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Effects of pre- and post-mating feed intake on blastocyst size, secretory function and glucose metabolism in Meishan gilts

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Abstract. This experiment was designed to determine the effects of a nutritional regime, known to increase embryo survival, on blastocyst development and function. Day 12 blastocysts were recovered from Meishan gilts allocated in a 2×2 factorial design to receive either a high or a maintenance diet before or after mating (n = 4–6 gilts per group). The post-mating diet had no effect on individual blastocyst size, cell number, secretion of oestradiol-17β or retinol binding protein, glucose metabolism or on the within-litter variability in these measures. Blastocysts recovered from gilts consuming the high pre-mating diet had more cells (13.501 *v.* 13.006 log cells; SED = 0.23; P = 0.05), greater production of CO_2 from glucose (2.19 *v.* 1.23 log pmol^{-1} blastocyst^{-1} 3 h^{-1}, SED = 0.42; P = 0.05) and a lower within-litter standard deviation in blastocyst surface area (0.66 *v.* 1.18 log mm^2, SED = 0.24; P = 0.04) compared with gilts fed the maintenance pre-mating diet. Collectively, these data suggest that a nutritional strategy that increases embryo survival is also associated with an increase in individual blastocyst cell number and reduced within-litter variability in blastocyst size.

Introduction

Although it is widely accepted that nutritional status affects porcine embryo survival (reviewed by Foxcroft 1997; Ashworth and Pickard 1998), the underlying mechanisms are poorly understood. For example, there is much confusion over whether nutritional regimes that enhance embryo survival do so by altering maternal physiology, such that the uterus provides a more appropriate environment to nurture developing embryos, or by altering functional aspects of embryo development. Recent work from our laboratory has clearly shown that the pre-mating nutritional status has a greater impact on embryo survival than the post-mating nutrition (Ashworth *et al.* 1999), suggesting that pre-mating nutrition may influence oocyte maturation, which in turn could alter growth and the functional aspects of embryo development.

It is generally agreed that more developed porcine embryos have a greater chance of survival (reviewed by Wilmut *et al.* 1985). This is believed to be related to the secretion of oestradiol-17β and retinol binding protein by the more advanced Day-11 and Day-12 embryos, which stimulates changes in the maternal environment to support their continued development, but which are detrimental and sometimes toxic to their less developed littermates (Pope *et al.* 1990). In addition, it has been proposed that embryo survival would be higher in gilts in which embryo development within a litter was less variable (Wilmut *et al.* 1986; Pope *et al.* 1990). Few studies have assessed the effects on within-litter variability in blastocyst size of nutritional regimes known to improve embryo survival. In the only study published to date, Whaley *et al.* (1997) observed that increased embryo survival following a pre-mating injection of vitamin A was associated with reduced within-litter variability in porcine blastocyst size on Day 12.

The objectives of the present experiment were to determine whether the pre- or the post-mating diet had the greater impact on embryo growth, secretory function and glucose metabolism and to test the hypothesis that nutritional regimens that increase embryo survival do so by reducing within litter variability in blastocyst development.

Materials and methods

Experimental animals

Day 12 blastocysts were recovered from the four groups of Meishan gilts described by Ashworth *et al.* (1999) that had received different amounts of a pre- or post-mating diet. Briefly, 24 pure-bred Meishan gilts weighing 118.5 ± 3.25 kg, which had previously received 2.3 kg day^{-1} of a complete diet supplying 14 MJ DE kg^{-1}, received either 1.15 kg day^{-1} (maintenance; n = 12) or 3.5 kg day^{-1} (high, i.e. ~3× maintenance; n = 12) of the same diet for an entire oestrous cycle. Gilts were checked daily for oestrous behaviour and naturally mated at 0, 12 and 24 h after the onset of the following oestrus by at least two different Meishan boars. Each group was subdivided such that six gilts from each group received the high diet after mating while the remaining six gilts received the maintenance diet. The post-mating diet commenced on the first day after the onset of behavioural oestrus and continued until slaughter on Day 12. The reproductive tract was collected aseptically immediately after exsanguination and each uterine horn was irrigated with 30 mL 0.9% sterile sodium chloride (Aquapharm 1, Animalcare Ltd, York,

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Yorkshire, UK) to collect conceptus tissue. Upon recovery from the uterus, blastocysts were measured in their shortest and longest dimension, and cultured individually in 3 mL minimum essential medium (MEM; Life Technologies Ltd, Paisley, Scotland, UK) on a rocking platform in a controlled atmosphere chamber gassed with 47.5% O_2, 50% N_2 and 2.5% CO_2. After 6 h, 750 μL of MEM was removed for subsequent oestradiol-17β determination. After 24 h the culture media were aspirated, clarified by centrifugation at 3000*g* for approximately 2 min and stored at –20°C. All blastocysts recovered from three randomly selected gilts from each treatment group were individually transferred to open eppendorf tubes containing 1 mL sterile glucose-free synthetic oviducal fluid medium (SOFM; Tervit *et al.* 1972) containing 32 g L^{-1} BSA and buffered with 25 mM Hepes, to determine blastocyst glucose metabolism. All remaining conceptuses were resuspended in 1 mL sterile phosphate buffer containing 2 M NaCl (LaBarca and Paigen 1980) and frozen at –70°C. All experimental procedures were approved by the appropriate ethical committee and conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986.

Blastocyst glucose metabolism assays

A modification of the methods described by McEvoy *et al.* (1997) to determine glucose utilization by ovine embryos was used. Individual pig blastocysts were washed with several changes of SOFM and incubated at 38°C in 200 μL of SOFM containing 1850 Bq D-[5-^{3}H]glucose (specific activity 607 GBq $mmol^{-1}$; Amersham International, Amersham, Bucks., UK) and 1850 Bq D-[U–^{14}C] glucose (specific activity 10.9 GBq $mmol^{-1}$; Amersham International). Each eppendorf tube was placed in a sealed vial containing 1 mL of 0.1 M NaOH, which served to trap CO_2 produced by glucose oxidation and to act as a reservoir for the exchange of tritiated water produced during glycolysis. The incubation was terminated after 3 h when the NaOH reservoir was added to vials containing 8 mL of liquid scintillation cocktail (Emusifier-Safe, Packard Instruments, Groningen, The Netherlands). Levels of ^{3}H and ^{14}C were determined by scintillation counting. NaOH aliquots from tubes containing 200 μL of identically radiolabelled SOFM, but not blastocysts, served as background controls. The SOFM surrounding each blastocyst was discarded and replaced with 1 mL of sterile phosphate buffer containing 2 M NaCl, which was frozen at –70°C.

DNA analysis

The DNA content of each conceptus was determined using a modification of the methods described by LaBarca and Paigen (1980). Conceptus tissues were thawed and sonicated on ice for three, 7-s bursts to solubilize the tissue. A standard curve was prepared using calf thymus DNA (Sigma Chemical Co., Poole, Dorset, UK) ranging from 0.001 to 20 μg mL^{-1}. The concentration of DNA in each standard or sample was measured in duplicate by adding Hoechst 33258 dye (bis-benzimide, Sigma Chemical Co.), at a concentration of 2 μg mL^{-1} assay buffer. The assay was performed in black 96-well microtitre plates and the resultant fluorescence measured using a Dynatech Fluorolite 1000 microfluorimeter (Dynatech Laboratories Ltd, Billinghurst, West Sussex, UK.) through an excitation filter of 450 ± 32.5 nm. Background fluorescence was accounted for by subtracting that of a sample of assay buffer. The number of cells per conceptus was calculated assuming that each porcine cell contained 5 pg DNA (Pusateri *et al.* 1990).

Constituents of conceptus culture media

Levels of oestradiol-17β were determined by radioimmunoassay according to the method of Webb *et al.* (1985) incorporating the modifications described by Ashworth *et al.* (1996). The mean inter- and intra-assay co-efficients of variation were 9.71% and 6.12%, respectively. The minimum detectable dose was less than 7.5 pg mL^{-1}. Levels of retinol binding protein were determined by radioimmunoassay as described by Vallet (1994), the mean inter- and intra-assay co-efficients of variation being 19% and 5%, respectively.

Data analysis

Data describing blastocyst glucose metabolism were available from blastocysts recovered from 12 gilts (n = 3 per treatment), whereas data describing blastocyst size, cell number and secretion of oestradiol-17β and retinol binding protein were available from 20 gilts (n = 4–6 per treatment group), which carried blastocysts that could be cultured individually. Measures of blastocyst glucose metabolism and secretion of oestradiol-17β and retinol binding protein were expressed per blastocyst and on a per cell basis. Blastocyst surface area was estimated from the equation

$$(2(\max \times \min) + \min^2) \times 0.1$$

where min and max correspond to the minimum and maximum dimensions, respectively, of each blastocyst. This equation estimates the surface area of an ovoid. It is derived from the observation that the ratio of the surface area of a sphere to that of an enclosing cube is $\pi/6$ and assumes that approximately the same relationship would apply to the ratio of the surface area of an ellipsoid and the enclosing parallelepiped (S. Ferris, personal communication).

Estimates of individual blastocyst surface area, cell number, ^3H_2O and $^{14}CO_2$ production and oestradiol-17β and retinol binding protein secretion were log transformed in order to normalize the data and the within-litter standard deviation in these measures calculated to assess within-litter variability.

The effects of the pre- and post-mating diets and their interaction on within-litter standard deviations in blastocyst surface area, cell number, ^3H_2O and $^{14}CO_2$ production and oestradiol-17β and retinol binding protein secretion were determined by analysis of variance. The effects of the pre- and post-mating diets and their interaction on individual blastocyst size, cell number, glucose metabolism and secretory function were determined by analysis of variance using a block structure that included the effect of gilt. Sequentially fitting both the total number of embryos per gilt and the number of embryos per uterine horn as covariates did not consistently enhance the statistical model and these terms were omitted from the final analysis. All analyses were performed using the Genstat 5 Statistical Package (Genstat 5 release 3.1; Genstat Committee of the Statistics Department, Rothamsted Experimental Station, Harpenden, Hertfordshire, UK).

Results

Of the 24 gilts used in the present experiment, one was not pregnant at the time of slaughter and three carried filamentous blastocysts recovered from the uterus as a tangled mass of tissue that could not be cultured individually. Of these three gilts, one was from the group receiving the maintenance diet throughout the experimental period, and the other two had received the high diet both before and after mating.

The post-mating diet had no significant effect on any aspect of blastocyst growth or function measured, or on the within-litter variability in these measures. However, the within-litter variation in blastocyst oestradiol-17β secretion per cell and ^3H_2O production tended to be lower in gilts consuming the high post-mating diet (Tables 1, 2). Blastocysts recovered from gilts consuming the high pre-mating diet had more cells (Table 3) and higher levels of $^{14}CO_2$ production (Table 4) than blastocysts recovered from gilts consuming the maintenance diet prior to mating. Furthermore, the high pre-mating diet reduced the within-litter variability in blastocyst surface area (Table 1). The interaction between the pre-

Table 1. Effect of pre- and post-mating diet on within-litter standard deviation in conceptus size, cell number and secretion of oestradiol-17β and retinol binding protein

Pre-mating diet	Maintenance		High			
Post-mating diet	Maintenance	High	Maintenance	High		
No. gilts	5	6	5	4	Average SED	Significance
Surface area (log mm^2)	1.291	1.138	0.781	0.501	0.335	Pre-mating $P = 0.04$ Post-mating $P = 0.48$
No. cells $conceptus^{-1}$ (log)	0.531	0.821	0.556	0.630	0.374	Pre-mating $P = 0.71$ Post-mating $P = 0.47$
Conceptus oestradiol-17β secretion (log ng $conceptus^{-1}$)	1.162	1.025	1.002	0.786	0.364	Pre-mating $P = 0.49$ Post-mating $P = 0.51$
Oestradiol-17β secretion (log pg 1000 $cells^{-1}$)	0.902	0.520	0.800	0.533	0.245	Pre-mating $P = 0.94$ Post-mating $P = 0.07$
Retinol binding protein (log ng $embryo^{-1}$)	0.409	0.530	0.530	0.465	0.201	Pre-mating $P = 0.85$ Post-mating $P = 0.80$
Retinol binding protein (log pg $cell^{-1}$)	0.657	0.764	0.658	0.808	0.308	Pre-mating $P = 0.97$ Post-mating $P = 0.57$

There were no significant effects of the pre-mating × post-mating diet interaction. SED, standard error of the difference.

Table 2. Effect of pre- and post-mating diet on within-litter standard deviation in conceptus production of ^3H_2O and $^{14}CO_2$

Pre-mating diet	Maintenance		High			
Post-mating diet	Maintenance	High	Maintenance	High		
No. gilts	5	6	5	4	Average SED	Significance
^3H_2O production (log pmol $blastocyst^{-1}$ 3 h^{-1})	0.488	0.294	0.588	0.341	0.141	Pre-mating $P = 0.48$ Post-mating $P = 0.06$
$^{14}CO_2$ production (log pmol $blastocyst^{-1}$ 3 h^{-1})	0.865	0.800	0.930	0.812	0.398	Pre-mating $P = 0.89$ Post-mating $P = 0.75$

There were no significant effects of the pre-mating × post-mating diet interaction. SED, standard error of the difference.

mating and the post-mating diets had no significant effect on any of the variables studied.

Discussion

This is believed to be one of the few studies of the nutritional effects on embryo survival in the pig that has specifically examined the effects of maternal nutrition on individual blastocyst size, secretory activity and metabolism. Although the high post-mating diet tended to reduce within-litter variability in both blastocyst oestradiol-17β secretion and ^3H_2O production, the pre-mating diet had a greater impact on embryo growth, metabolic function and within-litter uniformity. Day 12 blastocysts recovered from gilts fed a pre-mating diet, previously shown to be associated with increased embryo survival and total embryo numbers (Ashworth *et al.* 1999), had more cells and reduced within-litter variation in surface area compared with contemporary blastocysts recovered from gilts consuming maintenance rations prior to mating. These data support our initial hypothesis that a nutritional strategy that increases embryo survival may do so by enhancing blastocyst growth and decreasing the within-litter variability in blastocyst size.

Three gilts carried blastocysts that were more developed than the vast majority collected and could not be separated and individually cultured. The three gilts were not all from the same treatment group and therefore this range in developmental stages is considered to reflect natural between-gilt variation in blastocyst development, rather than a specific treatment effect. The data presented are from the remaining 20 gilts, which produced Day 12 Meishan blastocysts of comparable developmental stages to those observed previously in our laboratory (Ashworth *et al.* 1997).

Table 3. Effect of pre-and post-mating diet on mean conceptus size, cell number and secretion of oestradiol-17β and retinol binding protein

Pre-mating diet	Maintenance		High			
Post-mating diet	Maintenance	High	Maintenance	High		
No. gilts	5	6	5	4		
No. blastocysts	70	72	94	71	Average SED	Significance
Surface area (log mm2)	1.513	1.068	2.092	1.748	0.663	Pre-mating P = 0.17 Post-mating *P* = 0.52
No. cells conceptus^{-1} (log)	13.13	12.96	13.55	13.52	0.335	Pre-mating *P* = 0.05 Post-mating *P* = 0.70
Conceptus oestradiol-17β secretion (log ng conceptus^{-1})	–1.75	–2.237	–1.349	–1.264	0.631	Pre-mating *P* = 0.72 Post-mating *P* = 0.96
Oestradiol-17β secretion (log pg 1000 cells^{-1})	–0.943	–1.325	–0.984	–0.898	0.497	Pre-mating *P* = 0.16 Post-mating *P* = 0.95
Retinol binding protein (log ng embryo^{-1})	4.806	5.237	6.340	5.780	1.198	Pre-mating *P* = 0.29 Post-mating *P* = 0.49
Retinol binding protein (log pg cell^{-1})	–1.405	–1.140	–0.338	–0.837	0.923	Pre-mating *P* = 0.57 Post-mating *P* = 0.47

There were no significant effects of the pre-mating × post-mating diet interaction. SED, standard error of the difference.

Table 4. Effect of pre-and post-mating diet on mean conceptus production of $^{3}H_2O$ and $^{14}CO_2$

Pre-mating diet	Maintenance		High			
Post-mating diet	Maintenance	High	Maintenance	High		
No. gilts	3	3	3	3		
No. blastocysts	31	39	67	32	Average SED	Significance
$^{3}H_2O$ production (log pmol blastocyst^{-1} 3 h^{-1})	0.863	0.442	0.513	1.275	0.518	Pre-mating *P* = 0.94 Post-mating *P* = 0.90
$^{3}H_2O$ production (log pmol cell^{-1} 3 h^{-1})	1.460	1.472	0.829	1.754	0.638	Pre-mating *P* = 0.13 Post-mating *P* = 0.42
$^{14}CO_2$ production (log pmol blastocyst^{-1} 3 h^{-1})	1.962	0.622	2.060	2.655	0.671	Pre-mating *P* = 0.05 Post-mating *P* = 0.38
$^{14}CO_2$ production (log pmol cell^{-1} 3 h^{-1})	2.556	1.796	2.367	3.025	0.660	Pre-mating *P* = 0.33 Post-mating *P* = 0.70

There were no significant effects of the pre-mating × post-mating diet interaction. SED, standard error of the difference.

The present studies have confirmed that porcine embryos metabolize glucose. Flood and Weibold (1988) demonstrated that before the 8-cell stage the majority of glucose metabolism by pig embryos occurred by the pentose phosphate pathway, whereas between the 8-cell to early blastocyst (Day 8) stage, glucose metabolism occurred almost exclusively via the glycolytic pathway. The present data show similar levels of production of $^{3}H_2O$ and $^{14}CO_2$ by Day 12 blastocysts, suggesting that at this later stage of pregnancy blastocysts metabolize glucose by both glycolysis and glucose oxidation. The present data also show that embryos recovered from gilts fed the high pre-mating diet, which had the highest embryo survival, produced more $^{14}CO_2$. The physiological significance of this observation is not clear, and may merely reflect the greater numbers of cells in these blastocysts. It is important to note that increased glucose metabolism is not necessarily a positive trait, but may rather be an indicator of cellular stress (Pasternak *et al.* 1991; Sviderskaya *et al.* 1996). McEvoy *et al.* (1997) showed that cleavage-stage embryos recovered from ewes fed high levels of supplementary urea for the preceding 12 weeks were developmentally retarded, less viable in culture and suffered

increased mortality compared with embryos recovered from contemporary control-fed ewes. However, both $^{3}H_2O$ and $^{14}CO_2$ metabolism was increased in embryos recovered from ewes fed the diets high in urea.

In the present study, blastocysts recovered from gilts fed the high pre-mating diet had greater numbers of cells and reduced within-litter variation in surface area compared with blastocysts recovered from gilts consuming maintenance rations prior to mating. These characteristics are known to enhance embryo survival; for example, embryo transfer studies have clearly shown that more developed pig embryos have a greater likelihood of survival (reviewed by Ashworth and Pickard 1998). Furthermore, it is generally agreed that a reduction in the within-litter variability in embryo development would be associated with increased embryo survival (Wilmut *et al.* 1985; Pope *et al.* 1990), as a greater proportion of the embryos would be at an appropriate stage of development for the changes occurring in the uterine environment. It is of interest that improvements in porcine embryo survival following pre-mating injections of retinol were also associated with increases in individual blastocyst diameter and reduced within-litter variability (Whaley *et al.* 1997), suggesting that these may be common factors mediating the effects of nutrient status on embryo survival.

The observation that the pre-mating diet had a greater impact on embryo growth and function than the post-mating diet suggests that nutritional effects on pre and peri-ovulatory events, such as oocyte maturation, may influence subsequent embryo development. In the only study performed to assess this possibility in the pig, Zak *et al.* (1997) showed that sows fed to appetite immediately prior to mating had more large follicles, more oocytes that had matured to metaphase II and a greater rate of oocyte maturation than sows that had been fed to appetite from farrowing until Day 21 of lactation, but received only 50% rations from Day 22 to 28, suggesting that increased feed intake prior to mating may enhance oocyte quality. In addition, embryo survival on Day 28 of gestation was higher in the re-fed sows, thereby highlighting the proposed link between oocyte quality and embryo survival in the pig.

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