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The effects of season and moderate nutritional restriction on ovarian function and oocyte nuclear maturation in cycling gilts

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ABSTRACT

The fertility of female pigs is impaired during summer and in response to restriction of feed intake, resulting in reduced productivity of the breeding herd. This study determined the effect of season and moderate nutritional restriction on ovarian function and oocyte developmental competence of cycling gilts. Eighty prepubescent gilts were used across two seasons—summer (S: January to March) and winter (W: June to August)—and received either a high (2.5× maintenance) or a moderately restricted (1.5× maintenance) feeding level for the first 19 days of their second estrous cycle. On Day 19, ovaries were collected post-slaughter. Diameters of all surface follicles over 1 mm were measured. All follicles ≥4 mm were aspirated and cumulus–oocyte complexes underwent *in vitro* maturation for ∼44 hours to assess oocyte developmental competence on the basis of metaphase II (MII) attainment. Moderate dietary nutrition reduced daily liveweight gain but did not affect the ovarian follicle population or oocyte developmental competence. The number of large follicles (≥6 mm) was lower during summer (S: 10.7 ± 1.74 vs. W: 15.5 ± 1.15, $P < 0.05$), as was the proportion of oocytes at the germinal vesicle stage of meiosis (S: 0.06 ± 0.02 vs. W: 0.08 ± 0.02, $P < 0.05$). However, the proportion of oocytes attaining MII was similar in summer and winter (S: 0.72 ± 0.04 and W: 0.69 ± 0.06, $P > 0.05$). Intrafollicular concentrations of luteinizing hormone were higher in summer (S: 43.05 ± 6.44 vs. W: 12.05 ± 5.12 ng/mL, $P < 0.001$), whereas estradiol was lower (S: 1.27 ± 0.36 vs. W: 27.52 ± 5.59 ng/mL, $P < 0.001$). In conclusion, our data demonstrated that in summer, follicle growth beyond 6 mm is impaired during the periovulatory period, without affecting oocyte meiotic competence. Importantly, these data also demonstrated that ovarian follicle growth and the capacity of oocytes to reach MII *in vitro* appear unaffected by moderate nutritional restriction during the preceding estrous cycle.

1. Introduction

A major determinant of profitability in the Australian pig industry is the reproductive efficiency of the breeding herd, which is measured by farrowing rate and litter size [1]. Litter size is determined by the number of oocytes released during ovulation that are fertilized and successfully develop into a viable piglet [2]. Between 5% and 15% of

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the oocytes released at ovulation are not fertilized, and up to 30% of the embryos do not develop after Day 30 of pregnancy [3,4], reproductive losses that represent lost economic potential for the pig production industry [5]. Recent studies suggest that oocyte quality may be a primary determinant of embryo viability and survival [1,6]. Oocyte quality is affected by the maternal environment, especially the nutritional status of the animal; the external environment such as season and temperature; and the age of the animal [7,8].

The link between nutrition and reproductive performance has been well established, with severe feed restriction significantly impairing reproduction in pigs [9]. More specifically, ovarian follicle growth and oocyte quality are impaired in cycling gilts and sows fed at maintenance levels or below compared with those fed close to *ad libitum* [1,10–12]. Although moderate nutritional restriction reduced ovarian follicle growth beyond 3 mm and decreased oocyte meiotic competence in prepubertal gilts [13], the effect of moderate nutritional restriction on ovarian function has not been studied in cycling gilts.

The adverse effects of seasonal infertility on the reproductive efficiency of breeding sows are well established [14–16]. However, there is little information on how season affects the ovarian physiology and oocyte developmental competence [17]. Previous studies on seasonal infertility have concentrated on weaning-to-estrus intervals [18], conception rates [19], early disruption of pregnancy [20], and decreased litter size [21] as measures of reproductive outcomes, and there has been limited work on the effects of season on oocyte development before fertilization [22–24]. As a result, the underlying effects of season on oocyte developmental competence in cycling gilts are not well understood, and the physiological mechanisms through which season disrupts early pregnancy are yet to be established [17]. In addition, male seminal characteristics such as semen quality and volume are also impaired during summer or periods of increased temperature and humidity [25–27], which presents a confounding factor when determining gilt reproductive performance during the seasonal infertility period.

In order to determine whether the seasonal effects on reproductive efficiency in cycling gilts relates to the quality of the developing oocyte before ovulation, and whether ovarian function and oocyte quality could contribute to seasonal infertility, a more detailed examination of the oocyte is required. Further, the effects of the interaction between season and nutrition have yet to be established. Therefore, this study tested the hypothesis that ovarian function and oocyte quality would be impaired in cycling gilts in late summer/early autumn and that this impairment would be further exacerbated by a moderate feed restriction in late summer/early autumn. The aim of the study was to investigate the effects of season and moderate nutritional restriction on ovarian function and oocyte developmental competence in cycling gilts.

2. Materials and methods

All animal procedures were conducted at the University of Adelaide piggery, Roseworthy, South Australia, with approval from the Animal Ethics Committee of the University of Adelaide (Ethics code S-2012-011). The experimental design was a 2 $\times$ 2 factorial comparing the main effects of season and feed intake on oocyte developmental competence in cycling gilts. The two seasonal blocks were SUMMER; S (15th February to 10th March) versus WINTER; W (7th July to 4th August) and two feeding levels; 2.5 times maintenance (HIGH), which was the control, versus a moderately restricted diet; 1.5 times maintenance (MOD). Throughout the trial period, from selection until slaughter, the gilts were fed the same commercial diet containing 16% crude protein and 13.0 MJ DE, 0.45% lysine sulfate, and 5.1% fiber (Table 1). Maintenance feed intake was calculated as $0.444LW^{0.75}$ (Feeding Standards for Australian Livestock Pigs).

2.1. Animals, housing, and management

Eighty Large White $\times$ Landrace terminal sire line gilts ($n = 40$ gilts/season) were stimulated to attain puberty at 178 days (107.2 $\pm$ 1.0 kg) using a single intramuscular injection of PG600 (400 IU pregnant mare serum gonadotrophin and 200 IU human chorionic gonadotrophin (Intervet Australia Pty Ltd)) and 15 minutes of daily, full physical contact with a mature boar (>11 months of age). Puberty occurred at 182.4 $\pm$ 3.7 days of age, with estrus defined as the exhibition of a standing reflex, either in response to the manual application of pressure to the gilt's back (back pressure test) or mounting by the boar as described by Bartlett et al. [28].

Following puberty attainment, gilts were allocated to their treatment groups based on their liveweight (LW) and predicted day of second estrus to attain similar mean LW and predicted slaughter days between the treatment groups. Until their second estrus, gilts were group-housed and had *ad libitum* access to food and water.

From the onset of their second estrus, the gilts were individually housed and fed their experimental diets at either HIGH (mean 3.3 kg/day) or MOD (mean 1.9 kg/day) feeding levels from Day 1 until Day 19 after second estrus (Day 0 was determined as the first expression of behavioral estrus). Gilts received 15 minutes fence line boar exposure

Table 1
Main ingredients and chemical analysis of commercial diet fed to gilts.

Ingredients	%	Chemical analysis	
Barley	15.00	Dry material (%)	90.26
Wheat	41.49	Digestible energy (MJ)	12.99
Millrun	20.00	Protein (%)	16.00
Peas	7.40	Fat (%)	3.60
Canola expeller	8.40	Fiber (%)	5.10
Meatmeal	3.13	Calcium (%)	0.85
Tallow	0.50		
Limestone	1.25		
Sodium betonite	1.10		
Sodium chloride	0.20		
Lysine sulfate	0.45		
Threonine	0.11		
Avizyme 2100	0.02		
Betaine liquid	0.20		
Lienerchrom 400 PMX	0.05		
HP Grower PMX	0.15		
Biofix Select	0.05		

Maintenance feed intake ($0.444^{LW,\ 0.75}$) calculated using "Feeding Standards for Australian Livestock Pigs."