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

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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 *C. hircus, S. scrofa* and *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 *HEXA, PTTG1* and *TIMP1*. Hexosaminidase-α (*HEXA*) and its isozymes HEXB and 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 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]. 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 PTTG1 is highly expressed in various tumors, especially ovarian tumorigenesis. However, the exact involvement of PTTG1 in fertilization and with respect to litter size has not yet been reported. TIMP1 gene encodes for tissue inhibitor of metalloproteinase, it is found to play a key role in ovulation. Rosewell *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].

Biosynthesis and Metabolism of Lipids, Cholesterol and Steroidogenesis

Genes involved in steroid biogenesis and ovarian steroidogenesis pathways such as *Cyp11A1, Msmo1, Star, Dhrs7/Hsd17b7*, and *Hmgcs1* were up- regulated in high litter group samples. *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 hyperadrogenaemia 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]. *Msmo1* 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 [43]. 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

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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.

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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 follicles of 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	logFC
Alcohol Dehydrogenase Iron Containing 1	ADHFE1	GSE23985 Zhang et al [2015]	2	0.04	0.8
			10	0.05	3.3
Cyclin G1	CCNG1	GSE23985 Zhang et al [2015]	2	0.05	0.8
			3	0.05	1.6
Decay Accelerating Factor for Complement-55	CD55	GSE23985	3	0.04	1.6
		GSE21383	2	0.02	0.7
Cytochrome P450 Family 11 Subfamily A Member 1	CYP11A1	Zhang et al [2015] Chen et al [2015]	16	0.05	4.0
			18	0.05	4.2
Family with Sequence Similarity 167 Member B	FAM167B	Zhang et al [2015] Chen et al [2015]	5	0.05	2.3
			5	0.05	2.4
Hexosaminidase Subunit Alpha	HEXA	GSE23985 Zhang et al [2015]	2	0.02	0.9
			2	0.05	1.3
3-Hydroxy-3-Methylglutaryl-CoA Synthase 1	HMGCS1	Zhang et al [2015] Chen et al [2015]	16	0.05	4.0
			4	0.05	2.1
Haptoglobin	HP	Zhang et al [2015] Chen et al [2015]	5	0.05	2.2
			128	0.05	7.0
L-2-Hydroxyglutarate Dehydrogenase	L2HGDH	GSE23985 Zhang et al [2015]	2	0.02	1.1
			3	0.05	1.8
Unknown	LOC100517722	GSE23985 Zhang et al [2015]	2	0.00	1.2
			3	0.05	1.4
Lipin 1	LPIN1	GSE23985 Zhang et al [2015]	2	0.05	0.6
			8	0.05	3.0
Membrane Metallo-endopeptidase	MME	GSE23985 Zhang et al [2015]	3	0.03	1.7
			5	0.05	2.3
Microseminoprotein Beta	MSMB	Zhang et al [2015] Chen et al [2015]	24	0.05	4.6
			24	0.05	4.6
Methylsterol Monooxygenase 1	MSMO1	Zhang et al [2015] Chen et al [2015]	18	0.05	4.2
			7	0.05	2.9
Neurocalcin Delta	NCALD	GSE23985 Zhang et al [2015]	2	0.05	0.7
			4	0.05	1.9
2'-5'-Oligoadenylate Synthetase 1	OAS1	GSE21383 Chen et al [2015]	3	0.00	1.8
			7	0.05	2.8
Phytanoyl-CoA 2-Hydroxylase	PHYH	Zhang et al [2015] Chen et al [2015]	4	0.05	2.1
			4	0.05	2.1
Peroxiredoxin 3	PRDX3	Zhang et al	7	0.05	2.8

		[2015] Chen et al [2015]	4	0.05	2.0
Prostaglandin F Receptor	PTGFR	Zhang et al [2015] Chen et al [2015]	12	0.05	3.6
			11	0.05	3.4
Glutaminyl-Peptide Cyclotransferase	QPCT	Zhang et al [2015] Chen et al [2015]	7	0.05	2.9
			4	0.05	2.1
Retinol Dehydrogenase 11	RDH11	Zhang et al [2015] Chen et al [2015]	6	0.05	2.5
			5	0.05	2.3
Regucalcin	RGN	Zhang et al [2015] Chen et al [2015] GSE23985	4	0.05	2.1
			24	0.05	4.6
			2.3	0.05	1.15
Scavenger Receptor Class B Member 1	SCARB1	Zhang et al [2015] Chen et al [2015]	12	0.05	3.6
			4	0.05	2.1
Solute Carrier Family 35 Member F5	SLC35F5	Zhang et al [2015] Chen et al [2015]	6	0.05	2.5
			6	0.05	2.5
Steroidogenic Acute Regulatory Protein	STAR	Zhang et al [2015] Chen et al [2015]	12	0.05	3.6
			91	0.05	6.5
Tandem C2 Domains, Nuclear	TC2N	GSE23985 Zhang et al [2015]	6	0.04	2.6
			23	0.05	4.5
TIMP Metallopeptidase Inhibitor 1	TIMP1	Zhang et al [2015] Chen et al [2015]	12	0.05	3.6
			5	0.05	2.4
Transmembrane 7 Superfamily Member 2	TM7SF2	Zhang et al [2015] Chen et al [2015]	45	0.05	5.5
			7	0.05	2.8
Wnt Ligand Secretion Mediator	WLS	GSE23985 Zhang et al [2015]	2	0.05	0.7
			3	0.04	1.5
Glutathione S-transferase A2	GSTA2	Zhang et al [2015] Chen 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	Log2FC
Centromere Protein U	CENPU	GSE23985 Zhang et al [2015]	1.6	0.04	-0.7
			3.2	0.05	-1.7
GLI Family Zinc Finger 1	GLI1	GSE23985 Zhang et al [2015]	1.6	0.05	-0.7
			19.7	0.05	-4.3
Nexilin F-Actin Binding Protein	NEXN	GSE23985	2.6	0.03	-1.4
		GSE21383	1.9	0.01	-0.9
Ras Association Domain Family Member 2	RASSF2	GSE23985	1.6	0.01	-0.7
		GSE21383	1.7	0.04	-0.8
Solute Carrier Family 16 Member 3	SLC16A3	GSE21383	2.0	0.01	-1.0
		Chen et al [2015]	4.3	0.05	-2.1
PTTG1 Regulator of Sister Chromatid Separation, Securin	PTTG1	GSE23985 Zhang et al [2015]	2.5	0.05	-1.3
			2.6	0.05	-1.4
Epidermal growth receptor	EGFR	Zhang et al [2015]	19.69	0.05	-4.3
		GSE23985	1.6	0.05	-0.6
Aquaporin 1	AQP1	Zhang et al [2015]	2.5	0.05	-1.4
		GSE23985	1.6	0.05	-0.6
Pyrroline-5-carboxylate reductase	HDC	Zhang et al [2015]	3.0	0.05	-1.6
		GSE23985	2.7	0.05	-1.4
Tight Junction Protein 3	TJP3	Zhang et al [2015]	-2.9	0.05	-1.6
		Chen et al [2015]	-2.8	0.05	-1.6

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; HMGCS1; L2HGDH; TM7SF2; LPIN1; HEXA; 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 KOBAS 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

Figures

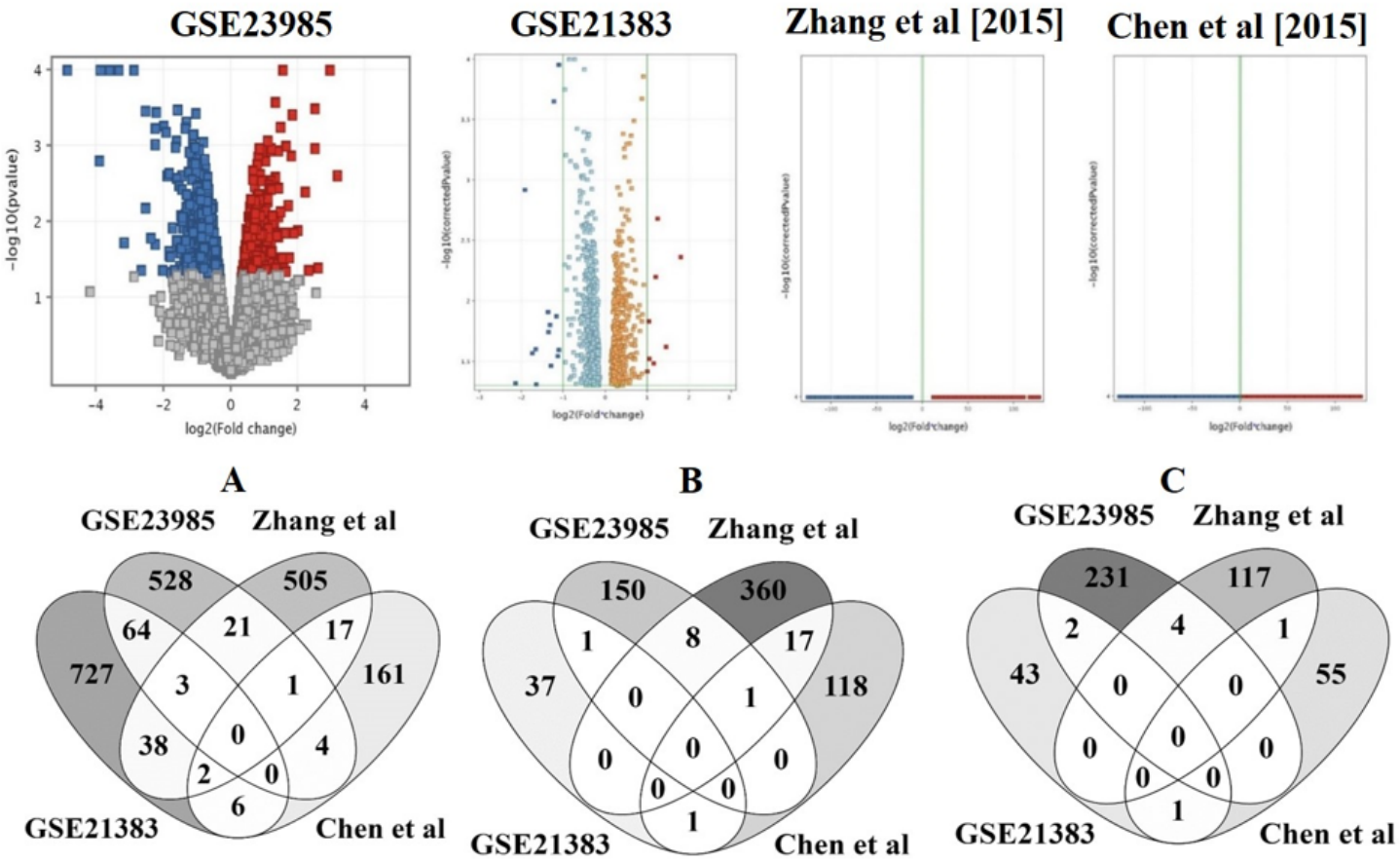

Figure 1

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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