The role of gene discovery, QTL analyses and gene expression in reproductive traits in the pig

S.K. Onteru, J.W. Ross and M.F. Rothschild

Department of Animal Science and Center for Integrated Animal Genomics, Iowa State University, Ames, IA, USA

The reproductive performance of the sow is one of the key factors affecting production profitability of the pig industry. Reproductive traits are in general, lowly heritable, and with reliable markers, they can be used to enhance current selection procedures for improvement of these traits. To find potential markers, large scale quantitative trait loci (QTL) and candidate gene studies have been conducted for reproductive traits. The present review discusses QTL and candidate gene discovery, large scale SNP association studies, gene expression profiling and discovery of miRNA regulation of pig reproductive tissues. Many QTL have been found for reproduction traits and a limited number of useful genes (e.g.: ESR1, PRLR, FSHB, EPOR and RBP4) have been found to have significant associations with reproductive traits. Expression studies with reproductive tissues have revealed differential expression within a few gene networks which need further mapping and association analyses to select prospective gene markers. The near completion of the pig genome sequence and the development of high density SNP chips will allow for large scale SNP association studies for pig reproductive traits in the future. Collection of appropriate phenotypes in large numbers and in broad populations representative of the swine industry are required if such genomic studies will ultimately be successful.

Introduction

Reproductive efficiency in pig breeding herds can best be measured as pigs per sow per year among all breeding females. Pork producers are also increasingly concerned with the length of sow productive life in a herd. Productive sows represent those animals which can farrow a litter of pigs, lactate for ~ 21 days, return to estrus, successfully conceive, complete gestation, and finally farrow again and again over many parities. The recent advances in pig genomics including whole genome sequencing have provided the identification of useful candidate genes and QTL (quantitative trait loci), expressed sequence tags (ESTs), single nucleotide polymorphisms (SNPs) and microRNAs; which all may affect reproductive phenotypes. This review focuses on the studies previously conducted and the roles they may play in improved reproductive performance.

E-mail: mfrothsc@iastate.edu

Quantitative approaches to improved reproduction

Reproductive traits in the male and female differ considerably. In males, reproductive traits or performance may be measured by testis size, semen volume, sperm concentration of the ejaculate, sperm quality and libido or breeding aggressiveness. Reproductive traits in females include age at puberty, estrous cycles and expression, litter size, weaning to estrus interval and farrowing interval. The component traits of litter size are ovulation rate, fertilization rate, embryo survival and uterine capacity. Fertilization rate is contributed in part by the boar. Embryo survival and uterine capacity have also been viewed in part as under the genetic control of the embryo/fetus. Hormone levels and control of hormone receptors are also important traits under consideration. Genetic differences have been observed both among breeds and lines. Those differences can be most effectively exploited through the use of crossbreeding. Within breed or line, heritability estimates are measures of the additive genetic variation that can be manipulated via selection of superior animals. Estimates of genetic parameters, heritabilities and genetic correlations vary for several reasons, including the breed(s) studied, method of analysis and sampling variation. Estimates of heritabilities for several traits are summarized (Lamberson 1990, McLaren & Bovey 1992). Estimates for most male traits are moderate (e.g. 0.4, testis wt.) and would be expected to respond to selection while most of the heritabilities for the female traits (e.g. 0.07, number born alive) are low and progress utilizing selection is expected to be more limited. Hence, while progress can be made using conventional selection, marker assisted selection (MAS) using useful genes and markers offer an opportunity to improve selection programmes for reproductive traits and reduces generation interval and enhances the accuracy of selection (Spotter & Distl 2006).

Candidate genes and QTL

To identify genetic markers the first approach has been to use genome scans using microsatellites to find quantitative trait loci (QTL) (Rathje *et al.* 1997, Rohrer *et al.* 1999, Wilkie *et al.* 1999) and the second approach has been to use candidate genes thought to play a role in controlling phenotypes (Rothschild *et al.* 1996, Drogemuller *et al.* 2001, Jiang *et al.* 2001). QTL analysis is the identification of genomic regions that are responsible for genotypic differences in a desired trait. Most QTL analyses have used at least three generations hence it is time-consuming to produce such pig populations for reproductive traits. Several QTL were found for both male and female reproductive traits (Table 1 and 2). However, initial studies revealed that chromosomes 8 and X harbored many QTL for female and male reproductive traits, respectively. Generally, QTL regions cover 10-20cM regions which are difficult to use in selection programmes. Thus fine mapping of QTL is necessary to develop markers to use in marker assisted selection programmes (Distl 2007). Several QTL have been found for reproduction traits but further research is required to find the causative genetic variation in the gene influencing the trait. Studies on the association of positional candidate genes are progressing and these studies require useful commercial populations for validation. The requirement for the candidate gene approach is to test the gene variants in different populations (Rothschild *et al.* 2000). Because lowly heritable reproductive traits are influenced greatly by management and other environmental influences, it is important to test the association of candidate genes with phenotypic traits in different populations under different farm conditions. There may be inconsistencies in associations of candidate genes with phenotypes in different studies but this does not mean that the gene marker does not work. Failure to be predictive of a trait may be the result of the small sample size used to test the association. In addition, association studies are usually affected by differences in the frequency of alleles and genotypes responsible for the candidate gene effects, different linkage phases between the marker and causal mutation in different populations and epistatic effects (Distl 2007).

Table 1. QTL for female reproductive traits in pigs.

Trait	SSC	Population*	Reference**
Age at puberty	1, 10	WC x M	Rohrer *et al.* 1999
	7, 8, 12	LW x Lr	Cassady *et al.* 2001
	7, 8, 12, 15	LW x Lr	Holl *et al.* 2004
Ovulation rate or Number of corpora lutea	4, 8, 13, 15	LW x Lr	Rathje *et al.* 1997
	8	Y x M	Wilkie *et al.* 1999
	8, 3, 10	WC x M	Rohrer *et al.* 1999
	8	Y x M	Braunschweig *et al.* 2001
	9	LW x Lr	Cassady *et al.* 2001
	9	LW x Lr	Holl *et al.* 2004
	3	M x D	Sato *et al.* 2006
Uterine capacity	8	WC x M	Rohrer *et al.* 1999
Gestation Length	9	Y x M	Wilkie *et al.* 1999
Litter size	6	GMP x M	Yasue *et al.* 1999
	7, 12, 14, 17	LW/Lr x M	De Koning *et al.* 2001
Total number born	8	LW x M	King *et al.* 2003
Number born alive	11	LW x Lr	Holl *et al.* 2004
	1	(LW x Lr) x Lc	Buske *et al.* 2006a
	7, 16, 18	LW x F Lr	Tribout *et al.* 2008
Number of still born	4	Y x M	Wilkie *et al.* 1999
	5, 13	LW x Lr	Cassady *et al.* 2001
	5	LW x Lr	Holl *et al.* 2004
	6, 11, 14	LW x F Lr	Tribout *et al.* 2008
Teat number	1, 3, 10	WC x M	Rohrer 2000
	1, 7	GMP x M	Wada *et al.* 2000
	1, 8, 6, 7, 11	LW x Lr	Cassady *et al.* 2001
	2, 10, 12	M x DP	Hirooka *et al.* 2001
	8	LW x M	King *et al.* 2003
	1, 8	M, P, WB crosses	Beeckmann *et al.* 2003
	5	M, P, WB crosses	Lee *et al.* 2003
	10	M, P, WB crosses	Dragos-Wendrich *et al.* 2003
	12	M, P, WB crosses	Yue *et al.* 2003
	X	M, P, WB crosses	Cepia *et al.* 2003
	5, 10, 12	Ib x M	Rodriguez *et al.* 2005
	3, 7, 8, 16, 17	LW x M	Bidanel *et al.* 2008

* F Lr: French Landrace; LW: Large white; Lr: Landrace; Y: Yorkshire; M: Meishan; WC: White composite; D: Duroc; GMP: Gottingen miniature pig; Lc: Leicoma, P: Pietrain; WB: Wild boar; DP: Dutch piglines; Ib: Iberian
** Most of the data were obtained from http://www.animalgenome.org/QTLdb/pig.html.

A list of some associations of candidate genes with female reproductive traits in different populations is presented in Table 3. The first discovered and perhaps most important being *ESR1*, which is a steroid hormone receptor mediating the actions of estrogens. The association of *ESR1* with litter size was first reported by Rothschild *et al.* (1996) who found a PvuII polymorphism in intron 9 of *ESR1* in Meishans, Meishan Synthetic lines and Large White populations. Among the PvuII genotypes (AA, AB and BB), the BB sows farrowed 2.3 and 1.5 piglets more than the AA sows for both the total number of piglets born (TNB) and the number born alive (NBA) traits respectively in Meishan synthetics and larger differences among purebred Meishan. Similar results of *ESR1* associations were found in Large White and Yorkshire populations by subsequent studies (Table 3) though the effects were smaller but still quite significant. Short

Table 2. QTL for male reproductive traits*

Trait	SSC	Population	Reference
Testicular weight			
300 d	X, 1, 7, 5	White Duroc x Erhualin	Ren *et al.* 2008
220 d	X	Meishan x White composite	Rohrer *et al.* 2001
180 d	X	Meishan x Large White	Bidanel *et al.* 2001
90 d	X, 1	White Duroc x Erhualin	Ren *et al.* 2008
60 d	X, 3	Meishan x Duroc	Sato *et al.* 2003
Epididymal weight			
300 d	7, 3	White Duroc x Erhualin	Ren *et al.* 2008
180 d	4, 10, 13, 15 and X	Meishan x Large White	Bidanel *et al.* 2001
90 d	2	White Duroc x Erhualin	Ren *et al.* 2008
Seminiferous tubular diameter			
300 d	16	White Duroc x Erhualin	Ren *et al.* 2008
90 d	X, 14, 13, 5	White Duroc x Erhualin	Ren *et al.* 2008
Serum testosterone concentration			
300 d	7, 13	White Duroc x Erhualin	Ren *et al.* 2008
Plasma FSH levels	3, 10, X	Meishan x White composite	Rohrer *et al.* 2001

* The data were obtained from http://www.animalgenome.org/QTLdb/pig.html.

et al. (1997) showed an additive effect of the B allele with average effect of 0.8 piglets in first parity and approximately 0.7 piglets in later parities using four Large White-based commercial pig lines with more than 4200 first parity records and over 4700 later parity records. This large population is a good example of the population sizes needed for reproductive trait studies. A favorable meta-analysis reported an association of the B allele with TNB and NBA using 15 studies in more than 9000 sows (Alfonso 2005). On the contrary, some much smaller studies reported an association of superior litter size with the A allele rather than the B allele (e.g. Van Rens *et al.* 2002). In addition, no significant association of *ESR1* with litter size was mentioned in different swine populations (Depuydt *et al.* 1999, Drogemuller *et al.* 2001, Isler *et al.* 1999). Many of these were small studies and environmental effects may have prevented seeing the significant effect of *ESR1*. Recently, Muñoz *et al.* (2007) reported five silent mutations in the coding region of *ESR1* and found an association of a SNP C1227T with litter size. Because of unaltered amino acid sequence by the reported polymorphisms of *ESR1*, the associated *ESR1* polymorphisms may not be causal mutations, and instead might be linked with the causative SNP. In addition, the genetic background (associations found only in Large White and Yorkshire populations) may be important to consider when using *ESR1* as a possible marker for marker assisted selection programmes (Rothschild *et al.* 1996). Similarly *ESR1* (AvaI) and *ESR2* (PvuII) polymorphisms showed significant differences in semen volume and live sperm concentration (Terman *et al.* 2006). *ESR1* is used by many breeders and breeding companies worldwide.

An essential process to establish pregnancy in pigs is a shift in endometrial prostaglandin (PG) F secretion from an endocrine (toward the myometrium and uterine vasculature) to an exocrine (toward the uterine lumen) orientation (Bazer & Thatcher 1977). This is mediated by interactive effects of estrogens and prolactin (Gross *et al.* 1990). Therefore, another important candidate gene associated with reproductive traits is the prolactin receptor (*PRLR*). In pigs, this gene was found to be associated with age at puberty, ovulation rate, uterine length and litter size (Table 3). Initial results for litter size were first presented by Vincent *et al.* (1998) and later confirmed by Van Rens & Van der Lende (2002) and Van Rens *et al.* (2003). These genotypes

Table 3. Candidate genes associated with female reproductive traits in pigs.

Trait	Associated genes†	SSC	Polymorphism	Polymorphism location	Population*	Reference
Age at puberty	*PRLR*	16	Alu site	-	LW x M	Van Rens & Vander Lende 2002
	AKR1C2	10	Ile16 Phe	Nt179 in coding region	¼ M	Nonneman *et al.* 2006
	PAX5	1	C/T	Intron 9	D x BT and Lr x BT	Kuehn *et al.* 2008
Ovulation rate	*PRLR*	16	Alu site	-	Lr x M	Van Rens *et al.* 2003
	*GNRHR***	8	-	3'UTR	M x LW	Jiang *et al.* 2001
	NCOA1	3	T/T	Exon 11	M x LW	Melvile *et al.* 2002
	MAN2B2	8	A/G	Nt1574 mRNA	M x WC	Campbell *et al.* 2008
Uterine length	*PRLR*	16	Alu site	-	Lr x M	Van Rens *et al.* 2003
	FSHB	2	FSHBMS microsatellite	5'flanking region	LW x M	Li *et al.* 2008
Uterine capacity	*EPOR*	2	C/T	Intron 4	Yx Lr x CW x LW	Vallet *et al.* 2005a
	sFBP	-	Ser-Arg	Exon 1	M x W	Vallet *et al.* 2005b
Litter size	*ESR1***	1	PvuII site	Intron	M x SL and LW	Rothschild *et al.* 1996
					LW	Short *et al.* 1997
					Lr	Chen *et al.* 2000
					M x LW	Van Rens *et al.* 2002
					LW	Matousek *et al.* 2003
					Czech LW	Goliasova & Wolf 2004
			C/T	Exon 5	M x LW	Muñoz *et al.* 2007
	PRLR	16	-	-	LW	Vincent *et al.* 1998
					SL	Droggemuller *et al.* 2001
					M x LW	Van Rens & Vander lende 2002
	*FSHB***	2	-	Intron	Y x EL	Li *et al.* 1998
					Lr and Y	Zhao *et al.* 1999
			Alu site	Promoter	LP, DP and Lr	Du *et al.* 2002
			FSHBMS microsatellite	5'flanking region	LW x M	Li *et al.* 2008
	RBP4	14	-	-	LW	Olliver *et al.* 1997
				Intron	SL	Rothschild *et al.* 2000
				Intron	GW	Spotter *et al.* 2009
	BF	7	-	Intron	(LW x Lr) x Lc	Buske *et al.* 2005
	LIF	8	-	Exon 3	GL	Spotter *et al.* 2009
			C/T	Intron 1	LW	Lin *et al.* 2009
	FUT1		Hha1	-	(LW x Lr) x Li	Buske *et al.* 2006b
	RNF4	6	C/T	Intron 5	CQ	Niu *et al.* 2009

† *AKR1C2*: Aldo keto reductase 1C2; *BF*: Properdin; *EPOR*: Erythropoietin receptor; ESR1: Estrogen receptor 1; FSHB: Follicle stimulating hormone beta; *FUT1*: fucosyl transferase 1; *GNRHR*: Gonadotropin releasing hormone receptor; *LIF*: Leukemia inhibitory factor; *MAN2B2*: Mannosidase 2B2; NCOA1: Nuclear receptor coactivator 1; *PAX5*: Paired box 5; *PRLR*: Prolactin receptor; RBP4: Retinol binding protein 4; *RNF4*: ring finger protein 4 gene; *sFBP*: Secreted folate binding protein.

* BT: Yorkshire x maternal Landrace composite; CQ: Chinese Qingping; CW: Chester White; D: Duroc; DP: Duli pigs; EL: Erhualian line; GL: German landrace; GW: German large white; LP: Laiwu pigs; Lr: Landrace; Lc: Leicoma; LW: Large white; M: Meishan; SL: Synthestic lines; Y: Yorkshire; W: White European breed cross; WC: White composite

**Some of the mentioned gene information can be seen in http://www.animalgenome.org/QTLdb/pig.html

were associated with significant ($P < 0.01$) differences in male reproductive traits such as ejaculate volume and spermatozoa concentration in ejaculate (Kmiec & Terman 2004).

During pregnancy, the pig uterus secretes a large amount of proteins in response to progesterone. These proteins are required to nurture the litter (Roberts & Bazer 1980). Among them, retinol binding protein (RBP) is secreted by endometrial epithelial cells to deliver retinol to the uterine lumen (Roberts *et al.* 1993). High levels of RBP secretion on day 12 of pregnancy verify its importance during that period of time (Trout *et al.* 1991). The *RBP4* gene was investigated as a candidate gene and initial results, based on a limited number of sows, indicated an additive gene effect for the favorable allele of 0.52 ± 0.30 pigs per litter in a Large White Hyperprolific line and 0.45 ± 0.43 in the control (Ollivier *et al.* 1997). It was again verified as a useful candidate gene for litter size (Rothschild *et al.* 2000) using an *MSP1* PCR-RFLP assay and the favorable A allele had an approximate additive effect of 0.23 pigs per litter ($P < 0.05$) for TNB and 0.15 pigs per litter for NBA in commercial Landrace lines. However, other small studies were unable to identify a significant association of *RBP4* with litter size in selected lines (Blowe *et al.* 2006) and synthetic lines (Drogemuller *et al.* 2001). This gene has been suggested for use only in Landrace populations.

The association of follicle stimulating hormone (*FSH*) with reproductive traits has been well studied. The association of a retroposon element in intron 1 of the follicle stimulating hormone-β (*FSHB*) gene with litter size was studied and it was found that the non-retroposon homozygous allele (BB) females produced on average 2.53 piglets more than the retroposon homozygous allele (AA) sows for total number born (TNB) and 2.12 for number born alive in the first parity in Landrace and Yorkshire pigs (Zhao *et al.* 1999). An Alu element with a difference in poly A length in the regulatory element of the *FSHB* gene was also found to be associated with litter size. These results indicated that *FSHB* was associated with pig litter size or it is linked with other genes (Du *et al.* 2002). Li *et al.* (2008) conducted a study on the association of a microsatellite 4Kb upstream of the *FSHB* gene in a Large white x Meishan F2 population and found a significant association with higher number of piglets at weaning and greater litter weight at weaning ($P < 0.05$).

In addition, the associations of polymorphisms in aldo keto reductase 1C2 (*AKR1C2*), erythropoietin receptor (*EPOR*), fucosyl transferase 1 (*FUT1*), gonadotropin releasing hormone receptor (*GNRHR*), leukemia inhibitory factor (*LIF*), mannosidase 2B2 (*MAN2B2*), nuclear receptor coactivator 1 (inhibin beta A), paired box 5 (*PAX5*), properdin (*BF*), ring finger protein 4 gene (RNF4) and secreted folate binding protein (*sFBP*) with different female reproduction traits have been reported. Using 119 SNPs from 95 genes, Fan *et al.* (2009) carried out association analyses for six reproductive traits (total number born, TNB; number born alive, NBA; still born number, SBN; mummy number, MN; gestation length, GL and non productive days, NPD) recorded in 2,066 animals for six parities. It was found that 23 genes were significantly ($P < 0.05$) associated with at least three reproductive traits. In parity 1, *COL9A1, NST, ADAM12, WARS2, DKFZ* and *LRP5* were significantly ($P < 0.05$) associated with both TNB and NBA while *COL1A2, CALCR* and *IGFBP5* were significantly ($P \leq 0.01$) associated with SBN; and *IL6* and *ESR2* were significantly ($P \leq 0.01$) associated with MN and NPD, respectively. During later parities, *CASR, ESR2, WARS2, NST, IFNγ* and *BMP8* had significant association ($P < 0.05$) with TNB and NBA. The genes *MC4R, FBN1, IGFBP2* and *SFRP4* were significantly ($P < 0.05$) associated with GL in several parities. It should be noted that ESR1 was not tested in this initial study. For male reproduction traits Lin *et al.* (2006) mentioned the association of *GNRHR* with motility, plasma droplet ratio and abnormal sperm rate; inhibin beta A (*INHBA*) with plasma droplet ratio and abnormal sperm rate and inhibin beta B (*INHBB*) with sperm concentration.

Though many candidate genes examined so far have direct physiological roles in different stages of reproduction, this is not mandatory. Instead, a QTL could be the result of genetic

variation in regulatory protein or initiation factor genes that then affect the expression of genes involved in pig reproduction. This concept of "polygenic paradox" was illustrated by Pomp *et al.* (2001) using the example of putative regulation of FSHB gene.

ESTs and gene expression in pig reproductive tissues

Gene expression analysis is also a useful approach to understanding the biological basis of reproduction and large numbers of expressed sequence tags (EST) to study gene expression for these traits are essential. The Midwest consortium (Tuggle *et al.* 2003) isolated ESTs from female reproductive organs and deposited 21,499 ESTs representing 10,574 genes in public databases. Out of these ESTs, 3,183 sequences were from the anterior pituitary; 3,900 were from term placenta; 4,505 were from the peri-implantation uterus; 4,165 were from embryo/fetus tissue; 1,544 were from hypothalamus and 1,643 were from ovary. Gorodkin *et al.* (2007) presented an expression study based on 35 tissues representing 98 cDNA libraries and 1,021,891 sequences assembled by the Distiller assembly program, and concluded that gene diversity is greater in the brain and testis, which are major components of reproduction. The expression profiles of the hypothalamus-pituitary-gonadal axis between animals with different reproductive performance are extremely useful in exploring variation among reproductive traits in a given situation. Using differential display PCR, Bertani *et al.* (2004) reported 125 EST sequences were differentially expressed in the anterior pituitary gland between control line and a line selected for ovulation rate and embryo survival. The differential expressions of the genes G-beta like protein, ferritin heavy chain and follicle stimulating hormone beta subunit, were confirmed by northern blotting of anterior pituitary RNA between the above lines. The expression levels of ferritin heavy chain and G beta like protein were less in a selected line for enhanced reproduction compared to the control line, whereas the FSH beta gene was increased in the selected line.

To date only a limited number of detailed transcriptome analyses on pig folliculogenesis have been conducted. Two of them (Caetano *et al.* 2004, Gladney *et al.* 2004) used whole follicles and were performed on pigs from lines selected for reproductive traits. It was found that follicle sizes were bigger in the selected line than control line. By using differential display PCR, Gladney *et al.* (2004) found that 152 genes were up regulated and 20 genes were down regulated in follicles of the selected line. Three differentially expressed genes were confirmed by northern hybridization. These genes were calpain 1 light subunit (*CAPN4*), cytochrome P450 aromatase and cytochrome P450 side chain cleavage enzymes. Similarly, microarray analysis of pooled follicles with two versions of human cDNA arrays (UniGem V1.0 and V2.0) resulted in 33 and 21 differentially expressed probes between selected and control line. Northern hybridization of differentially expressed mRNA resulting from microarray analysis confirmed the reduced expression of follistatin (*FST1*) and increased expression of nuclear receptor subfamily 4, group A, member 1 (*NR4A1*) in the line selected for ovulation rate and embryo survival. It was hypothesized that less expression of *CAPN4* is favorable to decreased follicular degradation and apoptosis, which promotes the recruitment of a larger pool of follicles for ovulation (Gladney *et al.* 2004). Similarly, low follistatin increases the bioavailability of activins, which are required for follicular growth and differentiation (Hasegawa *et al.* 1994). Higher expression of *NR4R1* increases the sensitivity of follicles to steroid hormones (Gladney *et al.* 2004). To know the expression profile of the ovary and follicle in the above mentioned selected and control lines, a microarray with 4608 probes was prepared at the Nebraska Medical Center Core Facility using the GMS417 Arrayer (Genetic Microsystems). Mixed model analysis of these microarray data (Caetano *et al.* 2004) found evidence for differential expression of 71 and 59 genes in

the whole ovary and ovarian follicles during the follicular phase of the estrous cycle between animals from the selected and control lines. The genes involved in steroid biosynthesis (e.g., cytochrome P450 side chain cleavage enzyme, steroidogenic acute regulatory protein and others), tissue remodeling (plasminogen activator inhibitor II) and apoptosis (calpain light chain I) were all differentially expressed between the lines. It was suggested that the differential expression of ovarian genes between the select and control lines was due to the effect of selection for increased reproduction on the frequency of allelic variation within the genes themselves or from allelic variation in genes that control the genes found to differ between lines (Caetano *et al.* 2004).

An ovarian transcriptome analysis was conducted on the effect of luteinization on preovulatory follicles (Agca *et al.* 2006). This microarray analysis detected the decreased regulation of 107 genes and increased regulation of 43 genes during the transition from preovulatory estrogenic to luteinized follicles. The decreased regulated genes belonged to cytoskeletal proteins, regulators of cytoskeleton, nuclear proteins, nucleic acid binding proteins, metabolic enzymes, mitochondrial transporters, proteins involved in the oxidative stress response, ligands, receptors and receptor pathways (predominantly cAMP response system), and cell proliferation/differentiation functional groups. The increased expression of certain genes during luteinization were for proteins that are involved in cell adhesion and migration, cell growth inhibition or angiogenesis. Another ovarian transcriptome study (Bonnet *et al.* 2008) found 79 differentially expressed transcripts associated with terminal follicular growth. These genes were involved in functional networks required for cellular growth, cell cycle, proliferation, cancer, reproductive system development and function, molecular transport, protein synthesis and lipid metabolism. Genes in glutathione metabolism (e.g. *GSTA1*, glutathione S-transferase alpha 1; and *MGST1*, microsomal glutathione S-transferase 1) and lipid metabolism (e.g. *CYP19A*, P450 aromatase A and many others) were up regulated, and the ribosomal protein genes (e.g. *CALU*, calumenin; *SLC40A1*, solute carrier family 40 and others) and cell shape genes (e.g. *TUBA1B*, tubulin, *CAPNS1*, calpain, small subunit 1 and others) were down regulated in terminal antral follicular development especially in large follicles compared to small and medium ovarian follicles. A quantitative RT-PCR study found significant ($P < 0.05$) correlations between oocyte number and the expression levels of *ESR1* and *IGFR1* in ovaries of Duroc x Meishan and PIC lines (Hu *et al.* 2006). Whitworth *et al.* (2005) compared expression profiles of germinal vesicle stage oocytes to that of 4-cell stage and blastocyst stage embryos produced from *in vitro* fertilization and culture and *in vivo* derived embryos. *In vivo* blastocyst stage embryos had higher expression of gene networks pertaining to ribosomal function and ion transport than that of *in vitro* blastocyst stage embryos. Both *in vitro* embryo stages had lower expression of plasma membrane-protein-tyrosine-phosphatase activity, and increases in expression of the nucleolus, small nucleolar ribonucleoprotein complex, and RNA binding and processing gene networks than *in vivo* embryo stages. The complete list of differentially expressed genes in these stages of embryos can be obtained from http://genome.rnet.missouri.edu/Swine/Publications. The utilization of these transcript profiles allows the identification of differentially expressed genes associated with embryogenesis as well as developmental competency associated with *in vitro* fertilization systems.

Studies of the endometrium transcriptome were conducted to determine the differential expression of genes in the uterus during the estrous cycle and pregnancy (Green *et al.* 2006). A total of 4,827 genes were significantly differentially expressed at some time during the estrous cycle. Clustering of these genes identified six main patterns across the estrous cycle. These patterns are related to the functions of the endometrium such as sperm maturation, blastocyst growth and position, and conceptus development and attachment. These data may be useful for transgenic and cell transfer approaches to improve reproduction efficiency. Ross *et al.* (2007) identified numerous endometrial genes aberrantly expressed following exogenous estrogen exposure prior to implantation that is associated with total pregnancy loss. In addition to different periods

of estrous cycle and pregnancy, the transcriptome profile of uterine epithelium was studied in comparison to other tissues using a 20,400 70 mer second generation pig oligonucleotide array (Pigoligoarray; www.pigoligoarray.org), Steibel *et al.* (personal communication). This study found that 286 transcripts were differentially expressed in uterine epithelium relative to liver, muscle and brain stem.

Because fertilization is an efficient process in pigs, mortality can be assessed throughout gestation by comparing conceptus/fetus numbers to the number of corpora lutea. Pigs suffer from a high incidence of prenatal mortality, ranging from 20 to 46% at term (Pope 1994). The occurrence of embryonic mortality can be broadly broken into two phases; the peri-implantation stage of development, Days 10-18 of gestation; and post-implantation development between Day 18 and 114 of gestation. In gilts where ovulation rate is not sufficient to exceed uterine capacity, the majority of prenatal mortality is thought to occur during the peri-implantation period of development (Anderson 1978). Transcript discovery and/or profiling during conceptus transition from a 9-10 mm sphere on Day 11-12 to a transient tubular shape (15-20 mm) and into a long filamentous thread (greater than 150 mm) over the course of a few hours has been achieved through quantitative RT-PCR (Green *et al.* 1995, Kowalski *et al.* 2002, Yelich *et al.* 1997a,Yelich *et al.* 1997b), differential display RT-PCR (Wilson *et al.* 2000), suppression subtractive hybridization (SSH) (Ross *et al.* 2003a), expressed sequence tag (EST) library construction and analysis (Smith *et al.* 2001), utilization of embryonic based cDNA array (Lee *et al.* 2005), serial analysis of gene expression (SAGE) (Blomberg *et al.* 2005) and Affymetrix GeneChip microarray (Ross *et al.* 2009). Several transcripts have been consistently identified as differentially expressed during transition through these critical developmental stages such as interleukin 1β (Ross *et al.* 2003b, Lee *et al.* 2005), steroidogenic acute regulatory protein (STAR) (Blomberg *et al.* 2005, Lee *et al.* 2005) and cyclooxygenase-2 (Wilson *et al.* 2000, Ross *et al.* 2009). The utilization of the Affymetrix GeneChip identified 192 transcripts with the putative ability to serve as molecular markers due to transient up or down regulation during early stages of trophoblastic elongation (Ross *et al.* 2009). In addition 482 transcripts were differentially expressed in filamentous day 12 conceptuses compared to large spherical conceptuses representing biological processes associated with cell motility, ATP utilization, cell growth, metabolism and intracellular transport (Ross *et al.* 2009). Expression of numerous genes and gene products characterize this developmental process, which is associated with the production of steroids (primarily estrogen), prostaglandins, cytokines and morphogenic factors having a tremendous influence on maternal recognition of pregnancy, immunological tolerance and growth of the conceptus.

Following the peri-implantation period, rapid fetal growth ensues and during the period from Days 21 to 45, uterine capacity becomes limiting. Uterine capacity is defined as the maximum number of piglets carried to term when potentially viable conceptuses are not limiting (Christenson *et al.* 1987). Uterine crowding significantly affects fetal weight, placental weight and protein secretion on both Days 25 and 35 of gestation (Vallet & Christenson, 1993), suggesting the expression and regulation of transcripts in placental and fetal tissue during this stage of gestation is responsive to environmental stresses. Wesolowski *et al.* (2004) conducted a fetal transcriptome study using pig fetuses at 21, 35 and 45 days of gestation and identified 17 differentially expressed genes which play a major role in musculoskeletal growth, immune system function, and cellular regulation. Comparison between Meishan and European Large White composite gilts identified numerous single nucleotide polymorphisms within the transcriptome of day 25 placental tissues (Bischoff *et al.* 2008).

Using human microarrays, differential expression of genes in testis between boars of high and low steroidogenesis was studied and results indicated that seven genes were over expressed in boars with high steroidogenesis. Among them three (*CYB5, CYP19A1,* and *CYP11A1*) are involved in steroidogenesis (Stewart *et al.* 2005).

MicroRNAs

The numerous transcriptional profiling experiments conducted in pig reproductive tissues assess transcript diversity and genomic regulation of gene expression, but do not assess post transcriptional regulation of mRNAs and how it influences cellular phenotypes in these tissues. MicroRNAs (miRNA) are single-stranded RNA molecules of about 18–24 nucleotides in length, and through their ability to confer post transcriptional gene silencing (PTGS) (Bartel 2004), may have a significant regulatory role in reproductive tissues. MicroRNAs are organized throughout the genome in multiple ways that allow the expression of an individual miRNA alone (Mendell 2005), clustered with other miRNAs (Lee *et al.* 2002) or, expressed within introns of transcribed mRNA (Kim 2005). RNASEN, DGCR8, exportin 5 and DICER are all critical enzymes for production of mature miRNA capable of conferring PTGS (Hutvagner & Simard 2008). Conditional knockout of DICER, the enzyme responsible for final processing of a mature miRNA, during oogenesis causes infertility through major disruption of the miRNA repertoire in the oocyte (Tang *et al.* 2007). In addition to the importance of miRNA in regulating oogenesis, reproductive tract development is also dependent on miRNA function (Hong *et al.* 2008). During implantation in mice, miR-101a and miR199a post-transcriptionally regulate cyclooxygenase 2 (COX2) (Chakrabarty *et al.* 2007), an enzyme whose transcript is also differentially expressed during the opening of the implantation window in the pig (Ashworth *et al.* 2006).

Because miRNA:mRNA complementation sites are relatively short and often imperfectly paired, slight changes in sequence, due either to RNA editing or the presence of a SNP in either the recognition site of the target gene or the miRNA could significantly alter PTGS in reproductive tissues. RNA editing can also produce variations in miRNA function, particularly, adenosine-to-inosine editing (Pfeffer *et al.* 2005, Athanasiadis *et al.* 2004). For example, the miRNA, miR-376 undergoes *tissue specific* RNA editing that converts an adenosine to an inosine. In addition to RNA editing, SNPs also affect miRNA function. Recent screening of miRNAs has revealed the occurrence of 323 SNPs in 227 known human miRNA genes (Duan *et al.* 2007). This study further demonstrated the ability of a SNP in miR-125a to prevent DROSHA recognition, in so doing blocking its ability to be processed into a pre-miRNA, thereby reducing its effectiveness in conferring PTGS (Duan *et al.* 2007).

In essence, miRNA influence, which can be impacted by appropriate enzyme expression and function, RNA editing and SNPs in both target genes and miRNAs; have a distinct ability to dramatically alter the phenotype of cells by influencing the mRNA and protein repertoire through PTGS, potentially altering the efficiency of function. The importance being that very few miRNAs have the ability to post-transcriptionally influence hundreds of mRNAs (Rajewsky 2006). The reports on miRNAs in different pig reproductive tissues are very limited. MicroRNAs are expressed in porcine reproductive tissues such as ovary (Kim *et al.* 2006, Pratt *et al.* 2008), oocytes (Ross and Prather, unpublished data), and day 33 fetus and extra-embryonic membranes (Huang *et al.* 2008). Further research is required to examine miRNA expression profiles in other reproductive tissues of the pig that require significant transcript turnover and proteome reorganization to function efficiently.

Whole genome sequencing, SNP discovery and SNP chips

The development of advanced methods to improve genotyping in large scale projects and the reduction of genotyping costs have allowed for large scale SNP discovery. These studies will allow genotyping of hundreds of thousands of SNPs and genes. Such large scale SNP association studies pertaining to pig reproduction traits are still in infancy. Following efforts to sequence the

human, cow and chicken genomes, sequencing for the pig genome began. Initial sequencing was done by a Sino-Danish team (Wernersson *et al.* 2005) and resulted in a .66X coverage of the swine genome. Since then an international effort led by individuals at several U.S. universities and centers around the world have focused on sequencing the pig genome at the Sanger Institute in the UK. Now in its third year, the International Swine Genome Sequencing committee has raised nearly 20 million dollars and nearly 75% of the pig genome is sequenced (http://www.sanger.ac.uk/Projects/S_scrofa/) to 6X coverage. It is expected that the sequencing will be completed by the end of 2009. This sequence information can be further mined for SNPs as was the case when nearly 100,000 SNPs were identified from the existing 1.2 Gb of porcine sequence (Kerstens *et al.* 2009). Initial work by the Danish-Sino team led to the first 7K SNP chip (Vingborg *et al.* 2008). Furthermore, the next generation sequencing technology revolutionized the ability to identify many more SNPs With the help of such technology, the International Swine SNP Consortium designed a 60K Illumina Infinium iSelect™ SNP Bead Chip for pigs and produced over 1 million SNPs. The SNPs used for this chip were a small group of previously validated SNPs in the public domain and the SNPs identified de novo by second generation sequencing on the Illumina Genome Analyzer (Solexa) and Roche 454 FLX sequencer (Groenen *et al.* 2009). These were then chosen on the basis of minor allele frequency and spacing when known from existing sequence information. Initial work suggests the chip have well over 50,000 useful SNPs with excellent minor allele frequency and is already being employed. This chip has the potential to modernize association trials and lead to effective genomic selection (Solberg *et al.* 2008).

Phenomics

The development of gene expression arrays, sequence information, SNP chips and other genomic tools is relatively well advanced. But if we are to better use high density SNP chips for association trials or investigate other gene action such as imprinting and epigenetics then clearly more useful and varied phenotypes must be collected. This includes the usual traits like number born and number born alive but also ovulation rate, embryo survival, hormone levels and other traits of interest. This area of collecting new and interesting phenotypes is called phenomics (Freimer & Sabatti, 2003). These traits must be measured not on tens of animals or hundreds of animals but on thousands. Furthermore, utilization of the available tools and resources to impact specific reproductive traits in swine requires cognizance that selection and improvement of single traits, such as number born alive, can have deleterious effects on other traits such as post-natal performance (Foxcroft *et al.* 2007). An effort is needed to determine the relevance of various alternative measurable traits to improvements in swine reproduction. Reproductive physiologists and animal geneticists need to participate more collaboratively to accomplish such data sets.

Acknowledgements

Efforts and information supplied by colleagues around the world is appreciated. Work discussed here has been supported in part by PIC USA, Monsanto Choice Genetics, Newsham Choice Genetics, the National Pork Board and by Hatch and State of Iowa Funds and funding provided by the College of Agriculture and Life Sciences at Iowa State University.

References

Agca C, Ries JE, Kolath SJ, Kim J, Forrester LJ, Antoniou E, Whitworth KM, Mathialagan N, Springer GK, Prather RS & Lucy MC 2006 Luteinization of porcine preovulatory follicles leads to systematic changes in follicular gene expression. *Reproduction* **132** 133–145.

Anderson LL 1978 Growth, protein content and distribution of early pig embryos. The Anatomical Record **190** 143-154.

Alfonso L 2005 Use of meta-analysis to combine candidate gene association to study the relationship between the ESR PvuII polymorphism and sow litter size. *Genetics Selection Evolution* **37** 417–435.

Ashworth MD, Ross JW, Hu J, White FJ, Stein DR, DeSilva U, Johnson GA, Spencer TE & Geisert RD 2006 Expression of porcine endometrial prostaglandin synthase during the estrous cycle and early pregnancy, and following endocrine disruption of pregnancy. *Biology of Reproduction* **74** 1007-1015.

Athanasiadis A, Rich A & Maas S 2004 Widespread A-to-I RNA editing of Alu-containing mRNAs in the human transcriptome. *PLoS Biology* **2** e39.

Bartel DP 2004 MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* **116** 281-297.

Bazer FW & Thatcher WW 1977 Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F2 alpha by the uterine endometrium. *Prostaglandins* **14** 397-400.

Beeckmann P, Schröffel Jr J, Moser G, Bartenschlager H, Reiner G & Geldermann H 2003 Linkage and QTL mapping for Sus scrofa chromosome 1. *Journal of Animal Breeding and Genetics* **120(1)** 1-10.

Bertani GR, Gladney CD, Johnson RK & Pomp D 2004 Evaluation of gene expression in pigs selected for enhanced reproduction using differential display PCR: II. Anterior pituitary. *Journal of Animal Science* **82** 32-40.

Bidanel JP, Prunier A, Iannuccelli N & Milan D 2001 Detection of quantitative trait loci for male and female reproductive traits in Meishan × Large White F2 pigs. In *Book of abstracts of the 51st Annual meeting of the European Association for Animal Production* **7, pp** 54. Wageningen, The Netherlands: Wageningen Pers.

Bidanel JP, Rosendo A, Iannuccelli N, Riquet J, Gilbert H, Caritez JC, Billon Y, Amigues Y, Prunier A & Milan D 2008 Detection of quantitative trait loci for teat number and female reproductive traits in Meishan x Large White F2 pigs. Animal **2 (6)** 813–820.

Bischoff SR, Shengdar T, Hardison NE, York AM, Freking BA, Nonneman D, Rohrer G & Piedrahita JA 2008 Identification of SNPs and INDELS in swine transcribed sequences using short oligonucleotide microarrays. *BMC Genomics* **9** 252.

Blowe CD, Boyette KE, Ashwell MS, Eisen EJ, Robison OW & Cassady JP 2006 Characterization of a line of pigs previously selected for increased litter size for RBP4 and follistatin. *Journal of Animal Breeding and Genetics* **123**389-395.

Blomberg LA, Long EL, Sonstegard TS, Van Tassell CP, Dobrinsky JR & Zuelke KA 2005 Serial analysis of gene expression during elongation of the peri-implantation porcine trophectoderm (conceptus). *Physiological Genomics* **20** 188-194.

Bonnet A, Le Cao KA, SanCristobal M, Benne F, Robert-Granie C, Law-So G, Fabre S, Besse P, De Billy E, Quesnel H, Hatey F & Tosser-Klopp G 2008 In vivo gene expression in granulosa cells during pig terminal follicular development. *Reproduction* **136** 211–224.

Braunschweig MH, Paszek AA, Weller JI, Da Y, Hawken RJ, Wheeler MB, Schook LB & Alexander LJ 2001 Generation and exploration of a dense genetic map in a region of a QTL affecting corpora lutea in a Meishan×Yorkshire cross. *Mammalian Genome* **12** 719–723.

Buske B, Brunsch C, Zeller K, Reinecke P & Brockmann G 2005 Analysis of properdin (BF) genotypes associated with litter size in a commercial pig cross population. *Journal of Animal Breeding and Genetics* **122** 259–263.

Buske B, Sternstein I, Reißmann M & Brockmann G 2006a Detection of novel single-nucleotide polymorphisms (SNPs) in the CYP21 gene and association analysis of two SNPs for CYP21 and ESR2 with litter size in a commercial sow population. *Journal of Animal Breeding and Genetics* **123(5)** 343-348.

Buske B, Sternstein I, Reimann M, Reinecke P & Brockmann G 2006b Analysis of association of GPX5, FUT1 and ESR2 genotypes with litter size in a commercial pig cross population. *Archives of Animal Breeding* **49 (3)** 259-268.

Caetano AR, Johnson RK, Ford JJ & Pomp D 2004 Microarray profiling for differential gene expression in ovaries and ovarian follicles of pigs selected for increased ovulation rate. *Genetics* **168** 1529–1537.

Campbell EM, Nonneman DJ, Kuehn LA & Rohrer GA 2008 Genetic variation in the mannosidase 2B2 gene and its association with ovulation rate in pigs. *Animal Genetics* **39(5)** 515–519.

Cassady JP, Johnson RK, Pomp D, Rohrer GA, Van Vleck LD, Spiegel EK & Gilson K M 2001 Identification of quantitative trait loci affecting reproduction in pigs. *Journal of Animal Science* **79** 623–633.

Cepia S, Reiner G, Bartenschlager H, Moser G & Geldermann H 2003 Linkage and QTL mapping for *Sus scrofa* chromosome X. *Journal of Animal Breeding Genetics* **120(1)** 144-151.

Chakrabarty A, Tranguch S, Daikoku T, Jensen K, Furneaux H & Dey SK 2007 MicroRNA regulation of cyclooxygenase-2 during embryo implantation. *Proceedings of the National Academy of Sciences* **104** 15144-15149.

Chen KF, Huang LS, Li N, Zhang Q, Luo M & Wu CX 2000 The genetic effect of estrogen receptor (ESR) on litter size traits in pig (in Chinese, with English abstract). *Yi Chuan Xue Bao* **27** 853–857.

Christenson RK, Leymaster KA & Young LD 1987 Justification of unilateral hysterectomy-ovariectomy as

a model to evaluate uterine capacity in swine. *Journal of Animal Science* **65** 738-44.

De Koning DJ, Rattink AP, Harlizius B, Groenen MAM, Brascamp EW & Van Arendonk JAM 2001 Detection and characterization of quantitative trait loci for growth and reproduction traits in pigs. *Livestock Production Science* **72** 185–198.

Depuydt J, De Smet ST, Grijspeerdt K & Herman L 1999 Association study of an AvaI and PvuII polymorphism at the porcine estrogen receptor (ESR) gene, with litter size. *Archives of Animal Breeding* **42** 172–174.

Distl O 2007 Mechanisms of regulation of litter size in pigs on the genome level. *Reproduction in Domestic Animals* **42** Supplement 2 10–16.

Dragos-Wendrich M, Moser G, Bartenschlager H, Reiner G & Geldermann H 2003 Linkage and QTL mapping for Sus scrofa chromosome 10. *Journal of Animal Breeding and Genetics* **120** 82-88.

Drogemuller C, Hamann H & Distl O 2001 Candidate gene markers for litter size in different German pig lines. *Journal of Animal Science* **79** 2545–2570.

Du LX, Liu SF, Yan YC & Jiang YL 2002 Research on Alu element inserted mutation in porcine FSH beta subunit gene (in Chinese, with English abstract). *Yi Chuan Xue Bao* **29** 977–982.

Duan R, Pak C & Jin P 2007 Single nucleotide polymorphism associated with mature miR-125a alters the processing of pri-miRNA. *Human Molecular Genetics* **16** 1124-1131.

Fan B, Onteru SK, Nikkilä MT, Stalder KJ & Rothschild MF 2009 Large-scale association studies for reproductive traits in pigs. Poster 535 in *The International Plant & Animal Genome XVII Conference*, January 10-14, Town & Country convention center, San Diego, California, USA.

Foxcroft G, Bee G, Dixon W, Hahn M, Harding J, Patterson J, Putman T, Sarmento S, Smit M, Tse WY & Town S 2007 Consequences of selection for litter size on piglet development. In: *Paradigms of Pig Science*, J.Wiseman, M.A. Varley, S, McOrist and B. Kemp, Eds., Nottingham University Press, Nottingham, 207-229.

Freimer N & Sabatti C 2003 The human phenome project. *Nature Genetics* **34** 15-21.

Gladney CD, Bertani GR, Johnson RK & Pomp D 2004 Evaluation of gene expression in pigs selected for enhanced reproduction using differential display PCR and human microarrays: I. Ovarian follicles. *Journal of Animal Science* **82** 17-31.

Goliasova E & Wolf J 2004 Impact of the ESR gene on litter size and production traits in Czech Large White pigs. *Animal Genetics* **35** 293–297.

Gorodkin J, Cirera S, Hedegaard J, Gilchrist MJ, Panitz F, Jørgensen C, Scheibye-Knudsen K, Arvin T, Lumholdt S, Sawera M, Green T, Nielsen BJ, Havgaard JH, Rosenkilde C, Wang J, Li H, Li R, Liu B, Hu S, Dong W, Li W, Yu J, Wang J, Stærfeldt H, Wernersson R, Madsen LB, Thomsen B, Hornshøj H, Bujie Z, Wang X, Wang X, Bolund L, Brunak S, Yang H, Bendixen C & Fredholm M 2007 Porcine transcriptome analysis based on 97 non-normalized cDNA libraries and assembly of 1,021,891 expressed sequence tags. *Genome Biology* **8(4)** Article R45.

Green JA, Kim JG, Whitworth KM, Agca C & Prather RS 2006 The use of microarrays to define functionally-related genes that are differentially expressed in the cycling pig uterus. In 'Control of Pig Reproduction VII', Eds C.J.Ashworth & R.R.Kraeling, Nottingham University Press, *Society of Reproduction and Fertility Supplement* **62** 163-76.

Green ML, Simmen RC & Simmen FA 1995 Developmental regulation of steroidogenic enzyme gene expression in the periimplantation porcine conceptus: a paracrine role for insulin-like growth factor-I. *Endocrinology* **136** 3961-70.

Groenen MAM, Crooijmans RPMA, Ramos AM, Amaral AJ, Kerstens H, Bendixen C, Hedegaard J, Rohrer G, Smith T, Van Tassel C, Taylor JF, Rothschild M, Zhiliang H, Nonneman D, Beever J, Archibald A, Law A, Milan D, Hansen M & Schook L 2009 Design of the illumina porcine 50k+ SNP iselect™ beadchip and characterization of the porcine hapmap population. Swine Genome sequencing W493 in *The International Plant & Animal Genome XVII Conference*, January 10-14, Town & Country convention center, San Diego, California, USA.

Gross TS, Mirando MA, Young KH, Beers S, Bazer FW & Thatcher WW 1990 Reorientation of prostaglandin F secretion by calcium ionophore, estradiol, and prolactin in perifused porcine endometrium. *Endocrinology* **127** 637–642.

Hasegawa Y, Eto Y, Ibuki Y & Sugino H 1994 Activin as autocrine and paracrine factor in the ovary. *Hormonal Research* **41** 55–62.

Hirooka H, de Koning DJ, Harlizius B, van Arendonk JA, Rattink AP, Groenen MA, Brascamp EW & Bovenhuis H 2001 A whole-genome scan for quantitative trait loci affecting teat number in pigs. *Journal of Animal Science* **79** 2320-2326.

Holl JW, Cassady JP, Pomp D & Johnson RK 2004 A genome scan for quantitative trait loci and imprinted regions affecting reproduction in pigs. *Journal of Animal Science* **82** 3421–3429.

Hong X, Luense LJ, McGinnis LK, Nothnick WB & Christenson LK 2008 Dicer1 is essential for female fertility and normal development of the female reproductive system. *Endocrinology* **149** 6207-6212.

Hu Y, Pan Z, Xu D, Xu Y, Liu H, Huang R & Hu Z 2006 The correlation of reproduction-related gene expression with germ cell number in DM and PLL gilts. Acta Genetica Sinica **33** 800-807.

Huang T, Zhu MJ, Li XY & Zhao SH 2008 Discovery of porcine microRNAs and profiling from skeletal muscle tissues during development. *PLoS ONE* **3** e3225.

Hutvagner G & Simard MJ 2008 Argonaute proteins: Key players in RNA silencing. *Nature Reviews Molecular Cell Biology* **9** 22-32.

Isler BJ, Irvin KM, Neal SM, Moeller SJ, Davis ME & Meeker DL 1999 The effect of the estrogen receptor

gene on litter traits in swine. Research and Reviews published by Department of Animal Science, The Ohio State University, OH, USA 50–53.

Jiang Z, Gibson JP, Archibald AL & Haley CS 2001 The porcine gonadotropin-releasing hormone receptor gene (GNRHR): genomic organization, polymorphisms, and association with the number of corpora lutea. *Genome* **44** 7–12.

Kerstens HHD, Kollers S, Kommadath A, del Rosario M, Dibbits B Kinders SM, Crooijmans RP & Groenen MAM 2009 Mining for single nucleotide polymorphisms in pig genome sequence data. *BMC Genomics* **10** 4.

Kmieć M & Terman A 2004 Prolactin receptor gene polymorphism in polish Landrace boars. *Animal Science Papers and Reports* **22** 529-532.

King AH, Jiang Z, Gibson JP, Haley CS & Archibald AL 2003 Mapping quantitative trait loci affecting female reproductive traits on porcine chromosome 8. *Biology of Reproduction* **68** 2172–2179.

Kim VN 2005 MicroRNA biogenesis: coordinated cropping and dicing. *Nature Reviews Molecular Cell Biology* **6** 376-385.

Kim HJ, Cui XS, Kim EJ, Kim WJ & Kim NH 2006 New porcine microRNA genes found by homology search. *Genome* **49** 1283-1286.

Kowalski AA, Graddy LG, Vale-Cruz DS, Choi I, Katzenellenbogen BS, Simmen FA & Simmen RC 2002 Molecular cloning of porcine estrogen receptor-β complementary DNAs and developmental expression in periimplantation embryos. *Biology of Reproduction* **66** 760-769.

Kuehn LA, Nonneman DJ, Klindt JM & Wise TH 2008 Genetic relationships of body composition, serum leptin, and age at puberty in gilts. *Journal of Animal Science* (In Press).

Lamberson WR 1990 Genetic parameters for reproductive traits. In L.D. Young (Editor), *Genetics of Swine*. Univ. of Nebraska, Lincoln: NC-103 Publication.

Lee SH, Zhao SH, Recknor JC, Nettleton D, Orley S, Kang SK, Lee BC, Hwang WS & Tuggle CK 2005 Transcriptional profiling using a novel cDNA array identifies differential gene expression during porcine embryo elongation. *Molecular Reproduction and Development* **71** 129-139.

Lee SS, Chen Y, Moran C, Stratil A, Reinder G, Bartenschlager H, Moser G & Geldermann H 2003 Linkage and QTL mapping for Sus scrofa chromosome 5. *Journal of Animal Breeding and Genetics* **120** 38-44.

Lee Y, Jeon K, Lee JT, Kim S & Kim VN 2002 MicroRNA maturation: stepwise processing and subcellular localization. *The EMBO Journal* **21** 4663-4670.

Li N, Zhao YF, Xiao L, Zhang FJ & Chen YZ 1998 Candidate gene approach for identification of genetic loci controlling litter size in pigs. In *Proceedings of the Sixth World Congress on Genetics Applied to Livestock Production* **26** 183–190.

Li FE, Mei SQ, Deng CY, Jiang SW, Zuo B, Zheng R, Li JL, Xu DQ, Lei MG & Xiong YZ 2008 Association of a microsatellite flanking FSHB gene with reproductive traits and reproductive tract components in pigs. *Czech Journal of Animal Science* **53** 139–144.

Lin CL, Ponsuksili S, Tholen E, Jennen DGJ, Schellander K & Wimmers K 2006 Candidate gene markers for sperm quality and fertility of boar. *Animal Reproduction Science* **92** 349–363.

Lin HC, Liu GF, Wang AG, Kong LJ, Wang XF & Fu JL 2009 Effect of polymorphism in the leukemia inhibitory factor gene on litter size in Large White pigs. *Molecular Biology Reports* (In Press).

Matousek V, Kernerova N, Kolarıkova O, Krızova H, Urban T & Vrtkova I 2003 Effect of RYR1 and ESR genotypes on the fecundity of sows of Large White breed in elite herds. *Czech Journal of Animal Science* **48** 129–133.

McLaren DG & Bovey M 1992 Genetic influences on reproductive performance. *Veterinary Clinics of North American Food Animal Proceedings* **8** 435-459.

Melville JS, Gibbins AMV, Robinson JAB, Gibson JP, Archibald AL, Haley CS & Jiang Z 2002 A Meishan positive QTL for prolificacy traits found at the NCOA1 locus on SSC 3. Communication 08-26. In *Proceedings of the Seventh World Congress on Genetics Applied to Livestock Production*, August 19-23, 2002, Montpellier, France.

Mendell JT 2005 MicroRNAs: Critical Regulators of development, cellular physiology and malignancy. *Cell Cycle* **4** 9.

Muñoz G, Ovilo C, Estelle J, Silio L, Fernandez A & Rodriguez C 2007 Association with litter size of new polymorphisms on ESR1 and ESR2 genes in a Chinese-European pig line. *Genetics Selection Evolution* **39** 195–206.

Niu BY, Ye LZ, Li FE, Deng CY, Jiang SW, Lei MG & Xiong YZ 2009 Identification of polymorphism and association analysis with reproductive traits in the porcine *RNF4* gene. *Animal Reproduction Science* **110** 283–292.

Nonneman DJ, Wise TH, Ford JJ, Kuehn LA & Rohrer GA 2006 Characterization of the aldo-keto reductase 1C gene cluster on pig chromosome 10: possible associations with reproductive traits. *BMC Veterinary Research* **2** 28.

Ollivier L, Messer LA, Rothschild MF & Legault C 1997 The use of selection experiments for detecting quantitative trait loci with an application to the INRA hyperprolific pig. *Genetics Research* **69** 227–232.

Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grasser FA, van Dyk LF, Ho CK, Shuman S, Chien M, Russo JJ, Ju J, Randall G, Lindenbach BD, Rice CM, Simon V, Ho DD, Zavolan M & Tuschl T 2005 Identification of microRNAs of the herpesvirus family. *Nature Methods* **4** 269-276.

Pope WF 1994 Embryonic mortality in swine. *In Embryonic Mortality in Domestic Species*, pp. 53-78. Eds Zavy MT & Geisert RD, Boca Raton: CRC Press.

Pomp D, Caetano AR, Bertani GR, Gladney CD & Johnson RK 2001 Applying functional genomics research to the study of pig reproduction. *Reproduction Supplement* **58** 277–292.

Pratt SL, Curry E & Barton HM 2008. Detection of microRNA in porcine somatic and reproductive tissues. *Journal of Animal Science* **86** *E-Supplement* 2 457.

Rajewsky N 2006 MicroRNA target predictions in animals. *Nature Genetics* **38** Supplement S8-S13.

Rathje TA, Rohrer GA & Johnson RK 1997 Evidence for quantitative trait loci affecting ovulation rate in pigs. *Journal of Animal Science* **75** 1486–1494.

Ren DR, Ren J, Xing YY, Guo YM, Wu YB, Yang GC, Mao HR & Huang LS 2008 A genome scan for quantitative trait loci affecting male reproductive traits in a White Duroc × Chinese Erhualian resource population. *Journal of Animal Science* (In Press).

Roberts RM & Bazer FW 1980 The properties, function and control of synthesis of uteroferrin, the purple protein of the pig uterus. In: *Steroid Induced Proteins*. pp 133. M. Beato (Ed.), Amsterdam, The Netherlands: Elsevier-North Holland.

Roberts RM, Xie S & Trout WE 1993 Embryo-uterine interaction in pigs during week 2 of pregnancy. *Journal of Reproduction and Fertility Supplement* **48** 171–186.

Rodriguez C, Tomas A, Alves E, Ramirez O, Arque M, Muñoz G, Barragan C, Varona L, Silio L, Amills M & Noguera JL 2005 QTL mapping for teat number in an Iberian-Meishan pig intercross. *Animal Genetics* **36** 490-496.

Rohrer GA, Ford JJ, Wise TH, Vallet JL & Christenson RK 1999 Identification of quantitative trait loci affecting female reproductive traits in a multi generation meishan-white composite pigs population. *Journal of Animal Science* **77** 1385–1391.

Rohrer GA 2000 Identification of quantitative loci affecting birth characters and accumulation of back fat and weight in a Meishan-White composite resource population. *Journal of Animal Science* **78** 2543–2547.

Rohrer GA, Wise TH, Lunstra DD & Ford JJ 2001 Identification of genomic regions controlling plasma FSH concentrations in Meishan-White Composite boars. *Physiological Genomics* **6** 145–151.

Ross JW, Ashworth MD, Hurst AG, Malayer JR & Geisert RD 2003a Analysis and Characterization of differential gene expression during rapid trophoblastic elongation in the pig using suppression subtractive hybridization. *Reproduction Biology and Endocrinology* **1** 23.

Ross JW, Malayer JR, Ritchey JW & Geisert RD 2003b Characterization of the interleukin-1β system during porcine trophoblastic elongation and early placental attachment. *Biology of Reproduction* **69** 1251-1259.

Ross JW, Ashworth MD, White FJ, Johnson GA, Ayoubi PJ, DeSilva U, Whitworth KM, Prather RS & Geisert RD 2007 Premature estrogen exposure alters endometrial gene expression to disrupt pregnancy in the pig. *Endocrinology* **148** 4761-4773.

Ross JW, Ashworth MD, Stein DR, Couture OP, Tuggle CK & Geisert RD 2009 Identification of differential gene expression during porcine conceptus rapid trophoblastic elongation and attachment to the uterine luminal epithelium *Physiological Genomics* **36** 140-148.

Rothschild MF, Jacobson C, Vaske D, Tuggle C, Wang L, Short T, Eckardt G, Sasaki S, Vincent A, McLaren D, Southwood O, Van der Steen H, Mileham A & Plastow G 1996 The estrogen receptor locus is associated with a major gene influencing litter size in pigs. *Proceedings of the National Academy of Sciences of the United States of America* **93** 201-205.

Rothschild MF, Messer LA, Day A, Wahs R, Short T, Southwood O & Plastow G 2000 Investigation of the retinol binding protein (RBP4) gene as a candidate gene for litter size in the pig. *Mammalian Genome* **11** 75–77.

Sato S, Oyamada Y, Atsuji K, Nade T, Shin-ichi S, Kobayashi E, Mitsuhashi T, Nirasawa K, Komatsuda A, Saito Y, Terai S, Hayashi T & Sugimoto Y 2003 Quantitative trait loci analysis for growth and carcass traits in a Meishan × Duroc F2 resource population. *Journal of Animal Science* **81** 2938–2949.

Sato S, Atsuji K, Saito N, Okitsu M, Sato S, Komatsuda A, Mitsuhashi T, Nirasawa K, Hayashi T, Sugimoto Y & Kobayashi E 2006 Identification of quantitative trait loci affecting corpora lutea and number of teats in a Meishan X Duroc F2 resource population. *Journal of Animal Science* **84** 2895-2901.

Short TH, Rothschild MF, Southwood OI, McLaren DG, de Vries A, Van der Steen H, Eckardt GR, Tuggle CK, Helm J, Vaske DA, Mileham AJ & Plastow GS 1997 Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. *Journal of Animal Science* **75** 3138–3142.

Smith TPL, Fahrenkrug SC, Rohrer GA, Simmen FA, Rexroad CE & Keele JW 2001 Mapping of expressed sequence tags from a porcine early embryonic cDNA library *Animal Genetics* **32** 66-72.

Solberg TR, Sonesson AK, Woolliams JA & Meuwissen THE 2008 Genomic selection using different marker types and densities. *Journal of Animal Science* **86** 2447-2454.

Spotter A & Distl O 2006 Genetic approaches to the improvement of fertility traits in the pig. *The Veterinary Journal* **172** 234–247.

Spotter A, Muller S, Hamann H & Distl O 2009 Effect of polymorphisms in the genes for LIF and RBP4 on litter size in two German pig lines. *Reproduction in Domestic Animals (In Press).*

Stewart JD, Lou Y, Squires EJ & Coussens PM 2005 Using human microarrays to identify differentially expressed genes associated with increased steroidogenesis in boars. *Animal Biotechnology* **16** 139-51.

Tang F, Kaneda M, O'Carroll, Hajkova P, Barton SC, Sun YA, Lee C, Tarakhovsky A, Lao K & Surani MA 2007 Maternal microRNAs are essential for mouse zygotic development. *Genes and Development* **21** 644-648.

Terman A, Kmieć M & Polasik D 2006 Estrogen receptor gene (*ESR*) and semen characteristics of boars. *Archives of Animal Breeding* **49** 71-76.

Tribout T, Iannuccelli N, Druet T, Gilbert H, Riquet J, Gueblez R, Mercat MJ, Bidanel JP, Milan D & Le

Roy P 2008 Detection of quantitative trait loci for reproduction and production traits in Large White and French Landrace pig populations. *Genetics Selection Evolution* **40** 61-78.

Trout WE, McDonnell JJ, Kramer KK, Baumbach GA & Roberts RM 1991 The retinol-binding protein of the expanding pig blastocyst: molecular cloning and expression in trophectoderm and embryonic disk. *Molecular Endocrinology* **5** 1533–1540.

Tuggle CK, Green JA, Fitzsimmons C, Woods R, Prather RS, Malchenko S, Soares MB, Kucaba T, Crouch K, Smith C, Tack D, Robinson N, O'Leary B, Scheetz T, Casavant T, Pomp D, Edeal JB, Zhang Y, Rothschild MF, Garwood K & Beavis W 2003 EST-based gene discovery in pig: virtual expression patterns and comparative mapping to human. *Mammalian Genome* **14** 565-579.

Vallet JL & Christenson RK 1993 Uterine space affects placental protein secretion in swine. *Biology of Reproduction* **48** 575-584.

Vallet JL, Freking BA, Leymaster KA & Christenson RK 2005a Allelic variation in the erythropoietin receptor gene is associated with uterine capacity and litter size in swine. *Animal Genetics* **36** 97–103.

Vallet JL, Freking BA, Leymaster KA & Christenson RK 2005b Allelic variation in the secreted folate binding protein gene is associated with uterine capacity in swine. *Journal of Animal Science* **83** 1860-1867.

Van Rens BTTM, De Groot PN & Van Der Lende T 2002 The effect of estrogen receptor genotype on litter size and placental traits at term in F2 crossbred gilts. *Theriogenology* **57** 1635–1649.

Van Rens BTTM & Van der Lende T 2002 Litter size and piglet traits of gilts with different prolactin receptor genotypes. *Theriogenology* **57** 883–893.

Van Rens BTTM, Evans GJ & Van der Lende T 2003 Components of litter size in gilts with different prolactin receptor genotypes. *Theriogenology* **59** 915–926.

Vingborg RKK, Gregersen VR, Høj A, Panitz F, Zhan B, Hornshøj H, Sørensen KK & Bendixen C 2008 Whole Porcine genome linkage map based on the 7K porcine SNP chip. In *Proceedings of International Society for Animal Genetics Poster* 2081.

Vincent AL, Evans G, Short TH, Southwood OI, Plastow GS, Tuggle CK & Rothschild MF 1998 The prolactin receptor gene is associated with increased litter size in pigs. In *Proceedings of the Sixth World Congress on Genetics Applied to Livestock Production* **27** 15–18.

Wada Y, Akita T, Awata T, Furukawa T, Sugai N, Inage Y, Ishii K, Ito Y, Kobayashi E, Kusumoto H, Matsumoto T, Mikawa S, Miyake M, Murase A, Shimanuki S, Sugiyama T, Uchida Y, Yanai S & Yasue H 2000 Quantitative trait loci (QTL) analysis in a Meishan × Göttingen cross population. *Animal Genetics* **31** 376–384.

Wernersson R, Schierup MH, Jørgensen FG, Gorodkin J, Panitz F, Stærfeldt H, Christensen OF, Mailund T, Hornshøj H, Klein A, Wang J, Liu B, Hu S, Dong W, Li W, Wong GKS, Yu J, Wang J, Bendixen C, Fredholm M, Brunak S, Yang H & Bolund L 2005 Pigs in sequence space: A 0.66X coverage pig genome survey based on shotgun sequencing. *BMC Genomics* **6** 70.

Wesolowski SR, Raney NE & Ernst CW 2004 Developmental changes in the fetal pig transcriptome. *Physiological Genomics* **16** 268–274.

Whitworth KM, Agca C, Kim JG, Patel RV, Springer GK, Bivens NJ, Forrester LJ, Mathialagan N, Green JA & Prather RS 2005 Transcriptional profiling of pig embryogenesis by using a 15-K member unigene set specific for pig reproductive tissues and embryos. *Biology of Reproduction* **72** 1437-1451.

Wilkie PJ, Paszek AA, Beattie CW, Alexander LJ, Wheeler MB & Schook LB 1999 A genomic scan of porcine reproductive traits reveals possible quantitative trait loci (QTL) for number of corpora lutea. *Mammalian Genome* **10** 573–578.

Wilson ME, Sonstegard TS, Smith TP, Fahrenkrug SC & Ford SP 2000 Differential gene expression during elongation in the preimplantation pig embryo. *Genesis* **26** 9-14.

Yasue H, Mikawa S, Uenishi H & Wada Y 1999 Analysis of allele segregation distortion in a pig resource family. *Animal Biotechnology* **10** 147–152.

Yelich JV, Pomp D & Geisert RD 1997a Detection of transcripts for retinoic acid receptors, retinol binding protein, and transforming growth factors during rapid trophoblastic elongation in the porcine blastocyst. *Biology of Reproduction* **57** 286-294.

Yelich JV, Pomp D & Geisert RD 1997b Ontogeny of elongation and gene expression in the early developing porcine conceptus. *Biology of Reproduction* **57** 1256-1265.

Yue G, Schröffel Jr J, Moser G, Bartenschlager H, Reiner G & Geldermann H 2003 Linkage and QTL mapping for Sus scrofa chromosome 12. *Journal of Animal Breeding and Genetics* **120(1)** 95-102.

Zhao YF, Li N, Xiao L, Cao GS, Chen YZ, Zhang S, Chen YF, Wu CX, Zhang J.S, Sun SQ & Xu XQ 1999 Inserting mutation of retroposon into porcine FSH-β gene and its association with litter size in pigs. *Science in China Series* C **29** 81–86.