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Oocyte and embryo metabolomics

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There is still much we do not understand about the metabolic requirements of oocytes and embryos. Their remarkable metabolic plasticity during in vitro culture has hampered our ability to make significant advances in culture media design that would better support optimal physiology. A new tool, metabolomics, may revolutionize what we know about the interactions between embryos and their culture environment. Armed with this knowledge, we may design more effective culture systems as well as discover metabolic biomarkers that predict oocyte and embryo viability. The objectives of this review are to introduce oocyte and embryo metabolism, review the current state of knowledge in the field, discuss the possibility that oocyte and embryo metabolism is significantly more complex than we have previously realized, examine a metabolomics dataset, and discuss how metabolomics may play a role in furthering our understanding of this exciting field that so significantly impacts the success of in vitro embryo production.

Introduction

Although incremental improvements have been made in the culture of oocytes and embryos from domestic species in the last decade, significant progress in improving in vitro maturation (IVM), fertilization (IVF) and culture (IVC) technologies remains elusive. Optimizing embryo culture media has been difficult, in part because we do not fully understand what the embryo requires in vitro to support successful development. Surprisingly, identifying the nutrient requirements of the early embryo has been difficult due to the plasticity of the embryo, rather than an extreme sensitivity of the embryo to culture conditions. Although the proportion of embryos developing to the blastocyst stage is affected by the composition of the culture medium, in most species some embryos are capable of successful development in a wide variety of media that often bear little resemblance to the composition of fluids of the follicle, oviduct and uterus. However, the metabolic costs of adaptation to suboptimal culture conditions can compromise embryo viability, cryotolerance, maintenance of pregnancy, fetal growth, and offspring health. Therefore, it is essential that embryo culture conditions provide the appropriate mixture of nutrients to support normal embryo physiology and to minimize adaptive stress and the associated risks to establishing pregnancy (Lane & Gardner 2007).

Metabolomics technology has tremendous potential to expand our knowledge of embryo metabolism. In contrast to other methodologies currently in use, metabolomics provides a broader, more complex view of oocyte and embryo metabolism by examining multiple metabolic pathways within the context of an optimized culture system, rather than examining a single pathway or metabolite under artificial conditions. Metabolism of radiolabeled substrates, as well as fluorescent measurements of metabolite consumption and/or production, has been widely used to date to study the basic cellular metabolic pathways in embryos from multiple species. However, these

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Mechanisms affecting litter sex ratio and embryo quality

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Sex ratios that deviate from 1:1 have been observed in response to a number of stimuli. In this review we will discuss sex ratio biasing, and the evolutionary and molecular mechanisms thought to underlie this phenomena in mammals. The role of embryo quality will be discussed in relation to sex ratio modulation and epigenetic programing of the embryo. Sex ratio skewing has been studied in many species and several factors have been proposed as influencing secondary sex ratios (body condition, maternal dominance, nutrition and developmental asynchrony). In swine, maternal nutrition has repeatedly been shown to influence offspring sex ratios, while maternal dominance and body condition exhibit less consistent evidence supporting their influence. Based on current evidence, we hypothesize that sex ratio biasing is the result of sexual dimorphisms that result in sex specific differences in embryo quality, and these differences lead to sex specific embryonic loss. The mechanisms through which sex specific loss occurs are not fully understood, however sexual dimorphisms in metabolism, gene expression and epigenetic mechanisms during early embryo development suggest that sex ratio modulation might be mediated through these mechanisms. We hypothesize that there are a number of mechanisms for skewing sex ratios in mammals, and that specific mechanisms are elicited in response to specific stimuli.

Introduction

Numerous factors influence embryo quality and offspring sex ratios, and to discuss all of these would be beyond the scope of this review: therefore, this review will focus on sex ratio modulation, and will specifically address embryo quality in relation to sex ratio biasing and programming mechanisms.

For many years investigators have been trying to answer the question of how adaptable sex ratios are within animal populations. Alteration of the sex ratio in mammals from the standard 1:1 ratio has implications for fields as diverse as evolutionary biology and livestock science, and while our understanding of the underlying theory and mechanisms of sex ratio skewing have improved, many answers remain to be found. Manipulation of sex ratio could have several economic advantages in the pig industry. Drickamer *et al*. (1997,1999) showed that both fertility rate and teat numbers were higher in gilts born from litters with a higher proportion of females: whereas Lamberson *et al*. (1988) demonstrated that in litters with higher numbers of males, the females in these litters had a reduced age of puberty. These characteristics are in

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Boar seminal plasma proteins and their relevance to reproductive technologies

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Seminal plasma proteins participate in a number of events important for fertilization and the establishment of pregnancy. As a result, attempts have been made to use them to enhance reproductive performance associated with several swine reproductive technologies. Inclusion of seminal plasma into cryopreservation and sex-sorting protocols improved sperm viability and membrane integrity and suppressed capacitation-like changes which are considered to be major challenges associated with these techniques. Unfortunately, it has yet to be shown that these improvements consistently increase in vivo fertility. In contrast, pre-breeding administration of seminal plasma in conjunction with conventional breeding regimens improved farrowing rates and numbers of pigs born alive on commercial farms that already had very good reproductive performance. The best way to capture these beneficial effects in A.I. programs currently is being investigated. Finally, three seminal plasma proteins appear to have reasonable correlations with fertility in boars that normally produce sperm with excellent motility and morphology. They hold potential for development of prospective male fertility tests. However, there is some evidence that indicates consideration of the complete profile of a boar's seminal plasma proteins may be more appropriate for this purpose as opposed to concentrating on individual ones independently. Preliminary results from a field study indicate that farrowing rate and litter sizes are superior in boars with high levels of two seminal plasma proteins associated with fertility compared with their counterparts in which only one of these is elevated. All of these technologies will benefit from continued research efforts devoted to the additional characterization of proteins in seminal plasma and elucidation of their biological effects on swine reproductive physiology.

Introduction

During natural matings, spermatozoa along with a small volume of fluid leave the cauda epididymi; are transported to the urethra where they are mixed with large volumes of secretions from the secondary sex glands; and then are deposited directly into the cervix of the sow.

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Cryopreservation of female germplasm in pigs

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Cryopreservation of female germplasm has basic importance in preservation and distribution of genetic lines in farm animals. Although vitrification technology has been applied for the cryopreservation of porcine oocytes and ovarian tissues, reduced developmental competence of preserved oocytes and the lack of offspring produced from them underlines the importance for further developments in cryopreservation protocols for this purpose. This review discusses the problems of female germplasm cryopreservation in pigs and the possible strategies to overcome them and gives an update on the present status of cryopreservation of porcine oocytes and ovarian tissues.

Introduction

Cryopreservation of female germplasm has basic importance in preservation and distribution of genetic lines in farm animals. Recent revival of some indigenous pig breeds in the pork market underlines the importance of the preservation of genetic diversity in pigs (Ratky *et al*. 2007; Daza *et al*. 2008). For decades, development of cryopreservation methods of porcine embryos and oocytes was far behind those of other domestic species. This was caused by the high sensitivity of porcine embryos and oocytes to low temperatures, the delayed establishment of *in vitro* embryo production (IVP) systems and the low quality of resultant oocytes and embryos. Although several research groups have established cryopreservation protocols for porcine oocytes in the last decades, embryo development remained low and there has been no report on piglets produced from cryopreserved oocytes to date. Therefore, oocyte cryopreservation in pigs still represents a challenge to be solved. In the meantime, new technologies for the cryopreservation of ovarian tissues are emerging which provide future possibilities for the cryopreservation of female germline. In the chapters below, we will discuss the problems of the germline cryopreservation in pigs and possible strategies to overcome them and give an update on the present status of cryopreservation of porcine oocytes and ovarian tissues.

Cryopreservation-related damages in porcine oocytes and embryos

The unique characteristics of porcine oocytes and embryos fundamentally determine their sensitivity to low temperatures and their feasibility to cryopreservation techniques. Porcine oocytes contain 156 ng lipid (McEvoy *et al*. 2000), which is extremely high, even compared to those of cows (89 ng/oocyte) (Ferguson & Leese, 1999). In porcine oocytes and embryonic cells, lipid droplets are usually form complexes with cytoskeletal elements, membranes and cytoplasmic organelles and play an important role in metabolism as energy source during

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Transcriptional profiling of oocyte maturation and embryonic development elucidates metabolism and control of development

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With the advent of next generation sequencing platforms (RNA-seq), transcriptional profiling permits the characterization of millions of RNAs from even the most limiting samples like early embryos. High-throughput RNA-seq can generate over 600 gigabases (Gb) in a single sequencing run, providing a near-complete record of all of the genes expressed in a sample at the time of collection. Condensing and finding coherence in the immense amount of raw data generated by transcriptional profiling methods such as RNA-seq is a complex task, but necessary if useful information is to be gleaned. Here we review the current technology and describe how transcriptional profiling has been used to improve oocyte maturation and embryo culture conditions, to decrease polyspermy and to improve somatic cell nuclear transfer. Most recently, RNA-seq data has provided a unique framework for understanding metabolism of the early embryo, i.e. the Warburg Effect. Rapidly proliferating cells use glucose for synthesis of nucleotides that are necessary for DNA synthesis. They shunt metabolism away from the tri-carboxylic acid cycle and toward lactic acid production and the pentose phosphate pathway. Pathways identified by RNA-seq data show that early embryos, as it turns out, are quite similar and thrive in conditions that promote proliferation of cancer cells. Application of the Warburg Effect framework to early embryos has, and will continue to contribute to improved culture conditions for embryos in vitro.

Introduction

A comprehensive understanding of factors that control oocyte maturation and pre-blastocyst stage development are needed to reduce reproductive losses and to apply reproductive technologies. Reproductive loss in pigs has been estimated to be as high as 30% through the first month of pregnancy. Any alterations in management or genetic selection that could reduce this loss would have a significant economic impact on production agriculture and may help reduce pregnancy loss in other mammals. In addition to the direct potential impacts on human health, a better understanding of what the oocyte and embryo need for robust growth will enable other technologies that may impact production agriculture. These technologies include *in vitro* embryo production (oocyte maturation, *in vitro* fertilization, and embryo culture) and the technologies that rely on *in vitro* embryo production (cloning by somatic cell nuclear transfer, genetic engineering, sex selection, etc.). To gain an understanding of what regulates

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Effects of season and follicle size on the metabolomic profile of porcine follicular fluid

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The domestic sow exhibits a decline in reproductive performance during the late summer and early autumn months, which in production systems is manifested as a decrease in farrowing rates. A recent study found that impaired oocyte quality may contribute to the seasonal infertility phenomenon, as oocytes collected in summer displayed a reduced capacity to form blastocysts in vitro compared with oocytes collected in winter (Bertoldo *et al*. 2010). The effect of season on oocyte quality was apparent in oocytes from large antral follicles (Bertoldo *et al*. 2010), which normally have superior developmental potential compared with oocytes from small antral follicles (Bagg *et al*. 2007). During the latter stages of folliculogenesis the cumulus oocyte complex is bathed in follicular fluid (FF), which provides a specialised and dynamic microenvironment that supports the final stages of oocyte maturation. While FF is known to consist of serum exudates and locally produced factors secreted by the follicular cells, detailed characterization of the composition of FF may provide useful insights into follicle growth and differentiation and the acquisition of oocyte developmental competence.

Metabolomics is the non-targeted identification and quantification of all the metabolites present in a biological sample. In contrast to transcriptomic or proteomic analyses, metabolomic analysis has the advantage of identifying the biological endpoints that occur as a result of environmental change or altered gene expression (Baskind *et al*. 2011). High resolution proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy provides a unique tool for studying the composition of biofluids as it is capable of identifying and quantifying all the metabolites present in an untreated sample. To date there are relatively few reports of the use of this technology to analyse FF samples (Baskind *et al*. 2011). Therefore, using the previously observed seasonal and follicle size effects on oocyte quality to classify models of good and poor follicular environments, the aim of the present study was to characterize the metabolomic profiles of porcine FF samples collected from small and large follicles in summer and winter by ^{1}H-NMR spectroscopy.

The FF samples were obtained from adult Large White/Landrace cross-bred sows as described previously (Bertoldo *et al*. 2011). The ovaries of sows culled for non-reproductive reasons (eg. lameness) were collected at slaughter, 4 days after weaning, in summer and winter (on at least three separate occasions in each season). The contents of small (3-4 mm) and large (5-8 mm) diameter follicles were aspirated and pooled separately (small FF and large FF) for each ovary pair. Cellular material was removed by centrifugation and the FF samples were stored at -20°C. Only FF samples of sufficient volume (500 μl) were analysed (summer-small (n = 8), summer-large (n = 15), winter-small (n = 9) and winter-large (n = 14)). For ^{1}H-NMR spectroscopy, the FF samples were thawed at room temperature, centrifuged at 3000g for 5 min, and prepared by mixing 500 μl of FF, 100 μL of D_2O solution and 100 μL of phosphate buffer to obtain a pH value of 7.4 $\pm$ 0.5. The spectra were acquired using a Bruker DRX-500 spectrometer (Bruker SADIS, Wissembourg, France) operating at 11.7 T, with a Broad Band Inverse probe head equipped with a Z gradient coil, and recorded with 90° pulse (p1 = 10μs, pl = 0dB) using a pulse-and-acquire sequence with residual water pre-saturation. Spectra were collected with 128 transients (and 8 dummy scans) in 32 K data points with a spectral width of 7500 Hz, and a recycling time of 15 s, and processed using WinNMR version 3.5 software (Bruker Daltonik, Karlsruhe, Germany). All spectra were corrected for phase distortion and the baseline was manually corrected for each spectrum. Spectral peaks were assigned according to the literature values of chemical shifts in various media and biofluids and quantified by using the electronic reference to

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Comparative transcriptomic analysis between in vivo derived porcine 4-cell and morula embryos

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During the pre-implantation period of embryonic development, the porcine embryo exhibits dramatic morphological changes associated with key developmental events such as embryonic genome activation (EGA). In mammals, oocyte-derived mRNAs are degraded shortly after fertilization, hence embryonic genome activation and production of embryo-derived transcripts must occur during early embryonic development (Thompson, Legouy & Renard 1998, Schultz 2002). EGA is a gradual process: a small portion of the embryo genome activates early and the major embryonic genome activation of abundant transcription occur later (Oestrup *et al*. 2009). The molecular mechanisms underlying these events are not yet fully understood. To better elucidate these mechanisms at the gene expression level, a comparative transcriptomic analysis between in vivo-derived 4-cell and morula stage porcine embryos was performed with with a custom designed porcine embryo-specific microarray platform (EMPV1: EmbryoGENE Porcine Array Version1 [GPL14925]) (Tsoi *et al*. 2012) (http://embryogene.ca/). In this study, the microarray analysis was performed following a reference design (Novoradovskaya *et al*. 2004, Konig *et al*. 2004) using a reference RNA pool generated from 10 different porcine embryonic stages (GV, MII, 2-cell, 4-cell, 8-cell, morula, early blastocyst, expanded blastocyst, hatched blastocyst stages and day 11 embryos).

In-vivo derived porcine embryos from 4-cell and morula stages were collected from gilts as described previously (Degenstein *et al*. 2008). All embryo samples were placed on dry ice immediately after collection and stored at -80°C. Total RNA samples were extracted from pools of five embryos using Arcturus® PicoPure® RNA Isolation Kit (Applied Biosystems, CA, USA). Total RNA samples were amplified using RiboAmp HS^{Plus} kit (Applied Biosystems, CA, USA) following manufacturer's instructions and generated amplified antisense RNA (aRNA) targets for microarray reactions. Each aRNA sample was labelled with Cy5 dye and hybridized with Cy3 dye labelled reference RNA pool on EMPV1 Microarray. Three biological replicates from each group were included in the comparative microarray analysis. Agilent two-colour RNA Spike-In® (Agilent Technologies, Mississauga, ON, Canada) were amplified, labelled and utilized as positive control in each hybridization as previously described (Tsoi *et al*. 2012). Microarray data analysis was performed using FlexArray software 1.6.1 (http://genomequebec.mcgill.ca/FlexArray). Simple background subtraction and within array lowess global normalization was performed on raw data from each array. The limma software (Smyth 2005) was utilized to identify the differentially expressed genes between different stages. Ingenuity® Pathway analysis (IPA) software were used for the biological process and pathway analysis. IPA Upstream Regulator Analysis tool was used in transcription factor identification and activation status (activated or inhibited) prediction using the regulation z-score algorithm ($|z| \geq 2$ is considered significant), which is calculated based on the direction of expression changes (up-regulate or down-regulate) of known transcriptional regulation targets in the experimental dataset and the direction of expectations derived from literature.

The comparative analysis revealed 2,582 differentially expressed genes between 4-cell and morula stage embryos. A major portion of these differentially expressed genes are associated

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Current progress in non-surgical embryo transfer with fresh and vitrified/warmed pig embryos

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Embryo transfer (ET) should play a critical role in the pig industry because it allows the movement and introduction of new genetic material into a herd with minimal risk of disease transmission and reduced transportation costs. In addition, embryo movement could prevent the potential health and welfare problems associated with transporting live pigs. Although the first successful ET was reported more than 60 years ago, the commercial use of this procedure in pigs is still in its infancy. The surgical requirements for embryo collection and transfer and the difficulties with embryo cryopreservation have prevented its use in pigs, unlike other livestock. However, new methodologies have been developed in the past decade to enable successful non-surgical ET and embryo cryopreservation that could allow the commercial use of ET in the pig industry. This review focuses on the development of these technologies with emphasis on our own findings. Specifically, we discuss the basic aspects of a non-surgical deep-uterine ET procedure and describe several factors that affect its efficacy in the transfer of fresh and short-term cultured embryos. Finally, we conclude with a brief discussion on the use of this procedure with long-term stored embryos.

Introduction

Little information is available concerning the reproductive performance of recipients after surgical transfers of fresh embryos. Most of the reports described experimental studies evaluating the pregnancy rates and fetus numbers 30–35 days after transfer. In general, these studies reported pregnancy rates of 60–80% with 5–8 fetuses per pregnant recipient (Polge 1982, Blum-Reckow & Holtz 1991, Wallenhorst & Holtz 1999). In only a limited number of surgical ET studies were the recipients allowed to carry litters to term, achieving farrowing rates of 50–80% and litter sizes of 6–8 piglets (James *et al*. 1980, Cameron *et al*. 1989, Niemann *et al*. 1989). Although Polge and Day (1968) demonstrated that pregnancy could be established in pigs through non-surgical ET, the procedure was considered an inefficient technique for many years due to the complex anatomy of the porcine cervix and uterus. However, new non-surgical procedures for embryo deposition were developed in the 1990s, achieving farrowing rates of 5–41% and litter sizes of 5–7.5 piglets (reviewed in Cameron *et al*. 2006). Among these procedures, the most promising involved the placement of embryos into the uterine body of non-sedated sows (Hazeleger &

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Birth weight and its impacts on testicular development in boars

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Selection for prolificacy appears to have created an imbalance between the number of conceptuses surviving to the post-implantation period and uterine capacity. Limited placental development due to intra-uterine crowding in early gestation results in entire litters with characteristics of Intra-Uterine Growth Restriction at birth, due to a great competition among fetuses for nutrients and oxygen, resulting in lighter fetuses at term (Foxcroft et al., 2009). The implications of birth weight on postnatal development, such as growth performance, muscle accretion and intestine morphology, have been described previously (Alvarenga et al., 2013). However, there is little information on its effects on testicular development in pigs. In the present study, we investigated some biometrical and morphological parameters of testicular development in light birth weight boars.

New-born male pigs (n = 56; PIC genotype), born to 4th- 6th parity sows and in litters of 10 to 15 pigs, were identified as falling into two birth weight groups: high (HW: range 2.0 to 2.2 kg) and low (LW: range 0.8 to 1.0 kg) littermates. A sub-set of 28 males from each experimental group was castrated at 8 days post-partum for evaluation of testicular biometrical (testes weight and volume) and histomorphologic characteristics. The other sub-set of 28 animals was castrated at 8 months of age for evaluation of the same testicular parameters. Testes' samples from both experimental groups at the evaluated ages were fixed in a glutaraldehyde solution and embedded in glycol methacrylate plastic resin. Histological sections (3 μm) were cut from these resin blocks and stained with toluidine blue-sodium borate for histomorphometric analysis (Chiarini-Garcia et al., 2011). These analysis included measurement of the seminiferous cords/tubules diameter, number of Sertoli cells present in twenty cross sections of cords/tubules, and the total Sertoli cell number per testis. Finally, to estimate sperm production capacity, the number of spermatids was calculated per gram of testis using a Neubauer chamber. Data were analyzed as a randomized complete block design, where litter of origin was blocked, and treatment effects on the parameters evaluated were analyzed using the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC). Least square means were compared using the Student T test with $P < 0.05$ being considered significant. Important associations among characteristics measured were examined across treatment groups using correlation analysis (INSIGHT procedure of SAS).

At 8 days of age, LW males had lighter body and testicular weights, lower testicular volume, and also showed lower testis weight relative to body weight (GSI – Gonadossomatic Index) compared to their HW littermates (Table 1). A litter of origin effect was observed for testicular weight, GSI and testicular volume ($P < 0.05$). At 8 months of age, LW males also showed lower body and testicular weights, and testicular volume than their HW counterparts (Table 2). On the other hand, the GSI was similar to their HW littermates. A litter of origin effect was also observed for GSI at 8 months of age ($P < 0.05$), which revealed the importance of the use of littermates when designing experiments of this kind to account for the differences due to family. Moreover, birth weight did not affect the diameter of seminiferous cords and the number of Sertoli cells per seminiferous cords cross section in 8 days old males. Similarly, diameter of the seminiferous tubules and the number of Sertoli cells

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per seminiferous tubules cross section at stage I of the seminiferous epithelium cycle in 8 months old males were not affected by birth weight. However, LW males showed lower total Sertoli cell number per testis in both ages evaluated. At 8 days old, testicular weight was highly correlated with body weight ($r = 0.72$; $P = 0.003$) and Sertoli cell number per testis ($r = 0.65$; $P = 0.012$); body weight was also positively correlated with Sertoli cell number per testis ($r = 0.56$; $P = 0.04$). At 8 months old, the number of spermatids per testis was lower in LW boars, however, when calculated per gram of testis the result was similar between both experimental groups (Table 2).

Table 1. Biometrical and histomorphometrical data (LS means ± SEM) from HW and LW boars at 8 days of age

Parameter	*Treatment*		*P-value*
	HW (n = 14)	*LW (n = 14)*	
Body weight, kg	3.62 ± 0.12[a]	2.37 ± 0.12[b]	< 0.01
Testicular weight, g	2.42 ± 0.14[a]	1.36 ± 0.14[b]	< 0.01
GSI	0.67 ± 0.03[a]	0.56 ± 0.03[b]	< 0.05
Testicular volume, cm^3	3.94 ± 0.22[a]	2.28 ± 0.22[b]	< 0.01
Seminiferous cord (SC) diameter, μm	48.0 ± 1.16[a]	50.6 ± 1.16[a]	NS
Sertoli cell number per testis, x 10^9	1.24 ± 0.13[a]	0.60 ± 0.13[b]	< 0.01
Sertoli cell number per cross section SC	19.57 ± 0.53[a]	18.41 ± 0.53[a]	NS

[a,b] Different letters within a row differ ($P < 0.05$).

Table 2. Biometrical and histomorphometrical data (LS means ± SEM) from HW and LW boars at 8 months of age

Parameter	*Treatment*		*P-value*
	HW (n = 14)	*LW (n = 14)*	
Body weight, kg	177.00 ± 3.70[a]	156.00 ± 3.70[b]	< 0.01
Testicular weight, g	415.20 ± 15.30[a]	357.90 ± 15.30[b]	< 0.05
GSI	2.36 ± 0.09[a]	2.30 ± 0.09[a]	NS
Testicular volume, cm^3	681.90 ± 31.10[a]	580.60 ± 31.10[b]	< 0.05
Seminiferous tubule (ST) diameter, μm	248.30 ± 4.70[a]	241.60 ± 4.70[a]	NS
Spermatid number per testis, x10^6	713.60 ± 283.30[a]	465.90 ± 187.45[b]	< 0.05
Spermatid number per gram of testis, x10^6	1.70 ± 0.64[a]	1.29 ± 0.43[a]	NS

[a,b] Different letters within a row differ ($P < 0.05$).

Together, these findings showed that biometrical and morphological testicular parameters are compromised in LW neonatal and adult boars, which is mainly due to their small body and testicular sizes. Therefore, we infer that birth weight may be used as an important criterion for boar selection for artificial insemination studs. Further investigations are necessary to study the impact of birth weight on reproductive parameters in adult boars, such as age at puberty and sexual maturation, libido, semen quality, and reproductive hormone profiles.

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References

Alvarenga, A. L. N., Chiarini-Garcia, H., Cardeal, P. C., Moreira, L. P., Foxcroft, G. R., Fontes, D. O. and Almeida, F. R. C. L. 2013. Intra-uterine growth retardation affects birth weight and postnatal development in pigs, impairing muscle accretion, duodenal mucosa morphology and carcass traits. Reprod. Fertil. Dev. http://www.publish.csiro.au/paper/RD12021.htm

Chiarini-Garcia, H., Parreira, G. G., and Almeida, F. R. C. L. 2011. Glycol methacrylate embedding for improved morphological, morphometrical and immunohistochemical investigations under light microscopy: testes as a model. In 'Light Microscopy: Methods and Protocols'. (Eds H. Chiarini-Garcia and R. C. N. Melo) pp 3-18. (Humana Press: Totowa, New Jersey).

Foxcroft, G. R., Dixon, W. T., Dyck, M. K., Novak, S., Harding, J. C. S. and Almeida, F. R. C. L. 2009. Prenatal programming of postnatal development in the pig. In 'Control of Pig Reproduction VIII'. (Eds H. Rodriguez-Martinez, J. L. Vallet, and A. J. Zicik) pp 213-231. (Nottingham University Press, Thrumpton, Nottingham).

Seasonal effect on sperm motility characteristics and plasma membrane integrity in boar ejaculate fractions

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The quality of boar semen is influenced by many factors, such as age, season, and seminal plasma composition (Xu *et al*. 1996; Fraser *et al*. 2003; Koziorowska-Gilun *et al*. 2011). Among these factors, seasonal variations may be seen as the most noticeable factor that affects boar semen quality (Strzeżek *et al*. 2000). Moreover, boar seminal plasma, a complex mixture originating in different accessory sex glands, contains a wide variety of components that are implicated in sperm function (Muiño-Blanco *et al*. 2008; Rodríguez-Martínez *et al*. 2011). This study aimed to investigate the effects of seasonal-related variations on motility characteristics and membrane status of spermatozoa originating from different fractions of boar ejaculate.

Ejaculates were collected from five Polish Large White boars. At collections, the ejaculates were split into 3 fractions as follows: approximately 8 ml of the sperm-rich fraction, SRF (F1); the remaining part of the SRF (approximately 2 ml) and approximately 8 ml of the post-SRF (F2); and the remaining part of the post-SRF (F3). The ejaculates were collected during the autumn-winter (October through March) and spring-summer periods (April through September) periods (n = 50, respectively). Vesicular gland and cauda epididymidal fluids were retrieved from animals at the slaughterhouse during the seasonal periods. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to identify the protein profiles of each ejaculate fraction, vesicular gland and cauda epididymidal fluids. Sperm motility characteristics, evaluated with the computer-assisted sperm analysis (CASA) system (HTR-IVOS 12.1, Hamilton Thorne Biosciences, Beverley, MA, USA), included total and progressive motility (TMOT and PMOT, respectively), mean path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), mean amplitude of lateral head displacement (ALH) and beat cross frequency (BCF). The SYBR-14/propidium iodide (PI) assay and hypoosmotic swelling (HOS) test were used to monitor the sperm plasma membrane integrity. Also, the percentage of normal apical ridge (NAR) acrosome was determined and the susceptibility of the sperm membrane lipids to induced lipid peroxidation (LPO) was analyzed by measuring the production of malondialdehyde (MDA). The data were subjected to a mixed factorial design with ANOVA followed by analysis with the Neuman–Keuls *post hoc* test ($P < 0.05$).

Even though there were marked variations in the total protein content among the boars, no significant differences were observed between the autumn-winter and spring-summer periods. Seminal plasma of F3 yielded the highest total protein content, which was lowest in F1, regardless of the seasonal periods. SDS-PAGE analysis revealed differences in the protein composition of F1, F2 and F3, and fluids of the vesicular glands and cauda epididymidis during the autumn-winter and spring-summer periods. Electrophoretic analysis of F1 showed similarity in terms of the number of protein bands and their molecular masses to the cauda epididymidal fluid. Furthermore, the protein fractions detected in F2 appeared to originate in the vesicular gland and cauda epididymidal fluids, whereas F3 comprised protein fractions which were predominant in the vesicular gland fluid. When the data were analyzed by three-way ANOVA, there were effects of seasonal periods (autumn-winter × spring-summer), ejaculate fractions (F1 × F2 × F3) and individual boars ($P < 0.05$). Seasonal periods had a significant effect on TMOT, PMOT, VAP, VSL, VCL and the sperm membrane integrity ($P < 0.05$). Also, differences in the ejaculate fractions significantly affected ($P < 0.05$) VAP, VCL, ALH and BCF. The ejaculate fractions displayed different sperm concentrations, which were highest in F1 and lowest in F3. Table 1 shows the motility characteristics and membrane status of spermatozoa

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Sow influence on neonatal survival: a special focus on colostrum

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The main cause of early postnatal deaths in piglets is hypothermia due to an inadequate intake of colostrum. Colostrum consumption is the outcome of complex interactions between the sow, the piglet, the litter and the environment. The sow may have an impact on many factors that are determinant for colostrum intake and chances of survival, such as piglet weight, maturity and vitality at birth, or within-litter variation in birth weight. Colostrum intake also depends on the ability of the sow to produce colostrum in sufficient quantity to fulfill the needs of the whole litter. Maternal stress during gestation may increase piglet morbidity and mortality up to weaning, presumably by affecting the ontogeny of the fetal immune system, but also IgG contents in colostrum and IgG transfer to newborn piglets. Ways to reduce neonatal mortality through maternal feeding during gestation are largely investigated. Feeding strategies generally failed to increase piglet birth weight but led to more promising results on piglet maturity and vitality at birth and on the acquisition of passive immunity. There is some evidence that maternal feeding during the peripartal period may influence both the quantity and the quality of colostrum; this needs however to receive further attention.

Introduction

Polytocous species like the pig produce a large number of offspring relatively undeveloped (Edwards 2002). Neonates have to compete for maternal resources, and the least able to compete will die shortly after birth. During the last few decades, a substantial increase in piglet mortality before weaning was observed in association with selection of sows to increase prolificacy and carcass lean merit. Piglet mortality has become a major source of economic loss in pig production and a social and ethical problem related to welfare concerns.

There is a large body of evidence that the main cause for piglet postnatal deaths is the lack of recovery from neonatal hypothermia, which is itself due to a low consumption of colostrum by newborn piglets (Edwards 2002, Le Dividich *et al*. 2005). Ultimately, hypothermia leads to starvation and crushing. Colostrum consumption and thus piglet survival are the outcome of complex interactions between the sow, the piglet, the litter and the environment (Figure 1). Colostrum intake depends on the ability of piglets to suckle quickly after birth. It is therefore influenced by piglet birth weight, maturity and vitality. Factors related to litter mainly involve size and within-litter variation in birth weight. Factors related to the sow involve farrowing

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Determining piglet survival

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The sow, piglet and their environment interact together to determine whether or not a piglet will survive to weaning. The physiology of the mother and offspring, as well as the synchronized expression of their appropriate behaviours, is integral to piglet survival and both are governed by genetic and environmental components. This review discusses the multifaceted nature of piglet survival, concentrating on the environmental factors that pre-dispose prenatal (stillborn) and postnatal death and the progress made towards understanding and decreasing piglet mortality.

Introduction

A certain amount of piglet mortality might be considered an inevitable event. A percentage of piglets are expected to die before weaning (10-20%, Edwards 2002) and may reflect a form of natural selection implemented by the sow whereby only the fittest offspring survive in challenging conditions. The evolutionary strategy adopted by the sow is one of over-producing; a form of parental optimism where production of numerous neonates allows replacement offspring in the event of members of the litter dying (Mock & Forbes 1995; Forbes & Mock 1998) and prepares for an unpredictable lactation phase, where resources may be plentiful or sparse. The often disproportionate provision of resources (i.e. milk) to the litter, further results in intense sibling rivalry, likened to avian facultative siblicide (Fraser *et al*. 1995), and increased mortality. Over-supplying offspring and providing limited resources is evident long before birth; with high ovulation rates but 30-50% of released ova not surviving gestation in the finite uterine space (Pope 1994; Geisert & Schmitt 2002).

In a polytocous species, such as the pig, embryo mortality, parental optimism and "siblicide" are considered normal. The limited success of attempts to reduce piglet mortality under domestic conditions may reflect these hard-wired evolutionary strategies. Continued selection pressure for hyper-prolificacy has further increased the challenge of piglet survival. Understanding the causes of mortality and the risk factors that predispose different types of death is crucial to focus solutions on both environmental and biological elements.

What are the main causes of piglet mortality?

Pre-weaning mortality (PWM) of live-born piglets is, on average, 11.5% (BPEX 2011), which varies between countries (Figure 1). With the inclusion of those piglets that are born dead (approximately 8% - Leenhouwers *et al*. 1999), total mortality averages 16-20% (Knol *et al*. 2002a; BPEX 2012).

Attempts to decrease piglet mortality have mainly been directed at the farrowing environment and husbandry procedures. The farrowing crate was introduced in the 1960s (Robertson *et al*. 1966) to decrease piglet over-lays by restricting sow movements, and to improve ease of

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How does nutrition influence luteal function and early embryo survival

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The pre-ovulatory LH surge triggers luteinisation of follicle tissue, but subsequent development of corpora lutea to full size is independent of LH up to around day 12 of pregnancy. Thereafter, severe (pharmacological) inhibition of LH secretion for 3 to 5 days will result in luteal failure and loss of pregnancy. It is unlikely that nutritional circumstances will have a similar effect, although scenarios with severe undernutrition have hardly been studied during early pregnancy. Milder levels of pre- and postmating undernutrition (around maintenance requirements), do affect luteal tissue development, but whether this is related to variation in LH is not clear as studies are equivocal, and there are indications that other nutrition related factors, like IGF-1 and insulin, may mediate these effects. A high plane of nutrition seems to increase progesterone secretion by the ovaries, even though systemic progesterone is reduced at the same time. Since there is direct transfer of progesterone from ovarian veins to the uterus, this may explain why a high plane of nutrition may actually benefit embryo survival and pregnancy, although very early during luteal formation (first days after mating), secretion by the ovaries may be overridden by systemic clearance of progesterone on a high feed level. Direct measurement of progesterone secretion by the ovaries is poorly understood as is the transfer of progesterone to the uterus and effects of specific nutrition related mediators such as IGF-1 and insulin on these processes.

Introduction

After rupture of the pre-ovulatory follicles at ovulation, reformation and reorganization of tissue take place at a remarkable speed, ultimately resulting in the formation of fully functioning corpora lutea by 7-10 d after ovulation, and in sheep, the proliferation rate of different cells involved in luteal tissue formation (luteal cells, endothelial cells, fibroblasts) has been likened to that of rapidly growing tumors (Niswender *et al*., 2000). The pre-ovulatory LH surge triggers the cascade of luteinising processes but as will be discussed in this paper, other factors control the development and later on, the function of established corpora lutea. Angiogenic factors such as VEGF influence corpus luteum formation and function early on (Schams and Berisha, 2004). Factors other than angiogenic factors that have been described are cytokines in cattle (Webb *et al*. 2002), luteotrophic factors like LH (see below), and IGF-1 (Ptak *et al*., 2003, 2004; Miller *et al*., 2003; Schams *et al*., 1999). The importance of some factors in the development

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Roles of selected nutrients in development of the porcine conceptus during pregnancy

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Conceptus development in mammals depends on an intra-uterine environment filled with histotroph that includes molecules that are secreted by uterine epithelia and/or selectively transported into the uterine lumen. In pigs, total recoverable glucose, fructose, arginine, leucine and glutamine increase in histotroph with advancing days of the peri-implantation period of pregnancy and in allantoic fluid later in gestation. During pregnancy, the uterine luminal epithelium (LE) and trophectoderm of conceptuses each express specific transporters for glucose. The most abundantly expressed amino acid transporters in uterine LE and trophectoderm are those for glutamate, neutral amino acids and cationic amino acids. These nutrient transporters are also expressed in uterine epithelia and placental tissues of pigs throughout gestation and expression of transporters and accumulation of nutrients in the uterine lumen is affected by progesterone and estradiol. Treatment of porcine trophectoderm cells with glucose, arginine and leucine stimulates the mechanistic target of rapamycin nutrient sensing cell signaling pathway to increase phosphorylation of RPS6K, RPS6 and EIF4EBP1 in the nucleus or cytoplasm to stimulate proliferation, mRNA translation and protein synthesis. Glucose and fructose are equivalent in stimulating proliferation of pig trophectoderm cells and in inducing synthesis of hyaluraonic acid via the hexosamine pathway. The results of our research indicate mechanisms whereby select nutrients act differentially to affect translation of mRNAs for cell signaling molecules that affect conceptus growth, development, and survival during pregnancy in pigs.

Introduction

Embryonic mortality and fetal morbidity claim 20-50% of conceptuses (embryo/fetus and extra-embryonic membranes) in pigs (Bazer *et al*. 2009; Bazer *et al*. 2011a). Successful establishment and maintenance of pregnancy requires orchestrated communication between

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Application of RNA-seq transcriptomic analysis to reproductive physiology of the pig: Insights into differential trophoblast function within the late gestation porcine placenta

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Next generation DNA sequencing is a high throughput method of sequencing DNA samples in parallel. During the last 10 years, this technology has expanded to include sequencing and quantification of an entire transcriptome. The advantage of this method of transcriptome analysis is that it allows the investigator to detect previously unknown genes and splice variants as well as detect potential DNA polymorphisms. Application of this technology, especially when used to perform cDNA sequencing, allows for comprehensive characterization of transcriptomes between cell types, tissues or under different physiological states. In this review, we summarize high throughput transcriptome analysis, the sequencing platforms currently available, some of the software needed to handle the data generated and how to develop a picture of what the data means from a physiologist's point of view. Lastly, we describe an example of this type of analysis applied to porcine placental trophoblast cell types.

Microarray analysis

A derivative of Southern blotting, one of the earliest reports of transcriptome analysis using a microarray was performed in *Arabidposis* on 45 genes (Schena *et al*. 1995). After almost 20

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Maternal and fetal amino acid metabolism in gestating sows

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Among livestock species, swine exhibit the most severe naturally-occurring intra-uterine growth restriction (IUGR) primarily due to a reduction in net protein synthesis. Thus, new knowledge about fetal metabolism of amino acids (AA), which are building blocks for proteins and regulators of intracellular protein turnover, can provide a solution to this problem. Among all AA, requirements of glutamate and glutamine by fetal pigs are quantitatively the highest, but cannot be met through uterine uptake alone. Nearly all glutamate and ~70% glutamine in diets for gestating swine are degraded in the maternal small intestine and, therefore, do not enter the portal circulation. This necessitates interorgan AA metabolism involving maternal skeletal muscle, placenta, and fetal skeletal muscle to synthesize glutamate and glutamine from branched-chain AA, as well as storage of glutamate and glutamine in allantoic and amniotic fluids. The porcine placenta does not degrade arginine or glutamine, leading to their maximal transfer from maternal to fetal blood. Therefore, maternal sources of ornithine and proline play a major role in the placental synthesis of polyamines needed for placental growth including placental vascular growth. Likewise, during late gestation, uterine uptake of arginine, proline and aspartate/asparagine cannot meet requirements for optimal fetal growth. To provide sufficient arginine, the fetal small intestine synthesizes citrulline and arginine from glutamate and glutamine, and fetal kidneys convert citrulline into arginine. Collectively, glutamine and arginine are major sources of AA nitrogen transferred between mother and fetus. Results of recent studies indicate that dietary supplementation with these two AA can ameliorate IUGR in swine. These findings greatly advance the field of maternal-fetal AA metabolism in pigs, but also have important implications for improving reproductive efficiency in swine production worldwide.

Introduction

Domestic pigs have no brown adipose tissue (Satterfield & Wu 2011), but possess a high capacity for synthesizing triacylglycerides in white adipose tissue after birth (Smith *et al*. 1999). Excess white fat in the body causes insulin resistance, metabolic disorders, and impaired lactation performance in animals, including swine (Jobgen *et al*. 2006; Wu 2010a). Thus, during gestation, gilts or multiparous sows are usually fed a substantially reduced amount of

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Next Generation Sequencing for microRNA profiling in the porcine endometrium

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The maternal recognition of pregnancy and embryo implantation are the key events occurring during early stages of pregnancy. Sequential changes in expression of numerous genes including growth factors, cytokines and lipid mediators, released locally by endometrium or embryos are crucial for establishment of specific embryo-maternal dialogue (Cha *et al*. 2012). Asynchrony between developing embryos and the uterus may have a tremendous effect on pregnancy, disturbing the implantation and placenta growth, ultimately causing spontaneous abortions as shown in rodents and domestic animals (Song *et al*. 2002, reviewed in Geisert and Yelich 1997). At the physiological state plethora of strictly coordinated embryo-maternal interactions affect gene expression that can be manifested by differences observed at the level of transcriptome and proteome (Cha *et al*. 2012). Interestingly, gene expression may be regulated through posttranscriptional gene regulation mechanisms driven by microRNAs (miRNAs). These small (~22 nucleotides) non-coding RNAs bind to target transcripts leading to inhibition of protein synthesis and/or degradation of the mRNA. Although, miRNAs have been shown to regulate gene expression during implantation in rodents (Hu *et al*. 2008) and are associated with embryo implantation defects in humans (Revel *et al*. 2011), expression profiles and function of miRNAs in the female reproductive tract of domestic animals are poorly identified.

Our previous study has demonstrated differential expression of miRNAs in the porcine embryos/trophoblasts collected between days 10 and 20 of pregnancy (Krawczynski *et al*. 2012), suggesting possible involvement of miRNAs in embryo development and implantation. In the present study we have applied Next Generation Sequencing (NGS) technology to analyze miRNA expression in porcine endometrial samples. Gilts in third estrus were either inseminated or let to undergo the next estrous cycle. Samples were collected on days 12, 16 and 20 of either the estrous cycle or pregnancy (n = 6/day/status). Total RNA containing small RNA fraction was extracted by the mirVana miRNA isolation kit and used for sequencing. Small RNA libraries were constructed using the NEXTflex™ Small RNA Sequencing Kit. Briefly, 100 ng of total RNA containing miRNA was ligated with the 3′ and 5′ adaptors. Then, the reverse transcription mix was added to the adaptor-ligated RNA to synthesize the cDNA. In the PCR amplification step specific barcodes for multiplexing were added. Resulting PCR products were pooled and size fractioned on an agarose gel to obtain ~140 bp fragments, which were sequenced using TrueSeq SR Cluster Kit v. 2 and Illumina Genome Analyzer IIx.

Generally, 36 libraries generated 22,391 to 69,065 unique sequences, which demonstrated predominant length of 22 nt and GC content at the level of ~40%. Abovementioned sequences were further blasted against miRBase v.18, Ensembl and NCBI databases. As a result, 815 sequences were annotated to known/predicted porcine and mammalian, mature or stem-loop miRNA sequences. 115 sequences showed 100% homology to known porcine mature miRNAs, 518 sequences showed full homology to miRNA stem-loop structures present in the porcine genome, 68 and 103 sequences were annotated to known mammalian, mature and stem-

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Integrin αv is necessary for the attachment of trophectoderm cells to osteopontin to mediate adhesion of the conceptus to the uterus during implantation in pigs

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Pigs experience a significant loss of conceptuses during the attachment phase of implantation. The molecules that facilitate uterine-conceptus interactions in pigs are not fully understood, and this gap in knowledge limits our ability to improve reproductive efficiency. Osteopontin (OPN, also known as Secreted Phosphoprotein 1 or SPP1) is an emerging candidate marker for uterine receptivity to implantation in pigs. Expression of OPN by the uterus increases markedly during the peri-implantation period of pigs, as well as in other mammalian species, including sheep, goats, rabbits, mice and humans (Johnson '03, Johnson '09). In pigs, OPN mRNA is initially induced by conceptus estrogens in discrete regions of the uterine luminal epithelium (ULE) juxtaposed to the conceptus just prior to implantation on Day 13. OPN mRNA expands to the entire ULE by Day 20 when there is firm adhesion of conceptus trophectoderm to ULE (Garlow '02, White '05). OPN is a secreted extracellular matrix (ECM) protein that binds to cell surface integrins. Integrins are transmembrane glycoprotein receptors composed of non-covalently bound α and β subunits that promote cell-cell and cell-ECM adhesion, cause cytoskeletal reorganization to stabilize adhesion, and transduce signals through numerous signaling intermediates. OPN potentially binds multiple integrins including αvβ3, αvβ1, αvb5, αvb6, α4β1, α9β1 and α8β1. Our central hypothesis is that OPN secreted by the ULE binds integrins expressed by both ULE and trophectoderm to mediate implantation of the conceptus.

In a previous study, we tested whether αv, α4, α5, β1, β3, β5, and β6 integrin subunits expressed by porcine trophectoderm cells (pTr2) and ULE cells bound OPN. OPN bound the αvβ6 integrin heterodimer on pTr2 cells and αvβ3 on ULE cells (Erikson '09). Further, OPN promoted dose- and integrin-dependent attachment of pTr2 and ULE cells, and stimulated haptotactic pTr2 cell migration, meaning that cells migrated directionally along a physical gradient of non-soluble OPN. Finally, OPN-coated microspheres revealed co-localization of the αv integrin subunit and talin to focal adhesions at the apical domain of pTr2 cells.

The objectives of the current studies were to: 1) determine mRNA expression and hormonal regulation of αv, β3, and β6 integrins in the uterus and placenta; 2) determine the temporal and spatial assembly of αv-, β3-, and β6-containing focal adhesions at the uterine-placental interface; and 3) determine whether integrin αv knockdown results in decreased attachment of pTr cells to OPN *in vitro*. In Experiment 1, gilts were hysterectomized on Day 9, 12 or 15 of the estrous cycle, or Day 9, 12, 15, 20, 25, 30, 35, 40, 50, 60 or 85 of pregnancy and uterine/placental tissues were subjected to *in situ* hybridization and immunofluorescence (IF) analyses to detect integrin subunit αv, β3, and β6 mRNA and αv and β3 protein at the maternal-conceptus interface. In Experiment 2, cyclic gilts were injected daily (Days 11-14) with 17-beta-estradiol benzoate (i.m.) or vehicle, and hysterectomized on Day 15 of pseudopregnancy. In Experiment 3, gilts were ovariectomized on Day 12, injected daily with progesterone (i.m.) or vehicle for 28 days, and hysterectomized on Day 40. For Experiments 2 and 3, uteri were subjected to *in situ* hybridization analyses to determine

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Prenatal stress in pigs: impact on growth, behaviour, neuroendocrine and immune functions in the offspring

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Studies in different animal models and humans give evidence that stress experienced by pregnant mothers affects foetal development and has long-term consequences on many physiological systems and behaviour in the offspring, thus facilitating the risk for disorders later in life. In farm animals, housing conditions or inadequate management practices during gestation may be potential stressors for the mother, which could affect growth, vitality, health and welfare of the dam and its offspring, and can also have economic implications. This paper gives a survey of results from different studies in pigs on the impact of maternal stress during gestation on growth, behaviour, neuroendocrine and immune functions in the offspring. Different experimental models using either elevated maternal cortisol levels or stress paradigms are introduced and major results are presented. The survey reveals that also in pigs prenatal stress can impair growth and modify immune functions, stress reactivity and behaviour in the offspring. The materno-foetal cortisol regulation is a major determinant of the alterations in the offspring, and mid- and late gestation seems to be sensitive gestational periods of increased vulnerability to prenatal stress. Neuroendocrine and behavioural results indicate that prenatally stressed pigs can express an over-reactive phenotype characterised by an increased HPA axis reactivity, altered emotionality, more fearfulness in a novel environment and disturbed social behaviour. Further research in this area should focus on the potential consequences of prenatal stress in offspring used for breeding as reproductive and behavioural characteristics may be affected in the long-term.

Introduction

Numerous studies with rodents, non-human primates and humans have shown that stress experienced by pregnant females has long-term consequences on many physiological systems thereby affecting the disease risk of their offspring. Maternal glucocorticoids are major candidates for the mediation of maternal stress to the foetus because they can cross the placental barrier and exert many organisational effects on foetal tissue maturation and differentiation (see reviews by Fowden & Forhead, 2004; Harris & Seckl, 2011). Their importance in processes of foetal programming was demonstrated in rats where maternal adrenalectomy prevented the effects of prenatal restraint stress in the offspring, which could be re-established after maternal

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Importance of pre-mating nutritional and metabolic conditions for litter uniformity

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For piglet survival, as well as for piglet performance before and after weaning, high piglet birth weights and litter uniformity are crucial (Quiniou *et al*. 2002). Within-litter variation in birth weight seems to be the consequence of within-litter variation in early embryo development (Van der Lende *et al*. 1990), which in turn reflects variation in follicle and oocyte development (Pope *et al*. 1990). Insulin-stimulating sow diets before mating can improve litter uniformity (Van den Brand *et al*. 2006, 2009), probably through beneficial effects of insulin on IGF-1 and follicle development. Plasma insulin and IGF-1 levels and follicle development are also influenced by the metabolic state of the sow. Plasma insulin and IGF-1 levels and follicle development are suppressed in sows with severe body condition loss during lactation, and restoration of plasma insulin and IGF-1 levels and follicle development occurs in sows with a recovery period after weaning (Quesnel 2009). We studied in multiparous sows effects of (i) insulin-stimulating diets during the weaning-to-estrus interval (WEI) on follicle development and development and uniformity of pre-implantation embryos at day 10 of pregnancy (Wientjes *et al*. 2012a,b) and fetuses and placentas at day 42 of pregnancy; and (ii) pre-mating conditions related with sow metabolic state during lactation (body condition loss) and after weaning (length of weaning-to-pregnancy interval; WPI) on litter uniformity at birth.

Multiparous sows (n = 32) were fed an insulin-stimulating diet (dextrose plus lactose, both 150 g/day) at 4h-intervals or an isocaloric control diet at 12h-intervals during WEI. Total insulin secretion, absolute insulin levels and plasma IGF-1 levels were not affected by the dietary treatments, although contrasts were created in the insulin secretion patterns during WEI (6 short insulin peaks/day vs. 2 sustained insulin peaks/day). The dextrose plus lactose diet fed at 4h-intervals during WEI resulted in a lower pre-ovulatory LH surge (3.0 vs. 3.7 ng/ml, $P = 0.03$), smaller follicles at day 4 after weaning (6.1 vs. 6.5 mm, $P = 0.08$) and subsequently smaller corpora lutea (mean diameter: 9.6 vs. 10.0 mm, $P = 0.06$) and less developed embryos at day 10 of pregnancy (mean diameter: 6.4 vs. 7.1 mm, $P = 0.07$) compared with the control diet fed at 12h-intervals. Independent of treatment, positive relations were found between mean insulin levels during WEI and subsequent progesterone levels ($\beta = 0.27$ (ng/ml)/(μU/ml), $P = 0.05$) and embryo development (mean diameter: $\beta = 0.06$ mm/(μU/ml), $P = 0.09$), but not embryo uniformity at day 10 of pregnancy. Whether and how this results in a more uniform development of fetuses and piglets at later stages of pregnancy was the focus of a follow-up experiment, in which multiparous sows (n = 54) were isocalorically fed (2x/day) an insulin-stimulating diet supplemented with dextrose plus corn starch (both 375 g/day or both 172 g/day) or a control diet during WEI. Postprandial insulin responses, both insulin peaks (<1h after feeding) and long-term insulin level (~4h after feeding), were successfully stimulated during WEI by the insulin-stimulating diets in a dose-dependent manner, but plasma IGF-1 levels during the first three days after weaning were not affected. Follicle development and subsequent development and uniformity of fetuses and placentas at day 42 of pregnancy were not affected by these insulin-stimulating diets, nor related with plasma insulin and IGF-1 levels during WEI. Because uniformity of embryos or fetuses and placentas was not influenced by the insulin-stimulating diets during WEI, nor related with plasma insulin and IGF-1 levels during WEI, these experiments do not confirm that nutritionally increased plasma insulin and IGF-1 levels during only WEI can improve subsequent litter uniformity in multiparous sows.

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Stem cells: Perspectives for the pig in relation to other species

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The derivation of pluripotent stem cells from mouse and human embryos and the reprogramming of somatic cells into induced pluripotent stem cells (iPSC) has initiated a new era of research in the field of regenerative medicine. The need for these cells to be tested in relevant animal models, as well as their potential to be used in the genetic engineering of livestock, has generated significant interest in the establishment of pluripotent stem cell lines from farm animals, including from the porcine species. Despite that to date no true porcine pluripotent stem cell lines have been established from cultured embryonic stem cells, there have been some significant advances in the area of iPSC and mesenchymal stem cells. This review examines the current state of porcine stem cell culture, with focus on the challenges that still need to be overcome in order to allow the wider use of these cells in biomedical models or in the field of animal biotechnology.

Introduction

The early embryonic development in all vertebrates begins with the totipotent zygote (which can form all the embryonic and placental tissues), and proceeds in highly organized and controlled fashion whereupon the ability of most cells to form multiple cell types is lost during the process of differentiation. At blastocyst stage, a relatively small group of cells, named inner cell mass (ICM), has retained the capability of specializing into all embryonic lineages but is not able to form the placenta, and is therefore designated as pluripotent. This characteristic is lost in most cells after the onset of gastrulation, where the main germ layers are formed. From this stage on, pluripotency (as induced in certain in vitro conditions) is retained in the germ line (primordial germ cells (PGC) and spermatogonial stem cells (SSC)), while throughout the somatic tissues many cells preserve the capability to form a limited number of different cell types (multipotency) and serve mainly as a source of tissue renewal (the so-called adult, or somatic, stem cells).

The preservation and maintenance of the pluripotent or multipotent characteristics of stem cells in vitro has been a subject of intense research. In 1981, two groups reported the establishment on the first murine embryonic stem cell (ESC) lines from cultured ICM (Evans & Kaufman 1981, Martin 1981). Seventeen years later, James Thompson and co-workers reported the establishment of the first human ESC (Thompson *et al*. 1998). Usually, the term "ESC" applies for pluripotent cells isolated from ICM in vitro; however, embryonic cells with pluripotent potential have been derived also from mouse embryos at later stages, such as epiblast stem cells (EpiSC) (Brons *et al*. 2007, Tesar *et al*. 2007). These cells resembled human rather than mouse ESC in their morphology and culture requirements, and could not form chimeras despite their ability to form teratomas in immunodeficient mice.

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Porcine pluripotent stem cells and their differentiation

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In some situations, the pig has advantages over the mouse as a model in biomedical research. The availability of pluripotent cell lines is likely to broaden this appeal. Here we review progress in the derivation and characterization of embryonic stem cells (ESC) and induced pluripotent lines (iPSC) from pigs. Until recently, most porcine ESC failed to meet the full criteria for pluripotency, but that may be changing as more becomes known about the culture conditions required to maintain epiblast outgrowths from early porcine conceptuses in an undifferentiated, self-renewing state. In addition, porcine iPSC cells have been generated, some with the features of FGF2-dependent epiblast-type cells, typified by human ESC, and others that require LIF and resemble the "ground state", naïve-type mouse ESC. Despite these successes, incomplete reprogramming and loss of pluripotency when selection conditions are relaxed continue to be problems that must be overcome if the full potential of iPSC is to be realized. The most immediate value of iPSC may relate to their ability to proliferate almost indefinitely in culture, thus enabling more complex genetic manipulations of the genome through growth selection than could be performed in other cell types. The "undifferentiated" state of iPSC may also allow improved cloning efficiency, although this remains to be proved. Finally, the pig will likely prove useful in testing stem cell-based therapies, although only a limited number of experiments demonstrating that the porcine iPSC can be directed to transform into more specialized sub-lineages and then form functional grafts have been performed.

Embryonic stem cells (ESC) from swine

The pursuit of ESC from swine has a long history originating back to the early 1990s (Notarianni *et al*. 1990), a time not long after the introduction of mouse ESC, which had first been reported earlier in the decade (Evans & Kaufman 1981, Martin 1981). Since their establishment, mouse ESC began to revolutionize developmental genetics by permitting changes, usually loss-of-function mutations, but later a variety of other modifications, to be introduced at preselected genetic loci in the mouse genome through homologous recombination (Capecchi 1989, Koller & Smithies 1992). In this regard, mouse ESC exhibited three crucial properties. First, they were able to differentiate into derivatives of all three germ layers (ectoderm, endoderm, and mesoderm) within embryoid bodies and teratomas. Second, their more-or-less infinite lifespan provided the extended times required for positive and negative selection to ensure that a

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Pigs as model systems for biomedical research

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Pigs have a long standing and very successful history as biomedical model for studying human diseases and developing novel therapies mainly attributed to the many genetic, anatomical and physiological similarities with humans. Non-transgenic pig models have long been used for a wide range of human organ systems and diseases, and even complex metabolic disorders and have served as model for developing novel surgical techniques and endoscopic approaches, such as NOTES (natural orifice transluminal endoscopic surgery). The availability of the porcine genome and novel tools to add or delete specific genes significantly expands the potential for transgenic pig production. Somatic cell nuclear transfer has emerged as the preferred method for transgenesis. Well characterized transgenic pig models have been reported for Cystic fibrosis, the eye disease Retinitis Pigmentosa, atherosclerosis and diabetes. Transgenic pigs have been produced for modeling neurological diseases, including Alzheimer and Huntington disease, specific forms of cancer, and skin diseases. Transgenic pigs play an important role in developing functional porcine xenografts to combat the growing shortage of appropriate human organs for transplantation. Other important transgenic pig models include immunodeficient pigs and Oct4/GFP transgenic pigs for studies of reprogramming. Pig models will not replace the already existing mouse models but can provide significant novel insight into a variety of diseases, as mouse models frequently do not mimic the human situation. Transgenic pigs will also soon play an increasing role in the development of novel therapies based on stem cell technology. The biomedical use of pigs will also facilitate transgenic pig production for agricultural production.

Introduction

Pigs have a long standing and very successful history as biomedical model for studying human diseases and developing novel therapies. Domestic pigs and minipigs are the main categories that have been used as biomedical models. Usually minipigs are in shorter supply than domestic pigs and thus more expensive compared with domestic pigs, which cost more due to housing, feed and medication (Litten-Brown *et al.*, 2010). The preferred use of pigs as model in biomedical research is attributed to the many anatomical and physiological similarities with humans. As humans, the pig is a monogastric omnivore. As result of a long domestication process a great variety of pig phenotypes exists worldwide that could be relevant for current human health research priorities, including obesity, diabetes and cardiovascular diseases. Given the high degree of similarity, many diagnostic, surgical or other medical techniques can be directly

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Mapping of quantitative trait loci for reproductive traits in pigs

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This paper reviews the major approaches used to map quantitative trait loci (QTL) and current knowledge about QTL affecting reproductive trait loci in pigs. Three different approaches, i.e. functional candidate gene analyses, genome-wide linkage studies (GWLS) and genome-wide association studies (GWAS) have been used to map QTL in pigs. The interest and limits of each of the three approaches are discussed. Candidate genes and QTL have been reviewed based on PigQTLdb at http://www.animalgenome.org. A total of 29 candidate genes affecting reproductive traits located on 14 autosomes have been considered. Some of them are well established results, but most results originate from single studies of limited scale and need to be confirmed. Several thousands of QTL affecting 15 male and 15 female reproductive traits have been identified on 17 and 19 different chromosomes, respectively, using GWLS. Yet, the majority of them are only putative QTL and few QTL regions overlap between studies. Epistatic interactions between QTL appear as rather important in the single study investigating the effects of epistasis on pig reproductive traits. A large number of QTL distributed over almost all pig chromosomes have been identified in the single GWAS study published so far for pig reproductive traits. Use of sequence data, of more complex genetic models and of integrative biology approaches should be considered for more thoroughly investigating the genetic architecture of pig reproductive traits in the future.

Introduction

The development of genomics in the last 25 years has revolutionized the research methods and tools in genetics and, more generally, in biology. This revolution has been largely driven by the human genetics area. Considerable resources have indeed been mobilized to decipher the human genome and its variations. The huge progress obtained in humans has largely contributed to the advancement of livestock genetics and genomics due to both technological leaps (genotyping, microarrays, sequencing, bioinformatics...) and to the input of comparative genomics on the knowledge of animal genomes.

Genomics has not only allowed the whole sequence of most livestock genomes to be deciphered, but has also allowed to get a much better understanding of the genetic architecture of complex traits. An increasing number of polymorphisms responsible for variations in simple Mendelian traits (e.g. Andersson and Plastow 2011), or having major effects on quantitative traits (Milan, *et al*. 2000, Van Laere, *et al*. 2003) have been or are about to be identified. Several thousands of Quantitative Trait Loci (QTL) associated with phenotypic variations have been mapped on livestock genomes (e.g. Bidanel and Rothschild 2002). In parallel, the other

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Transcriptome analyses of porcine endometrium during the pre-implantation phase

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The porcine conceptus undergoes rapid differentiation and expansion of its trophoblastic membranes between days 11 and 12 of gestation. The production of estrogen, the porcine embryonic pregnancy recognition signal, by the conceptus increases with trophoblast elongation. A complex interplay of estrogen signaling and prostaglandin (PG) metabolism in the endometrium finally results in prevention of luteolysis. Conceptus attachment to the uterine surface epithelium starts around day 14 of pregnancy preceded by a pronounced vascularization at the implantation zones, initiating the epitheliochorial placentation. To characterize the transcriptome changes in the porcine endometrium in the course of maternal recognition of pregnancy (MRP) and initial placentation, several transcriptome analyses using DNA microarrays and RNA sequencing (RNA-Seq) have been performed. This review summarizes and compares the results of these studies. Particularly, the studies where RNA-Seq has been used, revealed more than 2,500 and 1,900 differentially expressed genes (DEGs) for days 12 and 14 of pregnancy, respectively, in comparison to corresponding cyclic controls. Analysis of the results of these two studies revealed distinct differential gene expression, reflecting the different functions of the endometrium during these stages. The comparison of RNA-Seq and microarray data for day 14 of pregnancy revealed a good agreement of the results. Moreover, results of microarray studies investigating local responses of the uterine horn to embryos in the blastocyst stage and effects of premature exposure of pregnant gilts to exogenous estrogen on endometrium during early pregnancy are discussed and compared to the results from day 12 and day 14 of pregnancy.

Introduction

Establishment of pregnancy in mammals requires prolongation of luteal life span for sustained progesterone (P4) production. P4 stimulates secretory functions of the endometrium required for conceptus development and implantation. Maternal recognition and establishment of pregnancy in pigs requires a biphasic pattern of estrogen (E2) secretion (Geisert *et al*. 1990). Between days 11 and 12 of gestation, the porcine conceptus undergoes rapid differentiation and expansion of its trophoblastic membranes (Geisert *et al*. 1982, Stroband & Van der Lende 1990, Yelich *et al*. 1997). Coordinate with trophoblastic elongation, conceptus secretion of E2 increases (Ford *et al*. 1982, Stroband & Van der Lende 1990). The second phase of increased E2 secretion is between days 15 and 30 of pregnancy. Luteoprotective action of E2 is complex.

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Vitrification procedure decreases inositol 1,4,5-triphosphate receptor expression, resulting in low fertility of pig oocytes

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Although cryopreservation of mammalian oocytes is an important technology, it is well known that unfertilized oocytes, especially in pigs, are highly sensitive to low temperature and that cryopreserved oocytes show low fertility and developmental ability (Wu *et al*. 2006). To avoid these detrimental effects caused by exposure to low temperature, many attempts have been done. It is recognized that one of the important issues contributing to successful vitrification is to maximize the rate of cooling and warming (Kuwayama *et al*. 2007). In our previous study, we demonstrated that Cryotop yields high post-warming survival and developmental ability of vitrified oocytes in the mouse (Kohaya *et al*. 2011) and rat (Fujiwara *et al*. 2010).

As for vitrification of metaphase-II (MII) oocytes, it is thought that kinetics of intracellular calcium is also an essential factor for the success. During fertilization, rises of intracellular calcium released from the endoplasmic reticulum via inositol 1,4,5-triphosphate receptor, (IP_3R1) occur in oocytes (Ito *et al*. 2010; Ito & Kashiwazaki 2012). The rises of intracellular calcium induce exocytosis of cortical granules, resulting in the occurrence of zona hardening known as the zona reaction (Ducibella *et al*. 1988). In the mouse, Larman *et al*. (2006) demonstrated that exposure to vitrification solution containing cryoprotective agents causes the rise of intracellular calcium in MII oocytes, inducing zona hardening as well as normal fertilization. Indeed, our previous data showed that vitrification using calcium-free media significantly improved penetration and fertilization rates of vitrified oocytes in the mouse (Kohaya *et al*. 2011) and rat (Fujiwara *et al*. 2010). However, in pigs, the effect of calcium during vitrification in not well examined. In the present study, we focused on the kinetics of IP_3R1 during vitrification in pig oocytes and tried to clarify the reason why vitrified pig oocytes show low fertility and developmental ability.

Cumulus oocyte complexes (COCs) were cultured for 44 h and then used for vitrification. Vitrification-warming was carried out by the Cryotop method as previously described (Fujiwara *et al*. 2010). Vitrified-warmed COCs were used for *in vitro* fertilization (IVF). For immunostaining, the germinal vesicle (GV) stage and MII stage oocytes were collected at 0 h and 44 h, respectively. For western blotting, GV, MII, exposed MII and vitrified-warmed MII oocytes were collected. *In vitro* matured COCs were vitrified with Cryotop and then evaluated for fertility through IVF. Pronuclear formation rates of vitrified-warmed oocytes were significantly ($p < 0.05$) lower regardless of calcium supplementation (9.0 ± 1.3% (Ca+) and 8.0 ± 2.6% (Ca-)) compared to that of fresh ones (62.4 ± 13.0%). Most of the fertilized fresh oocytes reached the 2-cell stage (53.3 ± 10.1%). Some of the 2-cell embryos also developed to blastocysts (22.4 ± 1.8%). However, none of the fertilized oocytes in the vitrified groups developed to

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The use of stirred suspension bioreactors as a novel method to enrich germ cells from prepubertal pig testis

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Spermatogonial stem cells (SSCs) are the foundation of male fertility. Their low abundance and the lack of reliable markers for SSCs are major road blocks in their isolation and use in germ line modification.

Differential adhesion of germ and somatic cells to tissue culture surfaces (differential plating) has been used to enrich undifferentiated spermatogonia, that include SSCs, from heterogeneous testicular samples on a large scale (Luo et al. 2006). However, this approach is very labor intensive which creates a need for a protocol that can yield significant enrichment of spermatogonia with less handling where scalability is possible. Suspension bioreactors have been routinely used to culture homogenous cell populations in large quantities, and they have advantages over static cultures in that they utilize standardized and controlled culture conditions in a scalable environment (Krawetz et al. 2011).

The objective of this study was to investigate if germ cells could be enriched in stirred suspension bioreactors based on the differential adhesion properties of testicular cells. We also investigated if stirred suspension cultures, followed by differential plating, could provide additional enrichment. Methods: testicular cells were harvested from pre-pubertal (10 week old) pigs by a two-step enzymatic protocol (Luo et al. 2006). At this age, spermatogonia and Sertoli cells are the only cell types present in the seminiferous tubule. Testicular cell suspensions were submitted to three different enrichment methods: stirred suspension culture (A), differential plating (B) and stirred suspension culture followed by differential plating (C), n = 3 replicates each. For enrichment method A: $5x10^6$ cells/ml were cultured in 100 ml stirred suspension bioreactors (NDS Technologies, Vineland, USA) as previously reported (Shafa et al., 2012) and agitated for 48 h at 100 r.p.m. in high-glucose DMEM (Invitrogen, Carlsbad, USA) supplemented with 50 IU/ml penicillin, 50 U/ml streptomycin and 5% FBS (Invitrogen). Every 24 h the cell suspension was filtered through a 40 μm mesh to remove large aggregates of somatic cells and such attain enrichment of germ cells. For method B: $7x10^6$ cells/ml cells were plated on 100 mm tissue culture dishes in 15 ml DMEM + 5% FBS supplemented with 50 U/ ml penicillin, 50 U/ ml streptomycin, and incubated at 37°C in 5% CO_2 in air. After 18 and 48 h, cells remaining in suspension and those slightly attached were removed by trypsinization (1:10 dilution of trypsin-EDTA), and plated on new dishes. For method C: cells enriched by stirred suspension for 48 h were plated on 100 mm tissue culture dishes for 24 h under the same conditions as treatment B. Cell recovery was recorded at all time points and cells were characterized by immunocytochemistry for Vasa and Vimentin to identify germ cells ($Vasa^+$, $Vimentin^-$) and somatic cells ($Vasa^-$, $Vimentin^+$), results were compared by ANOVA. Cells obtained from group A were also characterized by immunocytochemistry using antibodies against Gata4, P450c17, α smooth muscle actin and vimentin to identify Sertoli cells ($Gata4^+$, $Vasa^-$), Leydig cells ($P450c17^+$, $Vimentin^+$) and myoid cells (α $smooth\ muscle\ actin^+$, $Vimentin^+$) to investigate the effect of shear forces on testicular cells after 48 h, paired student t test was used to analyze these results.

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Genomics-based selection for reproduction and adaptation in pigs

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Recent publication of the sequence of the pig genome allows for faster and more accurate selection for traits expressed late in life, sex limited or difficult to measure. Especially reproduction and adaptation traits fall in this category. Existing genetic variation in reproduction traits is substantial and polygenic selection has resulted in genetic trend of around half a piglet per sow per year. A very significant increase in genetic trend is anticipated through genomic selection. Genomic selection capitalizes mainly on Mendelian sampling, variation between full sibs is half of the total genetic variation; estimation of genetic merit based on markers yields, because of quantification of this Mendelian sampling, far higher accuracies then on the basis of parental average. For both polygenic and genomic selection, application appears to follow theory. Current research focuses of application of full sequence per animal, on non-additive genetic effects, and on genomic testing of embryos in order to increase selection intensity. Gradually traits relating to genotype-environment interactions will be added to the selection index, using genomic based methods.

Introduction

Genetic variation helps a population to adapt to changing environments. A population adapts its reproductive performance to a level which can be sustained given available resources. Pork production systems lower cost price by increasing reproduction and minimizing resource constraints through more and better feed, improved climate control and reduction of disease challenges. Pig breeding organizations have exploited genetic variation in reproductive traits by applying selection programs and extensive data recording systems for several decades now (Dekkers et al., 2011). Pigs have been selected based on quantitative polygenic assumptions for the traits of interest. This selection has been quite successful, but has led to noticeable changes in the physiology of the sow (Foxcroft, 2012). The publication of the porcine genome (Groenen et al., 2012) creates the potential for a much higher genetic trend in existing and in new reproduction traits.

Goals of this paper are (1) to quantify the basics of genetic selection for reproductive traits; (2) explore potential changes in reproduction traits through the application of genomic techniques; and (3) explore the role of genetic variation under challenged environmental conditions.

Basics of genetic change

Genetic variation

Litter size, total number born piglets per litter, can vary between zero and thirty. The litter size at a farm depends upon environment, parity, season, nutritional status etc. After correction for

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Intravaginal application of Ovugel®, a product containing the GnRH agonist triptorelin acetate (TA), stimulates LH secretion in gilts

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Intravaginal administration of OvuGel®, a proprietary gel formula containing the GnRH agonist, triptorelin acetate (TA), effectively synchronizes ovulation in weaned postpartum sows, which allows for fixed-time artificial insemination. GnRH stimulates LH release from the pituitary, thereby stimulating ovulation. Two trials with an ovariectomized (OVX) estradiol (EB)-treated gilt model and one trial with gilts after last feeding of MATRIX® were conducted to determine the optimum viscosity of the gel preparation and concentration of TA to induce surge like secretion of LH and ovulation. Estradiol treatment, in OVX gilts, initially suppresses LH secretion followed by a preovulatory-like LH surge 48-72 h later (Asonavich *et al*., 1998, Britt *et al*. 1991, Elsaesser *et al*. 1998). Thus, serum LH was assayed (Kraeling *et al*., 1982, 1998) to characterize the LH response to TA. Response parameters were: 1) magnitude (maximum serum LH concentration), 2) time to maximum serum LH concentration (T-Max) and 3) area under the LH response curve (AUC: 0-30 h). Treatments were administered during EB induced suppression of LH secretion.

Trial 1 was designed to determine which of three gel formulations having differing viscosities optimized LH release; 16 gilts about 200 d of age were OVX and fitted with jugular vein cannulae. Estradiol benzoate (15 μg/kg BW) was injected i.m. and 48 h later 4 gilts each received 2 ml of: 1) 0 μg TA in Viscosity B (Control), 2) 100 μg TA in Viscosity A, 3) 100 μg TA in Viscosity B or 4) 100 μg TA in Viscosity C intravaginally. Sequential blood samples were taken for 48 h after treatment. The proportion of gilts, which responded to TA within 2 h was 4/4, 2/3 and 1/3 for treatment 2, 3 and 4, respectively. LH surge secretion was captured in 3/4 Control, 4/4 treatment 2, 3/4 treatment 3, and 3/4 treatment 4 gilts. T-Max for Controls and treatments 2, 3 and 4 was 14.0 $\pm$ 3.6, 7.0 $\pm$ 5.8, 5.3 $\pm$ 5.8, and 9.3 $\pm$ 4.6 h, respectively. Magnitude was 3.4 $\pm$ 1.2, 9.7 $\pm$ 4.4, 8.0 $\pm$ 0.4, 8.7 $\pm$ 5.3 and AUC was 69.8 $\pm$ 18.8, 94.1 $\pm$ 30.7, 87.6 $\pm$ 28.1 and 87.6 $\pm$ 28.1 for Controls, and treatments 2, 3, and 4, respectively. Viscosity A gel was selected for subsequent dose titration trials. Two wks. later, in trial 2, the same gilts were prepared as in trial 1 and 4 each received 2 ml of Viscosity A gel containing 1) no TA (Control), 2) 25 μg TA, 3) 50 μg TA or 4) 100 μg TA intravaginally. Blood samples were taken for 30 h after treatment and evaluated as in trial 1. The proportion responding to TA within 2 h was 0, 33, 100 and 100% for Controls and treatment 2, 3 and 4, respectively. LH surge secretion was captured in 0/4 Control, 3/4 treatment 2, 4/4 treatment 3, and 4/4 treatment 4 gilts. T-Max for Controls and treatments 2, 3 and 4 was 16.0 $\pm$ 1.6, 7.0 $\pm$ 7.0, 3.5 $\pm$ 1.7, and 3.0 $\pm$ 0.0 h, respectively. Magnitude was 1.8 $\pm$ 0.7, 5.4 $\pm$ 4.9, 13.4 $\pm$ 4.8, 12.9 $\pm$ 2.4 and AUC was 16.5 $\pm$ 3.5, 46.6 $\pm$ 18.6, 52.8 $\pm$ 23.5 and 62.9 $\pm$ 11.6 for Controls, and treatments 2, 3, and 4, respectively. Number of gilts responding increased with TA concentration; 100 μg dose resulted in 100% response. All TA treatments decreased T-Max and increased AUC. T-Max was shortest and least variable in treatment 4 gilts. The optimum dose for further dose titration trials was 100 μg.

In trial 3, sexually mature gilts were individually fed 15 mg MATRIX®/d for 14 d and received: (Control) vehicle gel (n = 8) or gel containing (1) 100 μg (n = 6), (2) 200 μg (n = 7), or (3) 400 μg

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Potential sexing of spermatozoa using new antibodies to sperm surface proteins

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Current husbandry practices in the pig industry favour same-sex groups on welfare grounds (to prevent fighting) and because of a more even live-weight gain, with all members of the group reaching slaughter weight within a specified time. However, if male pigs are left entire, the meat develops "boar taint" as the animal reaches sexual maturity, which is considered undesirable by some consumers. Furthermore, there may be husbandry problems because of fighting. Therefore, young males must be castrated, which has attendant welfare considerations. An alternative to surgical castration would be to produce mainly female piglets by using sexed sperm doses for artificial insemination. Thus far, the only method of sexing spermatozoa which has been shown to work reliably is that of selection and separation of spermatozoa whose DNA is stained with a bis-benzimidazole dye, H33342 (Keeler *et al*, 1983), using the sorting capacity of a flow cytometer (Morrell *et al*, 1988; Johnson *et al*, 1989). However, the process of sorting sufficient numbers for an insemination dose in the flow cytometer takes too long since the stained spermatozoa must pass one at a time through a laser beam for detection of their DNA content. Although flow cytometric sexing of spermatozoa is 70-90% reliable, the method is slow and expensive, and sperm fertility may be reduced.

An alternative method of sperm sexing could be to use antibodies to sperm surface proteins, such as those produced by Cattle Logic Ltd (UK). The objective of the present study was to determine whether spermatozoa can be aggregated by antibody to sperm surface protein 1 and then separated into sub-populations enriched for either X- or Y-chromosome bearing spermatozoa.

The experiment was conducted in three consecutive stages: i) Antibody to a sperm surface protein 1 (Antibody 1; Cattle Logic Ltd, UK) was added to extended boar semen at a sperm concentration of $50x10^6$ spermatozoa/mL to see if agglutination of the spermatozoa occurred. ii) Boar sperm samples treated with different concentrations of antibody 1 were either allowed to precipitate and the supernatant was removed ("fall down"), or the samples were centrifuged at 100 or 200 g to pellet all the spermatozoa and non-aggregated spermatozoa were allowed to swim-up from the sperm pellet into fresh extender ("swim-up"). The X:Y ratio was measured by quantitative polymerase chain reaction (qPCR). iii) Using the optimum concentration of antibody 1, the experiment was repeated, analyzing the X:Y ratio in the sperm pellets and subsequently in the supernatant by qPCR. In all cases, controls (non-antibody treated semen from the same boars) were included. To determine the X:Y ratio in the separated sperm, SYBR-green based quantitative real-time PCR was performed Genomic DNA isolated from sperm samples was analyzed using primers designed to amplify the chromosome X-linked gene ProteoLipid Protein 1 or the chromosome Y-linked gene Sex-determining Region Y.. To calculate the percentage of X-bearing versus Y-bearing spermatozoa accurately, the cycle threshold for individual samples was compared to a standard curve generated from a serial dilution of a plasmid encoding the genes of interest.

The following results were obtained: i) Aggregation of boar spermatozoa was obtained with Antibody 1. Aggregated spermatozoa could be roughly separated from non-aggregated spermatozoa by allowing the sperm suspension to stand at room temperature for 30 mins, enabling the aggregated spermatozoa to sink to the bottom of the tube ("fall down"). A few non-aggregated spermatozoa were present with the aggregated spermatozoa and vice versa, hence the description "roughly". When

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What research is needed to improve commercial pig reproduction

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Artificial insemination with fresh or stored semen is currently the only sperm technology used at a commercial scale in the pig industry. Attention should therefore be given for further improvement of the functionality and fertilizing ability of cryopreserved semen, as well as for sperm sorting for gender pre-selection. During the last two decades various proteins and polypeptides have been identified in boar seminal plasma, and the relevance of some of them to reproductive technologies has been discussed at this conference. The long- term goal should be to isolate/synthetize those seminal plasma proteins proven important for the spermatozoa, and use them as ingredients in media used for e.g. cryopreservation and sex sorting of semen. A close cooperation between biochemists, molecular biologists and animal scientists is necessary to reach this goal.

The genomic revolution has brought us transcriptional profiling, allowing for the identification of many genes involved e.g. in mammalian gametogenesis, fertilization, and preimplantation embryo development. We do expect further progress within this field of research during coming years. The new information has also capacity to revolutionize the genetic progress within animal breeding.

The time of insemination in relation to ovulation is of great importance for fertility. Promising results were presented when using single insemination with fresh semen at fixed-time ovulation. However, further research is needed to find out if the same model also works in females given deep insemination with lower sperm number, or inseminated with sexed semen.

Piglet mortality is multifaceted in nature. To be successful in improving piglet survival in commercial pig production, a balanced selection program should be coupled with environmental and nutritional interventions.

Welfare and ethical aspects of commercial production are of growing interest for the society and for consumer organisations and cannot be neglected. More attention should therefore be paid to introduction/ improvement of different loose-/group-housing systems for sows all over the world.

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