ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

REVIEW ARTICLE

An Update of *In Vitro* Fertilization-Intra Cytoplasmic Sperm Injection: A Review of Sperm Selection Methods

Marianne Ingrid Aror[1*], Petty Narulita [1], Reny I'tishom[2], Silvia W Lestari[3]

1. *Andrology Specialist Program, Faculty of Medicine of Universitas Airlangga/Dr. Soetomo Hospital Surabaya, Indonesia.*
2. *Department of Medical Biology, Faculty of Medicine of Universitas Airlangga, Surabaya, Indonesia.*
3. *Department of Medical Biology, Faculty of Medicine of Universitas Indonesia, Jakarta, Indonesia.*

***Corresponding Author: Marianne Ingrid Aror**

Abstract

In Vitro Fertilization (IVF)-Intra Cytoplasmic Sperm Injection (ICSI) is a well-known infertility management, but the success rate remains low. One of etiologies is the sperm factor. The objective of this review is to explore the update of sperm selection methods for selecting the best sperm quality for IVF-ICSI. Initially, semen analysis should be conducted hence sperm motile and morphology data are obtained. If there is standard sperm morphology in semen analysis, then in the motile sperm organelle morphology (MSOME), important sperm organelles are also obtained, such as nuclues, which are supplemented with vacuole data. Then, due to limitation of semen analysis, the analysis of sperm DNA fragmentation exists to analyze the quality of sperm DNA integrity. Sperm DNA fragmentation is caused by apoptosis. Magnetic Activated Cell-Sorting (MACS) is a sperm selection method that selects non-apoptotic sperms for IVF-ICSI. Finally, Physiological ICSI (PICSI) method is performed to select qualified sperms based on its binding with hyaluronan, immitating the physiological process in natural fertilization. It is important to select the best sperm quality through the most appropriate selection method to increase the success rate of IVF-ICSI.

Keywords: *IVF-ICSI, Sperm selection, DFI, MSOME, MACS, PICSI.*

Introduction

It is having known that the success rate of In Vitro Fertilization (IVF)-Intra Cytoplasmic Sperm Injection (ICSI) remains low [1]. There are oocytes, sperm and endometrium factors involves in the IVF-ICSI. In the sperm factor, sperm should be screened prior the IVF-ICSI program in order to achieve an enormous success rate of this program. In sperm selection, there are a few methods that frequently performed. Nevertheless, there are some controversies related to the deterioration of these methods which may be used appropriately leading to successful IVF-ICSI.

Recent studies demonstrated methods that could select for better sperm quality by evaluate sperm motility, morphology, maturation, DNA fragmentation and apoptosis. This review aimed to summarize which method should be performed appropriately as an update to increase the success rate of IVF-ICSI program.

Semen Analysis

The diagnosis of male infertility is determined through history taking, physical examination and is completed by semen analysis. Conventional semen analysis evaluates semen samples including sperm concentration, motility, and morphology, specified as potential markers of male fertility. The standardize semen analysis was published by the World Health Organization (WHO) 2010 with reference values for normal semen analysis are shown in table 1 [2, 3].

However, although this semen analysis was performed to accurately predict male fertility, the results of standard semen analysis do not provide appropriate data [2]. In addition, the terminologies of abnormalities in the results

of semen analysis are shown in Table 2.

Table 1: Reference values for normal semen analysis [2, 3]

Sperm parameter	WHO, 1999	WHO, 2010 [2]
Volume (ml)	≥ 2	≥ 1.5
Sperm concentration (10^6/ml)	≥ 20	≥ 15
Total motility (%)	≥ 50	≥ 40
Progressive motility (%)	≥ 25 (grade a)	≥ 32 (grade a+b)
Morphology (%)	≥ 30	≥ 4
Vitality (%)	≥ 75	≥ 58
Leukocyte (10^6/ml)	< 1	< 1

Table 2: Terminologies in semen analysis [2]

Term	Results
Normozoospermia	Normal semen volume
Oligozoospermia	Sperm concentration < 15 x 10^6/ml
Asthenozoospermia	< 40% grade A or < 32% progressive
Teratozoospermia	< 4% normal morphology
Azoospermia	Absence of sperm in semen
Aspermia	Absence of semen/ejaculate
Leukocytospermia	Leukocyte present in semen

Semen analysis is commonly agreed to be required in determining the proper management of infertility, whether naturally by having regular sexual intercourse, intra uterine insemination (IUI) or in vitro fertilization (IVF) - intra-cytoplasmic sperm injection (ICSI) [5, 8]. Certain motile sperm counts have been used as guidelines to determine the management of infertility, namely 1) >40 million / ml to experience through sexual contact, 2) >5 - <40 million / ml for IUI and 3) <5 million / ml for IVF-ICSI [9].Studies have proved the prominent predictors of poor outcome of fertilization in IVF-ICSI such as the mitigate of sperm concentration and / or motility [10, 11].Especially for sperm morphology parameter with the standard criteria, it can be used on conventional IVF. In conventional IVF, insemination with normal standard criteria of sperm morphology <30%, the average fertilization rate is between 0-30 % [12]. On the other hand, sperm morphology parameters with strict criteria (< 4 %) are crucial for ICSI. The sperm morphology picture is shown in Figure 1. This parameter is essential to avoid failure of oocyte fertilization [13].

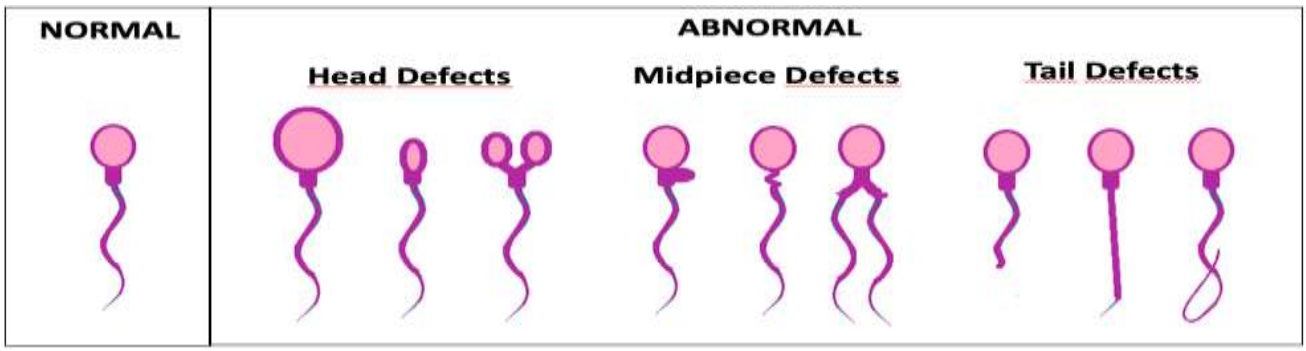

Figure 1: Sperm morphology

ICSI has become a preference for infertile men with abnormal semen parameter to gain offspring. Finding study by Palermo and strengthened by the following research stated that sperm concentration, motility, and morphology, further diagnosed as oligozoospermia, as then ozoospermia, teratoozospermia, or oligoasthenoteratoozospermia had any impact on fertilization in vitro or pregnancy rates in ICSI.

Followed by the leading edge studies claimed that ICSI may be ineffective in some cases with impaired semen parameters leading to adverse effect towards pregnancy rates, particularly in as then oozospermia, teratoozospermia, and cryptozoospermia [14, 15, 10].Good quality of sperm capable to fertilize on IVF ICSI, yet poor quality of sperm capable to fertilize on ICSI but not on IVF. Sperm parameter assessed by WHO giving the benefit for determining infertility

management, but are not good predictors for predicting the outcome of the program [16].

Sperm DNA Fragmentation

The limitation of semen analysis utilization is faced by 15% of men with normal semen encounter infertility, while men with abnormal semen able to have offspring naturally [17, 18]. This led to study on sperm DNA fragmentation as an alternative indicator for male infertility. Sperm DNA, located in head of sperm, is packed and bound by protamin through protamination. The failure during the protamination process is driving to damage or fragmentation of sperm DNA [19, 20]. A prospective etiology of sperm DNA fragmentation is imperfect apoptosis [21].An abnormal sperm is encoded to experience apoptosis, just like somatic cells. Nevertheless, because sperm are inert by transcription and translation, hence the apoptotic process does not keep on to accomplishment.

The apoptotic process initiated by reactive oxygen species (ROS), followed by the induction and the increase number of sperm DNA fragmentation [21]. In addition, the post testicular milieu can also be part of the cause of sperm DNA fragmentation, over the feat of ROS [20]. The findings proved that the upward level of sperm DNA fragmentation is higher in ejaculated sperm than sperm in the testes [22]. Sperm DNA fragmentation examination could be conducted by several methods, such as sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD), Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and comets, as well as others. SCD is the most frequent sperm DNA fragmentation examination method that applicable in the andrology laboratory. The examination of sperm DNA fragmentation is characterized by small or large halo images (DNA strands coming out of the pores of cell membranes).

Sperm with unfragmented DNA is characterized by large and medium halo, while sperm with fragmented DNA is characterized by small halo, without halo and degraded sperm. (Figure 2) The examination results are in the form of sperm DNA fragmentation index (DFI) obtained from the percentage of sperm with fragmented DNA in a total of 500 sperm cells.

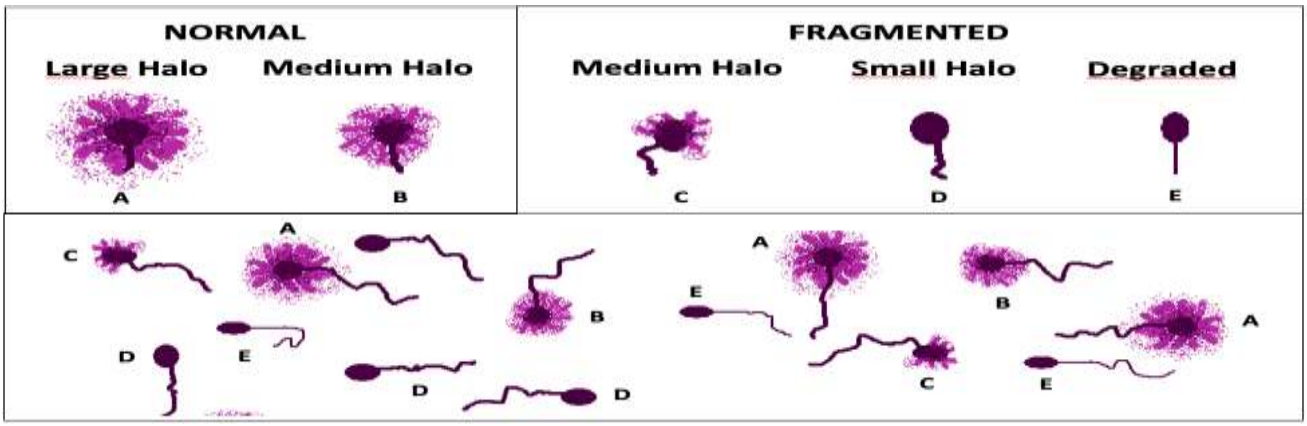

Figure 2: Sperm DNA fragmentation

Many systematic reviews seem to indicate an association between sperm DNA fragmentation and IVF and ICSI success rate; although these reviews are restrained by the heterogeneity of the original studies which make it strenuous to depict decisive conclusions from their analysis. This examination of sperm DNA fragmentation offers supplementary information about the important role of sperm in the growth and protection of pregnancy. A study has been able to map the value of DFI to help establish the procedure for infertility, namely 1) DFI <15% to experience through sexual activity, 2) 15-30% for IUI and 3)> 30% for IVF-ICSI [23]. DFI is expected to be able to select good sperm quality so that it can increase the success of each of these pregnancy programs. Nevertheless, at present, there is no conclusive indication to backing the sperm DNA fragmentation testing in the practice of infertility, routinely [24].

Motile Sperm Organelle Morphology

The measurement of motile sperm organelle morphology (MSOME) was first introduced in 2001 by Bartoov et al. In contrast to conventional semen analysis where sperm

morphology is examined at magnifications of 400x to 1000x and in a state of death, then in MSOME, sperm morphology is examined with even higher magnification and in a state of life at a moment's notice [25].

This magnification makes it possible to identify sperm with normal nuclei, determined from their oval shape with fine configuration and normal nucleus content [25] (Figure 3).

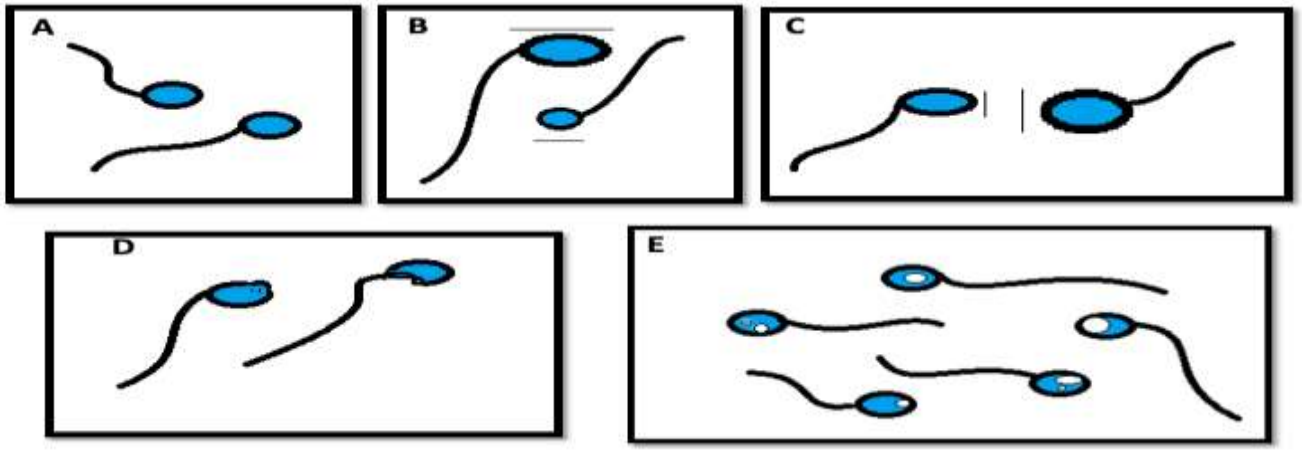

Figure 3: Motile sperm organelle morphology: A) Normal sperm, B) Long and short sperm, C) Wide and narrow sperm, D) Irregular oval sperm and D) Sperm with vacuoles

At first, MSOME evaluated six sperm organelles, namely the acrosome, lamina post-acrosome, nucleus, neck, mitochondria and tail. Nevertheless, the sperm nucleus is the most essential organelle in swaying ICSI results [26]. In addition to the normality of the nucleus in terms of shape and size, the vacuole of the nucleus must also be considered because it impacts the success of the pregnancy and the risk of abortion [27].

The classification of vacuoles has been compiled by Vanderzwalmen et al based on their presence and size, namely a) level I: no vacuoles, b) level II: <or = 2 small vacuoles; c) level III :> or = 1 large vacuole and d) level IV: large vacuole with other abnormalities [28]. Vacuole in the human sperm can be in sperm with normal and abnormal morphology. Study on Intracytoplasmic morpholologically selected sperm injection (IMSI) has reported that the frequency of normal sperm without vacuole is 18% with a magnification of 1500x, while study on reporting even less frequency is around 1.5 - 1.8% but with a magnification of 8400x [29, 32].

MSOME application in IVF-ICSI or IMSI, as an alternative to conventional ICSI is known to increase, but the presence of vacuoles in the sperm head is leaving a question regarding the impact towards the clinical results. The origin of vacuoles in sperm has been investigated lately. In all vacuole sperm, the acrosome is intact with a concave but intact plasma membrane [33].

Sperm vacuoles are associated to non-reacted acrosome conditions [34, 35].MSOME selection will eliminate vacuole sperm so that it supports ICSI with sperm that has reacted acrosomes. Several studies report contradictory results about the role of IMSI on embryonic development. Some previous comparative studies reported an increase in embryonic development with IMSI, while others reported embryonic development similar to IMSI and ICSI [36, 27, 37, 38].In addition, studies of congenital abnormalities were reported higher after IMSI compared to ICSI however, this difference was not statistically significant. This study must be further confirmed and carried out in the long run [39, 40].

Magnetic Activated Cell-Sorting (MACS)

It is known that one of the causes of ICSI failure is the use of sperm that undergo apoptosis during ICSI [41]. Apoptosis is one of the mechanisms involved in sperm DNA fragmentation. DNA-damaged sperm exhibit apoptosis characteristics, for example phosphatidylserine (PS) translocation, Caspase-3 activation and decreased mitochondrial membrane potential [42, 43].In the mean time, swim up and density gradient centrifugation are the most current sperm preparation in the clinical uses [44].This preparation method aims to select sperm that are suitable for achieving oocyte fertilization.

However, this method is based on sperm migration and / or sedimentation, which

depends on motility or density, without considering molecular factors such as apoptosis and / or sperm DNA fragmentation. Therefore, the development of innovative reproductive technologies for better sperm selection is urgently compulsory at this time. One of the new reproductive technologies discovered is Magnetic Activated Cell Sorting-MACS which works by eliminating apoptotic sperm cells exploiting Annexin V [45, 46].The principle of MACS is by using magnetic micro beads conjugated with specific proteins in the target cell membrane. Then there is a high power field filtration column which is used to maintain the targeted cell, so that the unbound cell can pass through the filtration column. Annexin V (AV) is a phospholipid binding protein in physiological conditions. On the other hand, translocation of phospholipids from inside to outside the plasma membrane is considered a marker of early apoptosis. Therefore, the use of MACS with AV eliminates apoptotic sperm thereby increasing sperm count with improved quality [47] (Figure 4).

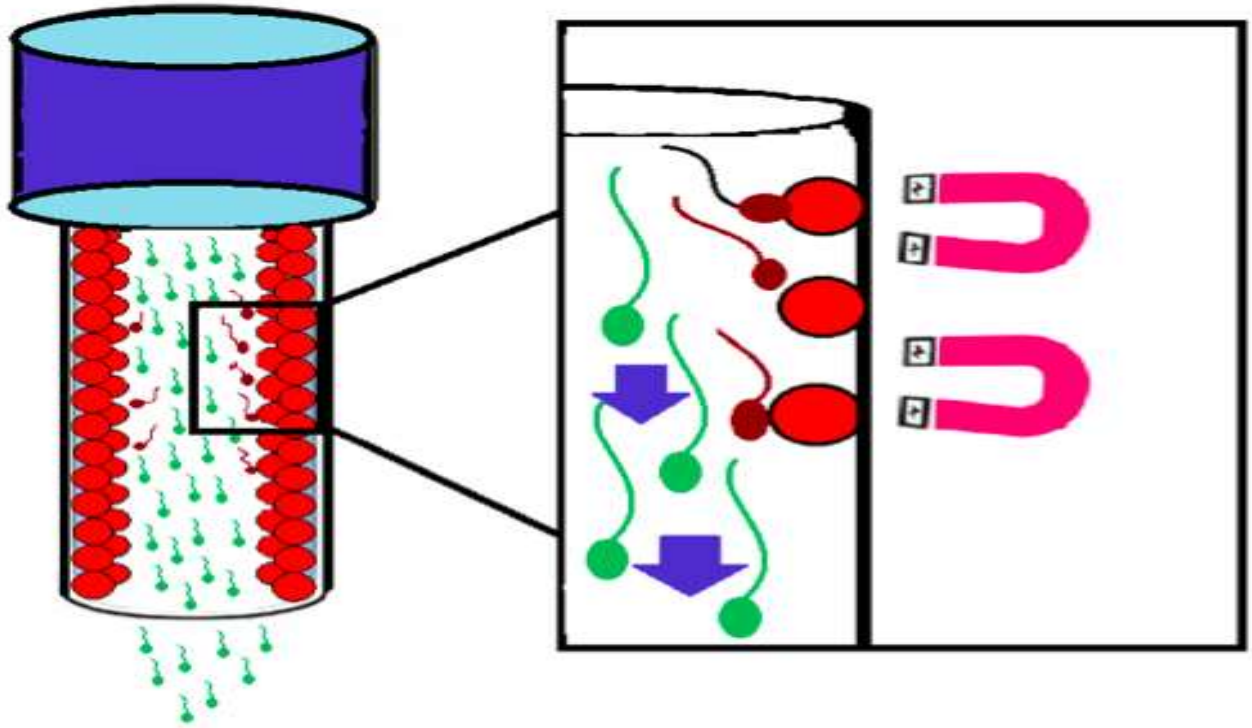

Figure 4: Magnetic Activated Cell Sorting-MACS. Green sperms are non-apoptotic sperms and red sperms are apoptotic sperms

Sperm selection using the MACS method is expected to increase the success of IVF-ICSI, compared to conventional sperm selection [48, 49].However, the MACS method still has limitations, namely in the case of too decreased sperm concentration (severe oligozoospermia) and sperm motility lower than 32% (asthenozoospermia)

Physiological ICSI (PICSI)

At present there is several sperm selection methods recognized to improve the quality of embryos [50, 51]. One of them is the use of hyaluronic acid (HA) which is considered to have a high success rate with low biological risk, compared to others [52].The selection of a functional sperm in vivo is hold by hyaluronan. While in vitro sperm selection followed by ICSI is considered for carrying the risk of genetic material damage. Increased sperm maturity intensity and genetic integrity associated with binding of hyaluronan binded (HB) sperm to ICSI can provide an increase in the quality of paternal genetic material in the embryo Hyaluronan, natural biopolymer expressed in all human cells, is the most important content of the cumulus oophorus oocyte layer in humans. In adult sperm heads there are hyaluronan-specific ligand receptors which facilitate adult sperm to bind to hyaluronan [53]. The working principle of this method is by inserting sperm in an IVF medium in a dish that has been added hyaluronan (PICSI-dish), so that mature sperm can be chosen by embryologist and used for ICSI procedures (Figure 5).

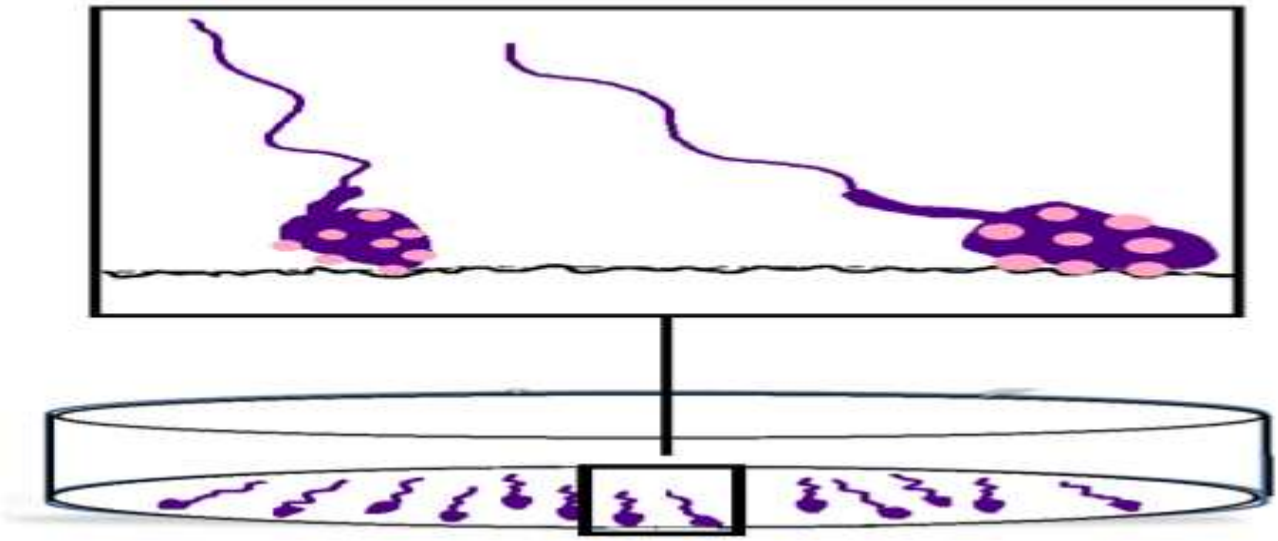

Figure 5: Physiologycal ICSI: The attached sperms are mature sperms

The binding between sperm and HA shows that the sperm is: a).Having a normal head morphology so that the potential for better fertilisation.12 b).Experience lower DNA fragmentation. c). Have a low risk of chromosomal Aneuploidy. PICSI is recommended in cases such as a) Slow embryonic development rate (low blastocyst rate), b) Low fertilized sperm with ICSI, c) Repetitive embryo transfer with implantation failure, d) Repetitive miscarriage and early pregnancy loss, e) Morphology and low sperm motility, f) Women over 38 years old, g) Sperm with low cryopreservation quality and h) Sperm obtained from PESA. Sperm selection with the PICSI method is expected to increase the success of ICSI. PICSI believes that sperm selected by this method have better quality to be injected into the oocyte and produce higher quality embryos [54].

Conclusion

At present, there are many sperm selection methods, but the most appropriate method should be chosen to obtain the best sperm quality to improve the success rate of IVF-ICSI. Indeed, the choice is depend on the capability of IVF laboratory, although ideally a good IVF lab should update its sperm selection method [55].

References

1. Greil AL, Slauson- Blevins K, McQuillan J (2010) The experience of infertility: a review of recent literature. Sociology of health & illness, 32(1):140-62.
2. World Health Organization WHO (2010) Laboratory annual for the examination and processing of human semen. 5th edition. WHO Press, Switzerland.
3. Esteves SC (2014) Clinical relevance of routine semen analysis and controversies surrounding the 2010 World Health Organization criteria for semen examination. Int. Braz. J. Urol., 40: 443-53.
4. Liu DY, Du Plessis YP, Nayudu PL, Johnsto WIH, Baker HWG (1988) The use of in vitro fertilization to evaluate putative tests of human sperm function. Fertility and Sterility 49(2):273-277.
5. Gardner Allison J, Evans Janice P (2005) Mammalian membrane block to polyspermy: new insights into how mammalian eggs prevent fertilization by multiple sperm. Reprod. Fertil. Dev., 18: 53-61.
6. Rath D, Töpfer-Petersen E, Michelmann HW, Schwartz P, Ebeling S (2005) Zona pellucida characteristics and sperm-binding patterns of in vivo and in vitro produced porcine oocytes inseminated with differently prepared spermatozoa. Theriogenology, 63: 352-362.
7. Huszar G, Jakab A, Sakkas D, Ozenci CC, Cayli S, Delpiano E, et al (2007) Fertility testing and ICSI sperm selection by hyaluronic acid binding: clinical and genetic aspects. Reprod Biomed Online, 14: 650-663.
8. Menkveld R, Holleboom CA, Rhemrev JP (2011) Measurement and significance of sperm morphology. Asian J. of Androl., 13: 59-68.

9. Turek PJ (2005) Practical approaches to the diagnosis and management of male infertility. Nature Clinical Practice Urology, 2: 226-238.
10. Strassburger D, Friedler S, Raziel A, Schachter M, Kasterstein E, Ron-el R (2000) Very low sperm count affects the result of intracytoplasmic sperm injection. J. Assist. Reprod Genet., 17(8):431-6.
11. Freeman MR, Archibong AE, Mrotek JJ, Whitworth CM, Weitzman GA, Hill GA (2001) Male partner screening before *in vitro* fertilization: preselecting patients who require intracytoplasmic sperm injection with the sperm penetration assay. *Fertil. Steril.,* 76: 1113-1118.
12. Kovac JR, Smith RP, Cajipe M, Lamb DJ, Lipshultz LI (2017) Men with a complete absence of normal sperm morphology exhibit high rates of success without assisted reproduction. Asian J. Androl., 19: 39-42.
13. Paul F, Obajini GO, Kolade CO (2018) Is semen analysis without strict criteria misleading decision in IVF? A prospective systematic study. Int. J. Reprod. Biomed., 16(7):459-462.
14. Mitchell V, Rives N, Albert M, et al (2006) Outcome of ICSI with ejaculated spermatozoa in a series of men with distinct ultrastructural flagellar abnormalities. Hum Reprod., 21(8):2065-74.
15. De Vos A, Van De Velde H, Joris H, Verheyen G, Devroey P, Van Steirteghem A (2003) Influence of individual sperm morphology on fertilization, embryo morphology, and pregnancy outcome of intracytoplasmic sperm injection. Fertil. Steril., 79(1):42-8.
16. Chen X, Zhang W, Luo Y, Long X, Sun X (2009) Predictive value of semen parameters in in vitro fertilization pregnancy outcome. Andrologia, 41(2).
17. Nallella KP, Sharma RK, Aziz N, Agarwal A (2006) Significance of sperm characteristics in the evaluation of male infertility. Fertil Steril., 85(3):629-34.
18. Agarwal A, Allamaneni SS (2005) Sperm DNA damage assessment: a test whose time has come. Fertil. Steril., 84(4):850-3.
19. Aitken RJ, De Iuliis GN, McLachlan RI (2009) Biological and clinical significance of DNA damage in the male germ line. Int. J. Androl., 32(1):46-56. doi:10.1111/j.1365-2605.2008.00943.x.
20. Aitken RJ, Bronson R, Smith TB, De Iuliis GN (2013) The source and significance of DNA damage in human spermatozoa; a commentary on diagnostic strategies and straw man fallacies. Mol. Hum. Reprod., 19(8): 475-85. doi:10.1093/molehr/gat025.
21. Aitken RJ, De Iuliis GN (2010) On the possible origins of DNA damage in human spermatozoa. Mol. Hum. Reprod., 16(1):3-13. doi:10.1093/molehr/gap059.
22. Greco E, Scarselli F, Iacobelli M, et al (2005) Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. Hum Reprod., 20(1):226-30.
23. Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J, Giwercman A (2006) Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod., 22(1):174-9.
24. Bach PV, Schlegel PN (2016) Sperm DNA damage and its role in IVF. Basic and clinical andrology, 26: 15.
25. Bartoov B, Berkovitz A, Eltes F (2001) Selection of spermatozoa with normal nuclei to improve the pregnancy rate with intracytoplasmic sperm injection. N. Engl. J. Med., 345:1067-1068.
26. Bartoov B, Berkovitz A, Eltes F, Kogosowski A, Menezo Y, Barak Y (2002) Real-time fine morphology of motile human sperm cells is associated with IVF-ICSI outcome. J. Androl., 23: 1-8.
27. Berkovitz A, Eltes F, Lederman H, Peer S, Ellenbogen A, Feldberg B, Bartoov B (2006a) How to improve IVF-ICSI outcome by sperm selection. Reprod Biomed Online, 12: 634-638.
28. Vanderzwalmen P, Hiemer A, Rubner P, Bach M, Neyer A, Stecher A, et al (2008) Blastocyst development after sperm selection at high magnification is associated with size and number of nuclear vacuoles. Reprod Biomed Online, 17: 617-627.
29. De Vos A, Polyzos NP, Verheyen G, Tournaye H (2013) Intracytoplasmic morphololologically selected sperm injection (IMSI): a critical and evidence-based review. Basic Clinical Andrology, 23: 10.

30. Oliveira JB, Cavagna M, Petersen CG, Mauri AL, Massaro FC, Silva LF, Baruffi RL, Franco JG Jr (2011) Pregnancy outcomes in women with repeated implantation failures after intracytoplasmic morphologically selected sperm injection (IMSI). Reprod Biol. Endocrinol., 9: 99-105.

31. Oliveira JBa, Petersen CG, Massaro FC, Baruffi RL, Mauri AL, Silva LF, Ricci J, Franco JG Jr (2010) Motile sperm organelle morphology examination (MSOME): intervariation study of normal sperm and sperm with large nuclear vacuoles. Reprod Biol. Endocrinol., 8: 56-62.

32. Oliveira JBb, Massaro FC, Baruffi RL, Mauri AL, Petersen CG, Silva LF, Vagnini LD, Franco JG Jr (2010) Correlation between semen analysis by motile sperm organelle morphology examination and sperm DNA damage. Fertil. Steril., 94:1937-1940.

33. Boitrelle F, Ferfouri F, Petit JM, Segretain D, Tourain C, Bergere M, Beilly M, Vialard F, Albert M, Selva J (2011) Large human vacuoles observed in motile spermatozoa under high magnification: nuclear thumbprints linked to failure of chromatine condensation. Hum Reprod., 26: 1650-1658.

34. Kacem O, Sifer C, Barraud-Lange V, Ducot B, De Ziegler D, Poirot C, Wolf JP (2010) Sperm nuclear vacuoles, as assessed by motile sperm organellar mor- phological examination, is mostly of acrosomal origin. Reprod Biomed Online, 20:132-137.

35. Montjean D, Belloc S, Benkhalifa M, Dalleac A, Ménézo Y (2012) Sperm vacuoles are linked to capacitation and acrosomal status. Hum Reprod., 27: 2927-2932.

36. Bartoov B, Berkovitz A, Eltes F, Kogosovsky A, Yagoda A, Lederman H, Artzi S, Gross M, Barak Y (2003) Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection. Fertil. Steril., 80: 1413-1419.

37. Hazout A, Dumont-Hassan M, Junca A, Cohen Bacrie P, Tesarik J (2006) High-magnification ICSI overcomes paternal effect resistant to conventional ICSI. Reprod Biomed Online, 12: 19-25.

38. Berkovitz A, Eltes F, Ellenbogen A, Peer S, Feldberg D, Bartoov B (2006b) Does the presence of nuclear vacuoles in human sperm selected for ICSI affect pregnancy outcome? Hum Reprod., 21: 1787-1790.

39. Junca AM, Dumont M, Cornet D, Douard S, De Mouzon J, Prisant N (2010) Is intracytoplasmic morphologically sperm injection (IMSI) detrimental for pregnancy control? Fertil. Steril., 94: S31.

40. Said TM and Land JA (2011) Effects of advanced selection methods on sperm quality and ART outcome: a systematic review. Hum Reprod Update, 17: 719-733.

41. Henkel R, Hajimohammad M, Stalf T, Hoogendijk C, Mehnert C, et al (2004) Influence of deoxyribonucleic acid damage on fertilization and pregnancy. Fertil. Steril., 81: 965-972.

42. Glander HJ, Schaller J (1999) Binding of annexin V to plasma membranes of human spermatozoa: a rapid assay for detection of membrane changes after cryostorage. Molecular human reproduction, 5: 109-115.

43. Paasch U, Grunewald S, Agarwal A, Glandera HJ (2004) Activation pattern of caspases in human spermatozoa. Fertil. Steril., 81(1):802-809.

44. Henkel R, Schill WB (2003) Sperm prepartion for ART. Reprod. Biol. Endocrionol., 1: 108.

45. Miltenyi S, Muller W, Weichel W, Radbruch A (1990) High gradient magnetic cell separation with MACS. Cytometry, 11: 231-238.

46. Grunewald S, Paasch U, Glander HJ (2004) Cryopreservation and thawing is associated with varying extent of activation of apoptotic machinery in subsetes of ejaculated human spermatozoa. Biol. Reprod., 71: 1828-1837.

47. Said TM, Grunewald S, Paasch U, Rasch M, Agarwal A, et al (2005) Effects of magnetic-activated cell sorting on sperm motiity and cryosurvival rates. Fertil. Steril., 83: 1442-1446.

48. Makker K, Agarwal A, Sharma RK (2008) Magnetic activated cell sorting (MACS): utility in assisted reproduction. Indian J. Exp. Biol., 46: 491-497.

49. Lee TH, Liu CH, Shih YT, Tsao HM, Huang CC, et al (2010) Magnetic-activated

cell sorting for sperm preparation reduces spermatozoa with apoptotic markers and improves the acrosome reaction in couples with unexplained infertility. Hum Reprod., 25: 839-846.

50. Nijs M, Creemers E, Cox A, et al (2010) Relationship between hyaluronic acid binding assay and outcome in art: a pilot study. Andrologia., 42(5):291-296.
51. Ola B and Li TC (2006) Implantation failure following in vitro fertilization. Curr Opin Obstet Gynecol., 18(4):440-445.
52. Nasr-Esfahani MH, Razavi S, Vahdati AA, et al (2008) Evaluation of spem selection procedure based on hyaluronic acid binding ability on ICSI outcome. J. Assist. Reprod. Genet., 25(5):197-203.
53. Worrilow KC, Huynh HT, Bower J, et al (2006) The clinical impact associated with the use of PICSI-derived embryos. Fertil. Steril., 86(3): S62.
54. Worrilow KC, Eid S, Woodhouse D, et al (2012) Use of hyaluronan in the selection of sperm for intracytoplasmic sperm injection (ICSI): significant improvement in clinical outcomes-multicenter, double blinded and randomized trial. Hum Reprod. 28(2):306-314.
55. Palermo G, Joris H, Derde MP, Camus M, Devroey P, Van Steirteghem A (1993) Sperm characteristics and outcome of human assisted fertilization by subzonal insemination and intracytoplasmic sperm injection. Fertil. Steril., 59(4): 826-35. doi: 10.1016/S0015-0282(16)55867-1.

