Published in final edited form as:

Reprod Toxicol. 2009 November ; 28(3): 329–334. doi:10.1016/j.reprotox.2009.05.003.

Arsenite Exposure Compromises Early Embryonic Development in the Golden Hamster

Dave Unis,

Department of Biology, Colorado State University-Pueblo, 2200 Bonforte Boulevard, Pueblo, CO 81001

Cassandra Osborne, and

Department of Natural and Environmental Sciences, Western State College of Colorado, Gunnison, CO 81231

Moussa M. Diawara

Department of Biology, Colorado State University-Pueblo, 2200 Bonforte Boulevard, Pueblo, CO 81001

Abstract

The toxicity of arsenite to 8-cell stage hamster embryos was evaluated. Females were superovulated and mated; embryos were collected and grown for 72 hours in culture medium containing vehicle control, 25, 50, 250, 500, or 750 nM arsenite. Morphological observations were taken at 0 and 24 hour increments. A TUNEL assay was used for determining DNA damage. Survival was expressed by the ability to undergo zona escape. The control group had 78% survival and no evidence of deformities. Embryos in the 25, 50 and 250 nM groups had survival rates of 63%, 55% and 27%, respectively. Arsenite exposure caused total embryo lethality, major deformities, complete failure to undergo zona lysis, and significantly higher number of cells with fragmented DNA in embryos at the 500 and 750 nM concentrations. The study underscores the sensitivity of preimplantation stage embryos to the presence of even relatively small amounts of arsenic in luminal fluid.

Keywords

Mesocricetus auratus; arsenic toxicity; zona escape; blastomere; blastocyst morphology; DNA fragmentation; preimplantation embryo

Introduction

Arsenic has long been known to humankind and is found naturally in concentrations of approximately 5 grams per ton of bed rock; however, since the dawn of the industrial age, the concentration of arsenic, particularly the more toxic inorganic forms such as sodium metaarsenite, has been steadily increasing [1]. Arsenic is ranked #1 on the United States Agency for Toxic Substances and Disease Registry priority list of hazardous substances, and is among

Corresponding author: Moussa M. Diawara, Department of Biology, Colorado State University-Pueblo, 2200 Bonforte Boulevard, Pueblo, CO 81001; Tel/FAX: 719.549.2813/2993; email: moussa.diawara@colostate-pueblo.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the US Superfund Top 10 Priority Hazardous Substances considered to pose the greatest hazard to human health [2-4].

Arsenic-induced reproductive toxicity has been known for decades. Lugo et al. [5] reported as early as 1969 a case study of neonatal death associated with maternal consumption of arsenic trioxide. Chronic exposure to arsenic has been associated with decreased birth weight as well as increased spontaneous abortions in women or partners of males working at or living in close proximity of smelters [6]. These spontaneous abortion rates were higher in cases where both the women and males worked at the smelters. There have been several animal studies on the effect of this compound on fetal development. In an *in vitro* study performed on somite stage (8 day gestational stage) mouse embryos, sodium arsenite was shown to have lethal effect at a concentration of 15 μ M and severe malformations in concentrations as low as 4 μ M [7]. Administration of 5 mg/kg arsenite in female hamsters on day 8 of gestation using intraperitoneal (*i.p.*) injection caused abnormalities such as cranioschisis, malformations of the ribs and kidney, or death of the embryos [8,9]. Gestational time point-dependent fetal malformations as well as increased fetal resorptions and reduced fetal body weight as results of exposure of female rodents to arsenic have been reported [10,11]. Navarro et al. [12] showed that administration of 8 or 16 mg/kg sodium metaarsenite in mice by *i.p.* injection every 2 days for 14 days reduced the percentage of embryos able to lyse the zona and become blastocysts. In contrast, Holson and colleagues [13] observed no treatment-related fetal malformations as a result of exposure to arsenic trioxide of female rats when dosed from 14 days prior to mating to gestational day 19.

While the studies mentioned above provide literature on the effects of arsenic on somite stage embryos and preimplantation embryos, they all used an *in vivo* approach to determine dose response levels, with the exception of work by Chaîneau et al. [7] where embryos were harvested for treatment from mice at 8 days of gestation. It is also critical to note that, in 1998, DeSesso and co-workers (14) extensively reviewed existing literature and argued that the majority of research showing relationship between exposure of pregnant experimental animals to inorganic arsenic and reproductive/developmental toxicity were not appropriately designed to yield reliable conclusions. The authors explained that many studies reporting cranial neural tube defects and other forms of malformations in rodents as a result of exposure to arsenic used very high doses and routes (*i.v.* or *i.p.*) unlikely to be seen in a human model. During a series of experiments where they used rodents models, DeSesso et al. (14) and Jacobson et al. (15) examined *i.p.*, gavage, dermal and inhalation routes of arsenic exposure and found no dose-response relationship between exposure to even high doses of inorganic arsenic and neural tube defects or other malformations.

Use of animal models in assessing risk associated with human exposure to xenobiotics must at minimum take into consideration the route of exposure, the dose range, species-related differential metabolism of the xenobiotic, and other factors that may validate use of the model (15). To address these concerns, we evaluated the effects of inorganic arsenic using an *in vitro* model that examines embryos during preimplantation stage of hamster embryo development, using very low doses and a biologically plausible route. We chose the Syrian Golden Hamster (*Mesocricetus auratus*) because it has proven to be a very accurate model [9]. Specifically, we aimed to determine 1) if arsenic exposure compromises embryo morphology and zona escape in a dose-dependent manner; and 2) if arsenic exposure results in DNA damage of embryonic cells.

Materials and Methods

Animal Care and Breeding Techniques

Sexually mature Syrian golden brown hamsters (*Mesocricetus auratus*) were purchased from Charles River Laboratories INC (St Louis MO, USA) and acclimated for at least three weeks on a 14-hour light 10-hour dark schedule, according to guidelines established by the Institutional Animal Care and Use Committee at Colorado State University-Pueblo. After three months of age the females were cycled by checking the postovulatory discharge secreted by the vagina, a method found to be most reliable for cycling hamsters [16]. Once this has been observed, females were labeled as being in day 1 of the 4-day estrous cycle and were injected with 50 IU of Pregnant Mare's Serum Gonadotropin (PMSG, Sigma-Aldrich, St Louis, MO) via *i.p.* injection. On day 4 of this cycle the female was considered in ovulation and mated. Once copulation was observed the female was marked as pregnant. Thus, the next morning was considered day 1 of pregnancy.

Culture Media Preparation

All reagents were purchased from Sigma-Aldrich Chemical Company (St Louis, MO, USA). The Hamster embryo culture media with 2hrs of equilibration in the incubator prior to use (HECM-2h) was prepared as previously described [17]. The media contained 116.5 mM NaCl, 3.2 mM KCl, 2 mM CaCl₂, 0.5 mM MgCl₂·6H₂O, 25 mM NaHCO₃, 10 mM sodium lactate, 0.25 mM sodium pyruvate, 1 mM glutamine, 0.5 mM succinate, 0.1 mg/mL polyvinyl alcohol, 100 IU/mL penicillin G, 3 mg/mL bovine serum albumin (BSA, fraction V), 3 μM pantothenate, 5 μM choline chloride, 3 μM inositol and 0.1 mM MEM (minimum essential medium) nonessential amino acid solution. Sterile procedures were used in all media preparation.

Embryo Culture Techniques

On day 3 of pregnancy, the females were sacrificed and the uterine horns were removed and immediately taken to darkroom conditions. The broad ligament was separated to minimize the amount of red blood cells in the media. Each uterine horn was flushed with 1mL HECM-2 h using a 1mL syringe with a 22 gauge blunt tipped needle to collect embryos. Embryos were rinsed through 2 successive washes in HECM-2 h and transferred to individual treatments. Treatments consisted of complete culture media containing vehicle control (distilled deionized water), 25, 50, 250, 500, or 750 nM sodium metaarsenite (NaAsO₂). We used arsenite because it proved to be more potent than arsenate to rodent embryos [18]. In addition, arsenate (As⁺⁵) is metabolized to arsenite (As⁺³) in humans and many experimental animals (15), and a number of recent studies have reported that inorganic arsenic and its metabolites are readily transferred across the placenta from mother to fetal tissue in human and experimental animals (19-22). The range we used was selected because of the sensitivity of preimplantation stage embryos and considering that sodium arsenite was shown to have lethal effect at a concentration of 15 μM on somite stage (8 day gestational stage) mouse embryos [7]. In addition, inorganic arsenic is poorly metabolized and readily transferred across the placental membrane; in some cases this resulted in a higher concentration of the fetus than the mother [5]. A negative control (lacking bovine serum albumin BSA) was also used in each experiment to ascertain the reliability of the procedure [23].

To prepare the embryos for culture, 40 μL of the treatments or control media was placed in a 35×10 mm Corning center well Petri dish, and the well was overlaid with 1mL of mineral oil. Three to five embryos were injected into each 40 μL media fraction. All groups were maintained at 5% CO₂, 37°C, and 50-75% relative humidity for 72 h. A Nikon Eclipse TS100 inverted microscope interfaced with a camera and a PC was used to photograph embryos at 0 h and at 24 h increments for morphological evaluation. The 72 h growth period was chosen because it has been proven as a normal time frame for 8-cell stage embryos to undergo zona escape *in*

vitro [17]. Eight cell stage embryos were chosen due to their success in development to blastocyst stage, when compared with embryos in the 1- to 4-cell stage [24,25]. Experiments were repeated seven times for statistical purpose.

Morphological Observation

During the morphological examination embryos were monitored at 0 h and at 24-h intervals for classical signs of overall cell degradation [26,27]. These signs include decreased cleavage rates, blastomere shrinkage, disassociation of blastomeres, malformed blastocysts, and lack of zonolytic expression. Survivability of embryos was determined by the embryo's ability to undergo zona lysis, a process required for fetal development; absence of this process ultimately results in abortion [28,29].

Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick End-labeling (TUNEL) Assay

Separate experiments were conducted using embryos from vehicle control and the 500 nM concentration groups to determine if arsenite exposure would result in DNA damage in the embryos. These embryos (four in each group) were incubated for 36 h prior to TUNEL assay. For this assay we used the Dead End™ Fluorometric TUNEL System purchased from Promega INC (Madison, WI). Briefly, the embryos were fixed overnight, then washed and permeabilized in phosphate buffer saline (PBS). They were rinsed and equilibrated. The reaction was begun using Terminal Deoxynucleotidyl Transferase Recombinant enzyme (rTdT) incubation buffer at 37°C for 1 h and terminated by washing with 2× SSC (saline sodium citrate). The embryos were then counterstained with 1× propidium iodine in PBS, washed with DDH₂O, and mounted on a slide. The stained embryos were immediately examined and photographed using a Nikon Eclipse TS100 inverted microscope with a fluorescent filter. All embryos were photographed at 520 nm and at 620 nm for scoring of cells with DNA damage. Blastomeres containing cells with fragmented DNA were observed as having a green-yellow color at 520 nm and exhibiting a bright red fluorescence at 620 nm.

Statistical Analysis

Data on embryo survival during the morphological examination experiments and the number of cells with fragmented DNA during the TUNEL assay were analyzed using one-way analysis of variance [30] between control and treatment groups. Statistically significant means were separated at the 5% level using Protected LSD and presented as mean ± SEM.

Results

Effect of Arsenite Exposure on Embryo Morphology and Zona Escape

Figure 1 shows appearance of embryos in both positive and negative control groups, at the initial 8-cell stage and after 72 h. Embryos in the positive control group exhibited normal appearance from the initial 8-cell extraction stage (Fig. 1: A, B) and displayed very morphotypic azonal blastocysts, characterized by a compacted fully intact mass of cells (blastomeres) with no visible deformities after 72 h (Fig. 1: C, D). The negative control group showed normal 8-cell stage embryos at time of extraction (Fig. 1: E, F); however, 72 h later these embryos had failed to undergo zona lysis and showed visible signs of death characterized by deflated blastomeres and a non-compact cell mass (Fig. 1: G, H).

Appearance of embryos in the different treatment groups at initial stage and 72 h later is shown in Figure 2. Like with controls, there were normal 8-cell stage healthy embryos initially in all treatment groups, regardless of concentration (Fig. 2 A to J). However, by day 3 (72 h), these embryos showed deformities in a dose-dependent manner. Embryos in the 25 nM and 50 nM arsenite treatment groups displayed blastocysts relatively comparable to controls, but an

incomplete zona escape and/or non-compact cell mass were observed in some of these (Fig. 2: K, L, M, N). In the 250 nM arsenite treatment group, the majority of embryos showed deflated blastomeres and intact zona pellucida (Fig. 2: O, P) and what appears to be a small blastocoel stage embryo within them, or a blastocoel like cavity (Fig. 2: O) at 72 h. Others exhibited blastocoel stage embryos with deformities such as a misshapen trophoctodermal layer and unevenly distributed inner cell mass (Fig. 2: P). Embryos exposed to 500 nM arsenite exhibited major signs of toxicity after 72 hr (Fig. 2: Q, R). Nearly all the blastomeres became deflated and there was no visible sign of a blastocyst (Fig. 2: Q). All embryos in the 750 nM group showed extreme signs of deformities after 72 h of exposure; these deformities were characterized by strange “blastocoel stage like cavities” within blastomeres and blastomeres that were almost completely disassociated or disintegrated (Fig. 2: S, T).

Effect of Arsenite Exposure on Embryo Survival

Arsenite exposure affected embryo survival in a dose-dependent manner (Fig. 3). The control group had a $78\% \pm 11$ survival rate ($n = 37$). In the group treated with 25 nM of arsenite, the percentage of embryos surviving (or undergoing lysis of the zona pellucida) was not statistically different from the control group ($63\% \pm 21$; $P < 0.05$; $n = 18$). Embryos in the 50 nM group had a lower survivability rate, but still statistically comparable to that of the control group ($55\% \pm 13$; $P < 0.05$; $n = 21$). However, when the embryos were exposed to 250 nM arsenite, significantly fewer of them hatched ($27\% \pm 7$; $P < 0.05$; $n = 29$). Embryos treated with the 500 or 750 nM concentrations completely failed to undergo zona lysis (0% ; $P < 0.05$; $n = 26$ for 500 nM; $n = 18$ for 750 nM).

Effect of Arsenite Exposure on DNA using TUNEL assay

A significantly higher number of blastomere cells with fragmented DNA were observed in embryos treated with arsenite at 500 nM compared with controls (9.7 ± 3.0 vs. 2.1 ± 1.1 ; $n = 4$; $P < 0.05$). As shown by the arrows, very few blastomeres with damaged DNA were visible in the control embryos (Fig. 4: A, B). In the 500 nM treatment group, however, DNA damage was visible in almost every blastomere of these embryos (Fig. 4: C, D). Furthermore, the fact that nearly all the blastomeres in the 500 nM treatment group had fragmented DNA supports our morphological data and explains the 0% survival rate observed in this group. The study demonstrates that arsenic-induced toxicity to preimplantation stage embryo is due, in part, to DNA damage, based on TUNEL assay. However, it is unclear if the DNA fragmentation of embryonic cells observed during the study is a direct result of apoptosis or necrosis.

Discussion

Our results are consistent with and explain, in part, previous observations of arsenic-induced neonatal death [5] and its implication in decreased birth weight as well as increased spontaneous abortions in women or partners of males working at or living in close proximity of smelters [6]. The study also explains, in part, why chronic exposure of females to arsenic for periods including pregnancy has been linked to conceptus mortality and postnatal growth retardation in a dose-dependent manner [31]. As stated earlier, the low doses (25-50 nM) appeared to have little or no effect on the embryo; however, these conclusions may be misleading due to the fact that exposure in our study was only evaluated up to the preimplantation blastocyst stage. It is unclear whether these low doses could have detrimental effects later during pregnancy. One indicator of this is the lack of compaction of cells of the morula expressed in the low treatment, where there was a dissociation of adhesive cells of the morula (see Fig. 2: K, L). The lack of compaction has been reported to have severe effects on preimplantation embryos, including malformed blastocysts, irregular polarization of blastomeres, and failure of zona lysis [32], all of which have been expressed in our treatments ranging from 250-750 nM. This lack of compaction of morula cells could also adversely impact embryos during post implantation by

causing cardiac dysmorphogenesis and failure of vasculogenesis [33]. Lack of compaction has recently been reported in human aortic endothelial cells exposed to sodium arsenite [34].

In the TUNEL assay we have shown that the control group had fewer blastomeres with fragmented DNA compared with the 500 nM treatment group where nearly every blastomere present was dying. This increase in cell death as a result of arsenite exposure is consistent with *in vivo* experimentation on morula formation stage embryos previously reported by Navarro and colleagues [12]. In addition there have been *in vitro* studies that have also shown a marked increase of apoptosis in preimplantation embryos when exposed to such xenobiotics as *o,p'*-DDT [23]. The increased number of embryos with damaged DNA, as well as failure of zona lysis and deformities associated with the very low concentrations of arsenite (i.e. within the nano molar range), are a good indication of how sensitive preimplantation stage embryos are to this xenobiotic. The study gives light as to how even small concentration of arsenite within luminal fluid could cause damage and provides a good indication of the importance of studying this compound as well as other xenobiotics at the embryo level *in vitro*.

Acknowledgments

We thank Stacy Righini, James Carsella, Kayleigh Zerr and Jennifer Cozzetta for their assistance in the laboratory; Davi Gonzales and Marcos Unis for their technical advice. We are also grateful to Patricia Diawara, Janna McLean, Annette Gabaldon, Jeff Smith, and Dan Unis for their review of the manuscript.

Grant support: Research supported by grant #2S06 GM008197-20 MPRC-B of the national Institutes of health and Colorado State University-Pueblo.

References

1. Adriano, DC. Trace elements in terrestrial environments – Biogeochemistry, bioavailability, and risks of metals. Vol. 2nd. New York: Springer-Verlag; 2001. p. 885
2. DeRosa CT, Johnson BL, Fay M, Hansen H, Mumtaz MM. Public health implications of hazardous waste sites: findings, assessment and research. Food Chem Toxicol 1996;34:1131–1138. [PubMed: 9119326]
3. U.S DHHS/ATSDR (U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry). Top 20 hazardous substances. 2007 [29 April 2008]. Available: <http://www.atsdr.cdc.gov/cxcx3.html>
4. U.S DHHS/ATSDR (U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry). ToxFAQs™. 2007 [29 April 2008]. Available: <http://www.atsdr.cdc.gov/toxfaqs.html>
5. Lugo G, Cassady G, Palmisano P. Acute maternal arsenic intoxication with neonatal death. Am J Dis Child 1969;117:328–330. [PubMed: 5765153]
6. Nordstrom S, Beckman L, Nordenson I. Occupational and environmental risks in and around a smelter in northern Sweden. V. Spontaneous abortion among female employees and decreased birth weight in their offspring. Hereditas 1979;90:291–296. [PubMed: 437992]
7. Chaîneau E, Binet S, Pol D, Chatellier G, Meininger V. Embryotoxic effects of sodium arsenite and sodium arsenate on mouse embryos in culture. Teratology 1990;41:105–112. [PubMed: 2305370]
8. Willhite CC. Arsenic-induced axial skeletal (Dysraphic) disorders. Exp Mol Pathol 1981;34:145–158. [PubMed: 6894125]
9. Hood R, Harrison WP. Effects of prenatal arsenite exposure in the hamster. Bull Environ Contam Toxicol 1982;29:671–678. [PubMed: 7159777]
10. Machado AF, Hovland DN Jr, Pilafas S, Collins MD. Teratogenic response to arsenite during neurulation: relative sensitivities of C57BL/6J and SWV/Fnn mice and impact of the splotch allele. Toxicol Sci 1999;51:98–107. [PubMed: 10496681]
11. Wang A, Holladay SD, Wolf DC, Ahmed SA, Robertson JL. Reproductive and developmental toxicity of arsenic in rodents: a review. Int J Toxicol 2006;25:319–331. [PubMed: 16940004]

12. Navarro P, Liu L, Keefe DL. In vivo effects of arsenite on meiosis, preimplantation development and apoptosis in the mouse. *Biol Reprod* 2004;70:980–985. [PubMed: 14656726]
13. Holson JF, Stump DG, Clevidence KJ, Knapp JF, Farr CH. Evaluation of the prenatal developmental toxicity of orally administered arsenic trioxide in rats. *Food Chem Toxicol* 2000;38:459–466. [PubMed: 10762732]
14. DeSesso JM, Jacobson CF, Scialli AR, Farr CH, Holson JF. An assessment of the developmental toxicity of inorganic arsenic. *Reprod Toxicol* 1998;12:385–433. [PubMed: 9717692]
15. Jacobson CF, Stump DG, Nemec MD, Holson JF, DeSesso JM. Appropriate exposure routes and doses in studies designed to assess developmental toxicity: a case study of inorganic arsenic. *Int J Toxicol* 1999;18:361–368.
16. Siegel, H. The Hamster: reproduction and behavior. New York: Plenum Publishing Company; 1981. p. 440
17. Archana M, Seshagiri PB. Evidence for the involvement of a species-specific embryonic protease in zona escape of hamster blastocysts. *Mol Human Reprod* 2000;6:1005–1012.
18. Tabocova S, Hunter ES 3rd, Gladen BC. Developmental toxicity of inorganic arsenic in whole embryo: culture oxidation state, dose, time, and gestational age dependence. *Toxicol Appl Pharmacol* 1996;138:298–307. [PubMed: 8658531]
19. Kubota R, Kunito T, Fujihara J, Tanabe S, Yang J, Miyazaki N. Placental transfer of arsenic to fetus of Dall's porpoises (*Phocoenoides dalli*). *Mar Pollut Bull* 2005;51:845–849. [PubMed: 16291195]
20. Jin Y, Xi S, Li X, Lu C, Li G, Xu Y, Qu C, Niu Y, Sun G. Arsenic speciation transported through the placenta from mother mice to their newborn pups. *Environ Res* 2006;101:349–355. [PubMed: 16458287]
21. He W, Greenwell RJ, Brooks DM, Calderon-Garciduenas L, Beal HD, Coffin JD. Arsenic exposure in pregnant mice disrupts placental vasculogenesis and causes spontaneous abortion. *Toxicol Sci* 2007;99:244–53. [PubMed: 17569693]
22. Waalkes MP, Liu J, Diwan BA. Transplacental arsenic carcinogenesis in mice. *Toxicol Appl Pharmacol* 2007;222:271–280. [PubMed: 17306315]
23. Greenlee AR, Quail CA, Berg RL. Developmental alterations in murine embryos exposed in vitro to an estrogenic pesticide, *o,p'*-DDT. *Reprod Toxicol* 1999;13:555–565. [PubMed: 10613404]
24. Seshagiri PB, Bavister BD. Relative developmental abilities of hamster 2- and 8-cell embryos cultured in hamster embryo culture medium-1 and -2. *J Exp Zoo* 1991;257:51–57.
25. Ulloa Ulloa CM, Yoshizawa M, Yamashita A, Hama S, Mitsui A, Hashi C, Abe H, Hoshi H, Fukui E, Matsumoto H. Blastocyst Production from In Vitro-produced Day-2 Bovine Embryos Classified by Cleavage Stage, and Cytogenetical Evaluation of the Resultant Day-8 Blastocysts. *J Reprod Dev* 2008;54:465–72. [PubMed: 18957827]
26. Liu L, Trimarchi JR, Keefe DL. Haploidy but not parthenogenetic activation leads to increased incidence of apoptosis in mouse embryos. *Biol Reprod* 2002;66:204–210. [PubMed: 11751284]
27. Hao Y, Lai L, Mao J, Im GS, Bonk A, Prather RS. Apoptosis in parthenogenetic preimplantation porcine embryos. *Biol Reprod* 2004;70:1644–1649. [PubMed: 14766725]
28. Lin SP, Lee RK, Tsai YJ. In vivo hatching phenomenon of mouse blastocysts during implantation. *J Assist Reprod Genet* 2001;18:341–345. [PubMed: 11495411]
29. Petersen CG, Mauri AL, Baruffi RL, Oliveira JB, Massaro FC, Elder K, Franco JG Jr. Implantation failures: success of assisted hatching with quarter-laser zona thinning. *Reprod Biomed Online* 2005;10:224–229. [PubMed: 15823228]
30. Minitab. Statistical Software for Windows. Minitab Inc.; New York: State College; 2004.
31. Golub MS, Macintosh MS, Baumrind N. Developmental and reproductive toxicity of inorganic arsenic: animal studies and human concerns. *J Toxicol Environ Health B Crit Rev* 1998;1:199–241. [PubMed: 9644328]
32. Riethmacher D, Brinkmann V, Birchmeier C. A targeted mutation in the mouse E-cadherin gene results in defective preimplantation development. *Proc Natl Acad Sci USA* 1995;92:855–859. [PubMed: 7846066]
33. Houghton FD, Barr KJ, Walter G, Gabriel HD, Grummer R, Traub O, Leese HJ, Winterhager E, Kidder GM. Functional significance of gap junctional coupling in preimplantation Development. *Biol Reprod* 2002;66:1403–1412. [PubMed: 11967204]

34. Pereira FE, Coffin JD, Beall HD. Activation of protein kinase C and disruption of endothelial monolayer integrity by sodium arsenite – Potential mechanism in the development of atherosclerosis. *Toxicol Appl Pharmacol* 2007;220:164–177. [PubMed: 17306850]

Figure 1. Development of Golden hamster (*Mesocricetus auratus*) embryos in complete control media (A, B, C, D) and in negative control lacking Bovine Serum Albumin (E, F, G, H). Embryos were collected three days after mating and cultured for 72 h in pre-equilibrated complete hamster embryo culture media. For each control group, embryos were photographed immediately after collection (0 h) and 72 h later.

Figure 2.

Development of Golden hamster (*Mesocricetus auratus*) embryos in complete control media supplemented with arsenite.

Embryos were collected three days after mating and cultured for 72 h in pre-equilibrated complete hamster embryo culture media supplemented with arsenite at 25 nM (A, B, C, D); 50 nM (E, F, G, H); 250 nM (I, J, K, L); 500 nM (M, N, O, P); and 750 nM (Q, R, S, T). For each treatment group, embryos were photographed immediately after collection (0 h) and 72 h later.

Figure 3. Survival of Golden hamster (*Mesocricetus auratus*) embryos in arsenite solutions. All embryos were collected three days after mating and cultured for 72 h in pre-equilibrated complete hamster embryo culture media (positive control), complete control media without Bovine Serum Albumin (negative control), complete control media supplemented with arsenite at 25, 50, 250, 500 or 750 nM. Bars above the means denote standard errors. Means with * are statistically significantly different from the positive control at the 5% level according to Protected LSD test. Error bars represent means $\pm$ SEM; n = 17-37.

Figure 4. Staining of a control embryo (A and B) and an embryo grown in arsenite at 500 nM (C and D), using Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick End-labeling (TUNEL) assay.

Embryos were collected three days after mating and cultured in pre-equilibrated complete hamster embryo culture media (control) or media supplemented with 500 nM arsenite. Embryos were incubated for 36 h prior to TUNEL assay. For the assay, the Dead End™ Fluorometric TUNEL System purchased from Promega INC (Madison, WI) was used. The embryos were photographed at 520 nm and at 620 nm for determination of DNA damage.

Blastomeres with fragmented DNA had a green-yellow color at 520 nm (A and C) and a bright red florescence at 620 nm (B and D).