RESEARCH ARTICLE

Ozcan Budak[1]
Mehmet Suhha Bostanci[2]
Osman Kose[3]
Nermin Akdemir[2]
Ahmet Gokce[3]
Arif Serhan Cevrioglu[2]

[1] Sakarya University, Faculty of Medicine, Department of Histology and Embryology, Sakarya, Türkiye
[2] Sakarya University, Faculty of Medicine, Department of Obstetrics and Gynecology and Artificial Reproductive Techniques, Sakarya, Türkiye
[3] Sakarya University, Faculty of Medicine, Department of Urology, Sakarya, Türkiye

Corresponding Author:
Ozcan Budak
mail:ozcanbudak@sakarya.edu.tr

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konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Evaluation of the Clinical Results of Using Microfluidic Channel System for Sperm Selection in IVF Cycles in Patients with Low Sperm Concentration

ABSTRACT

Objective: Microfluidic channel system (MAC), a new generation method, gives the chance to select better quality spermatozoa with lower DNA fragmentation indices. This study evaluated the treatment results in patients who underwent ICSI-ET due to the MAC technique's male factors.

Methods: Sakarya University ART Center carried out this retrospective study. Patients with 35 male factor indications were included in our study. In these patients, swim-up (SU) was used in the first of two consecutive IVF cycles, and the MAC sperm preparation technique was used in the second. Our study compared fertilization, quality embryo counts, implantation after fresh embryo transfer, pregnancy rates, fifth-day embryo, and frozen embryo numbers.

Results: Fertilization rate was higher in the MAC group than in the SU group (P=0.009). The number of 3rd and 5th Day Grade 1 embryo in the MAC group was statistically higher than in the SU group (p=0.000 for both parameters). The number of quality embryos frozen on day 5 was higher in the MAC group than in the SU group (P=0.000).

Conclusions: It is thought that MAC application does not make a statistically significant contribution on implantation and pregnancy in IVF cycles performed due to the malefactor. However, it may positively affect fertilization rate and embryo quality. In addition, we think that it increases the number of embryos frozen at the end of the cycle, and for this reason, the MAC technique may provide positive benefits to IVF treatments.

Keywords: Embryo Implantation, Infertility, Microfluidics.

Düşük Sperm Konsantrasyonu Olan Hastalarda Tüp Bebek Döngülerinde Sperm Seçiminde Mikroakışkan Kanal Sistemi Kullanmanın Klinik Sonuçlarının Değerlendirilmesi

ÖZET

Amaç: Yeni nesil bir yöntem olan mikroakışkan kanal sistemi (MAC), daha düşük DNA fragmantasyon indekslerine sahip daha kaliteli spermatozoa seçme şansı vermektedir. Bu çalışmada MAC tekniğinin erkek faktörleri nedeniyle ICSI-ET uygulanan hastalarda tedavi sonuçları değerlendirilmiştir.

Gereç ve Yöntem: Çalışmamız Sakarya Üniversitesi ART Merkezinde retrospektif olarak gerçekleştirdi. Çalışmamıza 35 erkek faktörü endikasyonu olan hastalar dahil edildi. Bu hastalarda, ardışık iki IVF döngüsünün ilkinde swim-up (SU), ikincisinde MAC sperm hazırlama tekniği kullanıldı. Çalışmamızda fertilizasyon, kaliteli embriyo sayısı,embriyo transferi sonrası implantasyon, gebelik oranları, beşinci gün embriyo sayısı ve dondurulmuş embriyo sayıları karşılaştırılmıştır.

Bulgular: Döllenme oranı MAC grubunda SU grubuna göre daha yüksekti (P=0.009). MAC grubundaki 3. ve 5. Gün Grade 1 embriyo sayısı SU grubuna göre istatistiksel olarak daha yüksekti (her iki parametre için p=0.000). 5. günde dondurulan kaliteli embriyo sayısı MAC grubunda SU grubuna göre daha yüksekti (P=0.000).

Sonuç: Erkek faktörü olan tüp bebek sikluslarında MAC uygulamasının implantasyon ve gebelik üzerine istatistiksel olarak anlamlı bir katkı sağlamadığını düşünmekteyiz.. Ancak fertilizasyon oranı ve embriyo kalitesini olumlu yönde etkileyebileceğini düşünmekteyiz. Ayrıca döngü sonunda dondurulan embriyo sayısını arttırdığını ve bu nedenle MAC tekniğinin tüp bebek tedavilerine olumlu katkı sağlayabileceğini düşünüyoruz.

Anahtar Kelimeler: Embriyo İmplantasyonu, İnfertilite, Mikroakışkan.

INTRODUCTION

Success rates in assisted reproductive techniques (ART) have increased significantly since treatment, especially in the last ten years (1). Similar to in vitro fertilization (IVF) cycles, in the success of treatment in intrauterine insemination (IUI) cycles; Factors such as female age, duration of infertility, ovarian reserve, and sperm parameters play a role (2). The importance of sperm parameters in ART treatments has been better understood by decreasing fertilization success in intra-cytoplasmic sperm injection (ICSI) procedures performed with sperm with low sperm count and motility (1). Therefore, selecting functionally normal sperm with fertilization ability is an essential need in ART treatments. Density gradient (DG) and swim-up (SU) techniques procedures are generally used as standard sperm preparation techniques in ART treatments (3). In both techniques, the centrifuge is used during sperm preparation and selection. However, centrifuges have a detrimental effect on sperm viability and can cause sperm DNA fragmentation (3). When sperms with DNA damage prepared by these methods are used in ICSI procedures, fertilization success and the possibility of obtaining good quality embryos may be adversely affected. When SU and DG methods are compared, it has been reported that a lower rate of standard chromatin structure is obtained in sperm prepared by DG method (4, 5). Although there is no consensus, using centrifuges for shorter times in the SU method is recommended. MAC, a new method developed recently for sperm selection, aims to increase high oocyte fertilization and pregnancy (6). Although DNA fragmentation has been shown to decrease in sperm selected by the MAC method, there is no clinical study showing that embryo quality and pregnancy rates increase (7, 8). While preparing sperm samples in the classical SU method, sperm are exposed to chemicals and the adverse effects of centrifugation methods, while sperm preparation in the MAC method imitates the natural sperm selection pathways in the female reproductive system (9, 10).

This retrospective cohort study aims to compare the effects of the MAC method and the SU method on the number of fertilized oocytes, quality embryos, the number of frozen embryos, and pregnancy outcomes in ART cycles due to the male factor. This study will be the first in the literature because it compares the treatment results of patients whose sperm preparation method was SU in the previous ART trial in the same patient, instead of comparing the effects of the MAC method in ART cycles with the results of different patients.

MATERIAL AND METHODS

Ethics Statement: This study was carried out retrospectively with the approval of the Sakarya University Faculty of Medicine Non-Invasive Ethics Committee dated 14.12.2020 and numbered 633.

Patients: The couples in this study consisted of patients who applied to Sakarya University Medical Faculty IVF center for treatment between 2019-2020. In our study, 35 couples who did not achieve pregnancy by using SU as the sperm preparation technique in the first trial of two consecutive ART treatments and who used the MAC method in the subsequent trial were included. Laboratory conditions, embryo culture and media used, sperm immobilization media, cumulus cell extraction media, sperm injection needles, and oocyte fixation needles (holding needles) were the same in both applications. The same embryologist performed ICSI procedures of both applications. Our study will compare the blastocyst numbers obtained on the fifth day and the blastocyst numbers frozen on the fifth day. Therefore, to obtain a sufficient number of blastocysts on the fifth day, attention was paid to the fact that the female patients included in our study were younger than 42 years old and had at least 5 MII oocytes in the ICSI procedure. When we used the MAC method for sperm preparation in male patients in previous treatments at our ART center, we experienced that a sufficient number of sperm could not be obtained in patients with a sperm count less than 1 million/ml. Therefore, we did not include male patients with a sperm count less than 1 million/ml in the study. Couples with acute or chronic diseases in both trials in ART cycles were not yet included in the study. The flow chart of the patient selection included in the study is given in Figure 1.

Sperm Preparation: Semen samples were taken by masturbation method after 2-5 days of sexual abstinence. For semen samples, sperm preparation methods were used after 15-60 minutes of liquefaction. Sperm analysis was performed according to the parameters of the world health organization (WHO) (11). Only fresh sperm samples were used in the study.

Swim-up Technique: All sperm samples were incubated for liquefaction in a 37 °C incubator for 15-60 minutes. Liquefied semen samples were then diluted 1:1 with culture medium and centrifuged at 1500 rpm for 10 minutes. After centrifugation, the supernatant was removed, 1 ml of fresh medium was carefully transferred onto the sperm pellet collected at the bottom, and it was incubated for 1 hour (37 °C, 6% CO2), kept tilted at 45 degrees. After incubation, the supernatant is collected in a sterile tube. Thus, the semen sample is ready for ICSI processing.

MAC Technique: Using a sterile micropipette, 13 µL of sperm sorting solution was added to the microfluidic chip inlet port, and then 2 µL of liquefied sperm sample was slowly added to the same port.

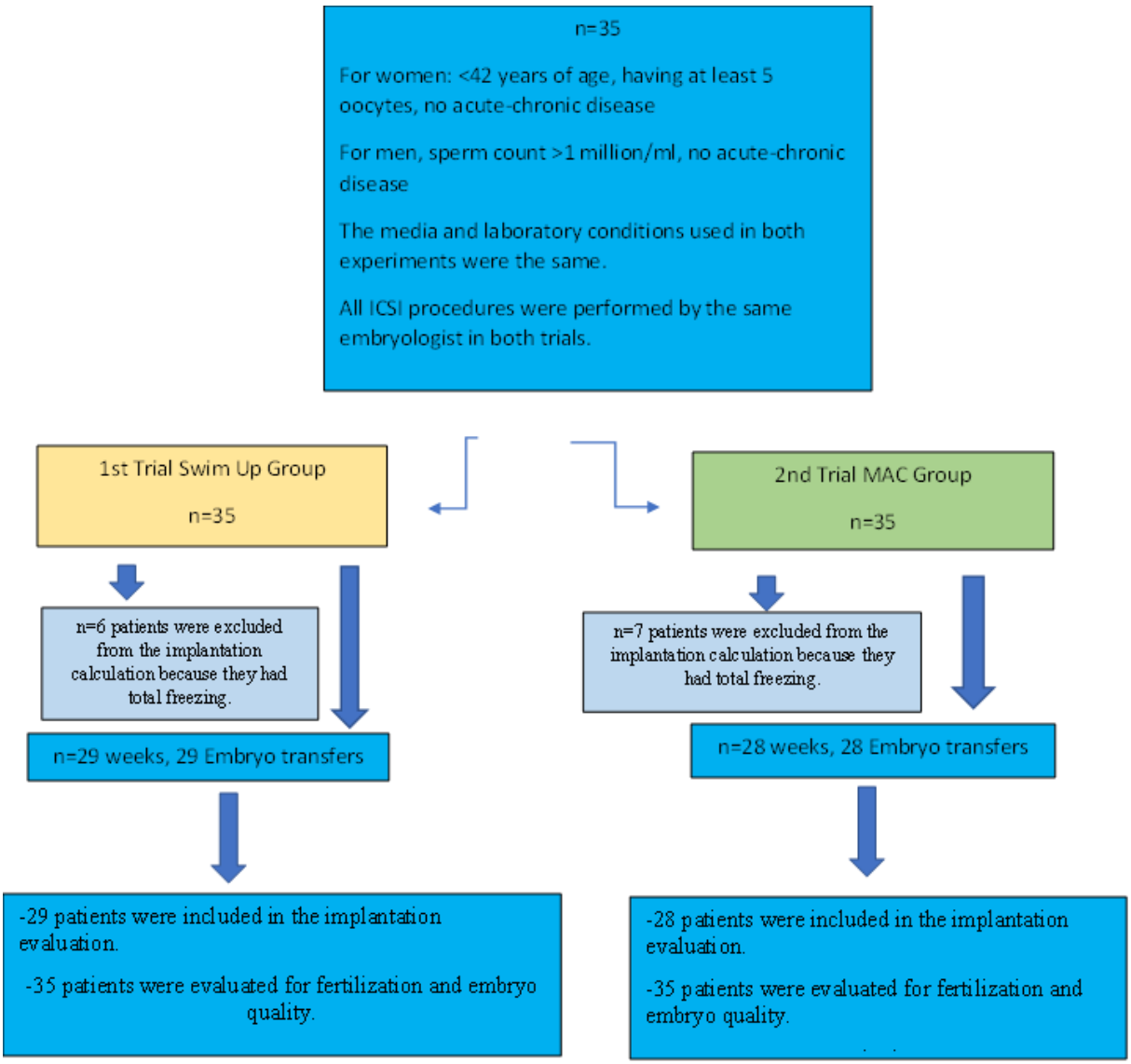

Figure 1. Patient selection flowchart.

Then, approximately 2 µL of mineral oil was added to the outlet port to protect the sperm cells against evaporation. For the same purpose, the inlet port will be covered with mineral oil and incubated at 37 degrees for about 30 minutes. After incubation, sperm cells containing solution from the outlet port under the mineral oil were examined under an inverted microscope. Sperm cells are ready for the ICSI procedure.

Controlled Ovarian Hyperstimulation and ICSI Procedure: Antagonist protocol (Cetrotide; Serono) was used as a method of controlled ovarian stimulation. Gonadotropin dose was determined according to the patients' ovarian reserve and response to the gonadotropins used. Human chorionic gonadotropin (HCG) injection was administered when the mean diameter of at least two follicles reached 17 mm. Oocytes were collected under general anesthesia by transvaginal-USG 36 hours after hCG injection (6). A single embryologist performed the ICSI procedure. Prepared sperm samples were transferred to a sterile culture dish with polyvinylpyrrolidone (PVP), ICSI, and ICSI dishes coated with sperm pool mineral oil. ICSI procedure was performed with the sperms with the best motility and morphology under the appropriate objective. After ICSI, oocytes were transferred into the culture medium, and fertilization and embryo follow-up were performed(12).

Embryo Evaluation: Fertilization control was performed according to the presence of pronucleus 16-18 hours after the ICSI procedure. Fertilized oocytes were incubated in a single-stage culture medium. According to previous studies, embryos were graded on the third day for their quality(13). Embryo culture was continued until the fifth day. The transfer status and embryos to be frozen were made according to blastocyst score grading(14).

Clinical Results: In the study, we compared the fertilization rates between the two cycles, the

number of good quality embryos on Day 3 and Day 5, the number of quality embryos frozen, and pregnancy outcomes. We compared the clinical pregnancy rates by comparing the patients who achieved pregnancy due to transfer and all the patients.

Statistical Analysis: Statistical analyzes were performed using the SPSS 24.0 package program (SPSS Inc. and Lead Tech. Inc. Chicago. USA). The Kolmogorow-Simirnow test was used for the normal distribution of the data. In our study, SU and MAC techniques, sperm preparation techniques, were applied to the same patient pairs at different time intervals. Since two sperm preparation techniques were compared, dependent groups with normal distribution were compared using the Paired-sample test. All results are presented as Mean±SD. Results with $p<0.05$ were considered significant.

RESULTS

Thirty-five patients who underwent IVF due to the male factors were included in our study. We compared the contributions of the SU technique as a sperm preparation method in the first trial and the MAC technique as a sperm preparation method in the subsequent trials to IVF treatments.

The mean age of the women included in the study was 32.01±0.96 years; the mean age of men was found to be 35.02±0.98 years. The mean BMI was found to be 24.7±5.7 in women and 28.1±3 in men. There was no statistically significant difference between the total amount of gonadotropin used in the treatment process, sperm concentration, sperm morphology, total sperm motility, and progressive sperm motility in terms of both treatments ($p>0.05$) TableI.

Table I. Comparison of total gonadotropin and sperm parameters used in two consecutive trials in the study.

Parameters	Swim-up	MAC	*P* value
Total Gonadotropin	2405±73	2556,41±69	*P*=0.181
Sperm concentration	32.5±4.3	33.1±4.1	*P*=0.872
Total sperm movement	58.3±3	62.1±2.4	*P*=0.346
Progressive sperm movement	45.3±2.6	46.2±2.1	*P*=0.811
Sperm morphology (%)	1.2±0.2	1.1±0.4	*P*=0.121

Analysis was performed with the paired sample test ($p<0.05$ was considered statistically significant).

In our study, comparisons of total oocytes, MII oocytes, fertilized oocytes, third and fifth-day quality embryo counts, third and fifth-day frozen quality embryo counts of two consecutive trials are presented in Figure 2. There was no statistically significant difference between the numbers of total oocytes, MII oocytes, and embryos frozen on day three collected between the two groups ($p>0.05$). Significant differences were observed between the number of fertilized oocytes, the number of good quality embryos on the third day, the number of good quality embryos on the fifth day, and the number of frozen good quality embryos on the fifth day in the direction of the MAC technique (Figure 2).

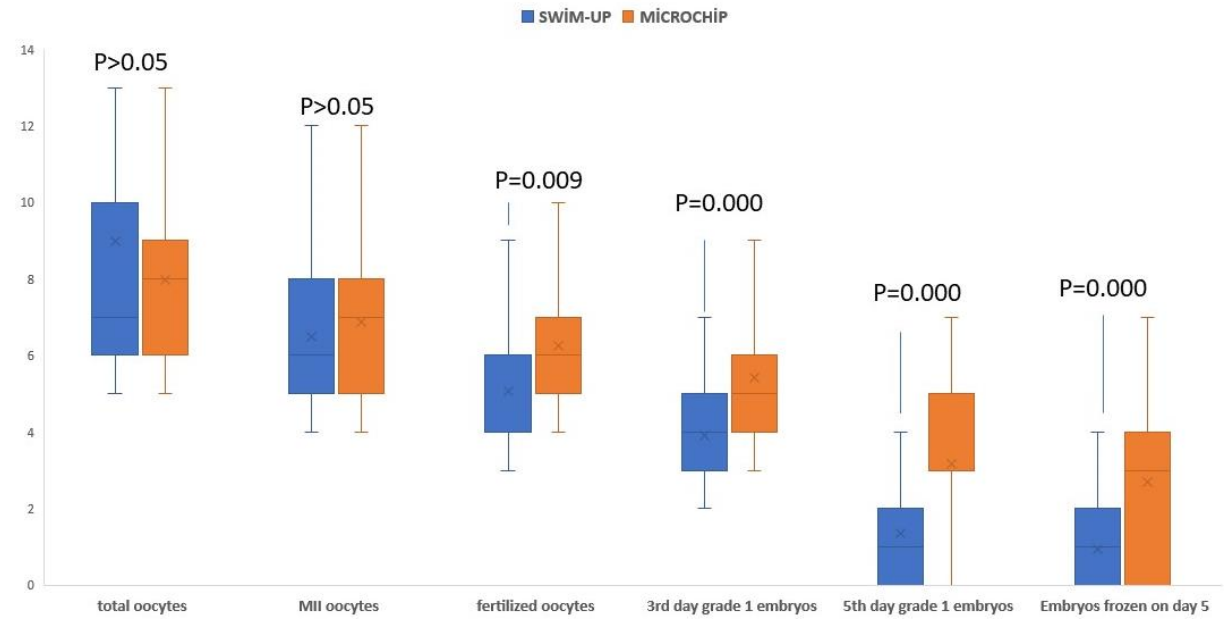

Figure 2. Comparison of oocyte and embryo values between groups.
There was no difference between the collected oocyte and MII oocyte numbers between the two groups. There were significant differences between the numbers of fertilized oocytes, 3rd and 5th day good quality embryos and 5th day frozen good quality embryos. Analysis was performed with the paired sample test ($p<0.05$ was considered statistically significant).

Since all embryos were frozen without embryo transfer in 6 patients in the SU group and seven patients in the MAC group, these patients were not included in the implantation and pregnancy evaluations. Implantation positivity was observed in 8 of 29 cases in the SU group. However, although pregnancy occurred in only one of them, it resulted in miscarriage in the following weeks. In the Mac method, implantation positivity was seen in 9 out of 28 cases. Among these cases, in cases prepared with microchip methods, pregnancy was positive in 6 of them, and pregnancy was observed in three. There was no statistically significant difference between implantation and pregnancy rates between the groups (P>0.05).

DISCUSSION

New technological developments have influenced ART. One of these new techniques is the MAC method, which imitates the physiological and biochemical environment of sperm and provides more opportunities for natural physiological sperm selection (15). In our study, we compared our patients' laboratory and clinical results whose sperm preparation method was SU in the first of two consecutive trials and whose sperm preparation method was MAC in the second trial. Different types of micro-chip types are used in ART treatments by the characteristics of sperm, such as motility and morphology. Our study used the type of sperm isolator according to sperm motility (16). The type used for the motility of the microchip is in principle similar to the SU technique in sperm separation. However, the microchip features such as long narrow channels and fluid flow differ from the SU method (17).

The contribution of microchip application to clinical outcomes is still not demonstrated (16). For these reasons, in our study, we examined the contribution of the microchip to implantation and clinical pregnancy outcomes in ART treatments, as well as to fertilization, obtaining quality embryos, the number of embryos on the fifth day, and the number of embryos frozen on the fifth day. Achieving a high fertilization rate and increasing the number of quality embryos in ART treatments are not dependent on a single cause or condition. Sperm preparation methods and good sperm isolation are important among the conditions that directly affect fertilization and obtaining quality embryos. There are very few studies investigating the effects of new technologies on embryo quality (6, 18, 19). It has been reported that sperm selection with the help of an inverted microscope causes an increase in the number of embryos of higher quality and ongoing pregnancies compared to the classical ICSI method (20). In our study, when we compared the MAC method, which is a recent innovation in sperm preparation method, and the SU method, which has been used conventionally for a long time; We observed that fertilization rates and the number of quality embryos on the third and fifth days increased with the MAC method. However, we observed no difference in implantation and clinical pregnancy rates. Similar to our study, only two studies in the literature compare and evaluate the contribution of MAC and SU methods to ART treatments (18, 19). Similar to our study, in one of these studies; It has been reported that the MAC method increases the number of quality embryos compared to the SU method and does not affect pregnancy outcomes (19). Unlike our study, it was reported that the Microchip method did not contribute to fertilization rates in this study (19). However, incomplete information was given about the days of the transferred embryos in the same study (19). In the other study, MAC and SU methods were compared, and they reported that they did not find any difference between laboratory and clinical findings in these two applications (18). However, in this study, it is understood that different periods are used in the sperm preparation process with the MAC method than the MAC manufacturer's working procedure (18). This may have affected the results of the study.

The current method for selecting high-quality embryos in embryology laboratories in vitro fertilization and embryo transfer (IVF-ET) practice is based on the quality criteria of day three embryos (21). This criterion is generally based on the number of cells, symmetry, and degree of fragmentation in the day three embryo after fertilization (22). According to this criterion, day three embryos containing eight blastomeres are preferably selected for fresh embryo transfers because a high rate of blastocyst formation and clinical pregnancy were associated (22). The advantages of this situation are that day five embryos have a lower risk of aneuploidy and a higher physiological synchronization with the endometrium (23, 24). In addition, the transfer of embryos at the blastocyst stage is hypothesized to prevent early exposure of the embryo to super physiological levels of estrogen and progesterone resulting from controlled ovarian stimulation(25). Besides these advantages, there are differences between the number of embryos obtained at the cleavage stage and between advancing the embryo culture and extending it to the blastocyst stage (25). Therefore, fewer embryos can be obtained because of the number of embryos remaining after transfer or in cycles extended to the blastocyst stage for all milled embryos since not every embryo can develop until the blastocyst stage. Therefore, there is still a persistent debate about whether to delay the transfer or advance embryo culture to the blastocyst stage (18). In our study, the blastocyst embryos obtained on the fifth day were higher in the MAC group than in the SU group. In a similar study, no significant difference was found between the embryos obtained at the blastocyst stage between the MAC and SU groups (18). Yetkinel et al.(19) obtained more quality embryos with the

MAC method than the SU method, but they did not give clear information about the embryo days. We think that the use of MAC will help select quality sperm and reduce the hesitations of advancing embryo culture to the blastocyst stage after fertilization. Due to the high embryo survival rates of the cryopreservation method in recent years, vitrification has been preferred over the slow-freezing method in embryo freezing-thawing processes (26). Fernandez-shaw et al.(25) reported that cumulative pregnancy rates in vitrified freeze-thaw cycles were significantly higher when blastocyst embryos were used than in cleavage stage embryos. When embryo transfer from fresh and thawed IVF cycles is evaluated, it has been reported that cumulative pregnancy rates are more successful in cases advanced to the blastocyst stage (23). In our study, after day 5 embryo transfer in fresh IVF cycles, more Grade 1 embryo were obtained in the MAC group than in the SU group. The resulting embryos were frozen for use in subsequent freeze-thaw cycles. Similar to our study, studies are reporting that more embryos are frozen with the MAC technique than with the SU technique after transfer (19). Unlike our study, there is also a study where it was reported that there was no difference between the number of frozen Day 5 embryos in the MAC and SU groups (18). Our study observed that the MAC method provided more Day 5 embryos to be used in Freeze-thaw cycles. Thus, we think that obtaining more quality embryos in ET with MAC or milling-all cycles may be beneficial in ART cycles.

In a study on sperm selection, it was reported that the importance of extending embryo development to the blastocyst stage should not be underestimated, as paternal effects are more decisive on the third day and beyond (24). The MAC method is thought to differentiate spermatozoa with higher DNA integrity (27). We think that this situation increases the blastocyst retrieval rates. In addition, it has been reported that the MAC method may be helpful to reduce the miscarriage rates, which are thought to occur due to high sperm DNA fragmentation (28, 29). In line with the information, we obtained in our study, we recommend the MAC method to infertile couples with male factor indications who have clinical difficulties in obtaining quality embryos. Although a very high pregnancy rate could not be obtained in the MAC method trials, we saw that the number of quality embryos obtained in the cycles using MAC increased considerably. Freezing of quality embryos has positive effects in ART trials. We think that frozen embryos will increase the number of pregnancies with appropriate endometrium and luteal phase support in obtaining pregnancy in later trials.

CONCLUSION

Our study observed that the MAC technique did not positively contribute to implantation and pregnancy outcomes in ART cycles performed due to the male factors, but it increased the fertilization rate, the number of quality embryos, and the number of frozen fifth-day embryos after ICSI. The higher number of frozen embryos will allow infertile couples to achieve pregnancy when the subsequent cycles are considered.

REFERENCES

1. Sakkas D, Ramalingam M, Garrido N, Barratt CL. Sperm selection in natural conception: what can we learn from Mother Nature to improve assisted reproduction outcomes? Hum Reprod Update. 2015;21(6):711-26.
2. Erdem A, Erdem M, Atmaca S, Korucuoglu U, Karabacak O. Factors affecting live birth rate in intrauterine insemination cycles with recombinant gonadotrophin stimulation. Reprod Biomed Online. 2008;17(2):199-206.
3. Zini A, Finelli A, Phang D, Jarvi K. Influence of semen processing technique on human sperm DNA integrity. Urology. 2000;56(6):1081-4.
4. Marchetti C, Obert G, Deffosez A, Formstecher P, Marchetti P. Study of mitochondrial membrane potential, reactive oxygen species, DNA fragmentation and cell viability by flow cytometry in human sperm. Hum Reprod. 2002;17(5):1257-65.
5. 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.
6. Ozcan P, Takmaz T, Yazici MGK, Alagoz OA, Yesiladali M, Sevket O, et al. Does the use of microfluidic sperm sorting for the sperm selection improve in vitro fertilization success rates in male factor infertility? J Obstet Gynaecol Res. 2021;47(1):382-8.
7. Asghar W, Velasco V, Kingsley JL, Shoukat MS, Shafiee H, Anchan RM, et al. Selection of functional human sperm with higher DNA integrity and fewer reactive oxygen species. Adv Healthc Mater. 2014;3(10):1671-9.
8. Zhang X, Khimji I, Gurkan UA, Safaee H, Catalano PN, Keles HO, et al. Lensless imaging for simultaneous microfluidic sperm monitoring and sorting. Lab Chip. 2011;11(15):2535-40.
9. Parrella A, Keating D, Cheung S, Xie P, Stewart JD, Rosenwaks Z, et al. A treatment approach for couples with disrupted sperm DNA integrity and recurrent ART failure. J Assist Reprod Genet. 2019;36(10):2057-66.

10. Samuel R, Feng H, Jafek A, Despain D, Jenkins T, Gale B. Microfluidic—based sperm sorting & analysis for treatment of male infertility. Translational Andrology and Urology. 2018:S336-S47.
11. World Health O. WHO laboratory manual for the examination and processing of human semen. 5th ed ed. Geneva: World Health Organization; 2010.
12. Mangoli E, Khalili MA, Talebi AR, Agha-Rahimi A, Soleimani M, Faramarzi A, et al. IMSI procedure improves clinical outcomes and embryo morphokinetics in patients with different aetiologies of male infertility. Andrologia. 2019;51(8):e13340.
13. Halvaei I, Khalili MA, Razi MH, Agha-Rahimi A, Nottola SA. Impact of different embryo loading techniques on pregnancy rates in in vitro fertlization/embryo transfer cycles. J Hum Reprod Sci. 2013;6(1):65-9.
14. Gardner DK, Schoolcraft WB. Culture and transfer of human blastocysts. Curr Opin Obstet Gynecol. 1999;11(3):307-11.
15. Lara-Cerrillo S, Ribas-Maynou J, Rosado-Iglesias C, Lacruz-Ruiz T, Benet J, García-Peiró A. Sperm selection during ICSI treatments reduces single- but not double-strand DNA break values compared to the semen sample. Journal of Assisted Reproduction and Genetics. 2021;38(5):1187-96.
16. Samuel R, Feng H, Jafek A, Despain D, Jenkins T, Gale B. Microfluidic-based sperm sorting & analysis for treatment of male infertility. Transl Androl Urol. 2018;7(Suppl 3):S336-s47.
17. Gode F, Bodur T, Gunturkun F, Gurbuz AS, Tamer B, Pala I, et al. Comparison of microfluid sperm sorting chip and density gradient methods for use in intrauterine insemination cycles. Fertil Steril. 2019;112(5):842-8.e1.
18. Yalcinkaya Kalyan E, Can Celik S, Okan O, Akdeniz G, Karabulut S, Caliskan E. Does a microfluidic chip for sperm sorting have a positive add-on effect on laboratory and clinical outcomes of intracytoplasmic sperm injection cycles? A sibling oocyte study. Andrologia. 2019;51(10):e13403.
19. Yetkinel S, Kilicdag EB, Aytac PC, Haydardedeoglu B, Simsek E, Cok T. Effects of the microfluidic chip technique in sperm selection for intracytoplasmic sperm injection for unexplained infertility: a prospective, randomized controlled trial. J Assist Reprod Genet. 2019;36(3):403-9.
20. Gianaroli L, Magli MC, Ferraretti AP, Crippa A, Lappi M, Capitani S, et al. Birefringence characteristics in sperm heads allow for the selection of reacted spermatozoa for intracytoplasmic sperm injection. Fertil Steril. 2010;93(3):807-13.
21. Wang S, Ding L, Zhao X, Zhang N, Hu Y, Sun H. Embryo Selection for Single Embryo Transfer on Day 3 Based on Combination of Cleavage Patterns and Timing Parameters in in Vitro Fertilization Patients. J Reprod Med. 2016;61(5-6):254-62.
22. Awadalla M, Vestal N, McGinnis L, Ahmady A. Effect of Age and Morphology on Live Birth Rate After Cleavage Stage Embryo Transfer. Reprod Sci. 2021;28(1):43-51.
23. Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev. 2016(6):Cd002118.
24. Neyer A, Zintz M, Stecher A, Bach M, Wirleitner B, Zech NH, et al. The impact of paternal factors on cleavage stage and blastocyst development analyzed by time-lapse imaging-a retrospective observational study. Journal of assisted reproduction and genetics. 2015;32(11):1607-14.
25. Fernández-Shaw S, Cercas R, Braña C, Villas C, Pons I. Ongoing and cumulative pregnancy rate after cleavage-stage versus blastocyst-stage embryo transfer using vitrification for cryopreservation: Impact of age on the results. Journal of Assisted Reproduction and Genetics. 2015;32(2):177-84.
26. Wong KM, Mastenbroek S, Repping S. Cryopreservation of human embryos and its contribution to in vitro fertilization success rates. Fertility and Sterility. 2014;102(1):19-26.
27. Quinn MM, Jalalian L, Ribeiro S, Ona K, Demirci U, Cedars MI, et al. Microfluidic sorting selects sperm for clinical use with reduced DNA damage compared to density gradient centrifugation with swim-up in split semen samples. Hum Reprod. 2018;33(8):1388-93.
28. Carlini T, Paoli D, Pelloni M, Faja F, Dal Lago A, Lombardo F, et al. Sperm DNA fragmentation in Italian couples with recurrent pregnancy loss. Reprod Biomed Online. 2017;34(1):58-65.
29. Kumar K, Deka D, Singh A, Mitra DK, Vanitha BR, Dada R. Predictive value of DNA integrity analysis in idiopathic recurrent pregnancy loss following spontaneous conception. J Assist Reprod Genet. 2012;29(9):861-7.