Effects of ooplasm transfer on paternal genome function in mice

Cheng-Guang Liang1, Zhiming Han1, Yong Cheng1, Zhisheng Zhong1, and Keith E. Latham1,2,3

1The Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, 3307 North Broad Street, Philadelphia, PA 19140, USA 2The Department of Biochemistry, Temple University School of Medicine, 3307 North Broad Street, Philadelphia, PA 19140, USA 3Correspondence address. 3307 North Broad Street, Philadelphia, PA 19140, USA. Tel: þ1-215-707-7577; Fax: þ1-215-707-1454; E-mail: klatham@temple.edu

BACKGROUND: The ooplasm plays a central role in forming the paternal pronucleus, and subsequently in regulating the expression of paternally inherited chromosomes. Previous studies in mice have revealed genetic differences in paternal genome processing by ooplasm of different genotypes. Ooplasm donation coupled to intracytoplasmic sperm injection (ICSI) has been used in human assisted reproductive technology (ART). This procedure exposes the developing paternal pronucleus to 'foreign' ooplasm, which may direct aberrant epigenetic processing. The potential effects of the foreign ooplasm on epigenetic information in the paternal pronucleus are unknown; however, some human progeny from ooplasm donation procedures display abnormalities.

METHODS: In this study, we employed inter-genotype ooplasm transfer followed by ICSI using two mouse strains, C57BL/6 and DBA/2, to explore the influence of foreign ooplasm on paternal pronucleus function. In order to assay for effects on the paternal genome without masking effects of the maternal genome, we examined ooplasm effects in diploid androgenones, which are produced by pronuclear transfer to contain exclusively two paternal sets of chromosomes, in combination with ICSI.

RESULTS: There was no significant effect of intra-strain ooplasm transfer among androgenones made with either C57BL/6 or DBA/2 oocytes. There was a significant negative effect on androgenone blastocyst development with inter-genotype transfer (10% volume) of DBA/2 ooplasm to C57BL/6 oocytes ($P < 0.05$). The reciprocal inter-genotype ooplasm transfer had no significant effect.

CONCLUSIONS: Thus, inter-genotype ooplasm transfer in conjunction with ICSI can alter the function of the paternal genome. However, the effect of foreign ooplasm is restricted to a negative effect, with no evidence of a positive effect. This study provides important new information about the possible consequences of ooplasm donation in human ART.

Key words: ooplasm donation / androgenone / parthenogenone / IVF / ICSI

Introduction

The oocyte cytoplasm plays a fundamental role in creating the embryonic genome from two gamete genomes, and preparing it for transcriptional activation and execution of the developmental program. A key part of this process involves epigenetic modifications during the first few hours after fertilization, when parental genomes are still separated in the distinct pronuclei (PN). These modifications appear to modify or extend epigenetic modifications (e.g. imprinting) established in the gametes, and both kinds of modification (pre- and post-fertilization) are subject to genetic variation (Latham, 1999).

Strain-specific differences exist between C57BL/6 and DBA/2 (denoted as B6 and D2 hereafter) mouse strains with respect to embryonic genome modification. Nuclear transfer (NT) studies have revealed that the oocytes of these two strains differentially modify paternal PN after fertilization, so that androgenetic embryos (two paternal PN, achieved by pronuclear transfer) display very different developmental abilities (Latham and Solter, 1991; Latham, 1994; Latham and Sapienza, 1998). Genetic studies have revealed specific loci (Egm1 and Egm2) that control these processes. Other studies with other mouse strains have revealed other strain-specific effects on genome function in the early embryo (Latham and Solter, 1991; Sapienza et al., 1992; Latham, 1994, 1999; Latham and Sapienza, 1998; Pardo-Manuel De Villena et al., 2000; Pickard et al., 2001). These results are consistent with a model in which the different strains of mice differentially modify the paternal genome after...
fertilization, perhaps to make it compatible with the oocyte epigenotype (Latham, 1999). Disruption of this process could result in altered epigenetic modification of the paternal pronucleus, and an incompatibility with the epigenetic state of the maternal pronucleus, and subsequent epigenetic defects in development.

Studies in mice have reported that the ooplasm genetic strain of origin can exert subtle effects on blastomere fragmentation and that the ooplasm likely interacts with the paternal genome (Han et al., 2002). Other studies have revealed subtle negative effects of ooplasm donation on development in early stage fertilized embryos; however, long-term effects on growth were not manifested (Cheng et al., 2009). These results collectively indicate that the paternal genome may be especially susceptible to epigenetic alteration by foreign ooplasm.

Novel microsurgical procedures in the human, including ooplasm transfer, have been developed and applied for clinical treatment of infertility (Cohen et al., 1997, 1998; Zhang et al., 1999; Barritt et al., 2001a, b; Hawes et al., 2002). One of the innovations involves the augmentation of recipient ooplasm of patients who have repeatedly failed to achieve pregnancy, with ooplasm from a supposedly ‘healthy’ donor oocyte (Cohen et al., 1997, 1998; Barritt et al., 2001a, b). With this procedure, the donor ooplasm is microinjected simultaneously with the sperm (cytoplasm transfer-coupled intracytoplasmic sperm injection, CT-ICSI). A more extreme version of the procedure involves the total ooplasm replacement via germinal vesicle transfer or NT (Zhang et al., 2002). These results indicate that the paternal genome may be especially susceptible to epigenetic alteration by foreign ooplasm.

Materials and Methods

Animals and reagents

Male (C57BL/6 x DBA/2)F1 mice (B6D2) at 10–14 weeks of age (National Cancer Institute, Frederick, MD, USA) were used for spermatogonia zoa collection. Inbred strains B6 (Harlan Aprague-Dawley, Indianapolis, IN, USA) and D2 (The Jackson Laboratory, Bar Harbor, ME, USA) female mice at 8–12 weeks of age were used as a source of MI stage oocytes. Studies adhered to procedures consistent with the National Research Council Guide for the Care and Use of Laboratory Animals. All chemicals used in this study were purchased from Sigma-Aldrich Company (St Louis, MO, USA) unless otherwise noted.

Ooplasm transfer affects paternal genome function

Oocyte collection, embryo construction and embryo culture

Adult B6 and D2 females were superovulated by sequential administration of 5 IU equine chorionic gonadotrophin (Calbiochem, San Diego, CA, USA) and human chorionic gonadotrophin (hCG) 48 h apart. Oocytes were isolated at ~14–15 h post-hCG injection, and cumulus cells were removed by hyaluronidase treatment in HEPES-buffered M2 medium as described (Chung et al., 2002).

Cytoplasm transfer was performed with MI oocytes in M2 medium containing 5 μg/ml cytochalasin B and 0.2 μg/ml demicoline. Approximately 10% of the total oocyte volume was removed from each recipient oocyte. This permitted an equivalent volume of donor ooplasm to be transferred. A 10% volume was chosen to mimic procedures employed in the human (Cohen et al., 1998; Barritt et al., 2001a). The inner diameter of the pipette was 10 μm. Calculating the cylindrical volume of the transfer pipette \( \pi r^2 h \) refers to the radius of the oocyte and pipette, respectively, the length of space in the pipette \( h \) to approximate 10% oocyte volume should be 85 μm, or approximately one oocyte diameter, which was used to estimate the ooplasm volume transferred (Fig. 1A). Ooplasms approximating 10% ooplasm volume were transferred from a donor MI oocyte to the perivitelline space of the recipient oocyte. After recovery in CZB medium (Han et al., 2008) for 1 h, the donor ooplast and recipient oocyte were fused by electrofusion using an electro cell manipulator (BTX, San Diego, CA, USA), with a single DC pulse of 0.9 kV/cm DC for 10 μs in 0.27 M mannitol medium supplemented with 0.1 mM MgSO4, 0.05 mM CaCl2 and 0.3% BSA. One hour after fusion, the fused oocytes were selected and cultured for further use.

Six kinds of oocytes were produced with B6 and D2 oocytes in this study: oocytes without ooplasm transfer (denoted as 100% B6 or 100% D2), oocytes with intra-strain ooplasm transfer (denoted as 90% B6 + 10% B6 or 90% D2 + 10% D2) served as controls and oocytes with inter-strain ooplasm transfer (denoted as 90% B6 + 10% D2 or 90% D2 + 10% B6) used to investigate the effects of ‘foreign’ ooplasm on early embryo development. Each kind of oocyte was used to make androgenones, parthenogenones, and control embryos by in vitro fertilization (IVF) or ICSI techniques (Fig. 1B).

ICSI to obtain fertilized control embryos was performed as described (Yoshida and Perry, 2007) using a piezoelectric micromanipulator (PMM Primer Tech, Ltd, Ibaraki-ke, Japan). ICSI was also performed to construct diploid androgenones with or without additional ooplasm fusion. The spindle–chromosome complex was first removed, and then two sperm heads were injected. Sperm were injected into the oocyte near the...
point of ooplasm transfer, as marked by the notch in the zona remaining after ooplast fusion. IVF embryos were constructed as described (Janssenswillen et al., 1997). Oocyte activation for parthenogenetic control embryos was achieved by 6 h incubation in calcium-free CZB-Glutamine supplemented with 10 mM SrCl₂ and 5 μg/ml cytochalasin B (Janssenswillen et al., 1997; Gao et al., 2004).

Embryos were examined for pronucleus formation at 6 h after sperm injection or oocyte activation, and those with PN were cultured in the CZB medium for 2 days followed by 3 days of culture in Whitten’s medium supplemented with 100 μM EDTA as described (Latham and Solter, 1991; Latham, 1994) and as in previous studies (Abramczuk et al., 1977; Chatot et al., 1989). Embryos were checked at 48, 72, 96 and 120 h post-hCG injection to record the 2-cell, 4-cell, morula and blastocyst development, respectively.

**Figure I** Illustration of ooplast transfers.

(A) Volume of 10% ooplasm of total oocyte. The volume of donated ooplasm can be controlled. The inner radius of pipette used for ooplasm transfer is 5 μm (r), the average radius of a mouse oocyte is 42.5 μm (R). Then the formula is: \( \pi r^2 h = (4/3) \pi R^3 / 10 \), thus \( h = 85 \) μm, which equals the oocyte diameter. (B) C57BL/6 (B6) components are dark-gray and DBA/2 (D2) components are light-gray. Resulting combinations from cytoplasm transfers and denotations are below. Six kinds of cytoplasm combinations were produced with B6 and D2 oocytes. The oocytes without ooplasmic transfer [denoted as 100% B6 (number 1) or 100% D2 (number 6)] and those with intra-strain ooplasmic transfer [denoted as 90% B6 + 10% B6 (number 2) or 90% D2 + 10% D2 (number 5)] were used as controls. The oocytes with inter-strain ooplasmic transfer [denoted as 90% B6 + 10% D2 (number 3) or 90% D2 + 10% B6 (number 4)] were used to investigate the effects of ‘foreign’ ooplasm on early embryo development. Each kind of construct was subjected to make androgenone, parthenogenone, IVF or ICSI embryos.

**Embryo staining, cell number counting and diameter measurement**

After 120 h post-hCG injection, the cultured embryos were stained with Hoechst 33342 (25 μg/ml) for 15 min, and mounted on a glass slide for image record under the microscope equipped with UV light. The images were analyzed with Image-Pro Plus software (Media Cybernetics, Inc., Bethesda, MD, USA) for cell number counting and blastocyst or blastocoel diameter measurement.

**Statistical analysis**

Each group consisted of at least 30 constructed embryos, with a minimum of three replicates for every experiment. The statistical significance of differences in development was evaluated using a \( \chi^2 \) test. Student's
t-test was used for cell number and blastocyst or blastocoel diameter comparison. A probability value \( P < 0.05 \) was considered statistically significant.

**Results**

**Experimental design**

One could postulate that ooplasm transfer in mammalian oocytes should have little or no effect on the formation, processing and subsequent function of the embryonic genome, because this process should be driven largely by the recipient ooplasm, which is present in great excess over the donor ooplasm. However, because genetic differences exist in genome function between unmanipulated embryos of different strains and because the transferred ooplasm might fail to disperse rapidly after transfer, there exists the potential for unexpected effects of the donor ooplasm on embryonic genome function, particularly with respect to the paternal pronucleus, which is extensively remodeled after fertilization. The overall objective of these studies was to determine whether foreign ooplasm of one genotype could either positively or negatively affect the function of paternal genomes within recipient oocytes of an opposing genotype. In order to detect such effects, it is necessary that the paternal genome function be assayed in the absence of any maternal genetic contribution that could mask such effects. The androgenetic mouse embryo provides an ideal model for this analysis, because ooplasm from genetically distinct mouse strains is known to modify the paternal genome differently. Androgenones can be produced readily with and without inter-genotype ooplasm transfer, and the developmental potentials of manipulated embryos can be compared. Our overall experimental design is summarized in Fig. 1B. Oocytes were collected from females of the two strains, B6 and D2. These oocytes are then either subjected to ICSI, IVF or parthenogenetic activation, with or without prior ooplasm exchange. Comparisons of embryos prepared from intra-strain ooplasm transfer oocytes to embryos produced from non-manipulated oocytes provide a control for possible effects of the ooplasm transfer itself. Comparisons between inter- and intra-strain ooplasm transfer embryos provide the ability to assess effects of foreign ooplasm on genome function. Comparisons between androgenones, parthenogenones and fertilized (IVF or ICSI) embryos provide the ability to determine whether the effects of foreign ooplasm are specific for maternal or paternal genomes. Last, it is important to note that for all androgenone, IVF and ICSI studies, the sperm are derived from (B6D2) F1 males, and hence the results are unaffected by genetic variation in the genotype of sperm donor.

**Effect of oocyte genotype on androgenetic, parthenogenetic, IVF and ICSI embryo development**

To determine whether the oocytes derived from B6 and D2 mice have the same capacity to support androgenetic, parthenogenetic, IVF and ICSI embryo development, the developmental efficiency of each kind of embryo was evaluated without ooplasm transfer (denoted as 100% B6 and 100% D2) (Fig. 2). We compared the ratio of embryos attaining each stage. In androgenones, there were no significant differences between B6 and D2 at the 2-cell, 4-cell and morula stages. However, androgenones produced with B6 oocytes showed the ‘higher’ rate (high relative to D2 androgenones) of blastocyst formation, an average of 32.9% (23 of 70), with the majority of these being expanded blastocysts of fairly normal morphology. Androgenones prepared with D2 oocytes developed poorly, with only 9.6% (9 of 94) forming abnormal blastocysts of poor morphology and with rudimentary blastocoels (\( P < 0.0002 \)). Hence, the difference in the blastocyst formation between the two strains was due to differences in progression from the morula to blastocyst stage (Fig. 2A). These results confirmed results from earlier studies of androgenone production by pronucleus transfer (Latham and Solter, 1991).

Parthenogenones constructed with B6 oocytes displayed a higher potential to develop to blastocyst stage compared with D2 oocyte (70.4% (143 of 203) versus 52.9% (164 of 310), \( P < 10^{-10} \)). However, the developmental differences in the parthenogenetic embryos were manifested earlier than with the androgenones, with lesser cleavage beyond the 2-cell stage for D2 parthenotes (94.6% (192 of 203) versus 87.1% (270 of 310), \( P < 0.01 \)) (Fig. 2B).

Embryos constructed with IVF or ICSI methods were also compared between B6 and D2 oocytes. There was no significant difference between these two strains in the development of these embryos from the 2-cell stage to blastocyst stage (Fig. 2C and D).

**Effect of ooplasm donation on androgenetic, parthenogenetic, IVF and ICSI embryo development**

In order to study the effect of ‘foreign’ ooplasm donation on the early developmental efficiency of different kinds of embryos, 10% of the ooplasm was transferred from donor oocytes of one strain to recipient oocytes of the other (denoted as 90% B6+10% D2 and 90% D2+10% B6). As controls, 10% ooplasm was exchanged between oocytes of the same strain (denoted as 90% B6+10% B6 and 90% D2+10% D2).

Among androgenones, intra-strain transfer of B6 ooplasm to B6 oocytes or D2 ooplasm to D2 oocytes diminished the development to the morula stage, but had no significant effect on blastocyst formation compared with the non-cytoplasm transferred groups (Figs 3A and 4A, Table I), indicating that the ooplasm transfer procedure itself was not producing any significant deficiency in development for these embryos. The transfer of 10% ooplasm from D2 to B6 oocytes significantly decreased the 2-cell, 4-cell and blastocyst rates for androgenones, when compared with the control group, in which 10% ooplasm was transferred from B6 to B6 oocytes (2-cell: 57.1% (52 of 91) versus 85.1% (80 of 94), \( P < 10^{-5} \); 4-cell: 48.4% (44 of 91) versus 65.9% (62 of 94), \( P < 0.05 \); blastocyst: 9.8% (9 of 91) versus 31.9% (30 of 94), \( P < 0.0002 \); Fig. 3A). Because the rates of development to both 2-cell and blastocyst stages were lower in androgenones constructed with 90% B6+10% D2 than that of the controls, we tested whether this decrease was the result of a deficiency in progression beyond the 2-cell stage (i.e. ‘2-cell block’), or a deficiency in development thereafter. Normalizing developmental data to the number of 2-cell stage embryos in each group (Fig. 3B) revealed no significant difference at the 4-cell and morula stages, but a significant difference in blastocyst formation as a fraction of 2-cell stage embryos, when comparing inter- and intra-strain cytoplasm transfer androgenones [17.3% (9 of 52) versus 37.5% (30 of 80)
indicating that the deficiency in development was attributable primarily to deficient transition from morula to blastocyst stage. Interestingly, the reciprocal inter-strain transfers, 10% ooplasm from B6 transferred to D2 oocytes, did not enhance D2 androgenone development (Fig. 4A). Thus, whereas D2 ooplasm can exert a dominant negative effect on development in embryos prepared with B6 recipient oocytes, B6 ooplasm was not able to overcome the negative effects of a recipient D2 ooplasm on androgenone development. The effects of inter-strain ooplasm donation on parthenogenetic, IVF and ICSI embryos were also investigated. There were no significant differences between inter- and intra-strain ooplasm donation groups amongst parthenogenetic, IVF and ICSI embryos (Figs 3B–D and 4B–D). The ooplasm transfer protocol itself had no effect on blastocyst formation since we did not find any significant difference between the intra-strain cytoplasm transferred and non-cytoplasm transferred parthenogenetic, IVF and ICSI embryos (Table I).

**Effect of ooplasm donation on embryo quality**

Embryo quality was compared between groups with respect to total embryo cell number, number of cells in embryos attaining the blastocyst stage, average blastocyst diameter and average blastocoel diameter (Table II). Diploid androgenones developed to the blastocyst stage at a high frequency when intra-strain ooplasm transferred B6 oocytes were used (Fig. 5A). These blastocysts were relatively normal in morphology, with larger cell numbers (30.73 ± 2.47 versus 19.36 ± 3.66, \( P < 0.05 \)) (Fig. 5C), larger blastocyst diameters (102.67 ± 7.74 versus 83.94 ± 5.72, \( P < 0.005 \)) and better-expanded blastocoel cavities (69.69 ± 8.94 versus 43.70 ± 6.39, \( P < 10^{-5} \)) than their D2 counterparts (Fig. 5B). Diploid androgenetic embryos formed blastocysts poorly when made with intra-strain ooplasm transferred D2 oocytes (Fig. 5D). The few androgenetic blastocysts that developed from D2 oocytes had very abnormal morphologies, with small blastocoel cavities (Fig. 5E), reduced cell numbers and many excluded cells compared with their B6 counterparts (Fig. 5F). However, for inter-strain ooplasm donated androgenones, 10% ooplasm donation from D2 to B6 oocytes significantly decreased the cell number (Fig. 5I) compared with intra-strain controls (16.57 ± 1.70 versus 26.09 ± 4.59, \( P < 10^{-5} \)), as well as the blastocyst (90.31 ± 3.50 versus 102.67 ± 7.74, \( P < 0.01 \)) and blastocoel (42.36 ± 6.95 versus 69.69 ± 8.94, \( P < 0.001 \)) diameters (Fig. 5H) of the androgenones. In contrast, 10% ooplasm donation from B6 to D2 oocytes did not increase the quality of the androgenones (Fig. 5J–L). A previous study reported cell numbers for non-ooplasm transfer androgenones prepared with C57BL/6 and DBA/2 oocytes as 25 and 15, respectively (Latham and Solter, 1991), similar to our current study. The combined data indicate that inter-strain ooplasm transfer of DBA/2 ooplasm to C57BL/6 oocytes diminished embryo quality for androgenones, whereas the reciprocal inter-strain transfer had no significant effect, and the intra-strain ooplasm transfer had no significant effect. For other kinds of embryos, including parthenogenetic, IVF and ICSI embryos, the inter-strain ooplasm donation had a significant effect on embryo quality (Table II).
Discussion

Abundant data exist to indicate significant epigenetic effects of the ooplasm on the paternal genome during the early post-fertilization period, as well as genetic diversity in these effects (Latham, 1999). Unexpected and undesired epigenetic modifications could thus arise via effects of ‘foreign’ ooplasm of one genotype on the formation of the paternal pronucleus in oocytes of a different genotype, and the subsequent function of those paternal genomes may also be affected. Investigating this possibility is crucial for evaluating the safety of ooplasm donation procedures in humans. We report here the differential effects of foreign ooplasm on paternal genome function following ooplasm transfer combined with ICSI. Our data reveal that ooplasm from D2 oocytes can negatively affect the efficiency and quality of blastocyst development from androgenetic mouse embryos prepared by dual sperm injection into B6 oocytes. These embryos provide a sensitive indicator of paternal genome function,
with no maternal genome present to obscure phenotype. The observed effect on paternal genome function is quite specific. No effect of inter-strain ooplasm transfer is observed in parthenogenetic, IVF or ICSI controls, and no negative effect is associated with intra-strain ooplasm transfer. Additionally, the inter-genotype effect in androgenones is restricted to a negative effect of D2 ooplasm in B6.

**Figure 4** Development of different kind of embryos constructed with D2 oocytes coupled with ooplasm donation.

Androgenone, parthenogenone, IVF and ICSI embryos were constructed with D2 oocytes coupled with intra- (square) or inter-strain (triangle) ooplasm donation. Embryos were checked for the ratio of 2-cell, 4-cell, morula and blastocyst stages at 48, 72, 96 and 120 h post-hCG. (A) Development of androgenetic embryos (number of embryos: 90% D2 + 10% D2 = 106; 90% D2 + 10% B6 = 79; 100% D2 = 94); (B) development of parthenogenetic embryos (number of embryos: 90% D2 + 10% D2 = 204; 90% D2 + 10% B6 = 96); (C) development of IVF embryos (number of embryos: 90% D2 + 10% D2 = 136; 90% D2 + 10% B6 = 70); (D) development of ICSI embryos (number of embryos: 90% D2 + 10% D2 = 105; 90% D2 + 10% B6 = 60). Within the same stage, percentages without a common letter are different (P < 0.05). To compare non-cytoplasm transferred androgenones with intra-strain cytoplasm transferred androgenones, the data of 100% D2 (circle) were reproduced from Fig. 2.

**Table I** Comparison of blastocysts formation between intra-strain cytoplasm transferred and non-cytoplasm transferred embryos

<table>
<thead>
<tr>
<th>Type of embryo</th>
<th>Blastocyst formation*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-cytoplasm transfer</td>
<td>10% intra-strain cytoplasm transfer</td>
</tr>
<tr>
<td>B6 Androgenone</td>
<td>32.9% (23)</td>
<td>31.9% (30)</td>
</tr>
<tr>
<td>Parthenogenone</td>
<td>70.4% (143)</td>
<td>62.1% (110)</td>
</tr>
<tr>
<td>IVF</td>
<td>77.5% (55)</td>
<td>74.4% (90)</td>
</tr>
<tr>
<td>ICSI</td>
<td>65.6% (63)</td>
<td>53.4% (55)</td>
</tr>
<tr>
<td>D2 Androgenone</td>
<td>9.6% (9)</td>
<td>8.5% (9)</td>
</tr>
<tr>
<td>Parthenogenone</td>
<td>52.9% (164)</td>
<td>54.9% (112)</td>
</tr>
<tr>
<td>IVF</td>
<td>85.4% (105)</td>
<td>82.4% (112)</td>
</tr>
<tr>
<td>ICSI</td>
<td>58.6% (99)</td>
<td>57.1% (60)</td>
</tr>
</tbody>
</table>

Numbers in parentheses show the numbers of blastocysts scored.

*Blastocysts defined by the presence of a fluid-filled cavity. Embryos were scored at 120 h post-hCG injection.
that occupied by the injected ooplasm. The studies here approximated the expression of imprinted genes that are required for blastocyst formation, which is mediated by ooplasmic factors and that these modifications affect the paternal genome by the D2 ooplasm, rather than a lack of positive effect of B6 ooplasm. The simplest explanation for these observations is that strain-dependent differences in male pronucleus modifications are a result of aberrant epigenetic processing, rather than a metabolic insufficiency. Knowledge of the specific molecular basis for reduced oocyte quality, to ‘cure’ poor oocyte quality may not be effective in certain instances. We did not observe a significant effect of inter-strain versus intra-strain ooplasm transfer on paternal genome function. Moreover, it appears that this negative effect is long-lasting, as the negative effect of transferred D2 ooplasm indicates that this procedure by injecting sperm nuclei into the region of the transferred ooplasm, as marked by the position of the slit in the zona pelucida. The negative effect of transferred D2 ooplasm indicates that the injected sperm nuclei are negatively affected by the surrounding D2 ooplasm even within a larger volume of recipient B6 ooplasm. Moreover, it appears that this negative effect is long-lasting, as the inter-strain ooplasm effect persists even after normalization to the number of 2-cell stage embryos obtained. As with earlier pronuclear transfer studies (Latham and Solter, 1991), these results support a stable epigenetic effect on paternal genome function. The lack of any beneficial effect of B6 ooplasm on D2 androgenes indicates that negative attributes of the native ooplasm cannot always be overcome by transferring a small amount of positive acting foreign ooplasm. This observation indicates that clinical procedures seeking to ‘cure’ poor oocyte quality may not be effective in certain instances. Knowledge of the specific molecular basis for reduced oocyte quality, therefore, would be needed in order to judge a priori whether ooplasm donation could be beneficial.

We did not observe a significant effect of inter-strain versus intra-strain ooplasm transfer upon parthenogenetic development. Thus, the foreign ooplasm itself does not perturb development, and neither does the foreign ooplasm compromise paternal genome function. The specificity of the foreign ooplasm effect for the paternal genome could reflect an effect upon target genes that are regulated by gamete genomic imprinting, or genes that are differentially modified by the D2 ooplasm, with no reciprocal positive effect of B6 ooplasm observed with D2 oocytes.

Earlier pronuclear transfer studies revealed that the reduced developmental capacity of androgenes made with D2 oocytes was the result of aberrant epigenetic processing, rather than a metabolic insufficiency of the D2 cytoplasm; i.e. paternal genomes that form in D2 oocytes are then transferred to B6 cytoplasm still cannot support the higher rate of blastocyst formation (Latham and Sapienza, 1998). Those earlier studies, however, could not distinguish whether the D2 ooplasmic effect was a negative effect upon the paternal PN, or the absence of a positive effect otherwise imposed by the B6 strain of ooplasm. The studies performed here are distinct from these earlier studies because they test for an effect of a small fraction (10%) of foreign ooplasm, rather than testing an effect of total ooplasm replacement, as with nuclear (including GV or pronuclear) transfer, on paternal genome function. Moreover, the data presented here reveal that the inter-strain difference in androgenone development is the result of a negative or unfavorable modification of the paternal genome by the D2 ooplasm, rather than a lack of positive effects of B6 ooplasm. The simplest explanation for these observations is that strain-dependent differences in male pronucleus modifications are mediated by ooplasmic factors and that these modifications affect the expression of imprinted genes that are required for blastocyst formation and must be expressed from the paternal genome.

Clinical procedures have combined foreign ooplasm injection with ICSI, so that the paternal pronucleus forms in the same region as that occupied by the injected ooplasm. The studies here approximated this procedure by injecting sperm nuclei into the region of the transferred ooplasm, as marked by the position of the slit in the zona pelucida. The negative effect of transferred D2 ooplasm indicates that the injected sperm nuclei are negatively affected by the surrounding D2 ooplasm even within a larger volume of recipient B6 ooplasm. Moreover, it appears that this negative effect is long-lasting, as the inter-strain ooplasm effect persists even after normalization to the number of 2-cell stage embryos obtained. As with earlier pronuclear transfer studies (Latham and Solter, 1991), these results support a stable epigenetic effect on paternal genome function. The lack of any beneficial effect of B6 ooplasm on D2 androgenes indicates that negative attributes of the native ooplasm cannot always be overcome by transferring a small amount of positive acting foreign ooplasm. This observation indicates that clinical procedures seeking to ‘cure’ poor oocyte quality may not be effective in certain instances. Knowledge of the specific molecular basis for reduced oocyte quality, therefore, would be needed in order to judge a priori whether ooplasm donation could be beneficial.

We did not observe a significant effect of inter-strain versus intra-strain ooplasm transfer upon parthenogenetic development. Thus, the foreign ooplasm itself does not perturb development, and neither does the foreign ooplasm compromise paternal genome function. The specificity of the foreign ooplasm effect for the paternal genome could reflect an effect upon target genes that are regulated by gamete genomic imprinting, or genes that are differentially modified between the paternal and maternal genomes post-fertilization, which would mimic the effects of gamete imprinting (Latham, 1999).
Figure 5 Morphology analysis of embryos developed to 120 h post-hCG.

Androgenetic embryos coupled with ooplasm donation were constructed. After culture for 120 h post-hCG, the morphology of each embryo was analyzed with the criterion of ratio of blastocysts formation (A, D, G and J), diameter of blastocyst/blastocoel (B, E, H and K) and cell number (C, F, I and L). The blastocysts derived from parthenogenetic embryos were also analyzed as controls (M–R). Diploid androgenones constructed with 90% B6+10% B6 develop to the blastocyst stage at a high frequency (A). These blastocysts are relatively normal in morphology, with well-expanded blastocoel cavities (B) and larger cell numbers (C). Diploid androgenetic embryos form blastocysts poorly when made with 90% D2+10% D2 ooplasm (D). The few androgenetic blastocysts that develop from D2 oocytes have very abnormal morphologies, with small blastocoel cavities (E), reduced cell numbers and many excluded cells (F). For inter-strain ooplasm donated androgenones, 10% ooplasm donation from D2 oocytes to B6 oocytes significantly decreased the cell number (I), blastocyst and blastocoel diameter (H) of the androgenones. In contrast, 10% ooplasm donation from B6 oocytes to D2 oocytes did not increase the androgenones quality (J–L). For other kinds of constructions such as parthenogenetic embryos, the blastocysts quality is much better than the androgenones (M–R).
We also failed to observe a significant effect of inter-strain ooplasm transfer on the development of IVF or ICSI embryos. Other studies from this laboratory have revealed that a similar ooplasm transfer procedure can yield subtle effects on embryo survival and post-term growth, but not yield pronounced effects in inter-strain versus intra-strain transfers (Cheng et al., 2009). Collectively, the results from parthenotes, ICSI and IVF embryos indicate that the paternal genome may be more highly affected by inter-strain ooplasm transfer than the maternal genome and that the presence of a maternal genome may mitigate the deleterious effects during later stages of development. However, this does not negate the fact that the inter-strain ooplasm transfer can alter paternal genome function and that this alteration could contribute to an altered phenotype after birth. It is becoming increasingly apparent that imprinted genes regulate a wide range of phenotypic characteristics other than growth and morphogenesis (Massa et al., 1992; Brenton et al., 1995; Polychronakos et al., 1995; Davies et al., 2005; Reiner, 2005), any of which could lead to long-term health consequences and possible behavioral or cognitive effects not readily assayed in the mouse model. It is also becoming widely appreciated that early epigenetic effects in the embryo can exert long-lasting effects on a range of characteristics such as imprinted gene expression, behavior, cognition and hypertension (Doherty et al., 2000; Khosla et al., 2001; Ecker et al., 2004; Fernandez-Gonzalez et al., 2004; Mann et al., 2004; Lee et al., 2005; Fetita et al., 2006; Kwong et al., 2006; Sinclair et al., 2007; Chelbi and Vaiman, 2008; Woo and Patti, 2008). Hence, our demonstration here that foreign ooplasm transfer can alter paternal genome function reinforces the view that the potential risks of such procedures have not yet been defined, and thus cannot yet be reliably evaluated for clinical application.

**Acknowledgements**

We thank Bela Patel for her technical assistance. We thank Carmen Sapienza for his helpful comments on the manuscript.

**Funding**

This work was supported by a grant from the National Institutes of Health, National Center for Research Resources (ROI RR18907 and HD 41440).

**References**


Pardo-Manuel De Villena F, de La Casa-Esperon E, Williams JW, Malette JM, Rosa M, Sapienza C. Heritability of the maternal meiotic drive system linked to Om and high-resolution mapping of the Responder locus in mouse. Genetics 2000; 155:283–289.


Submitted on June 3, 2009; resubmitted on July 9, 2009; accepted on July 13, 2009.