Animal Reproduction Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Anti-Müllerian hormone and Oestradiol as markers of future reproductive success in juvenile gilts

Alicia Steel[a,*], Rebecca Z. Athorn[b], Christopher G. Grupen[a]

[a] Faculty of Veterinary Science, School of Science, The University of Sydney, 425 Werombi Road, Brownlow Hill, NSW, 2570, Australia
[b] Australian Pork Ltd. ACT, Australia

ARTICLE INFO

Keywords:
Gilt retention
Anti-Mullerian Hormone (AMH)
Estradiol (E2)
Gonadotropin responsiveness
Fertility
Stillbirth
Gestation length

ABSTRACT

There is a need for an early marker for reproductive success in gilts as the traditional process for selecting breeding females is inefficient. There is evidence that circulating anti-Müllerian hormone (AMH) is indicative of ovarian reserve, antral follicle populations, gonadotropin responsiveness and fertility in various species other than the pig. Additionally, oestradiol (E2) has been shown to mark antral follicle populations in cattle and pregnancy outcomes in women, after gonadotropin treatment. The aims of this study were to determine whether 1) serum levels of AMH or E2, prior to or after gonadotropin injection at 60, 80 or 100 days of age, and 2) hormonal changes in response to gonadotropin stimulation (i.e. declining, plateauing or increasing hormone levels), are associated with future reproductive success in juvenile gilts. Serum samples were obtained at 0, 2 and 4 days after injection and mating and litter data were collected until parity three. Results showed that, regardless of age group and parity, Day 0 E2 levels were positively associated with the probability of stillbirth ($P = 0.035$) and E2 levels on Day 0 ($P = 0.032$), Day 2 ($P = 0.045$) and Day 4 ($P = 0.019$) were negatively associated with the number of piglets born alive. Further, both a single measurement of serum AMH levels at Day 2 ($P = 0.048$) and the AMH response type were associated with gestation length ($P = 0.012$). These findings suggest that serum AMH and E2 levels can be used to inform the selection of gilts for the breeding herd.

1. Introduction

Poor sow retention rates are a major source of economic loss for pork producers. In Australia, it is estimated that only around 60% of female pigs are retained to parity three (The Australian Pig Annual, 2014). This is concerning as female pigs do not reach optimal reproductive performance until parity three (reviewed by Engblom et al. (2007)). Moreover, gilt progeny have lower growth and sale weights, higher fat deposition and higher pre-weaning mortality rates than sow progeny (Smits and Collins, 2009). Poor reproductive performance is the most prevalent contributing factor for removal of breeding females prior to parity three and gilts are the most vulnerable age group to culling for this reason (Stalder et al., 2004; Hughes et al., 2010; Plush et al., 2016). This indicates that the traditional process for selecting replacement gilts (based on body conformation, number of teats and dam performance) is inadequate.

Ovarian characteristics such as follicular populations and gonadotropin responsiveness are positively linked with future fertility in females. However, up until recent decades, these parameters could not be measured without the use of ultrasound or surgery. Research shows that circulating anti-Müllerian hormone (AMH) is a good marker for ovarian reserve and antral follicle populations in

* Corresponding author.
E-mail address: alicia.steel@sydney.edu.au (A. Steel).

https://doi.org/10.1016/j.anireprosci.2018.05.024
Received 15 December 2017; Received in revised form 15 April 2018; Accepted 23 May 2018

cattle (Hirayama et al., 2012; Batista et al., 2014), sheep (Torres-Rovira et al., 2014); mares (Claes et al., 2014), humans (reviewed by La Marca et al. (2009)) and mice (Kevenaar et al., 2006) as well as a strong indicator of responsiveness to gonadotropins in cattle (Guerreiro et al., 2014; Souza et al.,2015; Ghanem et al., 2016; Hirayama et al., 2017), sheep (Lahoz et al., 2014; Torres-Rovira et al., 2014), goats (Monniaux et al., 2011) and humans (reviewed by La Marca et al. (2009)).

Oestradiol (E2) has also been shown to be associated with reproductive qualities in cattle and humans. The hormone is responsible for the growth and development of reproductive organs including ovarian follicles and the uterus. Clinical studies show that women with elevated basal E2 levels have poorer reproductive capacity (Royster et al., 2016). Cattle with low antral follicle counts have concentrations of E2 that are twice as high in follicular fluid compared with cattle with high antral follicle counts (Ireland et al., 2008). Furthermore, E2 hormonal profiles (declining, plateau or increasing) in response to gonadotropin administration has been linked with reproductive potential in women. Previous studies have shown that embryos produced in vitro from women who showed a declining E2 profile after gonadotropin administration resulted in lower pregnancy and live birth rates when compared to those that a plateau or increase in circulating E2 (Laufer et al., 1986; Kondapalli et al., 2012). Furthermore, women who display a plateau in E2 have been shown to have fewer oocytes recovered and women who had experienced a plateau or decrease in E2 had reduced ovarian reserve and implantation rates (Kondapalli et al., 2012).

This study aimed to quantify circulating AMH in juvenile gilts and to determine whether a single measurement of serum AMH and/or E2 in juvenile gilts (prior to, or after exogenous gonadotropin stimulation) or different hormonal profiles in response to exogenous gonadotropin can be used to predict future breeding characteristics.

2. Methods

2.1. Animals and ethics

All animal procedures were conducted with prior institutional ethical approval by the Rivalea Australia Animal Ethics Committee under the requirements of the NSW Prevention of Cruelty to Animals Act 1985, in accordance with the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organisation/Australian Animal Commission Code of Practice for the Care and Use of Animals for Scientific Purposes.

Eighty-five multiplier gilts (F1: Large White x Landrace, PrimeGro™ Genetics, Corowa, NSW) aged between 60 and 100 days of age were included in the study (D60: n = 16; D80: n = 37; D100: n = 32). Between the age of 28 and 70 days (approximately) gilts were housed in conventional weaner pens in groups of 45. At approximately 70 days of age gilts were moved to large commercial grower pens and housed on concrete slatted floors in groups of 200 until they were approximately 130 days old. At 130 days of age gilts were re-grouped into pens of 50 and housed in conventional finisher pens on concrete slatted and solid floors (50:50). When all gilts reached 160–170 days of age, they were evaluated and selected to enter the breeding herd. Selection criteria at this time point included live weight (gilts must have been heavier than 70 kg at selection to be used for breeding); body, vulva and udder conformation; teat number; and absence of physical defects such as hernias or lameness.

Once selected, gilts were kept for approximately five weeks in commercial finisher pens before being transferred to the mating shed for boar exposure and oestrus detection (approximately 190 days age). Gilts were mated (by artificial insemination with 2.3×10^9 sperm cells) at their first or second observed oestrus depending on the recommendation indicated by the estimated weight at each observed oestrus (measured by the Allometric Growth Tape for Gilts; SRDP, University of Alberta, Canada). The growth tape approximates the live weight of the animal at oestrus according to the circumference of the girth of the animal at the level of the shoulder, and recommends mating or measuring again at the next observed oestrus (101–135 kg), mating at the observed oestrus (136–150 kg), or not mating (< 100 kg or > 150 kg) based on this approximation. After mating, all gilts were housed for the duration of their gestation in group pens of 40–45 (space allowance approximately 1.8 m^2 per sow) and fed via and electronic sow feeding system

The feeding regime varied throughout the gilts' lifetime. Gilts were given ad libitum access to several commercial weaner and grower diets from weaning until around 130 days of age. Gilts were then fed a specific gilt developer diet ad libitum until entry into the breeding herd at approximately 190 days of age. Once transferred to the breeding herd they were fed a commercial lactation diet ad libitum up until they were mated. In gestation, gilts and sows were restrict-fed approximately 2.3 to 2.5 kg per day of a commercial gestation diet up until farrowing. Access to feed was ad libitum during lactation, except in the first four days after farrowing where they were fed on a step-up program. Pregnancy and farrowing outcomes were recorded for each successive mating for up until parity three. If a sow was removed from the herd prior to the end of the recording period, the reason for and age of the gilt/sow at removal was recorded.

2.2. Gonadotropin stimulation, blood collection and storage

Juvenile gilts aged 60, 80 or 100 days (Rep 1: n = 64; Rep 2: n = 21) were injected with a low dose (200 IU) of PG600 (1:2 PMSG:hCG; Intervet, Holland). Blood samples were collected at 0, 2 and 4 days after the injection into serum separator tubes using 18 *g* x 25 mm vacutainer needles and left to clot for two hours at room temperature. The tubes were then centrifuged at 1000 rcf for twenty minutes and sera separated and then stored at -80 °C prior to assay.