005; Auvigne *et al.* 2010). Summer infertility primarily manifests as either (1) difficulty in coming into oestrus, expressed as delayed puberty in gilts, extended weaning-to-oestrus interval in sows, or increased anoestrus in both gilts and sows; or (2) higher rates of pregnancy failure with irregular returns to service, which may be attributed to untimely ovulation or early embryonic loss (Paterson *et al.* 1978; Hughes and van Wettere 2010); and/or (3) reduced fertility potential in the male (Wettemann and Bazer 1985; Boma and Bilkei 2006; Auvigne *et al.* 2010). Although the domestic pig may breed throughout the year, the seasonal reproductive activity of wild boars/sows (*Sus scrofa ferus*) is attributed to either decreasing day length, summer rainfall and/or the availability of food (Ahmad *et al.* 1995; Rosell *et al.* 2012).

Several tropical countries are among the top 10 pig producers in the world including Brazil, Vietnam, The Philippines, and Mexico (National Pork Board 2014). Although different genetic lines/breeds of boars and sows show different tolerance to heat stress reflected in their reproductive performance (Bloemhof *et al.* 2008; Flowers 2008), the use of high-yield exotic white breeds from temperate countries have become commonplace in the tropics. As such, commercial farm animals particularly in these regions can inadvertently suffer from summer infertility when ambient temperatures rise beyond the animal's zone of thermal comfort (18–23°C; Stone 1982; Prunier *et al.* 1997; St-Pierre *et al.* 2003). The negative impact of heat stress on productivity is becoming increasingly important to developed and developing nations due to decreasing profit margins. On average, at least \$300 million are lost annually in swine alone and billions across the US livestock industry due to heat stress (St-Pierre *et al.* 2003). Longer weaning-to-conception intervals and reduced over-all reproductive performance in sows have been reported in large confinement units during hotter months from June to October in North Carolina (Britt *et al.* 1983). In a 5-year study in France, season was shown to clearly impact the fertility rate of pigs; with the lowest mean fertility of 81.2% occurring during the end of August (end of summer), compared with the highest mean fertility of 86.8% during the end of March (end of winter; Auvigne *et al.* 2010). However, it is pig producers particularly in equatorial countries that are likely to be the most sensitive to the impacts of summer infertility. Reproductive problems associated with heat stress and other concomitant factors have been reported involving small, medium and large

commercial pig farms in The Philippines. Small to medium farms are most severely affected, particularly in relation to the weaning to conception interval, farrowing index, farrowing interval and non-productive days (Vega *et al.* 2010). Seasonal variation in the reproductive performance of sows has also been observed in Thailand (Suriyasomboon *et al.* 2006) along with negative effects of high temperature and high humidity on the sperm production of Duroc boars (Suriyasomboon *et al.* 2004). Several strategies can be adopted to minimise the effects of heat stress on the animal's reproductive performance. These include modification of the diet, breed selection, provision of floor and roof cooling systems, and varying building orientation, among others (Gourdine *et al.* 2006; Silva *et al.* 2006; Gholami *et al.* 2011).

### Effect of heat stress on boar fertility

The processes of spermatogenesis and subsequent sperm maturation are highly sensitive to temperature. In fact, the scrotum, pampiniform plexus, and cremaster and dartos muscles in mammals are specific adaptations to ensure sperm production in a regulated environment 4–6°C below internal body temperature (Nakamura *et al.* 1987; Setchell 2006). Pigs are known to be inefficient at using sweat to cool their body during high ambient temperatures. Although cutaneous water loss over the general body surface appears to be similar to man and domestic species, the pig's ability to sweat is considerably limited (Ingram 1964, 1965; Einarsson *et al.* 2008). Stone (1981) reported that the thermal characteristics of the testis and epididymis of conscious boars ranged from 35.0°C to 36.6°C and 35.0°C to 37.0°C, respectively. These temperatures were 2.5°C to 1.9°C lower than the animal's rectal temperature of 38.2°C. Moreover, Stone (1982) found that an ambient temperature of 29°C appears to be the critical limit above which Large White boars are unable to produce normal numbers of motile spermatozoa. Specific breeds and/or genetic backgrounds also tend to influence normal sperm production (Huang *et al.* 2000; Flowers 2008). Landrace boars tend to have better semen quality than Yorkshire and Duroc boars during hot seasons (Huang *et al.* 2000). Unlike in rams and bulls, the boar scrotum is non-pendulous and is much closer to the body wall, which could limit its ability to regulate testicular temperature and thus potentially make this species sensitive to the effects of environmental heat stress on semen production (Knox 2003). Prolonged exposure of testes to high temperature (i.e. testicular temperature at 38°C) can predispose boars to significantly reduced basal concentrations of peripheral testosterone along with hypertrophy and impaired function of the Leydig cells (Stone and Seamark 1984).

The effect of heat stress on semen production and reproductive efficiency has been extensively studied as early as the 1950s and 1960s in various farm animals, including rams (Moule and Waites 1963), bulls (Casady *et al.* 1953) and boars. In the boar, the detrimental effects of heat stress on sperm quality and boar fertility can manifest several days or weeks post-heat treatment. These include decreased volume in seminal plasma (Cameron and Blackshaw 1980), decreased sperm concentration (Egbunike and Dede 1980), decreased

motility and increased abnormal sperm (McNitt and First 1970; Wettemann *et al.* 1979; Heitman *et al.* 1984; Malmgren 1989; Huang *et al.* 2000), disturbance in androgen biosynthesis (Wettemann and Desjardins 1979; Stone and Seamark 1984), prolonged ejaculation time (Egbunike and Dede 1980) and reduced libido (Flowers 1997). Recently, Zasiadczyk *et al.* (2015) reported the effect of seasonal variations (autumn–winter vs spring–summer) on the quality of ejaculates collected from individual boars. Ejaculates collected during spring–summer had significantly lower volume, sperm concentration and number of spermatozoa with functional mitochondria and intact plasma membrane (Zasiadczyk *et al.* 2015). By contrast, Petrocelli *et al.* (2015) observed photoperiod to be more important than housing temperature in affecting most boar semen characteristics.

Despite this extensive focus on classical parameters of sperm quality, there is a growing body of evidence that suggests damage to sperm DNA could invariably reduce male fertility and subsequent embryo survival (Evenson 1999; Paul *et al.* 2008b; Pérez-Crespo *et al.* 2008; Didion *et al.* 2009; Evenson *et al.* 2009). That is, sperm may swim and fertilise eggs normally but embryos that have acquired a damaged paternal genome could die *in utero*. Thus, new approaches to breeding soundness evaluation in the boar (i.e. DNA fragmentation analysis and biomarkers for normal sperm phenotypes) may yield a better understanding of the underlying factors causing poor reproductive performance, thereby leading to a robust solution to the problem of summer infertility (Sutovsky 2015).

### Impact of heat stress on sperm DNA integrity

The DNA of mature sperm is uniquely condensed and tightly packaged primarily with protamines and to a lesser extent with histone-bound chromatin attached to a nuclear matrix (Wykes and Krawetz 2003; Ward 2010). This unique framework allows structural protection to spermatozoa but also includes molecular regulatory factors and several gene clusters that are important to successful embryo development (Hammoud *et al.* 2009). In boars, this DNA transitions from a weak structure at the late spermatid stage in the testis, to a very rigid structure in mature spermatozoa from the caudal epididymis; suggesting significant change in histone-to-protamine content during sperm development (Ashikawa *et al.* 1987; Fortes *et al.* 2014). Protamine deficiency in bull sperm is closely associated with higher DNA fragmentation index as determined by Sperm Chromatin Structure Assay (Fortes *et al.* 2014). Moreover, scrotal heat stress can significantly reduce protamine disulfide bonding in stallion sperm resulting in sperm DNA with higher susceptibility to denaturation (Love and Kenney 1999).

In general, mammalian spermatozoa are particularly sensitive to oxidative damage due to the limited endogenous antioxidant systems inherent in these cells, which is compounded by the presence of large amounts of unsaturated fatty acids in the plasma membrane that are exposed to free radical attack (lipid peroxidation; Aitken and De Iuliis 2010). Furthermore, the loss of cytosolic machinery from these sperm cells during spermatogenesis makes them transcriptionally and translationally inactive, and results in a deficiency of repair

mechanisms once such damage has occurred (Henkel *et al.* 2004; Lewis and Aitken 2005; Paul *et al.* 2008a; Aitken *et al.* 2012). By comparison, the epididymis secretes both intraluminal free radical scavengers and extracellular antioxidant enzymes to help protect spermatozoa during the 12–14 days of epididymal transit and maturation, but these are absent during manufacture in the testis (Vernet *et al.* 1996; Aitken and De Iuliis 2010).

Spermatozoa immersed in caudal fluid further mix with secretions from the accessory sex glands, collectively called the seminal plasma, upon ejaculation. Unlike other species, the boar ejaculates large volumes of semen reaching up to 200–400 mL/ejaculate. Many studies have reported that seminal plasma contains lectin-like molecules belonging to the spermadhesin group of proteins. These proteins coat the plasma membrane of the sperm head during ejaculation and act as receptors to carbohydrate ligands present on the oviduct epithelium (Dostálová *et al.* 1994; Dostalova *et al.* 1995; Ekhlas-Hundrieser *et al.* 2005), thus facilitating the sperm reservoir in the oviduct. Seminal plasma also contains several biochemical components, which may facilitate overall fertility of boar sperm (López Rodríguez *et al.* 2013; Sancho and Vilagran 2013). One of which is glutathione peroxidase that protects sperm membranes from oxidative stress. Novak *et al.* (2010) found that fertility index and farrowing rate appear to correlate with the presence of glutathione peroxidase in the sperm-rich fraction of the boar ejaculate. Moreover, there was a significant improvement in conception rates and litter size when seminal plasma was added to thawed epididymal spermatozoa during artificial insemination (Okazaki *et al.* 2012).

Exposure of the scrotum to 40–42°C for 30 min in the mouse causes damage to spermatogonia, spermatocytes, spermatids or spermatozoa resulting in a significant increase in DNA damage and a distortion in sex-ratio of offspring born (Paul *et al.* 2008b; Pérez-Crespo *et al.* 2008). Moreover, embryo development is blocked between the 4-cell and blastocyst stages, resulting in abnormal embryo development and loss (Paul *et al.* 2008b). This detrimental effect might be attributed to heat stress causing testicular germ cell loss and abnormal gene expression (Rockett *et al.* 2001) as well as dissociation in the chromosomes leading to chromosomally unbalanced gametes (van Zelst *et al.* 1995). Rockett *et al.* (2001) showed that heat stress downregulates the expression of several DNA repair genes such as *Ogg1* (involved in base excision repair), *Xpg* (involved in nucleotide excision repair) and *Rad54* (involved in double-strand break repair), as well as polyADP ribose polymerase that is responsible for detection and signalling of strand breaks (Tramontano *et al.* 2000). Moreover, a reduction in the expression of oxidative stress-induced antioxidants due to heat stress (Rockett *et al.* 2001), may lead to increased susceptibility of spermatozoa to oxidative damage.

In humans, sperm DNA damage is significantly higher in infertile men, with ~20–30% DNA damage (depending upon the test) used as the demarcation between infertile and fertile groups (Gandini *et al.* 2000; Evenson and Wixon 2006; Schulte *et al.* 2010). In addition, *in vitro* fertilisation by human spermatozoa with greater than 8% DNA damage results in

reduced blastocyst development (Ahmadi and Ng 1999) and lower pregnancy rates (Henkel *et al.* 2004). Fertilisation using DNA-damaged sperm reduces the rate or completely blocks blastocyst formation in cattle (Fatehi *et al.* 2006; Fernandes *et al.* 2008), and causes embryonic loss in the mouse (Paul *et al.* 2008b).

Studies examining sperm DNA integrity in boars highlight the potential for using sperm DNA tests for boar fertility assessment. The percent DNA Fragmentation Index (%DFI) of boar spermatozoa showed a significant negative correlation to farrowing rate and average total number of pigs born (Didion *et al.* 2009). Similarly, there was a strong relationship whereby fertility of boars that are used for AI can be attested upon evaluation of both sperm morphology and DNA integrity (Tsakmakidis *et al.* 2010). Examination of sperm DNA structural damage in cryopreserved extended boar semen was able to correctly predict between potentially high and low fertility boars based on DNA integrity (Evenson *et al.* 2009). In other studies, DNA fragmentation in undiluted boar semen maintained at 37°C was significantly higher and occurred much earlier (as early as 2 days) than semen maintained at 16°C (Pérez-Llano *et al.* 2010), whereas storage of extended liquid boar semen at 18°C for 3 days resulted in reduced sperm DNA integrity (Boehansen *et al.* 2005). Interestingly, a subsequent study by Boehansen *et al.* (2008) reported a reduction in litter size by as much as 0.5–0.9 piglets per litter if the %DFI of semen is greater than 2.1%. Other studies suggest that a sperm sample with greater than 6% DFI results in decreased farrowing rate and average number of pigs born (Didion *et al.* 2009; Evenson *et al.* 2009).

Exposure to heat treatments (i.e. testicular insulation, scrotal heating, dipping of testes into hot water, heated incubation of spermatozoa, and so on) have been shown to cause significant fragmentation of sperm DNA in animals (Karabinus *et al.* 1997; Fatehi *et al.* 2006; Fernandes *et al.* 2008; Paul *et al.* 2008b). Boars that have been exposed to a controlled hot-room environment, direct sunlight or ambient temperatures ranging from 30°C to 40°C for between 3–90 days (McNitt and First 1970; Wettemann *et al.* 1976; Cameron and Blackshaw 1980; Stone 1982) have demonstrated a significant decrease in sperm motility, normal morphology, and sperm concentration; with one study reporting more than 1.5 times fewer embryos surviving the first month of pregnancy in gilts bred with semen from heat-stressed boars than gilts bred with semen from Control boars (Wettemann *et al.* 1976). Despite this work, the important link between heat stress and sperm DNA damage is still missing in the pig. Using TUNEL assay and flow cytometry techniques, preliminary results in our laboratory show a significant increase in the mean percentage of DNA damage in boar sperm from less than 2% during spring and winter to over 16% during summer in the dry tropics of Townsville, Queensland, Australia (Peña *et al.* 2016). This supports an earlier study by Zasiadczyk *et al.* (2015) in which sperm DNA fragmentation is markedly higher in spring–summer than in autumn–winter in fractionated ejaculates (particularly F<sub>1</sub> and F<sub>2</sub>) using neutral comet assay. Moreover, results by Petrocelli *et al.* (2015) suggest possible seasonal DNA damage to boar spermatozoa.

Although sperm DNA assays have their limitations (Barratt *et al.* 2010), the above studies suggest that examination of sperm

DNA integrity may provide important answers to male-factor causes of summer infertility in the pig that would otherwise go undetected by routine sperm assessment (Evenson *et al.* 1994; Enciso *et al.* 2006). Of equal importance, is an understanding of the downstream mechanism by which heat-stressed sperm may cause early embryo loss.

### **Mechanisms by which heat-stressed spermatozoa can affect blastocyst formation and early embryo loss**

The formation of the blastocyst is an essential step in embryo development that facilitates proper implantation and establishment of pregnancy (Bruce and Zernicka-Goetz 2010). It involves the formation of three distinct cell lineages that include the pluripotent epiblast that forms the embryo itself, and the trophectoderm and primitive endoderm that comprise the extra-embryonic tissues supporting the development of the embryo (Cockburn and Rossant 2010). Although our understanding of the mechanisms involved during these preimplantation events are still limited, it is believed that several factors and signalling events including transcriptional regulation, epigenetic regulation (such as DNA methylation, histone modifications and chromatin modelling; Shi and Wu 2009), cell position and cell polarity, and cell–cell contact/positional relationships precede the eventual segregation of these three distinct populations of cells (reviewed in Zernicka-Goetz *et al.* 2009; Bruce and Zernicka-Goetz 2010; Gasperowicz and Natale 2011; Oron and Ivanova 2012).

*In vitro* and cytogenetic studies in humans demonstrate that ~30% of embryos fail to complete implantation, with anomalies in the DNA of gametes or embryos being the main reasons for this failure (reviewed in Macklon *et al.* 2002). Despite the high fertilisation rates in the pig (90–100%), prenatal mortality of 30–40% can significantly limit the litter size and dramatically impact economic profitability. The majority of these losses (20–30%) occur during the preimplantation period of development (Anderson 1978; Bolet 1986; Geisert and Schmitt 2002; Spencer 2013) at a time when the embryo is forming a blastocyst and secreting maternal recognition of pregnancy signals. Embryonic oestradiol (E<sub>2</sub>) plays a crucial role in porcine maternal recognition of pregnancy signalling by shifting the secretion of prostaglandin F<sub>2</sub>α into the uterine lumen where it rapidly deteriorates; thus preventing transport to, and luteolysis of the corpus luteum via uterine vein-ovarian artery counter-current exchange (Bazer and Thatcher 1977; Zavy *et al.* 1980; Geisert *et al.* 1989; Stefańczyk-Krzyszowska *et al.* 1990).

Blastocyst formation is regulated by specific genes that directly influence the organisation and differentiation processes. *Oct4* expression in internally positioned populations of cells in the morula-stage murine embryo specifies differentiation of the inner cell mass, whereas *Cdx2* in externally positioned cells specifies differentiation of the trophectoderm. *Nanog* and *Gata6* are responsible for the formation of the epiblast (from inner cell mass cells) and primitive endoderm respectively (Ralston and Rossant 2005, 2010). Identifying the timing and expression patterns of these genes is important as this appears to differ among species, indicating a different role for such genes in other mammals. Kuijk *et al.* (2008) have

demonstrated significant differences in expression patterns of these genes in porcine and bovine embryos compared with that of the mouse. Although expression of *CDX2* and *GATA6* were similar, variation existed in the expression of *NANOG* and *OCT4* between species and during different stages of development. In the pig and cow, *OCT4* is not present in morulae but can be detected in both the trophoctoderm and inner cell mass of the blastocyst. *NANOG* expression is completely absent in porcine embryos during blastocyst formation.

However, in subsequent studies, *NANOG* was found to be expressed in the inner cell mass and epiblast of porcine embryos at 7.5 embryonic days (E7.5) by which time, the embryos have already arrived in the uterus. Moreover, expression of *NANOG* by the epiblast appears to be extended for a few days after the blastocyst has formed (Hall *et al.* 2009; du Puy *et al.* 2011). These findings differ considerably to the timing of *Nanog* expression in the mouse (i.e. early stage of mouse blastocyst; around E3.5; Chazaud *et al.* 2006). This early expression is believed to be indispensable for proper differentiation of the murine inner cell mass leading to epiblast and primitive endoderm formation (Silva *et al.* 2009; Messerschmidt and Kemler 2010). In the pig however, primitive endoderm appears to have already formed before *NANOG* is expressed. Recently, Wolf *et al.* (2011) found what appears to be a sequential expression of *OCT4* and *NANOG* in the pig. *OCT4* but not *NANOG* appears initially in the ICM and is followed later by co-localised expression of both of these genes in the epiblast; with subsequent downregulation of *NANOG* by the time the primitive streak develops.

Interestingly, although a seemingly healthy looking sperm according to classical measures of sperm quality, may swim and fertilise an oocyte normally (Ahmadi and Ng 1999; Fernandes *et al.* 2008), structural abnormalities in its DNA can lead to serious problems during pronuclear formation, embryonic genome activation, and early embryo development (Evenson 1999). Sperm DNA damage may manifest itself at the time of embryonic genome activation, in the form of altered or arrested expression of important developmental genes that lie in regions where damage is present. Understanding of the normal pattern of expression of these key developmental genes can serve as a guide to investigating altered expression in developing embryos fertilised *in vitro* using artificially heat-stressed spermatozoa and/or semen collected from boars exposed to environmental heat stress. In fact, one study in the mouse has demonstrated the link between heat stress, sperm DNA damage and arrested embryo development consistent with aberrant expression of key genes involved in blastocyst formation (Paul *et al.* 2008b). Compared with control blastocysts, Oct3/4 immunostaining of embryos retrieved from females mated to 42°C-heated males showed aberrant staining patterns associated with grossly abnormal embryos that lacked a blastocoel and had fragmented nuclei. Several embryos from females mated to 40°C-heated males were also developmentally delayed, lacking a blastocoel and still expressed Oct3/4 staining in all cell nuclei (Paul *et al.* 2008b).

Furthermore, the impact of heat stress may not only be limited to disturbing the integrity of paternal genomic DNA but could broadly alter epigenetic constituents, activation factors and a host of mRNAs and microRNAs. These factors appear to influence the

survival of the embryo post-fertilisation through participation in various molecular functions, such as signal transduction, cell proliferation and transcriptional proliferation (Krawetz 2005; Yamauchi *et al.* 2011; Kumar *et al.* 2013).

Normal and timely formation of the blastocyst is paramount not only to subsequent development of the embryo but in preparing the maternal environment to recognise the impending pregnancy (Leibfried-Rutledge 1996; Latham 1999; Latham and Schultz 2001; Bettegowda and Smith 2007; Minami *et al.* 2007; Jeanblanc *et al.* 2008). Any delay or arrest of embryo development will result in the delay or absence of properly timed maternal recognition of pregnancy signalling by the trophoctoderm. In porcine embryos, major morphological transformation occurs between 12 and 16 days of gestation when blastocysts elongate and reach their final length of ~800 mm to 1100 mm at Day 16 of pregnancy (Perry and Rowlands 1962; Anderson 1978; Bazer and Johnson 2014). At this time, the trophoctoderm secretes significant amounts of E<sub>2</sub> along with interferons gamma and delta (Spencer 2013). This is essential for preventing luteolysis of the corpus luteum, as this structure is the primary source of progesterone production necessary to support pregnancy for the entire period of gestation in the pig (Meyer 1994). Moreover, the surge of E<sub>2</sub> from the trophoctoderm is believed to influence gene expression in the endometrium and is responsible for promoting uterine receptivity and elongation of the conceptus (Johnson *et al.* 2009). In this regard, fertilisation of oocytes with DNA-damaged sperm may disrupt the organisation of genes required in the formation of cell lineages (trophoctoderm among others), distorting the sequence of events leading to the formation of the blastocyst (Ralston and Rossant 2005, 2010). As a consequence, embryonic development may be delayed and/or arrested resulting in disrupted implantation, the loss of properly timed maternal recognition of pregnancy signals and subsequent loss of the corpus luteum, and ultimately pregnancy failure. Using an *in vitro* fertilisation system, ongoing research in our laboratory seeks to demonstrate the definitive link between heat stress in the boar and summer infertility in the sow; warranting a closer look at boar management strategies during periods of elevated ambient temperature.

## Conclusion

Although several sow-specific factors play a crucial role in sustaining embryo development in the pig, there is a strong case for the hypothesis that reduced fertility and embryo survival associated with summer infertility in the sow may be due in significant part to a reduction in DNA integrity of spermatozoa in the boar. If oocytes are fertilised by heat stress-induced, DNA-damaged sperm, it is highly probable that subsequent embryo development will be affected. This may include decreased cleavage rates; decreased blastocyst formation due to the disruption of specific genes responsible for early lineage formation and eventually delayed embryo development or early embryonic death, disrupted implantation and pregnancy loss. This has important implications for the proper management of boars from housing conditions to nutritional requirements during summer.

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