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Research Article

Embryotoxicity in Unexplained Infertility: A Retrospective Study of 1129 Women

Jeyendran RS*
Androlab Inc., Oakbrook Terrace, IL 60181, USA

***Corresponding author:** Jeyendran RS, Androlab Inc., Oakbrook Terrace, IL 60181, USA
Tel: 312 335 0075; **Email:** jeyendran@sbcglobal.net

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Abstract

The objective was to determine the prevalence of embryotoxicity in sera from 1129 women experiencing unexplained infertility (UI). A minimum of 15 two-cell mouse embryos were incubated in the presence of 10% heat-inactivated serum obtained from women experiencing UI, and cultured for 72 hours. The frequency of atretic embryos was calculated and recorded as a percentage. The result was considered positive for embryotoxicity if the atretic embryos were 12.0% or greater. Of the 1,129 sera tested, 44 (3.90%) were positive. The low prevalence of embryotoxicity in the sera of women experiencing UI does not appear to justify routine embryotoxicity testing of these patients.

Keywords: Embryotoxicity; Mouse embryo assay; Unexplained infertility

Introduction

Approximately 30% of infertile couples are considered to experience 'unexplained infertility' (UI) [1,2]. UI is inevitably a diagnosis by exclusion and is often made when no abnormalities of the female and male reproductive systems are identified. However, there may be several undetectable defects or factors such as the circulating embryo toxins present that might prevent conception.

Prior reports have suggested the presence of circulating embryo toxins as a possible factor in women experiencing UI [3-7]. However, embryotoxicity results in sera of women experiencing UI were inconsistent, controversial, or ambiguous at best, probably due to differences in the assay techniques, procedures, or patient selection [3,4]. Embryotoxicity assays were time consuming and cost prohibitive, and not readily available, which may explain why this condition was not verified as a factor in UI until now.

Since the mouse embryo bioassay has been validated and established as a tool for identifying embryo toxins in human serum [3], to assess the true value of testing for embryotoxicity, the present retrospective study was undertaken to determine the overall prevalence of embryotoxicity in the sera of 1,129 women experiencing UI.

Materials and Methods

Patient Sera

Serum obtained from 1,129 women experiencing UI was heat inactivated at 56^{0}C for 30 minutes and kept frozen at -20^{0}C in aliquots until used. Similarly treated fetal bovine serum was used as control sera, known to support mouse embryo growth. A serum sample from a patient known to inhibit mouse embryo growth served as a positive control for embryotoxicity in each assay.

Embryotoxicity Assay

The mouse embryo bioassay has been validated and the result was considered positive for embryotoxicity if the quantity of atretic embryos were more than12.0% [3]. Briefly, the CB6F1/J Female mice, 2-4 months of age were super ovulated, mated, and sacrificed 3 days later. Two-cell embryos obtained from the fallopian tubes were cultured for 72 hours in 1ml Ham's F-10 medium supplemented with 10 % (v/v) test or control sera in 5% CO_2 at 37^{0}C. At least five embryos each and a total of 15 embryos from three different mice were incubated with every tested serum sample. The stage of the embryo development was evaluated and the result was considered positive for embryotoxicity if the atretic embryos were 12% or more.

Results

Forty-four of the 1,129 (3.90%) sera from UI, were positive for embryotoxicity while none of the controls known to support mouse embryo growth using fetal bovine serum were positive, and the serum sample from an individual known to inhibit mouse embryo growth always resulted in more than 12% atretic embryos.

Discussion

Embryotoxicity should inhibit embryo growth rather than embryo development, and therefore the percent of atretic embryos was used to establish embryotoxicity in our study. These results indicate that less than 4% of women with UI have circulating embryo toxins, which is less than what was previously reported [4-7].

We used a standardized assay protocol [3] and trained personnel performed the assays throughout the study period, excluding the variation due to interpersonal handling of the assay. Based on the results obtained, it is reasonable to conclude that embryotoxicity is

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present in under 4% of women experiencing UI. Although newer biotoxicity assays using stem cell technology are being introduced [8], based on the results presented, it appears that the routine testing of sera of women experiencing UI is not warranted. However, in very select population of women experiencing UI, who have failed to respond to standard therapy and have exhausted all other diagnostic procedures and assays, it may be beneficial to test for embryotoxicity in their serum.

References

1. Infertility Workup for the Women's Health Specialist: ACOG Committee Opinion, Number 781. Obstet Gynecol. 2019; 133: e377-e384.
2. Practice Committee of the American Society for Reproductive Medicine. Practice Committee of the American Society for Reproductive Medicine. Evidence-based treatments for couples with unexplained infertility: a guideline. Fertil Steril. 2020; 113: 305-322.
3. Roussev RG, Stern JJ, Thorsell LP, Thomason EJ, Coulam CB. Validation of an embryotoxicity assay. Am J Reprod Immunol. 1995; 33: 171-175.
4. Thomason EJ, Roussev RG, Stern JJ, Coulam CB. Prevalence of embryotoxic factor in sera from women with unexplained recurrent abortion. Am J Reprod Immunol. 1995; 34: 338-341.
5. Cameo M, Fontana V, Cameo P, Vauthay LG, Kaplan J, Tesone M. Similar embryotoxic effects of sera from infertile patients and exogenous interferon-gamma on long-term in-vitro development of mouse embryos. Hum Reprod. 1999; 14: 959-963.
6. Sargent IL, Dokras A. Embryotoxicity as a marker for recurrent pregnancy loss. Am J Reprod Immunol. 1996; 35: 383-387.
7. Dokras A, Sargent IL, Redman CW, Barlow DH. Sera from women with unexplained infertility inhibit both mouse and human embryo growth in vitro. Fertil Steril. 1993; 60: 285-292.
8. Zhang X, An S, Liu S, Qiu J, Zhang W, Zhou Q, et al. Comparative assessment of embryotoxicity of 2,4,6-triiodophenol to mouse blastoid and pre-implantation embryo models. Ecotoxicol Environ Saf. 2023; 252: 114608.