Stimulation of mitochondrial embryo metabolism by dichloroacetic acid in an aged mouse model improves embryo development and viability

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Objective: To determine whether supplementation of embryo culture media with a substrate to stimulate mitochondrial activity improves embryo viability and pregnancy establishment in aged mice.
Design: Female mice were superovulated and mated. Zygotes were collected and cultured in either G1/G2 or G1/G2 with 1.0 mM dichloroacetic acid (DCA), a stimulator of pyruvate dehydrogenase complex. Embryos were cultured to the blastocyst stage and transferred into pseudopregnant female mice.
Setting: University research facility.
Animal(s): Swiss female mice 26- to 28-week-old.
Intervention(s): The addition of DCA to the embryo culture media.
Main Outcome Measure(s): Embryo development, total, trophectoderm, inner cell mass (ICM) and epiblast cell number, mitochondrial membrane potential, reactive oxygen species, pyruvate oxidation, adenosine triphosphate (ATP) output, implantation rates, and fetal and placental size and weights.
Result(s): Supplementation of the embryo culture medium with DCA significantly increased blastocyst development rates in vitro, significantly improved total, trophectoderm, and ICM cell numbers and pluripotency of the ICM, significantly increased pyruvate oxidation and ATP output, and significantly increased fetal weights and size comparable to in vivo conditions.
Conclusion(s): This study demonstrates that the addition of DCA to embryo culture media improves mitochondrial output in embryos produced from aged mice. Although DCA itself may be of limited therapeutic value in a clinical setting due to its low threshold of dosage and high toxicity, this proof of concept study does suggest that the addition of a physiological-based mitochondrial stimulator to embryo culture media for aged women may potentially improve IVF outcomes. (Fertil Steril® 2014;101:1458–66. ©2014 by American Society for Reproductive Medicine.)
Key Words: Embryo development, IVF, mitochondrial medicine

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Epidemiological data of the past 20 years have indicated that the age of women at their first pregnancy has increased (1). It is well understood that fertility declines as women age. There is a marked reduction in ovarian reserve and pregnancy rates (PRs) in women >35 years (2). As a result there is an increase in the number of couples seeking infertility treatment (3). Although, at present, there have been significant improvement in PRs after IVF treatment, this increase is almost exclusively present in younger women aged <35 years, whereas live birth rates for women >40 years of age remain low (43% vs. 6%, respectively) (3). Given that PRs are not subject to the same age related decline in women of advanced maternal age when oocytes are obtained from a young donor (4), the reduction in oocyte viability is likely the reason for diminished fertility in older women.

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Increased rates of aneuploidy in oocytes from women >40 years of age significantly contribute to the reduced chances of achieving an ongoing pregnancy (5, 6). However, even when euploid embryos are transferred, the PRs for older women remain lower compared with younger women (3). Interestingly, it has also been shown that oocytes from older women have reduced mitochondrial function (7), with decreased inner mitochondrial membrane charge and energy production that is associated with a subsequent decrease in embryo viability (7). Furthermore, in the mouse model it has been shown that oocytes from older mice have perturbed expression of genes involved in mitochondrial function, as well as those associated with control of oxidative stress (8), concordant with changes seen in oocytes from older women (9). Collectively, these data suggest that impaired mitochondrial output is also associated with advanced maternal age.

Animal models have established that limiting mitochondrial energy output results in decreased rates of blastocyst development, impaired implantation potential, alterations to placental development, and fetal growth rates (10, 11). This study investigated the effect of stimulating mitochondrial metabolism in embryos from aged mice to improve mitochondrial health and increase embryo developmental competency. Dichloroacetic acid (DCA) is an indirect stimulator of the enzyme complex pyruvate dehydrogenase by direct inhibition of the enzyme pyruvate dehydrogenase kinase (12). This activates the oxidation of pyruvate, accelerating the uptake of pyruvate into the tricarboxylic acid cycle (TCA) cycle and increasing adenosine triphosphate (ATP) output (12). Dichloroacetic acid has previously been shown to stimulate mouse blastocyst development when embryos were cultured in a medium lacking physiological metabolic regulators, such as amino acids, vitamins, and chelators, conditions that also reduce mitochondrial function (13). The aim of this study was to use an aged mouse model to establish whether addition of DCA to the embryo culture medium improves development and viability of embryos, and subsequent pregnancy and fetal development.

MATERIALS AND METHODS

Animals

Mice were maintained in a 14:10 light:dark cycle and fed an ad libitum diet for the period of the study. Swiss (SWR/J) female mice aged 26–28 weeks of age were used for this study. We have previously determined that oocytes from mice this age have increased levels of oxidative stress and reduced mitochondrial metabolism as assessed by pyruvate uptake, mitochondrial membrane potential, and reactive oxygen species (Supplemental Table 1, available online). Female mice were given an intraperitoneal (IP) injection of 10 IU pregnant mare serum gonadotropin (PMSG) (Folligon; Invervet), followed 48 hours later with an IP injection of 10 IU hCG (Pregnyl; Organon) to induce ovulation. After the hCG injection female mice were placed with a Swiss (SWR/J) male and successful mating was assessed the next morning by the presence of a vaginal plug. The use and care of all animals used in this study were approved by the Animal Ethics Committee, The University of Adelaide.

Media/Reagents

All reagents and chemicals were obtained from Sigma Aldrich unless specified elsewhere. Dichloroacetic acid is soluble in water and therefore was added during media preparation without the need for a vehicle. Embryos were collected in (MOPS-G1) medium and cultured in published formulations of sequential G1/G2 media (14, 15) with or without 1.0 mM DCA from the zygote stage (Supplemental Table 2, available online). This concentration has previously been used for mouse embryo culture (13) and does not alter the intracellular pH of zygotes ($P>.05$; Table 1). Supplementation with 10 mM DCA resulted in zygote arrest (data not shown) and was therefore eliminated from further experimentation. All media was

TABLE 1

The effect of DCA mitochondrial homeostasis, function, and blastocyst cell number and apoptosis.

	Control	DCA	*P* value
Intracellular pH			
Zygote (2-h culture)	7.23 ± 0.20	7.22 ± 0.30	NS
Pyruvate oxidation			
2-cell (20-h culture, pmol/embryo/h)	0.31 ± 0.05	0.38 ± 0.03	NS
Blastocyst (96-h culture, pmol/embryo/h)	0.17 ± 0.02	0.27 ± 0.03	<.01
Blastocyst (96 h culture, pmol/embryo/per cell)	0.00229 ± 0.0003	0.00373 ± 0.0004	<.01
Mitochondrial membrane potential (20-h culture)			
Area 3 (perinuclear)	1.85 ± 0.04	2.34 ± 0.04	<.01
Area 2 (intermediate)	2.05 ± 0.05	2.46 ± 0.05	<.01
Area 1 (cortical)	2.02 ± 0.06	2.16 ± 0.05	<.01
Oxidative stress (20-h culture)			
Intracellular ROS levels (arbitrary values)	600.8 ± 17.7	264.1 ± 14.1	<.01
ATP (pg/mL)			
Blastocyst (96-h culture)	8.59 ± 0.20	9.20 ± 0.19	.03
Blastocyst (per individual cell)	0.116 ± 0.003	0.127 ± 0.003	.02

Note: All data are expressed at mean ± SEM. Ten zygotes per treatment group for intracellular pH. Twenty embryos at both 2-cell and blastocyst were used for pyruvate oxidation per treatment group per time point. Fifteen two cells were used for mitochondrial membrane potential per treatment group (arbitrary units). Twelve DCA two cells and 14 control two cells were used for oxidative stress. Sixteen DCA blastocysts and 11 control blastocyst were used for adenosine triphosphate (ATP) measure. DCA = dichloroacetic acid; NS = not significant; ROS = reactive oxygen species.

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