Review Article

A narrative review of sperm selection technology for assisted reproduction techniques

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Background and Objective: In-vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) has become increasingly prevalent even in cases without significant male factor infertility; however, stagnant live-birth rates, both nationally and internationally, have driven more research into sperm selection. To date, nothing has replaced swim-up and density-gradient preparation methods and therefore we sought to review the state of the science.

Methods: A PubMed search was performed between years of 1989 and 2024 for English research articles reporting data on sperm selection technology in assisted reproductive technology.

Key Content and Findings: IVF with ICSI is increasingly prevalent even in men with normal semen parameters. Despite technologic advances and widespread use, reproductive outcomes with ICSI have been stagnant. This market for opportunity growth has allowed for sperm selection techniques to grow exponentially with heterogeneity in utilization and a paucity of positive reproductive outcomes. Swim-up and density-gradient centrifugation remain the most utilized sperm selection techniques. Various future technologies show promise including epigenetics, sperm biomarkers and a potential role of artificial intelligence; however, more research is needed.

Conclusions: Given unchanged IVF success rates, sperm selection technologies hold promise to improve reproductive outcomes beyond traditional ICSI. At present, no technique has shown superiority to swim up and density centrifugation.

Keywords: Intracytoplasmic sperm injection (ICSI); assisted reproductive technology (ART); sperm selection technology; in-vitro fertilization (IVF)

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Introduction

Infertility affects 15% of couples worldwide (1) with male factor involved half of the time (2). Regardless of the underlying etiology, assessment of male fertility by a semen analysis (SA) has long been the initial diagnostic tool. Ejaculated volume, sperm concentration, motility, and morphology are the foundational parameters with reassuring values assumed to be those above the 5th percentile according to the most recent iteration of the World Health Organization (WHO) (3). The WHO

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Table 1 Summary of search strategy

Items	Specification
Date of search	Searches performed between December 1, 2023 and April 18, 2024
Databases and other sources searched	PubMed
Search terms used	Sperm selection technology, assisted reproductive technology, in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI)
Timeframe	Between 1989 and 2024
Inclusion criteria	Only English language studies were included. While peer-reviewed published manuscripts were prioritized, abstracts and textbook chapters that fit our search criteria (found on google.com) were also included
Selection process	Each author was independently involved in literature search. The primary author (D.K.C.) reviewed all included articles

cautions however that these are not normative values and stresses the inherent limitations of the SA. Arguably, the most predictive semen parameter is the total motile sperm count (4). The decline of sperm counts worldwide has gained traction in mainstream media and literature with a recently updated, large systematic analysis reporting 40–60% sperm count decline (population variability) since 1973 with loss of nearly 1 million/mL/year (5). This has implications for assisted reproductive technology (ART) and its ever more prevalent use [predominantly in-vitro fertilization (IVF)] up 234%, from 413,776 cycles in 2021 compared to 176,274 in 2012. Despite the numerous technological advances in ART since the first successful embryo transfer (6), including intracytoplasmic sperm injection (ICSI), live-birth rates (e.g., live birth per embryo transfer) via IVF (when considering women of all ages, all infertility diagnoses) have been stagnant, with similar international trends (7,8). From its most recent peak in 2008 (36.7%), live-birth rates via IVF in the U.S. have been on a steady decline ever since with 29.1% in 2012 and 22.2% in 2021. For ideal women undergoing IVF (i.e., age <35 years), the 2022 national summary preliminary data from Society for Assisted Reproductive Technology reports live-birth rate per intended egg retrieval of 43.1%. Even when this is considered, there is room for improvement (9). Several suspected theories have been postulated (7) including sperm dysfunction. Assessment of sperm quality for ART includes objective measures of concentration and motility as well as the subjective evaluation of morphology per WHO criteria (3) and there is a large need for universal, unbiased selection criteria to afford selection of more robust sperm (10). We sought to evaluate the literature to review the current landscape of sperm selection and determine if any modern research holds promise with hope of optimizing sperm for IVF. We present this article in accordance with the Narrative Review reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-24-195/rc).

Methods

This manuscript is a qualitative review with use of PubMed as our search modality. Search terms included: sperm selection technology, assisted reproductive technology, in-vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI). The timeframe of included studies was between 1989 and 2024. Only English language studies were included. Priority was given to peer-reviewed, published manuscripts but abstracts, case reports, textbook chapters that fit search criteria were also included. Further, cited studies found within the above were also considered for use. Please see *Table 1* for a summary of our research strategy.

Discussion

Despite numerous technological advances in ART since the first successful embryo transfer in 1978 (6), IVF birth rates have been stagnant. As a result, attention was expanded to consider sperm factors and the need for more expansive selection techniques to select for the most suitable sperm. Herein, we describe the classic sperm selection methods, a collection of promising techniques not used, and then focus on the most modern techniques.

"Classic"

Shortly following the success of the first embryo transfer

with the boom of IVF, there was a need for sperm separation techniques. The early techniques focused on optimizing motility, whereas newer techniques have emphasized sperm morphology and function. These two classic methods are representative of their names, with each respective test evaluating a sperm's motility, designed to select out the most suitable.

Swim up

Mahadevan & Baker first described this technique, in which the spermatozoa are centrifuged or liquefied (mimicking the action of activated prostatic serine proteases in the female reproductive tract) and the remaining thin, watery concentrate is layered atop a sperm culture medium (11). The actively motile spermatozoa then navigate into this media and are collected for use.

It is one of the most widely used forms of sperm selection, in addition to density-gradient centrifugation (DGC), in IVF for patients with normozoospermia and female factor infertility. Advantages include very high recovery of motile sperm (>90%), user-friendly, and cost-effective while disadvantages include restricted use in patients with male factor infertility, potential for increased reactive oxygen species (ROS) creation (if using pelleted spermatozoa) and thus reduced motility (12,13).

DGC

There is a gradient material of gradual density increase from the top to the bottom of the gradient. Regardless of the material, the ejaculate is placed atop the media and centrifuged at 200–500 g for 20 minutes. Sperm cells will move through the gradient to different degrees, depending on their density, motility. As a result, only highly motile sperm with good morphology navigate to the bottom of the gradient to form a concentrate, known as the "pellet" while less motile, variable morphologic, or dead sperm, among bacteria or leukocytes do not (13).

Ricci *et al.* compared sperm outcomes between swim-up *vs.* DGC in a split-sample study and found significantly greater sperm viability (using flow-cytometry), total and progressive motility in swim-up relative to DGC but DGC had greater total motile sperm counts (12). Boomsma *et al.* evaluated swim-up *vs.* DGC in setting of intra-uterine insemination (IUI) via Cochrane review with 4 randomized controlled trials (RCTs) and concluded inadequate, low-quality evidence to recommend one over the other, given similar clinical pregnancy rate (CPR) between swim-up *vs.* gradient technique (22% *vs.* 24% respectively) (14). More recently, Rao *et al.* evaluated live birth rate (LBR) in IVF cycles between swim-up and DGC, concluding no difference (15). In reality, IVF clinics tend to use one or both methods for modern IVF protocols though some concern exists with these methods causing harmful effects on sperm. DGC may induce high centripetal pressures and forces on sperm, potentially disrupting sperm integrity and secondly, those in the resulting pellet may have higher deoxyribonucleic acid (DNA) fragmentation and ROS (16-19), though controversy exists. Raimondo *et al.* nicely reviewed the history of this and updated the data by attempting to answer the question of whether one separation technique is associated with more apoptotic spermatozoa (using p53 expression as surrogate), concluding that swim-up has actually has significantly higher apoptotic rates relative to DGC (20). Larger, randomized studies will need to clarify this debate (14).

These classic sperm separation techniques require at least 50,000 total sperm after washing, to optimize fertilization rates (21). In specific scenarios with oligospermia, there is limited published data with one study showing a minimum concentration of 12 million/milliliter for swim-up (22) while another required at least 20 million/milliliter (23). If densities were less, DGC was pursued. When total sperm counts are less, conventional ICSI is pursued as minimal sperm are required. When considering classic IVF, the only prerequisite is motility. With that said, motility is not 100% concordant with fertility potential. Teratospermia or abnormal shape was previously felt to be a strong indicator of subfertility with lower pregnancy rates a motile sperm with IVF (24,25). Subsequent research suggested no difference in IVF/ICSI with teratospermia (26-28). Zhou *et al.* attempted to close this debate with a recent case-control study, finding morphology to have limited predictive value on pregnancy outcomes in IVF (29). A severe form of teratospermia is globozoospermia, a rare sperm defect leaving sperm head devoid of acrosome, cannot fertilize an oocyte *in vivo* (30). ICSI enables these men to conceive. Aside from globozoospermia, there is no consensus on management of teratospermia and morphology remains largely a subjective assessment via embryologists.

Morphology

Motile sperm organelle morphology examination (MSOME)

Conventional ICSI uses magnification of 200–400× to select the 'best' morphologic and motile sperm. Bartoov described MSOME, a technique using ultra-high magnification

(>6,000×) to evaluate minor morphologic criteria (six cellular organelles: acrosome, post-acrosomal lamina, neck, mitochondria, tail and nucleus). His prospective study demonstrated increased morphological normalcy of the sperm cell and was positively associated with ICSI fertilization rate [area under the curve (AUC) 88%] but not uniformly with pregnancy rates (31). His follow-up prospective comparative study examined couples with repeated ICSI failure (mean =4 transfers) for routine ICSI *vs.* intracytoplasmic morphologically selected sperm injection (IMSI) (also known as ICSI with MSOME). IMSI showed increased CPR compared with MSOME (66% *vs.* 30%) (32). This led to a large Cochrane review of MSOME *vs.* ICSI more widely, concluding non-superiority of LBR in one study (33) and insufficient evidence to support IMSI (34).

Membrane surface charge

Magnetic-activated cell sorting (MACS)

MACS is a sperm selection technology that sorts sperm based on the relative burden of phosphatidylserine (PS) on sperm cell membranes, a known marker for selecting apoptotic cells (35). MACS functions via a sperm sample run through a column coated in magnetic microspheres conjugated to annexin V (a cellular protein known to have a high affinity for PS), and therefore are able to filter out any non-viable sperm (36). Multiple studies comparing MACS to alternative sperm selection methods have shown conflicting results, with very few reporting any statistically significant change in CPR or LBR (37-40). Moreover, a recent systematic review of the MACS literature did not find a statistically significant change in overall ability for filtered sperm to result in pregnancy relative to traditional methods (41).

Zeta-potential method

Sperm characteristically have negatively charged membranes and with maturation. Its membrane potential becomes increasingly more negatively-charged and therefore can be selectively sorted out using electrophoresis or other charge-based equipment (42). This group of processes is referred to as the Zeta-potential methods. Some of the initial studies investigating Zeta-potential reported improved embryo and DNA quality, both of which are desired qualities in ART (42,43). Only one double-blinded RCT trial exists with similar results, in addition to the observation that sperm selected using DGC combined with the Zeta group had a higher XY/XX sex ratio than DGC alone (44). A 2019 Cochrane review compared Zeta-potential *vs.* conventional ICSI, concluding with the lone RCT previously mentioned, the overall quality of data on zeta-potential to be "very low" (45). Given the paucity of subsequent data, this method has fallen out of favor in lieu of standard selection techniques.

Nuclear/membrane integrity

Zona pellucida (ZP) binding

The ZP is a glycoprotein matrix that surrounds an ovulated oocyte and is the last barrier a sperm has to overcome before fertilization, thus serving an important role in the selection process of fertilization. Since the discovery of the sperm-ZP interaction (46,47), multiple studies have compared ZP-binding assays *vs.* the "classic" methods of ART [1 RCT (48), 2 observational studies (49,50)], concluding that the ZP selects for sperm with higher DNA and chromatin integrity, and decreased levels of methylation and thus can lead to higher quality embryos though no difference in CPR. The reduced DNA fragmentation was further corroborated with recent basic science work (51); however, a recent meta-analysis concluded that no difference existed between standard ICSI outcomes and use of ZP-bound sperm (52). Further, Liu and company demonstrated that reduced number of sperm-ZP binding correlated with failure of conventional IVF (53).

Physiologic ICSI (PICSI) with hyaluronic acid (HA)

HA is a ubiquitous fatty acid of the extracellular matrix of the cumulus oophorus and mature sperm express HA-binding sites that allows for HA digestion as an initial step with intent of reaching the oocyte (14). Serving as a physiologic marker of mature sperm, numerous studies have evaluated its efficacy *vs.* gold-standard of ICSI, including four robust, RCTs (54-57) and subsequent Cochrane review (45) concluding little to no difference in CPR, LBR, miscarriage or embryo quality.

Microfluidics

Microfluidics is a constantly evolving technology within the biomedical field that has showed significant promise as a potential tool for sperm selection. Along with sperm motility, and morphology, DNA integrity is also of utmost importance (58). Reduced motility, concentration and morphology have been correlated with reduced DNA integrity (59). In patients with idiopathic infertility and normozoospermia, some patients are observed to have higher DNA fragmentation (60). Subsequently, it is known that increasing DNA damage correlates with lower probability of pregnancy and prolonged time to pregnancy (61,62). With the concerns for ROS production and DNA fragmentation with DCG, there was room to improve

with a new technique, microfluidics. The overall design of each chip can be highly variable, but the theory is that each microchip can be designed to isolate sperm based on a variety of different biomimetic interactions encountered in the female reproductive tract such as geometry, fluid flow (i.e., rheology) (63), chemotaxis (64) and thermotaxis (65). Given the significant heterogeneity across these approaches, direct head-to-head comparison between microfluidics and conventional sperm selection methods (i.e., swim-up or DCG) are difficult to generalize. With that said, multiple studies comparing various microfluidics designs to these older methods have shown higher sperm motility selection rates and minimal DNA fragmentation rates among sperm selected using microfluidic devices (66-68). Unfortunately, as with many of the previously described methods, multiple recent review articles have reported conflicting data on whether microfluidic sperm selection increases clinically relevant outcomes such as fertilization, CPR and LBR (69-72). Godiwala *et al.* recently published a large, double-blinded prospective RCT concluding similar euploid blastocyst rate though fertilization rate favored microfluidic sperm selection (73). One aspect of microfluidics that sets it apart from some of the other previously discussed methods is that there is the potential for multiple modalities of sperm selection to be combined onto one chip to compound its sorting capabilities. Additionally, some researchers have hypothesized that microfluidics can be combined with developing technologies such as interferometric phase microscopy and advanced spectroscopy to fully potentiate its capabilities (74,75).

Future of sperm selection

Epigenetic testing

Since sperm's first reported identification in 1,677, despite many initial debates of its role in conception, leaps and bounds have been made with its genetic role with egg in mammalian development (76). With that said, it is well established that tremendous heterogeneity exists within sperm of a single species, but also within a single ejaculate, owing to numerous intrinsic or extrinsic alterations related to environmental pressures (77-80), which can result in variable shape, DNA content, motility or membrane protein structure. Changes may include calcium influx or protein kinase activation as response to external signals within the female reproductive tract (81).

Epigenetic heterogeneity or altered gene expression within sperm is also established (79) with changes such as methylation or acetylation resulting in change to DNA-binding histones, ribonucleic acids (RNAs) (82) resulting in altered sperm development and maturation, therefore indirectly impacting fertility (83,84). Methylation specifically is vital to DNA gene regulation and therefore at least considered important for sperm functioning (85). For example, Miller *et al.* recently analyzed public databases to evaluate epigenetic methylation data and its correlation with disease states, finding men with least epigenetic dysregulation of methylation at gene promotor sites were nearly twice as likely to conceive relative to those with more dysregulation (86). A follow-up study to quantify the precise aberrant methylation with infertility demonstrated significantly more than double both CPR and LBR between upper 10^{th} and lower 10^{th} percentile of hypermethylated promotors when undergoing IUI (not IVF) (87).

Further, Carrell *et al.* reviewed the sperm epigenome, concluding aberrant epigenetic changes (e.g., histone modification) are present in patients with abnormal spermatogenesis and idiopathic infertility (88). Detection of changes in discrete epigenetic markers often requires staining with a subsequent cellular analysis and while effective in identification of epigenetic alterations, render the sperm unsuitable for ART (89).

Ideally having the ability to identify such epigenetic changes that could impact sperm quality could result in better sperm selection. Methods that could accomplish this without need for invasive techniques include aforementioned microfluidics, along with sperm nanopurification and Raman spectroscopy (75). Nanotechnology implies use of nanoparticles for a procedure, now widely used in science and medicine for diagnostics and chemotherapy delivery (90) and also considered as a non-invasive sperm selection method (91). Sperm nanopurification is manipulation of sperm based on their biomarkers and the best example of this in clinical practice is MACS, which was discussed previously (41). The principle behind the use is again to select out defective sperm. There has been some success in bull IVF, in which they use a ferritin nanoparticle used to target ubiquitin or various lectins, both which present on defective sperm (92). Similarly, ubiquitin is a well-known marker for elimination of defective sperm in humans via phagocytosis (93,94) with subsequent sperm ubiquitin tag immunoassay (SUTI) able to quantify ubiquitylation. Ozanon *et al.* found that after sperm were separated via discontinuous density gradient, patients with male infertility diagnosis had higher residual ubiquitylated sperm and this negatively correlated with

embryo development (95). Raman spectroscopy is a laser-based technology, obtaining molecular information based off of vibrational energy from the biochemical makeup (96). Subsequent use of a microscope with Raman spectroscopy is deemed Raman microspectroscopy and allows for assessment of single cells and their components (97). While its application in sperm selection is used widely in IVF bovine sex sorting (98), its use is humans is mainly in assessment of sperm DNA damage (99). Promise exists as the ability to evaluate DNA, RNA and respective proteins has allowed Raman spectroscopy to monitor leukemic cells and implement a treatment to monitor induced epigenetic change subsequently alter cell makeup (100).

Artificial intelligence (AI)-based algorithms

AI is a rapidly evolving field with seemingly infinite applications within the medical field—sperm selection is certainly no exception. As previously discussed, sperm morphology, motility and DNA integrity are some of the key qualities assessed when trying to manually identify the best sperm (3). Early reports of computer-aided sperm analysis (CASA) highlighted that computer assistance has the potential to provide an objective, high-throughput alternative to an otherwise highly subjective process (101). Over the years, research has improved on countless deep-learning algorithms trained on very large datasets that have primarily focused on differentiating normal sperm morphologies characteristics (102,103). Although many of the algorithms have reached near parity with the gold-standard of manual interpretation, a large review article identified that most comparisons to date are retrospective, thus lack the real-time assessment necessary for use in ICSI (104) with some concern for current programs lacking specificity and capabilities for andrology use (105). These shortcomings seem to have been at least partially addressed by newer deep learning algorithms promising real-time sperm selection (106-109). For example, the algorithm developed by Sato *et al.* reported a sensitivity and positive predictive value (PPV) of 0.794 and 0.689 respectively when assessing sperm morphology, and a tracking performance of 78.4% mostly tracked (108).

As with any algorithm, adding a second layer of complexity (in this case sperm tracking) will ultimately reduce the overall accuracy of the procedure based on the concept of conditional probability. Taking things one step further, global IVF company IVIRMA, known for AI research in embryos, recently published a pilot study evaluating spermatozoa using hyperspectral (measuring electromagnetic wave lengths) imaging, a technique that combines fine microscopy (4,000×) with chemical information provided by spectra (giving sperm unique signatures based on various wavelengths). Reproducibility was near perfect and classification by the model was both highly sensitive (93.8%) and specific (96.7%), giving hope to build on this to select a given sperm cell and be able to predict the outcome of a given ICSI cycle, thereby avoid ICSI failures due to male factor (110). Additionally, it is important to note that machine learning algorithms developed for sperm selection are susceptible to bias given that the initial training data sets are commonly evaluated by technicians with inherent subjectivity and thus with high inter-expert variability (111). Additionally, other challenges exist including data accuracy and standardization across models (112). With that said, AI continues to be a promising area of sperm selection and appears to be a significant factor in enhancing various ARTs moving forward.

Sperm vitality testing

Sperm use for IVF are largely based on 'normal' morphology and motility (113). Most sperm viability tests involve DNA binding dyes or killing the sperm, leaving them unavailable for clinical use. Motility is a proxy for viability and most often used a surrogate given lack of other reliable options. With advent of IVF, the realization that testis sperm is largely immotile (114) led to research of other methods to test viability. Pentoxifylline is a phosphodiesterase inhibitor that inhibits breakdown of cyclic adenosine monophosphate, a molecule integral to sperm motility (115). Tarlatzis *et al.* demonstrated modest motility improvements and fertilization in IVF protocols (116) along with several others (117,118) while others disagreed with its fertilization enhancement (119,120).

Hypo-osmotic swelling test (HOST) was introduced in 1984 as a supplemental assay (based on reactionary swelling of sperm in a hypoosmotic environment given semi-permeable nature of functional plasma membranes) off the premise that an intact spermatic plasma membrane doesn't necessarily correlate with a functional membrane. This is felt to correlate with more global sperm functions, DNA integrity and fertilizing potential (121) with subsequent realization of inverse relationship of degree of swelling and DNA integrity (122). With that said, disparities exist. One RCT (123) showed improved pregnancy rates while another demonstrated inferior results to pentoxifylline (124). Further, it fell out of consideration given time needed, chemical usage, prolonged incubation reducing viability and

sperm dilution induced in the HOST solution (125,126).

Lasers (i.e., light amplification by stimulated emission of radiation) have been used over the last six decades in various scientific fields and quickly became ubiquitous in the 1970's in medicine and thereafter in ART via reproductive endocrinology for gamete and embryo manipulation (127-129). Sato *et al.* were the first to publish their experience of sperm motility manipulation via lasers (108). While subsequently used in various laser types and settings of sperm manipulation, photobiomodulation (PBM) utilizes light at near-infrared and red wavelengths to modulate biological activity with numerous studies demonstrating its impact on beneficial effect on sperm function and motility (130-134). PBM's hypothesized effect surrounds its ability to alter sperm mitochondrial function, release of ROS, nitric oxide release and activation of various G-proteins (135). With these mechanisms in mind, subsequent work confirmed these theories via improved motility via above studies and importantly, preserved DNA integrity in several studies (130,136,137). Regarding clinical utility, several small observational studies on patients with oligospermia or asthenospermia demonstrated significantly improved motility (138,139). Notably, several animal models demonstrated promise for future human application, including PBM induction of spermatogenesis of a hyperthermia-induced azoospermia murine model (140,141) and PBM induction of enhanced sperm capacitation and fertilization potential in a boar model (142). Lasers have been used to assess for sperm vitality as well, when sperm are not motile. By targeting the sperm tail with a laser, subsequent curling of tail considered sign of viability as dead sperm lose dynamic membrane integrity (143). Birefringence of sperm along with polar microscopy has been researched and two studies demonstrated significantly improved CPR when compared to control ICSI sperm (144,145). When looking at the various methods of evaluating sperm viability, (HOST, pentoxifylline, laser use), pentoxifylline is most commonly used based on what was previously stated (119,124) and given the lack of standardization in larger studies along with disadvantage of a need for laser equipment. With that said, Chen *et al.* had a successful pregnancy via ICSI after an immotile frozen-thawed spermatozoa was selected by laser (146).

Sperm biomarkers

PS

Another key step in successful sperm fertilization is the recognition of certain ligands by the ovulated oocyte. However, sperm binding does not necessarily always lead to fusion of the sperm and oocyte (147,148). One promising biomarker, recently identified to be a significant component of proper sperm-oocyte fusion, is the exposure of PS on the sperm head of viable and motile sperm. This interaction appears to be vital to fertilization and presentation of PS (PtdSer) progressively increasing during sperm transit through the epididymis. PS was historically considered a marker for sperm non-viability and apoptotic cells (3,35). Despite this seemingly paradoxical relationship, research studies have shown that as a sperm matures and undergoes capacitation, PS expression may be increasing in viable, motile sperm (149). Rival *et al.* identified PS expression on the sperm head and PS-recognition receptors to engage the sperm on the oocyte in a murine model (150). Masking the PS expression on sperm via a murine genetic knockout or antibody-mediated blocking of PS oocyte receptors inhibits sperm:egg fertilization. PS may therefore be a marker for fertilization competent sperm (150). A follow-up study confirmed that human sperm can fuse with murine myoblasts in a PS-dependent manner. PS expression may have several clinical applications including a diagnostic marker of male infertility that extends beyond the traditional SA, a functional diagnostic test for egg fusion competent sperm and in sperm selection for ARTs (151).

RNA

The discovery of the azoospermia factor (AZF) region of the Y chromosome in 1976 was a profound insight into the genetics of spermatogenesis (152). The subsequent completion of the Human Genome Project in 2003 reinvigorated research into DNA and its byproducts has exploded, including that of spermatozoa. Despite the two decades of research that have passed, approximately 60–75% of infertile men are considered idiopathic with belief that genetic alterations are contributing at least 40% with no novel interventions to date (153,154). Various studies have elucidated some 3,000 genes involved in male reproduction and more than 500 identifiable genes confirmed to result in infertility in animal models (155-158). RNA studies established differential gene expression between fertile and infertile men (159,160) along with universal membrane display patterns (rather than packed in cells like DNA) with the possibility for use as fertility biomarkers (161). Despite this progress, research has suggested that the evolution of RNA processing during maturation, the presence of numerous RNA subtypes and the belief that minor regulatory RNA are vital to the RNA as a whole indicates that more research is needed (162). Hua *et al.* exemplified

the potential of RNA by establishing that human sperm (with normal density, morphology, motility and viability) demonstrate differential expression of several subtypes of RNA in higher quality embryos, thus indirectly serving as sperm biomarker for IVF (163). Mehta *et al.* reviewed sperm RNA selection techniques and concluded that the future of RNA as viable biomarkers is bright but decoding the sperm RNA and focusing on these smaller regulatory RNA will be critical to its development (164).

Quantitative phase imaging (QPI)

One emerging technology that has the potential to replace traditional microscopy in the future as the marquee option for direct live sperm visualization is QPI. Unlike traditional microscopy, QPI can rapidly visualize >1,500 human sperm simultaneously in 3D (165) as well as track multidimensional swimming patterns, with promising application of isolating the most favorable sperm (166,167). Modern QPI applications have expanded to identify varying degrees of stress in otherwise normal morphologic sperm using traditional microscopy (168,169), as well as accurately predict sperm-cell DNA fragmentation in live, unstained sperm cells with a deep learning model (170). Another aspect of QPI that is particularly valuable is that it can be combined with numerous other sperm selection techniques. For example, Kamieniczna *et al.* successfully combined digital holography, a specific form of QPI, with the traditional "swim up" and DGC methods on live, unlabeled sperm cells (171). Atzitz *et al.* combined QPI with microfluidics (172). As of today, QPI technology isn't ready for primetime as concerns exist surrounding it its cost and time-intensive requirements (173). However, as QPI techniques become more standardized and combined with other selection modalities, it presents an exciting alternative to the traditional microscopy techniques being used across the world.

Ethics consideration

For the most part, rapid advances in medically assisted reproduction (MAR) have been lauded for transforming reproductive opportunities for millions of couples around the world. These advancements have raised numerous ethical and social issues that require constant discussions between many members of our society including physicians, human rights groups, legislators and many more (174). Some argue that as MAR techniques continue to improve, reports of illegal "baby factories" in poor countries will continue to increase despite countries passing legislation directly targeting international reproductive exploitation (175). Further, some of the ART methods previously discussed like AI have already led to successful births (176), raising new ethical concerns that have yet to be formally discussed in the literature. Another aspect of MAR and ART that needs further investigation is long-term follow-up of children born through artificial reproduction given that there are conflicting reports regarding the relative health of these individuals (177,178). Lastly, recent litigation within the United States has put numerous reproductive technologies at risk, further highlighting the necessity for a comprehensive and multidisciplinary ethical review of all ART and MAR techniques.

Benefits/limitations

While this narrative review is a qualitative assessment of the literature, it is an exhaustive survey of the available literature with particular attention given to original research on the topic over the last 35 years. With that said, limitations include lack of original research, lack of standardization relative to a typical quantitative study, as well as potential for bias with interpretation of the various studies. We sought to give consistent summaries of pertinent studies to avoid this.

Conclusions

While IVF is nearing its 50-year anniversary, the success rates have been stagnant with ample room for improvement. The research into optimizing this has been expansive, including promise with sperm selection, though the mainstay continues to be swim-up and density centrifugation. Much promise exists with numerous techniques, though sperm epigenetics and biomarkers appear most encouraging.

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Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-24-195/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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