Evaluation of seminal parameters in the indication of in vitro fertilization or intracytoplasmic sperm injection in the treatment of the male factor fertility problem

Avaliação dos parâmetros seminais na indicação da fertilização *in vitro* ou injeção intracitoplasmática de espermatozoide para o tratamento do fator masculino

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Resumo

A fertilização *in vitro* (FIV) tem se mostrado muito eficiente no tratamento de casais inférteis. Com o passar dos anos, a indicação da reprodução assistida para o tratamento do fator masculino cresceu muito, tornando necessárias novas técnicas de fertilização, como a Injeção intracitoplasmática de espermatozoide (ICSI). De acordo com a Organização Mundial da Saúde, os parâmetros espermáticos (pós-processamento) mínimos para realização da FIV convencional são: concentração $\geq 5 \times 10^6$ /mL, 30% de motilidade progressiva e 30% de morfologia normal. Para homens com parâmetros espermáticos inferiores a esses, a ICSI estaria indicada. Entretanto, esses valores são bastante questionados e não são seguidos em muitos serviços. Por isso, o objetivo deste trabalho foi revisar literatura, visando a analisar os parâmetros seminais na indicação da fertilização *in vitro* convencional ou ICSI para o tratamento do fator masculino. Após análise dos artigos, concluiu-se que pacientes com parâmetros seminais no limite entre indicação para FIV ou ICSI devem optar pelo segundo método para que seu tratamento seja eficiente. Por outro lado, além dos parâmetros espermáticos, outros fatores também são importantes para a escolha da técnica como, por exemplo: falha prévia de fertilização, qualidade oocitária e idade materna. Atualmente, a técnica de ICSI vem sendo utilizada indiscriminadamente. Porém, uma análise melhor dos parâmetros espermáticos e outros fatores são essenciais para a escolha ou não dessa técnica.

Unitermos: Fertilidade masculina; infertilidade; injeção intracitoplasmática de espermatozoide; fertilização *in vitro*.

Abstract

In vitro fertilization (IVF) has proven to be an effective treatment for infertile couples. Over the years, assisted reproduction to treat male factor has grown, making new reproduction techniques necessary. Thus, in July 1992, the first pregnancy following intracytoplasmic sperm injections (ICSI) was reported. In method, one spermatozoon is injected into the cytoplasm of the oocyte. According to the World Health Organization, the minimal concentration of spermatozoa in the semen, using conventional IVF, is $\geq 5 \times 10^6$ /mL, and more than 30% of motility and 30% of normal morphology. Men with lower spermatic parameters should be submitted to ICSI. However, these values have been questioned in the literature and many clinics do not follow these parameters. Therefore, this study aimed to review the literature on sperm parameters used in the indication of conventional *in vitro*

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fertilization or ICSI in the treatment of male factor. It was concluded that ISCI should be used in patients with seminal parameters within the boundary between indication for IVF or ICSI. On the other hand, this review concluded that other parameters are important to help in the decision-making about the use of ICSI or IFV, such as previous fertilization failure, egg quality and female age.

Uniterms: Male fertility; infertility; intracytoplasmic sperm injection; *in vitro* fertilization.

Introduction

Assisted human reproduction

Since the birth of Louise Brown in July 1978, *in vitro* fertilization (IVF) has proven to be an effective treatment for infertile couples[1]. Originally, IVF was indicated to the treatment of infertility associated with tubal factor, but over the years the indications were broadened to include cases of infertility associated with endometriosis, male infertility and even the treatment of couples without clinical apparent cause for infertility[2].

However, when this technique is indicated for the treatment of male factor infertility, the results based on seminal parameters (sperm concentration, morphology and motility) are less significant and sometimes even disappointing. Therefore, new fertilization techniques are needed to treat some cases of male factor infertility[3].

Assisted human reproduction techniques

Sperm collection

Shortly after collection, the semen is analyzed and subjected to seminal processing, or "trained". Sperm processing aims to improve the choice of the sperm, remove toxins and contaminants, and also increase the fertilizing potential of the sperms. The "swim-up" and discontinuous gradient methodologies are generally used. The "swim-up" technique eliminates the seminal plasma, debris, amorphous, material, dead skin cells, dead and motionless sperm, and sperm without progressive motility. In the end, a clean sample is obtained containing spermatozoa of excellent motility. The "swim-up" technique recovers around 20% of motile sperm present in the initial ejaculate. The main advantage of the "swim-up" is the recovery of high-quality cells, although the number of spermatozoa is not so elevated, particularly when the initial motility of the sample is low. The techniques of discontinuous colloidal gradients, in turn, are based on the exertion of centrifugal force on the spermatozoa and other particulate elements of the semen, which forces them to overcome different density gradients. This method is faster and usually leads to greater recovery rates of sperm motility than the "swim-up" technique, in all kinds of male infertility problems. However, the final motility quality is lower than that obtained with the "swim-up" technique[4,5].

Oocyte fertilization

Following controlled ovarian stimulation (COS), ovule collection is performed by transvaginal ultrasound-guided needle follicular aspiration. Aspiration is performed using a puncture needle coupled to a sterile test tube containing buffered culture medium, which, in turn, is coupled to an aspirator with controlled pressure that is activated by a pedal control. The test tubes containing follicular fluid are immediately taken to the embryology laboratory for evaluation of oocyte presence. After sperm collection, oocyte fertilization is performed by the conventional *in vitro* technique or by ICSI.

In vitro *fertilization*

In *in vitro* fertilization (IVF), for each oocyte a dilution of around 1:100.000 spermatozoa is added. The embryos obtained are then transferred fresh or frozen and stored. Previous ovarian stimulation is required to perform this technique. After the oocytes are obtained, they are separated and acclimatized to the environment in an adequate culture medium, under constant temperature (37° C) and pressure (5% of CO_2) conditions. After two to four hours of environmental acclimatization, the oocytes are ready for fertilization.

Around 16-18 hours after fertilization, the zygote begins to undergo cellular division to form the embryo. The transfer of these embryos to the uterine cavity is performed with a transfer catheter two to five days after oocyte collection. Usually two to three embryos are transferred to the uterine cavity. Within 10 to 12 days, a pregnancy test is performed[6].

Intracytoplasmic sperm injection

ICSI (intracytoplasmic sperm injection) is a complementary technique to *in vitro* fertilization used in patients with a low amount of viable spermatozoa that is insufficient to achieve *in vitro* fertilization.

This technique consists of choosing a good quality spermatozoon (alive, morphologically normal and with good motility) and injecting it inside a mature oocyte, using micromanipulation equipment. After fertilization, the following procedures are similar to those described for the *in vitro* fertilization[7].

Several studies have investigated the efficiency of both techniques, particularly in the cases of severe teratozoospermia, comparing the results of IVF to high insemination concentration

and ICSI. Plachot et al.[8] observed that the greatest fertilization rates and the best embryonary quality were obtained with ICSI when compared to conventional IVF. Jain et al.[9] confirmed a clear tendency for better pregnancy rates and implant in the group of patients who have undergone ICSI, despite the consensus that the use of ICSI should be restricted to its primary indication: severe male factor.

Indications for IVF or ICSI

In general, according to the World Health Organization (WHO), the minimum sperm parameters (after processing) for performing conventional IVF are: concentration ≥ 5 x 10^6/ mL, 30% of progressive motility and 30% of normal morphology. For men with lower sperm parameters, ICSI is indicated[10]. Nevertheless, these values have been questioned in the literature and, thus, are not observed in several fertility centers, which consider other factors such as morphology, in the indication or non-indication of ICSI. Plachot et al.[8] found that sperm morphology can have a greater impact on the success of a conventional IVF than oligospermia and/or oligoastenospermia[8].

In the absence of the male factor, it is clear that both the IVF and the ICSI are equally efficient in the treatment of infertile couples[11]. In cases in which there is previous failure of fertilization or when only ≤10% of the oocytes are fertilized in the IVF, the ICSI can be suggested, even in the absence of male factor[10].

Some issues associated with ICSI, however, remain unsolved: the risk of transmission of genetic defects to descendants, malformations[12], chromosome anomalies, damage caused to the oocyte after rupture of oolemma, asymmetric decondensation of sperm chromatin and the greater difficulty to observe *in vitro* blastocyst formation[8].

Currently, however, due to the excellent fertilization rates, the ICSI has been indicated in practically all infertility cases, even without the presence of male factor. Between 1999 and 2004, in the United States, the practice of ICSI grew from 11 to 57.5%[9]. Nevertheless, its disadvantages and the successful use of IVF instead of ICSI in some cases suggest that the parameters for indication of ICSI and its indiscriminate use are questionable.

Discussion

Several studies were carried out comparing the IVF and ICSI techniques and aiming to help in the choice of the most appropriate technique according to the type of infertility problem of the couple[10,11,8]. There is much controversy about the use of semen parameters to guide the choice of one of the referred techniques.

As above mentioned, in the ICSI, only one good quality spermatozoon is needed to ensure the success of the procedure; In the IVF, on the other hand, a minimum concentration of (5 million/mL post seminal processing) of motile and morphologically normal spermatozoa is necessary.

Seminal parameters

According to WHO, the normality values for semen are above 1.5 mL; pH of 7.2 to 8; minimum concentration of 15 million spermatozoa per mL; 39 million (or more) spermatozoa in the ejaculate; 4% of normal forms; 58% of vitality; maximum of 1 million leukocytes per mL of semen and 32% or more progressive motility (category A + B)[13]. The A motility is defined as progressive at a speed of 25 µM/s (CASA, Hamilton Thorn Master C v.10, Beverly, MA, USA).

Concerning strict morphological features, as recommended by Kruger et al,[14], one semen sample with excellent prognosis presents at least 14% of normal spermatozoa with a head of smooth oval configuration. (5-6 µm long/ 2.5-3.5 µm wide with panoptic stain), acrosome percent is 40-70% of the head and no defects in the intermediate piece or tail; semen with at least 4% normal spermatozoa have good prognosis[13].

The choice of a good quality spermatozoon to be used in ICSI is mostly based on motility[15,16]. Low motility seems to be one of the causes of asymmetric decondensation of sperm chromosomes, as well as of the reduced capacity of the embryo to form the blastocyst *in vitro*[17-19].

Some studies have assessed the influence of morphology and ideal concentration of spermatozoa in the choice of ICSI or IVF. Hall et al.[20] compared high insemination concentration (1 x 10^6 to 1 x 10^7/ mL of motile spermatozoa after training) to ICSI for semen with severe teratozoospermia (2.9% of Kruger morphology). The results[17-19] concerning fertilization rates, embryo quality and implantation rates were similar for both techniques. Fishel et al.[21] demonstrated that spermatozoa with plasmatic membrane with alterations have greater fertilization rate after ICSI compared to IVF.

Besides, it has also been demonstrated that, for patients with oligoastheno-teratozoospermia, the fertilization rate is 3.9 times greater after ICSI than IVF[3].

Concerning patients with normal sperm, the risk of failure in fertilization is much lower in ICSI than IVF[22,23].

In cases of anejaculatory infertility resulting from spinal lesions, medical conditions (diabetes mellitus, multiple sclerosis) or physiological conditions, spermatozoa can be collected by electroejaculation. The processing and selection of semen for ICSI in this type of sample is not different from the selection performed with ejaculated semen. It has been demonstrated that both techniques are equally efficient with constant

fertilization rates, number of embryos for transfer and clinical pregnancies (Table 1)[4].

Together with electroejaculation, in cases of non-obstructive azoospermia, the PESA and TESA techniques are used. In these cases, as long as it is possible to recover sperm directly from either the testis itself or from the epididymis, ICSI can be performed[24-25-27]. One study carried out with frozen embryos comparing ejaculated semen with semen obtained by PESA/TESA showed that the indication of ICSI, widely used in these cases, ensures that the survival and morphology of the embryos, as well as clinical gestation and implantation, are similar to those cases in which the semen is collected by normal ejaculation[28].

Patients with seminal parameters within the boundary between indication for IVF or ICSI and patients with suspected male infertility were investigated by Aboulghar et al.[29]. The fertilization rate was significantly higher when ICSI was used compared to IVF. The present study demonstrated that 45% of the patients might have their embryonary transfer cancelled due to failure in fertilization if ICSI. Besides, regarding those patients with no clinical apparent cause for infertility, 23% could have had failure in fertilization if IVF was performed instead of ICSI. This occurs because many additional factors, such as female factors, may interfere with the results[29].

In many cases, the seminal quality is not considered, and the couple is directly referred to the ICSI technique. In one review, Rumste[11] compared the rate of births resulting from ICSI and FIV, in cases in which the seminal parameters were considered normal (sufficient for IVF). It was found that the fertilization rate was greater for couples treated with IVF, but there was no difference in the miscarriage, pregnancy or birth rates.

The choice of the best treatment for couples with male infertility is usually empirical, and fertilization may fail many times after the use of IVF or the unnecessary use of ICSI. In a study with 58 couples whose semen had oligo, asteno and/or moderate teratozoospermia, half of the oocytes were inseminated by IVF and half of them by ICSI. The technique used for the second cycle was chosen according to the results of the first cycle. Therefore, after the use of both IVF and ICSI, in the first cycle, the embryonary morphology was predominantly in good quality for both techniques. As for the subsequent cycles, the embryonary quality decreased with IVF and remained good with ICSI, which demonstrates that a previous failure of fertilization by IVF had greater impact on the use of ICSI than the male factor itself[8].Yoeli et al.[12], in a study with couples that had failure of previous fertilization with IVF was up to 40% or had male factor, evaluated 177 women for the fertilization rate and quality of embryo following ICSI or FIV. The study found that the fertilization rate by injected oocyte was 66% for ICSI and 40.9% for IVF. As for the rates of good or bad quality embryos, the results were similar for both techniques.

In another study, 447 aspirated oocytes were fertilized by CSI, with 258 using semen with male factor and 187 fertilized by semen of good quality, though with previous failure of fertilization by IVF. A significant decrease ($p<0.001$) in implantation and pregnancy per transfer was found in the group with previous failure of fertilization by IVF compared to the group with male factor. No difference was found in age, number of recovered oocytes, quality and number of transferred embryos. However, the rates of fertilization and cleavage were lower in the group with previous failure of fertilization by IVF. Therefore, it can be inferred that in cases of previous failure of fertilization by IVF, ICSI may not be the best choice, since the problem to be solved is not only related to the bad quality of semen, but also to the oocyte[30].

It is believed that the results obtained with ICSI in the treatment of male factor are not successful because of the type of technique used, but because the female gamete used in this technique are good; only the male gamete is bad. This does not occur with the IVF when infertility problem may be in the male or female individual[31].

Concerning multiple gestations, it was demonstrated that the probability is similar in the IVF or in ICSI. There is a greater number of twinning using ICSI because there are more ICSI procedures than IVF procedures[10].

On the other hand, due to some disadvantages, the use of ICSI has been questioned, even in the treatment of patients with bad quality sperm. Although it is a successful technique, it is more expensive and lengthy. Besides, it can cause damage to 5-10% of the oocytes after the rupture of the oolema and asymmetric decondensation of the sperm chromatin[18].

One study compared the neuromotor development of children aged 5-8 years. In total, 81 single children born following ICSI, from 1996 to 1999 were compared to 81 children of the same age generated by IVF and by natural conception. The results have demonstrated 53% of lower neurological dysfunction in children generated by ICSI compared to 49% of those generated by IVF and

Table 1 – Comparison of semen used in ICSI by normal ejaculation and electroejaculation[4]

	Electroejaculated semen	Ejaculated semen
Number of cycles	18	775
Percentage of fertilization	72	76
Number of recovered embryos	3.4	3.2
Rate of clinical pregnancy per transfer	55.6	53

43% in those naturally conceived. Thus, it can be seen that ICSI is not directly associated to neurological problems[32-33].

Despite the importance of the seminal criteria in the type of reproduction technique to be adopted, the use of ICSI has been extended to patients without male infertility. From 1995 to 2004, the percentage of IVF with IVF increased from 11 to 57.5% in the United States, whereas the diagnosis of male infertility remained the same[9].

The introduction of ICSI has drastically changed the treatment of male infertility. In cases without such type of infertility, the pregnancy rates are not better with ICSI than with IVF, because the ICSI failures are mostly explained by the bad quality of the semen. Regardless the technique used, genetic counseling is also indicated for patients with seminal disorders[34].

Conclusions

The choice of the best treatment for couples with male infertility is usually empirical, and fertilization may fail many times after the use of IVF or the unnecessary use of ICSI.

The quality of the embryos generated from gametes with seminal parameters of bad quality, as well as the fertilization and gestation rates, were found to be greater following the use of ICSI, when compared to IVF. All the same, due to the disadvantages of the ICSI technique, in a first cycle, all the couples that seek assisted reproduction must be examined, and, in case of favorable seminal parameters, IVF should be the choice method.

On the other hand, ICSI should be the choice for patients with seminal parameters in the boundary between indication of IVF or ICSI. Moreover, many couples without clinical apparent cause for infertility can only be successfully treated with the use of ICSI.

It is known, therefore, that the quality of the semen interferes directly with the choice of the reproduction technique, and, consequently, with the results of the assisted reproduction.

After literature review, which included analysis of the available articles on the subject, we also concluded that previous failure of fertilization had a greater impact on the fertilization rates and blastocyst formation than the seminal parameters

Thus, the present review may lead us to conclude that besides sperm parameters, other parameters are also important in the choice of the reproduction technique such as previous failure of fertilization, oocyte quality and maternal age.

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