in vivo *38*: 1384-1389 (2024)
doi:10.21873/invivo.13579

Determinant Factors for Ongoing Pregnancy in Homologous Intrauterine Insemination Cycles – A Prospective Study

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Abstract. *Background/Aim: Intrauterine insemination (IUI) is the most common assisted-reproduction treatment. However, it has lower success rate in comparison to other treatments. Therefore, determining factors that contribute to IUI success is of particular interest and this was the purpose of this prospective study. Patients and Methods: In this study, only homologous inseminations with fresh semen samples were included. All women received mild ovarian stimulation with clomiphene citrate and gonadotropins. Before IUI, basic semen analysis, evaluation of DNA fragmentation index (DFI), as well as measurement of sperm redox potential, were performed on each semen sample. Semen was processed with density-gradient centrifugation and 500 μl of processed sperm was used for insemination. Results: In 200 cycles, there were 36 pregnancies, six of them ectopic. Cycles with ongoing pregnancies were characterized by younger male and female age and higher number of follicles. Multivariate logistic regression analysis showed that only female age was significantly associated with ongoing pregnancy. DFI was positively correlated with male age and negatively correlated with sperm concentration and progressive motility. Semen redox potential showed a strong negative correlation with sperm concentration and positive correlation with DFI. Conclusion: Female age seems to be the most important determinant factor for the achievement of an ongoing pregnancy in homologous IUI cycles with fresh semen.*

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Key Words: Intrauterine insemination, semen, age, ongoing pregnancy, redox potential, DNA fragmentation.

Intrauterine insemination (IUI) is probably the most common assisted-reproduction treatment worldwide. The International Committee for Monitoring Assisted Reproduction Techniques reported 176,724 homologous IUI cycles and 52,337 donation IUI cycles for 2014, with a delivery rate of 8.9% and 11.7%, respectively (1). The *In-Vitro* Fertilization (IVF) Monitoring Consortium of the European Society for Human Reproduction and Embryology reported 139,870 IUI homologous cycles and 45,436 donation cycles for 2019, with a delivery rate of 9.2% and 12.1%, respectively (2).

IUI treatment has several advantages that makes it popular among patients and clinicians: It can be performed in a natural cycle or after mild stimulation, sperm processing is simple, the whole procedure is easy and safe, whilst it also has a lower cost than the other assisted-reproduction treatments; however, its effectiveness, in terms of pregnancy and delivery rate, is falling behind that of the other treatments (1, 2).

Therefore, it is of particular interest to determine the factors that contribute to the success of IUI, so as to improve the efficiency and success of the treatment. In previous studies, various factors have been suggested as determinants for the outcome of IUI cycles: Female age, and quality of native and processed sperm. During recent years, there is also a special interest in the impact of the DNA fragmentation index (DFI). However, opposing views have been reported regarding its impact on IUI outcome.

In this context, a prospective clinical study was conducted in order to determine factors associated with ongoing clinical pregnancy in homologous IUI cycles. The study focused on the age and the body mass index of the patients, as well as the quality of the sperm, including DFI and redox potential of the native semen.

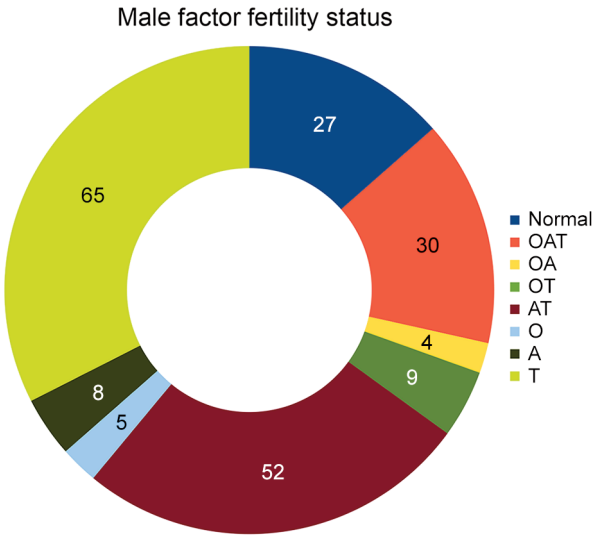

Figure 1. *Male factor fertility status (N=200). A: Asthenozoospermia; AT: asthenoteratozoospermia; Normal: normozoospermia; O: oligozoospermia; OA: oligoasthenozoospermia; OAT: oligoasthenoteratozoospermia; OT: oligoteratozoospermia; T: teratozoospermia.*

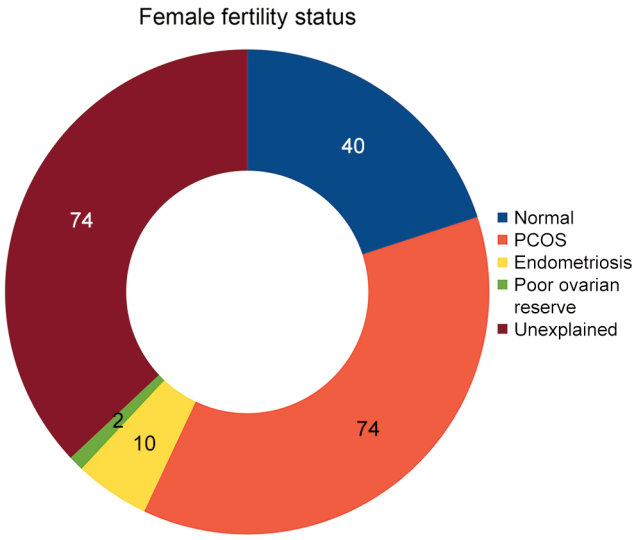

Figure 2. *Female fertility status (N=200). PCOS: Polycystic ovarian syndrome.*

Patients and Methods

Patients. In total, 200 hundred couples seeking treatment at the Athens IVF Center participated in the study during the years 2020-2023. The study received approval from the Research and Ethics Committee of the Democritus University of Thrace (ΔΠΘ/ΕΗΔΕ/51988/416) and the Supervisory Board of the Athens IVF Center (17/02/2020). All patients gave informed consent before participating in the study. The criterion for IUI treatment was failure to become pregnant after 12 months of regular intercourse. The exclusion criteria were: No patent fallopian tubes, anovulatory infertility, and the use of donor sperm.

Ovarian stimulation and follicle monitoring. All women received ovarian stimulation with clomiphene citrate (100 mg daily on days 3-7 of the menstrual cycle) and human menopausal gonadotropins (75 IU daily on days 4-8). Transvaginal ultrasound was used to monitor the development of follicles and the thickness of the endometrium.

Semen analysis and processing. Semen samples were obtained by masturbation. Basic semen analysis was performed on each sample according to the guidelines of World Health Organization (3). Additionally, the sperm redox potential was measured with a MiOXsys analyzer (Caerus Biotechnologies, Mies, Switzerland) and a small aliquot was analyzed for DNA fragmentation with a Halosperm sperm chromatin dispersion test (Halotech, Madrid, Spain). The rest of each semen sample was processed with density-gradient centrifugation, followed by washing in culture medium. The resulting fraction was injected in IUI with an Origio INCA 175-Q (Origio, Mälov, Denmark) insemination catheter. The total volume of the fraction injected was always 500 μl.

Statistical analysis. Initially, the sample was divided in two distinct groups, based on the achievement of ongoing clinical pregnancy after IUI. The distribution of each variable was assessed for normality using the Shapiro-Wilk test. Subsequently, descriptive statistics were calculated for both groups and comparisons between the two groups were made with a *t*-test when the variable in both groups was normally distributed, otherwise non-parametric Mann-Whitney *U*-test was used. Moreover, in order to examine the correlation between the parameters under investigation, we used Spearman's R correlation coefficient. Furthermore, the association between the clinical pregnancy and the independent parameters was investigated by utilizing multivariate logistic regression models. The analysis was conducted in Statistica 6.0 (StatSoft, Tulsa, OK, USA) and in R version 4.3.1. Statistical significance was set as α=5% (p<0.05).

Results

In the majority of the cases, male and female infertility factors coexisted (n=135, 67.5%); in 40 cases (20%), there was only male factor infertility and in 25 cases (12.5%) only female factor (Figure 1). In 27 cases (13.5%), the semen samples were within the World Health Organization reference values (3) whereas teratozoospermia was the most frequent pathology in the remaining cases (Figure 2). The female fertility status is presented in Figure 3; most of the patients suffered either from polycystic ovarian syndrome (37%) or unexplained infertility (37%).

Among the 200 IUI cycles, 43 showed an increase in human chorionic gonadotropin (21.5%); however, 36 were diagnosed with clinical pregnancy (18%). Among the 36 pregnancies, there were six miscarriages thus limiting the ongoing pregnancies to 30 (15%). The patients' demographic characteristics, the parameters of semen analysis, including DFI and redox potential, as well as the total number and the

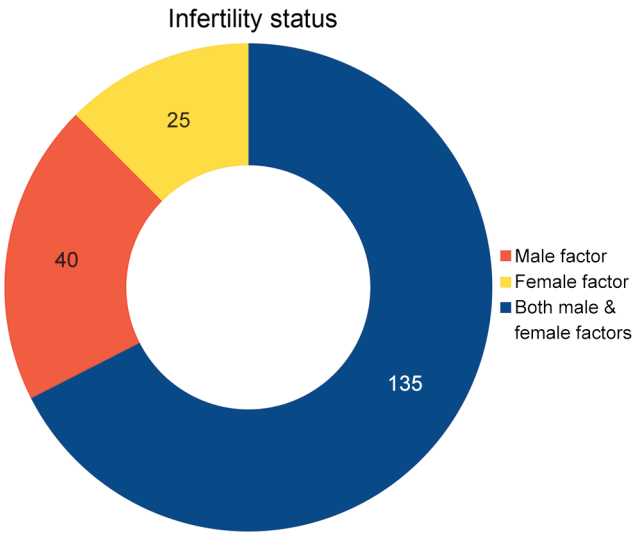

Figure 3. *Infertility status of the couples participating in the study (N=200).*

progressive motility of the injected spermatozoa in the IUI cycles with an ongoing pregnancy and those who failed are presented in Table I.

In the IUI cycles with an ongoing pregnancy, the female and male patients were 4 and 3 years younger than their counterparts, respectively; a difference which was statistically significant in both cases ($p<0.01$). The females with an ongoing pregnancy also had a higher median number of follicles ($p=0.04$). Regarding the rest of the explanatory parameters taken into consideration in the current study, there were no statistically significant differences between the two groups (Table I).

Correlation analysis gave some interesting results. Male age was weakly positively correlated with immotility (r=0.15; $p<0.05$) and DFI (r=0.19; $p<0.05$). Female age was negatively correlated with the number of follicles (r=–0.34; $p<0.05$). Body mass index of both males and females was not significantly correlated with any variable. Sperm concentration was positively correlated with progressive motility (r=0.26; $p<0.05$), vitality (r=0.38; $p<0.05$) and normal morphology (r=0.33; $p<0.05$) but on the other hand was negatively correlated with immotility (r=–0.39; $p<0.05$), DFI (r=–0.23; $p<0.05$) and redox potential r=–0.49; $p<0.05$). Progressive motility (r=0.80; $p<0.05$) and normal morphology (r=0.45; $p<0.05$) were also strongly and positively correlated with vitality. DFI was significantly negatively correlated with progressive motility (r=–0.28; $p<0.05$). Semen redox potential was significantly positively correlated with DFI (r=0.31; $p<0.05$). The total number of injected spermatozoa was strongly correlated with sperm concentration (r=0.71; $p<0.05$), progressive motility (r=0.31; $p<0.05$), vitality (r=0.37; $p<0.05$) and normal morphology (r=0.37; $p<0.05$). At the same time, the total number of injected spermatozoa was negatively correlated with redox potential (r=–0.43; $p<0.05$). Similarly, the progressive motility of the injected spermatozoa was positively correlated with sperm concentration (r=0.27; $p<0.05$), the progressive motility of the untreated semen sample (r=0.44; $p<0.05$), vitality (r=0.37; $p<0.05$) and the total number of the injected spermatozoa (r=0.32; $p<0.05$). There was a negative correlation between progressive motility of the injected spermatozoa and DFI (r=–0.36; $p<0.05$), as well as redox potential (r=–0.33; $p<0.05$).

The results from the multivariable logistic regression model showed that the association between IUI cycles and the model's predictors were not statistically significant for the majority of parameters (Table II). Nevertheless, female age seemed to play an important role, as a negative relationship between female age and IUI cycle was identified: a 1-year increase in the age of the female patients led to a 24% reduction of the odds of an IUI cycle being successful ($p=0.005$). As far as normal morphology is concerned, a tendency towards a positive association was also evident ($p=0.08$): a 1% increase in normal morphology led to an 86% increased chance of an IUI cycle being successful.

Discussion

Although IUI is a first-line assisted-reproduction treatment, there is controversy regarding the factors that determine its effectiveness. This is partly explained by the use of different ovarian stimulation regimens and by the use of different methods for sperm processing. In this study, all women received ovarian stimulation with the same protocol (clomiphene citrate plus gonadotropins) and sperm processing was exclusively performed with density-gradient centrifugation. This was a measure to homogenize the study population so that we would be better able to study the other parameters possibly determining the outcome. Ongoing pregnancies were defined as the primary outcome.

Numerous previous studies have focused on sperm quality as it is considered an important factor for the success of IUI cycles. Sperm morphology has been reported as significant determinant factor by many previous studies (4-20). In the present study, multivariate logistic regression analysis showed that normal morphology had a tendency towards a positive association with ongoing pregnancy, although not at a statistically significant level ($p=0.08$). Similarly, concentration and motility, whether of native or processed sperm, proved not to be significant determinants. At this point, it is worth mentioning that sperm processing is undoubtedly a factor able to even out differences between semen samples of different quality. In the present study, the

Table I. *Main characteristics of patients, semen samples and the processed sperm used in intrauterine insemination (IUI).*

		IUI cycles			
		Total (N=200)	Failed (N=170)	Ongoing pregnancy (N=30)	*p*-Value
Age, years	Males	39 (36-42)	39 (36-43)	36 (34.25-38.75)	**<0.01**
	Females	36 (33-38)	37 (34-38)	33 (31.25-36)	**<0.01**
BMI, kg/m^2	Males	26.5 (24.58-29.58)	26.5 (24.4-29)	27.67±3.74	0.37
	Females	22.8 (20.88-26.11)	22.8 (20.9-26.1)	22.7 (20.82-24.5)	0.99
Sperm	Volume (ml)	2.3 (1.7-3)	2.3 (1.7-3.1)	2 (1.5-2.98)	0.60
	Concentration (×10^6/ml)	28 (16-46.25)	28 (16-46)	33.72±19.89	0.64
	Progressive motility (%)	31 (22.75-38)	31 (22-38)	30.03±12.72	0.21
	C motility (%)	17.32±4.95	17.31±4.98	17.33±4.83	0.98
	Immotile (%)	45 (37-55)	47.1±14.21	43.6±10.9	0.29
	Vitality (%)	55 (45-63)	52 (42-62)	56.07±10.52	0.30
	Normal morphology (%)	2 (2-3)	2 (2-3)	3 (2-4)	0.13
	DFI (%)	24 (17-30)	24 (17-30)	23.12±7.63	0.64
	Redox potential (mV/10^6 sperm/ml)	1.01 (0.53-1.91)	1.08 (0.53-1.93)	0.88 (0.48-1.57)	0.66
	Progressive motility of sperm injected in IUI cycles (%)	72 (59-86.25)	72 (59-87)	72.7±15.68	0.62
	Total no. of sperm injected in IUI cycles (×10^6)	22 (12-39)	22 (12-39)	21 (15-35.25)	0.77
Follicles, n		3 (1-4)	2 (1-4)	4 (3-6.75)	**0.04**

BMI: Body mass index; C motility: local motility; DFI: DNA fragmentation index. Normally distributed variables are presented as mean±standard deviation, while non-normally distributed variables as median (lower quartile – upper quartile). Comparisons were made with *t*-test only wherever both variables were distributed normally, otherwise Wilcoxon test was used. Statistically significant *p*-values are shown in bold.

vast majority of semen samples had good enough parameters to give, after sperm processing, aliquots with a good total number of spermatozoa and progressive motility.

Sperm DNA fragmentation and consequently DFI is considered to be associated with low potential either for natural conception or after IVF (21). Although there are studies suggesting that a low DFI is associated with higher pregnancy rates after IUI (22, 23), the meta-analysis by Sugihara *et al*. (21) concluded that there is not enough evidence to consider DFI as a determinant factor in IUI and therefore to support sperm DNA fragmentation tests as part of the routine investigation of couples undergoing IUI. In the present study, all semen samples were tested for DNA fragmentation in an attempt to evaluate its usefulness as a possible determinant factor for IUI success. However, multivariate logistic regression analysis did not reveal a statistical significance for DFI. Moreover, regarding DFI, there was no significant difference between the cycles with ongoing pregnancies and those which failed. Correlation analysis showed that DFI was strongly and negatively correlated with progressive sperm motility and normal morphology; this finding indicates that progressively motile spermatozoa with good morphology have good DNA integrity.

DFI was also strongly and positively correlated with redox potential (r=0.31; $p<0.05$). This was expected as oxidative stress is considered a factor challenging sperm DNA integrity. Nguyen *et al*. (24) also reported higher DNA fragmentation in semen samples with high redox potential, finding a significantly positive correlation between the two parameters. However, in the present study, redox potential was not found to have an impact on IUI outcome; the cycles with an

Table II. *Results from the multivariable logistic regression model.*

		Adjusted OR	95% CI	*p*-Value
Age	Male	1.007	0.882-1.138	0.908
	Female	0.766	0.625-0.915	**0.005**
BMI	Male	0.986	0.834-1.153	0.863
	Female	0.996	0.848-1.147	0.956
Total number of sperm		0.976	0.945-1.003	0.106
Normal morphology		1.862	0.949-3.929	0.080
DFI		0.984	0.918-1.046	0.611
Redox potential		1.032	0.923-1.115	0.464
Follicles		1.119	0.904-1.391	0.300
Progressive motility in IUI		1.027	0.988-1.076	0.211

BMI: Body mass index; CI: confidence interval: DFI: DNA fragmentation index; OR: odds ratio. Statistically significant *p*-values are shown in bold.

ongoing pregnancy had a slightly lower, but not significantly different, redox potential than the failed cycles and the multivariate logistic regression analysis did not reveal redox potential as a determinant factor for IUI success.

Female age is considered a critical factor for the success of assisted-reproduction treatments. The results of the present study confirm its importance regarding the establishment of ongoing pregnancy in IUI cycles. Although there was a statistically significant difference regarding also age of males and the number of follicles between the cycles with an ongoing pregnancy and those which failed, the multivariate logistic regression analysis highlighted only female age as a determinant factor. In this study, the oldest woman with an ongoing pregnancy was 38 years old.

Conclusion

The present prospective study showed that in homologous IUI cycles, the most critical factor for the achievement of ongoing pregnancy is female age. Other parameters, such as male age, body mass index, sperm quality (including DFI and redox potential), do not seem to have a significant impact.

Conflicts of Interest

The Authors declare no potential conflicts of interest in relation to this study.

Authors' Contributions

BA: Study conception and design, supervision, revision of the article; GB: investigation, project administration, writing of the article; VA: investigation; AA: writing – review and editing; II: investigation; LOA: data analysis and revision of the article.

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Received November 13, 2023
Revised February 1, 2024
Accepted February 8, 2024