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ADVANCES IN SPERM SELECTION TECHNIQUES FOR IMPROVING OUTCOMES IN ASSISTED REPRODUCTIVE TECHNOLOGIES

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ABSTRACT

Male factors are a key reason for the increasing use of assisted reproductive technology (ART) and account for approximately half of all infertility cases worldwide. Even if their spermatozoa are of lower quality and function, most men who are subfertile may still generate them. The utilisation of methods that circumvent the natural selection barriers that prohibit low-quality spermatozoa from fertilisation is an essential component of assisted reproductive technology (ART). An extensive review of both traditional and cutting-edge sperm selection techniques utilised in reproductive medicine is presented in this article. Furthermore, cutting-edge methods including chemotaxis-based techniques, polarised light microscopy, artificial intelligence-based analysis, and Raman spectroscopy are covered. The goal of these methods is to minimise DNA damage and oxidative stress while isolating sperm that are better in motility, shape, and genomic integrity.

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I. INTRODUCTION

One in five couples of childbearing age will experience infertility at some point in their lives. Over 9 million infants have been born worldwide with assisted reproductive technology (ART) in the 40 years since the first in vitro fertilisation baby was born in 1978, an exponential increase in the number of individuals created through ART. In this period, our knowledge of reproductive biology in humans has grown, and it has become clear that infertility affects both sexes to a similar extent. One study found that men's reproductive issues account for half of all infertility cases, and another found that they account for 30% of all instances. These results may not come as a surprise, but it's still hard to get people to stop thinking infertility is just a female problem. Regular analysis of semen profiles has repeatedly shown that human beings create spermatozoa with incredibly bad shape and function; infertile or subfertile men are often defined by the generation of few spermatozoa that meet the diagnostic criteria for normal spermatozoa.

Men who have infertility often have low sperm counts, which is a result of other reduced semen factors such aberrant morphology and poor motility profiles. These imperfections have long been recognised as indicators of subpar sperm function because they hinder the capacity of sperm cells to pass through the female reproductive canal. Unfortunately, although these criteria do have a correlation with male fertility, they have not been validated as conclusive measures of competence. This is likely due to the fact that sperm still face a series of extra, resolute obstacles before they can fertilise an egg, even after they've been successfully delivered to the egg. Because of this, our knowledge of the basic sperm biology that supports natural conception has a lot to offer.

An ovulated egg is the end goal of millions of competing sperm cells during insemination into the anterior vagina, the first step in the process of natural conception. Only a tiny percentage of the sperm that are inseminated make it all the way to the distal end of the fallopian tube, where fertilisation takes place, due to the intense competition that exists. This proportion typically ranges from tens to hundreds. Even in a healthy or "fertile" human ejaculate, there are many low-quality sperm cells, and after passing through the female reproductive system, these cells face a series of highly specialised obstacles, leading to significant attrition. In addition to the functional traits of sperm, the most crucial parts of a spermatozoon are its paternal genome and epigenome. For decades, researchers have looked at the possibility that sperm populations with lower DNA integrity contribute to a variety of

negative reproductive outcomes, such as low embryo quality, spontaneous abortion, and developmental illnesses. Natural selection may have developed to prevent these cells from fertilisation and the possible transmission of a faulty paternal genome to progeny, according to new data. There is a high frequency of damaged DNA cargo in the spermatozoa of infertile men, as our molecular knowledge of sperm cell biology continues to advance. Importantly, for ART purposes, a spermatozoon with a defective genome can nonetheless fertilise an egg, as it might not be totally functionally impaired.

Surrounded by declining male fertility and an overabundance of "high intervention" assisted reproductive technology (ART) treatments for this illness, there is a chance of unintentionally using spermatozoa with damaged DNA. Indeed, new epidemiological evidence implicates ICSI-conceived infants, relative to their normally conceived counterparts, with an elevated risk of birth abnormalities and the maintenance of faulty semen characteristics. These findings are not conclusive, but they do raise the possibility that using spermatozoa with defects, such as damaged genomes or altered epigenomes, would increase the risk of poor reproductive and overall health outcomes in future generations. Concerns like this underscore the critical need to enhance our present arsenal of sperm selection technologies to reduce the accidental use of low-quality cells in assisted reproduction, with the "gold standard" being the intricate processes of natural conception. Because of this, we need to learn more about how sperm cells are able tofertilise eggs in vivo if we want to make the changes to artificial procedures that people want.

II. SPERM CHARACTERISTICS AND THE NEED FOR SPERM SELECTION

It is not unexpected that most sperm cannot be definitively classified as normal or abnormal, considering the numerous processes that must occur during a sperm cell's journey. The shape of the sperm might vary greatly even within the same ejaculate, going from perfectly normal to clearly aberrant. For instance, there is a wide range of motion characteristics in "normal" sperm motility, including different degrees of forward advancement. The spermatozoa undergo different kinds of tail swelling depending on the level of hypo-osmotic stress they are exposed to. There is a wide range of what is considered "normal" and "abnormal" for other sperm characteristics as well. The development of sperm selection procedures has allowed for the identification of this variance and the subsequent removal of potentially sterile sperm from samples.

Take the following case as an example: An ejaculate's motility, functional membrane integrity, acrosin content, and hamster oocyte penetration potential were all significantly higher in the initial aliquot sperm population following fractionation by silica ("glass") wool column filtration (8). According to these findings, it is possible to isolate a subset of higher quality sperm from a population of sperm that meets certain normality requirements.

A different experiment found that adding deceased sperm and sperm left in the filter reduced zona-free hamster oocyte penetration. This shows that even when competent cells were present, the presence of lower-quality sperm cells hampered the total fertilising capacity of the ejaculate. Therefore, it is necessary to evaluate and eliminate low-quality components when choosing sperm for assisted reproductive technology (ART) if we assume that defective or dead sperm reduce an ejaculate's total fertility potential. Through this process, even a low-quality sample of sperm may be prepared for use in reproductive technologies like IVF and IUI. In vitro fertilisation (IVF) utilising glass wool column-separated sperm results in a much higher success rate compared to swim-up-selected sperm. Actually, a subset of sperm with far better quality may be obtained with any standard sperm selection method by passing them via density gradients or adhesion column filters.

III. SPERM SELECTION METHODS

The results of assisted reproduction have been steadily increasing thanks to these traditional and modern sperm selection methods, which have opened up new avenues for diagnosing and treating male infertility. To increase fertilisation success rates and embryo quality in assisted reproductive technology (ART), efficient sperm selection approaches generally attempt to separate sperm cells with the highest motility, morphology, and genetic integrity.

Conventional Sperm Selection Methods

- **Swim-Up Technique**

Swim-Up has been around for a long time, is really easy to use, and doesn't cost a lot of money. It's popular because it's simple, and it's great at picking up motile sperm (>90%). This technique involves layering a sperm sample beneath a medium; thereafter, the viable sperm will swim upwards into the medium, while the less viable or immotile cells will remain behind. Swim-Up increases the percentage of active motile sperm in the sample, which is associated with better fertilisation potential, as motility is a natural selection criteria.

Thus, this method has the benefit of isolating sperm with less DNA fragmentation, which is especially useful for highly motile sperm since they sustain less DNA damage. In extreme cases of male factor infertility, where sperm motility is low, the Swim-Up procedure has several limitations and drawbacks, notwithstanding its effectiveness. Oligospermic and asthenozoospermic people are often not treated with this method due to low yields and the fact that only a small percentage of motile sperm swim up into the medium.

- **Density Gradient Centrifugation (DGC)**

Using cell density as a criterion, DGC is a popular sperm selection method that distinguishes between viable, mobile sperm and other types of detritus, leukocytes, and morphologically defective spermatozoa. The sample would be layered across a colloidal silica gradient during centrifugation, enabling the collection of high-quality motile sperm at the bottom layer. Several studies have demonstrated that DGC improves ART results compared to unprocessed semen by selecting sperm with good motility and intact DNA. A large output of motile and morphologically normal sperm is critical for effective fertilisation and embryo quality in IVF and ICSI settings, where DGC has proven particularly beneficial. Nonetheless, DGC isn't without its drawbacks; for example, the centrifugation process might produce oxidative stress. There is strong evidence that oxidative stress can cause spermatozoa DNA to fragment, which in turn affects fertilisation and embryonic development. Patients with DNA fragmentation levels more than 30% are ineffective candidates for DGC, which primarily targets shape and motility but not fragmentation. Despite these limitations, DGC is still a standard procedure in assisted reproductive technology (ART), but it is typically used in conjunction with improved selection procedures to guarantee even more purity of the chosen sperm.

Advanced Sperm Selection Techniques

- **Magnetic-Activated Cell Sorting (MACS)**

Apoptotic cell separation is accomplished by the production of phosphatidylserine, a marker for cell death, in the most modern and advanced method for sorting non-apoptotic sperm, Magnetic-Activated Cell Sorting (MACS). Annexin V attaches only to apoptotic sperm, and magnetic microbeads are coupled with it for MACS. The same holds true for humans; a magnetic field can separate their essential cells. This method has proven effective in situations of elevated oxidative stress and DNA fragmentation thus far.

It consists of removing damaged or dying sperm and replacing them with DNA-intact, viable sperm, which allows for a thorough assessment of the molecular features of eggs. Concerning MACS, there is contradictory data. Research has shown that there is a growing trend towards better ART outcomes in severe cases of male factor infertility, including higher fertilisation rates, shorter treatment durations, more cost-effective treatments, and better embryo quality. However, studies have also shown that MACS exhibits minimal statistically significant change in either CPR or LBR.

- **Microfluidic Sperm Sorting**

A new technique called microfluidic sperm sorting uses microfluidic tubes to mimic natural selection processes, selectively allowing only the most mobile sperm to pass through while exposing them to low levels of mechanical and oxidative stress. One of the most talked-about technologies in the field of infertility diagnosis and treatment is microfluidics, which is quickly becoming a tool for processing small samples. Microfluidics eliminates the requirement for centrifugation, which means it can significantly reduce the danger of DNA damage compared to conventional procedures. The production of sperm samples that are highly motile, morphologically entitled, and DNA-intact is a crucial need for effective IVF and ICSI outcomes, which is why this procedure is advantageous in ART settings. Because it concentrates samples with sperm that contain lower fragmentation indices and better morphology compared to other approaches, microfluidic sorting has proven to be quite beneficial in instances of male infertility with high levels of DNA fragmentation. Researchers Banti et al. found that compared to the DGC approach, using a sperm sorting microfluidic device improved blastocyst formation, utilisation, and euploidy rates after ICSI. Additionally, there was no statistically significant correlation between male factor infertility and its absence. Microfluidics has shown encouraging results in the field of assisted reproductive technology (ART), including higher rates of fertilisation, better embryo growth, and better live birth outcomes. This establishes microfluidics as a stress-free and highly successful method of sperm selection.

- **Zeta Potential Selection**

A new approach to sperm selection called zeta potential selection uses the sperm's electrical charge to differentiate between healthy, fully developed cells and those that are immature or damaged.

It is possible to use the greater negative zeta potential of mature sperm with intact membranes to retreat these cells. Zeta potential selection in research has demonstrated promise in several assisted reproductive technology (ART) applications because to its ability to reduce DNA fragmentation and generate sperm of high quality. Early studies have shown that using zeta-selected sperm improves fertilisation rates and embryo quality, however zeta potential selection is not as often employed in clinical settings as microfluidics or MACS. In certain instances of sperm selection, our findings suggest that zeta potential is a determinant.

- **Hyaluronic Acid (HA) Binding Assay (PICSI)**

The HA Binding Assay allows mature sperm to naturally select for HA, which ensures that the sperm's DNA is intact and that it is functionally mature. In this method, HA-coated plates are used to simulate the natural selection processes in the female reproductive canal by preferentially binding mature, viable sperm. Studies have consistently demonstrated that sperm with little DNA fragmentation tend to bind HA, making them excellent for in vitro fertilisation (ICSI), where the quality of the embryos and the pregnancy outcomes are influenced by DNA integrity. One way in which the HA Binding Assay helps ART work better is by raising the quality of embryos and the success rate of pregnancies. In situations where male infertility is caused by excessive DNA fragmentation, the incorporation of HA into in vitro fertilisation processes allows for the non-invasive extraction of mature sperm, which aids in both therapy and embryo development.

IV. SPERM SELECTION TECHNOLOGIES

Raman spectroscopy and confocal RAMAN micro spectroscopy

Molecular vibrations provide the basis for these non-invasive methods that employ laser light to evaluate sperm biochemical composition without tagging or harming the cells. Researchers have shown that these techniques can differentiate between sperm with substantial DNA fragmentation and those with normal DNA integrity and membrane structure (Huser et al., 2009). Due to the requirement for specialised equipment and knowledge, their clinical usage is limited, keeping them mostly within the research arena. However, they show promise for real-time and label-free sperm quality testing.

Polarized light microscopy

The birefringence of the sperm head, which indicates the degree of DNA packing, may be evaluated using polarised light microscopy, a non-invasive method, to determine the integrity of the chromatin in the sperm. Mature sperm with tightly packed chromatin show symmetrical birefringent patterns when illuminated by polarised light; aberrant patterns are associated with DNA damage and chromosomal abnormalities. Selecting birefringent sperm may improve fertilisation results and embryo quality, according to limited clinical research. However, its routine clinical application is still limited due to the need for specialised equipment and experience and the lack of large-scale validation.

Thermotaxis and chemotaxis-based selection

By separating sperm that react to temperature gradients or chemical compounds like progesterone, sperm selection techniques based on thermotaxis and chemotaxis try to imitate the natural guiding mechanisms of the female reproductive canal. In order to increase the likelihood of fertilisation, these techniques use specialised chambers to entice sperm that are functionally competent enough to traverse these gradients. Despite good preclinical evidence and theoretical promise, these methods are still in their early stages and have not yet made it into clinical practice owing to difficulties in standardisation and repeatability. Their practicality and effectiveness in assisted reproductive technology require more study.

V. CONCLUSION

Important decisions on sperm methods must be made based on the quality of each male partner's sperm. Improved chances of fertilisation, blastocyst development, implantation, and a successful pregnancy in in vitro fertilisation (IVF) are associated with optimal sperm cell selection, which is defined by less DNA damage and the avoidance of reactive oxygen species (ROS) generation. Artifactual reproductive technology (ART) methods can increase fertility rates, embryo quality, and reproductive safety for generations to come by enhancing the capacity to detect and extract healthy sperm with complete genetic material and ideal functional characteristics.

REFERENCES: -

[1] A. Williams *et al.*, "Hyaluronan sperm selection during ICSI (HA-ICSI) increases embryo utilization in couples with male factor infertility," *Fertil Reprod.*, vol. 6, no. 1, pp. 28–36, 2024.

[2] Y. Zeng, S. Yan, and X. Zhu, "Advancements in sperm selection: Overview of techniques and their clinical applications," *Journal of Clinical Reproduction*, vol. 28, no. 1, pp. 12–22, 2022.

[3] M. Sadeghi, M. Bakhtiari, and M. Ghasemi, "Advanced sperm selection techniques: An overview and impact on ART outcomes," *Journal of Assisted Reproduction and Genetics*, vol. 38, no. 3, pp. 617–626, 2021.

[4] T. M. Said *et al.*, "Hyaluronic acid sperm selection in ICSI outcomes," *Arch. Gynecol. Obstet.*, vol. 303, no. 1, pp. 149–159, 2021.

[5] M. L. Pedrosa, M. H. Furtado, M. C. F. Ferreira, and M. M. Carneiro, "Sperm selection in IVF: The long and winding road from bench to bedside," *JBRA Assist. Reprod.*, vol. 24, no. 3, pp. 332–339, 2020.

[6] F. Wu, X. Wang, and Z. Lu, "Sperm selection for ART: A new horizon," *Journal of Reproductive Health*, vol. 12, no. 6, pp. 312–320, 2020.

[7] M. H. Nasr-Esfahani, M. R. Deemeh, M. Tavalaee, M. H. Sekhavati, and H. Gourabi, "Zeta sperm selection improves pregnancy rate and alters sex ratio in male factor infertility patients: A double-blind, randomized clinical trial," *Int. J. Fertil. Steril.*, vol. 10, no. 2, pp. 253–260, 2016.

[8] H. N. Highland, A. S. Rishika, A. S. Almira, and P. B. Kanthi, "Ficoll-400 density gradient method as an effective sperm preparation technique for assisted reproductive techniques," *J. Hum. Reprod. Sci.*, vol. 9, no. 3, pp. 194–199, 2016.

[9] A. Volpes, F. Sammartano, S. Rizzari, S. Gullo, A. Marino, and A. Allegra, "The pellet swim-up is the best technique for sperm preparation during in vitro fertilization procedures," *J. Assist. Reprod. Genet.*, vol. 33, no. 6, pp. 765–770, 2016.

[10] M. Yamanaka *et al.*, "Combination of density gradient centrifugation and swim-up

methods effectively decreases morphologically abnormal sperms," *J. Reprod. Dev.*, vol. 62, no. 6, pp. 599–606, 2016.

[11] L. Simon, K. Murphy, K. I. Aston, B. R. Emery, J. M. Hotaling, and D. T. Carrell, "Micro-electrophoresis: A noninvasive method of sperm selection based on membrane charge," *Fertil. Steril.*, vol. 103, no. 2, pp. 361–366, 2015.

[12] M. Gil, V. Sar-Shalom, Y. Melendez Sivira, R. Carreras, and M. A. Checa, "Sperm selection using magnetic activated cell sorting (MACS) in assisted reproduction: A systematic review and meta-analysis," *J. Assist. Reprod. Genet.*, vol. 30, no. 4, pp. 479–485, 2013.

[13] L. Gianaroli, M. C. Magli, G. Collodel, E. Moretti, A. P. Ferraretti, and B. Baccetti, "Sperm head's birefringence: A new criterion for sperm selection," *Fertil. Steril.*, vol. 90, no. 1, pp. 104–112, 2008.

[14] P. J. Chan, J. D. Jacobson, J. U. Corselli, and W. C. Patton, "A simple zeta method for sperm selection based on membrane charge," *Fertil. Steril.*, vol. 85, no. 2, pp. 481–486, 2006.

[15] C. De Jonge, "The clinical value of sperm nuclear DNA assessment," *Hum. Fertil.*, vol. 5, no. 2, pp. 51–53, 2002.