Dielectrophoretic behavior of \textit{in vitro}-derived bovine metaphase II oocytes and zygotes and its relation to \textit{in vitro} embryonic developmental competence and mRNA expression pattern

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Abstract

Selecting developmentally competent oocytes and zygotes based on their morphology is more often influenced by personal judgments and lacks universal standards. Therefore, this experiment was conducted to investigate the rate of development and mRNA level of dielectrophoretically separated oocytes and zygotes to validate dielectrophoresis (DEP) as non-invasive option for selection of oocytes and zygotes. In the first experiment, metaphase II oocytes with (PB\(^+\)) and without (PB\(^-\)) first polar body and zygotes were subjected to DEP at 4 MHz and 450 \(\mu\)m electrode distance and classified into fast, very fast, slow, and very slow depending on the time elapsed to reach one of the electrodes in the electric field. Parthenogenetic activation was employed to monitor the embryonic development of dielectrophoretically classified oocytes. The result revealed that at 6 and 7 days of post-activation, the blastocyst rate of very slow dielectrophoretic PB\(^+\) and PB\(^-\) oocytes was significantly \((P<0.05)\) lower than other groups. Similarly, in zygotes, the blastocyst rate at 7 days post-insemination was higher \((P<0.05)\) in the very fast dielectrophoretic categories when compared with the slow and very slow categories. In the second experiment, mRNA level was analyzed in the very fast and very slow dielectrophoretic PB\(^+\) oocytes and zygotes respectively using the bovine cDNA microarray. The result showed that 36 and 42 transcripts were differentially regulated between the very fast and very slow dielectrophoretic categories PB\(^+\) oocytes and zygotes respectively. In conclusion, dielectrophoretically separated oocytes and zygotes showed difference in the rate of blastocyst development accompanied by difference in transcriptional abundances.

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Introduction

Oocytes retrieved from cattle ovaries collected from slaughterhouse are used as a source for embryo \textit{in vitro} production (IVP). However, these oocytes are extremely heterogeneous in developmental competence and ultimately reduce the efficiency of embryo transfer, nuclear transfer, and the blastocyst yield.

With advancement of IVP, different methods have been employed to select oocytes that yield the highest percentages of blastocysts after fertilization or parthenogenetic activation. The majorities of these methods emphasize on categorization of the cumulus–oocyte complex into different morphological groups to correlate with their developmental competence (Blondin & Sirard 1995, Mayes & Sirard 2001). Moreover, the numbers of blastomeres, the ratio between inner cell mass and trophectoderm cell numbers, metabolic rates, and cryotolerance (Mayes 2002) have been used as indicators of developmental competence of oocytes. Furthermore, selection of oocytes relative to the reproductive status of the animal also increases the \textit{in vitro} development rates (Chohan & Hunter 2003).

Similar to the oocytes, morphological criteria, namely the position of second polar body, the pronuclear morphology, and the nuclear morphology have been employed to correlate with the developmental capacity of zygotes. For instance, development of good-quality
human embryos can be obtained from zygotes with centralized and juxtaposed pronuclear zygotes (Gianaroli et al. 2003).

Although morphological evaluation of oocytes or zygotes is easier to accomplish the routine IVP, selection of competent zygotes based on the morphological parameters, namely polar body enlargement, pronuclear and nuclear morphology (Tesarik & Greco 1999, Gianaroli et al. 2003) requires careful handling and observation under the microscope until both pronuclei and polar body are inspected. Moreover, zygotes with invisible pronuclear (Tesarik & Greco 1999) are excluded from evaluation. Furthermore, morphologically best looking oocytes do not necessarily have the highest developmental competence (Blondin & Sirard 1995) and those oocytes with better cumulus expansions during maturation may not necessarily show the highest blastocyst rate. In addition, morphological criteria are more often influenced by personal judgments and they lack universal standards. Therefore, investigating other non-invasive techniques is required to predict and select competent oocytes and zygotes to increase the efficiency of IVP.

Dielectrophoresis (DEP), the motion of neutral particles due to the application of an external non-uniform electric field, has been a useful non-invasive technique for the extensive manipulation of living cells and DNA (Kadaksham et al. 2004). This method does not require knowledge of cell surface antigens or the use of antibodies, or other reporter molecules to label bioparticles (Burt et al. 1996).

Living cells can be electrically polarized depending on their composition, morphology, and phenotype and frequency of the applied electrical field (Huang et al. 1996). Therefore, different cell types in different physiological states can possess distinctly different dielectric properties that can be utilized for separation (Gascoyne et al. 1992, Wang et al. 1993). DEP has been employed for the separation of dead and live bacteria (Lapizco-Encinas et al. 2004), gram-positive and gram-negative bacteria (Markx et al. 1994), differential analysis of human leukocytes (Yang et al. 2000), manipulation and characterization of red blood cells (Minerick et al. 2003), separation of viable and non-viable cells (Huang et al. 1992), and controlling sperm trajectories to characterize sperm motility and morphology before intracytoplasmic injection (Fuhr et al. 1998). However, the application of DEP in screening of developmentally competent oocytes and zygotes has not been documented. Hence, this experiment was conducted to 1) evaluate the dielectrophoretic behavior of oocytes and zygotes, 2) investigate the relationship between the dielectrophoretic mobility of metaphase II (MII) oocytes and zygotes and their developmental competence, 3) evaluate the size of the blastocyst derived from dielectrophoretically separated zygotes, and 4) investigate the dielectrophoretic behavior of MII oocytes and zygotes in relation to the mRNA expression to generate candidate genes related to the developmental competence.

Materials and Methods

Experiment 1: dielectrophoretic behavior of oocytes and zygotes

Oocyte collection and in vitro maturation

Bovine ovaries were obtained from a nearby slaughterhouse and transported to the laboratory within 2–4 h in a thermos flask containing a 0.9% saline solution supplemented with streptocombin. The cumulus–oocyte complexes (COCs) were aspirated from follicles of 2–6 mm in diameter using a sterilized 5 ml syringe fixed to 18 gauge needle. The COCs were washed thrice with modified parker medium supplemented with 15% estrus cow serum (OCS), 0.5 mM L-glutamine, 0.2 mM pyruvate, 50 mg/ml gentamicin sulfate, and 10 μl/ml follicle-stimulating hormone (Folltropin, Vetrepahrm, Canada, Belleville, Canada). A group of 40–50 COCs were then cultured in 400 μl maturation medium covered with mineral oil (Sigma) in four-well dish (Nunc, Roskilde, Denmark). Maturation was performed for 24 h at 39 °C in a humidified atmosphere of 5% CO2. At the end of maturation, the cumulus cells were removed by vortexing the COCs in 200 μl Dulbecco’s PBS (Sigma) supplemented with 2 mg bovine hyaluronidase enzyme/ml and washed thrice with TCM air (TCM 199 contains L-glutamine and 25 mM HEPES (Sigma)) supplemented with 22 mg/ml pyruvate, 350 mg/ml NaHCO3, 50 mg/ml gentamicin, and 0.1% BSA (Sigma). The oocytes were then classified into two groups based on the presence (PB+) or absence (PB−) of the first polar body for further classification according to their dielectrophoretic behavior.

Dielectrophoretic classification of oocytes and zygotes

Am/phase Lock Generator Model 7056 (Geodätisches Institute Bonn) was used for DEP. The generator was connected to the voltmeter and the dielectrophoretic chamber through coaxial cable 7056. The dielectrophoretic chamber (Krüss, Hamburg, Germany) consists of base plate with electric connection points, an interchangeable microscope slide, two collapsible holders with two electrodes, and adjusting screw for electrodes distance. The two holders were fixed to the base plate by laminated springs. The electrodes are platinum–iridium wire with a diameter of 200 μm and the two electrodes are rod-shaped and parallel to each other. The distance between the electrodes was adjusted using adjusting screw and for this experiment, it was adjusted to 450 μm.

Prior to DEP, the oocytes and the zygotes were washed thrice in 400 μl of 0.3 M sorbitol solution (osmolarity of
300 mOsm and conductivity of 80 μs/cm and pH 7.0) to avoid any incidence of electric shock in the chamber due to contamination of maturation or culture medium. Afterwards, 400 μl of 0.3 M sorbitol solution (osmolarity of 300 mOsm and conductivity of 80 μs/cm and pH 7.0) were decanted onto the microscope slide to submerge the electrodes. Each of the oocytes or zygotes was then placed on the microscope slide midway between the two electrodes. The center precision was determined by scratching the interchangeable microscope slide at the center, which is the midway between the two electrodes. Therefore, every time, the oocytes or the zygotes were placed at the scratched position (center) so as to keep the precision. One oocyte or zygote was treated at a time. A 14 peak-to-peak volt and 4 MHz frequency electric field was applied. This combination of voltage and electric field frequency was selected based on the preliminary experimental results. Moreover, the repeatability of movement of bovine oocytes after repeated exposure in the dielectrophoretic chamber was tested in the preliminary experiment. Accordingly, measurement of the dielectrophoretic behavior of a single oocyte up to ten times exposure in the electric field showed a (mean ± s.e.m.) 5.0 ± 0.3 s for very fast oocyte, 13.7 ± 0.4 s for fast oocyte, 23.5 ± 0.5 s for slow-moving oocytes, and 38.5 ± 0.3 s for very slow oocytes group. The low standard error mean has revealed the repeatability of measurements of a single oocyte in all the four dielectrophoretic categories. Therefore, in this study, the time elapsed by each of the oocytes or zygotes to reach one of the electrodes was recorded by one-time exposure in the electric field and one oocyte or zygote was treated at a time. Oocytes and zygotes were then classified into four different dielectrophoretic groups according to their speed in the electric field (Table 1). The time point to classify PB⁺, PB⁻, and zygotes into different dielectrophoretic groups for monitoring the embryonic development was based on the preliminary experimental results (data not shown).

**Parthenogenetic activation of oocytes and in vitro culture**

Due to low fertilization efficiency of denuded oocytes, parthenogenetic activation was used to assess the developmental competence of dielectrophoretically classified oocytes. For this, the dielectrophoretic categories of PB⁺ and PB⁻ (very fast, fast, slow, and very slow) and the control oocytes were washed thrice with TCM air and were parthenogenetically activated with 400 μl of 5 μM ionomycin and 400 μl of 2 mM 6-dimethylaminopurine. After the end of activation, parthenogenetic-activating agents were removed by washing the oocytes thrice using Charles Rosenkrans 1 (CR1) medium. Following this, the oocytes were cultured in CR1 medium supplemented with 10% OCS, 20 μl/ml Eagle’s basal medium (BME), and 10 μl/ml minimum essential medium (MEM). The culture condition was maintained at 39 °C in an incubator with a humidified atmosphere of 5% CO₂.

**IVP and dielectrophoretic categories of zygotes**

In vitro fertilization was performed as described in Tesfaye et al. (2004). Briefly, a group of 50 matured oocytes were transferred into a four-well dish containing 400 μl fertilization medium. Frozen-thawed spermatozoa were swim-up and added to the fertilization medium at a final concentration of 1×10⁶ spermatozoa/ml. Oocytes were co-incubated with spermatozoa for 20 h in a humidified atmosphere of 5% CO₂ at 39 °C. Afterwards, the cumulus cells were removed by vortexing for 90 s in CR1 culture medium. Cumulus-free zygotes were washed twice in CR1 culture medium and transferred into 400 μl TCM air covered with mineral oil. Following this, the zygotes were washed thrice with the dielectrophoretic emersion medium and subjected to the dielectrophoretic procedure and classified according to their speed of movement into four groups (Table 1). Each dielectrophoretically grouped zygote was incubated in CR1 culture medium supplemented with 10% OCS, 20 μl/ml BME, and 10 μl/ml MEM at 39 °C in a humidified atmosphere of 5% CO₂ to monitor the embryonic development.

**Data collection**

The dielectric behavior of oocytes and zygotes was evaluated by recording the time spent by oocytes and zygotes to reach the electrodes from the centre. The cleavage rate at 3 days of post-parthenogenetic activation (dpa) and the blastocyst rates at 6, 7, 8, and 9 at dpa were recorded. Similarly, for each dielectrophoretic groups of zygotes, the cleavage rate (at 2-day post-insemination (dpi)), the morula rate (at 5 dpi), and the blastocyst rates (at 6, 7, 8, and 9 dpi) were recorded. The diameters of oocytes, zygotes, and blastocysts derived from zygotes

<table>
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<th>Developmental stages</th>
<th>Dielectrophoretic categories</th>
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</tr>
<tr>
<td><strong>PB⁻ oocytes</strong></td>
<td>1–8</td>
</tr>
<tr>
<td>Zygotes</td>
<td>1–6</td>
</tr>
</tbody>
</table>

Control, oocytes and zygotes which were not exposed to dielectrophoresis; s, seconds.

Table 1 Dielectrophoretic categories of oocytes and zygotes depending on the time elapsed to reach one of the electrodes in the electric field.
were measured using image tool Version 3.0 (University of Texas Health Science, San Antonio, TX, USA).

**Experiment 2: transcriptional profiling of dielectrophoretically separated oocytes and zygotes**

In this experiment, the transcriptional abundance of the very fast and very slow dielectrophoretic categories of PB+ oocytes and zygotes was measured using aminooallyl labeling and dye coupling.

**RNA isolation and cDNA synthesis**

mRNA was isolated from each three biological pools of 20–30 very fast and very slow dielectrophoretic categories of oocytes and zygotes using Dynabead Oligo (dT)25 (Dynal Biotech, Oslo, Norway) following manufacturer’s recommendation. Briefly, each pool of oocytes or zygotes was lysated with 50 µl binding buffer (20 mM Tris–HCl (pH 7.5), 1 M LiCl, 2 mM EDTA (pH 8.0)) at 70 °C for 5 min in a water bath. The cell lysate was incubated with 20 µl Dynabead Oligo (dT)25 suspension at room temperature for 30 min. The beads hybridized with mRNA was washed thrice with washing buffer (10 mM Tris–HCl with pH 7.5, 0.15 mM LiCl, 1 mM EDTA with pH 8.0) and mRNA was eluted with 11 µl RNase-free water.

First-strand cDNA was synthesized in a 20 µl reaction volume. For this, 11 µl mRNA were incubated with 1 µl oligo (dT)21 primer, TCT AGT CGA CGG CCA GTG AAT TGTAATACG ACT CAC TATAGG GCG (T)21, for 3 min at 70 °C to which 8 µl RT mix (4 µl of 5× first-strand synthesis buffer (50 mM Tris–HCl (pH 8.3), 75 mM KCl, and 3 mM MgCl2), 2 µl of 0.1 mM dithiothreitol (DTT), 1 µl of 0.3 M dNTP, 0.1 µl of 10 U RNase inhibitor (Promega), and 0.5 µl of 200 U superscriptase II (Invitrogen)) was added. This reaction was incubated at 42 °C for 90 min followed by reaction termination at 70 °C for 15 min.

**Double-strand cDNA synthesis and in vitro transcription**

Double-strand cDNA was synthesized using degenerated oligonucleotide primer (DOP)-PCR master kit (Roche Diagnostics GmbH). For this, 2 µl DOP, 2 µl T7 oligo (dT)23 primer, 40 µl 2× DOP-PCR master mix, and 16 µl RNase-free water were added to 20 µl single-strand cDNA. The mix was then incubated at 95 °C for 5 min, 94 °C for 1 min, and 30 °C for 2 min. After the end of annealing time, the temperature was jumped to 72 °C within 3 min and continued for 15 cycles at 94 °C for 1 min, 62 °C for 1 min, and 72 °C for 2 min. The reaction was terminated after final extension at 72 °C for 7 min and subjected to phenol–chloroform purification.

In vitro transcription was performed using AmpliScribe T7 transcription kit (Epicentre technologies, Oldendorf, Germany). Briefly, 2 µl of 10× reaction buffer, 4 µl dNTP, 2 µl DTT, and 2 µl T7 RNA polymerase were added to the 10 µl double-strand cDNA and incubated at 42 °C for 3.5 h. After the end of incubation, 1 µl DNase was added and incubated further at 37 °C for 30 min. The amplified RNA (aRNA) was purified using RNeasy mini kit (Qiagen) according to manufacturer’s instruction.

**Aminoallyl labeling and dye coupling**

The amplified RNA (aRNA) samples of oocytes or zygotes representing very fast and very slow dielectrophoretic groups were first labeled with aminooallyl dUTP (AA-dUTP) using CyScribe post-labeling kit (Amersham Biosciences). Briefly, 1.5 µl anchored oligo (dT) primer, 1.5 µl random primer, and RNase-free water were added to 2 µg mRNA template to make a 10 µl reaction volume. This reaction was incubated at 70 °C for 5 min and at 25 °C for 10 min consecutively. Following this, 10 µl mix containing 4 µl of 5× CyScript buffer, 2 µl of 0.1 M DTT, 1.5 µl oligonucleotide mix, 1.5 AA-dUTP, and 1 µl CyScript reverse transcriptase were added to the 10 µl reaction volume and incubated at 42 °C for 90 min. At the end of this reaction, 2 µl of 2.5 M NaOH were added and incubated at 37 °C for 15 min to hydrolyze the mRNA template and then 10 µl of 2 M HEPES-free acid were added. Purification of aminooallyl-labeled cDNA was performed using the CyScribe GFX purification kit (Amersham Biosciences). Cy3 and Cy5 dye coupling and purification of the aminooallyl-dUTP-labeled very fast and very slow dielectrophoretic groups of zygotes or oocytes were performed using CyScribe post-labeling kit and CyScribe GFX purification kit (Amersham Biosciences) respectively. Three biological replicates were used for each experiment during hybridization. The dye effect during hybridization was controlled by performing dye swap. The cDNA concentration, Cy3, and Cy5 dye incorporation were measured at 260, 550, and 650 nm wavelength respectively using Ultraspec 2100 pro-u.v./visible spectrophotometer (Amersham Biosciences).

**Target clones**

The ready-made bovine cDNA array (BlueChip, Department of Animal Science, Laval University, Quebec, Canada; Sirard et al. 2005) was used in this study. This chip consisting of more than 2000 clones (4928 spots) was separated into two subarrays. In addition to the target clones, each subarray of the BlueChip consists of nine clones to be used as a negative control, namely vide (32 spots), alien1 (8 spots), alien2 (8 spots), GFP (8 spots), GFP1 (4 spots), GFP1/2 (4 spots), GFP1/4 (4 spots), GFP1/8 (4 spots), GFP1/16 (4 spots) H2O/DMSO (50 spots), and plant (8 spots). The housekeeping genes, namely tubulin (eight spots), ubiquitin (eight spots), and actin (eight spots) were included in each array to be used as positive controls.
Probe preparation and hybridization

Before hybridizing the samples, the arrayed slides were placed in a corning GAPS II slide container containing 50 ml pre-hybridization buffer (0.5 g BSA (Roche) +0.5 ml of 10% SDS +7.5 ml of 20% SSC +42 ml sterile water) and incubated for 20 min at 55 °C. After the end of incubation, the slides were sequentially washed with boiled water, cold water, and isopropanol followed by the centrifugation at 2000 g for 2 min. Immediately before hybridization, dye-labeled probes were dissolved in 55 µl formamide-based buffer (15 µl hybridization buffer + 30 µl of 100% formamide + 10 µl distilled water) to which 2.5 µl yeast tRNA and 2.5 µl human cot-DNA (Invitrogen) were added to avoid non-specific hybridization. After denaturing at 95 °C for 5 min, the mix was hybridized to the array and covered with cover slips (Roth, Karlsruhe, Germany). The hybridized array slide was then placed in the hybridization chamber (GFL, Dülmen, Germany) and incubated at 42 °C for 20 h in darkness. At the end of incubation, the slides were sequentially washed for 10 min with 2× SSC +0.1% SDS, 5 min each with 0.2× SSC and 0.1% SSC buffers, 1 min each with water and isopropanol and centrifuged at 2000 g for 2 min.

Image capture and array data analysis

Array scanning and image analysis were performed using Axon GenePix 4000B scanner and GenePix Pro-analysis software (version 4.0; Axon Instruments, Foster City, CA, USA) respectively. Locally weighted scatter plot smoothing (LOWESS) fit normalization method was applied to normalize the channel intensity using GPROCESSOR 2.0, a free software developed at Yale University (New Haven, CT, USA). From the merged data, a mean log2-transformed value of (Cy5/Cy3) was calculated for the replicates to obtain one value per clone. This result was submitted to statistical analysis for microarray (SAM), a free software developed at Stanford University (Stanford, CA, USA), to obtain the list of differentially expressed genes. Heat map and average linkage hierarchical clustering were performed using the PermutMatrix software (Caraux & Pinloche 2004).

Quantitative real-time PCR

A total of seven and nine differentially regulated genes in the very fast and very slow dielectrophoretic categories of PB⁺ oocytes and zygotes were selected respectively to validate the result of microarray data. Sequence-specific primers (Table 2) were designed using Primer Express v. 2.0 (Applied Biosystems, Foster City, CA, USA). For each primer, a serial dilution of 10⁻¹–10⁹ copy number or molecules was prepared from the plasmid DNA. Quantification reaction was carried out using independent cDNA samples from three biological pools of 30–40 very fast and very slow dielectrophoretic groups of oocytes or zygotes. The real-time PCR was performed in 20 µl reaction volume containing 9 µl of 2.5× RealM asterMix/20× SYBR (Eppendorf, Hamburg, Germany), the specific forward and reverse primers, distilled water, and the serial dilution in ABI PRISM 7000 sequence detection system (Applied Biosystems). The thermal cycling parameter was 2 min at 50 °C, 10 min at 95 °C, 45 cycles of 15 s at 95 °C and 60 s at 60 °C. The transcriptional abundance of the samples was calculated by the relative standard curve method using histone (H2A) as endogenous control.

Statistical analysis

The general linear model procedure of SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) was used to test the significant variation in the cleavage and blastocyst rates by considering dielectrophoretic categories as a fixed effect and the list significant difference t-test was employed to separate means between the dielectrophoretic categories of oocytes or zygotes.

Results

Experiment 1: the dielectrophoretic behavior of oocytes and zygotes

Uniform time point was selected for comparing the dielectrophoretic behavior between PB⁺, PB⁻, and zygotes. For this, the dielectrophoretic behavior data from 948 PB⁺, 468 PB⁻, and 1006 zygotes were considered. The result indicates that 15.7% of PB⁺ oocytes, 13.2% of PB⁻ oocytes, and 15.8% of zygotes reached the electrode within 1–5 s. Furthermore, 30% of PB⁺ oocytes, 24% of PB⁻ oocytes, and 23% of zygotes spent more than 20 s to reach the electrode indicating that PB⁺ oocytes have relatively higher proportion of slow-moving population than the PB⁻ oocytes and zygotes (Fig. 1).

Dielectrophoretic behavior in relation to the size of oocytes and zygotes

A correlation analysis was performed between the diameter of the cell and the speed of dielectrophoretic movement in order to validate whether the size of the cell is influencing the dielectrophoretic behavior of oocytes and zygotes. The result showed that the dielectrophoretic speed has no significant correlation to the diameter of both the PB⁺ (n=49, P=0.118, r=0.23) and PB⁻ (n=63, P=0.75, r=0.63) oocytes, indicating that the movement of the oocytes in the electric field was not related to the size of the cell itself. On the other hand, the speed of zygotes in the electric field tends to show a negative correlation (n=37, P=0.06, r=−0.31) to the size of the cell. Nevertheless, the correlation was not statistically significant (P>0.05).
Dielectrophoretic behavior and developmental competence of oocytes

For investigating the dielectrophoretic behavior of PB⁺ oocytes in relation to developmental competence, a total of 457 oocytes derived from four biological replicates were subjected to DEP, in which 152, 121, 90, and 94 were classified as very fast, fast, slow, and very slow categories respectively. Moreover, 152 PB⁺ oocytes were used as control (not exposed to the electric field). The embryonic developmental competence of these dielectrophoretically classified oocytes and the control group was monitored after parthenogenetic activation. Results have shown that the cleavage rate (mean ± S.E.M.) was 89.9 ± 0.3 in very fast, 91.3 ± 1.3 in fast, 88.5 ± 2.7 in slow, 91.7 ± 6.2 in very slow, and 91.5 ± 0.8 in control groups of PB⁺ oocytes. These differences were not significant (P > 0.05). On the other hand, the very slow moving group had significantly (P < 0.05) lower blastocyst rate when compared with the very fast, fast and slow, and the control groups at 6 and 7 dpa (Fig. 2). However, at 8 and 9 dpa, the blastocyst rate was not statistically different (P > 0.05) but tended to be higher in the very fast dielectrophoretic categories.

Similarly, 210 PB⁻ oocytes obtained from a total of four biological replicates were subjected to the DEP procedure to study their dielectrophoretic behavior in relation to developmental competence, in which 54, 100, 50, and 16 were found to be very fast, fast, very slow and slow groups respectively. Moreover, 95 PB⁻ oocytes were used as control. The in vitro embryonic development of these dielectrophoretic groups indicates that the cleavage rate (mean ± S.E.M.) was 72.3 ± 1.9 in very fast, 77.1 ± 4.3 in fast, 93.3 ± 6.7 in slow, 76.4 ± 1.6 in very slow, and 91.5 ± 0.8 in control groups of PB⁻ oocytes.

Table 2

Sequence of primers used for quantification of differentially expressed genes.

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<th>Gene name</th>
<th>Accession no.</th>
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<th>Development stages</th>
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<td>For: GACAGTGAAAGCGACCTG</td>
<td>193</td>
<td>Zygotes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rev: ATATGAACTTGCTGCTGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPS18</td>
<td>AB098899</td>
<td>For: TTCTGGAGACACCCCACTG</td>
<td>189</td>
<td>Zygotes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rev: GGACGTGAAAGACAGGAAAATA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZP3</td>
<td>NM_173974</td>
<td>For: CCACCAGTGCTGGAACCTG</td>
<td>161</td>
<td>Zygotes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rev: TGCAGGATGCTGCTGCTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2a</td>
<td>NM_178409</td>
<td>For: CACGCTACATGCTAATTC</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rev: CAACGAGCTTTGACAGTCTC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Genes differentially regulated in oocyte and zygote categories.

Dielectrophoretic behavior and developmental competence of oocytes

For investigating the dielectrophoretic behavior of PB⁺ oocytes in relation to developmental competence, a total of 457 oocytes derived from four biological replicates were subjected to DEP, in which 152, 121, 90, and 94 were classified as very fast, fast, slow, and very slow categories respectively. Moreover, 152 PB⁺ oocytes were used as control (not exposed to the electric field). The embryonic developmental competence of these dielectrophoretically classified oocytes and the control group was monitored after parthenogenetic activation. Results have shown that the cleavage rate (mean ± S.E.M.) was 72.3 ± 1.9 in very fast, 77.1 ± 4.3 in fast, 93.3 ± 6.7 in slow, 76.4 ± 1.6 in very slow, and 91.5 ± 0.8 in control groups of PB⁺ oocytes. These differences were not significant (P > 0.05). On the other hand, the very slow moving group had significantly (P < 0.05) lower blastocyst rate when compared with the very fast, fast and slow, and the control groups at 6 and 7 dpa (Fig. 2). However, at 8 and 9 dpa, the blastocyst rate was not statistically different (P > 0.05) but tended to be higher in the very fast dielectrophoretic categories.

Similarly, 210 PB⁻ oocytes obtained from a total of four biological replicates were subjected to the DEP procedure to study their dielectrophoretic behavior in relation to developmental competence, in which 54, 100, 50, and 16 were found to be very fast, fast, very slow and slow groups respectively. Moreover, 95 PB⁻ oocytes were used as control. The in vitro embryonic development of these dielectrophoretic groups indicates that the cleavage rate (mean ± S.E.M.) was 72.3 ± 1.9 in very fast, 77.1 ± 4.3 in fast, 93.3 ± 6.7 in slow, 76.4 ± 1.6 in very slow, and 91.5 ± 0.8 in control groups of PB⁻ oocytes.

![Figure 1](https://www.reproduction-online.org)
in very slow, and 95.5 ± 2.8 in control groups showing significantly ($P < 0.05$) higher cleavage rate in the slow-moving category and the control groups. The blastocyst rate at 6, 7, 8, and 9 dpa was significantly higher in the very fast and fast categories compared with the slow and very slow groups (Fig. 3).

### Dielectrophoretic behavior and developmental competence of zygotes

A total of 940 zygotes from seven biological replications were subjected to dielectrophoretic procedure to study the dielectrophoretic behavior in relation to developmental competence and classified into very fast ($n = 329$), fast ($n = 329$), slow ($n = 97$), and very slow ($n = 245$) groups. Moreover, 323 zygotes were used as control group. After culturing these dielectrophoretic groups, the cleavage rate of zygotes was monitored at 2 dpi. The result showed that the cleavage rate was 74.3 ± 3.1 in very fast, 75.5 ± 7.1 in fast, 71.4 ± 6.7 slow, 72.2 ± 7.6 in very slow dielectrophoretic groups, and 77.0 ± 3.6 in control groups and differences were not significant ($P > 0.05$), indicating that although zygotes have different dielectric properties, separating the zygotes according to their dielectric properties has no significant effect on cleavage rates. The blastocyst rate at 7 dpi was significantly ($P < 0.05$) higher in the very fast (16.1 ± 2.2) than in the slow (9.1 ± 2.5) and very slow (10.6 ± 1.9) groups. However, it was not significantly ($P > 0.05$) higher than the fast (12.2 ± 4.1) and control (12.3 ± 2.7; Fig. 4) groups. The total blastocyst rate at 9 dpi was not significantly ($P > 0.05$) different between the dielectrophoretic categories of zygotes (Fig. 4).

### The dielectrophoretic behavior of zygotes in relation to blastocyst size

The very fast dielectrophoretic categories of zygotes yield significantly ($P < 0.05$) larger sized blastocysts (179.8 ± 1.9 μm, $n = 74$) at 7 dpi compared with fast (168.5 ± 1.8 μm, $n = 68$), slow (168.9 ± 1.7 μm, $n = 12$), very slow (168.8 ± 1.7 μm, $n = 52$), and control (173.7 ± 1.5 μm, $n = 55$) groups. Moreover, the diameter of blastocyst that appears at 8 dpi tends to be bigger in the blastocysts derived from the very fast (173.5 ± 1.9 μm, $n = 40$) group when compared with the fast (170.1 ± 3.7 μm, $n = 26$), slow (172.4 ± 3.9 μm, $n = 12$), and very slow (169.4 ± 2.3 μm, $n = 47$) groups but tends to be lower than the control (179.3 ± 4.0 μm, $n = 30$) group.

### Experiment 2: transcriptional profiling of dielectrophoretically separated oocytes

The microarray analysis between the very fast and the very slow oocytes revealed that out of 2000 transcripts, 713 were detected in both groups. The expression intensities (heat map) of these transcripts are shown in Fig. 5A. Scatter plot diagram was plotted for the log₂ value of Cy5 (very fast oocytes) total intensity against the log₂ value of Cy3 (very slow oocytes). Similar scatter plot was generated for the dye swap by plotting the log₂ value of Cy5 (very slow oocyte) total intensity against the log value of Cy3 (very fast oocytes). The coefficient of determination ($R^2$) for the target and dye reversal
hybridization was 0.941 and 0.933 respectively. Similar value of $R^2$ reveals the absence of the dye effect during the analysis.

The data analysis performed by SAM revealed that out of 713 commonly detected transcripts, 36 genes including the novel transcripts were found to be significantly differentially expressed in the very fast and very slow oocytes. Of these, 31 and 5 were up- and down-regulated respectively in the very fast group when compared with the very slow dielectrophoretic categories of oocytes. The average linkage clustering analysis (Fig. 5B) reveals that there were many subgroups within the up- or down-regulated genes (or clusters) sharing similar expression patterns. Such co-expressed genes may possibly be sharing common biological function. The magnitude of differentially regulated genes revealed by SAM showed that the expression level of eight genes was 3.2–5.0 times, 11 genes was 2.0–2.8 and 12 genes was 1.5–1.9 times higher in the very fast compared with the very slow dielectrophoretic categories of oocytes. Moreover, the expression level of five genes was from 1.7 to 2 times lower in the very fast compared with the very slow dielectrophoretic categories (Fig. 5B). The functions of differentially expressed transcripts were determined using gene ontology (http://www.geneontology.org/), NCBI (http://www.ncbi.nlm.nih.gov), and gene cards (http://www.genecards.org/index.shtml). Accordingly, 63.9% of the differentially regulated genes have known function involving in rRNA binding and structural constituent of ribosome, cell cycle regulation, protein and ion binding, metabolic regulation, including amino acid metabolism and other different functions (Fig. 6). The lists and functions of all differentially regulated genes are indicated in Tables 3 and 4.
Table 3 List of upregulated genes in the very fast dielectrophoretic categories of PB⁺ oocytes and their function.

<table>
<thead>
<tr>
<th>Accession no.</th>
<th>Gene bank name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC000047</td>
<td><em>Homo sapiens</em> ribosomal protein L8, mRNA (cDNA clone MAGE:3504599) (RPL8)</td>
<td>rRNA binding, structural constituents of ribosome</td>
</tr>
<tr>
<td>XM_372375</td>
<td>Predicted: <em>Homo sapiens</em> similar to 1-aminocyclopropane-1-carboxylate synthase (LOC390110)</td>
<td>Amino acid metabolism, biosynthesis</td>
</tr>
<tr>
<td>BC062798</td>
<td><em>Rattus norvegicus</em> chromosome 6 open reading frame 173 (C6orf173)</td>
<td>Unknown</td>
</tr>
<tr>
<td>XM_230305</td>
<td><em>Rattus norvegicus</em> similar to 2610203E10Rik protein (LOC311218)</td>
<td>Unknown</td>
</tr>
<tr>
<td>XM_601090</td>
<td>Predicted: <em>Bos taurus</em> similar to 1-aminocyclopropane-1-carboxylate synthase (LOC522802)</td>
<td>Amino acid metabolism, biosynthesis</td>
</tr>
<tr>
<td>BC103473</td>
<td><em>Bos taurus</em> similar to RNA-binding motif protein 3 (MGC128854)</td>
<td>RNA binding</td>
</tr>
<tr>
<td>BC004170</td>
<td><em>Homo sapiens</em> polymerase (DNA directed), epsilon 3 p17 subunit (POLE)</td>
<td>Protein binding, epsilon DNA polymerase activity</td>
</tr>
<tr>
<td>NM_174716</td>
<td><em>Bos taurus</em> annexin A2, mRNA (ANXA2)</td>
<td>Calcium- and phospholipid binding</td>
</tr>
<tr>
<td>BC032121</td>
<td><em>Homo sapiens</em> family with sequence similarity 58 member A (FAMS58A)</td>
<td>Regulation of cell cycle</td>
</tr>
<tr>
<td>BC011008</td>
<td><em>Homo sapiens</em> nucleolar and spindle-associated protein 1 (NUSAP1)</td>
<td>Cytokinesis, microtubule binding</td>
</tr>
<tr>
<td>X12877</td>
<td>Bovine mRNA fragment for cytokerin A8 (KRT8)</td>
<td>Protein binding</td>
</tr>
<tr>
<td>U38619</td>
<td>Sus scrota small intestine diadenosine tetraphosphatase (NUDT2)</td>
<td>Tetraphosphatase activity</td>
</tr>
<tr>
<td>BC007751</td>
<td><em>Homo sapiens</em> CDC28 protein kinase regulatory subunit 1B (CKS1B)</td>
<td>Cell division, protein kinase activity</td>
</tr>
<tr>
<td>NM_174788</td>
<td><em>Bos taurus</em> ribosomal protein, large (RPLP2)</td>
<td>rRNA binding, structural constituents of ribosome</td>
</tr>
<tr>
<td>BC003185</td>
<td><em>Homo sapiens</em> coenzyme Q7 homolog, ubiquinone (yeast) (COQ7)</td>
<td>Metabolic regulator</td>
</tr>
<tr>
<td>AY550044</td>
<td>Sus scrota 60S ribosomal protein L35(RPL35)</td>
<td>rRNA binding, structural constituents of ribosome</td>
</tr>
<tr>
<td>AY283766</td>
<td><em>Bos taurus</em> type 4 mucous-type core 2 β-1,6-N-acetylgalcosaminyltransferase (GCNT3)</td>
<td>Acetylgalcosaminyltransferase activity</td>
</tr>
<tr>
<td>AB098748</td>
<td><em>Bos taurus</em> mRNA similar to acidic ribosomal phosphoprotein PO (RPLP0)</td>
<td>rRNA binding and structural constituents of ribosome</td>
</tr>
<tr>
<td>BC030512</td>
<td><em>Homo sapiens</em> CDC91 cell division cycle 91-like 1 (S. cerevisiae) (CDC91L1)</td>
<td>Cell division control, GPI anchor binding</td>
</tr>
<tr>
<td>XM_230305</td>
<td><em>Rattus norvegicus</em> similar to 2610203E10Rik protein (RGG 390314_predicted)</td>
<td>Unknown</td>
</tr>
<tr>
<td>AF036198</td>
<td><em>Bos taurus</em> prostaglandin G/H synthase-2 (PGHS-2) or (PTGS2)</td>
<td>Ion binding, cell motility, and peroxidase activity</td>
</tr>
<tr>
<td>AB099917</td>
<td><em>Bos taurus</em> mRNA similar to 16S RNA clone</td>
<td>Unknown</td>
</tr>
<tr>
<td>AF091090</td>
<td><em>Homo sapiens</em> chromosome 669 unknown mRNA, complete sequence (RP11-82K18.3)</td>
<td>Carboxylase synthase activity</td>
</tr>
<tr>
<td>NM_182651</td>
<td><em>Bos taurus</em> DNA (cytosine 5) methyltransferase 1 (DNMT1)</td>
<td>Zinc ion binding, DNA methylation</td>
</tr>
<tr>
<td>X54703</td>
<td>Bovine mRNA for pancreatic anionic trypsinogen</td>
<td>Unknown</td>
</tr>
<tr>
<td>BC062798</td>
<td><em>Homo sapiens</em> cDNA clone IMAGE: 6495746</td>
<td>Structural constituents of ribosome</td>
</tr>
<tr>
<td>NM_031706</td>
<td><em>Rattus norvegicus</em> ribosomal protein S8 (Rps8)</td>
<td>Structural constituents of ribosome</td>
</tr>
</tbody>
</table>

**Transcriptional profiling of dielectrophoretically separated zygotes**

Similar to the oocytes, six hybridizations (three biological and three technical replicates as dye swap) were carried out between the very fast and the very slow dielectrophoretic categories of zygotes. After performing LOWESS normalization, the scatter plot diagram was plotted for the log₂ value of Cy5 total intensity against the log₂ value of Cy3 as described previously for the oocytes. The coefficient of determination (R²) for the target and dye reversal hybridization was 0.9137 and 0.9274 respectively, showing the absence of the dye effect during the analysis.

A total of 793 transcripts were commonly detected in both zygote groups. The expression intensities (heat map) of these transcripts are shown in Fig. 7A. The SAM analysis confirmed a total of 42 differentially expressed transcripts among all the detected transcripts. Of these, 25 and 17 genes were up- and down-regulated respectively in the very fast zygotes. The average linkage hierarchical clustering, relative fold change difference, and the q (%) value of differentially regulated genes are shown in Fig. 7B.

The function of differentially expressed genes was described using the same source described for oocytes. Accordingly, some transcripts are involved in ion binding (ZNFB5, ZNFS19, and NANO51), regulation of cell cycle (NAP5, DDX10, SMARCA5, and AURKA), and signal transduction (RALA). The lists and functions of all differentially expressed genes are presented in Tables 5 and 6 and their ontological classification is shown in Fig. 8.

Table 4 List of downregulated genes in the very fast dielectrophoretic categories of PB⁺ oocytes and their function.

<table>
<thead>
<tr>
<th>Accession no.</th>
<th>Gene bank name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC083812</td>
<td><em>Homo sapiens</em> 12 BAC RP11-288D9</td>
<td>Amino acid dephosphorylation</td>
</tr>
<tr>
<td>BC039831</td>
<td><em>Homo sapiens</em> intragellar transport 52 homolog (Chlamydomonas), mRNA (cDNA clone MGC:48784 IMAGE:6095634) (IFT52)</td>
<td>Unknown</td>
</tr>
<tr>
<td>BC000852</td>
<td><em>Homo sapiens</em> RAN, member RAS oncogene family (RAN)</td>
<td>Protein binding, transcription coactivator activity</td>
</tr>
<tr>
<td>XM_595660</td>
<td>Predicted: <em>Bos taurus</em> similar to PHD finger protein 22 (LOC517489)</td>
<td>Unknown</td>
</tr>
<tr>
<td>NM_130809</td>
<td><em>Homo sapiens</em> hypothetical protein MGC12103 (LOC133619)</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

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Validation of differentially regulated genes with quantitative real-time PCR

In oocytes, the validation results confirm that seven out of seven transcripts were found to be in accordance with the microarray data indicating higher validity of the expression pattern which was observed in the microarray data. This higher confirmation rate was brought by the fact that differentially regulated genes were selected after it was confirmed by three dye-swap replications. However, the fold change difference obtained by real-time PCR is much higher than the microarray data. In microarray data, the maximum fold change difference between the very fast and the very slow oocytes was fivefold upregulation in the very fast oocytes. Nevertheless, the maximum fold change obtained in real-time PCR was 72 and the minimum was 6 (Fig. 9) and the correlation coefficient of the microarray data and the real-time PCR was 0.458.

Similarly, in zygotes, the real-time PCR results revealed that eight out of nine transcripts were in accordance with the microarray data (Fig. 10). Furthermore, the coefficient of correlation between the microarray result and the real-time PCR was 0.552.

Discussion

Dielectrophoretic behavior of oocytes and zygotes

Understanding the mechanism of dielectrophoretic mobility of oocytes and zygotes enables appropriate validation of DEP technique to be used as one of the non-invasive tools for selecting developmentally competent oocytes or zygotes. The movement of the cells toward the higher electric field was driven by the higher polarizable ability of oocytes and zygotes compared the sorbitol solution used as dielectrophoretic emersion medium (conduction medium). It is also stated that the cell or particle can be pulled toward the higher electric field or repelled away depending on the medium used during DEP (Ramı´rez et al. 2003). As the conductivity of the medium is lower than the cells, the cells are more polarized than the medium and therefore the cells migrate toward the electrode (higher electric fields; Green & Morgan 1997, Goater & Pethig 1998). The movement toward the higher electric field is usually known as a positive DEP (Poh et al. 1981, Ikeda et al. 2005, Lao & Hsing 2005). Therefore, in our experimental setup, the oocytes and the zygotes also exhibited a positive DEP because the medium used during DEP has a conductivity of 80 μs/cm which triggers the oocytes and the zygotes to be more polarizable than the medium.

In view of the dielectrophoretic behavior, both the oocytes and zygotes tended to exhibit a similar dielectric behavior. However, the PB oocytes have relatively higher proportion of slow-moving population when compared with zygotes and PB oocytes (Fig. 1). This may be due to the lower electrical conductivity of the PB oocytes associated with the declining porosity in oocyte membrane during maturation by losing the K permeable channel (Lansman 1983). Moreover, a
be useful to understand the electrical and physiological dielectrophoretic mobility of oocytes and zygotes can maturational process and entered the MII stages. During maturation were the ones which completed the Hence, the oocytes that extruded the first polar body AC150515 Bos taurus BC039858 Homo sapiens BK001637 TPA: NM_004398 Homo sapiens AC058629 (RPS8) Structural constituents of ribosome... properties of the cells. The difference in dielectrophoretic mobility within bovine oocyte and/or zygotes can be driven by their intrinsic property within the cell. As reviewed by many authors, the features of a cell, including the morphology, the cytoplasm content (Arnold & Zimmerman 1988), the conductivity and permittivity of the cytoplasm (Wanichapichart et al. 2002), the membrane morphology and properties...
(Wang et al. 1997), the cell size (Pethig 1991, Goater & Pethig 1998), shape and composition of the plasma membrane, and the cell cytoplasm and nucleus (Archer et al. 1999) can be the major factors resulting in differences in dielectrophoretic mobility. However, in this experiment, it has been shown that the speed of oocytes and zygotes in the electric field was not related to the size of the cells as confirmed by the correlation analysis.

Difference in the cytoplasmic conductivity is the major contributor for the variation in cell conductivity at frequencies higher than 1 MHz (Docoslis & Alexandridis 2002). Hence, bovine oocytes and zygotes with different cytoplasmic contents can possess different tendencies of polarization and speed of migration in the electric field. Furthermore, the variability in the speed within the zygotes can be attributed by difference in the accumulation of ions during and after fertilization. Fertilization causes a constant increase in the ionic conductance of the plasma membrane (Jaffe et al. 1983). The concentration of ions may foster some of the cells to acquire charges and induce dipole. Based on this notation, we can explain that higher ionic concentration on the plasma membranes after fertilization in some of the zygotes may have triggered the motion of the zygotes. Similarly, a report by Arnold et al. (1989) revealed an increase in the cell membrane conductivity of rabbit oocyte when compared with the unfertilized oocyte as a result of changing the membrane structure due to fertilization. This may lead to the conclusion that zygotes which moved very fast in the electric field might be those derived from successfully fertilized oocytes.

**Dielectrophoretic behavior, developmental competence, and mRNA expression of oocytes and zygotes**

Unpublished data in our laboratory on bovine oocytes show that zona-free and zona-intact oocytes have the same moving time in the electric field showing the absence of the zona pellucida effect on the dielectrophoretic mobilities of oocytes. This in turn can suggest that the cytoplasmic content may govern the dielectrophoretic mobility. Therefore, the difference in dielectrophoretic mobility of individual oocytes or zygotes can be driven by variation in the abundance of transcripts in the cytoplasm that can be associated with the modification in morphology of a cell. For instance, Cristofanilli et al. (2002) noted the difference in dielectric properties that have occurred in MCF-7 sublines because of the overexpression of HER-2/neu oncogen. Moreover, after separating cell membrane, dielectric properties of five different cultivated cell lines and human peripheral blood mononuclear cells, it was shown that the gene expression profiles of the post-separated cells were significantly different from those of the pre-separated cell mixtures (Huang et al. 2002). Similarly, the expression analysis in our experiment showed that PTGS2, DNA (cytosine 5) methyltransferase 1 (DNMT1), and ANXA2 (Table 3) were abundant in fast-moving oocytes; and ZNF85, ZNF519, and NANOS1 (Table 5) were enriching very fast zygotes and all these genes are believed to play in ion binding. In addition, IQGAP1, which is enriched in the very fast dielectrophoretic zygotes, is involved in cell motility. As reported by Mataraza et al. (2003), the overexpression of IQGAP1 in mammalian cells can enhance cell migration and cell motility. However, the involvement of IQGAP1 in passive migration of oocytes in the electric field needs further investigation.

In addition to investigating the electrical behavior of the bovine MII oocytes and zygotes, looking into the developmental behavior is paramount important to predict the validity of the dielectrophoretic technique as the one non-invasive option for selecting...
Dielectrophoretic separation of oocytes/embryos

Figure 10 Relative abundance of differentially regulated genes in the very fast versus very slow dielectrophoretic categories zygotes.

Developmentally competent oocytes and zygotes. In this regard, our result revealed a significant difference in the rate of development at 6 and 7 dpa of dielectrophoretic separated PB+ oocytes and at 6, 7, 8, and 9 dpa of PB− oocytes. Furthermore, at 7 dpi, the very fast dielectrophoretic categories of zygotes yielded significantly higher number of blastocysts compared with the very slow dielectrophoretic groups of zygote. Therefore, this variation in the rate of development of oocyte and zygote of different dielectrophoretic categories is an indicator of the tendency of dielectrophoretic procedure to discriminate oocytes and/or zygotes according to the physiological status.

The critical point remaining as a challenge is to find out the fundamental causes which trigger the very fast moving dielectrophoretic categories of PB+ oocytes to become developmentally competent at 6 and 7 dpa compared with the very slow moving counterpart, despite the total blastocyst yield at 9 dpa, which was not significantly different.

In living cells, transmembrane enzymes can likely absorb free energy from an oscillating electric field and transduce it to chemical energy (Westerhoff et al. 1986). Similarly, the application of a 1 or 10 Hz electric field to human hepatoma cells was found to induce a fourfold increase in Ca2+ within 30 min of continuous field exposure (Cho et al. 1999). However, these assertions cannot signify the differences for developmental competence of the very fast and very slow dielectrophoretic groups of oocytes and zygotes as both groups were exposed to electric field. Therefore, investigating their molecular basis was essential to evaluate the differences in the cytoplasmic content. In order to get insight of the molecular differences between these groups of oocytes and zygotes, transcriptional analysis of the very fast and very slow oocytes or zygotes was performed using the bovine cDNA microarray.

The transcriptional abundance and developmental competence of oocytes has been assessed by many authors (Lonergan et al. 2003, Lequarre et al. 2004). High rates of RNA and protein synthesis, storage of ribosome mRNAs and protein in ooplasm during the oocyte growth are needed in high developmental capacity (Eichenlaub-Ritter & Peschke 2002). Since the molecular mechanism related to the development involves mRNA and protein synthesis, degradation and modification are required to enrich the oocyte with sufficient molecular stores for triggering the development to the start of embryonic genome activation (Donnison & Pfeffer 2004). Similarly, our microarray experiments showed that 86 and 60% of the differentially regulated genes were found to be more abundant in the very fast moving oocytes and zygotes respectively revealing the differences in the accumulation of specific transcripts in oocytes and zygotes with different developmental competences.

Among the transcripts enriching the very fast moving oocytes, RPL2, RPL8, RPL35, RPLP0, LOC52280L, and LOC390110 (Table 3; Fig. 5B) are known to be involved in amino acid metabolism, biosynthesis biological process, and encode ribosomal protein.

ANXA2, a Ca2+- and phospholipid-binding protein (Dreier et al. 1998, Filipenko et al. 2004), found to be higher in competent oocytes (Costa et al. 2006), in virally transformed cell lines and in human tumors (Filipenko et al. 2004) and involved in ion channel activity (Burger et al. 2006). Similarly, ANXA2 was abundant in the very fast moving categories of oocytes which exhibited higher blastocyst rate at 6 and 7 dpa.

Others, namely CDC91L1, NUSAP1, FAM58A, and CKS1B which are known to be involved in cell division were also upregulated in the very fast dielectrophoretic categories (Table 5). For instance, cells transfected with CDC91L1 gene grew at a faster rate and had increased anchorage-independent growth capability compared with control cells (Guo et al. 2004). Furthermore, the suppression of NUSAP1 in a cell by RNA interference has shown mitotic defects, aberrant mitotic spindles, defective chromosome segregation, and cytokinesis that interfere with normal cell cycle progression (Raemaekers et al. 2003). In addition, these authors noticed the upregulated NUSAP expression in proliferating cells during G2/M-phase of the cell cycle. The higher expression pattern of these genes in the very fast dielectrophoretic oocytes might have fostered the rate of embryonic development at days 6 and 7 dpa due to co-ordinated and proper cell programing and cell division compared with the very slow groups where these genes are downregulated.

Similarly, differentially expressed genes, including NASP, DDX10, IQGAP, SMARCA5, RGS2, and AURKA (aurora kinase A) were more abundant in the very fast zygotes that exhibited significantly higher blastocyst rate at 7 dpi compared with the very slow dielectrophoretic categories. These genes are believed to be involved in cell division and progression, transcriptional regulation, and other similar functions. For instance, the nuclear auto-antigenic sperm protein (NASP), a homolog of the N1/N2 gene expressed in oocytes of Xenopus laevis, was found in all dividing cells that is regulated by the cell cycle (Richardson et al. 2000). Moreover, the human somatic and testis NASP mRNAs are expressed in all the transformed cell lines and human tumors revealing the
involvement of NASP in the cell cycle of dividing cells (Richardson et al. 2001).


SMARCA5, which contains an open reading frame of 3156 nucleotides encoding a 1052 amino acid peptide (Aihara et al. 1998), also upregulated in the very fast moving zygotes and plays a central role in eukaryotic transcriptional regulation (Mohrmann et al. 2004).

IQGAP binds calmodulin, E-cadherin and involves a crucial role in transducing Cdc42 signaling to the cytoskeleton (Sokol et al. 2001, Swart-Mataraza et al. 2002). The overexpression of IQGAP1 can significantly increase the amount of active Cdc42 in embryonic cells which in turn control the organization of the cytoskeleton, cell cycle progression, and gene transcription (Sokol et al. 2001). The higher expression of IQGAP in the very fast zygote may be one of the transcripts for fast development of the very fast zygotes compared with the very slow zygotes. Moreover, RGS2, a regulator of G-protein signaling family member (Zmijewski et al. 2001), possibly involved in regulating the intracellular Ca$^{2+}$ mobilization and T-cell proliferation at the maternal–fetal interface during embryo implantation (Huang et al. 2003). The higher expression level of this gene in the very fast moving zygotes exhibited higher rate of development at 7 dpi may indicate the involvement of RGS2 in growth and development of pre-implantation embryo.

The very fast zygotes are also enriched with AURKA. It is involved in cell cycle control by regulating M-phase events. In mammals, AURKA is located at the mitotic spindle to regulate the centrosome and mitotic microtubules (Udayakumar et al. 2006). Furthermore, it is believed to be involved in mitotic control (Privé & Giet 2003), which means that the AURKA is required for centrosome maturation, proper chromosome segregation, and assembly and maintenance of a bipolar spindle (Satinoever et al. 2006).

DNMT1 is one of the genes enriching both the very fast moving oocytes and zygotes. DNMT1 is thought to maintain genomic methylation through DNA replication (Grohmann et al. 1997). The loss of phenotype associated with reduction in DNMT1 has been reported to reveal the importance of DNMT1 for mammalian cell development and growth. For instance, the loss of DNMT1 from somatic cells causes p53-dependent apoptosis (Jackson-Grusby et al. 2001). Depletion of DNMT1 from X. laevis embryos causes embryonic lethality and inappropriate gene expression (Stancheva & Meehan 2000). Similarly, the lower level of blastocyst of the very fast oocytes at 6 and 7 dpa and at the very slow zygotes may be attributed by deficient reprogramming and inefficient demethylation as a result of low level of DNMT1.

In conclusion, the difference in the rate of development between the dielectrophoretically categorized oocytes and zygotes is accompanied by the differences in transcriptional abundance. Furthermore, the results showed the potential of DEP to be applied as a non-invasive tool to screen developmentally competent oocytes and embryos.

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