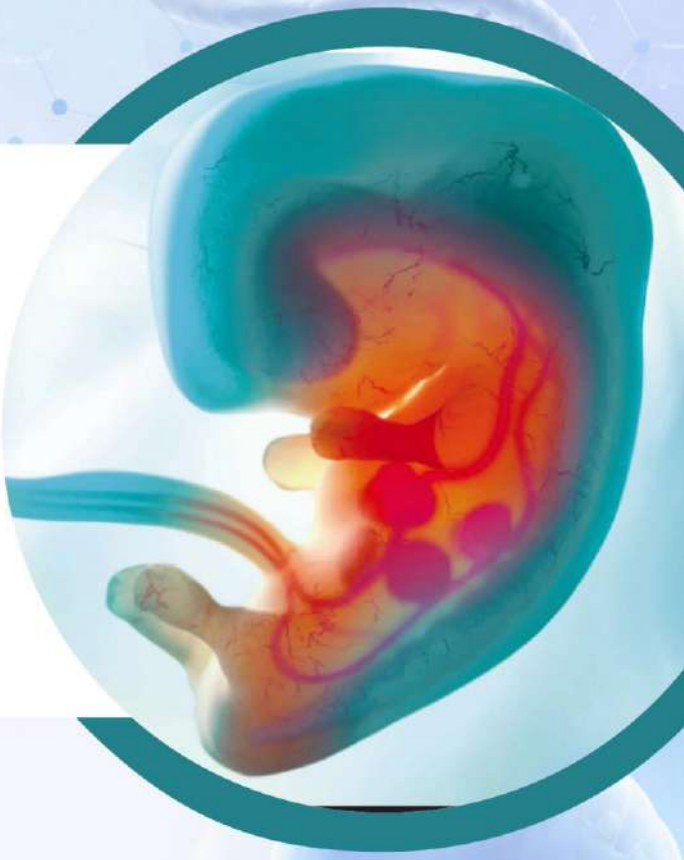
INDIAN FERTILITY SOCIETY



SIG Newsletter

May 2026

Embryology

Dr. (Prof) Neena Malhotra
President, IFS

Dr. Sweta Gupta
Secretary General, IFS

Dr. Leena Wadhwa
Editor, IFS

Dr. Garima Kapoor
Joint Editor, IFS

Dr. Sarabpreet Singh
Chair Embryology

Dr. Sanjay Shukla
Mentor - SIG

Dr. Dayanidhi Kumar
Convenor - SIG

Dr. Surabhi Kashyap
Co Convenor - SIG

Dr. Shipra Bharati
Co Convenor - SIG

Hyaluronic Acid–Containing Transfer Media (EmbryoGlue®) in Assisted Reproductive Technology: Biological Basis, Clinical Evidence, and Future Perspectives

Dr. Dayanidhi Kumar, MCE, PhD

Chief Embryologist & Bihar Head, Indira IVF Hospital Limited

Abstract

Embryo implantation remains one of the most critical yet inefficient steps in assisted reproductive technology (ART). Despite major advancements in embryo culture systems, vitrification, embryo selection, and preimplantation genetic testing, implantation failure continues to limit IVF success. Hyaluronic acid–containing transfer media (EmbryoGlue®) has emerged as one of the most scientifically studied embryo transfer adjuncts in modern reproductive medicine. Hyaluronic acid (HA), a naturally occurring glycosaminoglycan abundantly present within the female reproductive tract during implantation, plays an important role in cellular adhesion, extracellular matrix stabilization, tissue hydration, and embryo-endometrial interaction. Multiple randomized controlled trials, systematic reviews, and meta-analyses have evaluated the role of EmbryoGlue® in improving implantation, clinical pregnancy, and live birth outcomes. This review summarizes the biology of HA, mechanism of action, laboratory protocols, clinical applications, meta-analysis data, Indian experience, safety profile, limitations, and future perspectives regarding EmbryoGlue® in ART practice.

Keywords

EmbryoGlue®, Hyaluronic Acid, Embryo Transfer, IVF, Implantation, Frozen Embryo Transfer, Recurrent Implantation Failure, ART

1. Introduction

Embryo implantation is a highly synchronized and complex biological process involving continuous molecular communication between the embryo and the receptive endometrium. Despite remarkable advancements in assisted reproductive technology (ART), including time-lapse imaging, vitrification, preimplantation genetic testing (PGT), and optimized embryo culture systems, implantation failure continues to remain one of the major limiting factors affecting IVF success rates. Importantly, even morphologically high-quality or euploid embryos may fail to implant, highlighting the importance of implantation biology beyond embryo morphology alone. Consequently, increasing attention in modern reproductive medicine has shifted toward optimizing embryo-endometrial synchrony and improving the implantation microenvironment.

Hyaluronic acid (HA), also known as hyaluronan, is a naturally occurring glycosaminoglycan abundantly present within the female reproductive tract during the peri-implantation period. HA plays a critical physiological role in embryo adhesion, extracellular matrix stabilization, tissue hydration, angiogenesis, and implantation-related molecular signaling pathways (Heymann et al., 2020). Physiologically, HA concentration rises significantly during the implantation window, suggesting its important role in establishing endometrial receptivity and facilitating embryo apposition. EmbryoGlue® was specifically developed as a hyaluronan-enriched embryo transfer medium intended to mimic the physiological uterine environment during embryo transfer. Contrary to common misconceptions, EmbryoGlue® is not a mechanical adhesive or "glue," but rather a biologically optimized transfer medium containing functional concentrations of HA along with recombinant human albumin and supportive nutrients.

Over the past two decades, EmbryoGlue® has emerged as one of the most scientifically evaluated embryo transfer adjuncts in ART. Its use has gained increasing popularity among embryologists and clinicians, particularly in recurrent implantation failure (RIF), blastocyst-stage embryo transfer, repeated failed frozen embryo transfer (FET) cycles, and poor prognosis IVF populations. Importantly, the Cochrane systematic review by Heymann et al. (2020), involving more than 6,700 patients, demonstrated moderate-quality evidence supporting improved clinical pregnancy and live birth rates with the addition of functional concentrations of HA to embryo transfer media. This growing body of evidence reflects a

major conceptual shift in ART—from focusing solely on embryo selection toward optimizing the entire implantation ecosystem, including embryo-endometrial communication and transfer media composition.

2. Biology of Hyaluronic Acid (HA):

Hyaluronic acid (HA) is a naturally occurring high–molecular weight glycosaminoglycan abundantly present within extracellular matrices, uterine secretions, cervical mucus, follicular fluid, and tubal fluid. HA is highly expressed during the peri-implantation period and plays a fundamental role in embryo implantation, endometrial receptivity, and early placentation (Gardner et al., 2019; Atkinson et al., 2021). Unlike many extracellular matrix molecules, HA possesses remarkable hydrophilic properties that allow it to regulate tissue hydration, extracellular matrix viscosity, and cellular communication during implantation. During the implantation window, endometrial HA concentration increases significantly, supporting the hypothesis that HA contributes directly to embryo-endometrial interaction and implantation physiology (Heymann et al., 2020).

One of the most important biological properties of HA is its interaction with CD44 receptors expressed on trophoblast cells, cumulus cells, and endometrial epithelial cells (Bontekoe et al., 2014). The HA-CD44 interaction facilitates embryo adhesion and stabilizes embryo apposition during the earliest stages of implantation. Additionally, HA contributes to extracellular matrix remodeling, cellular migration, angiogenesis, cytokine modulation, and trophoblast invasion, thereby creating a favorable implantation microenvironment. Atkinson et al. (2021) demonstrated that HA-mediated signaling pathways enhance embryo-endometrial cross-talk and may improve implantation efficiency by promoting molecular communication and stabilization during the implantation window.

Experimental and clinical studies have further suggested that supplementation of embryo transfer media with HA may mimic the physiological uterine environment and optimize implantation conditions during assisted reproductive procedures. In addition to improving transfer media viscosity, HA may reduce embryo displacement following transfer while simultaneously supporting implantation-related molecular signaling pathways. This multifactorial biological role explains why HA-containing transfer media have attracted significant interest in modern IVF practice, particularly in patients with implantation-compromised conditions.

3. Composition of EmbryoGlue®:

EmbryoGlue® is a hyaluronic acid–enriched embryo transfer medium specifically designed to mimic the physiological uterine environment during the implantation window. Unlike conventional embryo transfer media, EmbryoGlue® contains a functional concentration of hyaluronic acid (HA) along with recombinant human albumin, amino acids, balanced salts, and energy substrates that collectively support embryo viability and implantation potential (Gardner et al., 2019; Heymann et al., 2020). The principal component of EmbryoGlue® is hyaluronic acid (0.5 mg/mL), a naturally occurring glycosaminoglycan abundantly present within uterine secretions during implantation. HA plays a major role in embryo adhesion, hydration, extracellular matrix stabilization, and embryo-endometrial interaction through CD44 receptor–mediated signaling pathways (Atkinson et al., 2021).

In addition to HA, EmbryoGlue® contains recombinant human albumin, which provides osmotic stability and protects embryos from environmental stress during transfer procedures. Amino acids and balanced salts support embryo metabolism, intracellular homeostasis, and pH regulation, whereas energy substrates help maintain embryo viability during the transfer phase (Morbeck et al., 2017). The combined composition of EmbryoGlue® is intended not merely to increase transfer media viscosity but to create a biologically optimized microenvironment that supports implantation-related molecular signaling and embryo-endometrial synchrony. This physiological approach may explain the improved implantation and clinical pregnancy outcomes observed in several randomized trials and meta-analyses (Bontekoe et al., 2014; Heymann et al., 2020).

Table 1. Biological Functions of Hyaluronic Acid During Implantation:

Biological Function	Mechanism	Clinical Relevance
Embryo adhesion	HA-CD44 interaction promotes embryo attachment	Enhances implantation potential
Cellular migration	Facilitates trophoblast movement and invasion	Supports implantation and placentation
Extracellular matrix stabilization	Maintains structural integrity of implantation site	Improves embryo-endometrial interaction
Tissue hydration	Retains water and maintains uterine microenvironment	Supports embryo survival
Cytokine modulation	Regulates implantation-related signaling pathways	Improves molecular cross-talk
Angiogenesis	Supports vascular remodeling during implantation	Enhances placental development
Endometrial receptivity	Creates favorable implantation environment	May improve clinical pregnancy outcomes

Table 2. Components of EmbryoGlue® and Their Biological Functions:

Component	Biological Function	Proposed Mechanism in Implantation
Hyaluronic Acid (HA)	Enhances embryo-endometrial interaction	Facilitates CD44-mediated embryo adhesion, stabilizes embryo apposition, improves implantation signaling
Recombinant Human Albumin	Maintains embryo stability and protection	Supports osmotic balance, protects embryos during transfer, maintains physiological microenvironment
Increased Viscosity of Transfer Medium	Reduces embryo displacement after transfer	Helps maintain embryo positioning within uterine cavity during immediate post-transfer period
Extracellular Matrix Support	Stabilizes implantation microenvironment	Promotes extracellular matrix remodeling and trophoblast interaction
Tissue Hydration Properties	Maintains receptive implantation environment	Improves hydration and endometrial receptivity during implantation window
Cytokine & Molecular Signaling Modulation	Enhances embryo-endometrial cross-talk	Supports implantation-related signaling pathways and cellular communication
Amino Acids	Nutritional and metabolic support	Provides essential substrates for embryo metabolism during transfer
Balanced Salts & Energy Substrates	Maintains physiological embryo environment	Supports osmolarity, pH stability, and embryo viability during embryo transfer
Angiogenesis Support	Facilitates early placentation	Promotes vascular remodeling and implantation support
Cellular Migration & Trophoblast Support	Enhances implantation efficiency	Supports trophoblast invasion and implantation stabilization

4. Mechanism of Action

EmbryoGlue® does not function as a mechanical adhesive or "physical glue." Instead, its beneficial effects on implantation are mediated through multiple biological and biochemical mechanisms that collectively enhance embryo-endometrial interaction during the implantation window (Heymann et al., 2020; Atkinson et al., 2021). The principal active component of EmbryoGlue® is hyaluronic acid (HA), a naturally occurring extracellular matrix glycosaminoglycan that plays a central role in cellular adhesion, tissue hydration, and implantation signaling. During embryo transfer, HA-rich media may recreate a physiological implantation microenvironment by improving embryo apposition, stabilizing embryo-endometrial communication, and supporting molecular cross-talk between the trophoblast and endometrial epithelium (Bontekoe et al., 2014).

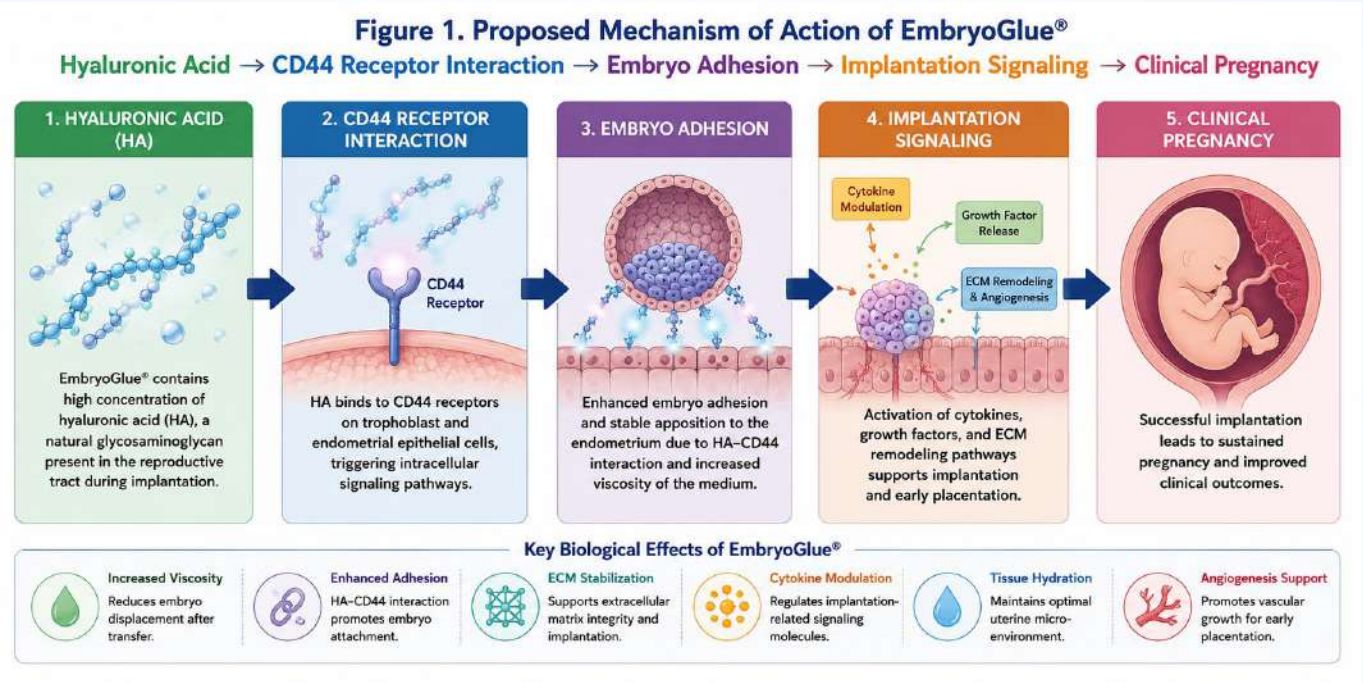
One of the most important biological pathways involves the interaction between HA and CD44 receptors expressed on trophoblast cells and endometrial epithelial cells. This HA-CD44 interaction facilitates embryo adhesion and may enhance implantation efficiency through modulation of cytokines, extracellular matrix remodeling, angiogenesis, and tissue stabilization (Simon et al., 2012). Additionally, the increased viscosity of HA-containing transfer media may reduce embryo displacement after embryo transfer, thereby maintaining closer embryo-endometrial contact during the critical early implantation period. HA also contributes to tissue hydration and extracellular matrix stabilization, which may improve endometrial receptivity and implantation support (Gardner et al., 2019). Collectively, these mechanisms suggest that EmbryoGlue® functions as a biologically active implantation-support medium rather than simply a transfer adjunct.

5. Clinical Evidence

Hyaluronic acid (HA)-containing transfer media, particularly EmbryoGlue®, have been extensively investigated for their potential role in improving implantation and pregnancy outcomes in assisted reproductive technology (ART). The biological rationale behind EmbryoGlue® is based on mimicking the physiological uterine environment during the implantation window while simultaneously enhancing embryo-endometrial interaction through HA-mediated molecular pathways. One of the largest and most influential evaluations was the Cochrane systematic review by Heymann et al. (2020), which included

26 studies involving more than 6,700 patients. The review demonstrated significantly improved implantation and live birth rates with HA-containing transfer media compared with conventional embryo transfer media. Moderate-quality evidence suggested improved clinical pregnancy and live birth outcomes, while low-quality evidence indicated a possible reduction in miscarriage rates.

Figure 1: Proposed mechanism (AI-Generated):



Clinical benefit appears more pronounced in fresh embryo transfer cycles, particularly during blastocyst-stage transfer, where embryo-endometrial synchrony is optimized. Several randomized studies reported improvements in implantation and live birth rates ranging from 10–15% in selected patient populations (Bontekoe et al., 2014). Blastocyst transfers may derive greater benefit due to increased HA receptor expression and enhanced implantation signaling during advanced embryonic development. Furthermore, HA-containing transfer media may help stabilize embryo apposition and reduce embryo displacement following transfer.

The role of EmbryoGlue® in frozen embryo transfer (FET) cycles remains somewhat controversial. Yung et al. (2021) reported variable benefit in FET populations; however, more

recent real-world studies have shown encouraging outcomes in selected patients, especially repeated failed FET cycles and poor prognosis populations. Importantly, the recent Indian multicentric study by Bhoi et al. (2024), involving 1,298 frozen blastocyst transfer cycles across 13 centers, demonstrated significantly higher clinical pregnancy rates (69.5% vs 57.6%) and live birth rates (60.6% vs 47.5%) in the EmbryoGlue® group. These findings further strengthened the growing evidence supporting the use of HA-containing transfer media in modern frozen blastocyst transfer practice. Recurrent implantation failure (RIF) represents another important clinical indication for EmbryoGlue®. Yan et al. (2023) demonstrated improved implantation outcomes in RIF populations, although improvement in live birth rate remained inconsistent. The beneficial effect in RIF may be related to enhanced embryo-endometrial apposition, extracellular matrix stabilization, and implantation-related cytokine signaling. Overall, cumulative evidence suggests that EmbryoGlue® may serve as a useful implantation-supportive adjunct in ART, particularly in blastocyst transfer, fresh cycles, recurrent implantation failure, and selected frozen embryo transfer populations. Nevertheless, further high-quality multicentric randomized trials using standardized laboratory protocols and euploid embryo transfer models are still required before universal recommendations can be established.

Table 3. Major Meta-Analyses and Clinical Studies Evaluating Hyaluronic Acid–Containing Transfer Media:

Author / Year	Study Population	Key Findings	Limitation
Bhoi et al., 2024	1,298 FET cycles	Higher CPR and live birth rates	Retrospective design
Yan et al., 2023	RIF patients	Improved implantation outcomes	No consistent live birth benefit
Yung et al., 2021	Frozen ET cycles	Variable benefit in FET	Mixed evidence

Heymann et al., 2020	26 studies; >6,700 patients	Improved clinical pregnancy and live birth rates	Study heterogeneity
Atkinson et al., 2021	Biological review	Improved embryo-endometrial signaling	Limited clinical data
Gardner et al., 2019	Implantation biology review	Highlighted implantation synchrony importance	Non-clinical review
Bontekoe et al., 2014	~4,000 ART patients	Better implantation and pregnancy outcomes	Variable protocols

6. Laboratory Protocol

The use of EmbryoGlue® can be seamlessly integrated into routine embryology laboratory workflow without major modification of standard embryo transfer procedures. The primary objective of HA-enriched transfer media is to expose the embryo to a physiologically optimized implantation-support environment immediately prior to embryo transfer (Gardner et al., 2019; Heymann et al., 2020). In clinical practice, embryos are typically exposed to EmbryoGlue® for a short incubation period before transfer into the uterine cavity. During this interval, the embryo is exposed to functional concentrations of hyaluronic acid (HA), recombinant human albumin, amino acids, and supportive metabolic substrates that may enhance embryo-endometrial interaction and implantation signaling (Morbeck et al., 2017).

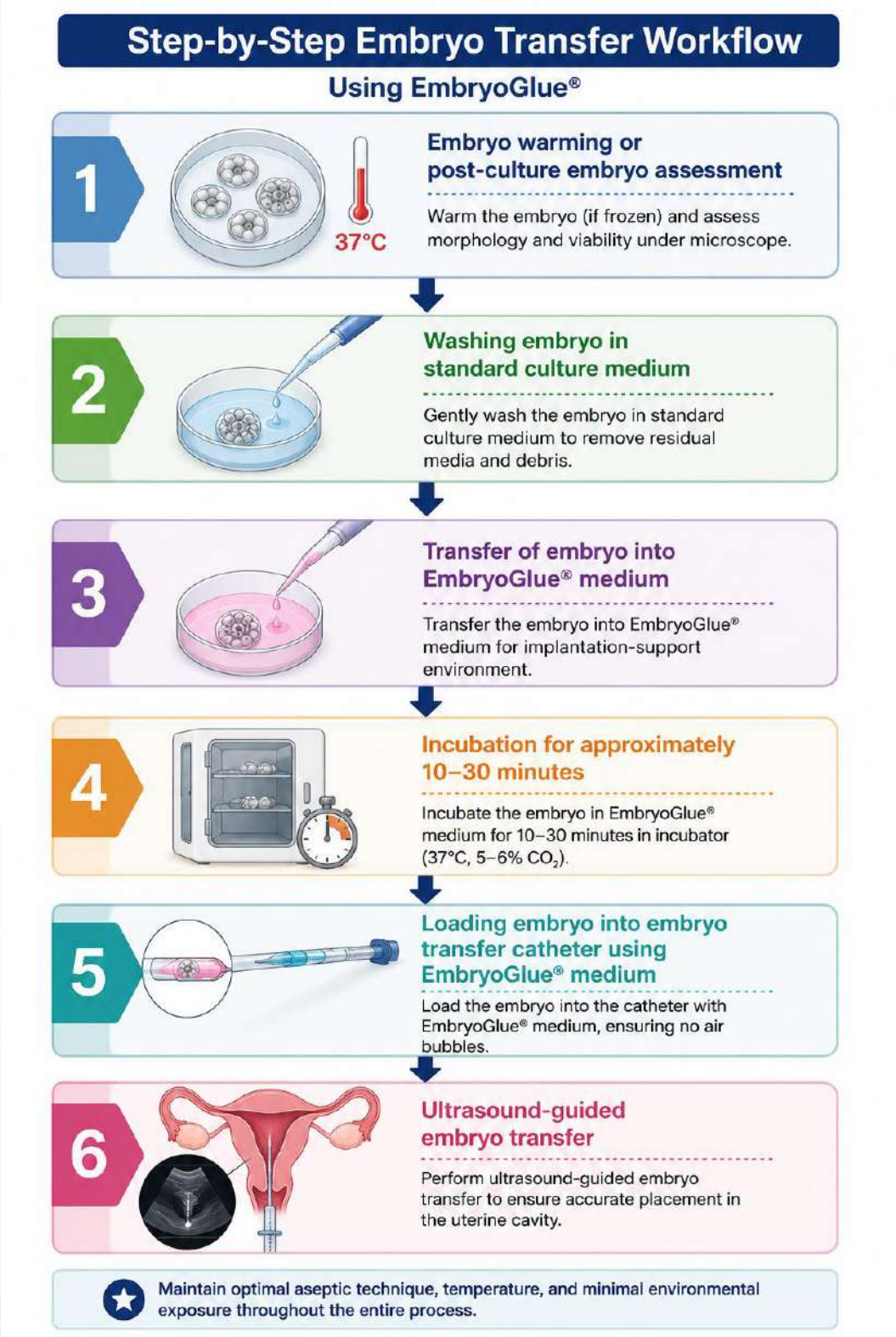
Proper laboratory standardization is critical for achieving optimal outcomes with EmbryoGlue®. Parameters such as temperature stability, pH maintenance, incubation duration, and minimal environmental exposure must be carefully controlled throughout the transfer preparation process. Excessive exposure outside controlled incubator conditions may adversely affect embryo physiology and implantation potential. Several studies have suggested that controlled pre-transfer incubation in HA-containing media for 10 minutes to 30 minutes may improve embryo apposition and stabilize embryo positioning within the uterine cavity following embryo transfer (Bontekoe et al., 2014; Heymann et al., 2020).

However, protocols regarding incubation duration remain variable across different IVF laboratories, highlighting the need for further standardization.

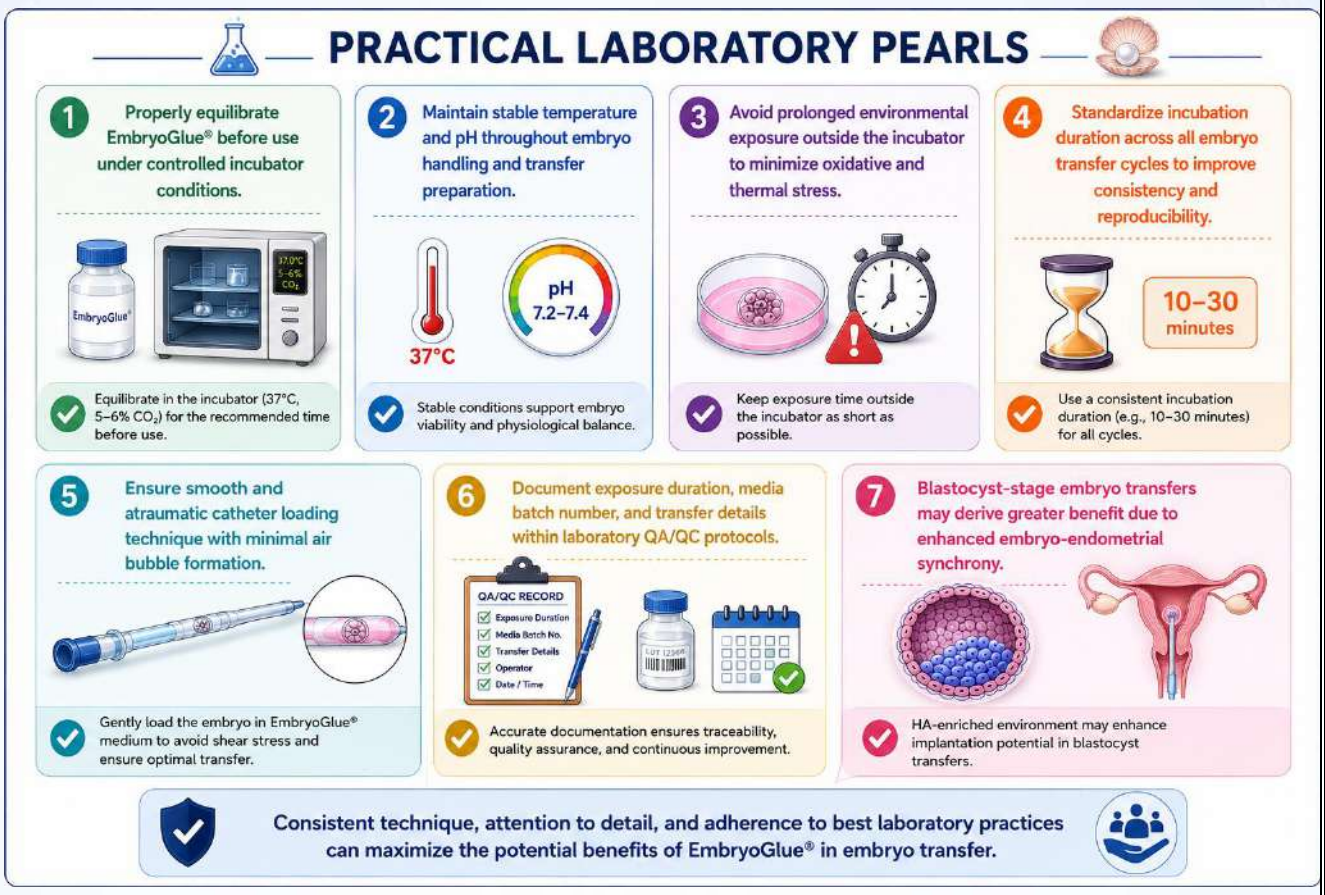
Table 4. Standard Laboratory Workflow:

Steps	Laboratory Procedure	Purpose	Critical Consideration
1	Embryo warming or culture assessment	Confirm embryo viability and morphology	Maintain optimal temperature
2	Washing embryo in standard culture media	Remove residual media and debris	Gentle handling to avoid stress
3	Transfer embryo into EmbryoGlue®	Exposure to HA-enriched environment	Proper media equilibration
4	Incubation for 10–30 minutes	Facilitate HA-mediated interaction	Standardize incubation duration
5	Catheter loading with EmbryoGlue®	Maintain implantation-support microenvironment	Avoid air bubbles and excessive manipulation
6	Ultrasound-guided embryo transfer	Precise embryo placement within uterine cavity	Smooth atraumatic transfer technique

Figure 2: Standard EmbryoGlue® Protocol (AI-Generated):



6.2: Key practical laboratory pearls:



7. Future Perspectives

The future of hyaluronic acid (HA)–containing transfer media in assisted reproductive technology (ART) lies in the development of more personalized and precision-based implantation strategies. As implantation failure continues to remain one of the major limiting factors in IVF success, future research is expected to move beyond generalized embryo transfer approaches toward individualized implantation-support protocols tailored to specific patient and embryo characteristics (Simon et al., 2012; Gardner et al., 2019). One of the most promising areas of investigation is the integration of molecular implantation biomarkers for identifying patients who are most likely to benefit from HA-enriched transfer media. Advances in transcriptomics, proteomics, and endometrial receptivity analysis may help define the optimal implantation window and improve embryo-endometrial synchrony (Atkinson et al., 2021).

Artificial intelligence (AI)–guided embryo-endometrial synchronization may further revolutionize implantation optimization. AI-based predictive models combining embryo morphokinetics, endometrial receptivity markers, patient clinical profiles, and laboratory variables may enable highly individualized embryo transfer strategies in the near future. Such approaches could potentially improve patient selection for EmbryoGlue® usage and maximize implantation efficiency. Another evolving concept is the development of next-generation implantation-support media containing additional bioactive molecules such as prolactin, cytokines, growth factors, and extracellular vesicles combined with HA. Prolactin-enriched HA media may enhance trophoblast invasion, implantation signaling, and endometrial receptivity, although clinical validation is still required. Optimization of HA exposure duration and standardization of incubation protocols also remain important future research priorities. Current protocols vary significantly among IVF laboratories, and the ideal exposure time for maximizing implantation benefit without adversely affecting embryo physiology remains unclear (Heymann et al., 2020).

Importantly, future evidence should ideally come from large multicentric randomized controlled trials using modern vitrification protocols, single euploid blastocyst transfer models, and standardized embryology workflows. Such studies are essential to establish definitive clinical guidelines regarding the universal or selective use of EmbryoGlue® in ART practice.

8. Conclusion

EmbryoGlue® is one of the most extensively studied embryo transfer adjuncts in assisted reproductive technology (ART). Current evidence suggests that hyaluronic acid–containing transfer media may improve implantation and clinical pregnancy outcomes with a reassuring safety profile. Although universal application remains debatable, cumulative literature increasingly supports its selective use in blastocyst transfer, recurrent implantation failure (RIF), and individualized embryo transfer strategies. With the evolution of precision reproductive medicine, EmbryoGlue® may become an important component of implantation optimization protocols in modern IVF practice.

Take-Home Messages

Hyaluronic Acid–Containing Transfer Media represent one of the most scientifically evaluated implantation-supportive adjuncts in assisted reproductive technology (ART). By mimicking the physiological uterine environment, these media may enhance embryo-endometrial interaction, implantation signaling, and embryo apposition during the critical implantation window.

Current evidence from randomized trials and meta-analyses suggests improved implantation, clinical pregnancy, and live birth outcomes, particularly in blastocyst-stage transfer, recurrent implantation failure (RIF), and selected frozen embryo transfer (FET) cycles. Proper patient selection, standardized laboratory protocols, and meticulous embryo transfer technique remain essential for optimal outcomes.

Overall, HA-containing transfer media reflect the evolving concept of implantation optimization in modern ART, where successful outcomes depend not only on embryo quality but also on endometrial receptivity and embryo-endometrial synchrony.

9. References

1. Atkinson B, Dellaire G, Chizen D. The use of hyaluronan in embryo transfer media: biological basis and clinical applications. *Hum Reprod Rev.* 2021;27(3):445–458.
2. Bhoi NR, Murdia N, Chandra V, Kumar D, et al. Effect of Hyaluronic Acid-Containing Transfer Media (EmbryoGlue®) on the Live Birth Rate in Frozen-Thawed Embryo Transfer Cycles. *Cureus.* 2024;16(1):e52713.
3. Bontekoe S, Heineman MJ, Johnson N, Blake D. Adherence compounds in embryo transfer media for assisted reproductive technologies. *Hum Reprod Update.* 2014;20(5):662–674.
4. Gardner DK, Lane M. Embryo culture systems and implantation potential. *Clin Obstet Gynecol.* 2019;62(2):285–295.
5. Heymann D, Fitzgerald CT, Mains L, et al. Hyaluronic acid in embryo transfer media for assisted reproductive technologies. *Cochrane Database Syst Rev.* 2020;CD007421.
6. Heymann D, Vidal L, Or Y, Shoham Z. *Hyaluronic acid in embryo transfer media for assisted reproductive technologies.* Cochrane Database of Systematic Reviews. 2021; Issue 9. doi:10.1002/14651858.CD007421.pub4.
7. Morbeck DE, Krisher RL, Herrick JR, et al. Composition of commercial media used for human embryo culture. *Fertil Steril.* 2017;107(4):1055–1062.
8. Simon A, Laufer N. Assessment and treatment of repeated implantation failure. *J Assist Reprod Genet.* 2012;29(11):1227–1239.
9. Yan Q, et al. Effect of hyaluronic acid-enriched transfer media in recurrent implantation failure. *Front Endocrinol.* 2023;14:1170727.
10. Yung SSF, et al. Hyaluronic acid-enriched transfer medium in frozen embryo transfer cycles. *Fertil Steril.* 2021;115(4):1024–1032.

Overcoming Cryptozoospermia with a Dual-Source Sperm Approach: From Combined TESA and Cryopreserved Ejaculated Sperm to Healthy Live Birth of DCDA Twins: A case Report

Nibhash Kumar, Ankul Kumar, Kumar Sourav, Surabhi Kashyap, Sushant Kumar, Sunita Kumari, Shipra Bharati, Dayanidhi Kumar

Indira IVF Hospital Limited, Patna

Abstract

Background: Cryptozoospermia is a rare and severe form of male factor infertility characterized by the presence of extremely few spermatozoa detectable only after extensive semen centrifugation. The unpredictable availability of viable sperm on the day of oocyte retrieval poses a substantial risk of fertilization failure and cycle cancellation during assisted reproductive technology (ART) treatment. We report a successful ART outcome using a dual-source sperm strategy combining pre-cycle semen cryopreservation with backup testicular sperm aspiration (TESA).

Case Presentation: A 31-year-old woman with a normal ovarian reserve underwent controlled ovarian stimulation using a gonadotropin-releasing hormone (GnRH) antagonist protocol. Due to severe male factor infertility secondary to cryptozoospermia, multiple ejaculated semen samples were cryopreserved before treatment initiation. On the day of oocyte pickup (OPU), backup TESA was performed to mitigate the risk of sperm unavailability; however, only a limited number of spermatozoa were retrieved. Consequently, previously cryopreserved ejaculated semen samples were thawed and utilized for intracytoplasmic sperm injection (ICSI).

Results: A total of 23 oocytes were retrieved, of which 16 mature metaphase-II (MII) oocytes underwent ICSI. Normal fertilization (2PN) was achieved in 14 oocytes, corresponding to a fertilization rate of 87.5%. Extended embryo culture resulted in the development of two high-quality Day-5 blastocysts (graded 1AA and 1AB). Following a programmed frozen embryo transfer (FET), serum β-hCG measured 4781.07 mIU/mL at 14 days post-transfer, indicating successful implantation. Fifteen days later, transvaginal ultrasonography confirmed a viable dichorionic diamniotic (DCDA) twin pregnancy with normal fetal cardiac activity. The pregnancy progressed

uneventfully and culminated in the delivery of healthy twins at 36 weeks and 4 days of gestation, with both neonates weighing approximately 2.5 kg each at birth.

Conclusion: This case highlights the clinical utility of a dual-source sperm strategy integrating pre-cycle semen cryopreservation with backup TESA in patients with severe cryptozoospermia. The approach effectively minimized the risk of cycle cancellation and resulted in excellent fertilization, successful blastocyst development, establishment of a viable DCDA twin pregnancy, and favorable neonatal outcomes. This strategy may represent a practical and effective option for managing selected cases of severe male factor infertility.

Keywords: Cryptozoospermia, Testicular Sperm Aspiration, TESA, Intracytoplasmic Sperm Injection, Semen Cryopreservation, Blastocyst Culture, Male Factor Infertility, Live Birth

1. Introduction

Male factor infertility contributes to nearly 40–50% of infertility cases worldwide, with severe oligozoospermia and cryptozoospermia representing some of the most challenging conditions encountered in assisted reproductive technology (ART). Cryptozoospermia is defined as the presence of extremely rare spermatozoa identified only after extensive centrifugation and microscopic examination of semen samples. Due to the inconsistent and unpredictable availability of viable spermatozoa, these patients are at increased risk of fertilization failure and cycle cancellation during in vitro fertilization (IVF) procedures [1-3]. The introduction of intracytoplasmic sperm injection (ICSI) has significantly improved reproductive outcomes in severe male factor infertility by enabling fertilization with a single viable spermatozoon. Previous studies have demonstrated that even in extreme cases such as cryptozoospermia, satisfactory fertilization and pregnancy outcomes can be achieved through optimized laboratory techniques and appropriate sperm selection methods. Several studies have also reported improved embryo quality, blastocyst development, and pregnancy outcomes when testicular sperm retrieval techniques such as testicular sperm aspiration (TESA) are used in cryptozoospermic patients [4-6]. Meta-analyses have suggested that testicular sperm may provide better reproductive outcomes compared to ejaculated sperm in selected severe male infertility cases. To reduce the possibility of sperm unavailability during treatment cycles, semen cryopreservation before ovarian stimulation is frequently recommended in cryptozoospermia. Combining cryopreserved semen samples with surgical sperm retrieval techniques can provide an effective backup strategy and improve the likelihood of successful fertilization during ART cycles [7].

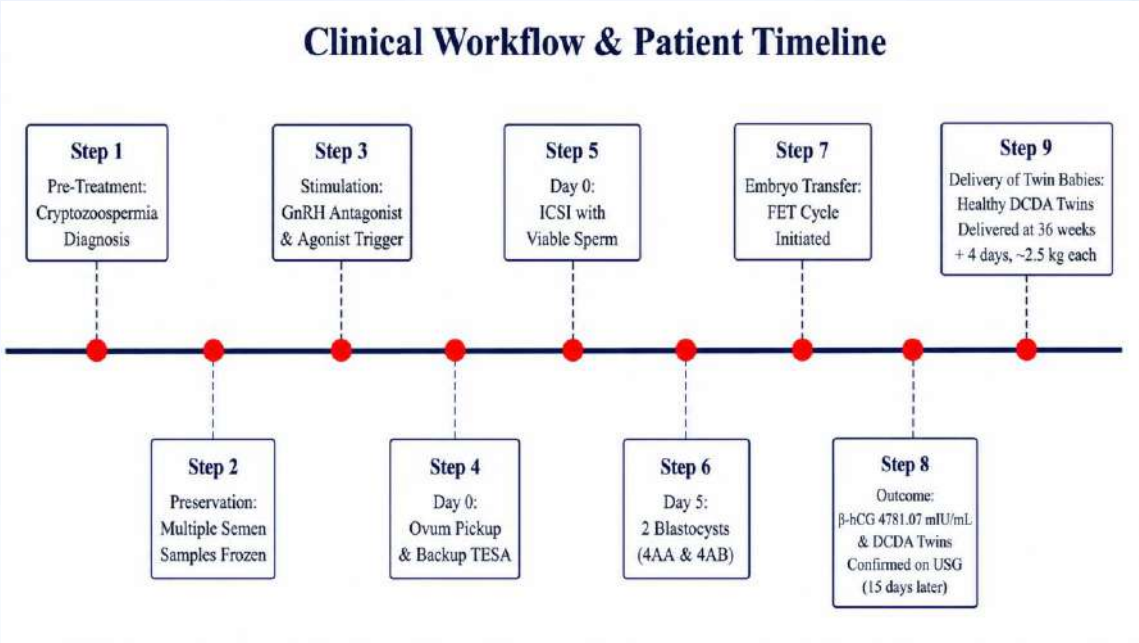
The present case study describes the successful management of a cryptozoospermic patient through a combined approach involving semen cryopreservation, TESA-assisted ICSI, blastocyst culture, and frozen embryo transfer, resulting in successful dichorionic diamniotic (DCDA) twin pregnancy.

2. Case Presentation:

A couple presented with primary infertility of five years' duration secondary to severe male factor infertility. The female partner was 31 years old with a body mass index (BMI) of 21.87 kg/m^2. Ovarian reserve assessment demonstrated an anti-Müllerian hormone (AMH) level of 4.00 ng/mL and an antral follicle count (AFC) of 20, indicating a favorable ovarian reserve. The male partner was diagnosed with cryptozoospermia, confirmed on repeated semen analyses performed according to World Health Organization (WHO) laboratory guidelines. Semen evaluation revealed a volume of 2.2 mL, with only rare spermatozoa identified following centrifugation and meticulous microscopic examination of the pellet. Owing to the extremely limited sperm concentration, comprehensive assessment of sperm motility, vitality, and morphology could not be reliably performed. Considering the high risk of sperm unavailability on the day of assisted reproductive treatment, multiple ejaculated semen samples were cryopreserved before initiation of the treatment cycle as a preventive fertility preservation strategy. Controlled ovarian stimulation was initiated using a gonadotropin-releasing hormone (GnRH) antagonist protocol. Recombinant follicle-stimulating hormone (rFSH) was administered throughout the stimulation phase, and cetrorelix acetate (0.25 mg/day) was introduced for pituitary suppression. The cumulative gonadotropin dose administered during stimulation was 2057 IU. Final oocyte maturation was triggered using a GnRH agonist trigger (0.2 mg). Hormonal assessment on the day of trigger demonstrated serum estradiol (E2) of 3450 pg/mL, progesterone (P4) of 0.92 ng/mL, luteinizing hormone (LH) of 1.8 mIU/mL, and follicle-stimulating hormone (FSH) levels within the expected range. Follicular monitoring revealed satisfactory bilateral follicular development with an anticipated oocyte yield of approximately 18 oocytes.

Transvaginal oocyte pickup (OPU) was performed 35.5 hours after trigger administration, resulting in the retrieval of 23 oocytes. Given the severe male factor infertility and the unpredictable availability of spermatozoa, testicular sperm aspiration (TESA) was simultaneously performed on the day of OPU as a backup sperm retrieval procedure. Fresh testicular sperm obtained through TESA and previously cryopreserved ejaculated sperm samples were evaluated by the embryology team. Due to the limited number and poor quality of sperm recovered through TESA, previously cryopreserved ejaculated sperm samples were thawed and

utilized for intracytoplasmic sperm injection (ICSI). Sperm selection was performed using conventional microscopic assessment based on sperm motility and morphology. Advanced sperm selection techniques, including intracytoplasmic morphologically selected sperm injection (IMSI), physiological ICSI (PICSI), microfluidic sperm selection, and hyaluronic acid-binding assays, were not employed in this case. Of the 23 retrieved oocytes, 16 were mature metaphase-II (MII) oocytes suitable for ICSI, while seven oocytes were immature or otherwise unsuitable for microinjection. ICSI was successfully performed on all 16 MII oocytes using selected viable spermatozoa. Fertilization assessment performed approximately 16–18 hours post-injection demonstrated normal two-pronuclear (2PN) fertilization in 14 oocytes, corresponding to a fertilization rate of 87.5%. Embryos were cultured under optimized laboratory conditions in a tri-gas incubation system with extended culture to the blastocyst stage. On Day 5, two blastocysts graded 1AA and 1AB according to the Gardner and Schoolcraft grading system were obtained and vitrified. A programmed frozen embryo transfer (FET) cycle was subsequently undertaken following endometrial preparation with oral estradiol valerate and vaginal progesterone supplementation. Both vitrified blastocysts survived warming with complete re-expansion and were transferred under ultrasound guidance according to institutional ART protocols. Standard luteal phase support was continued during the post-transfer period. Maternal serum β-hCG measured 4781.07 mIU/mL at 14 days following embryo transfer**,** indicating successful implantation. Fifteen days later, transvaginal ultrasonography confirmed a viable dichorionic diamniotic (DCDA) twin pregnancy with normal fetal cardiac activity. The pregnancy progressed without major antenatal complications and culminated in the delivery of healthy twins at 36 weeks and 4 days of gestation, with a birth weight of approximately 2.5 kg for each neonate**.**



3. Timeline of Clinical Interventions:

Procedure	Outcome
Repeated semen analysis	Cryptozoospermia confirmed
Multiple semen cryopreservation attempts	Backup sperm preservation
Controlled ovarian stimulation	GnRH antagonist protocol
Trigger administration	Agonist trigger (0.2 mg)
Oocyte pickup + TESA	23 oocytes retrieved
ICSI procedure	Fertilization achieved
Day-5 blastocyst culture	1AA and 1AB blastocysts
Blastocyst vitrification	Successfully performed
Frozen embryo transfer	Two embryos transferred

β-hCG assessment	4781.07 mIU/mL
Ultrasonography	DCDA twin pregnancy confirmed
Delivery	Twin baby delivered at 36 weeks 4 days

4. Discussion

Management of cryptozoospermia remains clinically challenging because of the extremely limited number of spermatozoa available for assisted reproductive procedures. Previous studies have demonstrated that sperm retrieval strategies using testicular sperm may improve reproductive outcomes in selected severe male factor infertility cases because of reduced sperm DNA fragmentation compared with ejaculated spermatozoa. Several reports have also suggested that cryopreservation of ejaculated sperm before initiation of ovarian stimulation may reduce the risk of treatment cancellation in cryptozoospermic patients. In the present case, the combined utilization of multiple semen cryopreservation attempts together with same-day TESA provided an effective backup strategy and ensured adequate sperm availability for successful ICSI and subsequent blastocyst development. The successful development of high-quality Day-5 blastocysts and subsequent dichorionic diamniotic (DCDA) twin pregnancy further supports the importance of optimized laboratory conditions, individualized sperm management, and coordinated embryology laboratory planning in severe male factor infertility cases. Although similar ART outcomes in cryptozoospermia have been previously reported, the present case is clinically significant because of the integrated application of semen cryopreservation and surgical sperm retrieval within the same treatment cycle.

Importantly, the successful live birth outcome further strengthens the clinical significance of this case. Despite severe cryptozoospermia and limited sperm recovery during TESA, the combined use of cryopreserved ejaculated sperm and surgical backup retrieval resulted not only in excellent fertilization and blastocyst development but also in the delivery of healthy DCDA twins at 36 weeks and 4 days of gestation, each weighing approximately 2.5 kg at birth, demonstrating favorable obstetric and perinatal outcomes. This successful continuum from sperm retrieval to live birth highlights the potential clinical value of a dual-source sperm strategy in carefully selected patients with severe male factor infertility and cryptozoospermia.

In such cases, the possibility of sperm unavailability on the day of oocyte retrieval can compromise the entire treatment cycle. Therefore, meticulous pre-treatment planning and coordinated laboratory management become essential for achieving successful reproductive outcomes. In the present case, semen cryopreservation was performed before initiation of ovarian stimulation to secure backup sperm samples and reduce the risk of cycle cancellation. Additionally, TESA was carried out on the day of oocyte pickup to ensure adequate sperm availability for ICSI. This combined approach provided greater procedural safety and flexibility during sperm selection and fertilization [8].

The patient demonstrated a favorable ovarian response, with retrieval of 23 oocytes, including 16 mature metaphase-II (MII) oocytes suitable for microinjection. Despite severe male factor infertility, successful fertilization and extended embryo culture resulted in the development of two high-quality Day-5 blastocysts graded 1AA and 1AB. These findings suggest that optimized laboratory conditions, careful sperm handling, and efficient embryo culture systems can support satisfactory embryonic development even in severe cryptozoospermia cases. The subsequent frozen embryo transfer resulted in successful implantation and establishment of a viable DCDA twin pregnancy.

The novelty of the present case lies in the integrated utilization of multiple semen cryopreservation attempts together with a same-day TESA backup strategy, resulting in successful high-grade blastocyst development and subsequent DCDA twin pregnancy following frozen embryo transfer. The case further highlights the importance of individualized sperm management, coordinated embryology laboratory planning, and optimized blastocyst culture in minimizing treatment failure risk in severe male factor infertility. The favorable live birth outcome observed in this case additionally supports the effectiveness of blastocyst vitrification and contemporary frozen embryo transfer protocols in achieving successful implantation, ongoing pregnancy, and healthy neonatal outcomes [9–10].

5. Conclusion

This case demonstrates that a coordinated dual-source sperm strategy incorporating pre-cycle semen cryopreservation and backup TESA can effectively mitigate the risk of cycle cancellation in severe cryptozoospermia. The approach resulted in excellent fertilization rates, successful blastocyst development, establishment of a viable DCDA twin pregnancy, and ultimately the live birth of two healthy neonates at 36 weeks and 4 days of gestation. These findings support the value of combining cryopreserved ejaculated sperm with surgical sperm retrieval as a practical and effective management strategy in selected cases of severe male factor infertility. Further

prospective studies are required to determine the optimal sperm retrieval approach and its impact on embryological, clinical, and neonatal outcomes.

6. Limitations:

Genetic investigations including karyotyping, Y-chromosome microdeletion analysis, and CFTR mutation testing were not available in the present retrospective case documentation. These limitations should be considered while interpreting the findings of this report.

7. Ethical Considerations

A written informed consent for treatment was obtained from the couple. All patient information has been fully anonymized to protect confidentiality. As this manuscript represents a retrospective case report describing routine clinical management without any research-related intervention, formal Institutional Ethics Committee approval was not required according to institutional policy and commonly accepted ethical guidelines for publication of case reports.

8. References

1. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet.* 1992;340(8810):17–18.
2. Esteves SC, Miyaoka R, Agarwal A. An update on the clinical assessment of the infertile male. *Clinics (Sao Paulo).* 2011;66(4):691–700.
3. Koscinski I, Elinati E, Fossard C, et al. DPY19L2 deletion as a major cause of globozoospermia. *Am J Hum Genet.* 2011;88(3):344–350.
4. Benchaib M, Braun V, Lornage J, et al. Sperm DNA fragmentation decreases the pregnancy rate in an assisted reproductive technique. *Hum Reprod.* 2007;22(4):1023–1028.
5. Hauser R, Yogev L, Paz G, et al. Comparison of efficacy of two techniques for testicular sperm retrieval in non-obstructive azoospermia. *Fertil Steril.* 2006;86(3):627–633.

6. Gil-Salom M, Romero J, Rubio C, et al. Intracytoplasmic sperm injection with cryopreserved testicular spermatozoa. *Mol Cell Endocrinol.* 2000;169(1-2):15–19.

7. Tournaye H, Verheyen G, Nagy P, et al. Are there any predictive factors for successful testicular sperm recovery in azoospermic patients? *Hum Reprod.* 1997;12(1):80–86.

8. Devroey P, Liu J, Nagy Z, et al. Normal fertilization of human oocytes after testicular sperm extraction and intracytoplasmic sperm injection. *Fertil Steril.* 1994;62(3):639–641.

9. Rienzi L, Gracia C, Maggiulli R, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis. *Hum Reprod Update.* 2017;23(2):139–155.

10. Esteves SC, Varghese AC. Laboratory handling of epididymal and testicular spermatozoa: what can be done to improve sperm injections outcome. *J Hum Reprod Sci.* 2012;5(3):233–243.

11. AbdelRaheem A, et al. Testicular sperm extraction and ICSI outcomes in cryptozoospermia: a systematic review and meta-analysis. Andrologia. 2021;53:e14145.

12. Kang HJ, et al. Clinical outcomes of testicular sperm versus ejaculated sperm in severe male infertility. Translational Andrology and Urology. 2022;11(4):512–520.

13. Colaco S, Modi D. Genetics of the human Y chromosome and its association with male infertility. Reproductive Biology and Endocrinology. 2018;16:14.

14. Agarwal A, Baskaran S, Parekh N, et al. Male infertility. The Lancet. 2021;397(10271):319–333.

22nd Annual National Conference of the Indian Fertility Society

INDIAN FERTILITY SOCIETY

FERTIVISION 2026

18th - 20th December 2026 | KOLKATA | INDIA

Theme: *Progress with Precision*

Dr. (Prof) Neena Malhotra
President, IFS
Organizing Chair

Dr. Sweta Gupta
Secretary General, IFS
Organizing Secretary

Dr. Surveen Ghumman
President Elect, IFS
Scientific Committee Chair

Dr. S.M Rahman
West Bengal Chapter Secretary, IFS
Organizing Chair (LOC)

Save the Date

18th - 20th December 2026

KOLKATA | INDIA

Secretariat Address

302, 3rd Floor, Kailash Building 26, Kasturba Gandhi Marg C.P. New Delhi - 110001
Phone: +91 1140018184, Mobile: +91-8505923904 ,+91-9136789307,
Email: indianfertilitysocietydelhi@gmail.com, Website: www.indianfertilitysociety.org

www.fertivision2026.com

INDIAN FERTILITY SOCIETY

Dr. (Prof.) Neena Malhotra
President IFS

Dr. Sweta Gupta
Secretary General IFS

How to Become an IFS Member?

01 Scan QR Code

02 Registration — 2 Min

03 UPI / Online Payments are made in Jiffy — 3 Min

04 E-Mail Notification — 4 Min

05 Download E Certificate & Membership Number — 7 Min

For More Information

+91-8505923904 (Ms. Faraha Khan)
indianfertilitysocietydelhi@gmail.com

www.indianfertilitysociety.org

INDIAN FERTILITY SOCIETY

IFS Special Interest Groups

(SIG 2026-28)

Special Interest Groups	Mentor	Convenor	Co Convenors	
Andrology	Dr Guruprasad Kalthur	Dr Sandeep Karunakaran	Dr Gaurav Kant	Dr Sanketh S Dhumal
Applied Genetics	Dr Jayesh S Amin	Dr Fiona D'Souza	Dr Sheetal Jindal	Mr Vakil Jagveer Singh
Counselling & Patient Support	Dr Poonam Nayar	Prof. Arvinder Kaur	Dr Rajvi Mehta	Dr Shikha Bathwal
Early Pregnancy	Dr Sohani Verma	Dr Renu Tanwar	Dr Neelam Bapna	Dr Namita Kotia
Embryology	Dr Sanjay Shukla	Dr Dayanidhi Kumar	Dr Surabhi	Dr Shipra Bharati
Endometriosis	Dr Roya Rozati	Dr Chitra T.	Dr Shalini Chawla Khanna	Dr S. Chitra
Endoscopy	Dr Kuldeep Jain	Dr Reeta Mahey	Dr Ritu Jain	Dr Amol Lukand
Environment and Infertility	Dr Ritu Khanna	Dr Nymphaea Walecha	Dr Anupama Bahadur	Dr Suma Varsha
Fertility Preservation	Dr Nalini Mahajan	Dr Shweta Mittal Gupta	Dr Kokila Desai	Dr Niti Vijay
Holistic Medicine	Dr Gauri Devi	Dr Syed Sajjad Hussain	Dr Anjali Patil	Dr Aanchal Agarwal
PCOS	Dr Surveen Ghumman	Dr Lavleen Sodhi	Dr Parul Garg	Dr Chaitra Nayak
POR (Poor Ovarian Response)	Dr KD Nayar	Dr Sangeeta Sharma	Dr Jitendra Behra	Dr Saumya Prasad
Reproductive Endocrinology	Dr Abha Majumdar	Dr Pikee Saxena	Dr Arti Kapoor	Dr Ruchica Goel
Research & Methodology	Dr Deepak Modi	Dr Ruma Satwik	Dr Bhawani Shekhar	Dr Archana Kumari
Ultrasound	Dr Bharti Jain	Dr Ladbans Kaur	Dr Juhi Bharti	Dr Maansi Jain
Laws & ART	Dr Sonia Malik	Dr Parag Nandi	Dr Neeti Tiwari	Adv Mohini Priya
Quality Assurance & Control in ART	Dr NC Chimote	Dr Gaurav Majumdar	Dr Pranay Ghosh	Dr Sarabjeet Singh
Public Awareness	Dr Pankaj Talwar	Dr Hitendra Somani	Dr Anita Sharma	Dr Garima Kapoor
Female Genital Tract Tuberculosis	Dr Sudha Prasad	Dr Rashmi Sharma	Dr Seema Mittal	Dr Nisha Bhatnagar
KPI & Audit	Dr PM Gopinath	Dr Priya Kannan	Dr Sankalp Singh	Dr Rahul Sen

Dr. (Prof.) Neena Malhotra
President IFS

Dr. Sweta Gupta
Secretary General IFS

For More Information

+91-8505923904 (Ms. Faraha Khan)
indianfertilitysocietydelhi@gmail.com

www.indianfertilitysociety.org