MSOME: Conventional Semen Analysis, Sperm Manipulation, and Cryopreservation

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MSOME and Conventional Semen Analysis

Evaluation of sperm morphology plays a crucial role in the diagnosis of male fertility potential and has demonstrated a predictive value for IVF-ICSI treatments [1–3]. MSOME provides an accurate description of spermatozoa abnormalities, particularly the presence of head vacuoles [4]. However, no consensus has been established concerning normal or abnormal MSOME criteria, despite being essential to transposing MSOME analysis into routine evaluation of male infertility [5]. Therefore, some studies have analyzed the relationship between sperm normalcy according to the World Health Organization (WHO) or Tygerberg criteria and MSOME.

Bartoov et al. [4] investigated the relationship between normal spermatozoa according to the WHO reference values [6] and MSOME in 20 patients and found no significant correlations between the percentage of morphologically normal spermatozoa as defined by the WHO and the percentage of morphologically normal spermatozoa as defined by MSOME. Conversely, a strong positive correlation between the percentage of normal sperm forms according to the Tygerberg criteria and MSOME was observed by Oliveira et al. [7]. Nevertheless, both studies found that MSOME was shown to be much more restrictive, presenting significantly lower normality percentages for the semen samples in comparison to those observed after analysis according to the Tygerberg or WHO criteria.

Later, Cassuto et al. [8] found significant correlations between the incidence of score-0 spermatozoa (presenting an abnormal head, one or several vacuoles, and an abnormal base) and sperm concentration, motility, and morphology.

Conventional semen analysis and MSOME evaluation were performed simultaneously in sperm samples from 440 patients [5]. The results showed that sperm head vacuoles were significantly larger in abnormal semen samples. Relative vacuolar area (RVA), defined as vacuole area (μm^2)/head area (μm^2) × 100, was the most discriminative MSOME criterion between normal and abnormal semen samples, and was negatively correlated with poor sperm morphology.

It is noteworthy that routine morphological examination is performed on the entire semen sample, whereas the most remarkable feature of MSOME is the focus on motile sperm fractions,

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providing information about the sperm fraction referred for ICSI. Moreover, MSOME is a reliable technique for analyzing semen and has been suggested as a routine technique for semen analysis [9].

MSOME and Sperm Preparation and Manipulation

During semen sample liquefaction, the spermatozoa are exposed to round cells and leukocytes, both potential sources of reactive oxygen species (ROS) that are positively correlated with sperm head morphological abnormalities [10]. Moreover, the concentration of ROS may produce crater defects in the form of deep vacuoles in mammals [11].

Despite the origin of sperm vacuoles remains disappointingly unknown, the use of MSOME may be a helpful tool for the selection of spermatozoa. However, whether or not specific in vitro conditions during sperm preparation and manipulation results in the formation of sperm vacuoles is still under debate.

It has previously been demonstrated that extended in vitro culture at 37 °C may reduce sperm viability [12]. Since the morphological evaluation of sperm under high magnification is a time-consuming technique [13], there has been some investigation regarding the impact of semen sample incubation at 37 °C on the sperm nucleus morphology. It has been demonstrated that after 2 h of incubation at 37 °C there was a significant increase in the frequency of vacuolated nuclei [14]. No significant morphological changes in sperm nuclei were observed upon prolonged incubation at 21 °C. Additionally, after 2 h of incubation, the incidence of spermatozoa with vacuolated nuclei was significantly higher at 37 °C compared with 21 °C [14]. Similarly, Schwarz et al. [15] reported a negative impact of temperature on the morphological integrity of sperm nuclei. Conversely, using the sperm-microcapture channels in a 24-h period, Neyer et al. [16] demonstrated that sperm vacuoles are not generated by incubation at 37 °C.

Several semen preparation techniques have been established to separate the sperm fraction for use in assisted reproductive techniques. The most commonly used protocols are density-gradient centrifugation and swim-up [17]. Several studies addressed whether there was any differences between these two methods regarding sperm motility and concentration after semen preparation and the outcomes of intrauterine insemination [18–22]. It is of great importance to select a processing technique that improves the sample with spermatozoids that show a low amount of nuclear vacuolization after preparation.

Monqaut et al. [23] evaluated sperm morphology under high magnification before and after swim-up and density gradient centrifugation and classified recovered spermatozoa according to the degree of vacuolization. Despite both methods showed a positive effect on sperm quality, the swim-up method produced significantly higher incidence of morphologically normal spermatozoa than gradient centrifugation.

Borges et al. [24] compared the results of intracytoplasmic morphologically selected sperm injection between cycles in which the swim-up or the density gradient centrifugation techniques were used for sperm preparation. Implantation, pregnancy, and miscarriage rates were not statistically different between the groups. Both techniques recovered improved sperm fractions and resulted in similar IMSI outcomes.

MSOME and Sperm Cryopreservation

Human sperm cryopreservation has been routinely practiced for several years. Despite the success of sperm cryopreservation technique, the freezing–thawing process has proven to be associated with modifications in seminal quality, particularly the decrease in sperm motility and increase in morphological abnormalities [25].

During the cryopreservation of spermatozoa, both the formation of intracellular ice crystals [26] and the crystallization of the extracellular medium [27] are associated with mechanical

damage and may result in rupture of the plasma membrane and disturbance of cellular organelles [28]. Moreover, sperm cryopreservation has been correlated with an increase in the levels of some apoptosis markers [29]. Lastly, cryopreservation was found to induce chromatin decondensation [30], DNA denaturation [31] and increased sperm DNA fragmentation [32]. However, it is still under debate whether or not cryopreservation can induce sperm nuclear damage. Most of the techniques used to evaluate sperm damage are invasive. It would be advantageous to recognize negative effects of cryopreservation that might appear in post-thaw spermatozoa. Hence, a few studies evaluated the sperm morphology by MSOME in frozen-thawed sperm.

Boitrelle et al. [33] evaluated whether or not cryopreservation modifies motile sperm morphology under high magnification and/or is associated with chromatin decondensation. Cryopreservation induced sperm nuclear vacuolization, decreased the incidence of grade I+II spermatozoa and the sperm viability rate and increased the incidence of sperm with noncondensed chromatin.

Conversely, Gatimel et al. [28] demonstrated that the cryopreservation has no effect on human sperm vacuoles. The main difference between the two studies is that Boitrelle et al. studied men from infertile couples, while only samples from recently fertile men were included in the study by Gatimel et al. Moreover, the dilution ratio with the cryoprotectant was different; and Boitrelle et al. used a morphological classification that included not only vacuoles but also other sperm abnormalities.

Conclusion

The available literature seems to support that MSOME is a much stricter criterion of sperm morphology evaluation, since it identifies vacuoles that are not identified by the conventional semen analysis. Any technique that increases the quality of recovered spermatozoa and/or decreases the extent of vacuolated sperm could present an advantage in treatment's outcomes. Nevertheless, it appears that both the swim-up and the density-gradient centrifugation techniques recover improved sperm fractions and result in similar IMSI outcomes. Sperm cryopreservation may result in the appearance of vacuoles due to mechanical stress during the procedure. As long as the precise reasons for vacuole formation are still unknown, it is important to avoid prolonged sperm manipulation.

References

1. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Steril. 1988;49(1):112–7.
2. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Veeck LL, et al. New method of evaluating sperm morphology with predictive value for human in vitro fertilization. Urology. 1987;30(3):248–51.
3. Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, van Zyl JA, et al. Sperm morphologic features as a prognostic factor in in vitro fertilization. Fertil Steril. 1986;46(6):1118–23.
4. Bartoov B, Berkovitz A, Eltes F, Kogosowski A, Menezo Y, Barak Y. Real-time fine morphology of motile human sperm cells is associated with IVF-ICSI outcome. J Androl. 2002;23(1):1–8.
5. Perdrix A, Saidi R, Menard JF, Gruel E, Milazzo JP, Mace B, et al. Relationship between conventional sperm parameters and motile sperm organelle morphology examination (MSOME). Int J Androl. 2012; 35(4):491–8.
6. WHO. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge: Published on behalf of the World Health Organization by Cambridge University Press; 1999.
7. Oliveira JB, Massaro FC, Mauri AL, Petersen CG, Nicoletti AP, Baruffi RL, et al. Motile sperm organelle morphology examination is stricter than Tygerberg criteria. Reprod Biomed Online. 2009;18(3):320–6.
8. Cassuto NG, Hazout A, Hammoud I, Balet R, Bouret D, Barak Y, et al. Correlation between DNA defect and sperm-head morphology. Reprod Biomed Online. 2012;24(2):211–8.
9. Oliveira JB, Petersen CG, Massaro FC, Baruffi RL, Mauri AL, Silva LF, et al. Motile sperm organelle morphology examination (MSOME): intervariation study of normal sperm and sperm with large nuclear vacuoles. Reprod Biol Endocrinol. 2010;8:56.
10. Agarwal A, Said TM. Role of sperm chromatin abnormalities and DNA damage in male infertility. Hum Reprod Update. 2003;9(4):331–45.
11. Tremellen K, Tunc O. Macrophage activity in semen is significantly correlated with sperm quality in infertile men. Int J Androl. 2010;33(6):823–31.

12. Calamera JC, Fernandez PJ, Buffone MG, Acosta AA, Doncel GF. Effects of long-term in vitro incubation of human spermatozoa: functional parameters and catalase effect. Andrologia. 2001;33(2):79–86.
13. Berkovitz A, Eltes F, Yaari S, Katz N, Barr I, Fishman A, et al. The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm. Hum Reprod. 2005;20(1):185–90.
14. Peer S, Eltes F, Berkovitz A, Yehuda R, Itsykson P, Bartoov B. Is fine morphology of the human sperm nuclei affected by in vitro incubation at 37 degrees C? Fertil Steril. 2007;88(6):1589–94.
15. Schwarz C, Koster M, van der Ven K, Montag M. Temperature-induced sperm nuclear vacuolisation is dependent on sperm preparation. Andrologia. 2012; 44 Suppl 1:126–9.
16. Neyer A, Vanderzwalmen P, Bach M, Stecher A, Spitzer D, Zech N. Sperm head vacuoles are not affected by in-vitro conditions, as analysed by a system of sperm-microcapture channels. Reprod Biomed Online. 2013;26(4):368–77.
17. Enciso M, Iglesias M, Galan I, Sarasa J, Gosalvez A, Gosalvez J. The ability of sperm selection techniques to remove single- or double-strand DNA damage. Asian J Androl. 2011;13(5):764–8.
18. Dodson WC, Moessner J, Miller J, Legro RS, Gnatuk CL. A randomized comparison of the methods of sperm preparation for intrauterine insemination. Fertil Steril. 1998;70(3):574–5.
19. Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. Cochrane Database Syst Rev. 2004;3, CD004507.
20. Xu L, Lu RK, Chen L, Zheng YL. Comparative study on efficacy of three sperm-separation techniques. Asian J Androl. 2000;2(2):131–4.
21. Allamaneni SS, Agarwal A, Rama S, Ranganathan P, Sharma RK. Comparative study on density gradients and swim-up preparation techniques utilizing neat and cryopreserved spermatozoa. Asian J Androl. 2005;7(1):86–92.
22. Sakkas D, Manicardi GC, Tomlinson M, Mandrioli M, Bizzaro D, Bianchi PG, et al. The use of two density gradient centrifugation techniques and the swim-up method to separate spermatozoa with chromatin and nuclear DNA anomalies. Hum Reprod. 2000; 15(5): 1112–6.
23. Monqaut AL, Zavaleta C, Lopez G, Lafuente R, Brassesco M. Use of high-magnification microscopy for the assessment of sperm recovered after two different sperm processing methods. Fertil Steril. 2011; 95(1):277–80.
24. Borges Jr E, Setti AS, Vingris L, Figueira Rde C, Braga DP, Iaconelli Jr A. Intracytoplasmic morphologically selected sperm injection outcomes: the role of sperm preparation techniques. J Assist Reprod Genet. 2013;30(6):849–54.
25. O'Connell M, McClure N, Lewis SE. The effects of cryopreservation on sperm morphology, motility and mitochondrial function. Hum Reprod. 2002;17(3):704–9.
26. Muldrew K, McGann LE. Mechanisms of intracellular ice formation. Biophys J. 1990;57(3):525–32.
27. Donnelly ET, McClure N, Lewis SE. Cryopreservation of human semen and prepared sperm: effects on motility parameters and DNA integrity. Fertil Steril. 2001;76(5):892–900.
28. Gatimel N, Leandri R, Parinaud J. Sperm vacuoles are not modified by freezing–thawing procedures. Reprod Biomed Online. 2013;26(3):240–6.
29. Said TM, Gaglani A, Agarwal A. Implication of apoptosis in sperm cryoinjury. Reprod Biomed Online. 2010;21(4):456–62.
30. Fortunato A, Leo R, Liguori F. Effects of cryostorage on human sperm chromatin integrity. Zygote. 2012; 21(4):330–6.
31. Dejarkom S, Kunathikom S. Evaluation of cryo-injury of sperm chromatin according to computer controlled rate freezing method part 2. J Med Assoc Thai. 2007; 90(5):852–6.
32. Gosalvez J, Nunez R, Fernandez JL, Lopez-Fernandez C, Caballero P. Dynamics of sperm DNA damage in fresh versus frozen-thawed and gradient processed ejaculates in human donors. Andrologia. 2011;43(6): 373–7.
33. Boitrelle F, Albert M, Theillac C, Ferfouri F, Bergere M, Vialard F, et al. Cryopreservation of human spermatozoa decreases the number of motile normal spermatozoa, induces nuclear vacuolization and chromatin decondensation. J Androl. 2012;33(6):1371–8.