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# Male Factor Infertility Outcomes Using Magnetic Activated Cell Sorting in Intra Citoplasmatic Sperm Injection Cycles

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## Abstract

Sperm DNA fragmentation can have negative consequences in clinical outcomes of couples undergoing Assisted Reproductive Technologies (ART). Sperm separation techniques are an important step in sperm selection for ART. The Magnetic Activated Cell Sorting (MACS) is a novel method that separates sperm by density gradient and molecular filtration to remove apoptotic sperm, which is associated to DNA damage. A decrease of DNA sperm fragmentation could improve ART outcomes. The main aim of this study was to assess the effect of MACS on fertilization, embryo development, implantation, clinical pregnancy and miscarriage rates, in couples undergoing intra cytoplasmic sperm injection (ICSI). Semen samples from 284 patients were divided in two groups; study group (n = 63) and control group (n = 221), analyzed by embryo transfer day (Day 3: ETD3 and day 5: ETD5) and male factor patients. Density gradients followed by MACS were used as sperm preparation method in the study group, while Swim up was the method used in the control group. Similar results were obtained between both groups for all parameters: fertilization rate of 77.18% versus 75.28%; blastulation rate of 46.66% versus 48.69%; implantation rate of 40.35% versus 35.52%; clinical pregnancy rate of 61.81% versus 59.31% and miscarriage rate of 2.94% versus 7.37%. However, statistical significant differences were found for implantation rate (study group 55.0% and control group 35.43%, p = 0.0138) in day 5 embryo transfers (ETD5). MACS technology does not improve general outcomes; however, it showed better results for ETD5. Further studies are required to identify real improvements in extended embryo culture in male infertility.

**Keywords:** Male infertility; Sperm DNA fragmentation; MACS; Sperm selection; ICSI; *In vitro* fertilization; Assisted reproductive technologies

## Introduction

One of the factors that has a great impact on the success of assisted reproductive technologies (ARTs) is sperm quality [1]. Kruger et al. [2] demonstrated the importance of sperm morphology in pregnancy rates of patients undergoing in vitro fertilization (IVF). However, with the introduction of intra cytoplasmic sperm injection (ICSI) [3], the majority of male factor cases were solved. Nevertheless, a significant number of unsuccessful cases of ARTs require new attempts before achieving pregnancy. In most of these cases sperm and/or oocyte quality could explain suboptimal results.

Indeed, one of the reasons of unsuccessful ICSI treatment cycles could be the use of apoptotic spermatozoa during ICSI [4,5]. Apoptosis is one of the mechanisms involved within the origin of sperm Deoxyribonucleic Acid (DNA) fragmentation. Spermatozoa which DNA damage show typical apoptosis-like features, such as phosphatidylserine (PS) translocation, Caspase-3 activation and decreased membrane mitochondrial potential [6-9]. Although, some studies have demonstrated that sperm with DNA damage maintain their fertilization capacity [10-12], other studies have identified a correlation with high levels of sperm DNA fragmentation and decreased fertilisation, implantation and pregnancy rates and an increased rate of spontaneous miscarriage [4,13-18].

Currently, there is clear evidence that normal spermatozoa used for ICSI can be chosen, having a negative impact on ARTs outcomes [19,20]. Several sperm preparation methodologies, such as swim-up and density gradient centrifugation, are currently useful for clinical purposes [21]. Overall, these methods aim to provide enough number of motile and viable sperms to reach the fertilization of the oocytes. However, these methods are based on migration and/or sedimentation of the spermatozoa, depending of their motility or density, without taking account of molecular features such as apoptosis and/or sperm DNA fragmentation. Therefore, the development of new reproductive technologies to allow better gamete selection is currently needed. Following this rationale, Magnetic Activated Cell Sorting-MACS [22] has been applied as a sperm preparation method to remove apoptotic cells using Annexin V [8,23,24]. Overall, MACS is a method to separate cells of interest from a mixed cell population. Their principle is the use of magnetic micro beads conjugated with specific antibodies or proteins to target cell's membranes. Then, a filtration column with a magnetic field of high power is used to retain cells that were targeted so that unbound cells maintain their initial pathway. In the case of Annexin V (AV), it is a phospholipid-binding protein which has high affinity to PS in physiological conditions. On the other hand, PS translocation from the cell inner to the outer leaflet on the plasma membrane is considered an early apoptotic marker. Thus, the use of MACS with AV removes spermatozoa with exposed PS, decreasing apoptosis-like cells to enrich sperm populations of better quality [25].

MACS utility has been widely studied over the last few years. Several studies show that MACS decreases apoptotic markers, such sperm DNA fragmentation, which increases fertilization potential and also sperm survival rates after cryopreservation [24-28]. However, few

clinical studies have been carried out using MACS sperm selection. Dirican et al. [29] found significant improvements in pregnancy rate and early developmental of gestation in oligoastenozoospermic patients undergoing ICSI in a prospective study. Moreover, two clinical reports have showed healthy born babies using MACS [30,31]. However, Romany et al. [32] showed an absence of significant differences for general reproductive outcome using MACS in an oocyte donation program (OD).

The positive effect of MACS sperm selection is still not clear, especially in patients with a male factor of infertility that could have increased apoptotic-like sperms. In this study, we aimed to compare the reproductive outcome of MACS with our standard sperm preparation method (swim up) in male factor and normozoospermic patients of selected subjects undergoing ICSI in a prospective manner, using selection criteria to avoid oocyte bias in male's couples.

## Subjects and Methods

This case-control study was performed in a prospective and comparative manner at the Andrology Laboratory and the Reproductive Medicine Laboratory, Unit of Reproductive Medicine, Clinica Las Condes, Santiago, Chile. Our endpoint was to compare fertilization, blastulation, implantation, pregnancy and miscarriage rates, as reproductive outcomes, for MACS sperm selection and Swim-up technique, as conventional method of sperm preparation in normozoospermic and male factor patients of selected couples undergoing ICSI treatment.

The inclusion criteria were couples with either primary or secondary infertility undergoing a first or second ICSI cycle. In the case of the female partner, women should be ≤37 years old and with a basal Follicle Stimulating Hormone (FSH) of ≤10 mIU/mL. On the other hand, normozoospermic and male factor patients (classified according to WHO Laboratory Manual, 2010) were also included. The exclusion criteria used were women with moderate to severe endometriosis, Polycystic Ovaries Syndrome (PCOS), Ovarian Hyperstimulation Syndrome (OHSS), women considered poor responders (classified as with ≤3 mature oocytes obtained in follicular aspiration) and women with more than 15 cumulus oocytes complexes (COC's) retrieved. Additionally, couples using cryopreserved sperm were also excluded of this study.

A total of 284 couples who met with the selection criteria were recruited and included in the study from November 2012 to December 2014. Sixty three couples were enrolled in the study group (MACS) and two hundred eighty four in the control group (Swim-up). Male partners were divided in four groups: A- normozooapermic using MACS: 32 patients; B- normozoospermic using Swim-up: 54 patients; C- male factors using MACS: 31 patients and D- male factors using Swim-up: 167 patients. Moreover, the effect of MACS sperm selection was also compared by embryo transfer day 3 (ETD3) and embryo transfer day 5/6 (EDT5/6) (Group E- day 3 embryo transfer with MACS: 29 subjects; Group F- day 3 embryo transfer with Swim-up: 116 patients; Group G- day 5 embryo transfer group with MACS: 21 subjects and H- day 5 embryo transfer with Swim-up: 70 patients).

In the MACS group, semen samples were prepared by a two layer discontinued, 80% and 40%, density gradient (Cook, Sydney IVF). The samples were centrifuged at 300 g for 15 minutes at room temperature (RT). Then, the pellets were washed twice for 5 minutes, first using wash media (Cook, Sydney IVF) and then 1x binding buffer (Binding buffer, Miltenyi Biotec). Then, the pellet was removed so that spermatozoa were incubated with annexin V-conjugated microbeads (Miltenyi Biotec, Germany) for 20 min at RT under continuous stirring. One hundred microliters of microbeads were used for each 10 million separated cells. The mix of sperm and microbeads were loaded into a separation column (MS columns, Miltenyi Biotec), which was allocated into a magnet (MiniMACS; Miltenyi Biotec). The apoptotic spermatozoa were retained in the separation column, while the non-apoptotic spermatozoa were eluted through the column and collected in wash media, to then perform a final washing.

In the control group, all sperm samples were prepared by the Swim-up technique. Semen samples were diluted 1:1 with washing media (Cook, Sydney IVF) and centrifuged at 300 g for 5 min at RT. Then, the pellet was washed once by centrifugation for 5 min, allowing spermatozoa swim-up into 0.5 ml of wash media for 30 min at RT.

Controlled ovarian stimulation and monitoring,, follicular aspiration, ICSI, embryo culture and embryo transfer were performed as described in previous studies published by our group [33]. Several characteristics of the couples included in this study were compared between the study and control groups (Table 1): Female partner age, body mass index (BMI), basal levels of FSH, percentage of primary or secondary infertility, the number of metaphase II oocytes inseminated, the number of oocytes fertilized, fertilization rate, blastulation rate (total number of blastocyst embryos on day 5/6 total number of zygotes), the number of optimal embryos on day 3, as described elsewhere by our group [34], the number of optimal embryos on day 5/6 (all blastocysts either vitrified or transferred) and the mean number of embryos transferred.

| Characteristics | MACS | Control | P value |
|---|---|---|---|
| Number of cycles (n) | 63 | 221 | |
| Number transferred cycles (n) | 55 | 204 | |
| **Females** | | | |
| Age (years) | 34.24 ± 0.25 | 34.05 ± 0.167 | NS |
| BMI (kg/m$^2$) | 21.72 ± 0.31 | 21.99 ± 0.26 | NS |
| FSH levels (mIU/mL) | 6.229 ± 0.23 | 6.421 ± 0.13 | NS |
| Number metaphase II oocytes inseminated (Mean) | 7.794 ± 0.35 | 7.158 ± 0.16 | NS |
| Number of oocytes fertilized (Mean) | 6.016 ± 0.35 | 5.414 ± 0.14 | NS |
| **Males** | | | |
| Age (years) | 37.16 ± 0.63 | 36.55 ± 0.29 | NS |
| Primary/secondary infertility | | | |
| Primary infertility (%) | 78.95 | 80.19 | |
| Secondary infertility (%) | 21.05 | 19.81 | |
| NS: Not significant; ± standard error. | | | |

**Table 1**: Cycle characteristics between MACS study group and control group.

Fertilization, implantation, clinical pregnancy and miscarriage rates were compared between the study and control groups (A versus B and C versus D; E versus F and G versus H).

## Ethics Approval and Statistical Analysis

This study was approved by the ethic committee of Clinica Las Condes (PI2011-013), allowing the use of our data base of patients involved in it. Cycle features, embryo development and clinical outcomes were compared between groups applying unpaired T-student, Fisher exact and chi square tests using Gradpad Prism software. For all statistics a P value lower than 0.05 was considered statistically significant.

## Results

A total of 284 couples met our selection criteria, 63 of them were treated using MACS sperm selection and 221 through the use of Swim-up technique as controls (Table 1). There were no statistical differences for cycles features such as female age, BMI, FSH basal levels, the number of metaphase II oocytes inseminated, the number of oocytes fertilized and male age (Table 1) between the study (MACS) and control (Swim up) groups. Moreover, the percentage of primary infertility and secondary infertility were similar between both groups (Primary infertility; MACS: 78.95%; Control: 80.19%; Secondary infertility; MACS: 21.05%; Control: 18.81%). Optimal embryo quality on day 3 and day 5 was similar in the study and control groups, without statistical differences for embryos transfers, either on day 3 or day 5 (Table 2). Blastulation rate (embryos that developed until day five or six, achieving the blastocyst stage and were, either transferred or vitrified) was slightly higher in MACS group, however, the difference was not statistically significant (Table 2).

| Parameter | MACS | Control | P value |
|---|---|---|---|
| **Embryo quality on day 3** | | | |
| Number of embryo transfer on day 3 (n) | 29 | 116 | |
| Optimal quality embryos (mean) | 3.172 ± 0.30 | 3.405 ± 0.16 | NS |
| Embryos transferred on day 3 (mean) | 2.207 ± 0.09 | 2.078 ± 0.03 | NS |
| **Embryo quality on day 5/6** | | | |
| Number of embryo transfer on day 5/6 (n) | 21 | 70 | |
| Optimal quality embryos (mean) | 3.333 ± 0.38 | 3.200 ± 0.15 | NS |
| Embryos transferred on day 5 (mean) | 1.905 ± 0.06 | 1.814 ± 0.05 | NS |
| Blastulation rate (utilisation on D5, %) | 52.74 | 47.61 | NS |
| Embryos transferred total (mean) | 1.966 ± 0.08 | 1.903 ± 0.04 | NS |
| Values are numbers, means or proportions. NS: Not significant; ± standard error. | | | |

**Table 2**: Embryo quality by embryo transfer day between MACS and control group.

The general clinical outcomes showed slightly higher trends in the study group, but there were not statistical differences for any of the parameters assessed (Table 3). In the case of normozoospermic patients (group A versus group B), there were slightly higher implantation and pregnancy rates in the MACS group, however, not significant differences were found for all parameters assessed.

Moreover, male factor patients showed not significant differences and/or clear higher trends between either group C or group D. Nevertheless, the MACS group showed a decreased miscarriage rate if compared to control group, but this was also not statistically significant (Table 3).

| | MACS | Control | P value | Odds ratio |
|---|---|---|---|---|
| General outcomes (n) | 63 | 221 | | |
| Fertilization rate (%) | 77.18 | 75.28 | NS | 1.111 |
| Implantation rate (%) | 40.35 | 35.52 | NS | 1.228 |
| Pregnancy rate (%) | 61.81 | 59.31 | NS | 1.111 |
| Miscarriage rate (%) | 2.94 | 7.37 | NS | 0.381 |
| Embryos transferred (mean) | 1.966 ± 0.08 | 1.903 ± 0.04 | NS | |
| **Normozoospermic group outcomes (n)** | **Group A (32)** | **Group B (54)** | | |
| Fertilization rate (%) | 77.63 | 80.39 | NS | 0.847 |
| Implantation rate (%) | 40.32 | 27.72 | NS | 1.762 |
| Pregnancy rate (%) | 62.06 | 52.94 | NS | 1.455 |
| Miscarriage rate (%) | 5.88 | 3.7 | NS | 1.625 |
| Embryos transferred (mean) | 2.067 ± 0.11 | 1.942 ± 0.06 | NS | |
| **Male factor group outcomes (n)** | **Group C (31)** | **Group D (167)** | | |
| Fertilization rate (%) | 76.77 | 73.53 | NS | 1.189 |
| Implantation rate (%) | 40.38 | 38.06 | NS | 1.102 |
| Pregnancy rate (%) | 61.53 | 62.09 | NS | 0.977 |
| Miscarriage rate (%) | 0 | 7.36 | NS | 0.358 |
| Embryos transferred (mean) | 1.857 ± 0.12 | 1.890 ± 0.049 | NS | |
| Values are numbers, means or proportions. NS: Not significant; ± standard error. | | | | |

**Table 3:** Reproductive clinical outcomes; general, male factor and normozoospermic outcomes between MACS and control group.

On the another hand, the reproductive clinical outcomes by embryo transfer day between MACS and the control groups showed similar results in the case of EDT3 (group E versus group F) (Table 4). However, implantation rate on EDT5/6 was significantly higher using MACS if compared with Swim up sperm selection (Group G: 55.5%; Group H: 35.43%; P <0.05) (Table 4). Additionally, it was also analyzed the embryos transferred for each group, which showed similar results for each group studied.

## Discussion

This study assessed the effect of MACS sperm selection for male factor and normozoospermic patients undergoing ICSI, in terms of clinical reproductive outcomes, such as fertilization, blastulation, implantation, pregnancy and miscarriage rates. In order to avoid the possible bias associated with oocyte quality a strict inclusion and exclusion criteria were used, removing infertility female factors from the study.

| | MACS | Control | P value | Odds ratio |
|---|---|---|---|---|
| **Embryo transfer day 3 outcomes (n)** | Group E (29) | Group F (116) | | |
| Fertilization rate (%) | 76.1 | 75.15 | NS | 1.053 |
| Implantation rate (%) | 29.68 | 37.75 | NS | 0.696 |
| Pregnancy rate (%) | 51.72 | 60.86 | NS | 0.689 |
| Miscarriage rate (%) | 0 | 5.71 | NS | 0.4767 |
| Embryos transferred on day 3 (mean) | 2.207 ± 0.09 | 2.078 ± 0.03 | NS | |
| **Embryo transfer day 5/6 outcomes (n)** | Group G (21) | Group H (70) | | |
| Fertilization rate (%) | 82.87 | 80.41 | NS | 1.178 |
| Implantation rate (%) | 55 | 35.43 | *0.0138 | 2.227 |
| Pregnancy rate (%) | 61.9 | 58.57 | NS | 1.149 |
| Miscarriage rate (%) | 0 | 9.75 | NS | 0.287 |
| Embryos transferred on day 5/6 (mean) | 1.905 ± 0.06 | 1.814 ± 0.05 | NS | |

Values are numbers, means or proportions; NS: Not significant; *Significant statistical differences; ± standard error.

**Table 4:** Clinical outcomes by embryo transfer day between MACS and control group.

In clinical terms, few studies have been carried out to date in ICSI cycles. The first prospective study was performed by Dirican et al. comparing fertilization, cleavage, implantation and pregnancy rates between density gradients and MACS as sperm preparation methods for ICSI cycles in oligoasthenozospermic patients [29]. Interestingly, this group found higher cleavage and pregnancy rates, using MACS as sperm selection method. Recently, Romany et al. has reported the first prospective randomized triple blinded study comparing embryo quality, fertilization, implantation, pregnancy, and live-birth rates in patients in which ICSI was performed as part of an ovum donation (OD) program [32]. However, this group did not found statistical differences in all parameters assessed using MACS as sperm selection method. Other studies have reported newborns, suggesting that MACS sperm selection is a safe method for couples with repeated ICSI failure [30,31]. Our study is the first prospective study in male factor infertility and normozoospermic patients undergoing ICSI cycles, comparing clinical outcomes. Our findings differ with the results reported by Dirican et al. but have similarities with those reported by Romany et al. study. In contrast with our original hypothesis, the effect of MACS on male factor infertility patients did not show different results if compared with normozoospermic patients, showing similar trends in overall clinical results. On the contrary hand, we founded an improved implantation rate on EDT5 in the MACS group. Moreover, in terms of optimal embryo quality, the removal of apoptotic sperms through MACS slightly enhanced the blastulation rate on EDT5, which could explain these higher trends in the MACS groups. However, in order to identify whether these outcomes are statistically significant or not, a total of 1188 patients are required (1-α = 95%; β = 80%; p1 = 48%; p2 = 53%). Thus, to confirm the effect of MACS, further randomized controlled trial with a greater number of patients is required. The design of these studies is crucial, especially considering the study groups, because all current clinical studies have been focused in different groups of patients.

MACS sperm selection can specifically remove spermatozoa cells with damaged membranes that show PS externalization as an expression of apoptosis. Considering this fact, this technique can complement the conventional routine techniques through a molecular selection instead of just sorting sperm according motility and density [27]. Moreover, better sperm quality for ART can be obtained if spermatozoa with apoptotic features are removed by MACS, if compared with conventional protocols [35,36]. However, it is important to notice that there are limitations involved in this technique. In case of severe oligozoospermic patients, it is not always possible their use, particularly in counts lower than 1 million/ml and severe oligoasthenozoospermia (progressive motility lower than 32%). Moreover, this technique works over sperm populations removing apoptotic cells, therefore, embryologists still have the chance to select spermatozoa with DNA fragmentation that were not removed before ICSI. On the other hand, there is also evidence that acrosome-reacted sperm, which can be sperm without DNA fragmentation, could be labeled and removed using MACS due to exposition of the inner leaflet membrane [37].

Although MACS sperm selection has few technical limitations, in our experience, we conclude that MACS after density gradients is a safe and suitable technique for selection of quality spermatozoa due to comparable outcomes with standard methods routinely used for ART. However, severe oligoasthenozoospermic patients could not be included for practical reasons, thus, this technique cannot be applicable easily for this group of patients that can make a difference in a male factor infertility group.

Oocytes fertilized by sperms with DNA damage still have developmental potential due to DNA repair capacity of the oocyte, which is able to repair DNA strand breaks before the initiation of the first cleavage in the early embryo development [38]. However, the capacity of the oocyte machinery to repair DNA damage depends of various factors, such as the level of sperm DNA damage, type of damage and the integrity of the functional repair machinery in the oocyte. This aspect of oocyte function has been poorly studied. DNA repair by the oocyte is believed to rely on the maternal repair pathway of messenger Ribonucleic acids (mRNAs) that are stored in the oocyte before ovulation. However, previous studies suggest that with increasing maternal and/or oocyte age these stored mRNAs decrease resulting in a decrease in the efficiency of DNA repair and negative downstream effects on embryo development [38,39]. The ability to repair DNA damage could be more effective in younger than older women. Actually, the higher implantation rates and differences in optimal embryo quality observed on day 5/6 after MACS could be explained because this was a group with patients with higher quality oocytes. Indeed, it has been previously described that sperm DNA fragmentation effect depends on oocyte quality [40]. On the another

hand, Jin et al. [41] have found that sperm DNA damage could have a higher impact on patients with low ovarian reserves (older oocytes) undergoing ARTs.

The potential benefits of the use of MACS remain to be determined. In fact, this method may positively enhance results in specific group of patients [32]. However, particularly in the study of sperm selection for ICSI, it is important to understand the possible bias in the results if damaged sperm are selected, regardless of whether or not a method as MACS has been applied. Indeed, there are reports showing improvements in terms of sperm function tests [36] and reproductive outcomes [42] when MACS was applied before intra uterine insemination (IUI) procedures.

We developed this study to identify possible benefits of MACS sperm selection in specific a group such male factor patients, avoiding include female factor in the study group. However, we are aware of our limitations such as a lack of randomization and an absence of sperm DNA fragmentation assessment for the cohort of patients. Considering this point, sperm DNA fragmentation tests are complex, involving high cost technology and strict quality controls, elements that we could not be sure during the sample collection. Nevertheless, confirm patients with different levels of apoptosis and/or DNA fragmentation could definitely be beneficial for further clinical application and research. Moreover, it could really important to study specific group of patients taking account the oocyte repair capacity, especially in young women due to this sperm selection improvements may could benefit older women with decreased DNA repair capacity.

## Conclusion

The MACS sperm selection of human spermatozoa is a safe, simple and suitable method for sperm preparations for ICSI use in a clinical setting due to it shows comparable results with our standard routine methods. However, it did not show general outcomes improvements compared to our conventional sperm preparation method swim-up. Although the results of the present study suggest that there are no differences in reproductive outcomes for either male factor or normozoospermic patients we found differences in implantation rate on ETD5, that should be further studied in a controlled and randomized manner. Studies in certain specific group of patients should be carried out in order to identify benefits of novel sperm selection methods, especially considering potential benefits in women depending on age and their oocyte quality. Thus, further studies are required to identify real improvements in extended embryo culture and male infertility factor.

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## References

1. Ombelet W, Deblaere K, Bosmans E, Cox A, Jacobs P, et al. (2003) Semen quality and intrauterine insemination. Reprod Biomed Online 7: 485-492.
2. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, et al. (1987) New method of evaluating sperm morphology with predictive value for human in vitro fertilization. Urology 30: 248-251.
3. Palermo G, Joris H, Devroey P, Van Steirteghem AC (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340: 17-18.
4. Seli E, Gardner DK, Schoolcraft WB, Moffatt O, Sakkas D (2004) Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. Fertil Steril 82: 378-383.
5. 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.
6. 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.
7. Weng SL, Taylor SL, Morshedi M, Schuffner A, Duran EH, et al. (2002) Caspase activity and apoptotic markers in ejaculated human sperm. Mol Hum Reprod 8: 984-991.
8. Paasch U, Grunewald S, Fitzl G, Glander HJ (2003) Deterioration of plasma membrane is associated with activated caspases in human spermatozoa. J Androl 24: 246-252.
9. Paasch U, Grunewald S, Agarwal A, Glandera HJ (2004) Activation pattern of caspases in human spermatozoa. Fertil Steril 81 Suppl 1: 802-809.
10. Lopes S, Sun JG, Jurisicova A, Meriano J, Casper RF (1998) Sperm deoxyribonucleic acid fragmentation is increased in poor-quality semen samples and correlates with failed fertilization in intracytoplasmic sperm injection. Fertil Steril 69: 528-532.
11. Twigg JP, Irvine DS, Aitken RJ, (1998) Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection. Hum Reprod 13: 1864-1871.
12. Gandini L, Lombardo F, Paoli D, Caruso F, Eleuteri P, et al. (2004) Full-term pregnancies achieved with ICSI despite high levels of sperm chromatin damage. Hum Reprod 19: 1409-1417.
13. Carrell DT, Liu L, Peterson CM, Jones KP, Hatasaka HH, et al. (2003) Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. Arch Androl 49: 49-55.
14. Virro MR, Larson-Cook KL, Evenson DP (2004) Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. Fertil Steril 81: 1289-1295.
15. Lewis SE, Aitken RJ (2005) DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell Tissue Res 322: 33-41.
16. Aitken RJ, De Iuliis GN, McLachlan RI (2009) Biological and clinical significance of DNA damage in the male germ line. Int J Androl 32: 46-56.
17. Sakkas D, Alvarez JG (2010) Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril 93: 1027-1036.
18. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, et al. (2012) The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod 27: 2908-2917.
19. Aitken RJ, De Iuliis GN (2007) Origins and consequences of DNA damage in male germ cells. Reprod Biomed Online 14: 727-733.
20. Avendaño C, Franchi A, Duran H, Oehninger S (2010) DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. Fertil Steril 94: 549-557.
21. Henkel RR, Schill WB (2003) Sperm preparation for ART. Reprod Biol Endocrinol 1: 108.
22. Miltenyi S, Müller W, Weichel W, Radbruch A (1990) High gradient magnetic cell separation with MACS. Cytometry 11: 231-238.
23. Grunewald S, Paasch U, Glander HJ (2001) Enrichment of non-apoptotic human spermatozoa after cryopreservation by immunomagnetic cell sorting. Cell Tissue Bank 2: 127-133.
24. Paasch U, Sharma RK, Gupta AK, Grunewald S, Mascha EJ, et al. (2004) Cryopreservation and thawing is associated with varying extent of activation of apoptotic machinery in subsets of ejaculated human spermatozoa. Biol Reprod 71: 1828-1837.
25. Said TM, Grunewald S, Paasch U, Rasch M, Agarwal A, et al. (2005) Effects of magnetic-activated cell sorting on sperm motility and cryosurvival rates. Fertil Steril 83: 1442-1446.

26. Said TM, Grunewald S, Paasch U, Glander HJ, Baumann T, et al. (2005) Advantage of combining magnetic cell separation with sperm preparation techniques. Reprod Biomed Online 10: 740-746.
27. Said T, Agarwal A, Grunewald S, Rasch M, Baumann T, et al. (2006) Selection of nonapoptotic spermatozoa as a new tool for enhancing assisted reproduction outcomes: an in vitro model. Biol Reprod 74: 530-537.
28. Said TM, Agarwal A, Grunewald S, Rasch M, Glander HJ, et al. (2006) Evaluation of sperm recovery following annexin V magnetic-activated cell sorting separation. Reprod Biomed Online 13: 336-339.
29. Dirican EK, Ozgun OD, Akarsu S, Akin KO, Ercan O, et al. (2008) Clinical outcome of magnetic activated cell sorting of non-apoptotic spermatozoa before density gradient centrifugation for assisted reproduction. J Assist Reprod Genet 25: 375-381.
30. de Fried EP, Denaday F (2010) Single and twin ongoing pregnancies in two cases of previous ART failure after ICSI performed with sperm sorted using annexin V microbeads. Fertil Steril 94: 351.
31. Rawe VY, Boudri HU, Alvarez Sedó C, Carro M, Papier S, et al. (2010) Healthy baby born after reduction of sperm DNA fragmentation using cell sorting before ICSI. Reprod Biomed Online 20: 320-323.
32. Romany L, Garrido N, Motato Y, Aparicio B, Remohí J, et al. (2014) Removal of annexin V–positive sperm cells for intracytoplasmic sperm injection in ovum donation cycles does not improve reproductive outcome: a controlled and randomized trial in unselected males. Fertility and sterility 102: 1567-1575.
33. MacKenna A, Crosby J, Zegers-Hochschild F (2013) Embryo early cleavage, number of blastomeres and morphology at day three as factors to predict blastocyst development. JBRA Assisted Reproduction 17: 158-161.
34. MacKenna A, Crosby J, Zegers-Hochschild F (2013) Sibling embryo blastocyst development as a prognostic factor for the outcome of day-3 embryo transfer. Reprod Biomed Online 26: 486-490.
35. Makker K, Agarwal A, Sharma RK (2008) Magnetic activated cell sorting (MACS): utility in assisted reproduction. Indian J Exp Biol 46: 491-497.
36. 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.
37. Grunewald S, Paasch U (2013) Sperm selection for ICSI using annexin V. Methods Mol Biol 927: 257-262.
38. Hamatani T, Falco G, Carter MG, Akutsu H, Stagg CA, et al. (2004) Age-associated alteration of gene expression patterns in mouse oocytes. Hum Mol Genet 13: 2263-2278.
39. González-Marín C, Gosálvez J, Roy R (2012) Types, causes, detection and repair of DNA fragmentation in animal and human sperm cells. Int J Mol Sci 13: 14026-14052.
40. Meseguer M, Santiso R, Garrido N, García-Herrero S, Remohí J, et al. (2011) Effect of sperm DNA fragmentation on pregnancy outcome depends on oocyte quality. Fertil Steril 95: 124-128.
41. Jin J, Pan C, Fei Q, Ni W, Yang X, et al. (2015) Effect of sperm DNA fragmentation on the clinical outcomes for in vitro fertilization and intracytoplasmic sperm injection in women with different ovarian reserves. Fertil Steril 103: 910-916.
42. Romany L, Meseguer M, Garcia-Herrero S, Pellicer A, Garrido N (2010) Magnetic activated sorting selection (MACS) of non-apoptotic sperm (NAS) improves pregnancy rates in homologous intrauterine insemination (IUI). preliminary data. Fertility and sterility 94: S14.