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ASSISTED REPRODUCTION TECHNOLOGIES

The gradient technique improves success rates in intrauterine insemination cycles of unexplained subfertile couples when compared to swim up technique; a prospective randomized study.

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Abstract

Purpose To compare the efficacy of gradient and swim-up semen preparation techniques on pregnancy rates in couples undergoing intrauterine insemination (IUI) cycles with low dose gonadotropin stimulation with the diagnosis of unexplained or mild male subfertility.

Methods Two hundred and twenty three couples were randomized into swim up or gradient technique groups for sperm preperation. The clinical and on going pregnancy rates per cycle and per patient were evaluated.

Results Both clinical and ongoing pregnancy rates per cycle were significantly higher in the “gradient” group (19 % and 16.9 %) in comparision with the “swim up” group (9.7 % and 6.9 %) ($p<0.05$). Clinical pregnancy and on-going pregnancy rates per patient were higher in the “gradient” group (26.1 % and 23.4 %) when compared to the “swim up” group (15.2 % and 10.7 %), ($p<0.05$). In the subgroup of 191 unexplained subfertile couples with 290 cycles; the “gradient” group also revealed significantly higher clinical and ongoing pregnancy rates per cycle (21.6 % and 17.9 %) when compared with the “swim up” group (10.3 % and 7.1 %) ($p<0.05$). In total of 48 treatment cycles upon 32 couples with mild male factor subfertility no significant difference were found between the two sperm preparation techniques in terms of clinical (% 5.3 vs %6.9, $p>0.05$) and ongoing (% 5.3 vs %6.9, $p>0.05$) pregnancy rates per cycle.

Conclusion The gradient technique significantly improves clinical outcome in IUI cycles of unexplained subfertile couples when compared to swim up technique. In male subfertile patients, both techniques yield similar clinical outcomes.

Keywords Intrauterine insemination · Sperm preperation · Swim up · Gradient

Capsule The gradient technique significantly enhanced pregnancy rates in unexplained subfertile patients with favorable sperm parameters when compared to swim up technique.

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Introduction

Intra uterine insemination (IUI) has been the standard first line treatment approach in infertility centers for those patients with normal or mild male factors and couples with unexplained infertility [1, 2]. Along the years, the progressive maternal advanced age and the greater improvements in assited reproductive technique laboratories and in cryopreservation has lead to a progressive shift towards in vitro procedures even in those categories of patients with normal or slightly abnormal semen profiles. The main reason attributed to this choice is related to the adoption of a standard stimulation protocol that allows to recover a higher number of eggs and consequently imply the opportunity to have more oocyte/embryos for further attempts . However, in most cases IUI could still have a place in infertility treatment giving an answer both to the anxiety of couples not wishing invasive treatments and to gradual approach at some stage indicated by national regulations, legal restrictions or personal ethical concern.

There are several factors infuencing on the success rate of IUI; some of these are clinical parametrs (i.e.; sperm parameters, female age, etc.) and some are related to IUI technique (i.e.; catheter type, time of insemination, etc.). Although the sperm preparation method is the crucial step of IUI, less attention has been paid to the effect of sperm preparation techniques on IUI outcome.

The basic premise of any sperm preparation technique is to (a) eliminate any factors detrimental to fertilization, (b) to block factors such as prostaglandins that would otherwise cause uterine contractions, (c) to increase sperm concentration, motility and (d) to form sperm capacitation. This is to be achieved by separating the seminal plasma from spermatozoas in a rapid and efficient fashion [3].

Up to now various sperm preparation techniques have been described. The most commonly used methods in use are; standard sperm wash, swim-up and density gradient centrifugation. Standard sperm wash removes seminal plasma from the semen specimen by centrifugation. Swim-up technique is based on migration of motile sperm from the bottom to the top fraction of the media, which separates motile sperms from the non-motile sperm and debris. Density gradient centrifugation is a procedure which selects motile sperms according to the their densities. Motile sperm have higher density than non-motile and dead sperm. Therefore, spermatozoa with high motility capacity and optimal morphology can be selected. It has been shown that sperm preparation for insemination yields better pregnancy rates compared to unprepared ejaculates [4]. However, there remains a discussion about which semen preparation procedure should be used as a first line procedure. Lack of large, randomized controlled trials comparing the effectiveness of sperm preparation techniques on IUI success might be the reason for this conflict. There are only a few studies in the literature that compare “swim-up” with “density gradient centrifugation” in which different conclusions were reached [5–7]. Heterogenity of these studies with regard to the infertility etiologies, study design and ovulation induction strategies might have weaken the power of results. Nonetheless in the meta-analysis investigating different sperm preperation techniques on IUI sucsess, it was concluded that data is insufficient to conclude any sperm preperation tecnique was superior [8].

The aim of this prospective randomized trial was to compare the efficacy of mostly used semen preparation techniques of density gradient and swim-up on the outcome of IUI cycles in unexplained and mild male factor subfertile couples. Unlike other studies in the current literature, this study encompasses a homogenous group treatment protocol in which low dose gonadotropin was used for ovarian hyperstimulation.

Materials and methods

Patients and study protocol

This was a single-center prospective randomized controlled trial comparing the effectiveness of “gradient” and “swim up” sperm preparation methods in couples undergoing ovarian stimulation and IUI cycles with the diagnosis of unexplained infertility or mild male subfertility. Two hundred and twenty three couples undergoing a total of 338 cycles involving ovarian hyperstimulation achieved with low dose recombinat FSH in our institution from January 2009 to April 2010 were included in the study. The study protocol was approved by our Faculty Ethics Committee and written informed consent was obtained from all patients.

All of the patients underwent a complete infertility evaluation including mid-luteal progesteron levels to assess ovulation, hysterosalpingogram (HSG), and semen analysis. Inclusion criteria for unexplained subfertility were as follows: failure to conceive for 12 months of unprotected regular intercourse, female age between 20-40 years,regular spontaneous menstrual cycles with mid-luteal progesteron levels of >3 ng/mL, basal FSH levels≤15 IU/l, bilateral tubal patency confirmed with HSG and normal semen features according to World Health Organization criteria [9]. Patients that fulfilled the above criteria but had a initial sperm count in the 5-15 $\times 10^6$/ml range were classified as patients with mild male subfertility. Patients with endocrine disorders (polycystic ovarian syndrome, abnormal thyroid function and prolactin levels, hypogonadotropic hypogonadism), prior ovarian surgery, prior IUI cycles with either CC or gonadotropins, moderate to severe endometriosis (American Fertility Society, stage III or IV), any contraindications for one of the investigated drugs or persistent ovarian cysts (>19 mm and >2 months) were excluded from the study.

Ovarian Stimulation Protocol

All patients underwent baseline transvaginal ultrasonography (TVU) on day 3 of the menstrual cycle and were then treated with a starting dose of 75-100 IU recombinant FSH (Gonal-F; Serono, Istanbul, Turkey; and Puregon; Organon, Istanbul, Turkey). Ovarian response and endometrial thickness was asseses with TVU starting from 7-8 day of cycles. If the leading follicle’sdiameter was<10 mm at the 8th day of stimulation, the dose of gonadotropin was increased by 50 %. The gonadotopin dose remained the same untill the day of hCG trigger after the leading follicle reached to 12 mm diameter. Cycles were triggered with 250µcg recombinant hCG (Ovitrelle; Serono, xxx, xxx) when at leastone dominant follicle had reached 18 mm in diameter. Cycles with more than three dominant follicles and/or estradiol levels>1500 pg/ml were cancelled to avoid ovarian hiperstimulation syndrome and high-order multiple pregnancy. IUI was performed 36 h after hCG administration with a disposable IUI catheter (Embryon; Rocket Medical, Washington,Tyne and Wear, U.K.) by two of the authors. Thepatient rested in a supine position for 15 min after theprocedure. Luteal phase progesterone support was initiated 2 days following insemination and carried on until a pregnancy test was performed. Luteal phase progesterone support was administered in the form of 600 mg/day micronized vaginal progesterone (Progestan, Kocak; Istanbul, Turkey). Patients were contacted on the 14th day post- insemination for a pregnancy test and were evaluated with a β-hCG sample. Patients that were pregnant continued to receive progesterone support up until the 8th week of gestation.

Semen preperation procedure

All semen samples used in insemination were prepared at the Andrology Laboratory of our University IVF clinic. Sperm samples were collected after 3-5 days of abstinence, on the day of the procedure. Basal sperm morphology, count and motility was assesed after the liquefaction of ejaculates. The sperm preparation technique was randomized in all patients; either "swim-up" or the "gradient" technique was used. The sperm preparation method was based on a randomization table in an SPSS Statistical Package for Social Science, version 11.0 for Windows) statistical data-base. The sperm preparation technique for each patients was choosen by the laboratory staff in order to the randomization table and the physicians conducting the insemination were blinded to which preparation technique was to be performed. Once a couple was randomized to one of the two sperm preperation techniques they remained in the same group during the entire study. The randomization results (the sperm preparation technique appointed) were revealed and evaluated after the study was completed.

Swim-Up Technique

Semen samples were kept in an incubator for 20 min during which liquifaction was completed. Following this, the sperm preparation process was initiated. 5 μL of semen was taken from the sample in order to assess sperm count and motility prior the preparation process. After the microscopic evaluation of the sample, the remaining semen was washed with the "swim-up" technique. This method, based on the "swimming-up" or separation of progressively motile sperm in a medium, starts by placing the semen sample in a conical tube and adding a 1:1 dilute of medium (Sperm Rinse Solution, Vitro Life, Sweden) then centrifuging the mixture at 900 rpm for 10 min. The supernatant was extracted with the aid of a micropipette. The tube was then placed on a stand and tilted at an angle of 45°; 0.25 ml of medium was again added onto the pellet, then incubated for 1 h at 37 °C without changing this angle. A sample was again taken from the supernatant and sperm count and motility was checked and noted. Lastly, 0.3-0.5 ml of the upper sperm solution was extracted without disturbing the angle of the tube and this solution was placed in a 5 ml falcon tube ready for insemination.

The gradient technique

A mixture of bicarbonate and hepes- buffered silane coated colloid silica solution (Sperm Grad-125, Vitrolife) was used as a gradient solution. Firstly, this solution was diluted with G-IVF Plus (Vitrolife, Sweden) in order to produce two different concentrations (40 % and 90 %) of solution. The 90 % dilute was placed at the bottom of a conical falcon tube and the 40 % dilute was placed upon this. Care was taken to avoid mixture of both solutions. The tube was incubated at 37 °C for 10–15 min, after which the semen was gradually added with a micropipette. The solution was then centrifuged at 1400 rpm for 10 min. The upper part was then carefully removed and disposed of with a pipette. The pellet at the bottom was the placed into a 4 ml falcon tube. After adding 3 ml of sperm washing solution (G-IVF plus, Vitrolife, Sweden) the solution was centrifuged at 1400 rpm for 10 min. After the upper portion was removed, the remaining 0.3–0.5 ml sperm solution was placed into a falcon tube with a pipette, ready for insemination.

Detection of pregnancy

Pregnancy testing was performed by determining the quantitative serum β- hCG level at 14 days after hCG administration, hCG>50 IU/L were considered as biochemical pregnancy. Transvaginal ultrasound was performed to confirm intrauterine pregnancy 1 week later, and fetal viability was assessed 3 weeks later. A clinical pregnancy was defined as the presence of a gestational sac on TVU or by histologic examination of products of conception in patients who were aborted. Ongoing pregnancy was defined as presence of a viable fetus detected after 12 weeks of pregnancy.

Outcome measures and statistical analysis

The primary outcome measures were clinical pregnancy and ongoing pregnancy. The Statistical Program for Social Sciences (SPSS, version 11.5; SPSS, Chicago, IL) was used for statistical analysis. Demographic data was expressed as mean±SD, and comparison of these data was performed by Student-*t* and *chi*-squared tests. Comparison of clinical pregnancy and ongoing pregnancy rates between groups were performed by chi-squared test. A P value of $<.05$ was considered to be statistically significant. The sample size was computed at the beginning of the study by an online analyser (http://homepage.stat.uiowa.edu/~rlenth/Power). For an expected difference of 10 % between the two methods (10 % vs 20 %) a sample size of 280 patients was required for a statistical power of 90 % at a level of a 5 % significance. Estimating that each couple should complete two cycles for the study, half the estimated sample size (approximately 140 patients for each arm) was estimated for each arm.

Results

Three hundred and thirty eight controlled ovarian hiperstimulation cycles with IUI were performed in 223 patients. One hundred and ninty one (85.6 %) of 223 patients had unexplained and 32 (14.4 %) had male subfertility. After randomization, 112 (50.5 %) couples received the"'swim-up" technique and 111 (49.5 %) couples had the "'gradient "technique as a semen preparation procedure. Demographic and cycle characteristics of the two groups are shown in Table 1. There were no significant differences noted between the two groups in terms of age, duration of subfertility, basal sperm count and morphology. Basal sperm motility measured was significantly higher in the swim-up group when compared to the gradient group (64.7±16.1 vs 60.1±16.9, p=0.03). The total dose of gonadotropin throughout the cycle, the duration of ovarian stimulation, the number of dominant follicles (≥ 16 mm) and the endometrial thickness on the day of hCG were not significantly different between the two groups.

Data retrieved from the entire group of participants (unexplained plus male factor subsets) were demonstrated in Table 2,3 . Biochemical, clinical and ongoing pregnancy rates per cycle were significantly higher in the "gradient" group (20.9 %, 19 % and 16.9 % respectively) in comparision to the "swim up" group (12.6 %, 9.7 % and 6.9 %, respectively) (p<0.05). (Table 2) Biochemical pregnancy, clinical pregnancy and on-going pregnancy rates per patient were higher in the"gradient" group (16.9 %, 15.2 % and 10.7 %, respectively) when compared to the "swim up" group (28.8 %, 26.1 % and 23.4 %, respectively) (p<0.05). (Table 3)

A hundred and ninety one couples with unexplained subfertility underwent a total of 290 treatment cycles. The "gradient" group revealed significantly higher biochemical pregnancy, and ongoing (17.9 %) pregnancy rates per cycle (23.9 %, 21.6 % and 17.9 %,respectively) when compared with the "swim up" group (12.2 %, 10.3 % and 7.1 % respectively) (p<0.05). A total of 48 treatment cycles were performed upon 32 couples with mild male factor subfertility. When both methods of sperm preparation were compared in this group, data did not vary significantly in biochemical,clinical or ongoing pregnancy rates (p>0.05).

No significant difference was observed between swim-up and gradient grups with regard to sperm concentration, normal sperm morphology percentage, progressive sperm motility percentage after sperm preparation procedure (19.8±14.1vs 20.6±16.2; 4.2±8.4 vs 4.05±4.01 and 79.4±14.5 vs 76.4±16.6, respectively, p 0.05). There was no statistically significant difference in normal sperm concntration, morpohology and progressive motility percentage changes either sperm preparation was achieved by gradient or swim-up technique (Table 4).

Discussion

Sperm preparation is the crucial procedure in IUI treatment. The sperm preparation method used in IUI may have an important impact on IUI success. However, studies evaluating the effect of sperm preparation methods on IUI prognosis are quantitively and qualititively inconclusive [8]. The lack of evidence based data on this topic prompted us to design a prospective randomized study that included a homogenous study population. We aimed to compare the effectiveness of the two mostly used sperm preparation techniques (swim-up vs dansity gradient) on IUI sucsess. The study group comprised a homogenous group of patients undergoing ovarian stimulation with a low dose gonadotropin protocol, where the majority had unexplained subfertility and some had defined mild male factor subfertility. By selecting a clearly defined

Table 1 Demographic and ovarian stimulation cycle characteristics of patients undergoing semen preparation with swim-up or gradient technique for IUI

	Swim-up (*N*_112)	Gradient (*N*_111)	*P* value
Female Age (years)	29.2±4.7	28.9±4.9	0.7
Male Age (years)	32.4±4.6	31.9±5.4	0.4
Duration of Infertility (years)	4.5±7.0	3,8±2.3	0.3
Primary Infertility (n/%)	92(%82)	93(%84)	0.8
Unexplained Infertility (n/%)	100(%89)	91(%82)	0.1
Male Factor Infertility (n/%)	12(%11)	20(%18)	0.1
Duration of stimulation (days)	9.3±3.2	9.2±3.1	0.3
Total gonadotropin dose (IU)	820.6±462.5	798.5±446.0	0.7
Follicle number (≥16 mm) on the day of hCG	1.5±1.2	1.4±0.9	0.6
Endometrial thickness on the day of hCG (mm)	10.8±1.8	10.7±1.4	0.5
Basal Sperm Concentration (x10^6/ml)	46.6±24.9	40.4±26.3	0.7
Basal Sperm Motility (%)	64.7±16.1	60.1±16.9	0.03*
Basal Normal Sperm Morphology (%)	1.4±2.2	1.5±2.0	0.8

Shows significant difference

Table 2 Biochemical, clinical and on-going pregnancy rates per cycle in total study group and unexplained, male subfertile subgroups

	Swim-Up	Gradient	*P* value
Study Group			
Biochemical pregnancy rate per cycle (%)	22/175 (%12.6)	34/163 (%20.9)	0.049*
Clinical pregnancy rate per cycle (%)	17/175 (% 9.7)	31/163 (%19.0)	0.019*
On going pregnancy rate per cycle (%)	12/175 (% 6.9)	26/163 (%16.0)	0.010*
Unexplained Infertility			
Biochemical pregnancy rate per cycle (%)	19/156 (%12.2)	32/134 (%23.9)	0.013*
Clinical pregnancy rate per cycle (%)	16/156 (%10.3)	32/134(%21.6)	0.009*
On going pregnancy rate per cycle (%)	11/156 (% 7.1)	32/134 (%17.9)	0.006*
Male Subfertility			
Biochemical pregnancy rate per cycle (%)	3/19 (%15.8	2/29 (%6.9)	0.37
Clinical pregnancy rate per cycle (%)	1/19 (% 5.3)	2/29 (%6.9)	0.37
On going pregnancy rate per cycle (%)	1/19 (% 5.3)	2/29 (%6.9)	0.37

Shows significant difference

subgroup of patients we have tried to minimalize the impact of other variables.

Our study clearly demonstrated the effectiveness of the gradient technique compared to swim up as a sperm preparation method with a favorable IUI sucsess in a group of unexplained subfertile couples. On the other hand, in the "mild male factor" subfertile group- comprising a small subset of the study group- the success rates were not significantly differed by the two sperm preparation techniques used. This confirms that the efficacy of the gradient method is more pronounced in those couples with the diagnosis of unexplained subfertility where sperm parameters are in the normal range.

A few studies comparing these two sperm preparation methods show inconsistent results. In a prospective study including 363 patients and a total of 898 treatment cycles, five different sperm preparation techniques were evaluated with respect to IUI success [6]. The methods compared were; percoll gradient (double layer), swim-up, swim- down, simple washing and the refrigeration/heparin techniques. According to this study, the ongoing pregnancy rates were significantly higher in the swim-up and percoll gradient groups (13.2 % and 12.7 % respectively) in comparison to the swim-down and simple washing techniques (6.1 % and 7 % respectively). However, no significant difference was demontrated when the efficiency of the swim- up and Percoll gradient techniques were compared [6]. In this study, patients with a sperm concentration of under $20x10^6$/ml were not included. The limitation of this study is that it included a non-homogenous group of patients with different etiologies of infertility and that the patients underwent various different hyperstimulation protocols. In addition to this, a major diasadvantage is the "cross-over" design enabled patients to receive a different method of treatment in every cycle.

In a study conducted by Dodson et al. the double centrifuge, multiple- tube swim-up and Percoll gradient techniques were compared [5]. In this study the sperm preparation

Table 3 Biochemical, clinical and on-going pregnancy rates per patient in total study group and unexplained, male subfertile subgroups

	Swim-Up	Gradient	*P* value
Study Group			
Biochemical pregnancy rate per patient (%)	19/112(%16.9)	32/111(%28.8)	0.035*
Clinical pregnancy rate per patient (%)	17/112(%15.2)	29/111(%26.1)	0.048*
On going pregnancy rate per patient (%)	12/112 (%10.7)	26/111 (%23.4)	0.013*
Unexplained Infertility			
Biochemical pregnancy rate per patient (%)	17/100 (%17.0)	30/91(%33.0)	0.012*
Clinical pregnancy rate per patient (%) On-	16/100(%16.0)	27/91(%29.7)	0.026*
going pregnancy rate per patient (%)	11/100(%11.0)	24/91(%26.4)	0.008*
Male Subfertility			
Biochemical pregnancy rate per patient (%)	2/12(%16.7)	2/20(%10)	0.06
Clinical pregnancy rate per patient (%)	1/12(% 8.3)	2/20(%10)	0.43
On-going pregnancy rate per patient (%)	1/12(% 8.3)	2/20(%10)	0.43

Table 4 Concentration, morphology and progressive motility percentage changes after sperm preperation with swim-up or gradient techniques

	Swim-Up	Gradient	*P*
Total Group			
Concentration (n x 10^6/mL) (SCASP- BSC)	19.8±14.1	20.6±16.2	0.68
Morphology (%) (NSMASP-BNSM)	3.1±9.8	2.7±2.7	0.72
Progressive Motility (%) (PSMASP-BPSM)	23.2±15.4	24.7±13.4	0.43
Unexplained Infertility			
Concentration (n x 10^6/mL) (SCASP- BSC)	22.7±18.1	21.5±18.2	0.77
Morphology (%) (NSMASP-BNSM)	3.2±10.1	3.1±2.8	0.85
Progressive Motility (%) (PSMASP-BPSM)	23.2±15.8	24.4±13.6	0.56
Male Subfertility			
Concentration (n x 10^6/mL) (SCASP- BSC)	6.5±5.9	7.1±5.1	0.8
Morphology (%) (NSMASP-BNSM)	0.7±1.6	0.7±0.8	0.9
Progressive Motility (%) (PSMASP-BPSM)	23.1±11.2	26±12.9	0.53

BSC Basal Sperm Concentration, *SCASP* Sperm Concentration After Sperm Preperation, *BNSM* Basal Normal Sperm Morphology (%), *NSMASP* Normal Sperm Morphology After Sperm Preperation (%), *BPSM* Basal Progressive Sperm Motility (%), *PSMASP* Progressive Sperm Motility After Sperm Preperation (%)

technique was selected via a computer based randomized table and the IUI indications included; 49 % unexplained, 33 % endometriosis, 13 % minor pelvic adhesions and 6 % male factor. It was concluded that non of the sperm preparation techniques were found to be superior to any other with respect to cycle fecundity [5]. Posada et al. compared the swim-up and gradient techniques prepared for IUI in terms of cost effectiveness and clinical pregnancy rates [7]. The results of this study showed that gradient technique cost twice the price as swim-up, while the clinical pregnancy rate was significantly higher in the swim-up group (28.2 %) as compared to gradient group (8.3 %) [7]. However, higher number of motile sperms found in the swim-up group before washing in this study might possibly effect the pregnancy rates in favor of the swim up group

In contrary to our own results that neither methods was superior to each other in male factor cases, it has become a well accepted general view that the gradient technique should be preferred in IUI cycles where the sperm count is either low or sperm motility is restricted. In a study conducted by Morshedi et al. including 311 couples with 676 cycles comparing the simple washing and the gradient method no significant difference was obseved in pregnancy rates. However in the subgroup including patients with a low sperm count (sperm concentration <22 million/ml), the gradient technique yielded greater pregnancy rates [10]. On the other hand; randomization based on particular days of the month and the use of varying protocols (ie, natural cycles, clomiphen citrate, clomiphen citrate+gonadotropins) comprising a non-uniform study population are the main limitations of this study. In another study, a commercial percoll gradient kit was compared to simple sperm washing preparation, and the percoll gradient technique yielded beter results in patients with severe male factor infertility [11]. These results confirm the superiority of the gradient method, however the aforementioned studies do not compare the efficacy of the gradient and swim-up techniques and therefore do not resemble our study. Although, our data do not show any difference between either methods of sperm preparation on IUI success in mild "mild male factor" subgroup, it might not be possible to make a certain conclusion as the number of cases in this group was limited. In fact, mild male factor subfertility have recently shown to have a negative impact on IUI outcome reflected by live birth rates [12].

Despite our evidence suggesting an increase in pregnancy rates with the gradient method, our results do not reveal any probable clues associated with its explanatory mechanism. An efficient sperm preparation technique involves the removal of cell debris and immotile sperm whilst separating and making the more progressively motile sperm available for use, and the selection of morpholologicaly/chromosomally normal sperms with high fertilazition capacity. The swim-up sperm preparation method is based on the ability of the spermatozoa to swim. In this procedure, the motile spermatozoa swim up to the culture medium layered over the liquified semen. Density gradient centrifugation separates spermatozoa according to their density. By this way the motile, morphologically normal spermatozoa can be selected in the solution with the highest concentration of gradient [13]. Studies show that the gradient method effieciently selects sperm with better DNA and chromatin structures, that is to say, sperm with a higher fertilization potential [14, 15]. Prakash et al's study comparing the swim up and percoll gradient methods' success in selecting sperm with normal morphology for IVF cycles revealed different results. In this study each sperm sample was divided into two and preapared with both methods. Their data suggested that the sperm prepared with the percoll gradient technique collected sperm with better morphology in comparison to the swim up technique [16] . In contradiction to other studies, there was no statistically significant difference in normal sperm morpohology and progressive motility percentage changes either sperm preparation was achieved by gradient or swim-up technique according to our study data. This finding was also evident in the "unexplained infertility" group. In conclusion, the gradient method may have effects on other sperm properties that may increase ferilization capacity. As the effects of washing technique on sperm chromatins and DNA characteristics, which alter the fertilization potential indirectly were not investigated in our study, it is not possible to comment on these issues. The only study that compared sperm chromatin and DNA content after gradient and swim-up preparation showed that the gradient technique was much more successful [15].

As a result, the gradient technique significantly enhanced pregnancy rates in a group of unexplained subfertile patients with favorable sperm parameters when compared to swim up tecnique. In contrast to current literature, our data did not support the view that the gradient technique was more efficient in patients with male factor subferility. In male subfertile patients both techniques yield similar pregnancy rates. However, our male subfertile population limits the value of this finding.

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