Transgenerational effects of binge drinking in a primate model: implications for human health

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Objective: To determine if binge ethanol consumption before ovulation affects oocyte quality, gene expression, and subsequent embryo development.
Design: Binge levels of ethanol were given twice weekly for 6 months, followed by a standard in vitro fertilization cycle and subsequent natural mating.
Setting: National primate research center.
Animal(s): Adult female rhesus monkeys.
Intervention(s): Binge levels of ethanol, given twice weekly for 6 months before a standard in vitro fertilization cycle with or without embryo culture. With in vivo development, ethanol treatment continued until pregnancy was identified.
Main Outcome Measure(s): Oocyte and cumulus/granulosa cell gene expression, embryo development to blastocyst, and pregnancy rate.
Result(s): Embryo development in vitro was reduced; changes were found in oocyte and cumulus cell gene expression; and spontaneous abortion during very early gestation increased.
Conclusion(s): This study provides evidence that binge drinking can affect the developmental potential of oocytes even after alcohol consumption has ceased. (Fertil Steril® 2015;103:560–9. ©2015 by American Society for Reproductive Medicine.)
Key Words: Cumulus cells, granulosa cells, reproduction, fetal alcohol syndrome, transcriptome, cDNA array

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The incidence of binge drinking in the United States continues to increase, and in 2010, occurred in 28% of people aged 18 to 34 years, with an average drink consumption per episode of 9 drinks for men and 5.9 drinks for women (1). On college campuses, 44% of students report binge drinking, which accounts for 91% of alcohol consumed on campuses (2). Overall, 90% of alcohol consumption by underage youth, and 75% of total alcohol consumption, occurs during binge drinking (www.cdc.gov/alcohol/fact-sheets/binge-drinking.htm).

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Heavy alcohol use has been associated with alcohol-related neurodevelopment disorders and fetal alcohol syndrome (FAS) (3). Avoidance of alcohol during pregnancy will prevent FAS, but by the time pregnancies are confirmed, major embryonic events may have already occurred (4). Case reports documenting FAS pregnancies in women that drink ethanol during only the first trimester, or only until a positive pregnancy test (5), support the hypothesis that the early stages of pregnancy are significantly affected by alcohol consumption.

The timing of the origin of FAS has been difficult to determine, in part because of the challenge associated with in vivo studies of the peri-implantation stage of embryonic

development. Recent studies add further weight to the likelihood that effects can arise prior to fetal development. A growing body of evidence in rodent and domestic animal models indicates that maternal nutritional state and other environmental factors influence follicle selection, oocyte quality, and embryo growth, with exposures during the periconceptional period being especially important (6–8). These observations highlight the importance of understanding the effects of maternal exposure, both preconception and periconception.

Only a few studies evaluating the effects of ethanol on oocyte/early embryo function have been performed, all of which have utilized the mouse model. In vitro exposure of murine embryos to ethanol and acetaldehyde impairs embryo development (9). Embryos derived from ovulated oocytes exposed to ethanol in vitro exhibit an elevated rate of nondisjunction of chromosomes during the first mitotic division (10). Follow-up studies of ethanol exposure in vivo, at a time that would ensure exposure of recently ovulated oocytes, resulted in similar levels of nondisjunction that were confined exclusively to the oocyte-derived chromosome set (11). It has long been recognized that drinking as little as 1 ounce of absolute ethanol just twice per week can increase the risk of spontaneous abortion in women (12). These murine studies provide a glimpse into 1 possible mechanism of that effect, but additional mechanisms may apply.

Oocyte growth and maturation is a process that occurs over many months in primates, including humans and monkeys. Therefore, binge ethanol consumption has the potential to affect oocytes well before the time of ovulation and the menstrual cycle in which pregnancy may be initiated. The potential for ethanol to affect meiosis and early mitotic divisions of the embryo has a significant impact on the approach that must be taken to prevent adverse effects of alcohol consumption on pregnancy. Therefore, an important step is to determine whether ethanol affects oocytes and associated follicle cells even before fertilization.

This study reports the effects of ethanol on gene expression patterns in cumulus and mural granulosa cells that have been recovered from rhesus monkey females after at least 6 months of binge ethanol dosing. Using oocytes from these animals, effects on subsequent in vitro embryo growth and survival are demonstrated. After the cycle of controlled ovarian stimulation, the animals were naturally mated, and early pregnancy loss is observed. These data reveal a previously unappreciated severe effect of binge ethanol drinking on oocyte quality and early embryogenesis.

MATERIALS AND METHODS

Adult female rhesus macaques (Macaca mulatta), housed at the California National Primate Research Center, were housed as described elsewhere (13), except that the animals were not socially paired on days when dosing occurred. All procedures for maintenance and handling of the animals were reviewed and approved in advance by the Institutional Animal Use and Care Administrative Advisory Committee at the University of California at Davis. The criteria for selection included age range from 6 to 12 years, history of successful pregnancy, and normal menstrual cycles. The control group consisted of 7 females, age 8.5 ± 3.1 (mean ± SD) years, weighing 8.01 ± 1.78 kg. The treatment group consisted of 9 females, age 8.2 ± 1.9 years, weighing 8.08 ± 1.57 kg. Menstrual bleeding was monitored daily, and body weights were recorded weekly for the duration of study.

Binge Ethanol Treatment

For the ethanol treatment group, 2 days per week, animals were hand-caught, and were administered ethanol in water via a nasogastric tube. A regimen with a gradually increasing dose was used to initiate treatment. Animals were given a low dose the first week, a midrange dose the second week, and a high dose for the remainder of the study. The low, midrange, and high doses of ethanol were 0.75, 1.125, and 1.50 g/kg, respectively. The high-dose level was continued for the remaining treatment time.

The high-dose level was selected to be equivalent, on an alcohol/body-weight basis, to the 4- to 5-drink consumption that usually defines a binge-drinking episode in women. Animals received ethanol treatment for ≥6 months before controlled ovarian stimulation to obtain oocytes, and before natural mating, to assure that all phases of follicular growth and development were exposed to ethanol. Dosing continued through the cycle of controlled ovarian stimulation, but did not occur on the day of, or the day before, oocyte retrieval.

Controlled Ovarian Stimulation, Embryo Culture, and Natural Mating

Oocytes were obtained from ethanol-treated (n = 9) or untreated (n = 6) female rhesus monkeys, by controlled ovarian stimulation with twice-daily injections of human recombinant follicle-stimulating hormone (37.5 IU) for 7 days, and 1,000 IU of human chorionic gonadotropin (hCG) on day 8. On day 9, oocytes were obtained by ultrasound-guided needle aspiration, described in detail elsewhere (14). Oocyte-cumulus complexes were recovered from the follicular aspirate, dissociated, and the oocytes and cumulus cells were processed according to established procedures (15, 16). Granulosa cells were recovered from the follicular aspirate and processed as described elsewhere (17).

Oocytes were observed for maturation status, and a randomly selected subset (usually n = 8) of mature oocytes (MII) were placed in PicoPure (Life Technologies) buffer and stored frozen for gene expression analysis. The remaining oocytes were used for in vitro fertilization and subsequent embryo culture, and blastocyst stage embryos were fixed, stained, and analyzed for differential cell counting, as described elsewhere (14). The percentage of blastocysts was calculated for each animal based on the number of MII oocytes per animal.

After resting for 1 menstrual cycle after oocyte retrieval, females were naturally mated (n = 7 control; n = 9 ethanol) with proven males each month at midcycle until the female became pregnant, or for up to 6 months. Ethanol dosing was discontinued once pregnancy was confirmed by ultrasound examination (18), at approximately 19–20 days of gestation. This time of pregnancy confirmation is similar to