Original Article

Relationship between Intrauterine Bacterial Infection and Early Embryonic Developmental Arrest

Shao-Fei Yan[1], Xin-Yan Liu[2], Yun-Fei Cheng[3], Zhi-Yi Li[2], Jie Ou[2], Wei Wang[1], Feng-Qin Li[1]

[1]Laboratory of Microbiology, Key Laboratory of Food Safety Risk Assessment of Ministry of Health, China National Center for Food Safety Risk Assessment, Beijing 100021, China

[2]Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China

[3]Department of Family Planning, Tianjin Central Hospital of Gynecology and Obstetrics, Tianjin 300052, China

Shao-Fei Yan and Xin-Yan Liu contributed equally to this work.

Abstract

Background: Early embryonic developmental arrest is the most commonly understudied adverse outcome of pregnancy. The relevance of intrauterine infection to spontaneous embryonic death is rarely studied and remains unclear. This study aimed to investigate the relationship between intrauterine bacterial infection and early embryonic developmental arrest.

Methods: Embryonic chorion tissue and uterine swabs for bacterial detection were obtained from 33 patients who underwent artificial abortion (control group) and from 45 patients who displayed early embryonic developmental arrest (trial group).

Results: Intrauterine bacterial infection was discovered in both groups. The infection rate was 24.44% (11/45) in the early embryonic developmental arrest group and 9.09% (3/33) in the artificial abortion group. Classification analysis revealed that the highest detection rate for *Micrococcus luteus* in the early embryonic developmental arrest group was 13.33% (6/45), and none was detected in the artificial abortion group. *M. luteus* infection was significantly different between the groups ($P < 0.05$ as shown by Fisher's exact test). In addition, no correlation was found between intrauterine bacterial infection and history of early embryonic developmental arrest.

Conclusions: *M. luteus* infection is related to early embryonic developmental arrest and might be one of its causative factors.

Key words: Artificial Abortion; Bacterial Infection; Early Embryonic Developmental Arrest; *Micrococcus Luteus*; Spontaneous Abortion

Introduction

Early embryonic developmental arrest is a state of embryonic growth arrest in early pregnancy in which either no developed embryonic bud can be detected through B-scan or no embryonic heartbeat is detected despite the presence of embryonic bud.[1] This condition is a complication commonly observed in early pregnancy and accounts for a significant proportion of all cases of spontaneous abortion. Its occurrence has gradually increased in recent years, and it has become a subject of interest among clinicians and researchers.

Diverse factors are associated with early embryonic developmental arrest, and some of these factors remain unclear.[2] Of the identified causes, hereditary factors, immune and endocrine systems, nutrition and environment, and infection have been the focus of studies. Of all known factors, genetic abnormality provides the highest contribution to early embryonic developmental arrest.[1] The second most potent factor is immune and endocrine system, followed by nutrition and environment and infection.[3-5] Despite its relatively low effect, increasing studies have reported a crucial status in infective causes among the remaining unknown causative components for early embryonic developmental arrest, with directly relevant microorganisms,

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.183411

Address for correspondence: Dr. Xin-Yan Liu, Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China
E-Mail: liuxymeng@163.com

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

© 2016 Chinese Medical Journal ¦ Produced by Wolters Kluwer - Medknow

Received: 22-01-2016 **Edited by:** Yi Cui

How to cite this article: Yan SF, Liu XY, Cheng YF, Li ZY, Ou J, Wang W, Li FQ. Relationship between Intrauterine Bacterial Infection and Early Embryonic Developmental Arrest. Chin Med J 2016;129:1455-8.

including *Gardnerella vaginalis*, *Mycoplasma urealyticum*, and *Mycoplasma hominis*.[6-9]

Studies have shown that *Listeria monocytogenes* can cause spontaneous abortion.[10] *L. monocytogenes* might enter the body through ingestion of contaminated food, as well as through contact with the eyes, abraded skin, and mucous membranes. When this pathogen persists in the vagina or cervix, the bacterium may infect a pregnant host and its fetus or newborn through placental or obstetric canal transmission. *L. monocytogenes* infection through sexual contact may also cause abortion.[11] Although the uterus is commonly regarded as an aseptic environment, intrauterine bacterial proliferation resulting from infection or other factors might adversely affect the embryonic development. This study aimed to investigate the relationship between intrauterine bacterial infection and early embryonic developmental arrest through comparative analyses of the conditions for bacterial infection in the early embryonic developmental arrest group and artificial abortion group.

Methods

Sample collection and ethical approval

Seventy-eight cases of early pregnancy (8–12 weeks) were identified at Peking Union Medical College Hospital between July 1, 2014 and September 30, 2014. Among these cases, 45 patients requesting for curettage because of embryonic developmental arrest were designated as the trial group and 33 patients with normal embryonic development and requesting for artificial abortion were designated as the control group. This study used the following criteria to constitute the control and trial groups. The artificial abortion group (control group) should meet all the following conditions: without history of spontaneous abortion (or embryonic diapause); regular menstrual cycle; consistency between menopausal days and results of gestational sac ultrasound; detection of fetal heart beat in preoperative ultrasonography; normal results of liver function tests; and negative result for infection index (hepatitis B, HIV, and syphilis). The following were the criteria for spontaneous abortion caused by embryonic developmental arrest (trial group): without history of spontaneous abortion (or embryonic diapause); regular menstrual cycle; and gestational sac observed through ultrasound, although embryo bud and embryo heartbeat were not detected or embryo heartbeat was detected early and eventually disappeared. If the reason for spontaneous abortion in certain cases was identified, such cases were excluded. No significant disparities in terms of age and gravidity were observed between the two groups. Vaginal cleanliness in both groups was examined prior to operation, and operation was not conducted in patients with vaginal infection or in patients recovering from postinfection treatment. This study was approved by the Ethics Committee of Peking Union Medical College Hospital. We obtained informed consent from each participant before we collected and tested the samples. Information obtained from each patient was kept strictly confidential. If the results indicated infection, the participants were treated subsequently.

Sample preparation

A routine vulval, vaginal, and cervical disinfection was performed, followed by aseptic cotton swabbing along the uterine cavity and then daubing for one cycle. Chorionic tissue was directly taken from the uterus, and the cotton swabs and tissue were placed in sterile centrifuge tubes containing 5 ml of preliminary *Listeria* enrichment broth 1 (LB_1) (Beijing Land Bridge Technology CO., Ltd., China) for testing. Chorionic tissue was cut, ground, and cultured at 30°C for 24 h in 10 ml of replenished LB_1. Following centrifugation at 200 × g for 5 s, the swab samples in the tubes were cultured at 30°C for 24 h in 10 ml of replenished LB_1.

Bacterial isolation

The national standard GB 4789.30-2010 test method for food safety was used to isolate *L. monocytogenes*. After 24 h of culture at 30°C in 10 ml of LB_1, 1 ml of each sample was transferred and sub-cultured in 10 ml of secondary *Listeria* enrichment broth 2 (LB_2) (Beijing Land Bridge Technology CO., Ltd., China) for 24 h at 30°C. LB_2 was obtained and streaked onto PALCAM agar (Beijing Land Bridge Technology CO., Ltd., China) and CHROMagar™ *Listeria* plates (Zhengzhou Biocell Biotechnology Co., Ltd., China) and then cultured for 24 h at 37°C. Five suspected colonies of *L. monocytogenes* were selected from each plate, streaked onto brain–heart infusion agar (Beijing Land Bridge Technology CO., Ltd., China) plates (in cases where no specific colonies exist, five single colonies with different morphologies were randomly chosen), and cultured for 24 h at 37°C. In accordance with the hospital's general sampling methods for assays in obstetrics and clinical gynecology, the cotton swabs or samples touched by inoculation loops were directly smeared onto China blue agar (Beijing Land Bridge Technology CO., Ltd., China) and blood agar (Beijing Land Bridge Technology CO., Ltd., China) plates for culturing without preliminary enrichment.

Bacterial identification

For observation of colony characteristics, the isolated bacteria were subjected to Gram-staining and microscopy. A Vitek 2 biochemistry identifier (bioMérieux, Marcy l'Etoile, France) was used to identify the isolated bacteria.

Statistical analysis

The Chi-square test and Fisher's exact test were used in this study. SPSS 19.0 (SPSS Inc., Chicago, USA) was used for statistical analysis, and significance was set at $P < 0.05$.

Results

Bacterial detection in intrauterine

Table 1 shows the eight bacterial species that caused infection in the uterine cavity of the trial or control group; these species included *Micrococcus luteus*, which was found in six samples and showed the highest detection percentage of 7.69% (6/78). *Sphingomonas paucimobilis* was the second highest at 2.56% (2/78), and each of the six other species was detected once. In addition, *S. paucimobilis* was detected both in the trial and control groups, whereas *Micrococcus kristinae* and *Gemella morbillorum* were found only in the

control group. *L. monocytogenes* was not detected in either the trial or control group.

Intrauterine infection ratio

Eleven cases of bacterial infection were detected among the 45 cases in the trial group, accounting for a detection rate of 24.44%. Three cases of infection were detected in the control group consisting of 33 cases, accounting for a detection rate of 9.09%. Chi-square test revealed an insignificant difference ($P > 0.05$) in intrauterine bacterial infection between the early embryonic developmental arrest group and artificial abortion group [Table 2].

Comparison of intrauterine *Micrococcus luteus* infection

Six cases (13.33%) of *M. luteus* infection were detected in the trial group, whereas no *M. luteus* infection was detected in the control group. Given that *M. luteus* infection was not detected in the control group, Fisher's exact test was employed. The result indicated a significant difference ($P < 0.05$) in intrauterine *M. luteus* infection between the early embryonic developmental arrest group and artificial abortion group [Table 3].

Early embryonic developmental arrest history and intrauterine infection

Table 4 shows no significant differences in the total infection rate or individual *M. luteus* infection rate among patients who have had early embryonic developmental arrest once, twice, and three times or more ($P > 0.05$).

Discussion

The causes of early embryonic developmental arrest in early pregnancy include genetic factors, immune and endocrine systems, nutrition and environment, and infective factors.[1,3,5] Studies have attributed the number of adverse occurrences in pregnancy, including spontaneous premature delivery, premature rupture of membranes, chorioamnionitis, and recurrent abortion, to embryonic tissue infection. However, whether intrauterine infection, including embryonic tissue infection, is a causative factor for early embryonic developmental arrest requires further investigation.

L. monocytogenes is a virulent food-borne pathogen recognized for its capability for contamination through ingestion of foodstuffs and sexual contact in pregnant women, leading to miscarriage in severe cases.[11] Through the method used to detect *L. monocytogenes*, this study isolated and identified cultivated bacteria from two groups of patients undergoing either early embryonic developmental arrest or artificial abortion; this work attempted to elucidate the relationship between *L. monocytogenes* infection and early embryonic developmental arrest. A clinical method for bacterial testing was used simultaneously, namely direct smearing onto China blue agar and blood agar plates without preliminary enrichment. No *L. monocytogenes* was detected, and this finding was possibly caused by the combination of inherently low morbidity of the pathogen and limited sample size. No *L. monocytogenes* was also detected through the clinical test method. However, given that the culture method for *L. monocytogenes* involved a two-time pre-enrichment step for 24 h each prior to plate cultivation, other bacterial species were detected. This finding might imply that: (1) intrauterine bacterial infections are present in certain patients; (2) low bacterial infection levels result in misdetection in samples directly smeared onto China blue and blood agar plates; and (3) certain bacteria require selective enrichment and suitable media for successful isolation. Thus, assay culture media and preliminary enrichment procedures are suggested for inclusion in clinical testing.

Table 1: Bacterial detection in intrauterine of patients with either spontaneous or artificial abortion ($N = 78$)

Bacterium	Gram stain (+/−)	Cases (spontaneous/ artificial), *n*	Infection ratio (%)
Micrococcus luteus	+	6 (6/0)	7.69
Sphingomonas paucimobilis	−	2 (1/1)	2.56
Acinetobacter baumannii	−	1 (1/0)	1.28
Micrococcus kristinae	+	1 (0/1)	1.28
Staphylococcus epidermidis	+	1 (1/0)	1.28
Pseudomonas aeruginosa	−	1 (1/0)	1.28
Ochrobactrum anthropi	−	1 (1/0)	1.28
Gemella morbillorum	+	1 (0/1)	1.28

Table 2: Comparison of intrauterine bacterial infection in spontaneous abortion induced by embryonic developmental arrest and artificial abortion

Experimental group	Total cases, *N*	Bacterium positive	
		Cases, *n*	Infection rate (%)
Spontaneous abortion	45	11	24.44
Artificial abortion	33	3	9.09

Table 3: Comparison of the incidence of intrauterine bacterial infection by *Micrococcus luteus* in spontaneous abortion induced by embryonic developmental arrest and artificial abortion

Experimental group	Total cases, *N*	*Micrococcus luteus*-positive	
		Cases, *n*	Infection rate (%)
Spontaneous abortion	45	6	13.33
Artificial abortion	33	0	0

Table 4: Frequency of spontaneous abortion induced by embryonic developmental arrest and intrauterine bacterial infection

Frequency of spontaneous abortion	Total cases, *N*	Bacterium-positive		*Micrococcus luteus*-positive	
		Cases, *n*	Infection rate (%)	Cases, *n*	Infection rate (%)
1	31	6	19.35	4	12.90
2	8	3	37.50	1	12.50
≥3	6	2	33.33	1	16.67

Seven species of intrauterine infective bacteria were detected in this study, all being conditioned pathogens. *M. luteus* is a Gram-positive coccus that belongs to the family *Micrococcaceae* and found in soil, dust, water, and air; *M. luteus* is also one of the normal floras found in mammalian skin.[12] However, *M. luteus* can cause illness when it colonizes other areas of the body. *M. luteus* infection induces insidious manifestation of pyogenic liver abscess,[13] meningitis,[14] and myocarditis.[15] Our results demonstrated that the history of early embryonic developmental arrest carried no significant bearing on infection, supporting the previously reported noncorrelation between mycoplasma infection and number of early embryonic developmental arrests.[8] No distinct disparity in intrauterine bacterial infection was observed between the artificial abortion group and early embryonic developmental arrest group. By contrast, six cases of *M. luteus* infection were detected in the early embryonic developmental arrest group, whereas none was found in the control group; this disparity was statistically significant. This finding hinted at the correlation between the presence of *M. luteus* in the intrauterine cavity and early embryonic developmental arrest. Based on all of the findings presented, the role of *M. luteus* in early embryonic developmental arrest must be continuously investigated to reduce the incidence of spontaneous abortion.

We used the same method to isolate bacterium from vaginal and cervical secretions. The bacteria found in the vaginal and cervical secretions were quite different from those isolated from the intrauterine. This difference was possibly caused by the environment. For instance, *M. luteus* was isolated from a relatively closed environment. Although we did not find a relationship between vaginal infection and intrauterine infection, the results showed that unclean sexual activity might lead to intrauterine infection, even though examination of vaginal secretion yielded normal findings.

Studies have shown that bacteria can migrate to and thrive in the uterus where intrauterine infection is engendered by vaginal and cervical inflammation.[16] As miscarriage induced by early embryonic developmental arrest typically occurs during early pregnancy, preconception health education and health examinations should be vigorously addressed to facilitate advance treatment of any inflammation and reduce the effects of infection on embryonic development. Concurrently, further research on the functional mechanisms of infection in embryonic development should be undertaken to bolster the theoretical foundation for the prevention of early embryonic developmental arrest.

Financial support and sponsorship

This work was funded by National "Twelfth Five-Year" Plan for Science and Technology Support (No. 2013BAI12B01 and No. 2012BAI31B06).

Conflicts of interest

There are no conflicts of interest.

References

1. Jia CW, Wang L, Lan YL, Song R, Zhou LY, Yu L, *et al*. Aneuploidy in early miscarriage and its related factors. Chin Med J 2015;128:2772-6. doi: 10.4103/0366-6999.167352.
2. Rull K, Nagirnaja L, Laan M. Genetics of recurrent miscarriage: Challenges, current knowledge, future directions. Front Genet 2012;3:34. doi: 10.3389/fgene.2012.00034.
3. Sarkar D. Recurrent pregnancy loss in patients with thyroid dysfunction. Indian J Endocrinol Metab 2012;16 Suppl 2:S350-1. doi: 10.4103/2230-8210.104088.
4. Ticconi C, Giuliani E, Veglia M, Pietropolli A, Piccione E, Di Simone N. Thyroid autoimmunity and recurrent miscarriage. Am J Reprod Immunol 2011;66:452-9. doi: 10.1111/j.1600-0897.2011.01021.x.
5. Giakoumelou S, Wheelhouse N, Cuschieri K, Entrican G, Howie SE, Horne AW. The role of infection in miscarriage. Hum Reprod Update 2016;22:116-33. doi: 10.1093/humupd/dmv041.
6. Bretelle F, Rozenberg P, Pascal A, Favre R, Bohec C, Loundou A, *et al*. High *Atopobium vaginae* and *Gardnerella vaginalis* vaginal loads are associated with preterm birth. Clin Infect Dis 2015;60:860-7. doi: 10.1093/cid/ciu966.
7. Nigro G, Mazzocco M, Mattia E, Di Renzo GC, Carta G, Anceschi MM. Role of the infections in recurrent spontaneous abortion. J Matern Fetal Neonatal Med 2011;24:983-9. doi: 10.3109/14767058.2010.547963.
8. Ye LL, Zhang BY, Cao WL. Relationship between the endocervical mycoplasma infection and spontaneous abortion due to early embryonic death (In Chinese). Chin J Obstet Gynecol 2004;39:83-5. doi: 10.3969/j.issn.1007-8991.2010.02.004.
9. Bayraktar MR, Ozerol IH, Gucluer N, Celik O. Prevalence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* in pregnant women. Int J Infect Dis 2010;14:e90-5. doi: 10.1016/j.ijid.2009.03.020.
10. Kaur S, Malik SV, Vaidya VM, Barbuddhe SB. *Listeria monocytogenes* in spontaneous abortions in humans and its detection by multiplex PCR. J Appl Microbiol 2007;103:1889-96. doi: 10.1111/j.1365-2672.2007.03414.x.
11. Pezdirc KB, Hure AJ, Blumfield ML, Collins CE. *Listeria monocytogenes* and diet during pregnancy; balancing nutrient intake adequacy v. adverse pregnancy outcomes. Public Health Nutr 2012;15:2202-9. doi: 10.1017/S1368980012000717.
12. Kloos WE, Tornabene TG, Schleifer KH. Isolation and characterization of micrococci from human skin, including two new species: *Micrococcus lylae* and *Micrococcus kristinae*. Int J Syst Bacteriol 1974;24:79-101. doi: 10.1099/00207713-24-1-79.
13. Ioannou A, Xenophontos E, Karatsi A, Petrides C, Kleridou M, Zintilis C. Insidious manifestation of pyogenic liver abscess caused by *Streptococcus intermedius* and *Micrococcus luteus*: A case report. Oxf Med Case Reports 2016;2016:1-3. doi: 10.1093/omcr/omv071.
14. Fosse T, Peloux Y, Granthil C, Toga B, Bertrando J, Sethian M. Meningitis due to *Micrococcus luteus*. Infection 1985;13:280-1. doi: 10.1007/BF01645439.
15. Miltiadous G, Elisaf M. Native valve endocarditis due to *Micrococcus luteus*: A case report and review of the literature. J Med Case Rep 2011;5:251-6. doi: 10.1186/1752-1947-5-251.
16. Choi SJ, Park SD, Jang IH, Uh Y, Lee A. The prevalence of vaginal microorganisms in pregnant women with preterm labor and preterm birth. Ann Lab Med 2012;32:194-200. doi: 10.3343/alm.2012.32.3.194.