Whole Transcriptome Analysis of the Effects of Type 1 Diabetes on Mouse Oocytes

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Introduction

It has been estimated that diabetes affects 6.4% of adults (285 million) worldwide by 2010, and this number is expected to increase to 7.7% by 2030 [1]. Diabetes not only affects the health of adults, but maternal diabetes also affects female factor fertility including oocyte maturation and ovulation, and even embryonic and fetal development [2,3]. To investigate the effects of diabetes on oocytes, experimental animal models were utilized; streptozotocin administration induced diabetes mice (STZ mice) and genetically derived NOD diabetic mice or chemically induced STZ diabetic mice with that of corresponding normal mice. Differentially expressed genes were extracted from the two diabetic models. Gene set enrichment analysis showed that genes associated with metabolic and developmental processes were differentially expressed in oocytes from both models of diabetes. In addition, NOD diabetes also affected the expression of genes associated with ovulation, cell cycle progression, and preimplantation embryo development. Notably, Dnmt1 expression was significantly down-regulated, but Mbd3 expression was up-regulated in diabetic mouse oocytes. Our data not only revealed the mechanisms by which diabetes affects oocyte quality and preimplantation embryo development, but also linked epigenetic hereditary factors with metabolic disorders in germ cells.

Abstract

In mouse ovarian follicles, granulosa cells but not oocytes take up glucose to provide the oocyte with nourishments for energy metabolism. Diabetes-induced hyperglycemia or glucose absorption inefficiency consistently causes granulosa cell apoptosis and further exerts a series of negative impacts on oocytes including reduced meiosis resumption rate, low oocyte quality and preimplantation embryo degeneration. Here we compared the transcriptome of mouse oocytes from genetically derived NOD diabetic mice or chemically induced STZ diabetic mice with that of corresponding normal mice. Differentially expressed genes were extracted from the two diabetic models. Gene set enrichment analysis showed that genes associated with metabolic and developmental processes were differentially expressed in oocytes from both models of diabetes. In addition, NOD diabetes also affected the expression of genes associated with ovulation, cell cycle progression, and preimplantation embryo development. Notably, Dnmt1 expression was significantly down-regulated, but Mbd3 expression was up-regulated in diabetic mouse oocytes. Our data not only revealed the mechanisms by which diabetes affects oocyte quality and preimplantation embryo development, but also linked epigenetic hereditary factors with metabolic disorders in germ cells.

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Competing Interests

The authors have declared that no competing interests exist.
developmental delay of early embryos was also identified in both chemically induced and in genetic diabetic mice [2,14,15,16].

Although the negative effects of diabetes on female fertility are well recognized, we know little about the mechanisms by which metabolic disorders affect oocyte quality and early embryo developmental potential. For that, by using STZ diabetic mice and NOD diabetic mice as models, we compared the transcriptomes of diabetic mouse oocytes with that of normal mouse oocytes.

**Methods**

**Ethics Statement**

This study was approved by the Animal Research Committee of the Institute of Zoology, Chinese Academy of Sciences. All animal manipulations were according to manual of Animal Research Committee. Details of animal welfare and steps taken to ameliorate suffering are included in the section about oocyte collection.

**Diabetes Mouse Models and Oocyte Collection**

For the construction of diabetes model mice, 8–12 weeks old mice were used, and STZ diabetic ICR strain mice were produced according the methods of Bonnevie-Nielsen [17]. The NOD spontaneous diabetic mice were purchased from Experimental Animal Facility of Nanjing University. All ICR and NOD mice with a blood glucose concentration more than 22 mM were used as diabetes model mice, and those with a blood glucose concentration less than 8 mM were used as control (5–10 mice for each group). Both diabetic and normal mice were superovulated by injecting 5U PMSG followed by 5U hCG 48 hours later. Cumulus oocyte complexes were collected 14 hours post-hCG injection, and oocytes at the metaphase of the second meiosis (MII) stage were separated from cumulus cells by hyaluronidase treatment and used for transcriptome analysis.

**SOLiD Sequencing Library Preparation and Quantitative RT-PCR Validation**

The library preparation procedure mainly references the protocol of Saitou [18] and protocol provided by Applied Biosystems website (http://www.appliedbiosystems.com). The procedure is briefly summarized as follows. Each 15 oocytes were lysed in one tube and the total mRNAs were reversely transcribed to cDNAs by universe primer 1 (UP1) adaptor primers containing 24 bp oligo(dT) sequence. The remaining primers were removed and poly(A) tails to the 3' terminal of the cDNAs were added. Universe primer 2 (UP2) primers containing 24 bp oligo(dT) were used for the synthesis of the second cDNA strand. The double strand cDNA library was amplified by UP1 and UP2 for 18 cycles, and by amine-blocked UP1 and UP2 for 14 cycles. The final amplified cDNA libraries were sent to Genome institute of Beijing and sequenced by Applied Biosystem SOLiD sequencing system. Quantitative RT-PCR was performed to evaluate the RNA sequencing results (Fig S1), primers used in RT-PCR were listed in Table S1.

**Whole Transcriptome Sequencing and Data Analysis**

Total cDNAs were sequenced by SOLiD system and sequencing reads were mapped to mouse genome to extract the whole transcriptome information of oocytes. Mapped reads data were

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*Figure 1. Gene expression profiles in STZ and NOD diabetic mouse models. (A) Schematic illustration of the experimental procedure. (B) Statistics of transcriptome sequencing results. (C) Counts of differentially expressed genes in STZ and NOD diabetic mouse MII oocytes.*

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analyzed by DESeq package [19]. Firstly, we divided the detected genes with corresponding reads count more than 10, into two groups: highly expressed genes (corresponding reads counts more than 1/10 of the mean value of all detected genes reads counts) and lowly expressed genes (reads counts less than 1/10 of the mean value). Secondly, fold change selection method was used for differentially expressed genes selection. For highly expressed genes, whose log2 (fold change) values bigger than 0.8 or less than −0.8 were selected as up-regulated genes or down-regulated genes. For lowly expressed genes, the selection standard was set at 2 to reduce the false positive rate, and genes log2 (fold change) values bigger than 2 or less than −2 were selected as up-regulated genes or down-regulated genes, respectively. Fisher exact test was used for differentially expressed genes biological processes enrichment analysis [20]. KEGG pathway graphs were created from KEGG color map website. SOLiD sequencing data was submitted to SRA with accession id: SRA037765. All R scripts can be obtained on request.

Results

Screening Differentially Expressed Genes in Diabetic Mouse Oocytes

To compare the oocyte transcriptomes of diabetic and normal mice, STZ and NOD diabetic mice were used as diabetes models. MII stage oocytes were isolated from diabetic model mice and corresponding normal mice. Total mRNAs were extracted and used for SOLiD whole transcriptome analysis (Figure 1A). Sequenced mRNA reads were mapped to mouse genome, and the reads number mapped to each transcript was used to represent the expression level of the transcript (Figure 1B). As a result, 16457 genes were detected in all samples, in which the reads number of 8792 genes was more than 10.

By the selection standards described in the methods, we found that the mRNA levels of 1199 genes were up-regulated and 658 genes were down-regulated in NOD diabetic oocytes, whereas mRNA levels of 1016 genes were up-regulated and 325 genes were down-regulated in STZ diabetic oocytes. Among these, 535 genes’ transcription was up-regulated and 83 genes’ transcription was down-regulated in both STZ and NOD diabetic oocytes (Figure 1C). All differentially expressed genes in STZ diabetic oocytes and NOD diabetic oocytes are listed in Dataset S1. To evaluate the quality of the sequencing method, some differentially expressed genes were re-analyzed by quantitative RT-PCR. As a result, the RNA sequencing and RT-PCR correlation values of the STZ group and NOD group were both more than 0.97 (Figure 2), which reflected the reliability of high-throughput sequencing technology.

The Effects of Diabetes on Biological Processes of Mouse Oocytes

The effects of diabetes on oocytes from the two models were analyzed by gene set enrichment analysis. By our methods, we found that STZ induced diabetes mainly up-regulated the transcription of genes associated with the metabolic biological processes, such as molecular metabolic process, nitrogen compound metabolic process and oxidation-reduction process. The genes whose mRNAs decreased in STZ diabetic oocytes were enriched in proliferation, development, chemical stimulus response and biological quality regulation-associated processes (Table S2).

Compared to STZ-induced diabetes, spontaneous NOD diabetes caused more severe effects on oocytes. Not only enriched in metabolic processes, up-regulated mRNAs in NOD diabetic oocytes were also enriched in processes like cell cycle, translation initiation, chromosome segregation, and ovulation cycle process (Table 1). In addition to the similar effects induced by STZ diabetes, down-regulated mRNAs in oocytes from NOD diabetic mice were also enriched in processes such as transmembrane transport, sexual reproduction, embryo implantation, cell adhesion, and cell communication (Table 2).

To find the common features of diabetes mouse oocytes, we obtained the gene lists of up- or down-regulated mRNAs in both diabetic models. Results showed that genes whose mRNAs up-regulated in both diabetic models were enriched in numerous biological processes including protein activation cascade, biosynthetic process, cellular metabolic process, cellular component movement, macromolecule metabolic process, primary metabolic process, and activation of immune response. Whereas down-regulated mRNAs in both diabetic models were enriched in biological processes including response to chemical stimulus, multicellular organism development, hormone metabolic process, anatomical structure development, biosynthetic process, nitrogen compound metabolic process, neurotrophin production, vesicle-mediated transport, and anatomical structure arrangement.

The Effects of Diabetes on Cell Cycle-associated Genes in Mouse Oocytes

To find the details on how diabetes affects meiosis progression of mouse oocytes and further post-fertilization embryo cleavage, KEGG color map was created (Figure 3). From the KEGG meiosis pathway we can see that genes like mitogen-activated protein kinase 1 (Mapk1), and MAD2 mitotic arrest deficient-like 2 (Mad2l2) were up-regulated in diabetic mouse oocytes, whereas Moloney sarcoma oncogene (Mos) and Securin (Pttg1) were down regulated. Genes differentially expressed in diabetic mouse oocytes which associated with meiosis or cell cycle processes are listed in Dataset S2.

Cellular Localization Enrichment of Differentially Expressed Genes in both STZ and NOD Diabetic Mouse Oocytes

To find the cellular localization of differentially expressed genes in both diabetic models, cellular component enrichment was analyzed by our GO slim method [21]. Results showed that 89 genes whose mRNA levels were significantly changed in both diabetic oocytes were localized in mitochondria (Fisher exact test p value <0.01); all cellular components which differentially expressed genes enriched in NOD and STZ mouse oocytes are listed in Table S3.

Diabetes Affects Dnmt1 and Mbd3 Transcription in MII Oocytes

DNA methyltransferase 1 (Dnmt1), which is critical for the maintenance of DNA methylation during DNA replication [22]. The mRNA of Dnmt1 was down-regulated in both STZ and NOD diabetic MII oocytes. Genes for de novo DNA methylation (Dnmt3a, Dnmt3b, and Dnmt3l) expressed normally in STZ diabetic oocytes, but mRNAs of Dnmt3b and Dnmt3l were significantly down-regulated in NOD diabetic oocytes. In addition, we found that mRNAs of key methyl-CpG binding (Mbd) proteins were up-regulated in both STZ (Mbd3 and Mbd5) and NOD (Mbd2 and Mbd3) diabetic oocytes. The epigenetics-associated genes differentially expressed in two types of diabetic mouse oocytes are listed in Dataset S3.
Diabetes Affects Oocyte Maturation and Oocyte Quality

Communication between oocytes and cumulus cells is critical for oocyte meiosis resumption and ovulation [23,24,25,26]. Our results showed that genes significantly changed in NOD diabetic mouse oocytes were enriched in cell-cell communication, and most of which were down-regulated, indicating that diabetes weakens the communication between oocyte and cumulus cells. In addition, Bmp15 and Kit, which consisted of a negative feedback network regulating granulosa cell division [27], were down-regulated in STZ diabetic mouse oocytes, which may affect COC response to the LH, and further impair meiosis progression and oocyte ovulation [28].

Our results showed that diabetes strongly changed the transcription of cell cycle associated genes (Dataset S2). The
resumption of oocyte meiosis is mainly dependent on the activity of maturation promoting factor (MPF), which includes the regulatory subunit Ccnb1 and the catalytic subunit Cdk1 [29]. In our transcriptome data, the transcription of many MPF upstream genes was significantly changed in diabetic mice oocytes. For example, fizzy/cell division cycle 20 related 1 protein (Fzr1, also known as Cdh1) is an activator of anaphase promoting complex. The Fzr1 depleted oocytes can overcome the meiosis resumption inhibition by milrinone and promote the meiosis resumption of the not fully grown oocytes (diameter, 60–69 \( \mu \text{m} \)).

The increase of Ccnb1 in Fzr1 depleted oocytes indicates that Fzr1 is an upstream regulator of MPF [30]. The check point protein Bub1b (also known as BubR1) is also important for meiosis resumption. The Bub1b knocking down GV oocytes can partially break through the 3-isobutyl-1-methylxanthine induced GV stage arrest. Bub1b knocking down can also induce the decrease of Fzr1 in GV oocytes [31]. The mRNAs levels of Fzr1 decreased in both STZ and NOD diabetic oocytes, and mRNAs of Bub1b decreased significantly in NOD diabetic oocytes and slightly decreased in STZ diabetic oocytes. These results indicated that diabetic oocytes could not strictly control the meiosis resumption which might cause the low quality of oocytes. For NOD diabetic mouse oocytes, transcriptions of some key DNA damage responding genes were changed significantly. The genes whose transcriptions decreased in NOD diabetic oocytes included the base excision repair associated genes like Polb, Smug1, and Ccno, the double strand break repair associated genes like Smarca5, H2afx, Mms221, and Shfm1, the nucleotide excision repair associated Dclrela, and the single strand break repair associated Xrcc1. On the other hand, transcriptions of some DNA damage checkpoint genes were up regulated.

### Table 1. Gene set enrichment analysis of genes up regulated in NOD diabetes mice oocytes.

| GO Acc   | GO Term                              | P-value |
|----------|--------------------------------------|---------|
| GO:0044237 | cellular metabolic process            | 0.000   |
| GO:0044238 | primary metabolic process             | 0.000   |
| GO:0043170 | macromolecule metabolic process       | 0.000   |
| GO:0002253 | activation of immune response         | 0.000   |
| GO:0072376 | protein activation cascade            | 0.000   |
| GO:0044281 | small molecule metabolic process      | 0.000   |
| GO:0009056 | catabolic process                     | 0.000   |
| GO:006955  | immune response                       | 0.001   |
| GO:006807  | nitrogen compound metabolic process   | 0.001   |
| GO:0071841 | cellular component organization or biogenesis at cellular level | 0.001   |
| GO:006950  | response to stress                    | 0.001   |
| GO:0050789 | regulation of biological process      | 0.001   |
| GO:0009058 | biosynthetic process                  | 0.002   |
| GO:0016043 | cellular component organization       | 0.002   |
| GO:0045184 | establishment of protein localization | 0.003   |
| GO:0051707 | response to other organism            | 0.004   |
| GO:007155  | cell adhesion                         | 0.011   |
| GO:0022525 | immune effector process               | 0.011   |
| GO:0048869 | cellular developmental process         | 0.016   |
| GO:009628  | response to abiotic stimulus          | 0.016   |
| GO:002402  | cell cycle process                    | 0.017   |
| GO:0065009 | regulation of molecular function       | 0.019   |
| GO:0044085 | cellular component biogenesis          | 0.022   |
| GO:007049  | cell cycle                            | 0.025   |
| GO:006413  | translational initiation              | 0.025   |
| GO:007059  | chromosome segregation                | 0.026   |
| GO:0022602 | ovulation cycle process               | 0.026   |
| GO:0065008 | regulation of biological quality      | 0.027   |
| GO:0055114 | oxidation-reduction process           | 0.030   |
| GO:0051656 | establishment of organelle localization | 0.038 |
| GO:0033002 | muscle cell proliferation             | 0.038   |
| GO:0021700 | developmental maturation              | 0.042   |
| GO:0051301 | cell division                         | 0.046   |
| GO:009607  | response to biotic stimulus           | 0.046   |
| GO:0042698 | ovulation cycle                       | 0.048   |

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including Chek1, Rad9, Rad23b, Rad50, and Rad52. The DNA damage repair deficiency and higher expression of DNA damage check point genes may explain why the quality and maturation rates are lower in diabetic oocytes.

During oocyte maturation, mitochondria are important organelles for oocyte quality. Previous data have shown that maternal diabetes could cause the dysfunction of mitochondria, including mtDNA increase, narrowed intermembrane space, rupture of the outer membrane, decreased ATP yield, and induced decrease of tricarboxylic acid metabolites such as citrate, aspartate, and malate [12]. Here we found that expression of 89 mitochondria-associated genes was significantly changed in oocytes from both diabetic models, among which only four genes were down-regulated. These results may be caused by the increased number of mitochondria in diabetic oocytes. The increase in metabolic enzymes such as phosphoinositide dependent kinase 1 (Pdk1) may affect the metabolic processes during subsequent preimplantation embryo development.

**Diabetes Affects the Energy Production of Oocytes**

In mammalian cells, the production of ATP by glucose oxidation mainly relies on three biological processes: glycolysis in the cytoplasm; transformation of pyruvate to acetyl-coenzymeA and tricarboxylic acid (TCA) cycle in the mitochondrial matrix; and the oxidative phosphorylation process in the inner membrane of mitochondria. Both substrates of the last two processes depend on the products of glycolysis. In our results, expression level changes of enzymes associated with the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate and the transformation of 1,3-bisphosphoglycerate to 2-phosphoglycerate (Figure 4 and Figure S1), may retard the glycolysis process and finally, reduce the ATP production in oocytes. Evidence has been accumulated to show that important energy substrate absorption like pyruvate, fructose, and even glucose of oocytes partly relies on cumulus cells or on the environment [7,32,33]. Considering assisted reproductive technologies (ART), our results could provide new information on potential targets for increasing ATP content of diabetic oocytes and promote normal development of diabetic oocytes and embryos [34].

**Diabetes Affects Genes Associated with Preimplantation Embryo Development**

Maternal factors are essential for early embryo development. Previous reports showed that, fewer than 20% Zarl (zygote arrest 1) deleted embryos progressed to the 2-cell stage and none of the embryos developed to 4-cell stages [35]. Down-regulation of Zarl transcription in diabetic mouse oocytes indicated that diabetes may affect the zygote to embryo transition. Geminin (Gmn) gene

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**Table 2. Gene set enrichment analysis of genes down regulated in NOD diabetes mice oocytes.**

| GO Acc  | GO Term                                      | P-value |
|---------|----------------------------------------------|---------|
| GO:0007275 | multicellular organismal development       | 0.000   |
| GO:0055085 | transmembrane transport                      | 0.000   |
| GO:0001763 | morphogenesis of a branching structure       | 0.000   |
| GO:0048856 | anatomical structure development             | 0.000   |
| GO:0009653 | anatomical structure morphogenesis           | 0.001   |
| GO:0019953 | sexual reproduction                          | 0.003   |
| GO:0006810 | transport                                    | 0.003   |
| GO:0019725 | cellular homeostasis                         | 0.004   |
| GO:0016043 | cellular component organization              | 0.006   |
| GO:0042221 | response to chemical stimulus                | 0.008   |
| GO:0042445 | hormone metabolic process                    | 0.009   |
| GO:0007389 | pattern specification process                | 0.009   |
| GO:0007566 | embryo implantation                          | 0.014   |
| GO:0009790 | embryo development                           | 0.014   |
| GO:0050900 | leukocyte migration                          | 0.015   |
| GO:0048869 | cellular developmental process                | 0.015   |
| GO:0007155 | cell adhesion                                | 0.016   |
| GO:0071841 | cellular component organization or biogenesis at cellular level | 0.016 |
| GO:0071514 | cell communication                           | 0.017   |
| GO:0051674 | localization of cell                         | 0.019   |
| GO:0048870 | cell motility                                | 0.019   |
| GO:0006928 | cellular component movement                  | 0.024   |
| GO:0032504 | multicellular organism reproduction           | 0.035   |
| GO:0048609 | multicellular organismal reproductive process | 0.035  |
| GO:0006950 | response to stress                           | 0.039   |
| GO:0048646 | anatomical structure formation involved in morphogenesis | 0.041 |
| GO:0035264 | multicellular organism growth                 | 0.048   |

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is a cell cycle regulator which regulates the S phase to M phase transition. In our results, Gmnn was down-regulated in STZ diabetic mouse oocytes. Maternal Gmnn could not be detected until the 4-cell embryo stage, and the Gmnn deleted embryos showed developmental retardation only when the maternal Gmnn was exhausted [36]. The decrease of maternal Gmnn mRNAs may be one of the reasons to explain why preimplantation development is delayed in STZ diabetic mice.

**Epigenetic-associated Genes are Affected by Diabetes**

During oocyte growth and maturation, the original imprint memory will be wiped off and oocyte-specific imprints will be reconstructed [37]. After fertilization, the zygote genome DNA is globally demethylated by DNA replication-dependent passive pathway (maternal) or DNA replication-independent active pathway (paternal) [38,39,40,41]. To protect maternal and paternal imprint information from being destroyed, oocyte specific Dnmt1 (Dnmt1o) but not somatic Dnmt1, is used to maintain the methylation state of imprint-specific CpG sites in cleavage stage embryos [42,43].

From our results, expression of Dnmt1 decreased in oocytes from both diabetes models, indicating that diabetes may threaten normal imprint of the mouse genome. In addition, some methyl-CpG binding domain proteins were up-regulated in diabetic oocytes, such as Mbd3. Evidence showed that Mbd3 could maintain imprint of paternal H19, but with no effects on other imprinted genes [44]. The change of imprint memory inheritance-associated genes in diabetic mouse oocytes showed that epigenetic markers were affected by the metabolism disorders induced by diabetes.

![Figure 3. Cell cycle and meiosis KEGG pathway maps of differentially expressed genes in STZ and NOD diabetic MII oocytes.](image)

![Figure 4. Model of diabetic mouse oocyte energy production.](image)
Supporting Information

Figure S1 Oxidative phosphorylation and Glycolysis/ Gluconeogenesis KEGG pathway maps of differentially expressed genes in STZ and NOD diabetic MII oocytes. Red, genes up-regulated in both STZ and NOD diabetic mouse oocytes; Blue, genes down-regulated in both STZ and diabetic mouse oocytes; Yellow, genes up-regulated uniquely in NOD diabetic mouse oocytes; Green, genes down-regulated uniquely in NOD diabetic mouse oocytes; Orange, genes up-regulated uniquely in STZ diabetic oocytes; and Purple, genes down-regulated uniquely in STZ diabetic mouse oocytes.

Table S1 Samples for quantitative RT-PCR were extracted by the same methods as SOLiD sequencing library preparation.

Table S2 Gene set enrichment analysis of genes down or up regulated in STZ diabetic mice oocytes.

Table S3 Cellular components enrichment analysis of differentially expressed genes in diabetic MII oocytes.

Table S4 Genes Differentially Expressed in STZ and NOD diabetic Mice MII Oocytes.

Table S5 Expression Information of Cell Cycle Associated Genes in Diabetic Oocytes.

Author Contributions

Conceived and designed the experiments: QS JM ML. Performed the experiments: JG ML DT SS JY BZ YL. Analyzed the data: JG. Contributed reagents/materials/analysis tools: YO YH. Wrote the paper: JM QS HS. Participated in discussion of the experiment: XO ZL.

References

1. Shaw JE, Sicree RA, Zimmet PZ (2009) Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 87: 4–14.
2. Javerbaum A, White V (2010) Animal models in diabetes and pregnancy. Endocr Rev 31: 680–701.
3. Wang Q, Moley KH (2010) Maternal diabetes and oocyte quality. Mitochon-
drion 10: 403–410.
4. Junod A, Lambert AE, Staufferacher W, Renold AE (1969) Diabetogenic action of streptozocin: relationship of dose to metabolic response. J Clin Invest 48: 2129–2139.
5. Yoon JW, Jun HS (2001) Cellular and molecular pathogenic mechanisms of insulin-dependent diabetes mellitus. Ann N Y Acad Sci 928: 200–211.
6. Thomas HE, Kay TW (2006) Beta cell destruction in the development of autoimmune diabetes in the non-obese diabetic (NOD) mouse. Diabetes Metab Res Rev 16: 251–261.
7. Wang Q, Chi MM, Schell T, Moley KH (2012) An intercellular pathway for glucose transport into mouse oocytes. Am J Physiol Endocrinol Metab.
8. Sutton-McDowall ML, Gilchrist RB, Thompson JG (2010) The pivotal role of glucose metabolism in determining oocyte developmental competence. Reproduction 139: 685–695.
9. Sugiyama K, Su YQ, Daz FJ, Pangas SA, Sharma S, et al. (2007) Oocyte-derived BMP15 and FGFs cooperate to promote glycolysis in cumulus cells. Development 134: 2593–2603.
10. Chang AS, Dale AN, Moley KH (2005) Maternal diabetes adversely affects precocious oocyte maturation, development, and granulosa cell apoptosis. Endocrinology 146: 2445–2453.
11. Ratchford AM, Esguerra CR, Moley KH (2008) Decreased oocyte-granulosa cell gap junction communication and connexin expression in a type 1 diabetic mouse model. Mol Endocrinol 22: 2643–2654.
12. Wang Q, Ratchford AM, Chi MM, Schoeller E, Frolova A, et al. (2009) Maternal diabetes causes mitochondrial dysfunction and meiotic defects in marine oocytes. Mol Endocrinol 23: 1603–1612.
13. Li L, Zheng P, Dean J (2010) Maternal control of early mouse development. Development 137: 859–670.
14. Moley KH, Vaughn WK, DeCherney AH, Diamond MP (1991) Effect of diabetes mellitus on mouse pre-implantation embryo development. J Reprod Fertil 93: 325–332.
15. Diamond MF, Moley KH, Pellicer A, Vaughn WK, DeCherney AH (1989) Effects of streptozocin- and alloxan-induced diabetes mellitus on mouse follicular and early embryo development. J Reprod Fertil 86: 1–10.
16. Beebe LF, Kaye PL (1991) Maternal diabetes and retarded preimplantation development of mice. Diabetes 40: 457–461.
17. Bourenne-Niefenstiel V, Steffes MW, Lemmink A (1981) A major loss in inlet mass and B-cell function precedes hyperglycemia in mice given multiple low doses of streptozotocin. Diabetes 30: 424–429.
18. Kurimoto K, Yabuta Y, Ohinata Y, Saitou M (2007) Global single-cell cDNA amplification to provide a template for representative high-density oligonucleo-
tide microarray analysis. Nat Protoc 2: 739–752.
19. Wang L, Deng Z, Wang X, Zhang X (2009) DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. Bioinformatics 26: 134–136.
20. Manoli T, Gertz N, Grone HJ, Kenzelmann M, Eils R, et al. (2006) Group testing for pathway analysis improves comparability of different microarray datasets. Bioinformatics 22: 2500–2506.
21. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, et al. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102: 15545–15550.
22. Li E, Bostor TH, Jaenich R (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell 69: 915–926.
23. Gilula NB, Epstein ML, Beers WH (1978) Cell-to-cell communication and ovulation. A study of the cumulus-oocyte complex. J Cell Biol 78: 58–75.
24. Dekel N, Lavrence T, Gilula NB, Beers WH (1981) Modulation of cell-to-cell communication in the cumulus-oocyte complex and the regulation of oocyte maturation by LH. Dev Biol 86: 414–424.
25. Russell DL, Robker RL (2007) Molecular mechanisms of ovulation: co-ordination through the cumulus complex. Hum Reprod Update 13: 289–312.
26. Osuka F, Shimasaki S (2002) A negative feedback system between oocyte bone morphogenic protein 15 and granulosa cell kit ligand: its role in regulating granulosa cell mitosis. Proc Natl Acad Sci U S A 99: 8060–8065.
27. Moore RK, Erickson GF, Shimasaki S (2004) Are BMP-15 and GDF-9 primary determinants of ovulation quota in mammals? Trends Endocrinol Metab 15: 356–361.
28. Heinkheim G, Gibbons WL (1998) The molecular mechanisms of oocyte maturation and early embryonic development are unveiling new insights into reproductive medicine. Mol Hum Reprod 4: 745–756.
29. Holt JE, Tran SM, Stewart JL, Minahan K, Garcia-Higuera I, et al. (2012) The APC/C activator FZR1 coordinates the timing of meiotic resumption during prophase I arrest in mammalian oocytes. Development 139: 905–913.
30. Homer H, Gai L, Carroll J (2009) A spindle assembly checkpoint protein functions in prophase I arrest and prometaphase progression. Science 326: 951–954.
31. Sutton-McDowall ML, Gilchrist RB, Thompson JG (2010) The pivotal role of glucose metabolism in determining oocyte developmental competence. Reproduction 139: 685–695.
32. Wang Q, Chi MM, Moley KH (2012) Live imaging reveals the link between decreased glucose uptake in ovarian cumulus cells and impaired oocyte quality in female diabetic mice. Endocrinology 153: 1984–1989.
33. Van Blerkom J, Davis PV, Lee J (1995) ATP content of human oocytes and development potential and outcome after in vitro fertilization and embryo transfer. Hum Reprod 10: 415–424.
34. Wu X, Viveiros MM, Eppig JJ, Bai Y, Fitzpatrick SL, et al. (2003) Zygote arrest 1 (zar1) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. Nat Genet 33: 187–191.
35. Harra K, NakayamaKI, Nakayama K (2006) Geminiin is essential for the development of preimplantation mouse embryos. Genes Cells 11: 1281–1293.
36. Reik W, Dean W, Walter J (2001) Epigenetic reprogramming in mammalian development. Science 293: 1089–1093.
37. Santos F, Hendrich B, Reik W, Dean W (2002) Dynamic reprogramming of DNA methylation in the early mouse embryo. Dev Biol 241: 172–182.
38. Mayer W, Nivealea A, Walter J, Fundele R, Haaf T (2000) Demethylation of the zygotic paternal genome. Nature 403: 501–502.
39. Oswald J, Engemann S, Lane N, Mayer W, Oell A, et al. (2000) Active demethylation of the paternal genome in the mouse zygote. Curr Biol 10: 475–476.
41. Iqbal K, Jin SG, Pfeifer GP, Szabo PE (2011) Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine. Proc Natl Acad Sci U S A 108: 3642–3647.

42. Howell CY, Bestor TH, Ding F, Latham KE, Mertineit C, et al. (2001) Genomic imprinting disrupted by a maternal effect mutation in the Dnmt1 gene. Cell 104: 829–838.

43. Ratnam S, Mertineit C, Ding F, Howell CY, Clarke HJ, et al. (2002) Dynamics of Dnmt1 methyltransferase expression and intracellular localization during oogenesis and preimplantation development. Dev Biol 245: 304–314.

44. Reese KJ, Lin S, Verona RI, Schultz RM, Bartolomei MS (2007) Maintenance of paternal methylation and repression of the imprinted H19 gene requires MBD3. PLoS Genet 3: e137.