Fetal Exencephaly Arising as a Result of Preimplantation Exposure to Ammonium Chloride

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Abstract

Objectives : To investigate the effect of preimplantation exposure to 0.6 mM ammonium chloride on both preimplantation and postimplantation development of (F1 x F1) strain mouse embryos.

Method : Two-cell stage mouse embryos were randomly allocated to culture in either M16 medium or M16 added with 0.6 mM ammonium chloride for 2 days before being transferred to 2.5 day pseudopregnant recipients. Embryo morphology was assessed after 1 and 2 days of culture. The recipient females were sacrificed on day 15.5 of gestation. The number of implantation sites, fetuses, moles and any gross abnormalities found were noted.

Results : There was no significant difference in the number of embryos reaching morula stage after two days of culture between the two groups (X^2=0.86, P>0.05). Implantation and pregnancy loss rates between the two groups were within comparable ranges. Crown-rump length was significantly higher in the group of embryos exposed to ammonium chloride (t=2.46, P<0.05). There was one gross abnormality, exencephaly, detected in the experimental group (4.35% per fetus obtained).

Conclusions : Besides the abnormal increase in fetal size, preimplantation exposure to ammonium chloride also resulted in gross abnormality, exencephaly. If such effects occurred in the course of human *in vitro* fertilization, it could be devastating. Further study in this aspect is, therefore, clinically very important in preventing unwanted abnormalities that could arise from human *in vitro* fertilization.

Key word : Exencephaly, Ammonium Chloride, Preimplantation Exposure

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Normally the development of preimplantation stage mammalian embryos occurs within the protected environment of the female reproductive tract. At present, preimplantation embryos of numerous experimentally useful species, plus human embryos used in the treatment of infertility, can be routinely cultured to the blastocyst stage, usually with the production of normal offspring following embryo transfer. In spite of this, embryo development *in vitro* is far from satisfactory, and much remains to be discovered. In general, development of embryos in culture is slower than *in vivo*, as manifested by reduced cell number, consistent with a progressive loss of viability and reduced metabolism[1-4]. This indicates that media currently used for embryo culture are suboptimal. In addition, the culture environment used for preimplantation embryos can profoundly affect post-implantation events. A striking and serious example of this, the etiology of which is not understood, is the production of abnormally large fetuses or offspring following transfer of preimplantation embryos that may have spent only a few days in culture. These abnormally large fetuses were found to be associated with increased gestational length and mortality[5,6]. These findings, together with the more recent report by Lane and Gardner which demonstrated that the presence of ammonium ions in the medium of preimplantation mouse embryos was associated with fetal retardation and exencephaly in a time- and concentration-dependent manner[7], indicate that sub-optimal culture conditions may have long term effects on the offspring. If such effects occurred in the course of human *in vitro* fertilization, it could be devastating. This study was thus designed to test whether the presence of 0.6 mM ammonium chloride in M16 medium was associated with abnormality in both preimplantation and postimplantation development of (F1xF1) strain mouse embryos when the embryos were cultured from 2 cell stage for the duration of 2 days.

MATERIAL AND METHOD

Animals and superovulation procedure

Before the procedure of superovulation proceeded, all the mice were raised in light-cycle controlled rooms which they were in for at least a fortnight. Embryos were obtained from (C5BL/Ola x CBA/Ca) F1 hybrid females. Virgin females 4-6 weeks old were superovulated with an intraperitoneal injection of 5 iu pregnant mare's serum gonadotropin (PMSG) at 12.00 h, and followed 48 hours later by 5 iu of human chorionic gonadotropin (HCG). The superovulated females were placed with F1 males immediately following the second injection. The presence of a vaginal plug the following morning indicated that mating had taken place.

Pseudopregnant state

Pseudopregnant mice were prepared by mating 6-8 weeks old CF1 females in natural estrus with vasectomized CF1 males and the females with vaginal plugs the following morning were designated 0.5 day of pseudopregnancy. This procedure was performed on the same day that the donors of 2 cell stage embryos were checked for vaginal plugs.

Embryo collection

Collection of 2 cell stage embryos was performed 42-46 hours after HCG injection (day 2.5 of pregnancy). The 1-ml syringe, containing M2 medium, attached to a 32-guage hypodermic needle was used to flush the embryos out of the oviduct. The embryos were flushed from the oviduct using about 0.1 ml of M2 medium from the syringe. After the embryos were collected, they were washed three times in M2 medium before being randomly allocated to culture in either M16 medium or M16 added with 0.6 mM ammonium chloride.

Embryo culture

All culture dishes were prepared 1 day before embryo collection by dispensing 20 ul drops of either M16 medium or M16+0.6 mM ammonium chloride in an array on the bottom of the 35 mm petri dish and flooding the dish with paraffin oil. The dishes were equilibrated in a gas of 5 per cent CO_2 in air at 37°C overnight.

The embryos were transferred into the culture dishes the following morning after the culture dishes had been prepared. All embryos were cultured in a group of ten in 20 ul drops of the medium under a layer of lightweight paraffin oil.

Assessment of embryo morphology

Embryo morphology was determined after 22, 46, or 70 hours of culture using a phase contrast microscope. Before the eight-cell stage, development was judged by counting the number of blastomeres. Embryos showing compaction and blas-

Table 1. Stock solutions for making medium M2, M16 and M16 + 0.6 mM ammonium chloride.

Stock	Component	g/100 ml
A (10 x conc)	Nacl	5.534
	KCl	0.356
	KH_2PO_4	0.162
	$MgSO_4.7H_2O$	0.294
	NaLactate	2.608
	Glucose	1.000
	Penicillin	0.060
	Streptomycin	0.050
B (10 x conc)	$NaHCO_3$	2.106
	Phenol red	0.010
C (100 x conc)	NaPyruvate	0.036/10 ml
D (100 x conc)	$CaCL_2.2H_2O$	0.252/10 ml
E (10 x conc)	Hepes*	5.957
	Phenol Red	0.010
NH_4Cl	NH_4Cl	0.016

To make up Hepes, dissolve the solid in 40 ml H_2O + 30 ml M/5 NaOH (0.8 g/ml). Adjust with more NaOH to 7.4 before making up to 100 ml.

Table 2. Preparations of medium from stock solutions.

Stock	M2 (ml)	M16 (ml)	M16+0.6NH_4Cl (ml)
A	1.0	1.0	1.0
B	0.16	1.0	1.0
C	0.1	0.1	0.1
D	0.1	0.1	0.1
E	0.84	-	-
NH_4Cl	-	-	2.0
H_2O	7.8	7.8	5.8
BSA	40 mg	40 mg	40 mg

tocoel cavity formation were classified as morulae and blastocysts, respectively. Blastocysts in the process of emerging and having emerged from the zonae pellucidae were classified as hatching and hatched blastocysts, respectively.

Classification of the embryo morphology was as follows:

2 cell stage embryo
3-4 cell stage embryo
5-8 cell stage embryo
Compacted morula
Blastocyst

After assessment of embryo morphology was completed, the culture dishes were returned to the gas-equilibrated incubator and maintained at 37°C. The time periods in which the embryos were assessed were minimized as much as possible to ensure that the embryos were maintained at 37°C most of the time.

Embryo transfer

5-7 embryos at either morula or blastocyst stage from each treatment group were separately transferred to the uterine horn of 2.5 day pseudopregnant females. After the recipient females recovered from the embryo transfer process, they were kept in a light-cycle controlled room and left for another 13 days until day 15.5 of gestation at which they would finally be sacrificed.

Assessment of the fetuses

In rodent species it is often observed that most malformed fetuses would be destroyed by their mothers right after birth. In order to be certain about the number of abnormal fetuses detected, the recipient females in this study, therefore, were killed on day 15.5 of gestation. The uterine cavities were carefully examined to determine the number of implantation sites, fetuses and resorptions (or moles). Fetal growth was subsequently assessed by the scoring system developed by Wahlsten and Wainwright in 1977[8] which was based on the development of the external features including skin, limbs, eyes, and ears. Crown-rump length, weight of the fetus and other morphological presentations of the fetus were also evaluated and recorded. Crown-rump length of the fetuses was measured using the objective lens-attached scale under microscopy. Any morphological abnormalities being found were noted. Photography and bone staining of fetuses with limb abnormalities were also performed.

Media

Three media were used in this study. M2 medium was used for collecting and transferring embryos. Standard M16 medium was utilized to culture embryos in the control group while M16 medium added with 0.6 mM ammonium chloride was used to culture embryos in the experimental group.

M2, M16, and M16 +0.6 mM ammonium chloride were made up from individual stock solutions (Table 1 and 2). All stocks were pushed through a Millipore filter and stored in a refrigerator at 4°C in Falcon plastic tubes. All stocks can be kept for 3 months except stock B and C which

Table 3. Preimplantation development of embryos in M16 medium (summarized).

Stage	Day 1.5 %	Day 2.5 %	Day 3.5 %
2 cell	100	2.19	2.19
3-4 cell	-	4.38	0.73
5-8 cell	-	89.78	-
morula	-	3.65	86.86
blastocyst	-	-	10.22
Total number of embryo	137	137	137

Table 4. Preimplantation development of embryos in medium M16 + 0.6 mM NH_4CL.

Stage	Day 1.5 %	Day 2.5 %	Day 3.5 %
2 cell	100	0.71	0.71
3-4 cell	-	3.57	-
5-8 cell	-	89.29	-
morula	-	6.43	87.86
blastocyst	-	-	11.43
Total number of embryo	140	140	140

need to be prepared every other week. Bovine serum albumin (BSA) was prepared every time media were made up. All salts and glucose were of Analar grade (BDH, Poole, Dordet, UK) Sodium pyruvate, sodium lactate, glutamine, ammonium chloride, and phenol red were of cell culture grade (Sigma Chemical Co, Poole, UK) Bovine serum albumin, lot 90 (Miles Pentex Crystalline) was purchased from Bayer Diagnostics.

Data collection

The embryo morphology was scored daily, in the morning (from 10.30 am to 11.00 am) for the whole duration of culture. Postimplantation evaluation was performed in the morning (from 10.30 am to 11.30 am) of day 15.5 of pregnancy.

Experimental design

There were two experiments carried out in this study. In each experiment, 2 cell stage embryos from F1 strain donors were collected and randomly allocated to either M16 medium (control group) or M16+0.6 mM ammonium chloride (experimental group). The total number of embryos studied in the control and experimental groups were 137 and 140, respectively. Embryos were left in culture for two days before being transferred to 2.5 day pseudopregnant recipients of CF1 strain. Embryo morphology was assessed after 1 and 2 days of culture. The recipient females were kept until day 15.5 of gestation at which time they were sacrificed. The number of implantation sites, fetuses and moles were noted. The morphology of the fetuses was assessed to give the estimated age. Weight of the fetus, crown-rump length and any abnormality found were noted. Abnormal fetuses were kept for further evaluation.

Statistical analysis

All statistical analysis was done with the software package, Multistat (Biosoft, Cambridge), on a MacIntosh computer. Data was keyed in and type of statistical analysis chosen was performed on the data. Chi-square test was used to compare the number of embryos that achieved morula or more developmentally advanced stage, preimplantation pregnancy loss, postimplantation pregnancy loss, the number of fetuses obtained, and the number of fetus per implantation, between the treated group and the control group. The test gave a value for Chi-square (x^2) and also a probability (P). Yates correction was applied when appropriate. Fisher exact test was used where Chi-square test was inappropriate. This test gave us probability (P) directly. Student *t*-test (unpaired) was also used to determine whether or not there were significant differences between weight, crown-rump length, and estimated age of the fetuses in the treated group and those from the control group. This test provided t-value (t), and probability (P).

RESULTS

The results of preimplantation development in this study (Table 3 and 4) revealed that after 2 days in culture 97.08 per cent and 99.29 per cent of embryos cultured in M16 and M16+0.6 mM ammonium chloride, respectively, were at morula or more developmentally advanced stages. On day 3.5 p.c., 10.22 per cent and 11.43 per cent of embryos from M16 medium and medium M16+ 0.6 mM ammonium chloride group, respectively, had reached the blastocyst stage. The statistic analysis showed that there was no significant difference in the number of embryos achieving morula stage after two days of culture between the two groups

Table 5. Postimplantation development of fetuses previously cultured in M16 medium or medium M16 + 0.6 mM ammonium chloride (summarized results).

Parameters	M16 medium	M16 + ammonium chloride
1. Number of embryos transferred	56	98
2. Number of recipients	4	7
3. Number of pregnant recipients	4	5
4. Number of implantation sites	32	32
5. Number of fetuses	28	23
6. Number of moles	4	9
7. Number of abnormal fetuses	-	1
8. Number of embryos transferred to pregnant recipients	56	70
9. Preimplantation pregnancy loss	24	38
10. Percentage of implantation	57.14	45.71
11. Percentage of fetuses obtained	50.00	32.86
12. Percentage of fetuses per implantation	87.50	71.87
13. Percentage of preimplantation pregnancy loss	42.86	54.29
14. Percentage of postimplantation pregnancy loss	12.50	28.12
15. Weight of fetus (mean ± SEM), (g)	0.429 ± 0.009	0.454 ± 0.014
16. CRL (mean ± SEM), (mm)	14.159 ± 0.122	14.610 ± 0.141*
17. Average age (mean ± SEM), (days)	15.080 ± 0.002	15.070 ± 0.036

* Significantly different from the control value ($P<0.05$).

($x^2 = 0.86$, $P>0.05$). This indicated that development of (F1 x F1) strain mouse embryos in both M16 medium and medium M16+0.6 mM ammonium chloride, when first started in culture at 2 cell stage, were within comparable ranges in terms of development to morula and/or blastocyst stages after 2 days of culture.

The postimplantation assessments on day 15.5 of gestation from this study (Table 5) showed that implantation rate and percentage of fetuses obtained from embryos cultured in medium M16+ 0.6 mM ammonium chloride were lower than those cultured in M16 medium. However, statistic analysis did not show any significant differences in such aspects between the two groups ($X^2 = 1.63$, $P> 0.05$; and $X^2 = 3.79$, $P>0.05$, respectively). In addition, preimplantation and postimplantation pregnancy losses of embryos cultured in medium M16+ 0.6 mM ammonium chloride were not significantly different from those cultured in M16 medium ($X^2 = 1.62$, $P>0.05$; and $X^2 = 1.54$, $P>0.05$, respectively).

Crown-rump length (CRL) of the fetuses from the group of embryos cultured in medium M16+0.6 mM ammonium chloride was significantly higher than that of embryos cultured in M16 medium ($t = 2.46$, $P<0.05$). However, weight and estimated age of fetuses from these two groups were not significantly different ($t = 1.56$, $P>0.05$; and $t= 0.31$, $P>0.05$, respectively).

One grossly abnormal fetus was detected in this study. The abnormality found was exencephaly. The percentage of exencephalic fetus was 4.35 per cent per fetus obtained or 1.43 per cent per embryo transferred.

DISCUSSION

It has been known that the cleavage rate and viability of mammalian preimplantation embryos is greatly reduced by culture *in vitro*[1,2,9], indicating that the present culture systems are far from optimal. Over the past decade there have been several attempts to develop better culture media. These include the addition of several types of amino acids which result in both positive and negative effects to embryonic growth *in vitro*. The detrimental effects on the developing embryos *in vitro* due to the addition of some types of amino acids was thought to be attributed to ammonium ions, the waste product commonly broken down from protein and amino acids. More recently, Gardner and Lane (1993) demonstrated that, while started in culture from 1-cell stage, the presence of ammonium in mMTF medium significantly decreased the number of (F1 x F1) strain mouse embryos reaching the morula stage after 72 hours and the blastocyst stage after 96 hours of culture, at the concentration 0.62 and 0.15 mM, respectively[10].

The presence of ammonium in culture medium may affect the developing embryo in several ways: Ammonium could decrease the concentration of alpha-ketoglutarate by its conversion to glutamate. This would impair the flux through the TCA cycle leading to serious depletion of ATP in the cell. Furthermore, ammonium can activate the enzyme phosphofructokinase resulting in increased glycolytic activity, a pathway that appears to be detrimental to early cleavage stages[11,12]. Alternatively, ammonium as a weak base could elevate pHi, against which the mouse embryo appears to have no regulatory mechanism[13,14].

In this study another medium, M16, was investigated since it is a commonly utilized medium for mouse embryo culture in most laboratories. When exposure of (F1 x F1) strain mouse embryos to ammonium chloride was postponed from 1 cell to 2 cell stage, it was surprising to find that there was no retardation in preimplantation development of embryos cultured in M16+0.6 mM ammonium chloride in comparison to those being cultured in M16 medium.

Considering the results from this study together with those previously reported[7,10], the author hypothesize that the detrimental effects of ammonium chloride on preimplantation development of mouse embryos could possibly be varied by several factors including, developmental stage of the embryos and type of culture medium used.

Components of M16 medium used in this study were very similar to those present in mMTF medium except for a lower concentration of NaCl, and higher concentration of both NaLactate and glucose in M16 medium than in mMTF medium (94.70 *vs* 103.40 mMol/L, 23.30 *vs* 4.79 mMol/L, and 5.55 *vs* 3.40 mMol/L, respectively). Whether different culture media (M16 *vs* mMTF medium) could give rise to different effects of preimplantation exposure of mouse embryos to ammonium chloride and mechanisms behind such differences will need to be elucidated in future studies. The study utilizing M16 medium to culture embryos starting from 1 cell stage onwards is suggested to answer the previously asked question.

When the effects on fetuses were brought into consideration it was interesting to find that, being cultured in M16 medium, the exposure of 2 cell stage (F1 x F1) strain mouse embryos to 0.6 mM ammonium chloride significantly increased crown-rump length compared to that of control group. In addition, weight of fetuses from the group of embryos exposed to ammonium chloride during preimplantation period was consistently, though not significantly, higher than that of the control group. The author also found no significant developmental retardation in the group of fetuses previously exposed to ammonium chloride when compared to the control group. These consistent results lead the author to firmly conclude that preimplantation exposure of 2 cell stage embryos to 0.6 mM ammonium chloride resulted in a significant increase in crown-rump length of the fetuses.

There has been no previous report on the association between the presence of ammonium chloride in preimplantation culture medium and the increase in size and weight of fetuses or offspring. However, there is accumulating evidence that simple experimental manipulations of early embryonic stages of a wide range of mammals have resulted in a variety of startling growth phenotypes. *In vitro* fertilization, nuclear transfer, and embryo transfer of bovine embryos have resulted in dramatic overgrowth, lengthened gestation, and increased perinatal mortality, the so-called "large calf syndrome" (15-17). Manipulation of mouse embryos increases variability in body mass[18], or causes reduced body mass[1,19]. In addition, alterations in behavior and a range of morphophysiological parameters (including increased body mass) have been demonstrated in mice derived from frozen embryos[20]. Human babies resulting from *in vitro* fertilization and embryo transfer have exhibited low birth mass [21], and hypertension occurs in IVF-ET pregnancies more commonly than in pregnancies conceived spontaneously[22]. The primacy of growth defects observed across a range of species in these studies suggests that expression at a variety of growth related loci is adversely affected by manipulation of early embryos. However, it is not clear whether there is a common factor associated with all or some of these abnormalities. Neither the environmental factor (s), nor the underlying mechanism (s) resulting in the oversized phenotype have been elucidated.

Parental imprinting may explain why manipulation of early mammalian embryos *in vitro* frequently results in a range of abnormalities, with aberrant growth predominating[23]. Such growth abnormality may reflect genetic conflict between maternal and paternal alleles over growth program (conflict theory), and that manipulation during pre-

implantation development *in vitro*, such as exposure of embryos to ammonium chloride in this study, may coincide with a particularly sensitive period of programming of growth-related genes.

One of the likely molecular components of the imprinting mechanism is the methylation of DNA at CpG dinucleotide, a view that is supported by description of putative DNA methylation germline imprints in Igf2r[24], H19[25] and Xist[26] genes.

It has been reported that mice that are null mutants for Igf2, Igf2r and H19 exhibit marked growth phenotypes[27]. This suggests that imprinted gene can be strongly implicated in fetal and placental growth. It is also worth noting that, while allelic methylation of imprinted genes may be extensively (and variously) remodeled between fertilization and the blastocyst stage, non-imprinted genes undergo an invariant global demethylation by the morula to blastocyst stage, and remethylation is not observed until around gastrulation. This provides a basis for the differential susceptibility on imprinted and non-imprinted genes to environment in the preimplantation period.

The variability in growth responses following insults during preimplantation period in different species suggests that several genetic loci may be involved, or that aberrant expression of a smaller number of genes may vary depending on the precise nature or duration of the insults. Species differences in the identity of susceptible loci may also be expected. The author, thus, proposes that identification of the altered gene expression between normal and large fetuses may provide clues as to the nature of the underlying mechanism (s) of the large fetuses obtained after preimplantation exposure to ammonium chloride found in this study.

Besides the significant increase in crown-rump length and consistently increased weight of the fetuses, preimplantation exposure to 0.6 mM ammonium chloride also resulted in gross abnormality of the fetus, exencephaly. The incidence of fetal exencephaly found in this study was 4.35 per cent per fetus obtained which is very low when compared to the result reported by Lane and Gardner (1994) who, using the same (F1 x F1) strain embryos, demonstrated that after 69 hours of exposure to 0.3 mM ammonium chloride, 29 per cent of fetuses were exencephalic[7]. One possible explanation underlining this huge difference might be the difference in the stage of embryos being exposed to ammonium chloride since in the report by Lane and Gardner the embryos were exposed to ammonium chloride from one cell stage onwards but in this study the embryos were collected at two cell stage and exposure to ammonium chloride was thus begun at the later developmental stage. The author, therefore, suggest a larger scale study using the same (F1 x F1) strain of mice to investigate the effects of ammonium chloride exposure at different developmental stages to ensure that the correlation between preimplantation exposure to ammonium chloride and fetal exencephaly is certain, and possibly demonstrates a stage – specific pattern.

Some reports have addressed the possible causes of exencephaly in mice. These possible causes include (1) polygenic inheritance and (2) environmental factors.

In the view of genetic predisposition, the SELH strain of mice showed that 17 per cent of its offspring had exencephaly. However, it was only in the presence of a combination of the "mutant" genes that exencephaly was seen[28]. Future study using various strains of mice should be helpful in demonstrating that the presence of fetal exencephaly after preimplantation exposure to ammonium chloride found in the Lane and Gardner study, and the present study was not due to unusual susceptibility to ammonium chloride in a particular mouse strain.

Concerning teratogenesis, several agents have previously been reported to induce neural tube defect including exencephaly[29]. These teratogens may act through the final common pathways to disturb the neurulation process. Such interference may result in inhibition of cell proliferation and induction of cell death, thus, producing a dramatic effect on cranial neurulation, leading to exencephaly. Most, if not all, of the known teratogens which result in exencephaly, however, normally induces disruption when they are administered during the organogenetic stage, not in the predifferentiated period as has been observed in this study.

The results found in this study, together with those previously reported by Lane and Gardner in 1994[7], are very surprising since they obviously contradict the generally held convictions which indicate that preimplantation embryos are not susceptible to teratogens, a large insult may kill the embryos but the surviving embryos usually manifest no organ-specific anomalies. The author ulti-

mately proposes that exposing preimplantation embryos to insults, such as ammonium chloride, is capable of resulting in long-term adverse effects such as abnormal increase in body mass and fetal dysmorphogenesis. If such effects occurred in the course of human *in vitro* fertilization, it could be devastating. Further study in this aspect, therefore, is clinically very important in preventing the unwanted abnormalities that could arise from human IVF practices.

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ภาวะ fetal exencephaly ซึ่งเกิดขึ้นจากการสัมผัสของตัวอ่อนระยะก่อนการฝังตัวต่อแอมโมเนียม คลอไรด์

*สุพัชญ์ สีนะวัฒน์, พ.บ. วท.ม.**

วัตถุประสงค์การวิจัย : เพื่อศึกษาผลของการเลี้ยงตัวอ่อนหนูสายพันธุ์ (F_1 x F_1) ในน้ำยาเลี้ยงตัวอ่อนที่มีแอมโมเนียม คลอไรด์ขนาดความเข้มข้น 0.6 มิลลิโมลาร์

กระบวนการวิจัย : ตัวอ่อน (embryo) ของหนูระยะ 2 เซลล์จำนวน 277 ตัวได้ถูกแบ่งเป็นสองกลุ่มโดยวิธีการสุ่มเพื่อเลี้ยงในน้ำยาเลี้ยงตัวอ่อน 2 ชนิดคือ น้ำยา M16 (กลุ่มควบคุม) และน้ำยา M16 + แอมโมเนียม คลอไรด์ 0.6 มิลลิโมลาร์ (กลุ่มศึกษา) ตัวอ่อนทั้งสองกลุ่มถูกเลี้ยงในน้ำยาเลี้ยงตัวอ่อนเป็นเวลา 2 วัน ก่อนที่จะถูกย้ายฝากเข้าไปยังโพรงมดลูกของหนูพันธุ์ CF_1 ซึ่งทำหน้าที่เป็นแม่อุ้มบุญ แม่อุ้มบุญจะถูกเลี้ยงจนกระทั่งตั้งครรภ์ได้ 15.5 วัน ก็จะถูกฆ่าเพื่อศึกษาลักษณะภายในโพรงมดลูกว่ามีจำนวนตำแหน่งที่มีการฝังตัว (implantation site) จำนวนทารก (fetus) และจำนวนการแท้ง (mole) เท่าใด ลักษณะโครงสร้างภายนอกของทารก (fetus) ถูกบันทึกอย่างละเอียดเพื่อตรวจหาความผิดปกติทางโครงสร้าง

ผลการวิจัย : หลังจากเลี้ยงตัวอ่อน (embryo) ในน้ำยาเลี้ยงตัวอ่อนเป็นเวลา 2 วัน ไม่พบความแตกต่างในแง่ของจำนวนตัวอ่อนที่พัฒนาไปถึงระยะมอร์รูลา (morula) อัตราการฝังตัวของตัวอ่อน (implantation rate) และอัตราการสูญเสียของการตั้งครรภ์ (pregnancy loss rate) ระหว่าง กลุ่มควบคุมและกลุ่มศึกษา แต่เมื่อพิจารณาในแง่ของทารก (fetus) ระยะ 15.5 วันแล้วพบว่า กลุ่มที่สัมผัสกับแอมโมเนียม คลอไรด์มี crown-rump length สูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($t = 2.46$, $p < 0.05$) ยิ่งไปกว่านั้นยังพบว่าร้อยละ 4.35 ของทารก (fetus) ที่ได้จากกลุ่มศึกษามีความผิดปกติทางโครงสร้าง โดยแสดงออกในลักษณะของ exencephaly

สรุป : การสัมผัสต่อแอมโมเนียม คลอไรด์ของตัวอ่อนระยะก่อนการฝังตัว (preimplantation embryo) ก่อให้เกิดผลเสียที่ร้ายแรงต่อทารก (fetus) คือทำให้ทารกมีลักษณะพิกลรูป โดยแสดงออกในรูปของ exencephaly และมีความยาวของร่างกาย (crown-rump length) เพิ่มมากขึ้นอย่างผิดปกติ การศึกษาเพิ่มเติมในเรื่องนี้มีความจำเป็นอย่างยิ่ง ในการป้องกันผลอันไม่พึงประสงค์ที่อาจมีต่อทารกของมนุษย์ที่เกิดจากกระบวนการเด็กหลอดแก้ว (*in vitro* fertilization)

คำสำคัญ : Exencephaly, แอมโมเนียม คลอไรด์

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