The effects of heat stress on porcine oocyte maturation, fertilisation and embryo development and methods of alleviation

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Summary
During summer and early autumn, female pigs experience a decrease in fertility (seasonal infertility) (Lopes et al. 2014) which is caused by the combination of inappropriate photoperiods and elevated ambient temperatures (heat stress) (Auvigne et al. 2010). Seasonal Infertility accounts for an approximate annual loss of $40 million to the pig industry, this is exacerbated by heat stress which has been reported to cost the US pig industry $450 million annually (Lewis and Bunter 2011). These numbers alone warrant further research into methods to alleviate the effects of heat stress on pigs, as this will reduce seasonal infertility effects seen annually. This project aims to alleviate the negative effects of heat stress through supplementation of antioxidants in order to reduce damaging reactive oxygen species. This will be achieved using an in vitro model that will first assess the stage of oocyte maturation and embryo development most affected by heat stress, in order to target antioxidant treatment. Thus the second experiment involves supplementation with the antioxidants, melatonin or vitamin A, in an attempt to reduce the negative impact that heat stress has on oocyte maturation and early embryo development.

Introduction
Seasonal infertility is a major economic problem within the pork industry as it greatly impairs the reproductive performance of sows (Tast et al. 2002; Bertoldo et al. 2010), with lower reproductive rates resulting in significant financial losses for the production industry (Ross et al. 2015). A 3-7% decrease in fertility has been demonstrated between the late summer and early autumn periods (Bertoldo et al. 2010). Poor sow performance as a direct result of heat stress (excluding reduced offspring growth and carcass quality) costs the USA $450 million annually. The USA livestock industry holds heat stress accountable for between 1.6 and 2.4 billion dollars in economic losses, 15% of that is associated to pigs (Lewis and Bunter 2011), however this phenomenon is a global issue affecting the United Kingdom, Spain and Australia.

Reproductive efficiency is a key driver of profitability within the pork industry and is determined by litter size and farrowing rate, with both of these reduced in sows mated during summer-early autumn (Bertoldo et al. 2010). Although understanding is incomplete, the overarching premise is that high ambient temperatures depress the reproductive axis, with photoperiod having the prominent role and heat stress exacerbating the negative effects (Auvigne et al. 2010).

Seasonal infertility most commonly manifests as delayed oestrus (both pubertal and post weaning), reduced pregnancy maintenance and low litter sizes (Bertoldo et al. 2011). It has been determined that luteinising hormone (LH) (Tast et al. 2002; Bertoldo et al. 2011) oestradiol, follicle stimulating hormone (FSH) and gonadotrophin releasing hormone (GnRH) (Armstrong et al. 1989) are all produced in lower concentrations throughout the summer-autumn period, reducing reproductive capabilities.

Photoperiod is the principal regulator of fertility in seasonal breeders (Giraldo et al. 2014), and is detected by the transmission of light from the retina, inhibiting the production of melatonin (Prunier et al. 1996). The low levels of melatonin inhibit the release of gonadotrophin releasing hormone (GnRH) and this alters LH and FSH secretion (Malpaux et al. 1999 & Prunier et al. 1996). Pigs are particularly sensitive to rises in temperature because they are poor thermoregulators, as they lack functional sweat glands and have a thick layer of subcutaneous fat (Ross et al. 2015). This alone is enough to consider heat stress as a key area of focus for research. At a basic level heat stress compromises female fertility because the successful development of gametes and embryos is comprised during high ambient temperatures (Ross et al. 2015). Heat stress disrupted follicle development, oocyte maturation, embryo development and fetal growth (Fu et al. 2014).

Heat stress increases oxidative stress within cells, and this is a key factor in the failure of embryo development (Sakatani et al. 2004; Matsuzuka et al. 2005). Oxidative stress caused reactive oxygen species (ROS) to be produced. ROS carry reactive free radicals, and they react with other molecules and cells within the body, changing their natural mechanisms (Agarwal et al. 2012). When produced in excess ROS are incredibly disruptive to cellular function, causing losses to membrane integrity, induces structural changes to proteins and damage to nucleic acids (Tamura et al. 2012).
Antioxidants, in particular Melatonin and Vitamin A, are able to reduce the effects of ROS.

Melatonin is recognised as a universal antioxidant because it is highly hydrophobic and hydrophilic meaning it is readily passed through almost all organs and fluids (Tamura et al. 2015).
Melatonin can be transferred from the blood into the follicular fluid and its antioxidant properties allows melatonin to scavenge ROS (Tamura et al. 2012). Melatonin is also found in follicular fluid and directly influences functioning of the follicle-oocyte compartment (Cruz et al. 2014). Vitamin A is a natural retinooid that also has scavenger activity against free radicals (Ikeda et al. 2005). Vitamin A is a regulator of cell growth, cell differentiation and embryo morphogenesis and thus play a fundamental role in signalling and controlling cell proliferation (Hidalgo et al. 2005). The effects of each of these antioxidants on their ability to alleviate heat stress will be determined.

The primary aim of this project (to be undertaken in 2016) is to determine when heat stress has the greatest effect, during oocyte maturation or early embryo development. The secondary aim, is to determine whether supplementation of vitamin A or melatonin to the media will alleviate the impacts of heat stress. We hypothesise that heat stress will have the greatest impact during maturation of the oocyte as it has previously been shown by Kraeling and Webel (2015) that the most detrimental periods are during the first 30 days of gestation, due to a large increase in embryonic death. It is also hypothesised that melatonin and retinol will alleviate the negative effects of heat stress, because these two compounds have previously been shown to improve oocyte and embryo survival in pigs and have, to some degree, alleviated the effects of heat stress in other species.

**Materials and Methods**

Oocytes will be aspirated from abattoir derived ovaries and matured in vitro (IVM) for 44 hours at either 38°C (control) or 41°C (heat stress). Following IVM oocytes will be collected and stained to determine nuclear maturation: germinal vesicle, germinal vesicle breakdown, metaphase 1, anaphase to telophase and metaphase II will be assessed. The spent media will be tested for glucose consumption and lactate production and the ADP:ATP ratio of the pools of 5 oocytes will be assessed using a luminescence assay. A subset of oocytes will be assessed for polar body formation to determine the effect of heat on maturation rates.

The remaining matured oocytes will then be moved through hyaluronidase and then undergo in vitro fertilization (IVF) at either 38°C or 41°C for 6 hours. After this time the oocytes will be assessed for fertilisation based on cleavage rate. Excess sperm will be removed and the presumptive zygotes will be transferred to in vitro culture (IVC) media and incubated at either 38°C or 41°C. Blastocyst development will be assessed at day 8 and total cell counts determined using a Hoechst 33342 staining protocol. The percentage of 2 cell, morula and blastocyst formed will determined to assess the effects of heat stress on early embryo development. Following this, the stage of in vitro embryo production that is most affected by heat stress will be statistically identified and the experiment described above will be repeated with the addition of either melatonin or retinol during IVM, IVF and/or IVC.

**Discussion**

The current production and economic losses to pork production farmers during the summer-autumn period is the main purpose behind conducting this research. Defining the underlying causes of seasonal infertility in sows is an ongoing problem, and proof of the paramount significance of this research. Determining whether the detrimental effects of heat stress in vitro are most severe during porcine oocyte maturation, fertilisation or early embryo development, will allow a targeted on farm approach to overcoming this issue. Additionally, the research relating to the action of certain antioxidants may allow a practical method for farmers to combat the problem.


Bertoldo, M, Holyoake, PK, Evans, G and Gruppen, CG 2010, ‘Oocyte developmental competence is reduced in sows during the seasonal infertility period’, Reproduction, fertility, and development, vol.22(8), pp.1222-1229

Cruz, M, Leal, C, Cruz, J, Tan, D and Reiter, R 2014, ‘Essential actions of melatonin in protecting the ovary from oxidative damage’, Theriogenology, vol.82, pp.925-932


