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EFFECT OF STAPHYLOCOCCUS AUREUS CO CULTURE ON EMBRYONIC DEVELOPMENT AND BLASTOCYST FORMATION FOLLOWING INTRACYTOPLASMIC SPERM INJECTION (ICSI)

Sudhakar Cholan[2], Arun Muthuvel[2], Mopuru Thuheena Reddy[3], Satheesh Kumar Sabapathy[*1], Chitra Venkatachalam[4], Sumithra Maniraj[4] , Kayalvizhi Rangasamy[5]

[1*]Saveetha Institute of Basic Medical Sciences (SIBMS), Saveetha Institute of Medical and Technical Sciences. Saveetha University, Chennai-602105, India. Email. Satheeshkumars.sibms@saveetha.com, Orcid ID.0000-0003-4983-0542
[2]Chief, Embryologist, Iswarya Fertility Centre, Gandhi Nagar 1st Main Rd, Kasturba Nagar, Adyar, Chennai, Tamil Nadu 600020, India. Email. sumu7000@gmail.com
[2]Gynecologist and Reproductive Medicine Specialist, Iswarya Fertility Centre, Gandhi Nagar 1st Main Rd, Kasturba Nagar, Adyar, Chennai, Tamil Nadu 600020,India.Email. arunmuthuvel@gmail.com
[3]Embryologist, Iswarya Fertility Centre, Saraswathi Nagar, Dargamitta, Nellore, Andhra Pradesh -524003. Email. thuhinareddy175@gmail.com
[4]Department of Chemistry (PG), Vellalar College for women (Autonomous), Thindal, Erode-638012, India. Email.chitu.sv@gmail.com; sumithramaniraj@gmail.com
[5]Department of Science and Humanities, Sri Eshwar College of Engineering, kinathukadavu, Coimbatore-641202, India. Email.kayalvizhi.r@sece.ac.in
*Corresponding author address; Satheesh Kumar Sabapathy, satheeshkumars.sibms@saveetha.com,

ABSTRACT

In Assisted Reproductive Technology (ART), the microbiological environment of embryo culture systems has become more interesting as microbial metabolites and host-microbe interactions could affect the growth and implanting potential of embryos. In the present study, the effect of Staphylococcus aureus co-culture on embryo development following intracytoplasmic sperm injection (ICSI) was evaluated. Retrieved oocytes from infertile patients undergoing ART treatment were subjected to ICSI procedures they are cultured in standard culture media till on day 2 and on day 2 (i.e 2 cell or 4 cell) they are subsequently divided into two groups: embryos cultured in standard culture medium without bacterial supplementation (control group) and embryos cultured in medium containing S. aureus (test group). On day 4 and 5 in culture, the cleavage rate, embryo morphology, developmental progression and formation of blastocysts were evaluated. Its adverse effect on embryo was absent and it was observed that co-culture with S. aureus was inducing better embryonic progression and better blastocyst formation when compared to the control when it comes to embryos. Further studies would need to be conducted to confirm this. Based on the findings, it is hypothesized that metabolites or growth promoting factors produced by the microbes of S. aureus could have a beneficial effect on the cell activity and on the developmental competence of the embryos. The potential mechanisms behind these findings, however, will need more molecular study to prove safety, reproducibility and clinical applicability. This study demonstrates the growing relevance of microbiome-associated reproductive biology and offers preliminary results of microbial involvement in embryo culture conditions of ART laboratories.

KEYWORDS: Staphylococcus aureus; ICSI; Embryo culture; Blastocyst; ART; Embryonic development.

INTRODUCTION

Infertility is a significant health issue in many countries, including the United States, and is a problem that impacts millions of couples. In recent years, the use of assisted reproductive technologies (ART), such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), has greatly enhanced the results of treatment for infertile couples (Palermo et al., 1992; Steptoe and Edwards, 1978). One of the most important factors in achieving a successful fertilization, cleavage, blastocyst formation, implantation and pregnancy outcome is the embryo culture conditions (Gardner and Lane, 1997; Valan et al., 2024).

Optimization of embryo culture systems has been directed at the nutrient composition, pH, temperature, osmolarity and gas concentrations (Biggers and Summers, 2008). Recent studies, however, suggest that the microbiome of the reproductive tract can be a factor in fertility and implantation and that the microbiome can affect embryonic development (Franasiak and Scott, 2015; Moreno et al., 2016). A metabolic system of microbes in the female reproductive tract may generate metabolites, cytokines and signaling molecules that can regulate the growth and differentiation of cells (Koedooder et al., 2019, Adiga et al., 2024).

Staphylococcus aureus is a Gram-positive bacterium, it is the most prevalent and recognized microorganism in the human mucosal and skin flora (Lowy, 1998). S. aureus is typically linked to opportunistic infections and hospital-acquired infections, but there are some interactions that affect mammalian cell physiology by the secretion of metabolites and

bioactive compounds (Otto, 2014, Jijo et al., 2024). Co-culture systems with bacteria have been shown in other studies to affect the proliferation, oxidative state and extracellular signaling pathways of cells (Turnbaugh et al., 2007; Ravel et al., 2011).

Understanding of the microbial interactions occurring in embryo culture systems is still limited and few data exist on the effects of exposure to bacteria during the embryogenesis process. The new field of reproductive microbiomics has sparked a great deal of interest in the potential of microorganisms or their metabolites to positively or negatively influence embryo quality and implantation success (Molina et al., 2020; Baker et al., 2018).

The aim of this study was to determine if the presence of Staphylococcus aureus in embryo culture media has an effect on embryo or if it promotes embryonic growth and blastocyst formation after IVC using the ICSI technique. The effects of culturing embryos with S. aureus versus standard conditions (no bacteria) were compared in terms of pattern of cleavage and embryonic development.

MATERIALS AND METHODS

Study Design

The present experimental study was carried out in Embryology lab of Iswarya fertility hospital, Tamil Nadu, India. Before the study was conducted, it had been approved by the Institutional Ethics Committee. All patients involved provided informed consent.

Collection of Oocytes

The oocytes were retrieved from infertile women who have undergone controlled ovarian stimulation and ART. Mature metaphase II oocytes were chosen for ICSI procedures based on the normal embryology laboratory protocol.

Intracytoplasmic Sperm Injection (ICSI)

Standard micromanipulation technique was employed for ICSI in the presence of an inverted microscope. The cleavage of egg becomes visible after 44-45 hours from insemination on the second day after fertilization.

The preparation of Staphylococcus aureus Culture is described below. Here are the steps to prepare Staphylococcus aureus Culture.

Staphylococcus aureus were confirmed by the use of the conventional microbiological technique such as Gram staining, blood agar culture, catalase test, and coagulase test. Culture sensitivity testing was carried out for antibiotic susceptibility testing.

Embryo Culture Conditions

Embryos were split into two groups on day 2 after ICSI.

Control Group: Embryos cultured in standard embryo culture medium only and not supplemented with bacteria.

Culture condition 2: Embryos cultured in S. aureus containing medium.

Under controlled conditions, embryos, incubated at 37C in a humidified atmosphere with 6% CO2.

Embryo Assessment

The development of the embryos was followed daily with the aid of an inverted microscope. On days 4 and 5, the cleavage rate, the number of cells, fragmentation status, the morphology and blastocyst formation were noted.

Outcome Measures

The following were considered as primary outcome measures:

- Cleavage rate
- Embryo morphology score
- Blastocyst formation rate
- Embryonic developmental progression

RESULTS

Isolation and Identification of *Staphylococcus aureus*

The bacterial isolates were found to be Gram positive cocci in clusters and were found to be golden yellow in appearance on blood agar medium. S. aureus isolates used in the study were confirmed using culture sensitivity testing.

Embryonic Cleavage and Development

The embryos cultured in the medium supplemented with S. aureus showed better progress in the cleavage. The test group showed increased cell division and decreased developmental arrest.

Day 4 Embryonic Assessment

Embryos cultured with S. aureus showed better morphological features than the control group with higher progress of development and compaction on day 4.

Day 5 Blastocyst Formation

The test group showed greater blastocyst development than the control group. Embryos cultured with S. aureus exhibited better blastocyst expansion and morphology.

Comparative Outcome Analysis

Based on the comparative evaluation, the embryo developmental competence in the S. aureus co-culture environment might be improved following ICSI procedure.

Parameter	**Control Group**	***S.aureus* Group**

Number of fertilized oocytes	1	1
Cleavage rate (%)	100%	100%
Day 4 embryo quality	fragmented	Good
Blastocyst formation rate (%)	0%	100%
Day 5 expanded blastocysts	0%	100%

Table 1. Comparison of Embryo Developmental Outcomes Between Control and *Staphylococcus aureus* Co-culture Groups Following ICSI

Figure 1. *Staphylococcus aureus* growth on blood agar plates.

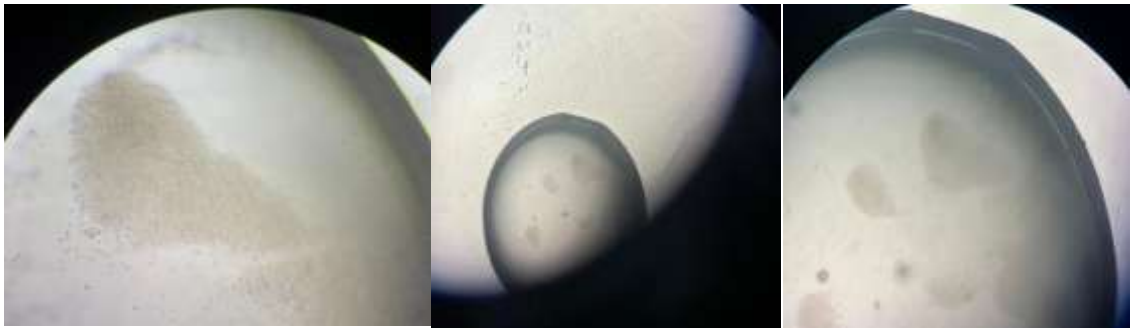

Figure 2. *Staphylococcus aureus* in culture dish

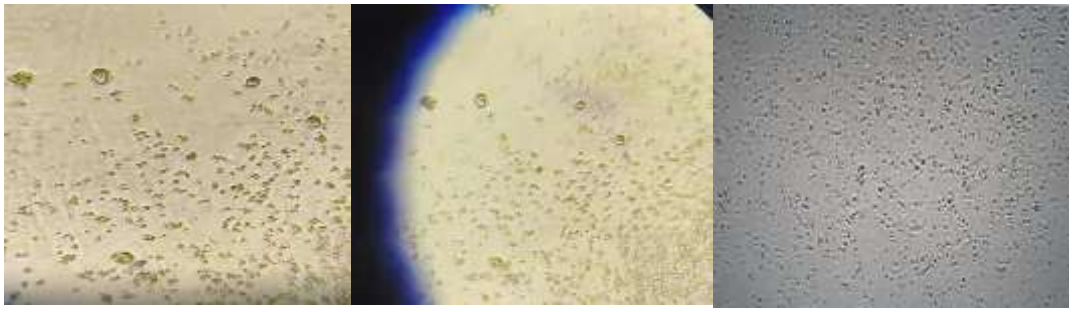

Figure 3. Microscopic appearance of *Staphylococcus aureus* under inverted microscopy.

Figure 4. Culture sensitivity and resistant report

Figure 5. Embryonic development in control culture conditions without bacterial supplementation.

Figure 6. Embryonic development in *Staphylococcus aureus* co-culture conditions.

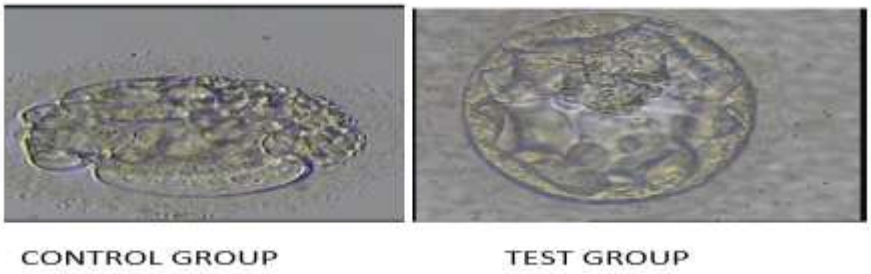

Figure 7. Comparative blastocyst formation on day 5 between control and test groups.

DISCUSSION

The success rates of ART depend on the embryo culture conditions. The current research showed embryo cultured with S. aureus did not lead to embryo damage and may have shown increased developmental progression and improved blastocysts formation compared to embryos cultured under conventional bacteria free conditions.

Embryo development has been studied in the past and it has been concluded that the outside signals, metabolites and micro environmental factors are very important (Gardner and Kelley, 2017). The microbiota of the reproductive tract may provide beneficial metabolic metabolites that affect cell growth, mitochondrial function and gene regulation (Moreno and Simon, 2019).

The results from the present study show that S. aureus may be producing metabolites or signaling molecules that can positively affect the communication and growth of the embryonic cells. Some bacterial metabolites such as short chain fatty acids and extracellular peptides have been shown to influence host cellular pathways and oxidative stress responses (Nicholson et al., 2012).

While S. aureus is thought to be an opportunistic pathogen, microbial host interactions are complex and context-dependent (Otto, 2014). Microbial exposure in laboratory settings under controlled conditions can be different to the metabolic effects of microbial infections. This has also been observed in other microbiome related reproductive studies, whereby certain microbial community patterns have been linked with positive reproductive outcomes (Koedooder et al., 2019).

It has been recently discovered that the reproductive tract microbiome is a key factor affecting fertility, successful implantation, and pregnancy maintenance (Franasiak et al., 2016). Overall, for positive reproductive outcome, microbial communities are dominant in the Lactobacillus genus, while dysbiosis can affect the process of implantation and trigger inflammatory responses (Moreno et al., 2016). However, there is a limited number of studies in direct bacterial interaction with embryo culture system.

This is a novel study on the concept of “Microbial-assisted embryo culture.” However, a few drawbacks should be noted. A limited number of bacteria were tested and detailed molecular analysis of bacterial metabolites was not conducted. Moreover, long-term embryonic safety, implantation potential and neonatal outcomes were not assessed. Additional transcriptomic, proteomic as well as metabolomic studies are needed to elucidate the precise molecular processes that can be observed.

Microbial co culture systems could also be investigated for parameters of oxidative stress, mitochondrial activity, cytokine profiles, epigenetic changes and embryo implantation rates in future studies. Careful biosafety assessment is crucial before entering clinical translation.

CONCLUSION

The present study showed that embryos treated with Staphylococcus aureus did not damage the embryos and suggest it may play more effective role in the cleavage progression and promote blastocyst formation than standard embryo culture conditions; further studies will focus on this. These results indicate that the microbial associated factors could affect embryonic developmental competence in ART. The study suggests preliminary evidence of the involvement of microbial interactions in reproductive biology and optimization of embryo culture. Further large-scale molecular and clinical studies are required however, to confirm these findings and prove their safety to be implemented in clinical use.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

The ethical approval has been issued by Saveetha Institute of Basic Medical Sciences; vide reference number 079/06/2025/IEC/FSR/SIBMS dated on 25th June 2025.

REFERENCES

1. Adiga SK, Predheepan D, Salian SR, Uppangala S, et al. 2024. Advanced maternal age affects their frozen-thawed embryo susceptibility to high oxygen environment. **Sci Rep** 14:23008.
2. Palermo G, Joris H, Devroey P, Van Steirteghem AC (1992). Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340:17-18.
3. Steptoe PC, Edwards RG (1978). Birth after reimplantation of a human embryo. Lancet 2:366.
4. Gardner DK, Lane M (1997). Culture and selection of viable blastocysts. Hum Reprod Update 3:367-382.
5. Biggers JD, Summers MC (2008). Choosing a culture medium: making informed choices. Fertil Steril 90:473-483.
6. Franasiak JM, Scott RT (2015). Reproductive tract microbiome in assisted reproductive technologies. Fertil Steril 104:1364-1371.
7. Moreno I, Codoner FM, Vilella F, Valbuena D, et al. (2016). Evidence that the endometrial microbiota has an effect on implantation success or failure. Am J Obstet Gynecol 215:684-703.
8. Koedooder R, Mackens S, Budding A, Fares D, et al. (2019). Identification and evaluation of the microbiome in IVF patients. Hum Reprod Update 25:298-325.
9. Lowy FD (1998). Staphylococcus aureus infections. N Engl J Med 339:520-532.
10. Otto M (2014). Staphylococcus aureus toxins. Curr Opin Microbiol 17:32-37.
11. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, et al. (2007). The human microbiome project. Nature 449:804-810.
12. Ravel J, Gajer P, Abdo Z, Schneider GM, et al. (2011). Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA 108:4680-4687.
13. Molina NM, Sola-Leyva A, Saez-Lara MJ, Plaza-Diaz J, et al. (2020). New opportunities for endometrial health by modifying uterine microbial composition. Hum Reprod 35:1-11.
14. Baker JM, Chase DM, Herbst-Kralovetz MM (2018). Uterine microbiota and reproductive outcomes. Front Immunol 9:1238.
15. Gardner DK, Kelley RL (2017). Impact of culture conditions on embryo metabolism. Reproduction 153:R49-R61.
16. Nicholson JK, Holmes E, Kinross J, Burcelin R, et al. (2012). Host-gut microbiota metabolic interactions. Science 336:1262-1267.
17. Franasiak JM, Werner MD, Juneau CR, Tao X, et al. (2016). Endometrial microbiome at embryo transfer. Fertil Steril 106:1299-1306.
18. Lane M, Gardner DK (2005). Understanding cellular disruptions during embryo culture. Hum Reprod Update 11:41-57.
19. Bavister BD (1995). Culture of preimplantation embryos: facts and artifacts. Hum Reprod Update 1:91-148.
20. Schoolcraft WB, Gardner DK (2001). Blastocyst culture and transfer. Fertil Steril 76:559-568.
21. Swain JE (2019). Optimizing embryo culture environments. Semin Reprod Med 37:127-135.
22. Magli MC, Jones GM, Gras L, Gianaroli L, et al. (2008). Chromosome mosaicism in embryos. Hum Reprod 23:598-603.
23. Meseguer M, Herrero J, Tejera A, Hilligsøe KM, et al. (2011). Embryo morphokinetics and implantation. Fertil Steril 95:2425-2430.
24. Brison DR, Sturmey RG, Leese HJ (2014). Metabolic heterogeneity in human embryos. Reproduction 147:R25-R35.
25. Krisher RL, Wheeler MB (2010). Towards understanding embryo culture. Reprod Fertil Dev 22:32-41.
26. Watson AJ, Natale DR, Barcroft LC (2004). Molecular regulation of blastocyst formation. Anim Reprod Sci 82:583-592.

27. Hardy K, Spanos S (2002). Growth factor expression in embryos. Hum Reprod Update 8:311-323.
28. Jijo A, Munshi I, Uppangala S, LakshmiKumar RVP, et al. 2024. Secretomes from conventional insemination and intra-cytoplasmic sperm injection derived embryos differentially modulate endometrial cells in vitro. **Reprod Sci** 31:2080-2091.
29. Leese HJ (2012). Metabolism of the preimplantation embryo. Reproduction 143:417-427.
30. Johnson MH, Nasr-Esfahani MH (1994). Radical solutions and embryo culture. BioEssays 16:31-38.
31. Wale PL, Gardner DK (2016). Oxygen regulation and embryo development. Hum Reprod Update 22:231-250.
32. Sakkas D, Gardner DK (2005). Noninvasive methods for embryo selection. Semin Reprod Med 23:336-347.
33. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, et al. (2017). International glossary on infertility and fertility care. Fertil Steril 108:393-406.
34. Simopoulou M, Sfakianoudis K, Rapani A, Giannelou P, et al. (2018). Microbiota and fertility. J Assist Reprod Genet 35:1075-1084.
35. Pelzer ES, Allan JA, Cunningham K, Mengersen K, et al. (2011). Microbial colonization of follicular fluid. Fertil Steril 96:704-706.
36. Benner M, Ferwerda G, Joosten I, van der Molen RG (2018). How uterine microbiota affect reproductive function. Reproduction 156:R25-R38.
37. Kyono K, Hashimoto T, Nagai Y, Sakuraba Y (2019). Analysis of endometrial microbiota. Reprod Med Biol 18:331-337.
38. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, et al. (2016). The third international consensus definitions for sepsis and septic shock. JAMA 315:801-810.
39. Human Microbiome Project Consortium (2012). Structure, function and diversity of healthy human microbiome. Nature 486:207-214.
40. Marchesi JR, Adams DH, Fava F, Hermes GD, et al. (2016). The gut microbiota and host health. Gut 65:330-339.
41. Sharma R, Agarwal A, Rohra VK, Assidi M, et al. (2013). Effects of oxidative stress on male reproduction. Reprod Biol Endocrinol 11:66.
42. Vyas P, Balakrishnan B, Joshi P, Nair S (2021). Role of microbiome in assisted reproductive technologies. Reprod Sci 28:2993-3004.
43. Valan AS, Krithikadatta J, Guru A, et al. 2024. Exploring periostracum as an alternative root canal irrigant: insights from zebrafish embryo experiments. **Cureus** 16:e56638.