Juniper Publishers
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Journal of
Gynecology and Women's Health

Research Article
Volume 1 Issue 1 - March 2016

J Gynecol Women's Health


# Use of Semen Quality to Predict Pregnancy in Couples Undergoing ICSI

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**Submission:** February 25, 2016; **Published:** March 10, 2016

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**Abstract**

The objective of this study was to determine how fertilization, implantation and pregnancy depend on two basic sperm parameters, sperm concentration and sperm motility in ICSI procedure. Primary outcome as fertilization rate (FR), implantation rate (IR), βhCG/cycle, clinical pregnancy rate (PR) and live births rate (LBR) to be stated. In our center 44 cycles of ICSI were carried out in natural and stimulated cycle. Male subfertility was defined as semen quality not meeting the criteria for normality as defined by the World Health Organization (WHO, 1999). FR, IR, βhCG/cycle, PR and LBR were not significantly different between the 5 form male subfertility (normo-, oligo-, astheno-, oligo-astheno- and oligoasthenoteratozoospermia) in ICSI ($P < 0.05$). In oligoasthenoteratozoospermia IR, βhCG/cycle, PR and LBR was higher than in other cases. The best results are achieved in the case of fertilization rate. PR and LBR had the lowest values in the case oligoasthenozoospermia. In normozoospermia were extremely low values PR and LBR. The only factor that obviously impacts on ICSI-related pregnancy is maternal age, impairing oocyte/embryo quality. The answer to the question of how to predict and increase the success of the ICSI procedure should be sought in assessment of sperm DNA integrity.

**Keywords**: Intracytoplasmic sperm injection; Semen quality; Male subfertility; Pregnancy

**Abbreviations:** FR: Fertilization Rate; IR: Implantation Rate; PR: Pregnancy Rate; LBR: Live Births Rate; WHO: World Health Organization; ICSI: Intracytoplasmic Sperm Injection; hMGs: Human Menopausal Gonadotropins; hCG: Human Chorionic Gonadotropin; MII: Metaphase II; OAT: Oligoasthenoteratozoospermia

## Introduction

Abnormalities in sperm production or function, alone or in combination with other factors, account for 35–50% of all cases of infertility. A multi center study conducted by the World Health organization (WHO) concluded that in 20% of infertile couples, the predominant cause of infertility is the male factor, while in another 27% of couples both partners contribute. While these statistics underline the importance of male factor in reproduction, the clinical methodology used to diagnose male infertility has depended on assessment of sperm concentration, motility and sperm morphology [1].

Male infertility is certainly multi-factorial. It is improbable that one sperm function test will prove to be a panacea, owing to the multiple steps involved in fertilization. In addition to arriving at the site of fertilization, sperm must undergo capacitation and the acrosome reaction; they must penetrate the cumulus, bind to the zona pellucida, penetrate through the zona, fuse with the oolemma, activate the oocyte, undergo nuclear decondensation, form the male pronucleus, and then fuse with the female pronucleus. As intracytoplasmic sperm injection (ICSI), more and more logical questions are being asked about the proper role for sperm function testing. For ICSI, live sperm with the ability to activate the oocyte and form a pronucleus are necessary, but morphology, motility, and acrosome status are generally not important [2-6]. It has been demonstrated that the positive outcome of ICSI is largely independent of the three basic sperm parameters – motility, morphology, and concentration in couples in whom these characteristics are severely impaired [7]. In the latter case, its successful application to surgically retrieved sperm proves that this micromanipulation technique is able to achieve fertilization regardless of the maturation of the gametes. The possibility to bypass the steps of testicular and epididymal sperm maturation, acrosome reaction, binding to the zona pellucida, and fusion with the oolemma now permits infertility due to a male factor to be addressed successfully. The

only factor that obviously impacts on ICSI-related pregnancy rates is maternal age, impairing oocyte/embryo quality.

Is it possible to predict what extent, whether and in what way the sperm quality affects on ICSI result? Is it possible to identify what are the sperm parameters that best predict result of ICSI procedure? Is there a correlation between the different forms of male infertility and fertilization, embryo development and ultimately the conception and birth of a healthy child? The most common reasons for rejection of potential donors are poor sperm count and poor motility [8]. However, individual semen parameters seem to have little accuracy in predicting pregnancy rates [9-14]. Most semen characteristics seem to be positively correlated, suggesting that the different parameters are not independent. That is a semen sample with poor values for one parameter (e.g., count) is likely to have poor values for other parameters as well, such as motility [15]. The purpose of our current study was to determine how fertilization, implantation and pregnancy depend on two basic sperm parameters, sperm concentration and sperm motility in ICSI procedure.

## Materials and Methods

In 2010, in our center 44 cycles of ICSI were carried out in natural and stimulated cycle. Age of women ranged from 24 to 44 years. Even 18 (41%) were women older than 39 years. 57% of our small sample, were women older than 35 years (Figure 1). The medical records of women were examined for relevant details. The pertinent obstetric history and gynecological history were recorded. Notes on the patients infertility, duration of infertility, presence and type of any contributing female factors, and type of contributing male factors. Any pregnancies were noted, along with the outcomes of the pregnancies (miscarriage or live birth). The semen samples were collected after 48 to 72 hours of abstinence. Sperm specimen was obtained by masturbation into sterile specimen cups. Ejaculates were left to liquefy for 20 to 30 min. Male subfertility was defined as semen quality not meeting the criteria for normality as defined by the World Health Organization (WHO, 1999). Primary outcomes as fertilization rate, implantation rate, βhCG/cycle, clinical pregnancy rate and live births rate to be stated.

Semen concentration and motility are assessed in a Makler counting chamber. The specimen is examined microscopically, and at least 100–200 spermatozoa are categorized. Semen samples were processed by the swim-up method from a washed sperm preparation (indirect method) to give a highly motile sperm population. After centrifugation, the supernatant was removed and the pellet re-suspended. The concentration of the assessed sperm suspension is adjusted to 1–1.5 × 106/ml. The tubes were loosely capped and placed in a 37 °C incubator, under 5% CO2 in air, at a 45° angle for 1h. During this period, motile spermatozoa migrated from the under layered sperm suspension to the upper layer. The top of the supernatant containing the actively motile spermatozoa was removed with extreme care.

Oocyte retrieval is performed after pituitary desensitization with a gonadotropin-releasing hormone agonist, with ovulation induction carried out by administering a combination of human menopausal gonadotropins (hMGs). Human chorionic gonadotropin (hCG) is administered when criteria for oocyte maturity are met, and oocyte retrieval by vaginal ultrasound-guided puncture is performed 35 hours later. Each oocyte is then examined under the microscope to assess the maturation stage and its integrity, metaphase II (MII) being assessed according to the absence of the germinal vesicle and the presence of an extruded polar body. ICSI is performed only in oocytes that have reached this level of maturity. A P value of < 0.05 was considered statistically significant.

## Results

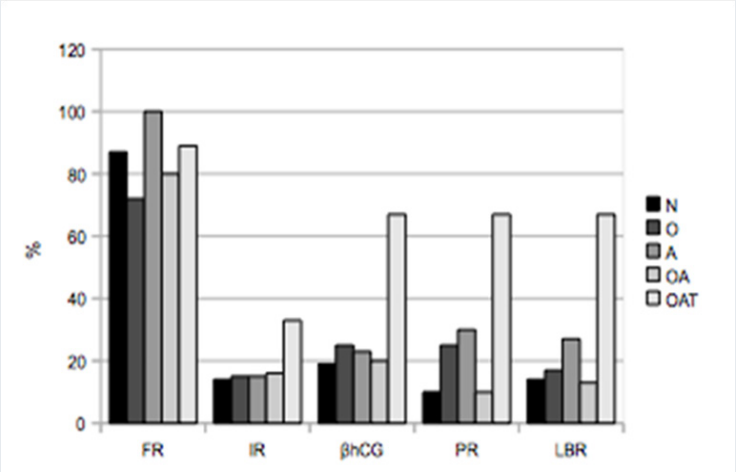
Results are shown in Table 1 and Graph 1. Fertilization rate (FR), implantation rate (IR), βhCG/cycle, pregnancy rate (PR) and live births rate (LBR) were not significantly different between the 5 form male subfertility (normo-, oligo-, astheno-, oligo-astheno- and oligoasthenoteratozoospermia) in ICSI. In Graph 1 it is easily observed deviation results in the case of the most severe form of male infertility (oligoasthenoteratozoospermia). Implantation rate, βhCG/cycle, pregnancy rate and live births rate was higher than in other cases, which are not expected. Research has shown that the best results are achieved in the case of fertilization rate. High values of this parameter were obtained in all cases of infertility. The biggest differences between the 5 forms of subfertility can be seen on the properties pregnancy rate and live births rate. These parameters had the lowest values in the case oligoasthenozoospermia what was expected. In case normozoospermia extremely low values PR and LBR were not expected.

**Table 1**: Success of ICSI in five abnormalities of spermatozoa.

| | Normozoospermia (N) | Oligozoospermia (O) | Asthenozoospermia (A) | Oligoasthenozoospermia (OA) | Oligoasthenoterato (OAT) |
|---|---|---|---|---|---|
| FR | 87% | 72% | 100% | 80% | 89% |
| IR | 14% | 15% | 15% | 16% | 33% |
| βhCG/cycle | 19% | 25% | 23% | 20% | 67% |
| PR | 10% | 25% | 30% | 10% | 67% |
| LBR | 14% | 17% | 27% | 13% | 67% |

P<0.05


How to cite this article: Gordana K, Ljiljana S. Use of Semen Quality to Predict Pregnancy in Couples Undergoing ICSI. J Gynecol Women's Health. 2016; 1(1): 555555. DOI: 10.19080/JGWH.2016.01.555555




**Graph 1:** Fertilization rate, implantation rate, βhCG, pregnancy rate and live births rate in normo-, oligo-, astheno-, oligoastheno- and oligoasthenoteratozoospermia.

In 2010, in our center 44 cycles of ICSI were carried out in natural and stimulated cycle. Age of women ranged from 24 to 44 years. Even 18 (41%) were women older than 39 years. 57% of our small sample, were women older than 35 years (Figure 1).

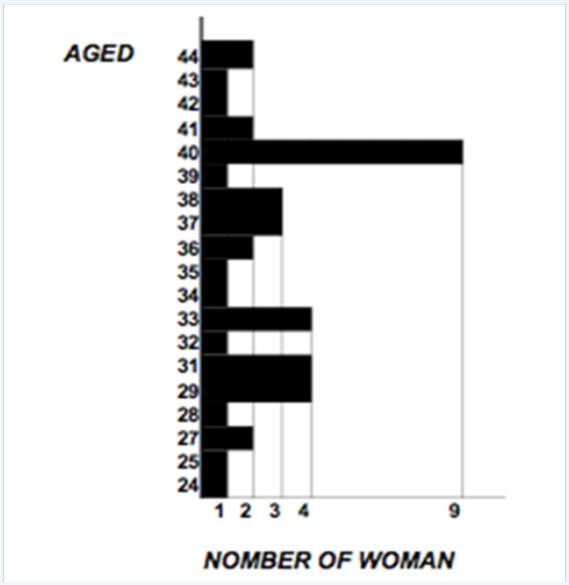



**Figure 1:** The age structure of women.

The number of oocytes obtained per patient ranged from 0 to 18 (Figure 1). Apparently, most of the women had a deficient response to stimulation. Among them, 25 (57%) received less than 5 oocytes during cycle. Supernumerary oocytes (more than 10) had 5 patients, which were reflected in the quality of their oocytes. These probably less quality cells make up 31% of the total number of collected cells. Number of mature oocytes was 195 (82%), as it is used for microfertilization.

## Discussion

The results from our experiments have shown that fertilization rate, implantation rate, βhCG/cycle, pregnancy rate and live births rate did not correlate with any of the forms of male infertility. This is in accordance with the [2-6]. These authors have found that for ICSI, live sperm with the ability to activate the oocyte and form a pronucleus are necessary, but morphology, motility, and acrosome status are generally not important. Also it has been demonstrated that the positive outcome of ICSI is largely independent of the three basic sperm parameters – motility, morphology, and concentration in couples in whom these characteristics are severely impaired [7]. This is consistent with our results. Although [8] found that the most common reasons for rejection of potential donors are poor sperm count and poor motility. These parameters in our case did not have a significant role to success. This can be explained by the fact that ICSI micromanipulation technique is able to achieve fertilization regardless of the maturation of the gametes. The possibility to bypass the steps of testicular and epididymal sperm maturation, acrosome reaction, binding to the zona pellucida, and fusion with the oolemma now permits infertility due to a male factor to be addressed successfully. The only factor that obviously impacts on ICSI-related pregnancy rates is maternal age, impairing oocyte/embryo quality. Sidhu [9-14] found that individual semen parameters seem to have little accuracy in predicting pregnancy rates. Also Agarwal [15] found that the most semen characteristics seem to be positively correlated, suggesting that the different parameters are not independent. That is, a semen sample with poor values for one parameter (e.g., count) is likely to have poor values for other parameters as well, such as motility [15]. Our results are not inconsistent with these statements. We have not examined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the correlation between semen parameters. The appearance that almost all parameters, except fertilization rate in case oligoasthenoteratozoospermia (OAT) were higher than in other forms of infertility can be explained by the following factors: 1) too small a sample, 2) unfavorable age structure of women and 3) poor response to stimulation, which led to a small number of oocytes in the sample studied. For the same reasons as in the case of normozoospermia obtained extremely low levels of PR and LBR, which were not expected.

Research has shown that best results are achieved in the case of fertilization rate. High values of this parameter were obtained in all cases of infertility. It has only confirmed that as ICSI, more and more logical questions are being asked about the proper role for sperm function testing. In addition to arriving at the site of fertilization, sperm must undergo capacitation and the acrosome reaction; they must penetrate the cumulus, bind to the zona pellucida, penetrate through the zona, fuse with the oolemma, activate the oocyte, undergo nuclear decondensation, form the male pronucleus, and then fuse with the female pronucleus.

## Conclusion

Fertilization rate (FR), implantation rate (IR), βhCG/cycle, pregnancy rate (PR) and live births rate (LBR) were not significantly different between the 5 form male subfertility (normo-, oligo-, astheno-, oligo-astheno- and oligoasthenoteratozoospermia) in ICSI. The possibility to bypass the steps of testicular and epididymal sperm maturation, acrosome reaction, binding to the zona pellucida, and fusion with the oolemma now permits infertility due to a male factor to be addressed successfully. The only factor that obviously impacts on ICSI-related pregnancy is maternal age, impairing oocyte/embryo quality. The answer to the question of how to predict and increase the success of the ICSI procedure should be sought in assessment of sperm DNA integrity. Standard measurements may not reveal subtle sperm defects such as DNA damage, and these defects can affect fertility. New markers are needed to better discriminate infertile men from fertile ones, predict pregnancy outcome in the female partner, and calculate the risk of adverse reproductive events.

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