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Letter to the Editor
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# Oocytes from female mice on MF1 genetic background are not suitable for assisted reproduction†



Dear Editor,

We write this Letter to share our experiences with applying assisted reproduction technologies (ARTs) to oocytes from mice on the MF1 genetic background.

In 2003, we initiated a long-term collaboration with Paul Burgoyne (Crick Institute, London, UK) and received cryopreserved sperm samples from male mice carrying a Y chromosome mutation ($Y^{Tdym1}$qdel). The mutation arose and was maintained on the MF1 genetic background. In the following years, we received from Burgoyne Lab other MF1 males with various Y chromosome deficiencies as well as male and female wild-type MF1 mice to establish the MF1 colony in Hawaii.

While using infertile MF1 mice in studies requiring ART, we observed that whenever we performed intracytoplasmic sperm injection (ICSI) with oocytes from MF1 females, the resulting zygotes deformed shortly after pronuclei formation and persisted in or expanded this deformity by the two-cell stage (Figure 1A) Thereafter, some embryos recovered and regained normal morphology while other proceeded through the preimplantation development with variable shape deformities or arrested. Although we obtained live offspring after embryo transfer, the efficiency was significantly lower than when spermatozoa were injected into oocytes from females of other strains (Figure 1B) [1].

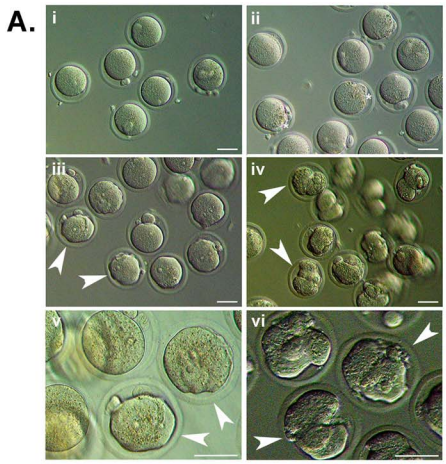
Faced with difficulties maintaining subfertile/infertile mice with Y chromosome deletions on the MF1 background, we tried to prevent embryo deformity and improve ART outcome by modifying experimental approach (Figure 1C). During rederivation of MF1 colony at Burgoyne Lab higher, success rate was achieved with females that were not hormonally stimulated as compared to primed females. We therefore performed ICSI with oocytes from an unprimed MF1 female. All oocytes that became activated after injection (6/7) deformed similarly as ICSI-fertilized oocytes from primed females. The oocytes and embryos from some species are sensitive to light [2]. One of the experiments included in the data shown in Figure 1B was carried out in darkness. All activated oocytes (43/43) deformed ~ 8 h after ICSI and 65% (25/40) of those that reached the two-cell stage remained amorphous. Only one pup was born after transfer of 39 embryos. To exclude a possibility that zygote/embryo deformity was due to the use of injection, we compared two-cell embryos produced by conventional in vitro fertilization (IVF, $n = 24$) and ICSI ($n = 24$), using the same pool of oocytes and sperm from the same male. Almost all IVF- and ICSI-derived embryos deformed. Twelve embryos from each group were transferred, and live offspring rate was similarly poor (ICSI: 0/12, IVF: 1/12). To test whether the presence of sperm is required for embryo deformation, we chemically activated MII oocytes from MF1 females with $SrCl_2$. About half of the oocytes became activated (25/49) and all those deformed (25/25); MII oocytes that failed to become activated and MII oocytes from the same pool that were not treated with $SrCl_2$ remained normal. We also checked chromosome integrity of abnormal zygotes resulting from oocyte activation, and all had normal and intact chromosomes.

The MF1 colony in Hawaii was derived from four wild-type MF1 females sent from Burgoyne Lab in 2004. In 2009, we requested and received 10 more MF1 females. Five of them were tested for oocyte deformation after IVF and compared to the females from our colony. Zygotes from both groups deformed similarly and embryo development in vitro measured as proportion of blastocysts developed from two-cell embryos was similarly impaired (21%, 8/38 and 35%, 16/44 for our colony and newly imported females, respectively, $P = 0.15$). Thus, what we observe is not a defect that arose specifically in Hawaii.

We then investigated in more detail embryo development in vitro. We generated embryos using oocytes from MF1 and B6D2F1 females and sperm from MF1 males, and cultured embryos in the CZB medium that we routinely use and the KSOM medium that we have previously shown to be beneficial for development of embryos from sensitive strains [3] (Figure 1D, Supplementary Table S1). MF1xMF1 embryos had decreased developmental potential when compared to B6D2F1xMF1. Culture in the KSOM medium improved developmental potential of MF1 embryos but did not prevent deformity at the zygote/two-cell stage. Interestingly, the zygotes resulting from in vivo fertilization after mating of primed females and males did not display deformity when collected at 24 h post-hCG injection. Although at 30 h post-hCG some of these zygotes slightly deformed, all recovered by the two-cell stage, and most developed to blastocyst in vitro (Figure 1D). The efficiency of in vivo fertilization was higher after mating of B6D2F1 mice than MF1 mice (oocytes activated/retrieved: 11/37 vs. 47/47, $P < 0.0001$).

Oxygen tension is known to influence embryo development [4]. We therefore cultured ICSI-derived MF1 zygotes under low (5% $CO_2$/5% $O_2$) and standard (5% $CO_2$/21% $O_2$) oxygen and measured developmental potential in vivo after embryo transfer of two-cell embryos and in vitro after culture to the blastocyst stage (Figure 1E, Supplementary Tables S2–S3). Under both conditions, all or almost all zygotes deformed. Under standard oxygen tension, more embryos recovered from deformity by the two-cell stage. Low oxygen tension positively influenced preimplantation in vitro embryo development but had no effects on live offspring rate.

To test which oocyte component, cytoplasm, or nuclei play a role in embryo deformity, we performed pronuclear transfer between MF1 and B6D2F2 embryos (Figure 1F). Although surgical pronuclear transfer per se had negative effects and resulted in 41% (7/17) oocytes being severely deformed, when two types of oocytes after reciprocal transfer were compared, more deformity was noted when




B.

The results of ICSI with oocytes from MF1 females and cryopreserved sperm from infertile males with severe Y chromosome deletion (9/10NPYq-).

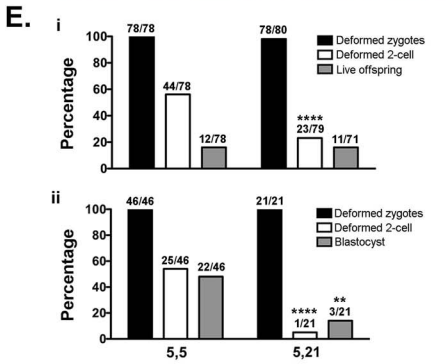
| Sperm source | Oocyte source | No. of oocytes injected* | No. of 2-cell embryos (%)† | No. of 2-cell embryos transferred†† | No. of live offspring (%)§ |
|---|---|---|---|---|---|
| MF1# | B6D2F1 | 163 (5) | 115 (71) | 115 (8) | 53 (46) |
| MF1 | MF1 | 264 (5) | 175 (66) | 162 (11) | 10 (6)[a] |

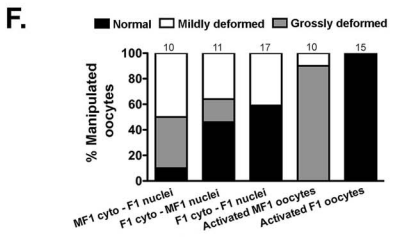
# Data reported in Ward and Burgoyne, Biology of Reproduction, 2006 ([1]. * Numbers in parentheses indicate number of experiments. † Proportions were calculated from oocytes injected. †† Numbers in parentheses indicate number of surrogates. § Proportions were calculated from embryos transferred. Statistical significance (Fisher's Exact test): [a] P<0.0001.

C.

Summary of ART modifications and outcome.

| Factor tested | Modification attempted | Outcome |
|---|---|---|
| Effect of hormonal stimulation | ICSI with oocytes from unprimed female | Deformity occurs |
| Effect of light | Oocytes and embryos manipulation in darkness | Deformity occurs |
| Effect of injection | Side-by-side IVF and ICSI | Deformity occurs |
| Effect of sperm | Chemical activation of oocytes | Deformity occurs |



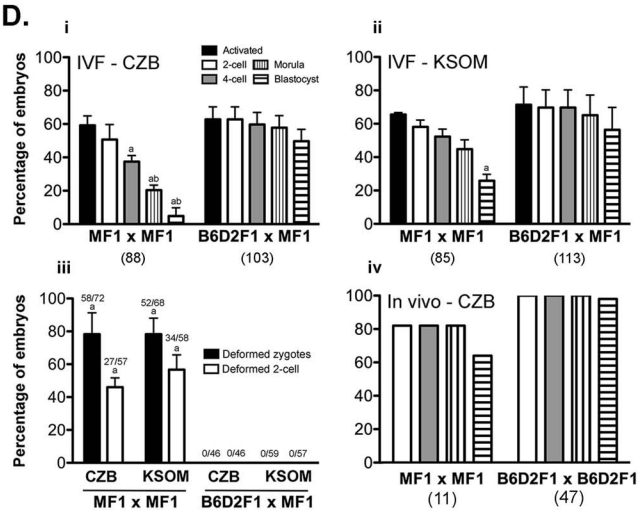




**Figure 1.** Oocytes from female mice on MF1 genetic background are not suitable for assisted reproduction. (A) Exemplary images of embryos produced after in vitro fertilization with gametes from MF1 mice. Metaphase II (MII) oocytes retrieved from MF1 females (i) were in vitro fertilized by sperm from MF1 males. Formation of fertilization cone (*) occurred normally (ii), followed by normal second polar body extrusion and formation of pronuclei. However, as the pronuclei stage progressed, the zygotes began to lose their smooth oval shape (iii, v, arrowheads). The deformity was the most apparent at the two-cell stage, with shape of blastomeres highly irregular and blebbing (arrowheads) (iv, vi). Scale = 50 µm. (B) Comparison of developmental potential of embryos produced with oocytes from MF1 and B6D2F1 females. (C) Summary of ART modifications attempted in order to overcome embryo deformity. (D) In vitro development of in vivo and in vitro generated embryos. Zygotes were obtained after in vitro (i–iii) and in vivo (iv) fertilization and were cultured for 96 h either in CZB (i, iii, iv) or KSOM (ii–iii) culture medium. The percentage of embryos was calculated from oocytes inseminated (i–ii), embryos of a given stage (iii), and activated oocytes retrieved from female reproductive track after mating (iv). The number of oocytes inseminated/retrieved is shown in parentheses under the X axis (i–iii) and the numbers from which percentage was derived are shown above the graphs (iii). In crosses, a female is shown first. The data in i-iii are shown as mean ± SEM, with $n = 3$ independent experiments. The data in iv represent a single experiment. Raw data are shown in Supplementary Table S1. Statistical significance (Fisher's exact test, $P < 0.05$): [a]significantly different than respective developmental stage in B6D2F1 × MF1; [b]significantly different than respective developmental stage in the same cross cultured in KSOM (shown in panel ii). (E) Effect of oxygen tension on in vitro generated MF1 embryos. Zygotes were obtained after in vitro fertilization and were cultured in CZB medium under two different oxygen tensions (5,5: 5% $CO_2$, 5% $O_2$; 5,21: 5% $CO_2$, 21% $O_2$), and were either transferred at the two-cell stage (i) or cultured for 96 h (ii). The percentages of deformed zygotes and deformed two-cell embryos were calculated from zygotes and two-cell embryos obtained, respectively. The percentages of live offspring and blastocysts were calculated from two-cell transferred or cultured, respectively. The numbers from which percentages were derived are shown above the bars. The data in i and ii are derived from two and three independent experiments, respectively. Raw data are shown in Supplementary Tables S2 and S3. Statistical significance (Fisher's exact test): different than respective category in 5,5: $^{**}P < 0.01$; $^{****}P < 0.0001$. (F) Pronuclear transfer. B6D2F1 (F1) and MF1 MII oocytes were chemically activated. Between 4 and 5 h post-activation, pronuclei (MF1 and F1 nuclei) were removed and transplanted reciprocally to enucleated MF1 or F1 oocytes (MF1 cyto or F1 cyto). F1 nuclei transplanted to F1 cyto served as a control for surgery effects and activated unmanipulated MF1 and F1 oocytes served as positive and negative control for deformity. Twenty-four hours after activation embryos were scored as normal, mildly deformed (one or more cytofragments smaller in size than the size of polar body and/or evident blebbings), and severely deformed (more small-sized fragments or fragments of larger size with total volume of fragments comprising an approximately equivalent of one-half of one blastomere of greater). The data represent pooled data from two independent experiments. The numbers above the graphs show the number of oocytes from which percentages were derived.

the B6D2F1 nuclei were transferred to the MF1 cytoplasm. This suggests that deformity originates from MF1 cytoplasm rather than nuclei. It has been shown that deformation of the zygotic cortex occurs in both nucleate and anucleate halves of bisected zygotes indicating that morphogenetic changes do not depend on cell nuclei [5]. It seems plausible that those cortical deformities are caused by abnormal regulation of actomyosin cytoskeleton enriched in the embryonic cortex. The fragmentation may be a consequence of cortical deformation caused by the loss of normal interplay between the spindle complex and cortical microfilaments [6]. In zygotes produced by IVF or ICSI with MF1 oocytes, and in chemically activated MF1 oocytes, the interplay between the microtubular and actin cytoskeleton may also be disturbed, possibly due to unique susceptibility of this strain to the artificial culture conditions, resulting in cortical deformation and fragmentation.

Together, our data point to unusual sensitivity of oocytes from MF1 females, and zygotes derived from them, to in vitro handling. We do not have a recipe on how to best manipulate these oocytes and embryos. When continuing our investigations of mice with Y chromosome deficiencies whenever possible, we tried to avoid using MF1 females in ART, and if we could not avoid it, we approached ART with awareness that the efficiency will be low. We are sharing our findings to warn those planning to use MF1 females in ART about difficulties they may encounter. Our results may also be of interest to those investigating morphogenesis events during early embryonic development and specifically cytoskeletal changes. Cytoplasmic fragmentations are common in human cleavage stage embryos, are traced primarily to the oocyte, are linked with cytoskeletal disorders, and bear negative clinical consequences [7]. Thus, MF1 mice may become a suitable model for studies on morphogenetic changes in response to environmental fluctuations during culture.

## Conflict of interest

None declared.

## Supplementary data

Supplementary data is available at *BIOLRE* online.

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