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: mrothsc@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, Droegenmuller 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 | 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 (Aval) and ESR2 (Pvull) 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| ¾ 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      | Melville 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                     |
|                      | 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   | Pvull site   | Intron                | M x SL and LW LW | Rothschild et al. 1996           |
|                      |                   |     |              |                       | LR          | Short et al. 1997                 |
|                      |                   |     |              |                       | M x LW      | Chen et al. 2000                  |
|                      |                   |     |              |                       | LW          | Matousek et al. 2003              |
|                      |                   |     |              |                       | Czech LW    | Goliasova & Wolf 2004             |
|                      |                   |     | C/T          | Exon 5                | M x LW      | Muñoz et al. 2007                 |
|                      |                   |     |              |                       | LW          | Vincent et al. 1998               |
|                      |                   |     |              |                       | SL          | Drogeymuller et al. 2001          |
|                      |                   |     |              |                       | M x LW      | Van Rens & Vander lende 2002      |
|                      |                   |     |              |                       |             |                                     |
|                      | FSHB**            | 2   | Intron       | Y x EL                | Y and Y     | Zhao et al. 1999                  |
|                      |                   |     |              |                       | LP, DP and LR | Du et al. 2002                   |
|                      |                   |     | Alu site     | 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   | -            | Intron 3              | GL          | Spotter et al. 2009              |
|                      |                   |     | C/T          | Intron 1              | LW          | Lin et al. 2009                   |
|                      | FUT1              |     | HhaI         | -                     | (LW x LR) x Li | Buske et al. 2006b              |
|                      | RNF4              | 6   | C/T          | Intron 5              | CQ          | Niu et al. 2009                   |

**Table entries:**
- **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
- **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
- **DW:** 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

**References:**
- **Van Rens & Vander Lende 2002**
- **Nonneman et al. 2006**
- **Kuehn et al. 2008**
- **Jiang et al. 2001**
- **Melville et al. 2002**
- **Campbell et al. 2008**
- **Van Rens et al. 2003**
- **Li et al. 2008**
- **Vallet et al. 2005a**
- **Vallet et al. 2005b**
- **Rothschild et al. 1996**
- **Short et al. 1997**
- **Chen et al. 2000**
- **Van Rens et al. 2002**
- **Matousek et al. 2003**
- **Goliasova & Wolf 2004**
- **Muñoz et al. 2007**
- **Valent et al. 1998**
- **Drogeymuller et al. 2001**
- **Van Rens & Vander lende 2002**
- **Li et al. 1998**
- **Zhao et al. 1999**
- **Du et al. 2002**
- **Li et al. 2008**
- **Olliver et al. 1997**
- **Rothschild et al. 2000**
- **Spotter et al. 2009**
- **Buske et al. 2005**
- **Spotter et al. 2009**
- **Buske et al. 2006b**
- **Niu et al. 2009**

*Some of the mentioned gene information can be seen in [http://www.animalgenome.org/QTLdb/pig.html](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 $0.52 \pm 0.30$ pigs per litter in a Large White Hyperprolific line and $0.45 \pm 0.43$ 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-1 ($FSHB$) 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, $COL1A2$, $NST$, $ADAM12$, $WARS2$, $DKFZ$ and $LRP5$ were significantly ($P < 0.05$) associated with both TNB and NBA while $COL11A2$, $CALCR$ 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, $CASR$, $ESR2$, $WARS2$, $NST$, $IFNy$ 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 (FSTI) 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 NR4A1 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 CMS417 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, steriodogenic 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 IGF1 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://genomesnet.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 (CYP5B5, 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
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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.

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