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COMPARISON OF THE EFFECTIVENESS OF DENSITY GRADIENT CENTRIFUGATION AND MICROCHIP SPERM PREPARATION METHODS ON PREGNANCY SUCCESS IN INTRAUTERINE INSEMINATION

INTRAUTERİN İNSEMİNASYONDA DANSİTE GRADİYENT SANTRİFÜGASYON VE MİKROÇİP SPERM HAZIRLAMA YÖNTEMLERİNİN GEBELİK BAŞARISI ÜZERİNE ETKİNLİKLERİNİN KARŞILAŞTIRILMASI

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ABSTRACT

Introduction: Intrauterine insemination technique is one of the most commonly used methods in assisted reproductive treatments. Semen parameters are one of the prognostic factors affecting the clinical success of the intrauterine insemination (IUI) method, and different sperm preparation techniques are applied to select quality sperm.

Methods: In this retrospective study, 217 couples who applied to the infertility center of our hospital between 2012 and 2020 were included in the study. Before the application of the intrauterine insemination method, semen samples taken from 178 patients were prepared by microchip method and semen samples taken from 39 patients were prepared by density gradient centrifugation (DGC) method, and the concentration and motility properties of sperm were evaluated.

Results: The clinical pregnancy success of sperm samples prepared with microchip and density gradient centrifugation method was evaluated after intrauterine insemination application. In the density gradient centrifugation method, motile sperm (79.46±12.48%) were obtained at a higher rate than the microchip method (57.48±20.24). While clinical pregnancy was observed in 46.15% of the patients included in the density gradient centrifugation group, it was determined as 23.03% in the microchip group. On the other hand, while pregnancy continued in 27.78% of the density gradient centrifugation group patients, the continuing pregnancy in the microchip group was determined as 53.66%. As a result, while the percentage of motile sperm and the incidence of clinical pregnancy increase in the density gradient centrifugation method compared to the microchip method, there is a significant decrease in the ongoing pregnancy rates.

Conclusions: It is seen that sperm samples prepared by microchip method have positive contributions to the clinical success of intrauterine insemination.

Keywords: Infertility, Intrauterine insemination, Density gradient centrifugation, Microchip, Sperm preparation

ÖZET

Giriş: Intrauterin inseminasyon tekniği yardımcı üreme tedavilerinde en sık kullanılan yöntemlerden biridir. Semen parametreleri intrauterin inseminasyon yönteminin klinik başarısını etkileyen prognostik faktörlerin başında gelmektedir ve kaliteli spermlerin seçilimi için farklı sperm hazırlama teknikleri uygulanmaktadır.

Yöntemler: Bu retrospektif çalışmada 2012 ve 2020 yılları arasında hastanemiz infertilite merkezine başvuran 217 çift çalışma kapsamına alınmıştır. Intrauterin inseminasyon yönteminin uygulaması öncesi 178 hastadan alınan semen örnekleri mikroçip yöntemi ve 39 hastadan alınan semen örnekleri dansite gradiyent santrifügasyon yöntemi ile hazırlanmış ve spermlerin konsantrasyonu ve motilite özellikleri değerlendirilmiştir. Intrauterin inseminasyon uygulaması sonrası mikroçip ve dansite gradiyent santrifügasyon yöntemi ile hazırlanan sperm örneklerinin klinik gebelik başarısı değerlendirilmiştir.

Bulgular: Dansite gradiyent santrifügasyon yönteminde motil sperm (%79.46±12.48) mikroçip yöntemine göre (%57.48±20,24) daha yüksek oranda elde edilmiştir. Dansite gradiyent santrifügasyon grubuna dâhil olan hastaların %46.15'ünde klinik gebelik gözlenirken mikroçip grubunda %23.03 olarak belirlenmiştir. Buna karşın dansite gradiyent santrifügasyon grubu hastaların %27.78'inde gebelik devam ederken mikroçip grubunda devam eden gebelik %53.66 olarak belirlenmiştir. Sonuç olarak mikroçip yöntemine kıyasla dansite gradiyent santrifügasyon yönteminde motil sperm yüzdesi ve klinik gebelik görülme oranı artarken, devam eden gebelik oranlarında ciddi oranda azalma görülmektedir.

Sonuç: Mikroçip yöntemi hazırlanan sperm örnekleri intrauterin inseminasyon'nun klinik başarısı üzerine olumlu katkılar verdiği görülmektedir.

Anahtar Kelimeler: İnfertilite, Intrauterin inseminasyon, Dansite gradiyent santrifügasyon, Mikroçip, Sperm hazırlama

INTRODUCTION

Infertility is an important reproductive health problem affecting 8-12% of couples in fertile age worldwide (1-3). Primary infertility is defined as no previous pregnancy, and secondary infertility is defined as inability to conceive after at least one pregnancy (4). Infertility is caused by men in 30-40% couples, women in 40-50% couples, and both men and women in 25% couples. However, 15% of cases are classified as unexplained infertility. Although there are common factors for spouses such as age, gender, socio-economic level, education level, and professional-working conditions in the etiology of infertility, mainly female and male specific causes come to the fore. Infertility is commonly caused by leiomyomas, endometriosis, ovarian

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cysts, polycystic ovary syndrome, adhesions in the pelvis and premature ovarian failure, low sperm count and motility, obstructions in the sperm-carrying ducts, hormonal imbalances, varicocele, and ejaculation problems. Infertility development is also observed in women and men with undetermined factors (5-7). In infertility, treatment of specific endocrine problems, tubal surgery, etiologic causes such as adhesiolysis or varicocele operation can be performed, and especially in recent years, important developments in assisted reproductive techniques enable infertile couples to have children (8).

Low sperm count, low motile sperm count despite normal sperm count, and anomalies in sperm morphology are among the most important causes observed in infertile men (9,10). Therefore, different sperm preparation techniques in IUI, in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedures are performed for increasing sperm motility, concentration, and fertilization power, removing prostaglandins and inhibitory substances that cause contractions in the uterus and inhibiting antifertility factors. The sperm preparation technique to be applied in the routine is determined according to the characteristics of the sperm sample (11-13). DGC and microchip methods are sperm preparation and selection methods used in IUI, IVF and ICSI methods (14). DGC method is a separation technique based on sperm density and motility using centrifuge. It is a technique generally applied to obtain motile sperm in cases of oligozoospermia, teratozoospermia or asthenozoospermia. Many studies reported that the centrifugation time and speed applied in this technique cause damage to sperm and DNA damage in healthy sperm. Decreased fertilization rates and pregnancy losses can be observed in patients with sufficient sperm count due to DNA damage (15,16). In order to eliminate these effects, microchip method has been developed in sperm preparation in recent years. In this method, healthy sperm can pass through a filter system without the need for centrifugation and sperm with smooth morphology, high motility and no DNA damage was collected (17,18).

In this retrospective study, it was aimed to determine the effects of DGC and microchip sperm preparation procedures used in the IUI method on clinical pregnancy and the continuation of pregnancy.

MATERIALS AND METHODS

Between 2012 and 2020, 217 couples who applied to the Gynecology and Obstetrics Clinic of our hospital with complaints of infertility were included in the study, and the clinical data of these cases were evaluated retrospectively. The study was approved by the Izmir Bakırçay University Non-Invasive Clinical Research Ethics Committee (decision no. 650, 29.06.2022). Age, number of cycles, duration of infertility, and type of infertility and causes of infertility of the women included in the study were recorded. All patients were evaluated by basal transvaginal ultrasound examination on day 3 of the menstrual cycle. Medical history, physical examination, and examinations by laparoscopy and/or hysterosalpingography were performed to confirm at least unilateral tubal patency. Recombinant follicle stimulating hormone (rFSH) was given to the patients included in the study as an initial dose of 75 IU/day (Gonal-F, Serono). If the patient shows monofollicular growth or excessive multiple follicular growth, the starting dose is increased or decreased. IUI was applied at > 17 mm follicular maturity and > 7 mm endometrial thickness. Ovulation was triggered by an injection of recombinant human chorionic gonadotropin (hCG) (250 µg; Ovitrelle, Serono). Cycles containing more than three dominant follicles were aborted. IUI was performed with a disposable IUI catheter 36 hours after hCG injection.

The preparation procedure of sperm samples taken from the couples was determined according to the characteristics of the semen sample. In order to obtain more motile sperm from cases diagnosed with oligozoospermia, teratozoospermia or asthenozoospermia, sperm were prepared by DGC method. In the remaining cases, sperm preparation procedure was applied by microchip method.

Semen samples were collected by masturbation after 2-5 days of sexual abstinence without any medical application and collected in a sterile container. The samples were incubated for liquefaction at 37°C for 45 min. After incubation, semen analysis was performed to determine the initial sperm concentration and the percentage of motile sperm. Puresperm® brand gradient kit was used for the preparation of sperm with the DGC method and processes were carried out according to the manufacturer's procedure. After the procedure, the sperm samples were centrifuged at 300-400 g for 8-10 min. and the supernatant was removed and 1 ml of sperm washing solution was added to the pellet and the semen was ready for insemination. In the preparation of sperm with the microchip method, 800 µL of sperm was carefully loaded onto the sample loading part of the microchip with sterile pipette. Then, 1 mL of sperm washing solution was added to the membrane filter part of the microchip and incubated at 37ºC for 30 min. After incubation, sperm samples were taken from the sample collection chamber on the microchip and ready for insemination. Semen analysis was performed on the sperm prepared by DGC and microchip methods, and the sperm concentration and percentage of motile sperm were determined.

Sperm prepared by microchip method was given to 178 of the women included in the study, and sperm prepared by DGC method to the remaining 39 patients were given using an intrauterine catheter. 1 mL of sperm sample was given from the cervical canal to the upper fundal region by the same doctor. After the IUI procedure, women were kept in the supine position for 30 min. Luteal phase was

supported with natural micronized progesterone (Koçak Farma) 100 mg (total dose 600 mg/day) until the 12th week of pregnancy. Pregnancy was determined by blood βhCG test on the 14th day after IUI and clinical pregnancy was confirmed by the detection of gestational sac in transvaginal ultrasonography. Ongoing pregnancy was defined as the presence of a viable fetus after the 12th week of pregnancy.

Descriptive statistics of the data obtained from the study are given with mean, standard deviation for numerical variables, and frequency and percentage analysis for categorical variables. Chi-square test and Analysis of Variance (ANOVA) test were used to compare the demographic variables obtained according to the study groups. Repeated Measurements Analysis of Variance was used to compare the sperm concentration and motility values obtained before and after the treatment according to the study groups. Dunnett multiple comparison test was used to determine the difference between groups. All analyzes were performed using SPSS 22.0 program (p value of <0.05 was accepted to demonstrate statistical significance).

RESULTS

A total of 217 couples (178 couples in the microchip group and 39 in the DGC group) were included in the final analyses. The mean age of the participants, number of cycles, and infertility durations were given in Table 1. There was no significant difference between the mean age, number of cycles and infertility duration in the patient groups (p>0.05).

The infertility type and the causes of infertility of the participants are given in Table 2. Primary infertility was observed more frequently in the patients included in the study. In addition, the cause of infertility in patients is polycystic ovary syndrome and unexplained reasons.

The mean sperm concentration from men before and after treatment was examined. A significant difference was found between the sperm concentration values before and after the treatment according to the sperm preparation techniques (p<0.05). Our findings demonstrated that a significant decrease in sperm concentration was observed in the microchip method compared to the DGC method. It was found significant between pre- and post-treatment motility values according to the preparation techniques (p<0.05). Accordingly, a higher increase in average sperm motility values was observed in the DGC method compared to the microchip method (Table 3).

When the clinical IUI success of sperm prepared by DGC and microchip methods was examined, clinical pregnancy was observed in 41 of 178 women included in the microchip group (clinical pregnancy rate 23.03%) and pregnancy continued in 22 patients (pregnancy continuation rate 53.66%). A significant relationship was determined between sperm preparation techniques and clinical pregnancy rate

Table 1. Demographic and infertility characteristics of women included in the study.

Variables (n=217)	Values	
Age (years) (mean ± sd, (min-max))	28.83±6.47 (17-45)	
Number of Cycles (mean ± sd, (min-max))	1.35±0.61 (1-4)	
Infertility Duration (years) (mean ± sd, (min-max))	3.02±2.50 (1-12)	
		Number of Patients
Infertility Type (n, %)	Primary	132 (60.83%)
	Secondary	85 (39.17%)
Cause of Infertility (n, %)	Unexplained	92 (42.40%)
	Polycystic ovary syndrome	84 (38.71%)
	Other	41 (18.89%)

Table 2. Comparison of demographic and infertility characteristics of women included in the study between DGC and microchip groups.

Microchip		Sperm Preparation Technique		
		DGC	*p* value	
Age (years) (mean ± sd)		28.65±6.36	29.64±6.99	0.610*
Number of Cycles (mean ± sd)		1.35±0.60	1.33±0.66	0.657*
Infertility Duration (years) (mean, [min-max])		2 [1-14]	2 [1-12]	0.054*
Microchip		Number of Patients		
		DGC	*p* value	
Infertility Type (n, %)	Primary	110 (61.80%)	22 (56.21%)	0.484#
	Secondary	68 (38.20%)	17 (43.59%)	
Cause of Infertility (n, %)	Unexplained	72 (40.45%)	20 (51.28%)	0.052#
	Polycystic ovary syndrome	68 (38.20%)	16 (41.02%)	
	Other	38 (%21.35)	3 (%7.69)	

*,#p value (<0.05) is statistically significant. Statistical analysis was performed using *ANOVA and #Chi-square tests.

Table 3. Change of semen analysis parameters after microchip and DGC method.

	Sperm Concentration (10^6/mL)		
	Before Preparation	After Preparation	*p* value
Microchip (n=178) (mean ± sd)	69.48±50.83	27.61±27.97	
DGC (n=39) (mean ± sd)	35.79±21.13	29.00±20.08	0.037$
	Sperm Motility (%)		
	Before Preparation	After Preparation	*p* value
Microchip (n=178) (mean ± sd)	30.43±17.79	57.48±20.24	
DGC (n=39) (mean ± sd)	39.03±10.05	79.46±12.48	<0.001$

$p value (<0.05) is statistically significant. Statistical analysis was performed using Dunnett test.

Table 4. Pregnancy rates of sperm samples prepared by microchip and DGC method after IUI.

		Sperm Preparation Technique		
	Microchip	DGC	*p* value	
	Number of Patients			
Clinical Pregnancy (n, %)	None (158)	137 (76.97%)	21 (53.85%)	0.012#
	Available (59)	41 (23.03%)	18 (46.15%)	
Progress of Pregnancy (n, %)	Continuing (27)	22 (53.66%)	5 (27.78%)	0.019#
	Discontinued (32)	19 (46.34%)	13 (72.22%)	

#p value (<0.05) is statistically significant. Statistical analysis was performed using Chi-square test.

(p<0.05). Accordingly, the clinical pregnancy rate was higher in sperm preparation with the DGC method compared to the microchip method. However, the continuation status of clinical pregnancy was determined higher in the microchip method compared to the DGC method (Table 4).

DISCUSSION

Today, in the treatment of infertility, it is quite common to use the IUI method as the initial treatment before the application of complex in-vitro methods such as IVF and ICSI. Numerous experimental and clinical studies reported that many clinical parameters related to men and women affect the success rate of IUI (19-21). In current study, the effects of DGC and microchip sperm preparation techniques were investigated on sperm parameters (concentration and motility) and clinical pregnancy success after IUI method. In order to investigate the prognostic factors affecting clinical pregnancy success in patients after IUI application, significant effects of sperm prepared by DGC and microchip methods on clinical success were determined in couples with clinical IUI indication.

The decrease in pregnancy rates after IUI with increasing female age has been reported in the literature. However, there are studies showing that age does not have a limiting effect on the success of IUI in women younger than 30 years of age (22,23). Alorf and co-workers indicated that IUI method may be implemented in women aged <40 years, while this method may be discouraged in women aged ≥43 years (24). Although there was no significance between the mean age of the patients included in the DGC and microchip groups in this study, the fact that the mean patient age was below 30 in both groups minimizes the age factor in the comparison of IUI and pregnancy outcomes.

It has been reported in the literature that infertility duration and IUI method are associated with clinical pregnancy and live birth rates (25). However, the difference in the mean infertility duration of the patients included in the DGC and microchip groups in our study was not significant. In this study, no correlation was found between age, number of cycles, and duration of infertility and IUI success in patients included in DGC and microchip groups. Yu et al. reported that the number of cycles did not affect the pregnancy rate in young women. However, the higher pregnancy rates detected in the second and third cycles in older women (26).

Primary infertility was detected in more than 50% of the patients included in the DGC and microchip group. In the study of Botchan et al., no significant difference was found between primary and secondary infertile on clinical pregnancy success after the application of the IUI method (27). Furthermore, the correlation between the cause of infertility and the success of IUI is not found in the literature.

Sperm concentration and motility are accepted as major factors affecting the success of IUI among semen parameters. In the literature, the critical values of sperm concentration and motile sperm percentage have been

reported in many studies for the success of the IUI method. In a study conducted by Richard P. Dickey et al. on 1841 fertile couples, 2x106/ml sperm concentration and 17% motile sperm ratio were determined as the threshold value for IUI success. The success of the IUI method is significantly increased if the sperm concentration is >5x106/ml and the motile sperm ratio is >30% (28). In a study by Yalti S. et al. determined that the incidence of clinical pregnancy increased approximately four times in those with sperm motility ≥30% compared to those with <30% (29). Ahmed Badawy et al. indicated that the clinical success of the IUI method increased in 389 fertile couples as the motile sperm count increased. When patients with motile sperm count >5x106 and patients with motile sperm count <5x106 were compared, it was determined that the clinical pregnancy rate per cycle increased from 5.55% to 24.28% (30). In summary, the incensement in sperm concentration and motility enhances the clinical pregnancy rates in the IUI method. In our study, sperm concentrations in the microchip and DGC groups were calculated as 27.61±27.97 106/ml and 29.0±20.08 106/ml, respectively. Also, the percentage of total motile sperm was determined as 57.48±20.24 and 79.46±12.48% in microchip and DGC group, respectively. The data indicated that sperm concentration and motile sperm percentage in sperm preparation with DGC and microchip methods are above critical values for the successful application of the IUI method.

In our study, the success of clinical pregnancy was higher in the DGC group (46.15%) compared to microchip group (23.03%) after IUI application. On the other hand, the continuation of pregnancy status was higher in the microchip group (53.66%) compared to the DGC group (27.78%). According to the results, the sperm prepared with the microchip method increased the ongoing pregnancy rate compared to the sperm prepared with the DGC method in the IUI method. Clinical studies indicated that more motile sperm are obtained in the DGC method compared to other sperm preparation techniques (31). In this method, the number of apoptotic sperm increases with the amount of reactive oxygen species formed during centrifugation at high speed. Thus, the success of IUI with sperm prepared by the DGC method shows serious decreases in the continuation of clinical pregnancy (15,32). In recent years, the use of microchip method in IUI applications for the elimination of DNA damaged and apoptotic sperm provides serious advantages in the continuation of clinical pregnancy (33). In a retrospective study, 112 couples diagnosed with infertility and IUI was applied with sperm samples prepared by DGC and microchip methods. According to the results, the rate of motile sperm was measured as 93±3.23% in the DGC method and this rate was determined as 88±4.91% in the microchip method. The incidence of pregnancy after IUI was 17.39% in the DGC group, while it was 22.73% in the microchip group. In parallel with our results, although more motile sperm were obtained in the DGC method, the continued pregnancy rate was higher in the microchip group (34). Recently, Mirsanei et al. reported that microchip sperm preparation method improves fertilization rate in patients with poor fertilization outcomes following intracytoplasmic sperm injection technique (35). In another study, the effect of microchip and DGC methods on ongoing pregnancy rates of patients in IUI. The results demonstrated that microchip method improved motile sperm rates and ongoing pregnancy (36).

CONCLUSION

In summary, more motile sperm were obtained with the DGC method and the percentage of clinical pregnancy after IUI application was higher in the DGC group when compared to the microchip method. On the other hand, the percentage of continuation of pregnancy was determined to be lower compared to the microchip method. Therefore, sperm preparation with microchip method in IUI applications provides more success in the continuation of pregnancy.

Ethics Committee Approval: The study protocol was approved by Izmir Bakırçay University Non-Invasive Clinical Research Ethics Committee (decision no. 650, 29.06.2022).

Informed Consent: Informed consent was provided from all patients who wanted participated in the study.

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