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Intrauterine air impairs embryonic postimplantation development in mice

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ABSTRACT

Objective: Although most embryologists load air bubbles into the catheter along with embryos during embryo transfer, the effects of these air bubbles on embryo transfer success rate are not clear.
Study design: Air bubbles were nonsurgically injected into unilateral uterine horns of mice to demonstrate the negative effects of intrauterine air bubbles on embryonic development.
Results: Our data showed that when air bubbles are nonsurgically injected into unilateral uterine horns of pregnant 4 days mice the litter size is significantly decreased. Four days after the introduction of air, abnormal decidua and dead conceptuses were detected in the uterine horns receiving the air bubbles. In addition, intrauterine air also significantly impaired murine embryo transfer success rates, and induced an increase in endometrial capillary permeability and decidualization in mice on day 4 of pseudopregnancy. These results strongly indicated that the air bubbles loaded into embryo transfer catheters to bracket the embryo-containing medium may have negative effect on embryonic implantation and development.
Conclusions: Intrauterine air impaired murine embryonic postimplantation development, and this provided some clues for improving embryo transfer techniques in human.

Introduction

Embryo transfer is the last decisive and critical step after in vitro fertilization treatment [1,2]. It is widely accepted that avoidance of blood, mucus, bacterial contamination, excessive uterine contractions, and trauma to the endometrium may improve clinical pregnancy and implantation rates [3], but there are a large variety of methods used to load embryos into the catheter [4–6]. A retrospective evaluation using the results of a web-based survey from 265 centers in 71 countries showed that 57% of centers load air bubbles into the catheter along with embryos to aid in sonographic visualization during transfer and to prevent embryo migration in the fluid column [4]. In the United States 80% of centers load air bubbles into the catheter [7]. However, the effects of air bubbles on implantation rates were controversial [8]. Some documents reported that the air loaded into the transfer catheter to bracket the embryo-containing medium can be helpful for improving pregnancy rates [9–12] while others reported that the air bubbles did not affect the implantation and pregnancy rates [1,2,13]. In mice, blastocysts were frequently accompanied by air bubbles when transferred into uteri. However, the effects of air bubbles on embryo implantation and development have been ignored [14]. There is an urgent need for well-designed and powered randomized trials to determine the beneficial or detrimental effect of air bubbles transferred into uteri during embryo transfer. In this study, we investigated the effects of intrauterine air bubbles on murine embryonic implantation and development and hope to provided some clues for improving embryo transfer techniques in human.

Materials and methods

Animals

Mice (CD-1 strain, 6–wk) were purchased from Beijing Vitalriver Laboratory Animal Technology Company Limited (Beijing, China) and housed at 25 °C under 50%–60% relative humidity and a 12:12 h light:dark photoperiod (lights on at 06:00) until they were

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required. Mice were fed commercial pelleted food and water *ad libitum*. All experimental protocols and animal handling procedures were reviewed and approved by the Laboratory Animal Care and Use Committee of Hebei Agricultural University.

Intrauterine air injection

The females were selected by visual indications to be in proestrus-estrus [15], and mated. The presence of a vaginal plug the morning after mating was taken as evidence of successful copulation and this was considered to be the first day of pregnancy. On the afternoon of the fourth day of pregnancy, the treatment group females were given a 2.0 μL intrauterine injection of air. The air was injected into a single uterine horn using a transcervical embryo transfer (TCET) device purchased from Elim Springs Biotech Limited (London, UK). The TCET device was used in accordance with the manufacturers instructions as described previously [16]. In brief, the pregnant females were anesthetized with 0.6 mL of Avertin solution and restrained on the top of a cage. The base of the tail was grasped by using the thumb and forefinger, and the tail was angled upward as the researcher lightly pressed the base of the tail with the opposite edge of the hand. An appropriate speculum was inserted gently into the vagina. An adjustable Multiposition Fiber Optic Illuminator System (Beijing Fukai Instrument, Beijing, China) was used to shine light into the speculum, which facilitated the identification of the cervical opening. The TCET device was gently inserted into the speculum, through the cervix, and into the uterine horn and air was injected using a pipettor set to 2 μL (Eppendorf). The control group females were given a sham injection which involved inserting the TCET device into a uterine horn, as above, but without an air injection. Uterine tissues were collected on the eighth, eleventh and fourteenth days of pregnancy. In addition, the number of live pups and birth weight were recorded at delivery.

Decidualization evaluation

Four days after the intrauterine air injection, the extent of decidualization was evaluated by separately weighing the injected and noninjected uterine horns at autopsy. The dilated uterine horn showing a non-beaded appearance was considered to be the horn injected with air. The difference in weight between the two horns was taken as an estimate of the size of the response. The maximal diameter and individual weights of uterine horns injected with air were also recorded. In addition, individual decidua was dissected from the endometrium and weighed, and each conceptus was mechanically isolated from decidua with fine forceps and their morphological structure was examined under stereomicroscope (SMZ 745, Nikon).

Histological examination

Uteri was fixed in 4% paraformaldehyde, embedded in paraffin wax and cut into 5 μm sections. The sections were mounted on slides, dewaxed, and rehydrated in a graded alcohol series and stained with hematoxyhin and eosin by a routine procedure [17].

Determination of early implantation sites by intravenous dye injection

Embryo implantation sites in mice can be detected on day 5 by intravenous injection of a Trypan Blue dye saline solution, normally via a tail vein. Briefly, a 1 mL syringe was filled with 1% Trypan Blue saline solution and attached to a 30 gauge needle. After anestheasia the tail veins were dilated by warming it with water (38 °C). The needle was then aligned with the plane of the tail vein. The needle was inserted into the vein and 0.1 mL of dye injected. Three minutes after the dye injection the animals were sacrificed and dissected to allow permit identification of the implantation sites (blue bands) in the uterus.

Air-induced endometrial decidualization

To induced decidualization, air (2.0 μL) was given into the single uterine lumen of pseudopregnancy day 4 mice by TCET device as described above. Four days after the air injection, laparotomy was performed and decidualization was evaluated.

Embryo recovery and transfer

Blastocysts were recovered from 6 week old donor female mice, which that had been mated naturally. Morphologically normal blastocysts were pooled after collection, washed twice in M2 medium and transferred to a droplet of KSOM medium. Six week old pseudopregnant recipient mice were prepared by mating with vasectomized males. On the morning of the 3rd day of pseudopregnancy, blastocysts (six to eight) with or without air were transferred into single uterine horn using a TCET device as described previously. Pregnant recipients were subject to a natural delivery. The number of live born pups was recorded.

Statistical analysis

Comparisons between the groups were performed using unpaired or paired Student's *t*-test. A *p*-value less than 0.05 were considered statistically significant. Results were presented as mean ± standard deviation (S.D.). Each experiment was repeated independently at least three times.

Results

Intrauterine air injection decreased litter size at birth

To demonstrate whether the air was injected into the uterine lumen, three females were killed immediately after injection. At this time point distinct air bubble can be located under stereomicroscope. However when the females (n = 8) were killed at 6 h after injection, air bubbles can not be detected.

Litter size and birth weight were recorded from 12 females in the treatment and control groups, respectively. Mean litter size and mean birth weight were 7.2 pups and 1.55 g (n = 20) in the treatment group compared with 11.0 pups and 1.49 g (n = 20) in the control (Table 1). The difference of birth weight in two groups was not significant ($P > 0.01$), but litter size was significantly different

Table 1
Effects of intrauterine air on litter size in pregnant mice.

Groups	No. of females used (day 4 of pregnancy)	Total number of pups born	Mean litter size (±S.D.)	Mean birth weight (±S.D.) (g)
Air treatment	12	86	7.2 ± 1.2[a]	1.55 ± 0.13
Control	12	121	11.0 ± 1.8	1.49 ± 0.08

[a] Significantly different from control.