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Improvement of embryo quality of infertile patients with in vitro fertilization failure using magnetic activated cell sorting for sperm selection

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Abstract

Magnetic-activated cell sorting (MACS) is an advanced sperm selection technique developed for patients with IVF failure related to sperm quality. For this reason, this study was conducted to show the efficiency, and selective power, of MACS associated with the double density gradient centrifugation technique (DGC) on sperm quality and embryological outcomes for 12 couples with unexplained IVF-ICSI failure. The group with DGC and MACS sperm treatment was considered the test group compared with the previous cycle of each DGC couple. The latter constitutes the control group. Spermogram-spermocytogram, embryological outcomes were evaluated for each group. Results showed that the MACS treatment significantly improved sperm morphology (2% vs. 5%) and the D3 and D5 embryo quality (52% and 25% vs. 47% and 6%) between the test group and the control group, respectively. With such selective power of MACS for couples with repeated IVF-ICSI failure doubling the embryo quality until the blastocyst stage, it is accurate to suggest that MACS could avoid micro-injecting spermatozoa with high DNA fragmentation or other masked abnormalities into the oocyte.

Keywords: Magnetic activated cell sorting, density gradient centrifugation technique, IVF-ICSI failure, spermatozoa.

Full length article MADKOUR Aicha, e-mail: a.madkour@um5r.ac.ma

1. Introduction

Infertility is the incapacity to conceive after one year of regular, unprotected intercourse without contraception, according to the World Health Organization [1]. Infertility affects more than 80 million couples who are childbearing age at a rate of 15% globally. In Morocco, infertility affects 11% of couples according to the investigation of the Moroccan society of medicine and reproduction [2], 20-35% of infertility is due to woman etiology, 20-30% due to man etiology, 25-40% due to both etiologies and 10% idiopathic etiology [3]. Therefore, many couples struggling with infertility are turning to assisted reproductive technologies as their therapy of choice. However, the success of assisted reproductive techniques (ART) is mostly dependent on sperm treatment in the laboratory [4]. Intracytoplasmic sperm injection (ICSI) is a procedure that consists of introducing a single sperm into an oocyte. Now, this technique is the in vitro fertilization (IVF) of choice, and this procedure regularly excludes traditional IVF, even in cases where there is no indication of ICSI and/or no male factor involved in the diagnosis [5]. However, particular sperm criteria, such as motility and morphology, do not reliably select the highest quality sperm. Moreover, sperm selection strategies evaluate sperm function and fertilization potential in order to maximize sperm quality and enhance ART results [4]. Nevertheless, the selection of sperm preparation and selection procedures should be appropriate for the couples' diagnosis and the sperm sample's quality. In order to maximize sperm

quality and enhance ART outcomes, sperm selection strategies increasingly depend on the evaluation of sperm function and sperm fertilization potential [4]. Our study aims to demonstrate the effectiveness of the MACS technique combined with the DGC, and its selective power on embryological outcomes for couples with repeated IVF-ICSI failures.

2. Materials and methods

2.1. Ethical Standards

The study was approved by the ethics committee of Faculty of Medecine and Pharmacy, University Hassan II, Casablanca, Morocco and patients provided written informed consent after being presented with the terms and issues of the study. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

2.2. Study design

The present study conducted over two years, was carried out at the Ghandi Fertility Center, Casablanca, Morocco. It included 68 couples who met the following inclusion criteria: at least one failed IVF attempt, unexplained male infertility, and the woman's age must not exceed 42 years. Moreover, the exclusion criteria adopted eliminated women with polycystic ovary syndrome (PCOS), endometriosis, and those with low ovarian reserve, which limited the number to 12 couple, the patients did not undergo any specific treatment. After being informed of the study's terms and issues, all 12 participants signed an informed consent. Indeed, this study is focused on embryological outcomes after two different systems of sperm selection: DGC alone as control for the first attempt with IVF failure then adding to DGC the MACS technic for the second attempt of the same couple to avoid the impact of bias. Subsequently, the oocytes of the first attempt patients were injected with spermatozoa treated with DGC alone (control group), and in the second attempt, after fertilization failure or having poor quality embryos in the first, the oocytes of the patients were injected with spermatozoa treated with DGC-MACS (test group). Then, the day 3 (D3) and day 5 (D5) embryos of each group were evaluated according to the Gardner classification [9] (Figure 1).

2.2.1. Sperm preparation with DGC and/or MACS

The sperm sample was obtained by masturbation after 3-5 days of sexual abstinence into sterile, non-toxic plastic vials, which were incubated for 30 minutes to facilitate liquefaction. In the control group, the sperm was treated by double density gradient centrifugation (DGC) (PureSperm: Nidacon, International AB, Gothenburg, Sweden). All samples were loaded onto a 40% and 80% discontinuous gradient and centrifuged at 1600 rpm for 20 min. The resulting pellets were washed with 2 ml of HTF, Irvine Scientific, Santa Ana, CA, USA, and centrifuged at 1200 rpm for 10 min. For the test group, sperm were treated with DGC and then with MACS® ART Annexin V System (Miltenyi Biotec), following the manufacturer's instructions. The resulting DGC pellet was incubated with 100 µl of MACS ART Annexin V reagent and 400 µl of MACS ART Binding Buffer for 15 min at room temperature. After rinsing the column with 1000 µl of MACS ART binding buffer, the sperm suspension was added to the column with 500 µl of MACS ART binding buffer, and thus the annexin fraction was obtained.

2.2.2. IVF-ICSI program

All included patients underwent the same antagonist protocol using r-FSH (Cetrotide 0.25 and Gonal-F) adjusted according to the usual parameters of follicle growth evaluated by serum estradiol levels and ultrasound monitoring. After reaching follicles ≥17 mm, human chorionic gonadotrophin (HCG, Ovitrelle) at 10000 IU (IM) and at 35-36h post-trigger, the oocytes were pick-up. Then, they are denuded by hyaluronidase (Cumulase), and their maturation is evaluated before ICSI. Under microscope ICSI, the spermatozoa after treatment (DGC or DGC and MACS) were injected by pipette into the mature oocyte, then incubated in microdroplets of 50µl Medium culture (Sage) of each box at 37°C, 5% CO2 and 5% O2. Thereafter, fertilization was confirmed by observing the second polar body expelled and two pronuclei (2PN) in the oocyte cytoplasm. Embryos are kept in culture until the D3, and their quality is evaluated, then kept in culture until D5 to evaluate the blastulation rate and its quality. Indeed, the classification of different obtained blastocysts at day 5 were evaluated morphologically according to Gardner Score (Gardner et al., 2016) [6]. The different resulting embryos were cryopreserved for the next cycle for embryo transfer.

2.3. Statistical analysis

The mean, standard deviation (SD), or percentage of the total are used to present data. Statistical Package, version 6.0 (Statistica), was used for data analysis. The Student's t-test was used to compare mean values, and the chi-squared test was used to compare percentages.

3. Results

The study involved 12 couples whose oocyte factor was insignificant, excluding the female impact on the results. Table 1 shows that men's and women's age, AMH, and sperm parameters (concentration, mobility) were compared in the control group (DGC only) and the test group (MACS-DGC), which does not detect any significant differences, whereas a significant difference was observed in sperm morphology (0.02 ± 0.01 against 0.05 ± 0.01; p-value 0.002), between DGC alone and DGC-MACS respectively. In addition, the number of oocytes retrieved (9.33 ± 2.72 versus 8.17 ± 2.03; p 0.36) and injected (6.92 ± 3.06 versus 6.25 ± 1.50; p 0.61) was not significant between the control group and the MACS - DGC group, respectively (p> 0.05). ICSI results such as fertilization, cleavage, embryo quality, and implantation rate were compared between the control and the test groups. As shown in Figure 2, the fertilization rates in the test, and the control groups, were not significantly different (0.67 ± 0.24 vs. 0.67 ± 0.22; p 0.59), respectively. In contrast, the embryonic quality D3 and D5 was significantly higher in the test group (0.53 ± 0.25 / 0.48 ± 0.27) compared to the control group (0.25 ± 0.27 / 0.06 ± 0.10), with p-value (0.04 / 0.01) respectively.

Table 1. Results of sperm parameters in Control group compared to Test group using DGC with MACS.

Parameters	Test group	Control group	p-value
Sperm concentration (M/ml)	50.15±50.83	31.18±27.54	0.43 (ns)
Sperm motility (%)	52%±0.26	55%±0.23	0.83 (ns)
Sperm morphology (%)	**5%±0.01**	**2%±0.01**	**0.002 (s)**

Results are presented as the mean n or the percentage (n%) ± standard deviation (SD). When p 0.05, a P-value is significant (s). The control group is including patients with DGC as sperm treatment while the Test group is representing DGC with MACS.

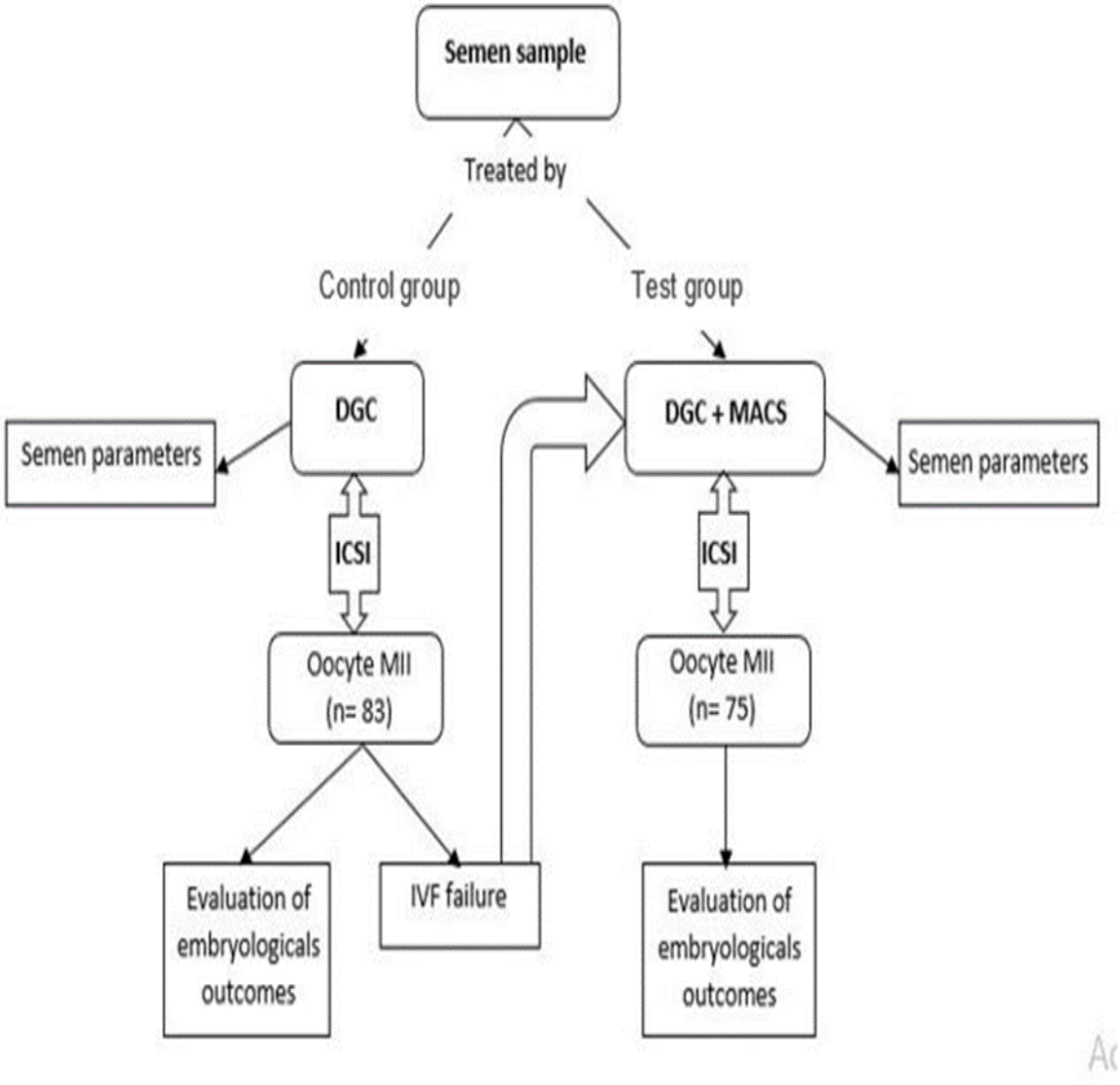

Figure 1: Study design

IVF failure: fertilization failure, poor embryo quality, prolonged culture failure (no blastocyst), or even more, no pregnancy

Table 2: Comparison of different patient characteristics between test and control groups

Parameters	Test group	Control group	P-value
Female age (years) (n)	34.42 ±3,25	34.42 ±3,25	1.00 (ns)
Male age (years) (n)	44.42±6.99	44.42±6.99	1.00 (ns)
AMH (ng/ml) (n)	3.94±1.58	3.94±1.58	1.00 (ns)
Oocytes number per patient (n)	8.17±2.03	9.33±2.72	+0.40 (ns)
MII per patient (n)	6.25±1.50	6.92±3.06	0.61 (ns)
Maturation rate (n %)	78%±0.14	72%±0.14	0.43 (ns)

Results are presented as the mean n or the percentage (n%) ± standard deviation (SD). When p 0.05, a P-value is significant (s). The control group is including patients with DGC as sperm treatment while the Test group is representing DGC with MACS

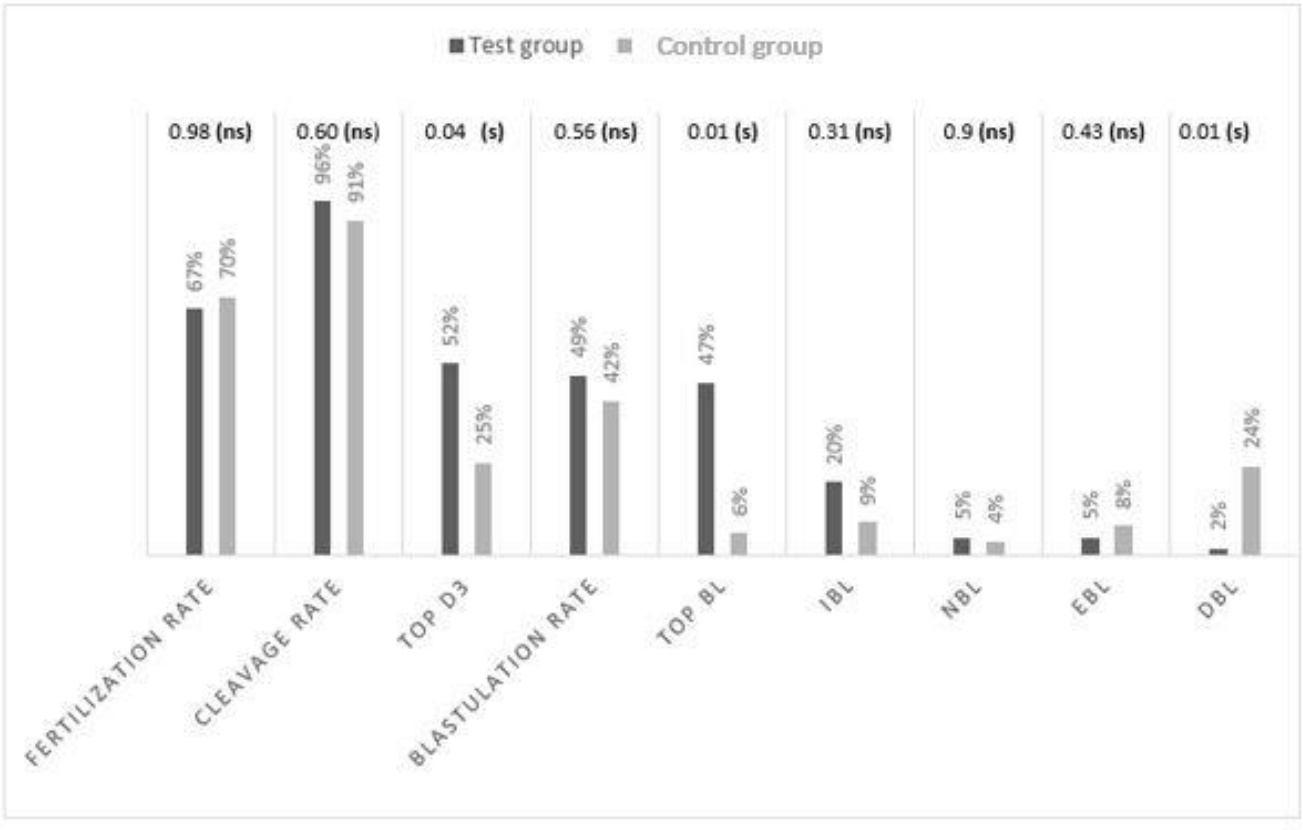

Figure 2: Comparison of embryological outcomes between of test and control groups, fertilizaton rate, cleavage rate, good quality of embryos Day 3 rate, blastulation rate, intermediate blastocyst (IBL) rate, non-top quality of blastocyst (NBL) rate, early blastulation (EBL) rate and delayed blastulation (DBL) rate.

Results are expressed as percentage (n %). P-value is significant when $p<0.05$ (s) and not significant (ns) when $p\geq0.05$

4. Discussion

In this research, we studied the cleavage rate, blastulation, and embryo quality associated with two sperm preparation methods, density gradient centrifugation (DGC) alone and density gradient centrifugation associated with the magnetically activated cell sorting technique (DGC-MACS). Sperm can have fragmented DNA, as well as impaired integrity of their membranes, despite normal appearance and motility. Using these sperm in assisted reproduction can adversely affect the results [5]. Successful fertilization requires, among other things, a sperm plasma membrane with normal integrity and function [7-10]. Density gradient centrifugation selects sperm cells based on their motility. However, apoptotic sperm cannot be removed by this technique, unlike magnetic cell sorting (MACS) with annexin V-conjugated microbeads [11]. Phospholipid-binding protein Annexin V has a high affinity for phosphatidylserine (PS) and is Ca2+-dependent. In fact, MACS conjugated with annexin V, focuses on spermatozoa with damaged membranes due to PS externalization to the outer membrane leaflet [12]. In other words, MACS works at the molecular level of the sperm [10]. By deducing from these data, different studies have proposed to combine the MACS technique with the DGC technique to prepare human spermatozoa for assisted reproduction techniques [13]. An earlier study Lukaszuk et al. 2015, demonstrated that the selection of viable spermatozoa with high motility and lower expression of apoptotic markers leads to better results when DGC and MACS techniques are combined [14]. This combination is better than other approaches for decreasing the proportion of apoptotic sperm following sperm preparation [10]. Our results showed (Table 2) that the morphology of the spermatozoa is improved after selection by the two combined techniques DGC-MACS. The subpopulations of non-apoptotic spermatozoa selected by the MACS annexin V technique have a significantly higher proportion of spermatozoa with normal morphology and SDI scores and significantly lower percentages of spermatozoa with acrosomal defects, in agreement with Aziz et al. (2007) study [15]. The results in figures 2 showed that the DGC-MACS combination enabled the selection of good quality D3 embryos. Indeed, paternal DNA abnormalities impact the quality of the embryo [16, 17]. The increase in sperm DNA damage is associated with a considerable decrease in the percentage of good quality embryos and an increase in the percentage of low-quality embryos on day 3 up to the blastocyst [18, 19, 20]. DNA fragmentation may activate dedicated additional DNA repairs and embryo development could be delayed, resulting in poor embryo quality [21, 22]. It should be noted that the particularity of our study is based on the comparison of the results of the same couple on two different cycles. It is true that the diagnosis of male infertility is based on the detection of sperm abnormalities. However, cases of normal sperm can occur [23]. 15% of men with infertility problems were classified with normozoospermia [24]. The MACS technique can circumvent unexpected fertilization failure and poor embryo quality with normozoospermia. In addition, the success of the fertility process and the development of the embryo depend in part on the integrity of the DNA of the sperm and good practice [25]. The control group provided D3 embryos and blastocysts of poor quality compared to the test group. This is probably due to the selection of non-apoptotic sperm using the MACS Annexin V technique (Figure 2). Indeed, it is reported that oocytes can repair damaged sperm DNA up to a threshold beyond which damaged sperm DNA seems irreparable and could negatively impact embryos [23].

5. Conclusions

The selection of human sperm by the DGC-MACS technique improve sperm morphology and the quality of day 3 embryos and blastocysts can be an interesting alternative to address unexplained infertility problems. This improvement observed could be linked to better elimination of apoptotic spermatozoa by the DGC-MACS technique. However, further studies on sperm are needed, including chromatin decondensation and DNA fragmentation, knowing that exposure to PS could be due to reasons other than apoptosis. This study deserves to be deepened with a larger cohort and more relevant evaluation criteria to improve and promote its success rates.

List of abbreviations

ART, assisted reproductive technologies; DBL, delayed blastulation rate; D3,Day 3; D5, Day 5; DGC, density gradient concentration; EBL, early blastulation rate; IBL, intermediate blastocyst rate; ICSI, intracytoplasmic spermatozoa injection; IVF, in vitro fertilization; MACS, Magnetic-activated cell sorting; NBL, non-top quality of blastocyst rate.

Funding Statement

There is no funding for the project.

Declaration of Interest Statement

Elmoutabi Kawtar, Madkour Aicha, Taghzouti Khalid, Messy Narjiss, Defort Naomi, Naitouahmane Fatima, Bennis Mohamed, Bennis Faiza, Blaghen Mohamed, Chegdani Fatima declare that they have no conflict of interest.

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References

[1] World Health Organization. (2021). WHO laboratory manual for the examination and processing of human semen. 6e éd. Geneva

[2] Madkour, A., Kaarouch, I., & Bouamoud, N. (2019). Immunologie de l'infertilité féminine en Fécondation In Vitro: Aspects immunitaires de l'infertilité féminine au stade pré et post-embryonnaire en Assistance Médicale à la Procréation. Éditions universitaires européennes.

[3] S. El Adlani, A. Benksim, Y.A.B. Kaddour, A. Soummani, M. Cherkaoui. (2021). Infertility: knowledge and attitudes of Moroccan young people—gender approach. Middle East Fertility Society Journal. 26(1): 1-5. https://doi.org/10.1186/s43043-021-00060-9.

[4] A. Mehta, M. Sigman. (2014). Identification and preparation of sperm for ART. Urologic Clinics. 41(1): 169-180.

[5] M. Simopoulou, L. Gkoles, P. Bakas, P. Giannelou, T. Kalampokas, K. Pantos, M. Koutsilieris. (2016). Improving ICSI: A review from the spermatozoon perspective. Systems biology in reproductive medicine. 62(6): 359-371..doi : 10.1080/19396368.2016.1229365.

[6] D.K. Gardner, B. Balaban. (2016). Assessment of human embryo development using morphological criteria in an era of time-lapse, algorithms and 'OMICS': is looking good still important? MHR: Basic science of reproductive medicine. 22(10): 704-718..doi : 10.1093/molehr/gaw057

[7] E.K. Dirican, O.D. Özgün, S. Akarsu, K.O. Akın, Ö. Ercan, M. Uğurlu, Ç. Çamsarı, O. Kanyılmaz, A. Kaya, A. Ünsal. (2008). Clinical outcome of magnetic activated cell sorting of non-apoptotic spermatozoa before density gradient centrifugation for assisted reproduction. Journal of assisted reproduction and genetics. 25: 375-381. https://doi.org/10.1007/s10815-008-9250-1.

[8] A. Sheikhi, M. Jalali, M. Gholamian, A. Jafarzadeh, S. Jannati, N. Mousavifar. (2013). Elimination of apoptotic spermatozoa by magnetic-activated cell sorting improves the fertilization rate of couples treated with ICSI procedure. Andrology. 1(6): 845-849.
doi : 10.1111/j.2047-2927.2013.00140.x.

[9] I. Vermes, C. Haanen, H. Steffens-Nakken, C. Reutellingsperger. (1995). A novel assay for apoptosis flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled annexin V. Journal of immunological methods. 184(1): 39-51.
doi : 10.1016/0022-1759(95)00072-i.

[10] M. Tavalaee, M. Deemeh, M. Arbabian, M. Nasr-Esfahani. (2012). Density gradient centrifugation before or after magnetic-activated cell sorting: which technique is more useful for clinical sperm selection? Journal of assisted reproduction and genetics. 29: 31-38.
doi : 10.1007/s10815-011-9686-6.

[11] K. Lukaszuk, M. Wcislo, J. Liss, A. Stachowicz, G. Jakiel, M. Lukaszuk, E. Pastuszek, I. Woclawek-Potocka, A. Galvao, D. Bialobrzeska. (2015). First pregnancy, somatic and psychological status of a 4-year-old child born following Annexin V TESA sperm separation. American Journal of Perinatology Reports. 5(02): e105-e108.
doi : 10.1055/s-0035-1548726.

[12] N. Aziz, T. Said, U. Paasch, A. Agarwal. (2007). The relationship between human sperm apoptosis, morphology and the sperm deformity index. Human reproduction. 22(5): 1413-1419.
doi: 10.1093/humrep/dem016.

[13] M. Benchaib, J. Lornage, C. Mazoyer, H. Lejeune, B. Salle, J.F. Guerin. (2007). Sperm deoxyribonucleic acid fragmentation as a prognostic indicator of assisted reproductive technology outcome. Fertility and sterility. 87(1): 93-100.
doi : 10.1016/j.fertnstert.2006.05.057.

[14] L. Simon, G. Brunborg, M. Stevenson, D. Lutton, J. McManus, S.E. Lewis. (2010). Clinical significance of sperm DNA damage in assisted reproduction outcome. Human reproduction. 25(7): 1594-1608.
doi : 10.1093/humrep/deq103.

[15] L. Simon, K. Murphy, M. Shamsi, L. Liu, B. Emery, K. Aston, J. Hotaling, D. Carrell. (2014). Paternal influence of sperm DNA integrity on early embryonic development. Human reproduction. 29(11): 2402-2412.
doi : 10.1093/humrep/deu228

[16] M. Ivec, B. Kovacic, V. Vlaisavljevic. (2011). Prediction of human blastocyst development from morulas with delayed and/or incomplete compaction. Fertility and sterility. 96(6): 1473-1478. e2.
doi: 10.1016/j.fertnstert.2011.09.015.

[17] D. Wells, M. Bermudez, N. Steuerwald, A. Thornhill, D. Walker, H. Malter, J. Delhanty, J. Cohen. (2005). Expression of genes regulating chromosome segregation, the cell cycle and apoptosis during human preimplantation development. Human reproduction. 20(5): 1339-1348.
https://doi.org/10.1093/humrep/deh778.

[18] M. Bazrgar, H. Gourabi, P.E. Yazdi, H. Vazirinasab, M. Fakhri, F. Hassani, M.R. Valojerdi. (2014). DNA repair signalling pathway genes are overexpressed in poor-quality pre-implantation human embryos with complex aneuploidy. European Journal of Obstetrics & Gynecology and Reproductive Biology. 175: 152-156.
doi : 10.1016/j.ejogrb.2014.01.010.

[19] C. Avendaño, A. Franchi, H. Duran, S. Oehninger. (2010). DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. Fertility and sterility. 94(2): 549-557.
doi : 10.1016/j.fertnstert.2009.02.050.

[20] S.M. Kim, S.K. Kim, B.C. Jee, S.H. Kim. (2019). Effect of sperm DNA fragmentation on embryo quality in normal responder women in in vitro fertilization and intracytoplasmic sperm injection. Yonsei Medical Journal. 60(5): 461-466.
doi : 10.3349/ymj.2019.60.5.461.

[21] A. Agarwal, S. Allamaneni. (2004). The effect of sperm DNA damage on assisted reproduction outcomes. Minerva Ginecol. 56(3): 235-245.

[22] G. Anifandis, T. Bounartzi, C. Messini, K. Dafopoulos, R. Markandona, S. Sotiriou, A. Tzavella, I. Messinis. (2015). Sperm DNA fragmentation measured by H alosperm does not impact on embryo quality and ongoing pregnancy rates in IVF/ICSI treatments. Andrologia. 47(3): 295-302.
doi: 10.1111/and.12259.