Large-Scale Metadata Analysis of Ovary Based Multi-Omics Datasets for Understanding the Genes Regulating Litter Size

CURRENT STATUS: UNDER REVIEW

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DOI: 10.21203/rs.3.rs-22487/v1

SUBJECT AREAS
Epigenetics & Genomics

KEYWORDS
Litter size/Fecundity/Prolificacy, Transcriptome, Metadata Analysis, Fertilization, Ovarian steroidogenesis, Ovarian follicular development
Abstract
Background: Litter size is a very important production index in the livestock industry, which is controlled by various complex physiological processes. To understand and reveal the common gene expression patterns involved in controlling prolificacy, we have performed a large-scale metadata analysis of five genome-wide transcriptome datasets of pig and sheep ovary samples obtained from high and low litter groups, respectively. We analyzed separately each transcriptome dataset using GeneSpring v14.8 software by implementing standard, generic analysis pipelines and further compared the list of most significant and differentially expressed genes obtained from each dataset to identify genes that are found to be common and significant across all the studies.

Results: We have observed a total of 62 differentially expressed genes common among more than two gene expression datasets. The KEGG pathway analysis of most significant genes has shown that they are involved in metabolism, the biosynthesis of lipids, cholesterol and steroid hormones, immune system, cell growth and death, cancer-related pathways and signal transduction pathways. Of these 62 genes, we further narrowed the list to the 25 most significant genes by focusing on the ones with fold change >1.5 and p<0.05. These genes are CYP11A1, HSD17B2, STAR, SCARB1, IGSF8, MSMB, SERPINA1, FAM46C, HEXA, PTTG1, TIMP1, FAM167B, CCNG1, FAXDC2, HMGCS1, L2HGDH, Lipin1, MME, MSMO1, PARM1, PTGFR, SLC22A4, SLC35F5, CCNA2, CENPU, CEP55, RASSF2, and SLC16A3.

Conclusions: Interestingly, comparing the list of genes with the list of genes obtained from our literature search analysis, we found only three genes in common. These genes are HEXA, PTTG1, and TIMP1. Our finding points to the potential of a few genes that may be important for ovarian follicular development and oocyte quality. Future studies revealing the function of these genes will further our understanding of how litter size is controlled in the ovary while also providing insight on genetic selection of high litter gilts.

Background
Increasing litter size and raising healthy, quality animals are crucial factors in the livestock industry [1]. Understanding the genetic traits controlling the reproductive physiology has become an important research topic in the last decade [1]. Litter size is a complex trait in multiparous mammals.
It is dependent on ovarian development, ovulation rate, placental health, uterine capacity, embryonic and foetal survival rate, many of which affect the availability and quality of the oocyte [2, 3]. Pigs (Sus scrofa) are one of the most important livestock. In addition, due to its similarities with humans, pigs are highly used as a model organism in human medical research. For more than 150,000 years, pigs have been considered one of the most highly divergent species. To date, 12 Sus scrofa subspecies have been reported worldwide with 142 different breeds, respectively. Although they have different meat production performance, previous studies have reported the Chinese Meishan breed of having an average litter size of 14.3 piglets, the Iberian breed having an average of 7 piglets per litter and the Berkshire breed with an average of about 8.9 piglets per litter [2]. Thus, it is desirable to develop a highly reproductive breeding line using an efficient set of prolificacy-related genes.

Metadata is the data associated with a corresponding article which is generally provided as supporting or additional data required for drawing valuable conclusions. Development of public repositories such as NCBI Gene Expression Omnibus (NCBI GEO), ArrayExpress, and many more are strongly encouraging metadata analysis by hosting large-scale genomic, transcriptomic and proteomic datasets obtained from various research platforms and research groups around the world. Recent genomic and transcriptomic studies have revealed the involvement of various genes in controlling litter size. Amanda et al. (2011) performed a transcriptome analysis of pregnant pig ovaries exhibiting high and low litter size [4]. This study has majorly reported genes encoding the immune system, maternal homeostasis and fatty acid metabolic enzymes involved in the steroidogenesis pathway [4]. Amanda et al. (2011) have also identified 27 differentially expressed genes which were found to be co-localized with quantitative trait locus (QTL) of litter size traits [4]. Another study compared gene expression patterns of ovarian follicles of Chinese Taihu (highly prolific sows) and large white low litter sows, and identified 133 differentially expressed genes that function in development and signal transduction processes [5]. Zhang et al. (2015) also performed a genome-wide expression analysis to reveal the gene expression differences between high and low litter size in Yorkshire pigs [6]. A similar study that was performed in sheep reported a total of 1252 genes that were differentially expressed in Hertian sheep (low litter) compared to Qira sheep (high prolificacy).
The KEGG pathway analysis of these genes found them to be associated with steroid biosynthesis, steroid hormone biosynthesis, TGF-β, insulin, Wnt, Notch, and other signaling pathways [7]. These studies provide first-line screening data on litter size-related gene expression in various breeds and conditions. However, further analysis is required to extract the most meaningful information from this vast amount of data. We hypothesize that large-scale metadata analysis of these datasets will allow comparison of ovarian litter size-related gene expression across different species, breed and conditions to ultimately help identify commonly observed genes among these datasets. This may provide insight on potential novel gene(s) and/or pathway(s) to focus on for understanding regulation of ovarian follicular development and competent oocyte availability.

Methods

Data Retrieval: We have retrieved six pig gene expression datasets based on ovary samples of high and low litter experimental groups from NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/). The gene expression datasets retrieved from NCBI GEO were a) GSE21383, b) GSE23985, c) Zhang et al. [2015] e) Chen et al. [2015] (Table-1). We have also retrieved the list of genes and proteins which were found to be involved in regulating the litter size in pig, mouse and sheep from UniProt and NCBI databases, respectively. A total of 96 genes related to litter size distributed among Sus scrofa, Capra hircus, Mus musculus, Ovis aries were retrieved from UniProt databases. From Harmonizome databases, (https://amp.pharm.mssm.edu/Harmonizome/) we have retrieved three gene sets: abnormal (270 genes), increased (11 genes) and decreased (260 genes) litter size gene sets. We further compared all the retrieved genes above and tried to retrieve the common genes among all the gene sets.

Data Analysis: We have performed metadata analysis of 5 gene expression datasets: GSE21383 [4], GSE23985 [5], Zhang et al., [2015] [6] and Chen et al., [2015] [7]. All the datasets mentioned above were retrieved based on their experimental study and design as they focused on understanding the differentially expressed genes in ovarian samples grouped as high and low litter experimental subjects of pigs and sheeps, respectively. The GSE21383 and GSE23985 datasets were imported into GeneSpring® version 14.8 software using “Import experiments from NCBI GEO” wizard. The samples
were log transformed, baseline transformed and normalized using the RMA normalization method. The experimental grouping information obtained from the available metadata was used to group the samples. All the samples were then subjected to quality control analysis using a filter based on expression values using the standard (100- higher percentile and 20- lower percentile). After quality control analysis, we performed a statistical analysis using a moderated t-test with asymptotic p-value method and Benjamini Hochberg multiple testing correction method for p-value correction. Statistically significant lists of genes obtained were tested for fold change calculation. The Zhang et al. (2015) and Chen et al. (2015) transcriptome datasets were imported into GeneSpring® using generic single-color experiments without normalization, baseline transformation or log transformation. The samples were grouped using the experimental design retrieved from the corresponding literature. The grouped conditions were subjected to fold change and volcano plot analysis using the moderate t-test with p-value correction (Benjamini Hochberg MTC correction method). Genes exhibiting FC > 2.0 were retrieved for further analysis. All the differentially expressed lists of genes obtained from all the above experiments were compared using the Venny and Jvenn web applications.

Pathway Enrichment Analysis using KOBAS: As obtained from our metadata analysis, the five datasets showed significant and differentially expressed genes and were further subjected to pathway enrichment analysis using KOBAS web software (http://kobas.cbi.pku.edu.cn/kobas3) [8]. We have used the “Gene List Enrichment” function and have selected the KEGG and Reactome pathway databases for the KOBAS analysis. The genes in the KEGG and Reactome pathways were enriched using the hypergeometric/Fisher’s exact statistical test method and the Benjamini Hochberg False Discovery Rate correction method, respectively.

Results
Litter size controlling genes from literature search: To understand the current gene networks involved in regulation of litter size, we have retrieved and categorized all the genes obtained from the UniProt and NCBI databases based on their source organism. Our search with the term “litter size” in UniProt databases resulted in a list of 96 genes (32- S. scrofa, 32- C. hircus, 16- M. musculus and 16- O. aries,
respectively). Similarly, our search within NCBI databases has resulted in a total of 288 genes (163- S. scrofa, 69- C. hircus, 13- M. musculus, 1- R. norvegicus and 39- O. aries, respectively). A recent study conducted by Lai et al. (2016) reported the genes controlling the fecundity in dairy goat, which include gonadotropins, ovarian steroid hormones, luteinizing hormones, follicle-stimulating hormone, 17β-estradiol (E2), progesterone, activin A, etc. [9]. Earlier studies have also reported that inactive homozygous mutations in transforming growth factor β, BMP15, and GDF9 superfamily genes leads to decreased ovulation rates which further leads to sterility in dairy goat [10, 11] (Supplementary Information-S1). Previous studies have reported that proteins such as insulin growth factor 1 (IGF1), oviductin (OVGP1), tissue inhibitor of metalloprotein (TIMP1), uteroglobin, leptin and plasminogen activator inhibitor 1 (PAI1) were reported to contribute to sperm capacitation, gamete fertilization and the facilitation of the entrance of the embryo into the uterus [12–17]. The genetic polymorphisms of the steroid hormone-related genes such as progesterone receptor (PR), estrogen receptor (ER), and steroid receptor co-activators (SRC1 and SRC2) were found to be associated with the risk of implantation failure [18–21]. Similarly, prostaglandins play an important role in various reproductive processes such as ovulation and implantation [22, 23]. We have also retrieved the MPO gene-phenotype associated gene sets [24, 25] encoding for abnormal, increased and decreased litter size from Harmonizome databases [26]. We have retrieved a total of 270 genes, which were common among the abnormal, increased and decreased litter size gene sets. Interestingly, when we compared the list of litter size-regulating genes of C. hircus, S. scrofa and M. musculus retrieved from UniProt and NCBI databases, 26 genes were found to be commonly identified in these studies. These genes are: GDF9, P4HB, MC1R, PPWD1, DPYSL2, HSPD1, NRP2, RBP4, YWHAZ, PDC, KLF7, HAT1, NR4A2, FLJ11457, TRIP12, FSHR, MMP9, EPHA4, KIT, KITLG, ESR2, FST, NOS2, ROPN1, SLC9A3R1, and SOD1.

Comparison of differentially expressed genes across databases: We have retrieved 5 transcriptome datasets focused on ovarian samples of high and low litter size, four of which come from pig experimental groups while one dataset comes from sheep. The transcriptome datasets of pigs GSE21383 and GSE23985 were developed using Affymetrix Porcine Genechip, comprised of 24,123 probe sets representing 124 controls and 23,999 transcripts. Zhang et al. [2015] developed the
transcriptome using Illumina HiSeq 2000 platform. The datasets GSE21383 and GSE23985 were analyzed using standard gene expression analysis pipelines of GeneSpring. The data was normalized, log transformed and filtered to remove low quality probe sets. The total number of significant differentially expressed genes obtained after statistical analysis (p-value: <0.05) was: 1191 (GSE21383) and 988 (GSE23985). We have sorted and retrieved the list of significantly expressed genes from the corresponding supplementary information of the dataset from Zhang et al. [2015] (Fig. 1). From the list of significantly expressed genes, we further narrowed down the list to include only genes with a fold change FC > 1.5. The distribution of those highly significant and differentially expressed genes is shown in Fig. 1. In addition, the significantly differentially expressed genes were further listed as up- or down-regulated genes. Their full gene names and fold change information are detailed in Table-2A and Table-2B, respectively.

**Pathway Enrichment Analysis**

We have performed the pathway enrichment analysis separately using the up- and down-regulated list of genes obtained from the metadata analysis. A total of 83 and 69 pathways were obtained for the up- and down-regulated genes, out of which 38 and 49 pathways were found to be significant (p-value < 0.05), respectively (Table 4A and 4B). Results obtained from our analysis have shown that the top significant enriched pathways obtained from up-regulated genes were metabolic pathways involving ovarian steroidogenesis, cortisol synthesis secretion, cholesterol biosynthesis, aldosterone synthesis secretion, steroid biosynthesis, butanoate metabolism, and the metabolism of steroids among other metabolic pathways. On the other hand, the top significant enriched pathways obtained from down-regulated genes were central carbon metabolism in cancer, proximal tubule bicarbonate reclamation, histidine metabolism, the Hippo signaling pathway (occurring in multiple species), bladder cancer, other pathways in cancer and the Hedgehog signaling pathway, respectively.

**Discussion**

In this study, we have retrieved a list of genes found to be involved in both positive and negative regulation of litter size in *S. scrofa, O. aries, Mus musculus, Rattus norvegicus* and *Capra hircus* from UniProt and NCBI repositories. Earlier studies have reported that the reproductive system of animals...
is regulated by an arsenal of hormones [5]. The litter size gene list retrieved from public repositories majorly represented genes encoding for hormones. Interestingly, when we compared the list of litter size-regulating genes of \textit{C. hircus}, \textit{S. scrofa} and \textit{M. musculus} retrieved from public repositories, it resulted in a total of 26 genes among the compared list of genes. Studies conducted in the past have mainly reported the involvement of the commonly observed genes mentioned above to be involved in regulation of litter size. We have also retrieved and compared the decreased, increased and abnormal litter size gene sets from Harmonizome databases. In comparison, all of the genes obtained from our literature analysis and differentially expressed genes obtained from our metadata analysis have shown 3 genes in common, which are \textit{HEXA}, \textit{PTTG1} and \textit{TIMP1}. Hexosaminidase-α (\textit{HEXA}) and its isozymes \textit{HEXB} and \textit{HEXS} together have the capacity to breakdown a variety of substrates such as G_{M2} gangliosides, glycolipids, glycosaminoglycans and glycoproteins, which for the most part contain β-linked N-acetylglucosamine and N-acetyl galactosamine residues [27]. According to Juneja (2002), the hexosaminidase knockout mice exhibited reduced fertility at a young age, which progressively decreased with increased age and ultimately lead to infertility [27]. However, the \textit{HEXB} knockout mice were found to develop normally and be fertile during the early stages of development. These results indicate that hexosaminidase is not required for sperm-ovum interactions and fertilization [27]. \textit{PTTG1} gene encodes for pituitary tumor-transforming gene, an oncogene which is found to play a key role in cell cycle regulation and sister chromatid separation [28]. Previous studies have reported that \textit{PTTG1} is highly expressed in various tumors, especially ovarian tumorigenesis. However, the exact involvement of \textit{PTTG1} in fertilization and with respect to litter size has not yet been reported. \textit{TIMP1} gene encodes for tissue inhibitor of metalloproteinase, it is found to play a key role in ovulation. Rosewell \textit{et al.} (2013) demonstrated that gonadotropin-induced increase in TIMP protein in human periovulatory follicles could help regulate the follicular extracellular matrix and other TIMP associated processes with ovulation with an increase in TIMP inhibitor [29].

\textbf{Biosynthesis and Metabolism of Lipids, Cholesterol and Steroidogenesis}

Genes involved in steroid biogenesis and ovarian steroidogenesis pathways such as \textit{Cyp11A1}, \textit{Msmo1}, \textit{Star}, \textit{Dhrs7/Hsd17b7}, and \textit{Hmgcs1} were up-regulated in high litter group samples. \textit{Cyp11A1}, also
known as cholesterol side-chain cleavage enzyme, is a mitochondrial enzyme which helps with the biosynthesis of various steroid hormones [30]. According to Gharani et al. (1997), allelic variants of Cyp11a1 might cause hyperadrenoeaemia which may further lead to changes in ovarian morphology [31]. This study also reported that Cyp11a plays a significant role in the progression of hirsutism in polycystic ovary syndrome conditions [31]. Msms1 gene is also involved in the biosynthesis of steroids and the production of zymosterol from lanosterol. The gene expression studies conducted by Dessie et al. (2015) have reported that the down-regulation of genes involved in the biosynthesis and metabolism of steroids, cholesterol and lipids in PCOS model rats (rats subjected to 5α-dihydrotestosterone (DHT)) was found to mimic a hyperandrogenic condition [32]. Dessie et al. (2015) also reported that genes involved in the synthesis of steroid hormones, such as Cyp11A1, Star, Dhrs7/Hsd17b7, and Hmgcs1 were significantly repressed in PCOS model rats but expressed within the control group [32]. Thus, expression of genes involved in the biosynthesis and metabolism of steroids, lipids and cholesterol in high litter group samples supports their involvement in fertility.

Similarly, we also observed various other genes involved in the biosynthesis and metabolism of lipids, such as Aldh1a1, Dhrs7, Faxdc2, HEXA, and Lipin1, which were up regulated in high litter group samples. Bowles et al. (2016) reported that Aldh1a1 expressed in fetal ovaries played an important role by providing retinoic acid, and that a lack of Aldh1a1 in fetal ovaries lead to delayed germ cell meiosis [33]. Dhrs7, also known as 17β-hydroxysteroid dehydrogenase, is involved in the conversion of estrone (E1) to estradiol (E2) and is highly expressed in the ovaries of pregnant animals. According to Pasi et al. (2000), spatial and temporal expression of Dhrs7/Hsd17b7 in the uterus indicates that locally-produced estradiol plays a crucial role in implantation [34]. Faxdc2 (fatty acid hydroxylase domain 2), which is involved in cholesterol synthesis, had megakaryopoiesis highly up-regulated in all high litter samples [35]. Lipin1 is a central metabolic regulator found to play an important role in lipid metabolism, especially the glycerolipid and glycerophospholipid metabolic pathways. Lipin1 gene and its polymorphisms were also found to be involved in the development of PCOS. Gowri et al. (2007) reported that Lipin1 is down-regulated by estradiol in the uterus and liver, and that the expression levels of Lipin1 is low and compromised in mouse models with diabetes and/or reduced fertility [36].
Earlier studies have reported that Lipin1 deficient mice (fld/fld) have less body fat and exhibit symptoms of diabetes and impaired fertility [37–39]. Regulation of Lipin1 by estrogen plays a critical role between reproduction, growth and metabolism [40].

**Genes exhibiting a role in the progression of polycystic ovary syndrome**

The above obtained list of differentially expressed genes were found to be play a minor to major role in the development and progression of PCOS. Previous studies have reported that genes encoding for CYP11A1, HSD17B7, STAR, INHA, PARM1, SCARB1, PTGFR, SLC22A4, and SLC35F5 were found to take part in the development and progression of PCOS. The gene expression studies conducted by Dessie et al. (2015) reported that down-regulation of genes involved in the biosynthesis and metabolism of steroids, cholesterol and lipids in PCOS model rats (rats subjected to 5α-dihydrotestosterone (DHT)) was found to mimic a hyperandrogenic condition [32]. Dessie et al. (2015) also reported that genes involved in the synthesis of steroid hormones such as Cyp11A1, Star, Dhrs7/Hsd17b7, and Hmgcs1 were significantly repressed in PCOS model rats but expressed in the control group [32]. Francisco et al. (2009) hypothesized that haptoglobin (HP2) polymorphisms may contribute to the conditions, such as oxidative stress and chronic inflammation, which are associated with polycystic ovary syndrome, obesity and glucose tolerance [41]. All the genes mentioned above that are involved in the metabolism of steroids, cholesterol, and lipids were found to be significantly down regulated in low litter group samples.

**Genes involved in the immune response, cancer, cell growth and death-related pathways**

Genes involved in immune responsive pathways such as CD55 (decay-accelerating factor for complement-55) and OAS1 (2'-5'-oligoadenylate synthetase 1) were highly up-regulated in high litter group samples. OAS1D is a cytoplasmic protein expressed in growing oocytes and early embryos (Wei et al., 2005). Mutant mice without OAS1D exhibited reduced fertility as they possessed defects in ovarian follicle development [42]. For the first time, Wei et al. (2005) revealed that OAS1D controls female fertility in mice, and that OAS1D non-enzymatic OAS1 proteins may suppress IFN/OAS/RNaseL and protect oocytes and early embryos from cell death [42]. Decay-accelerating factor (CD55) is a complement regulatory protein which protects the host cells through the innate immune response.
The function of CD55 in reproduction has been hypothesized based on its up-regulation in the fetoplacental trophoblast, which protects the fetus from maternal complement injury [43]. Kim et al. (2017) reported that CD55 was down regulated in the endometrium of subjects with repeated implantation failure. Similarly, genes encoding for CCNG1 (participates in p53-dependent G1–S and G2 checkpoints and might function as an oncogenic protein in the initiation and metastasis of ovarian carcinoma) [44] are PTGFR and ADHFE1 (breast cancer oncogene which induces metabolic re-programming) [45]. All the genes above were up-regulated in high litter group samples, whereas genes encoding for CCNA2, GLI1, and SLC16A3 involved in cancer progression were up-regulated in low litter group samples.

**Potential genes involved in prolificacy:** The quantitative trait locus (QTL) studies conducted in the past [4, 46] have tried to understand the list of candidate genes affecting litter size in pigs. These studies have reported a list of 18 genes as significant QTL: CYP19A1, C5, PTGDS, NOV, TST, KRT8, HP, CES1, SULT2A1, CD83, FKBP5, DHRS4, SERPINA1/3, FGA/B/G, SPP1, HPX, MSRB2, SLC16A3, SPHK1, VTN, AHSG, OAS1, RBP4, CYP2E1, NCKAP5, EPHA4, and HOXA9 [4, 46]. Amanda et al. (2011) reported that genes encoding for OAS1, CD55, and SERPINA1 were up-regulated in high litter samples, and genes encoding for FAM46C, SPP1, RBP4, TST, and VTN were highly up-regulated in low litter group samples [4]. Results obtained were in accordance with the findings of Amanda et al. (2015) [4], except genes encoding for FAM46C and FAM167B were found to be differentially up-regulated in high litter groups of the GSE23985 dataset. Genes encoding for CD55 and OAS1 were highly up-regulated in high litter samples and genes encoding for RASSF2, NEXN, and SLC16A3 were up-regulated in low litter samples of GSE21383 found in common with other datasets, respectively. Sun et al. (2011) reported genes involved in the p53 and Wnt signaling pathways such as CCNG1 (GSE23985 and Zhang et al., [2015]), GTSE1 (Zhang et al., [2015]) and WLS (GSE23985 and Zhang et al., [2015]). Zhang et al. (2015) has reported about 10 genes encoding for CO1, GPX3, MSMB, COX3, TIMP1, CYTB, STAR, HSD3B, CYP11A1, SCARB1, and HSD17B2 that were found to be differentially expressed between the high and low litter group samples. Results obtained from our metadata analysis are also in accordance with the findings of Zhang et al. (2015).
Finally, results obtained from the metadata analysis of prolificacy-based gene expression datasets of pig and sheep has revealed a list of 42 genes differentially expressed in high litter sow groups and 20 genes expressed in low litter sow groups. Previous reports have proposed the involvement of genes such as CYP11A1, HSD17B2, STAR, SCARB1, IGSF8, MSMB, and SERPINA1 in regulating fecundity. The functional involvement of several other genes such as HEXA, PTTG1, TIMP1, FAM46C, FAM167B, CCNG1, FAXDC2, HMGCS1, L2HGDH, Lipin1, MME, MSMO1, PARM1, PTGFR, SLC22A4, SLC35F5, CCNA2, CENPU, CEP55, RASSF2, and SLC16A3 were reported to play a role in fertilization and embryo development. However, their exact function regarding prolificacy rate must be uncovered in future studies.

Conclusions
In our present study, we have conducted a large-scale metadata analysis of genome-wide multi-omic datasets of pig and sheep in hopes of better understanding the factors affecting litter size. To the best of our knowledge, this is the first study to report on the metadata analysis of gene expression studies focused on litter size for understanding and revealing common gene expression patterns regulating fecundity. We found that 62 genes were significant and differentially expressed between the high and low proliferating groups of sows and sheep. KEGG pathway analysis of these significant genes indicates that some of these genes are involved in the metabolism of lipids and cholesterol, steroid hormone biosynthesis, especially ovarian steroidogenesis, immune system pathways, and cancer overview pathways. A literature search of 42 highly expressed significant genes among high prolificacy sows has revealed that these genes are involved in fertilization, implantation and embryo development. Results obtained in our study have also proposed that genes which are involved in the progression of PCOS were also found to exhibit a similar pattern in low prolificacy groups. From what we know, our study is the first attempt made to understand the common gene expression patterns between high and low prolificacy groups. Our present study provides highly significant genetic information that might contribute to a better understanding of the molecular mechanisms involved in high and low prolificacy variations.

Declarations
Ethics approval and consent to participate: Not Applicable

Consent for publication: Not Applicable

Availability of data and materials: Not Applicable

Competing interest: The authors declared that they have no competing interests.

Funding: This work was supported by Natural Sciences and Engineering Research Council Canada Ontario Research Chair Funding to JL.

Authors contributions:
AKSK was involved in designing, planning and analyzing the transcriptome data and representing the results, organizing and writing the manuscript. JL was the principal investigator of the project, designed and led the project, participated paper writing, revision, and finalized the paper for publishing as corresponding author. All the authors read and approved the final manuscript.

Acknowledgements: Not applicable

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Tables

Table-1: List of transcriptome and gene expression datasets of pig and sheep ovary tissues based on
litter size used in our current metadata analysis:

| S.no | GEO Accession | Experimental Details |
|------|---------------|---------------------|
| 1    | GSE21383      | **Objective**: Expression data from porcine ovary tissue of sows from two prolificacy levels  
Platform: GPL3533 Affymetrix Genome Array  
Organism, Tissue: **Pig**, Ovary  
Samples & Reference: 12 [4] |
| 2    | GSE23985      | **Objective**: Differential gene expression in PMSG-hCG stimulated preovulatory ovarian from Chinese Taihu and Large White sows  
Platform: GPL3533 Affymetrix Genome Array  
Organism, Tissue: **Pig**, Ovary  
Samples & Reference: 6 [5] |
| 3    | Zhang et al [2015] | **Objective**: Transcriptomic Analysis of Ovaries from Pigs with High and Low Litter Size  
Platform: Illumina HiSeq 2000  
Organism, Tissue: **Pig**, Ovary  
Samples & Reference: 6 [6] |
| 4    | Chen et al [2015] | **Objective**: Differential Gene Expression in Ovaries of Qira Black Sheep and Hetian Sheep Using RNA-Seq Technique  
Platform: Illumina HiSeq 2000  
Organism, Tissue: **Sheep**, Ovary  
Samples & Reference: 6 [7] |

Table 2A: List of significant differentially up-regulated genes and their associated values in high-litter group samples and down regulated in low litter group samples:

| Description                                             | Gene Name   | Datasets                          | FC  | P-val |
|---------------------------------------------------------|-------------|-----------------------------------|-----|-------|
| Alcohol Dehydrogenase Iron Containing 1                 | ADHFE1      | GSE23985                          | 2   | 0.04  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | GSE21383                          | 10  | 0.05  |
| Cyclin G1                                               | CCNG1       | GSE23985                          | 2   | 0.05  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | GSE21383                          | 3   | 0.05  |
| Decay Accelerating Factor for Complement-55             | CD55        | GSE23985                          | 3   | 0.04  |
|                                                        |             | GSE21383                          | 2   | 0.02  |
| Cytochrome P450 Family 11 Subfamily A Member 1          | CYP11A1     | GSE23985                          | 16  | 0.05  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | GSE21383                          | 18  | 0.05  |
|                                                        |             | Chen et al [2015]                 |     |       |
| Family with Sequence Similarity 167 Member B            | FAM167B     | GSE23985                          | 5   | 0.05  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | GSE21383                          | 5   | 0.05  |
|                                                        |             | Chen et al [2015]                 |     |       |
| Hexosaminidase Subunit Alpha                            | HEXA        | GSE23985                          | 2   | 0.02  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | GSE21383                          | 2   | 0.05  |
| 3-Hydroxy-3-Methylglutaryl-CoA Synthase 1               | HMGCS1      | GSE23985                          | 16  | 0.05  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | GSE21383                          | 4   | 0.05  |
|                                                        |             | Chen et al [2015]                 |     |       |
| Haptoglobin                                             | HP          | GSE23985                          | 5   | 0.05  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | GSE21383                          | 128 | 0.05  |
|                                                        |             | Chen et al [2015]                 |     |       |
| L-2-Hydroxyglutarate Dehydrogenase                      | L2HGDH      | GSE23985                          | 2   | 0.02  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | GSE21383                          | 3   | 0.05  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                          | LOC100517722| GSE23985                          | 3   | 0.05  |
|                                                          |             | Zhang et al [2015]                |     |       |
| Lipin 1                                                 | LPIN1       | GSE23985                          | 2   | 0.05  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | GSE21383                          | 8   | 0.05  |
| Membrane Metallo-endopeptidase                          | MME         | GSE23985                          | 3   | 0.03  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | GSE21383                          | 5   | 0.05  |
| Microseminoprotein Beta                                 | MSMB        | GSE23985                          | 24  | 0.05  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | Chen et al [2015]                 | 24  | 0.05  |
| Methylsterol Monooxygenase 1                            | MSMO1       | GSE23985                          | 18  | 0.05  |
|                                                        |             | Zhang et al [2015]                |     |       |
|                                                        |             | Chen et al [2015]                 | 7   | 0.05  |
| Gene Name                                      | Accession | Reference               | RPKM High litter | RPKM Low litter |
|-----------------------------------------------|-----------|-------------------------|------------------|-----------------|
| Neurocalcin Delta                            | NCALD     | GSE23985 Zhang et al [2015] | 2                | 0.05            |
| 2'-5'-Oligoadenylate Synthetase 1             | OAS1      | GSE21383 Chen et al [2015] | 3                | 0.00            |
|                                               |           |                         | 7                | 0.05            |
| Phytanoyl-CoA 2-Hydroxylase                   | PHYH      | Zhang et al [2015]      | 4                | 0.05            |
|                                               |           | Chen et al [2015]       | 4                | 0.05            |
| Peroxisome 3                                 | PRDX3     | Zhang et al [2015]      | 7                | 0.05            |
|                                               |           | Chen et al [2015]       | 4                | 0.05            |
| Prostaglandin F Receptor                      | PTGFR     | Zhang et al [2015]      | 12               | 0.05            |
|                                               |           | Chen et al [2015]       | 11               | 0.05            |
| Glutaminyl-Peptide Cyclotransferase           | QPCT      | Zhang et al [2015]      | 7                | 0.05            |
|                                               |           | Chen et al [2015]       | 4                | 0.05            |
| Retinol Dehydrogenase 11                      | RDH11     | Zhang et al [2015]      | 6                | 0.05            |
|                                               |           | Chen et al [2015]       | 5                | 0.05            |
| Regucalcin                                   | RGN       | GSE23985 Zhang et al [2015] | 4                | 0.05            |
|                                               |           | Chen et al [2015]       | 24               | 0.05            |
|                                               |           |                         | 2.3              | 0.05            |
| Scavenger Receptor Class B Member 1           | SCARB1    | Zhang et al [2015]      | 12               | 0.05            |
|                                               |           | Chen et al [2015]       | 4                | 0.05            |
| Solute Carrier Family 35 Member F5            | SLC35F5   | Zhang et al [2015]      | 6                | 0.05            |
|                                               |           | Chen et al [2015]       | 6                | 0.05            |
| Steroidogenic Acute Regulatory Protein        | STAR      | Zhang et al [2015]      | 12               | 0.05            |
|                                               |           | Chen et al [2015]       | 91               | 0.05            |
| Tandem C2 Domains, Nuclear                    | TC2N      | GSE23985 Zhang et al [2015] | 6                | 0.04            |
|                                               |           |                         | 23               | 0.05            |
| TIMP Metallopeptidase Inhibitor 1             | TIMP1     | Zhang et al [2015]      | 12               | 0.05            |
|                                               |           | Chen et al [2015]       | 5                | 0.05            |
| Transmembrane 7 Superfamily Member 2          | TM7SF2    | Zhang et al [2015]      | 45               | 0.05            |
|                                               |           | Chen et al [2015]       | 7                | 0.05            |
| Wnt Ligand Secretion Mediator                 | WLS       | GSE23985 Zhang et al [2015] | 2                | 0.05            |
|                                               |           |                         | 3                | 0.04            |
| Glutathione S-transferase A2                  | GSTA2     | Zhang et al [2015]      |                  |                 |

Table 2B: List of significant differentially down-regulated genes and their associated values in high-litter group samples and down regulated in low litter group samples.
| Description                                      | Gene Title                  | Dataset IDs                  | FC  | P-va |
|--------------------------------------------------|-----------------------------|-----------------------------|-----|------|
| Centromere Protein U                             | CENPU                       | GSE23985                    | 1.6 | 0.04 |
|                                                  |                              | Zhang et al [2015]          |     |      |
|                                                  |                              | GSE21383                    | 3.2 | 0.05 |
| GLI Family Zinc Finger 1                         | GLI1                        | GSE23985                    | 1.6 | 0.05 |
|                                                  |                              | Zhang et al [2015]          |     |      |
|                                                  |                              | GSE21383                    | 19.7| 0.05 |
| Nexilin F-Actin Binding Protein                   | NEXN                        | GSE23985                    | 2.6 | 0.03 |
|                                                  |                              | GSE21383                    | 1.9 | 0.01 |
| Ras Association Domain Family Member 2           | RASSF2                      | GSE23985                    | 1.6 | 0.01 |
|                                                  |                              | GSE21383                    | 1.7 | 0.04 |
| Solute Carrier Family 16 Member 3                | SLC16A3                     | GSE23985                    | 2.0 | 0.01 |
|                                                  |                              | GSE21383                    |     |      |
|                                                  |                              | Chen et al [2015]           | 4.3 | 0.05 |
| PTTG1 Regulator of Sister Chromatid Separation,  | PTTG1                       | GSE23985                    | 2.5 | 0.05 |
| Securin                                          |                              | Zhang et al [2015]          |     |      |
|                                                  |                              | GSE21383                    | 2.6 | 0.05 |
| Epidermal growth receptor                        | EGFR                        | Zhang et al [2015]          | 19.69| 0.05 |
|                                                  |                              | GSE23985                    | 1.6 | 0.05 |
| Aquaporin 1                                      | AQP1                        | Zhang et al [2015]          | 2.5 | 0.05 |
|                                                  |                              | GSE23985                    | 1.6 | 0.05 |
| Pyrroline-5-carboxylate reductase                 | HDC                         | Zhang et al [2015]          | 3.0 | 0.05 |
|                                                  |                              | GSE23985                    |     |      |
|                                                  |                              | GSE21383                    | 2.7 | 0.05 |
| Tight Junction Protein 3                         | TJP3                        | Zhang et al [2015]          | -2.9| 0.05 |
|                                                  |                              | Chen et al [2015]           | -2.8| 0.05 |

Table 3 not provided with this version.

Table 4A: List of significant pathways from up-regulated genes obtained from the metadata analysis using the “gene list enrichment analysis” function of KOBAS web software
| Pathway                                      | P-val | Adj P-val | Input                                                                 |
|----------------------------------------------|-------|-----------|----------------------------------------------------------------------|
| Metabolic pathways                           | 0.00  | 0.00065   | MSMO1; HMGC5; L2HGDH; TM7SF2; LPIN1; CYP11A1; RDH11; RGN              |
| Ovarian steroidogenesis                      | 0.00  | 0.00080   | SCARB1; CYP11A1; STAR                                                |
| Cortisol synthesis and secretion             | 0.00  | 0.00118   | SCARB1; CYP11A1; STAR                                                |
| Cholesterol biosynthesis                     | 0.00  | 0.00168   | HMGCS1; TM7SF2                                                       |
| Aldosterone synthesis and secretion          | 0.00  | 0.00226   | SCARB1; CYP11A1; STAR                                                |
| Steroid biosynthesis                         | 0.00  | 0.00300   | MSMO1; TM7SF2                                                       |
| Butanoate metabolism                         | 0.00  | 0.00396   | HMGCS1; L2HGDH                                                       |
| Metabolism of steroids                        | 0.00  | 0.00494   | HMGCS1; TM7SF2                                                       |
| Cushing syndrome                             | 0.00  | 0.00494   | SCARB1; CYP11A1; STAR                                                |
| Metabolism                                   | 0.00  | 0.00899   | HMGCS1; TM7SF2; ADHFE1; RDH11                                       |
| Cholesterol metabolism                       | 0.00  | 0.00899   | SCARB1; STAR                                                        |
| Hematopoietic cell lineage                   | 0.00  | 0.02734   | MME; CD55                                                           |
| Detoxification of Reactive Oxygen Species    | 0.01  | 0.04272   | PRDX3                                                               |
| Metabolism of fat-soluble vitamins           | 0.01  | 0.04272   | RDH11                                                               |
| Activation of kainate receptors upon glutamate binding | 0.01  | 0.04272   | NCALD                                                               |
| RA biosynthesis pathway                      | 0.01  | 0.04272   | RDH11                                                               |
| The canonical retinoid cycle in rods (twilight vision) | 0.01  | 0.04272   | RDH11                                                               |
| Pyruvate metabolism and Citric Acid (TCA) cycle | 0.01  | 0.04272   | ADHFE1                                                              |
| Synthesis and degradation of ketone bodies   | 0.01  | 0.04272   | HMGCS1                                                              |
| Hepatitis C                                  | 0.01  | 0.04272   | OAS1; SCARB1                                                        |
| Metabolism of lipids                         | 0.01  | 0.04272   | HMGCS1; TM7SF2                                                       |
| WNT ligand biogenesis and trafficking        | 0.01  | 0.04272   | WLS                                                                 |
| Signaling by Retinoic Acid                   | 0.02  | 0.05329   | RDH11                                                               |
| Visual phototransduction                     | 0.02  | 0.05329   | RDH11                                                               |
| Glycosphingolipid biosynthesis - ganglio series | 0.02  | 0.05454   | HEXA                                                               |
| Glycosphingolipid biosynthesis - globo and isoglobo series | 0.02  | 0.05569   | HEXA                                                               |
| Ascorbate and aldarate metabolism            | 0.02  | 0.05575   | RGN                                                                 |
| Glycosaminoglycan degradation                | 0.02  | 0.05575   | HEXA                                                               |
| Other glycan degradation                     | 0.02  | 0.05575   | HEXA                                                               |

Table 4B: List of significant pathways from down-regulated genes obtained from the metadata analysis using the “gene list enrichment analysis” function of KOBA5 web software
| Pathway                                                                 | P-val | Adj P-val | Input                  |
|------------------------------------------------------------------------|-------|-----------|------------------------|
| Central carbon metabolism in cancer                                    | 0.000 | 0.0150    | SLC16A3; EGFR           |
| Proximal tubule bicarbonate reclamation                                | 0.009 | 0.0797    | AQP1                   |
| Histidine metabolism                                                   | 0.009 | 0.0797    | HDC                    |
| Hippo signaling pathway - multiple species                              | 0.011 | 0.0797    | RASSF2                 |
| Bladder cancer                                                         | 0.015 | 0.0797    | EGFR                   |
| Pathways in cancer                                                     | 0.015 | 0.0797    | GLI1; EGFR             |
| Hedgehog signaling pathway                                             | 0.017 | 0.0797    | GLI1                   |
| Endometrial cancer                                                     | 0.022 | 0.0797    | EGFR                   |
| Metabolism of amino acids and derivatives                              | 0.022 | 0.0797    | HDC                    |
| Basal cell carcinoma                                                   | 0.024 | 0.0797    | GLI1                   |
| Non-small cell lung cancer                                             | 0.024 | 0.0797    | EGFR                   |
| Renin secretion                                                        | 0.026 | 0.0797    | AQP1                   |
| Melanoma                                                               | 0.026 | 0.0797    | EGFR                   |
| Bile secretion                                                         | 0.026 | 0.0797    | AQP1                   |
| Adherens junction                                                      | 0.026 | 0.0797    | EGFR                   |
| Glioma                                                                 | 0.027 | 0.0797    | EGFR                   |
| Pancreatic cancer                                                      | 0.027 | 0.0797    | EGFR                   |
| EGFR tyrosine kinase inhibitor resistance                               | 0.029 | 0.0797    | EGFR                   |
| ErbB signaling pathway                                                 | 0.031 | 0.0797    | EGFR                   |
| Gap junction                                                           | 0.032 | 0.0797    | EGFR                   |
| Colorectal cancer                                                      | 0.032 | 0.0797    | EGFR                   |
| GnRH signaling pathway                                                 | 0.033 | 0.0797    | EGFR                   |
| Endocrine resistance                                                   | 0.034 | 0.0797    | EGFR                   |
| PD-L1 expression and PD-1 checkpoint pathway in cancer                 | 0.035 | 0.0797    | EGFR                   |
| Prostate cancer                                                        | 0.035 | 0.0797    | EGFR                   |
| Choline metabolism in cancer                                           | 0.036 | 0.0797    | EGFR                   |
| Parathyroid hormone synthesis, secretion and action                    | 0.039 | 0.0804    | EGFR                   |
| HIF-1 signaling pathway                                                | 0.040 | 0.0804    | EGFR                   |
| Oocyte meiosis                                                         | 0.043 | 0.0804    | PTTG1                  |
| Cell cycle                                                             | 0.045 | 0.0804    | PTTG1                  |
| Relaxin signaling pathway                                              | 0.048 | 0.0804    | EGFR                   |
| FoxO signaling pathway                                                 | 0.048 | 0.0804    | EGFR                   |
| Estrogen signaling pathway                                             | 0.048 | 0.0804    | EGFR                   |
| Breast cancer                                                          | 0.053 | 0.0804    | EGFR                   |
Volcano plots showing highly significant and differentially expressed genes among the selected gene expression datasets. Venn diagrams showing: A) p-value <0.05 list of statistically significant genes obtained from the gene expression datasets, B) FC >1.50, p-value <0.05 list of up-regulated significant genes and C) FC >-1.50, p-value <0.05 list of down-regulated significant genes. [Note: The red and blue color in the volcano plots represents the up- and down-regulated genes. Each circle in the Venn diagram corresponds to a list of differentially expressed genes obtained from the dataset with the associated GSE dataset ID listed respectively]

Supplementary Files
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