JOURNAL OF FERTILITY AND REPRODUCTIVE HEALTH
Jan-Mar 2024, VOL. 2, ISSUE 1, pp. 20-23
https://doi.org/10.61577/jfrh.2024.100003

RESEAPRO JOURNALS

MINI REVIEW

OPEN ACCESS

Manual vs. laser associated sperm immobilization techniques in intracytoplasmic sperm injection (ICSI): A comprehensive review article

Rahul Kumar Sen, Ashish Sood, Simi Sood, Hiya Agarwal, Ritu Choudhary and Nikita Mohanty

Department of Embryology, Neelkanth IVF, Rajasthan, India

ABSTRACT

Intracytoplasmic sperm injection (ICSI) has emerged as a pivotal technique in the management of male factor infertility. One of the critical steps of ICSI is the immobilization of sperm prior to injection into the oocyte, which is essential for ensuring precision and optimizing fertilization. This review provides a detailed examination of current sperm immobilization methods, including traditional manual techniques, and LASER technology. The implications of these techniques on fertilization rates, embryo quality, and overall clinical outcomes are discussed. Furthermore, potential risks associated with each method are evaluated, highlighting the necessity for additional data, continued research, and addressing the current gaps in evidence.

KEYWORDS

Sperm immobilization; Sperm selection; Embryo, Fertilization; Blastocyst; Oocyte; Sperm

ARTICLE HISTORY

Received 18 January 2024;
Revised 20 February 2024;
Accepted 28 February 2024

Introduction

Infertility affects approximately 15% of couples worldwide, with male factor infertility contributing to about 50% of these cases [1]. Intracytoplasmic sperm injection (ICSI) is a specialized form of in vitro fertilization (IVF) that involves the direct injection of a single spermatozoon into the cytoplasm of a mature oocyte. This technique has revolutionized the treatment of male factor infertility, offering hope to couples facing issues related to abnormal sperm motility, morphology, and count [2]. A crucial step in the ICSI process is the immobilization of the selected sperm, which is necessary to prevent movement during the injection, thus enhancing precision and increasing the likelihood of successful fertilization. Before performing Intracytoplasmic sperm injection (ICSI), sperm tails are immobilized to avoid damage to the oocyte. This is achieved by either crushing the tail against the dish using an injection pipette or by manually drawing the tail down and across with a fine-tipped glass micro-tool. Many studies have shown that damaging the sperm membrane by compressing the tail can yield better outcomes as this may alter the acrosome and sperm head plasma membrane.

Additionally, immobilization increases the permeability of the sperm membrane, which can facilitate nuclear decondensation [3]. The choice of sperm immobilization technique can significantly influence fertilization outcomes, embryo development, and ultimately, live birth rates [4]. Over the years, various methods have been employed for sperm immobilization in ICSI, each with its advantages and limitations. Manual techniques, such as pipetting, squeezing, and mechanical manipulation using glass micropipettes, have been the mainstay of ICSI since its inception [2]. However, the effectiveness of these methods is highly dependent on the skill and experience of the embryologist, and variability in sperm behavior and inappropriate immobilization can lead to inconsistent results [5].

Furthermore, the use of LASER technology has emerged as a cutting-edge method for sperm immobilization in ICSI. LASER immobilization involves using focused LASER beams to halt sperm movement without causing significant damage to their structural integrity [6]. This technique allows for highly controlled immobilization, enhancing the selection of high-quality sperm. However, potential risks associated with LASER immobilization, such as thermal damage and DNA integrity concerns, require careful consideration. This review aims to provide an in-depth analysis of current sperm immobilization techniques, their impact on ICSI success, and future directions in research and clinical practice. By examining the advantages, limitations, and potential risks of each method, clinical Embryologists can make informed decisions to optimize ICSI outcomes and improve the treatment of male factor infertility.

Manual techniques

Manual sperm immobilization techniques have been the cornerstone of ICSI since its inception. These methods typically involve the use of glass micropipettes to manipulate and immobilize motile sperm through mechanical means. Techniques such as pipetting, squeezing, and mechanical manipulation are employed to achieve immobilization.

Advantages and limitations

While manual techniques are widely used due to their accessibility and simplicity, they are not without limitations. The effectiveness of these methods is highly dependent on the skill and experience of an embryologist. Variability in sperm behavior and inappropriate manipulation can lead to inconsistent results, affecting fertilization rates and embryo quality. Studies have shown that manual techniques may not always yield optimal outcomes, particularly in cases of severe male factor infertility where sperm quality is severely compromised [5].

***Correspondence:** Dr. Rahul Kumar, Department of Embryology, Neelkanth IVF, Rajasthan, India, e-mail: rk98298@gmail.com

Main Challenges in Traditional Sperm Immobilization Techniques

Operator dependency

The success of traditional sperm immobilization techniques heavily relies on the skill and experience of the embryologist. Variability in technique can lead to inconsistent results, affecting fertilization rates and embryo quality.

Human error

Manual techniques are prone to human error, which can result in improper immobilization of sperm. Errors such as misjudging the sperm's position or applying excessive force can lead to sperm damage or failure to immobilize effectively.

Sperm quality variability

The effectiveness of manual immobilization techniques can be significantly affected by the quality of the sperm being used. Sperm with poor motility or abnormal morphology may be more difficult to immobilize effectively.

Time-consuming process

Traditional methods can be time-consuming, as they often require careful handling and multiple attempts to achieve successful immobilization. This can delay the ICSI procedure and increase the risk of sperm degradation due to prolonged exposure to environmental conditions.

Limited precision

Manual techniques may lack the precision required for optimal sperm selection and immobilization. This can lead to the selection of suboptimal sperm, which may negatively impact fertilization rates and embryo development.

Stress on sperm

Mechanical manipulation can induce stress on sperm, potentially affecting their viability and function. Excessive force or improper handling can lead to physical damage, which may compromise the sperm's ability to fertilize the oocyte.

Environmental factors

Traditional sperm immobilization techniques often expose sperm to environmental factors such as temperature fluctuations and changes in pH, which can further affect sperm viability.

Inconsistent outcomes

Due to the variability in operator technique and sperm quality, traditional methods can yield inconsistent outcomes, complicating treatment planning for patients undergoing ICSI.

Non-standardized technique

Manual sperm immobilization is considered non-standardized due to the variability in technique among practitioners, leading to inconsistent outcomes, and the potential for unintentional sperm damage during the process.

Multiple Touch vs. Single Touch Immobilization

A randomized trial by Eustache and Auger (2014) compared the effectiveness of multiple-touch sperm immobilization and single-touch sperm immobilization in ICSI [6]. In the multiple-touch technique, the sperm tail was touched multiple times with the injection pipette, while in the single-touch technique, the sperm tail was touched only once. The study found no significant differences in fertilization rates, embryo quality, or pregnancy rates between the two techniques. However, the multiple-touch method resulted in a higher rate of complete sperm immobilization compared to the single-touch method. The authors concluded that both techniques are effective, with the multiple-touch method providing a slight advantage to ensure complete sperm immobilization [7].

LASER-assisted sperm immobilization

LASER technology has emerged as a cutting-edge method for sperm immobilization in ICSI. This technique involves using focused LASER beams to immobilize sperm without causing significant damage to their structural integrity.

Mechanism and efficacy

LASER immobilization operates by applying precise energy to the sperm, effectively halting its movement while preserving its viability. LASER diodes could immobilize sperm effectively while maintaining DNA integrity and normal sperm morphology. The use of LASER technology allows for highly controlled immobilization, which can enhance the selection of high-quality sperm. Research has shown that specific wavelengths of LASER light can optimize the immobilization process. For instance, a study investigating the use of a 1.48 µm wavelength diode LASER found that it achieved 100% immobilization rates when applied to various parts of the sperm (head, mid-tail, and tail) while preserving DNA integrity up to 90% [8]. This indicates that LASER immobilization can be finely tuned to maximize efficacy while minimizing potential damage to sperm.

Applications of LASER in Assisted Reproductive Technologies

LASER immobilization has several applications in ICSI, including:

- **Sperm Selection:** The precision of LASER technology allows for the selection of high-quality sperm based on motility and morphology.
- **Assisted Hatching:** LASER technology can be utilized to create an opening in the zona pellucida, facilitating breakage of oolemma in cases where it is hard to penetrate & on embryos and blastocyst to facilitate implantation.
- **Embryo Biopsy:** for pre-implantation genetic assessment of embryos & blastocyst before their transfer

Benefits of LASER-Assisted Sperm Immobilization

The use of LASER technology for sperm immobilization offers several advantages:

- **Enhanced Precision:** LASER immobilization allows for highly targeted application of energy to specific areas of the sperm, reducing the risk of damage to the sperm's structure and enhancing the likelihood of successful fertilization.
- **Reduced Time for Immobilization:** LASER techniques can significantly decrease the time required for sperm immobilization compared to traditional manual methods, which is crucial in minimizing exposure to potentially harmful environmental conditions.
- **Higher Sperm Viability:** Studies have indicated that LASER-immobilized sperm maintain higher viability and functional integrity than those immobilized using traditional mechanical methods, which can lead to

https://doi.org/10.61577/jfrh.2024.100003

improved fertilization rates and embryo quality [9].

- **Improved Embryo Quality:** The precision of LASER immobilization has been associated with better embryo development and higher morphological scores compared to sperm immobilized by traditional methods [10]. This suggests that using LASER technology may select sperm with better genetic and epigenetic integrity.
- **Versatility:** LASER technology can be adapted for various applications in assisted reproductive technologies, including sperm selection, assisted hatching, and biopsy procedures, making it a versatile tool in the reproductive laboratory.

Despite these advantages, the implementation of LASER systems requires significant investment in specialized equipment and training, which may not be feasible for all clinical settings.

Risks and considerations with LASER-assisted sperm immobilization

Despite its advantages, LASER immobilization is not without risks. Potential concerns include:

- **Thermal Damage:** The focused energy from LASERs can generate heat, potentially leading to thermal damage to the sperm and affecting its motility and viability.
- **DNA Integrity:** There is a risk that LASER exposure could cause DNA fragmentation, which is critical for successful fertilization and embryo development.
- **Cell Membrane Damage:** The focused energy may also damage the sperm cell membrane, hindering its ability to fuse with the oocyte.

Given these risks, careful calibration of LASER parameters and optimal settings are essential to ensure safety and efficacy. Using a specific wavelength of 1,480 nm could effectively immobilize sperm while minimizing thermal damage and preserving DNA integrity.

Comparative studies

Comparative studies have evaluated the effectiveness of LASER-assisted sperm immobilization versus traditional mechanical methods. A study by 11. Ebner et al. (2001) found no significant differences in fertilization rates, cleavage rates, or embryo quality between sperm immobilized by LASER and those immobilized mechanically [11]. However, the LASER technique significantly reduced the time required for sperm immobilization, allowing for quicker ICSI procedures. This reduction in exposure time is critical, as prolonged exposure of gametes to external environments can lead to irreversible damage.

Impact on fertilization and embryo outcomes

The method of sperm immobilization can significantly influence fertilization outcomes in ICSI. It is already indicated that different techniques affect the timing of calcium oscillations in oocytes post-ICSI, which are critical for successful fertilization. Calcium oscillations are essential for triggering the activation of the oocyte and initiating subsequent embryonic developmental processes. Studies have shown that the timing and method of sperm immobilization can impact these oscillations, ultimately affecting fertilization success rates. LASER immobilization, in particular, has been associated with better fertilization outcomes. A study by Chan et al. (2017) demonstrated that the use of LASER technology for sperm immobilization resulted in higher rates of embryo development and better morphological scores compared to traditional mechanical methods [8]. This suggests that LASER immobilization may select sperm with better genetic integrity, leading to improved embryo quality. The precision of LASER techniques allows for targeted immobilization, which minimizes mechanical stress on the sperm and enhances its functional integrity.

However, traditional manual techniques also have their merits. They are generally more accessible and do not require specialized equipment, making them easier to implement in many clinical settings [12]. Manual techniques can also be advantageous in scenarios where immediate sperm selection is necessary, as they allow for quick adjustments based on the embryologist's assessment of sperm quality at the moment of immobilization. Furthermore, the timing of sperm immobilization relative to oocyte injection plays a crucial role in fertilization outcomes. This highlights the importance of precise timing in the immobilization process to optimize ICSI success. Moreover, the impact of sperm immobilization techniques extends to the long-term outcomes of embryos. Research has indicated that embryos derived from LASER-immobilized sperm tend to exhibit enhanced developmental potential and improved implantation rates. This is particularly important in the context of assisted reproductive technology, where the quality of embryos plays a crucial role in the success of treatment. Both methods have their respective benefits and limitations, and the choice of immobilization technique should be tailored to the specific needs of the patient and the clinical context. Ongoing research and clinical trials will be crucial in further elucidating the optimal practices for sperm immobilization in ICSI, ensuring that couples facing infertility receive the best possible care.

Conclusions

Sperm immobilization is a critical step in the ICSI process that significantly impacts fertilization outcomes. The choice of immobilization technique—whether manual or LASER-based—requires careful consideration of the associated benefits and risks. While traditional manual techniques remain prevalent, advancements in LASER technology offer promising alternatives that may enhance the precision and success rates of ICSI procedures. As the field of assisted reproductive technology continues to evolve, ongoing research and innovation are essential to optimize sperm immobilization techniques and improve clinical outcomes for couples facing infertility. Future studies should focus on the long-term effects of various immobilization methods on embryo quality, pregnancy rates, and live birth outcomes to ensure the continued advancement of ICSI as a viable treatment for male factor infertility.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13:1-9. https://doi.org/10.1186/s12958-015-0032-1
2. Palermo GD, O'neill CL, Chow S, Cheung S, Parrella A, Pereira N,

https://doi.org/10.61577/jfrh.2024.100003

Rosenwaks Z. Intracytoplasmic sperm injection: state of the art in humans. Reproduction. 2017;154(6):93-110. https://doi.org/10.1530/REP-17-0374
3. Takeuchi T, Colombero LT, Neri QV, Rosenwaks Z, Palermo GD. Does ICSI require acrosomal disruption? An ultrastructural study. Hum Reprod. 2004;19(1):114-117. https://doi.org/10.1093/humrep/deg511
4. McDowell S, Kroon B, Ford E, Hook Y, Glujovsky D, Yazdani A. Advanced sperm selection techniques for assisted reproduction. Cochrane Database Syst Rev. 2014(10). https://doi.org/10.1002/14651858.CD010461.pub2
5. Rubino P, Viganò P, Luddi A, Piomboni P. The ICSI procedure from past to future: a systematic review of the more controversial aspects. Hum Reprod Update. 2016;22(2):194-227. https://doi.org/10.1093/humupd/dmv050
6. Eustache F, Auger J. Inter-individual variability in the morphological assessment of human sperm: effect of the level of experience and the use of standard methods. Hum Reprod. 2003;18(5):1018-1022. https://doi.org/10.1093/humrep/deg197
7. Xu ZP, Sun HX, Hu YL, Zhang NY, Zhao X. Laser-assisted immobilization causes no direct damage to sperm DNA. Zhonghua nan ke xue= National Journal of Andrology. 2007;13(3):216-218.
8. Chan DY, Li TC. Comparison of the external physical damages between laser-assisted and mechanical immobilized human sperm using scanning electronic microscopy. PloS one. 2017;12(11): e0188504. https://doi.org/10.1371/journal.pone.0188504
9. Diansyah AM, Yusuf M, Kaiin EM. The quality of sperm post-immobilization at some parts of FH sperm using laser diodes. InIOP Conference Series: Earth and Environmental Science. IOP Publishing. 2020;492(1): https://doi.org/10.1088/1755-1315/492/1/012074
10. Debrock S, Spiessens C, Afschrift H, Van der Auwera I, D'Hooghe TM. Application of the Fertilase®-Laser System versus the conventional mechanical method to immobilize spermatozoa for intracytoplasmic sperm injection: A randomized controlled trial. Gynecol Obstet Invest. 2003;56(2):102-105. https://doi.org/10.1159/000072995
11. Ebner T, Yaman C, Moser M, Sommergruber M, Hartl J, Tews G. Laser assisted immobilization of spermatozoa prior to intracytoplasmic sperm injection in humans. Hum Reprod. 2001; 16(12):2628-2631. https://doi.org/10.1093/humrep/16.12.2628
12. Sran MM. To treat or not to treat: new evidence for the effectiveness of manual therapy. Br J Sports Med. 2004;38(5):521-525. https://doi.org/10.1136/bjsm.2003.010876

https://doi.org/10.61577/jfrh.2024.100003