



# Clinical assessment of the male fertility

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The evaluation of infertility in males consists of physical examination and semen analyses. Standardized semen analyses depend on the descriptive analysis of sperm motility, morphology, and concentration, with a threshold level that must be surpassed to be considered a fertile spermatozoon. Nonetheless, these conventional parameters are not satisfactory for clinicians since 25% of infertility cases worldwide remain unexplained. Therefore, newer tests methods have been established to investigate sperm physiology and functions by monitoring characteristics such as motility, capacitation, the acrosome reaction, reactive oxygen species, sperm DNA damage, chromatin structure, zona pellucida binding, and sperm-oocyte fusion. After the introduction of intracytoplasmic sperm injection technique, sperm maturity, morphology, and aneuploidy conditions have gotten more attention for investigating unexplained male infertility. In the present article, recent advancements in research regarding the utilization of male fertility prediction tests and their role and accuracy are reviewed.

**Keywords:** Infertility; Semen analysis; Spermatozoa

## Introduction

Male factor infertility can be a health issue for males and is primarily responsible for inability to conceive a child after 1 year of regular, unprotected intercourse [1]. Particularly, male factor infertility affects nearly 50% of infertile couples worldwide who want a child and who require empirical therapy [2]. In a fertility clinic, physiologically abnormal spermatozoa are the prime target for male fertility evaluation and are tested for each step of successful conception, such as movement, fertilization, embryonic progress, and pregnancy. For these evaluations, distinct aspects of semen analysis such as sperm concentration, motility, morphology, acrosomal integrity, DNA damage, chromatin stability, oxidative stress, and genomic and proteomic composition have been thoroughly investigated. Nonetheless, the present understanding of abnormal sperm functions including their different physiological and pathological aspects remains limited and not well defined. Therefore, more extensive evaluation techniques are required to clarify the associations among certain diagnostic strategies and their evaluation of fertilizing capacity for males. Conventional semen analysis has been considered as the initial choice for fertility assessment and commonly tailored by more intensive and comprehensive sperm function tests. Recently, concerns have arisen to establish tests for selecting mature

spermatozoon containing regular number of chromosomes. Thus, SpermSlow, sperm aneuploidy analysis, proteomic and genomic investigations are getting more attention, which could provide highly accurate assessment of male fertility by determining the true fertilizing potential of spermatozoa. However, both comprehensive and conventional sperm function tests still lack accuracy and reproducibility. Successive introduction of intracytoplasmic sperm injection (ICSI), intracytoplasmic morphological sperm injection (IMSI), and physiological intracytoplasmic sperm injection (PICSi) for successful reproductive outcome encouraged to go for newer tests which should expect the successful fertilization *in vitro* and *in vivo*.

Received: 2017.06.01. Revised: 2017.09.06. Accepted: 2017.09.26.  
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## Sperm biology

Spermatogenesis is a differentiation process that transforms a spermatogonial stem cell into spermatozoa within 74 days in the seminiferous tubules of the testes [3]. Over 1 mitotic and 2 meiosis divisions, the spermatogonial cell is transformed through subsequent proliferation and differentiation into a primary spermatocyte, secondary spermatocyte, spermatid, and finally a spermatozoon. This spermatogenesis process is controlled by follicle-stimulating hormone. However, at the end of the spermatogenesis process, spermatozoa move forward from the lumen of the seminiferous tubule to the proximal end of the epididymis [4]. During the next journey from the proximal to distal end of the epididymis, spermatozoa undergo a process where they acquire maturity, motility, and fertilizing capacity. Eventually, matured spermatozoa are stored in the epididymis tail until ejaculation. After ejaculation, spermatozoa undergo the capacitation process in the female reproductive tract. During capacitation, spermatozoa undergo a series of biochemical and physiological modifications through which they gain fertilizing ability [5]. Subsequently, capacitation triggers hyperactivated motility. Once spermatozoa reach an oocyte, they start the acrosome reaction (AR) before penetrating the zona pellucida. Fertilization occurs through subsequent zygote formation and development. Any abnormality that could befall spermatozoa during movement from testes to oocyte can lead to infertility.

## Conventional semen analysis

Spermatogenesis and maturation processes can be affected

by fluctuations in hormones, temperature, dietary balance, and exposure to toxins due to habits or environmental pollutants (i.e., smoking, alcohol, cadmium, lead, radiation, pesticide, endocrine disruptor chemicals) [6-9]. Eventually, these factors can affect semen quality and result in abnormal spermatozoa. Conventional semen analysis is commonly used to define semen quality and to predict only quantitative values. Semen samples should be tested twice after an abstinence period of 2–7 days. After collection, the sample should be liquefied at room temperature and analyzed within 1 hour. Particularly, the number of spermatozoa present in each ejaculate, the percentage of motile spermatozoa or progressive motility, and the proportion of morphologically normal spermatozoa are evaluated based on standard reference values proposed by the World Health Organization (WHO) (Table 1) [10]. A large study was performed to evaluate male fertility based on sperm concentration, motility, and morphology among 765 males of infertile couples and 696 males of fertile couples. In that study, significant overlap was found between the fertile and infertile groups in all parameters (sperm concentration, motility, and morphology) [11]. Despite of the inaccuracy of these conventional semen analyses to evaluate male fertility was acknowledged in the mid-1980s [12], these are corner stone of infertility evaluation. Several recent studies have focused on establishing effective reference values for clinical use of semen analysis [13-15]. In 1998, Bonde et al. [16] proposed that sperm count and morphology were correlated with conception. Although a range of semen analysis methods are commonly used throughout the fertility clinics and laboratories across the world, the current quality assessment tools of semen are unable to provide accuracy for predicting fertility status of a man [10,17-19].

**Table 1.** Standard reference values for semen characteristics World Health Organization (WHO) (2010)

Volume	2 mL or more
pH	7.0–8.0
Sperm concentration	15 million or more/mL
Total No. of spermatozoa	39 million or more spermatozoa/ejaculate
Motility	40% or more progressive motility or 32% (a+b) (within 1 hour after ejaculation)
Morphology	4.0% or more (normal forms)
Viability	58% or more live spermatozoa
Leukocytes ( $10^6$ /mL)	<1.0
Mixed antiglobulin reaction	Less than 50% spermatozoa with adherent particle

Source: WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 5th edition. Geneva (Switzerland): World Health Organization; 2010.

Therefore, lower reference limits for semen parameters have been modified several times (1987, 1992, 1999, 2010) in the WHO manual to increase the clinical value of these parameters for evaluating male fertility.

## Sperm motion kinematics

Motile spermatozoa follow various specific movement patterns that are attained based on the sperm's functional requirement. Human spermatozoa ideally tend to generate propulsive forces, i.e., linear and progressive trajectories, during cervical barrier penetration. Therefore, several specific movement attributes (average path velocity, straightness, and amplitude of lateral sperm head displacement) including conventional analysis criteria (sperm count and morphology) are considered features of semen quality that can facilitate cervical barrier penetration [20,21]. Consequently, computer-assisted semen analysis (CASA) has been established for mechanical analysis of sperm kinematics of ejaculated semen [22]. Based on serial digital imaging, CASA determines the sperm head trajectory and movement by measuring the motion patterns of the sperm head in 2 dimensions. Several researchers have suggested that quantitative evaluation of sperm kinematics using CASA can assess human sperm fertility under *in vitro* conditions, and that conventional semen analysis provides values of limited accuracy [23,24]. In addition, many studies have demonstrated that quantitative assessment of sperm motion kinematics has diagnostic value for evaluating unexplained infertility [25,26].

Although CASA was initially accepted as an important method for semen diagnosis, the individual motion kinematic value for fertility assessment remains questionable. Moreover, a wide range of errors due to object selection, setup procedures, and different kinetic values among populations render this application unacceptable [27]. Hyperactivation is another form of spermatozoa movement defined by high velocity and asymmetrical flagellar waves. Hyperactivated motility is an indication of the capacitation process believed to facilitate the mechanical thrust from the tubal epithelium to penetrate the zona pellucida [28]. Although hyperactivated motility can have significant effect on sperm fertility, there is no established value for the proportion of hyperactivated spermatozoa that should be present in each semen sample of a fertile male; as a result, the universal criteria for hyperactivation remain un-

known. Therefore, although a previous study has established a relationship between hyperactivated sperm percentage and successful fertilization *in vitro*, this quantitative parameter is not recommended for clinical use [29].

## Sperm morphology

Sperm morphology is an integral part of routine analysis of human semen. Kruger et al. [30] proposed strict criteria for evaluation of sperm morphology, where spermatozoa with any slight defect in the head, neck, body, and tail regions are considered to have abnormal morphology under the strict criteria. Eventually, these strict criteria were included in the latest WHO laboratory manual for the prediction of normal sperm morphology [31-38]. If more than 4% of sperm show a normal sperm morphology, the semen is considered within 95% of the fertile reference range [32]. One recent study has shown that the percentage of morphologically normal spermatozoa influenced the time to pregnancy (TTP) [39]. After evaluating 501 couples, investigators reported that sperm head width and coiled tails were important predictors of TTP [40]. In addition, percentage of morphologically normal spermatozoa independent of sperm concentration provided significant predictive value for couple fecundity as measured by TTP [40,41]. However, after 1987, studies revealed that sperm morphology is a vital indicator of male fertility, controversies are also increasing. A study evaluated the consequences for men with 0% morphologically normal spermatozoa to determine the relationship between sperm morphology and reproductive success independent of assisted reproductive technologies (ART). Men with 100% abnormal spermatozoa achieved pregnancies in which only 25% required *in vitro* fertilization (IVF). Therefore, to refine the evaluation process, additional functional tests have been developed to explain the unanswered questions pertaining to male fertility [42].

## Sperm viability

Sperm viability is an evaluation parameter for investigating the percentage of viable spermatozoa in an ejaculate that contains <5%–10% motile spermatozoa. Ultrastructural defects in human spermatozoa produce dead or non-motile

spermatozoa. Moreover, sperm viability is used to identify viable spermatozoa that are appropriate for ICSI. Correa and Zavos [43] proposed that a positive correlation between viable and motile spermatozoa percentage in human semen could predict sperm fertility. Two methods are commonly used for sperm viability testing, the hypo-osmotic sperm swelling test (HOST) to differentiate dead or live sperm and flow cytometry to investigate sperm membrane integrity. The HOST procedure consists of placing spermatozoa into media with a lower osmotic potential than the spermatozoa. Thus, water enters the cytoplasm of live spermatozoa to achieve osmotic pressure equilibrium, which swells the spermatozoa tails. Supravital dyes (e.g., eosin or trypan blue) are also used to investigate sperm viability. Since the mixture of spermatozoa and supravital dye kills spermatozoa, the cells cannot later be used for ICSI. Therefore, the HOST is considered more suitable and allows the spermatozoa to subsequently be used for ICSI. Flow cytometry is another technique used to investigate the viability of spermatozoa by examining the integrity of both the plasma and mitochondrial membranes [44]. According to the WHO (5th edition), HOST is normal for a semen sample if  $>58\%$  of spermatozoa undergo tail swelling, indicating an intact membrane. Semen consisting of  $<50\%$  viable spermatozoa is considered abnormal. A recent study focused on both sperm viability and DNA fragmentation by testing 3,049 semen samples from 2008 to 2013 and showed a strong negative correlation between sperm viability and DNA fragmentation rate. The study reported that men with sperm viability  $\geq 75\%$  or  $\leq 30\%$  do not require DNA fragmentation index (DFI) [45]. Therefore, sperm viability tests are a valuable cost-effective measure for investigating male fertility, while DNA fragmentation tests are expensive as a routine test. Although motile spermatozoa could be defined as viable, viable spermatozoa are also related to an undamaged plasma membrane since the plasmalemma is essential for interactions between spermatozoa and oocyte. Therefore, sperm viability assay methods not only focus on cell viability, but also assess whether the plasmalemma is intact.

## Acrosomal integrity

Evaluation of acrosomal status in human spermatozoa is another method to predict male fertility. Ionophore A23187-

induced AR (acrosome reaction to ionophore challenge [ARIC]) is a good indicator of male fertility [46]. An infertile male who undergoes the ARIC test shows a significantly reduced number of acrosome-reacted spermatozoa. A study of 86 males with good fertilization rate ( $\geq 30\%$ ) and 39 males with poor fertilization rate ( $<30\%$ ) undergoing IVF and embryo transfer was conducted to verify the efficacy of the ARIC test. Significant reduction in induced AR rate and ARIC value was found in the poor fertilization group. Using the cutoff value of 8.5 for the ARIC test, sensitivity, specificity, positive predictive value, and negative predictive value were 83.7%, 92.3%, 96.0%, and 72.0%, respectively [47]. When using the ARIC test with ionophore A23187, clinicians can determine the difference between complete acrosome-reacted spermatozoa with and without treatment [48]. This percentage difference provides the evaluative value; a difference  $\leq 5\%$  indicates male infertility. If the acrosome-reacted spermatozoa represent 5%–10% of a given sample, high fertility and reproductive outcomes might be indicated [49]. Thus, this evaluative parameter could be useful to explain male infertility if a couple fails IVF, but is not yet suitable for assessing male infertility as the primary stage.

## Hemizona assay

Sperm-zona binding triggers the AR in mammalian spermatozoa. Therefore, during IVF, imperfect binding and penetration of spermatozoa with the zona pellucida result in unsuccessful fertilization. Overstreet and Hembree [50] introduced an assay for assessing human sperm-oocyte interactions. Because sperm-zona binding is a species-specific event, the bioavailability of this assay is limited [51]. However, 2 available assays are used to assess sperm-zona binding ability, the hemizona assay and the sperm-zona binding ratio. Human oocytes are used to isolate zona pellucida, which is divided in half during the hemizona assay. One half of the zona pellucida is incubated with a fertile male's spermatozoa (control group), and the other half is incubated with the patient's spermatozoa (evaluated group).

The binding ratio of patient spermatozoa to that of a fertile donor is evaluated; a ratio  $<30\%$  is considered abnormal/infertile [51]. In addition, the sperm-zona binding ratio is another method to assess male fertility. The patient and fertile male spermatozoa are labeled with 2 distinct fluorochromes.

After incubation with intact oocytes, the total number of bound spermatozoa is counted [49]. The lower binding ratio of spermatozoa with the zona pellucida has been correlated with lower fertilization rates during IVF. Therefore, these tests are beneficial mainly for male patients that have failed standard IVF and have limited utility in cases of primary infertility. However, the use of human oocytes raises ethical issues for these tests, while intra- and inter-assay variability during testing are 2 major factors that affect the assay efficiency.

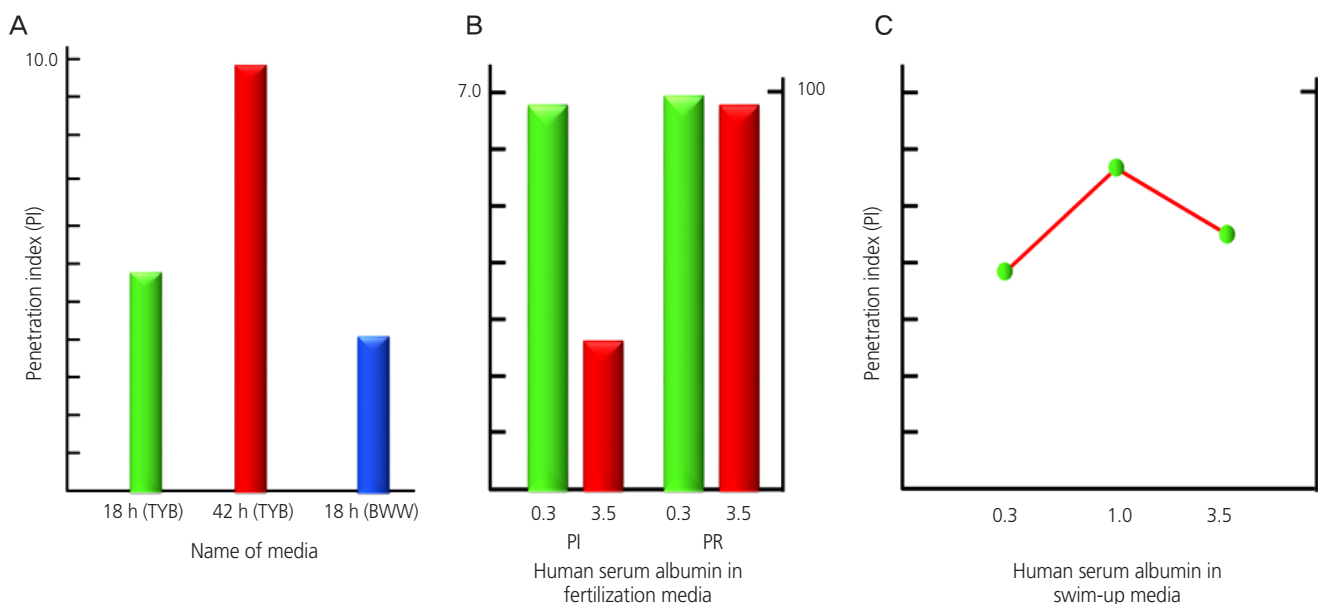
## Sperm penetration assay

In an earlier study, basic semen analysis showed a large percentage of false data with low accuracy to predict fertility potential in terms of both spontaneous and assisted conception [21]. Therefore, to obtain more efficient assessment tool, researchers focused on the ability of spermatozoa to penetrate an oocyte. Hence, sperm penetration assay (SPA) introduced by Yanagimachi [52] in 1976 is considered the most reliable bioassay for explaining non-defined male infertility [53,54]. Subsequently, this assay was utilized due to the reduced false data results and high accuracy [53,54]. However, hamster egg retrieval, semen sampling and liquefaction, sperm washing

and preincubation, insemination, sperm-egg co-incubation, as well as the protein source in the media are the main factors that affect the SPA test [55]. Various protein sources and their different concentrations have significant effects on SPA test results (Fig. 1) [56]. Several factors such as period of abstinence, sperm concentration during co-incubation, media constituents, trypsinization, and clinician experience have been reported as inter and intra-assay variables those can influence SPA accuracy [57]. Therefore, several studies have been done to optimize and standardize SPA test using other mammalian animal trials [56,58,59]. In the early stage of SPA, several studies have focused on the correlation (positive/negative) among quantitative parameters and SPA. Additionally, SPA combined with other tests, including strict sperm morphology criteria and the ARIC test, can provide more accurate values regarding fertilizing capacity [60]. Nonetheless, SPA is still difficult to standardize due to variation of culture condition. Moreover, this is a cumbersome, costly, time consuming method, which had lost its clinical usefulness after introducing ICSI.

## Reactive oxygen species

In recent years, oxidative stress has been recognized as one



**Fig. 1.** Effects of sperm treatment based on TEST-yolk buffer (TYB), Biggers-Whitten-Whittingham (BWW), and human serum albumin (HSA) on the outcome of the sperm penetration assay (SPA). (A) Effect of TYB and BWW on the outcome of SPA tests. (B) Human serum albumin concentrations in fertilization media. (C) Human serum albumin concentration in swim up method. The figure has been modified, and citing the original source published by Oh et al. [59]. PI, penetration index; PR, plasticity range.

of the main factors affecting sperm functions [52,53]. In the male reproductive system, production of antioxidant scavengers and reactive oxygen species (ROS) is required to maintain equilibrium; low ROS levels regulate capacitation, and elevated ROS levels affect sperm physiology by increasing oxidative stress [61]. Elevated ROS levels such as superoxide anions, hydrogen peroxide, and hydroxyl radicals or decreased antioxidant levels are the main factors for sperm malfunctions by affecting the sperm cell membrane lipid peroxidation, sperm motility, and DNA integrity [62]. Since human spermatozoa are very sensitive, these dysfunctions impair their fertilizing ability. To assess ROS levels in semen, the chemiluminescence assay is used [63], and total antioxidant capacity is detected using the colorimetric assay [64]. A recent study proposed an optimal cutoff value to differentiate between controls and infertile males of 102.2 relative light unit/s/ $10^6$  sperm, showing 76.4% sensitivity and 53.3% specificity [65]. However, the accuracy of this method to evaluate male fertility is not well-established. Further investigations are required to establish highly accurate ROS cutoff values in both fertile and infertile patients.

## Sperm DNA damage

DNA damage is responsible for causing apoptosis in spermatozoa and loss of embryo development and pregnancy. A considerable number of studies have shown the number of DNA-damaged spermatozoa is higher in infertile than fertile males [66]. Therefore, DNA damage is an important factor for sperm quality evaluation that correlates with both *in vivo* and *in vitro* development of an embryo [67]. DNA damage can be assessed by several assays including single-cell electrophoresis assay, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling assay, alkaline gel electrophoresis, sperm chromatin structure assay (SCSA), and quantitative polymerase chain reaction of nuclear and mitochondrial DNA. Previous studies have indicated that sperm DNA damage affects male fertility potential and has a higher predictive value for reproductive outcome in natural fertility [66,68,69]. In addition, poor results after intrauterine insemination (IUI) are associated with a high percentage of damaged DNA in a group of spermatozoa; however, the factors affecting fertilization rate or pregnancy can be avoided by ICSI [70,71]. Study results have indicated that

SPA optimized with a sperm head chromatin pattern was highly accurate [72], showing 96% sensitivity and 56% specificity, which could be useful for evaluating infertile males. Moreover, results from ICSI or routine IVF demonstrated that a DFI value <30% can decrease fertility success in infertile couples by 1.6-fold [73]. Conversely, a recent meta-analysis confirmed a minor but statistically significant relationship between sperm DNA damage and pregnancy rate in IVF and ICSI cycles, although the clinical utility of sperm DNA damage has not been determined [74].

## SCSA

The SCSA was proposed by Evenson et al. [75] in 1980 to investigate sperm DNA integrity. After its introduction, SCSA was considered a method that could potentially help with male factor infertility and obtain success in ART. SCSA is a flow cytometric method that tests the vulnerability of DNA to acid-induced denaturation by exposure to acridine orange media [76]. Double-stranded DNA binds with acridine orange and fluoresces green, while single-stranded DNA binds with acridine orange and fluoresces red. Using flow cytometry, the ratio of red to green fluorescence can be analyzed. The percentage of spermatozoa with red fluorescence as the ratio of red/red and green fluorescence is termed the DFI and can be visualized on a histogram. Conversely, spermatozoa with green fluorescence show the percentage of sperm with high DNA integrity (mature sperm). DNA damage in spermatozoa is considered to be associated with poor semen quality, which can lead to a lower preimplantation rate, higher abortion rate, and higher childhood cancer [77]. Combining the SCSA test with conventional sperm analysis can result in higher accuracy when assessing male fertility. If one of the traditional sperm parameters is abnormal, fertility is reduced, with a DFI >10%. Infertile males with a high DFI percentage have less potential for natural fertility and IUI outcome. However, ICSI does not consider DNA damage as a factor affecting the reproductive outcome [75,78].

## ICSI, IMSI, and PCSI for male fertility assessment

Following all sperm function tests, the percentage of sperm



that bind and penetrate the zona pellucida in oocytes cannot explain the approximately 25% of male infertility worldwide [69]. For couples who suffer from a low fertilization rate over several IVF cycles, ICSI helps to alleviate male factor infertility [69,74]. However, the outcome of ICSI depends primarily on the quality of the oocyte, female age, and sperm morphology. Although ICSI has been successful for overcoming unexplained infertility, studies showed that sperm morphology, motility, sperm-zona pellucida binding, sperm-zona pellucida penetration, zona pellucida-induced AR, and sperm DNA damage are still useful predictors before patients commence ICSI treatment [79-81]. However, substantial incidence of failed (0%) and suboptimal (<50%) fertilization after ICSI remains a challenge due to morphological defects in spermatozoa [82,83].

Motile sperm organelle morphology examination has provided an opportunity for intensive selection of spermatozoa for ICSI [84]. This examination offers improved morphological assessment of mitochondria, nucleus, acrosome, post-acrosomal region, neck, and tail of spermatozoa using high magnification >6,000x [85]. Thus, the inclusion of this method into ICSI led to a new technique termed IMSI [86]. After introduction of IMSI, numerous comparative and randomized studies were conducted, although the comparison between IMSI and conventional ICSI provided controversial data. A wide range of factors can contribute to the efficiency of IMSI over conventional ICSI such as dissimilar study design, lack of homogeneous inclusion criteria, and non-classified high magnification sperm morphology [87]. However, a recent study showed that IMSI in infertile couples enhanced the reproductive outcomes compared with conventional ICSI by increasing implantation rate (3 times), pregnancy rate (2 times), and miscarriage reduction rate (70%) [87]. However, a meta-analysis of current trials determined that IMSI has no significant effects on clinical pregnancy rate and live birth [88].

Using hyaluronic acid-containing media for selection of spermatozoa provided a new opportunity to sort mature spermatozoa with lower biological risk [89]. Binding ability of spermatozoa with hyaluronic acid depends on plasma membrane maturity and fertilizing ability of spermatozoa. This novel method of sperm selection based on the ability to bind with hyaluronic acid led to a new method termed PICSi [90,91]. Many recent studies have indicated that PICSi is effective for selecting spermatozoa by excluding fragmented DNA and abnormal nucleus [92,93]. However, conflicting results showed

that PICSi does not improve the fertilization and cleavage rate after ICSI [94-97].

## SpermSlow for assessment of spermatozoa

SpermSlow is used to decelerate the movement of spermatozoa to allow the selection of viable, mature, and non-fragmented DNA-containing spermatozoon for ICSI [98]. This technique allows the selection of the most mature spermatozoa during ICSI. A plastic culture dish with microdots of hyaluronic acid is used as a device for ICSI, and SpermSlow is used as a viscous media containing hyaluronic acid. A significant enhancement of embryo quality was observed in a study when SpermSlow was used to select spermatozoon [98]. Controversy has increased because several studies did not find any significant difference in fertilization rate or embryo quality after injecting SpermSlow-selected spermatozoa compared to other physiologic ICSI treatments [99,100].

## Sperm aneuploidy analysis for ICSI, IMSI, and PICSi

The presence of inadequate numbers of chromosomes in spermatozoa causes chromosomal abnormalities, defined as aneuploidy, reportedly occurring 3 times more frequently in spermatozoa of infertile males with azoospermia [99,101]. Notable improvements have been observed using ICSI, IMSI, and PICSi for infertility treatment in recent years since the treatments required spermatozoa without any cytogenetic abnormalities to ensure pregnancy and healthy live birth [102,103]. Therefore, to decrease aneuploid fertilization, combined HOST and fluorescence *in situ* hybridization (FISH) are used simultaneously as a cytogenetic assay to evaluate the rates of chromosomal abnormalities. Only a few studies reported a significantly reduced aneuploidy frequency in spermatozoa with tail-tip swelling pattern (using HOST) and FISH [104,105]. Due to the increased rate of aneuploid fertilization during ICSI [106], FISH in functionally live spermatozoa is considered the most accurate evaluation tool for excluding unhealthy fertilization in infertile men. Future studies are needed to evaluate both the HOST and FISH for consideration as routine tests of cytoplasmic abnormalities in human spermatozoa.

## Future diagnostic tests for male fertility: omics

Recently, more advanced research methods have provided an opportunity to investigate new prediction techniques based on genomics, proteomics, transcriptomics, and metabolomics. Combination of the current omics and conventional semen analysis could provide new methods for exploring potential predictors of male fertility [101]. Research focuses on the differentially expressed proteins and genes found under different conditions including fertility/infertility [107,108]. During the past decade, protein biomarkers have been the subject of extensive research for diversified diseases as well as male fertility. Several seminal plasma proteins have already been evaluated as potential biomarkers for genital duct patency. For example, a current study showed that the cysteine-rich secretory protein level in seminal plasma is a predictable proteomic biomarker that can identify fertile and infertile men with 85% specificity and 92% sensitivity [109]. Another study proposed that the level of lipocalin-type prostaglandin D synthase is significantly lower in infertile men with obstructive azoospermia compared to men with normal semen parameters [110]. However, intensive proteomic analysis of both semen and sperm is discovering functionally important proteins, protein-protein interactions during the path to the oocyte, and other various altered proteins associated with the fertilization process. Similarly, transcriptional profiling of spermatozoa is also a valuable method for males with unexplained infertility problems, and further studies are required to establish a true fertility/infertility predictor by investigating omics of human semen.

## Conclusion

Conventional semen analysis is considered as the initial step to investigate semen quality and male factor infertility; however, this method cannot always provide valid information regarding specific defects of sperm physiology. Therefore, novel predictors are needed for assessing semen quality to determine the reason for non-pregnancy in infertile couples. The current assays can determine specific imperfections based on sperm physiology, but newer predictive tests might reveal the precise reason for male infertility. Thus, the addition of new predictive methods can aid researchers to better understand sperm

potency. Implementation of such evaluation procedures might help clarify the unknown characteristics of spermatozoa to maximize successful reproduction.

## Acknowledgements

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries (iPET) through Agri-Bio Industry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (116172-3).

## Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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