Detection and quantification of maternal-effect gene transcripts in mouse second polar bodies: potential markers of embryo developmental competence

Ze-Xu Jiao, M.D., Ph.D. and Teresa K. Woodruff, Ph.D.

Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois

Objective: To test the hypothesis that quantification of messenger RNAs originating from the second polar body (PB_2) provides a non-invasive tool for assessing embryo quality.
Design: Prospective study.
Setting: Hospital-based academic research laboratory.
Animal(s): CD1 female mice.
Intervention(s): Metaphase II oocytes obtained from 7- to 8-week-old mice after pregnant mare's serum gonadotropin and hCG priming. After in vitro fertilization, the PB_2 was biopsied from zygote, followed by reverse transcription. Real-time polymerase chain reaction was performed to quantify gene expression levels in single PB_2. The sibling zygotes were continuously cultured to blastocyst stage.
Main Outcome Measure(s): Embryo developmental competence and six maternal-effect gene (*Dnmt1*, *Mater*, *Nobox*, *Npm2*, *Tcl1*, and *Zar1*) transcripts in the PB_2.
Result(s): Second polar body messenger RNA was detected in all candidate genes. Transcripts that were present in greater abundance in the zygote were more likely to be detected in quantitative polymerase chain reaction replicates from single PB_2. Four candidate genes (*Dnmt1*, *Nobox*, *Npm2*, and *Tcl1*) expression levels in PB_2 between two groups (two-cell embryo vs. blastocyts) approached statistical significance.
Conclusion(s): Second polar bodies may contain a representative transcript profile to that of the zygote after fertilization. Differences in gene expression in PB_2 may be potential biomarkers of embryo quality. (Fertil Steril® 2013;99:2055–61. ©2013 by American Society for Reproductive Medicine.)
Key Words: Infertility, meiosis, maternal-effect genes, embryo quality

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Despite remarkable progress in both the clinical and embryologic aspects of assisted reproductive technologies (ART), the live birth rate is still disappointingly low. The ratio of oocytes harvested to live-born babies is approximately 25:1 for young women (1). Eighty-five percent of embryos produced in vitro and transferred to the uterus fail to develop into an infant (2). The utilization of the most competent embryo during IVF is crucial to ensure a successful pregnancy. Currently the morphologic criteria and the standard cytogenetic methods used to select and classify embryo are not sufficient for predicting the IVF outcomes. The lack of reliable predictors of oocyte/embryo developmental competence hampers the effectiveness of ART (3, 4). Thus, there is an

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Z.-X.J.'s present affiliation is Center for Reproductive Medicine, Weill Cornell Medical College of Cornell University, New York, New York.
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Reprint requests: Teresa K. Woodruff, Ph.D., Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, 303 East Superior Street, Lurie Building 10-250, Chicago, Illinois 60611 (E-mail: tkw@northwestern.edu).

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urgent need to identify more objective, predictive, and noninvasive markers to choose the embryos with the highest implantation potential to be prioritized for transfer to the uterus (5).

Development of a mammalian embryo starts with fertilization, the fusion of sperm and egg, and formation of a totipotent zygote. After fertilization, the mouse sperm is incorporated into the cytoplasm of the egg and provides DNA for the male pronucleus, which is essential for egg activation. However, earlier studies have demonstrated that the sperm play no major role in cleavage-stage embryogenesis; the maternal genome controls virtually all aspects of early animal development (6, 7). The most conclusive evidence that stored maternal-effect determinants are required for embryonic developmental competence has come from loss-of-function studies in the mouse. Loss of maternal *Mater* (8), *Zar1* (9), and *Npm2* (10) transcripts causes the arrest of development at embryonic genome activation stage. Embryos derived from *Tcl1*-null females have delayed cleavage-stage progression with decreased fecundity (11). *Nobox* plays an important role in regulation of embryonic genome activation, pluripotency gene expression, and blastocyst cell allocation (12). Assessment of maternal-effect mRNA expression levels, as markers of embryo quality, may provide a way to more objectively assess and predict the developmental competence of the embryo; such an approach may ultimately aid in improving implantation rates in IVF (13). However, because of the drawback that assessing maternal-gene expression profile has the risk of damaging the oocyte during sampling, it is still not known whether maternal-effect molecular markers could be used to predict the developmental competence of the embryo.

A polar body (PB) is the byproduct of an oocyte meiotic division. The first PB (PB_1) is extruded as the oocyte matures and resumes meiosis I before ovulation. The second PB (PB_2) is extruded after fertilization and resumption of meiosis II. There is no clear evidence of the fate of the PB in any mammal, including the mouse, which is the commonly used research model. However, the PB is generally considered as waste material and therefore not essential to embryo development. In recent years the PB has gained prominence because it has been used as a DNA source representative of the oocyte for genetic testing (14). First polar body messenger RNA (mRNA) is also being considered as a proxy for the oocyte in tests for oocyte competence and embryonic viability (15–17). The ability to quantify mRNA in a single PB opens up the possibility that we can detect and compare individual differences in gene expression in the PB without harming the oocyte (18).

The PB_2 is produced by asymmetric cytokinesis approximately 2 hours after sperm–egg fusion, 10 hours before major zygotic transcription in mouse (19). The PB_2 contains approximately 4.5% of the zygote volume and one maternal chromosome set and a hemi-spindle. The PB_2 can be present in the perivitelline space of the developing embryo for several days and usually completely decays before the embryo reaches the blastocyst stage (20, 21). Isolation of the PB_2 after fertilization can be regarded as a noninvasive proxy for cytoplasmic sampling of the oocyte from which the zygote was formed (22).

In this study we have set out to examine the expression of six maternal-effect genes in the PB_2. The candidate genes were chosen on the basis of their important roles in early embryo development in mouse (Table 1). We first compared transcript abundance between PBs and their sibling zygotes and then we tested whether that relative abundance of mRNA transcripts in PB_2 is a marker of embryo developmental competence.

MATERIALS AND METHODS

Animals

We obtained institutional review board permission to perform the animal experiments in this study. CD1 mice were housed and bred in a controlled barrier facility within Northwestern University's Center for Comparative Medicine (Chicago, IL) in a temperature- and light-controlled environment (12 hours light/12 hours dark) and were provided with food and water ad libitum. All mice were maintained in accordance with the policies of Northwestern University's Animal Care and Use Committee and National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Collection and Culture of Metaphase II Oocytes

Metaphase II (MII) oocytes were collected from 7- to 8-week-old female mice after superovulation was induced by IP injection of a single dose (5 IU) of pregnant mare's serum gonadotropin (Calbiochem), followed by the same dose of hCG (Intervet) 46–48 hours later. Cumulus oophorous complexes were recovered from ampullae into Leibovitz L15 medium containing 1% fetal bovine serum 14 hours after hCG administration. Oocytes were dissociated from the surrounding cumulus cells using 0.3 mg/mL hyaluronidase (Sigma) to identify MII oocytes. After the removal of cumulus–corona cells, only those MII oocytes that had extruded a PB were collected. All oocyte and embryo culture was in humidified CO_2 (5% [vol/vol] in air) at 37°C.

Collection of Zygotes after IVF

For IVF of oocytes, motile sperm were prepared from a sperm suspension collected from the cauda epididymis of proven

TABLE 1

Maternal-effect genes selected for testing in individual PBs and sibling zygotes.

Gene	Function	Accession no.
Dnmt1	Necessary for embryogenesis	NM_001199431.1
Mater	Required for normal early embryogenesis	NM_011860.2
Nobox	Regulation of embryonic genome activation, pluripotency gene expression, and blastocyst cell allocation	NM_130869.3
Npm2	Control the activation of specific maternal mRNAs necessary for early embryogenesis	NM_181345.3
Tcl1	Participates in early embryonic development	NM_009337.3
Zar1	Play essential roles during the oocyte-to-embryo transition	NM_174877.3

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