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# INTRAUTERINE INSEMINATION OUTCOME USING TOW SPERM PREPARATION TECHNIQUES CENTRIFUGATION SWIM-UP AND DENSITY GRADIENT TECHNIQUES

**Dr. Sahar Muhssin Muhammad M.B.Ch.B., D.G.O.,**

**MSc.ART, Dr.Samira A MuhsinM.B.Ch.B., D.G.O., MSc. ART[2]**

[1]*Alimamain Alkahdimian Medical City, Um Albanin center for infertility treatment & IVF.*

[2]*Alimamain Alkahdimian Medical City, Um Albanin center for infertility treatment & IVF.*

**ABSTRACT**

***Background:*** *Artificial insemination (AI) is a procedure in which sperm are placed in the cervix or high in the uterine cavity through a transcervical catheter. The aim is to deposit sperm as close to the oocyte as possible (ASRM, 2006). It may be preceded by ovarian stimulation with gonadotropins or clomiphene to encourage multiple oocytes development, especially in cases of unexplained infertility (Mathuret al., 2007**).**Sperm separation procedures are able to significantly improve the sperm quality, such selection of spermatozoa separates motile sperm from nonmotile, removes seminal plasma, and infectious agents, other background materials and debris (Zavos, 1992) and also in the same time initiates the capacitation of sperm (Henkel and Schill, 2003).*

***Patients, materials and methods:*** *One hundred thirty-five infertile couples were participated in this study where the male partners needed in vitro sperm preparation and activation, while the female partners were normal and subjected to ovarian stimulation program. In vitro sperm activation was performed using two different techniques, the centrifugation swim-up technique with Global medium and density gradient technique with ALLGrad solution and Global medium. Randomly the infertile males shared in this study were grouped into two groups according to type of technique used in sperm preparation. In the first group the centrifugation swim-up technique was applied for 70 infertile males, while in the other group 65 infertile males have density gradient technique for their in vitro sperm activation. Both groups were subjected to IUI.*

***Results:*** *Intrauterine insemination was applied for the wives of all infertile males involved in this study after having in vitro preparation for their sperm samples, among the 135 couples involved in the present study, 16 couples have positive IUI outcome, 9 couples were in the group used density gradient technique with percentage of (13.8%) which is higher than the percentage of positive IUI outcome in the infertile couples used centrifugation swim-up method where it was (10%) and count of 7 from 70 patients used that technique.*

***Conclusions:*** *Density gradient techniques with ALLGrad solution has better IUI outcome compared to centrifugation swim-up technique. An improvement of percentages of sperm progressive motility, grade (A) progressive sperm motility, normal sperm morphology that were higher in density gradient technique were the main factors that enhance the IUI outcome.*

***Keywords:*** *Sperm preparation techniques, Density gradient techniques, centrifugation swim-up technique, IUI.*

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## INTRODUCTION:

Male infertility refers to the inability of a male to achieve a pregnancy in a fertile female, and it is commonly due to deficiencies in the semen and semen quality that is used as a surrogate measure of male infertility (AUA, 2010). Furthermore, assisted reproductive technologies not only permit the completion of sperm diagnostic testing, but also provide efficient means to overcome multiple sperm deficiencies.

For ART, fertilization and pregnancy are the main outcome measures; multiple sperm characteristics are presently being evaluated in an attempt to accurately predict results. (Sergio, 2000). Therefore, when a male factor is found during evaluation of a couple for infertility, a complete evaluation of the man is warranted. If treatable conditions causing the male factor are found, they should be corrected. If treatment is unsuccessful, or if the couple still does not conceive, then assisted reproduction is indicated (Harris and Sandlow, 2008).

More sophisticated techniques have been developed to separate functional spermatozoa from those that are immotile, have poor morphology or are not capable to fertilize oocytes (Hansen *et al.*, 2005). Initially, starting from simple washing of spermatozoa, separation techniques based on different principles like migration, filtration or density gradient centrifugation have hence evolved (Henkel and Schill, 2003).

Intrauterine insemination has been a method of infertility treatment utilized by physicians for the past several decades. Initially used only as a treatment for male factor infertility, the indications of IUI have broadened, as it is currently implemented for treatment of immunologic infertility (the presence of anti-sperm antibodies), unexplained infertility, cervical factor infertility, and as an adjunct to Clomid or FSH therapy (AUA, 2010). Also it is usually used in cases were the man has slight deficiency in the number or motility of the sperms, it is also done in cases of negative post coital test (due to either antibodies or thick cervical mucus) (Hurst, 2001)**.**

Intrauterine insemination or artificial insemination as it is commonly known, therefore, involves the preparation of a semen sample in the laboratory followed by its direct placement into the uterine cavity for fertilization (Clarke *et al.*, 1997). If the semen is not washed it may elicit uterine cramping, expelling the semen and causing pain, due to content of prostaglandine. The woman should rest on the table for 15 minutes after an IUI to optimize the pregnancy rate (Laurie, 2009).

## MATERIALS AND METHODS:

One hundred and thirty five infertile couples were participated in this study where the male partners were subjected to sperm activation in preparation for IUI, while the female partners were having normal ovulatory cycle and were subjected tocontroled ovarian stimulation program.The clinical assessment was evaluated for the couple. Complete history pubertal andpubertal mumps, alcohol consumption, and smoking habit are reported. History of surgical operation, occupation, were also reported.

Instruments and equipments used in the present study including: Centrifuge (D-78532), Disposable Petri-dish (D-810), Air incubator (B-6060), Air incubator (B-6060),Glass Pasteur Pipette (150 mm),Eppendorff pipette,Light microscope (N-200M),Vaginal ultrasound, intra-uterine catheter,Cusco's speculum, sponge forceps.

Different chemicals and culture media were used in this study: Global All Grad Solution, Global medium, Letozole tablets (2.5 mg), Human chorionic gonadotrophin (ovitrelle) 6500 IU, Duphastone tablets (10mg), Normal saline. Seminal fluid analysis:

The sample of seminal fluid was collected after 3-5 days of sexual abstinence directly in to a clean, dry and sterile disposable Petri-dish by masturbation in a private and quite room adjacent to the semen analysis laboratory. The container was labeled with the following information, name, age, abstinence period and time of sample collection. The specimens were placed in an incubator at 37ºC for 30 minutes to allow liquefaction. The liquefied semen is then carefully mixed for few seconds, and then the specimen was examined by macroscopic and microscopic examinations.

*In vitro* sperm activation techniques:

Two methods of *In vitro* sperm activation have been used in this study:

1- Centrifugation swim-up technique with the use of Global medium alone.

2- Density gradient centrifugation technique with the use of ALLGrad solution and Global medium.

**EXPERIMENTAL DESIGN:**

The present study was performed on 135 infertile couples where the male partners had the infertility factors and the female partners were usually normal. Both partners were subjected to clinical and laboratory assessment. The males semen samples were prepared for *in vitro* sperm activation and IUI. The semen samples were divided into two groups:

Group- 1: include 70 samples where the *In Vitro* sperm activation was done by centrifugation swim-up technique using Global medium.

Group- 2: The study group that include 65 samples where the *In Vitro* sperm activation was done density gradient centrifugation technique by using ALLGrad solution with Global medium.

Each group results were correlated to IUI out come. Statistical analysis:

Statistical analysis was performed using theStatistical Package for Social Sciences (SPSS; version 17.00) and also Microsoft Excel worksheet 2007. The data analysis was done using paired sample t-test for tables with mean and standard error of mean (S.E.M) tocompare between pre-and post-activation for sperm parameters. Independent sample t-test for tables with mean and standard error of mean (S.E.M) tocompare between Global media and ALL Grad solution for sperm parameters. P-value < 0.05was used as a level of statistically significance.

**RESULTS:**

The classification of the positive IUI outcome according to the technique of ISA used was noticed in figure (1). Among 135 patients participated in this study, density gradient technique was applied to (65) patients, (9) of them had positive IUI outcome with a percentage of (13.8%), while centrifugation swim up technique was applied to (70) patients and (7) of them had positive IUI outcome with percentage of (10%).

The sperm parameters classified according to the outcome of IUI, pre and post-activation, using centrifugation swim up technique were clarified in table (1). In that table it is clear that percentage of progressive sperm motility grade (B) was significantly decreased ($P<0.05$) in patients with positive IUI outcome compared with those of negative IUI outcome, the same results were obtained for percentage of progressive sperm motility grades (A and B), while there was a significant increment ($P<0.05$) in the percentage of sperm motility grade (C), however, all other parameters showed non significant differences ($P>0.05$) between pregnant and non pregnant patients before activation. However, after activation the percentage of progressive sperm motility grade (A) had a significant increment ($P<0.05$) in pregnant patients compared with non pregnant patients, while percentages of grades (C and D) motility were significantly decreased ($P<0.05$) in patients with positive IUI outcome compared with patients with negative results. On the other hand, in all other parameters there were non significant differences ($P>0.05$) between both groups.

The changes in sperm parameters before and after activation classified according to the IUI outcome using density gradient technique were shown in table (2), according to that table, in all sperm parameters there were non significant differences ($P>0.05$) between pregnant and non pregnant patients before activation, while after activation, sperm concentration increased with highly significant difference ($P<0.001$) in patients with positive IUI outcome compared with negative IUI outcome patients, and the same is true for the percentage progressive sperm motility grade (A). Furthermore, percentage of progressive sperm motility grades (A+B) showed a significant increment ($P<0.05$) in the pregnant patients than non pregnant patients, while the percentages of grade (B) progressive motility and grade (D) motility decreased significantly ($P<0.05$) in patients with positive IUI outcome compared to those with negative IUI outcome. The other sperm parameters showed non significant differences ($P>0.05$) between both groups post activation.

Sperm concentration for IUI positive groups using two techniques was represented in figure (2). It was noticed that the positive IUI outcome was achieved with sperm concentration (51.667±5.869 million/mL)

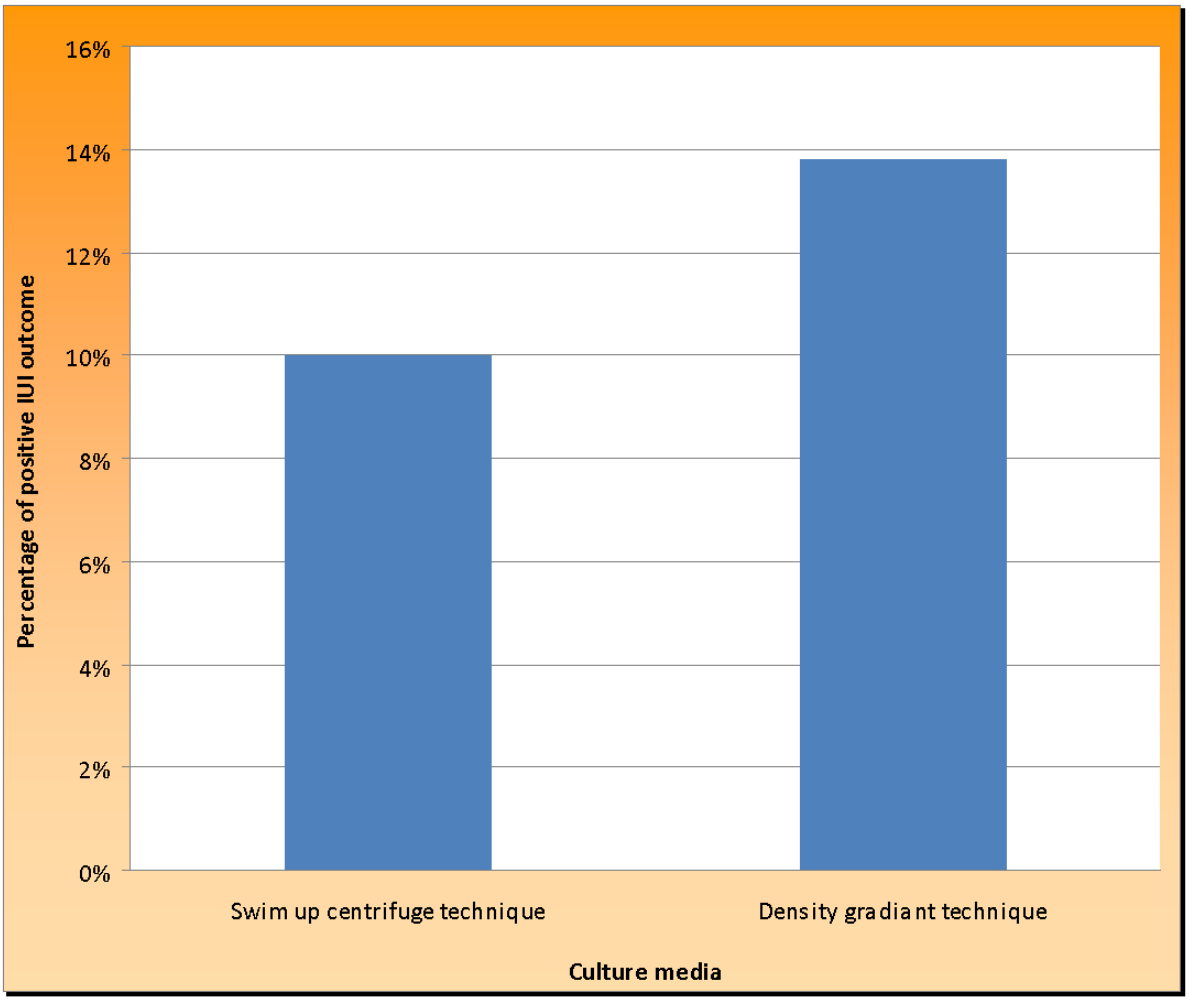
using density gradient technique that was obviously higher than the sperm concentration in positive IUI outcome using centrifugation swim up technique (25.600±4.864 million/mL)

Figure (3) showed the percentage of sperm motility post-activation in the positive IUI groups using the two different techniques, it was clear that the positive IUI outcome was gained with the higher sperm motility percentage (96.333±1.202) using density gradient technique than the lower percentage of sperm motility (93.000±3.742) in positive IUI patients using centrifugation swim up technique.

The post-activation percentage of the sperm motility grades A, B and C in the positive IUI groups using the two different techniques was shown in figure (4). The higher percentage of progressive sperm motility grade (A) (54.000±2.343) was observed in positive IUI groups using density gradient technique, while the percentage of progressive sperm motility grade (A) in positive IUI patients using cntrifugation swim up technique was (40.000±1.200). The percentage of progressive sperm motility grade (B) in positive IUI patients was higher (47.000±4.899) when the centrifugation swim up technique was used than the same percentage in IUI positive patients using the density gradient technique where the percentage was (38.833±2.007). However, the percentage of postactivation sperm motility grade (C) was also higher in positive IUI patients (6.000±1.000) using the centrifugation swim up technique than positive IUI groups when the density gradient technique was used (4.833±1.537).

Figure (5) showed the percentage of progressive sperm motility grades (A+B) in positive IUI patients using different techniques. It was higher (92.500±1.708) in positive IUI patients when the density gradient technique was used than those using the centrifugation swim up technique (87.000±6.63).

Regarding normal sperm morphology percent in positive IUI males , as it shown in figure (6), it was higher (75.000±4.472) in positive IUI groups using the density gradient technique than patients with positive IUI who used the centrifugation swim up technique (59.000±6.000).




**1: Percentage of positive IUI classified according to the two techniques. Centrifugation swim up technique=70 Infertile males, 7couples positive IUI (10%). Density gradient technique= 65 Infertile males, 9 couples positive IUI (13.8%).**

**Table 1: Sperm parameters, preand post activation, classified according to outcome of IUI using centrifugation swim uptechnique.**

| Parameters | Swim up centrifuge outcome | activat ion | *P*- value | activati on | *P*-value |
|---|---|---|---|---|---|
| Sperm concentration | Non pregnant | 57.631 ±2.294 | 0.481 NS | 29.703 ±1.871 | 0.551 NS |
| | Pregnant | 51.600 ±6.933 | | 25.600 ±4.864 | |
| | Non pregnant | 59.538 ±1.739 | | 90.077 ±1.406 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Sperm motility (%)** | | **Pregnant** | **53.000 ±4.062** | **0.310 NS** | **93.000 ±3.742** | **0.575 NS** |
| **Sperm grade activity (%)** | **Grade A** | **Non pregnant** | **2.231 ±0.559** | **0.549 NS** | **31.915 ±1.453** | **0.002*** |
| | | **Pregnant** | **1.000 ±1.000** | | **40.000 ±1.200** | |
| | **Grade B** | **Non pregnant** | **37.754 ±1.676** | **0.008*** | **45.062 ±1.659** | **0.255 NS** |
| | | **Pregnant** | **21.000 ±2.449** | | **47.000 ±4.899** | |
| | **Grade C** | **Non pregnant** | **19.708 ±1.229** | **0.018*** | **14.077 ±1.565** | **0.025*** |
| | | **Pregnant** | **31.000 ±5.339** | | **6.000 ±1.000** | |
| | **Grade D** | **Non pregnant** | **40.000 ±1.740** | **0.278 NS** | **9.154 ±1.415** | **0.034*** |
| | | **Pregnant** | **47.000 ±4.062** | | **7.000 ±2.000** | |
| **Progressive motility (%)** | | **Non pregnant** | **39.831 ±1.914** | **0.013*** | **76.231 ±2.394** | **0.228 NS** |
| | | **Pregnant** | **22.000 ±2.550** | | **87.000 ±6.633** | |
| **Sperm morphology (%)** | | **Non pregnant** | **36.031 ±1.379** | **0.057 NS** | **63.092 ±1.868** | **0.558 NS** |
| | | **Pregnant** | **46.200 ±6.406** | | **59.000 ±6.000** | |
| **Sperm agglutination (%)** | | **Non pregnant** | **14.921 ±1.362** | **0.795 NS** | **0.161 ±0.161** | **0.779 NS** |
| | | **Pregnant** | **13.600 ±5.609** | | **0.000 ±0.000** | |
| **round cell count** | | **Non pregnant** | **8.938 ±1.306** | **0.661 NS** | **0.000 ±0.000** | |
| | | **Pregnant** | **6.800 ±4.164** | | **0.000 ±0.000** | |

Data are mean ±SE.M.

Total number of infertile males used centrifugation swim up technique= 70. Number of positive IUI outcome= 7. Number of IUI outcome= 63.

NS= not significant idfference. (P> 0.05).

* Significant difference (P < 0.05).

**Table 2: Sperm parameters, pre and post-activation, classified according to outcome of IUI using density gradient technique.**

| Parameters | | Density gradient outcome | Pre-activation | *P*-value | Post-activation | *P*-value |
|---|---|---|---|---|---|---|
| Sperm concentration | | Non pregnant | 61.915 ±3.123 | 0.082 NS | 28.864 ±1.860 | ≤0.001** |
| | | Pregnant | 81.167 ±15.600 | | 51.667 ±5.869 | |
| sperm motility (%) | | Non pregnant | 61.102 ±1.911 | 0.535 NS | 93.864 ±1.065 | 0.468 NS |
| | | Pregnant | 65.000 ±5.477 | | 96.33 ±1.202 | |
| Sperm grade activity (%) | Grade A | Non pregnant | 5.000 ±1.121 | 1.000 NS | 44.015 ±1.943 | ≤0.001** |
| | | Pregnant | 5.000 ±1.291 | | 54.000 ±2.343 | |
| | Grade B | Non pregnant | 36.186 ±1.587 | 0.370 NS | 41.610 ±1.175 | 0.017* |
| | | Pregnant | 40.833 ±3.962 | | 38.833 ±2.007 | |
| | Grade C | Non pregnant | 20.424 ±1.258 | 0.757 NS | 7.288 ±1.220 | 0.377 NS |
| | | Pregnant | 19.167 ±2.713 | | 4.833 ±1.537 | |
| | Grade D | Non pregnant | 38.220 ±1.872 | 0.601 NS | 6.593 ±0.787 | 0.029* |
| | | Pregnant | 35.000 ±5.477 | | 3.333 ±1.054 | |
| Progressive motility (%) | | Non pregnant | 41.441 ±2.069 | 0.515 NS | 86.017 ±1.616 | 0.014* |
| | | Pregnant | 45.833 ±5.069 | | 92.500 ±1.708 | |
| Sperm morphology (%) | | Non pregnant | 33.746 ±1.066 | 0.902 NS | 68.241 ±1.586 | 0.194 NS |
| | | Pregnant | 34.167 ±1.537 | | 75.000 ±4.472 | |
| Sperm agglutination (%) | | Non pregnant | 13.276 ±1.189 | 0.423 NS | 0.038 ±0.038 | 0.737 NS |
| | | Pregnant | 16.600 ±2.135 | | 0.000 ±0.000 | |
| round cell count | | Non pregnant | 10.339 ±1.314 | 0.638 NS | 0.000 ±0.000 | |
| | | Pregnant | 12.333 ±2.445 | | 0.000 ±0.000 | |

**Data are mean ±SE.M., Total number of infertile males used swim up density gradient technique= 65., Number of positive IUI outcome =9., Number of negative IUI outcome= 56., NS= not significant difference (P>0.05)., * Significant difference (P < 0.05)., ** Highly significant difference (P≦0.001).**

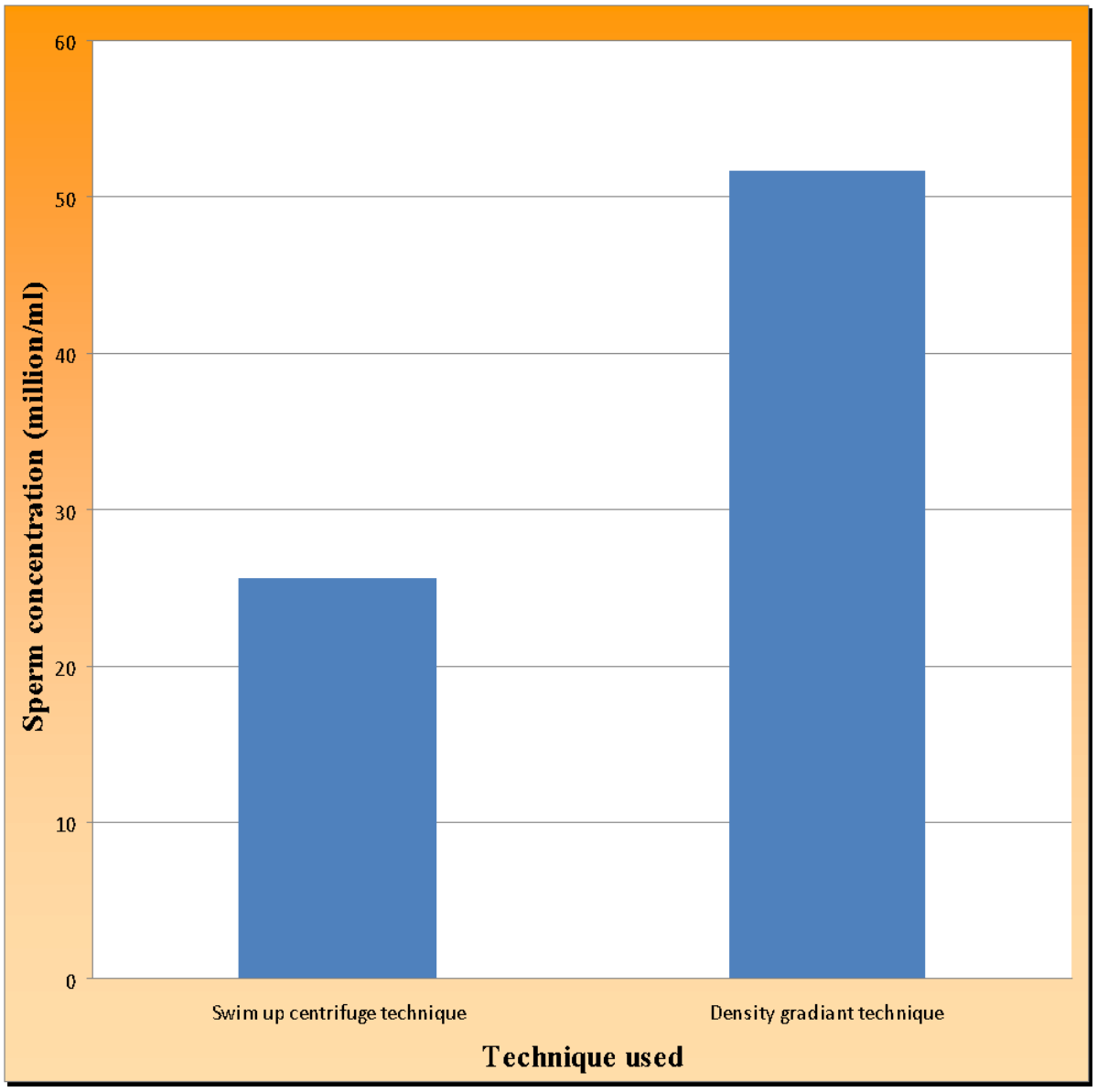



**Figure 2: Sperm concentration post-activation for positive IUI groups using two techniques.**

**Number of positive IUI outcome couples using centrifugation swim up technique= 7. Number of positive IUI outcome couples using density gradient technique=9.**

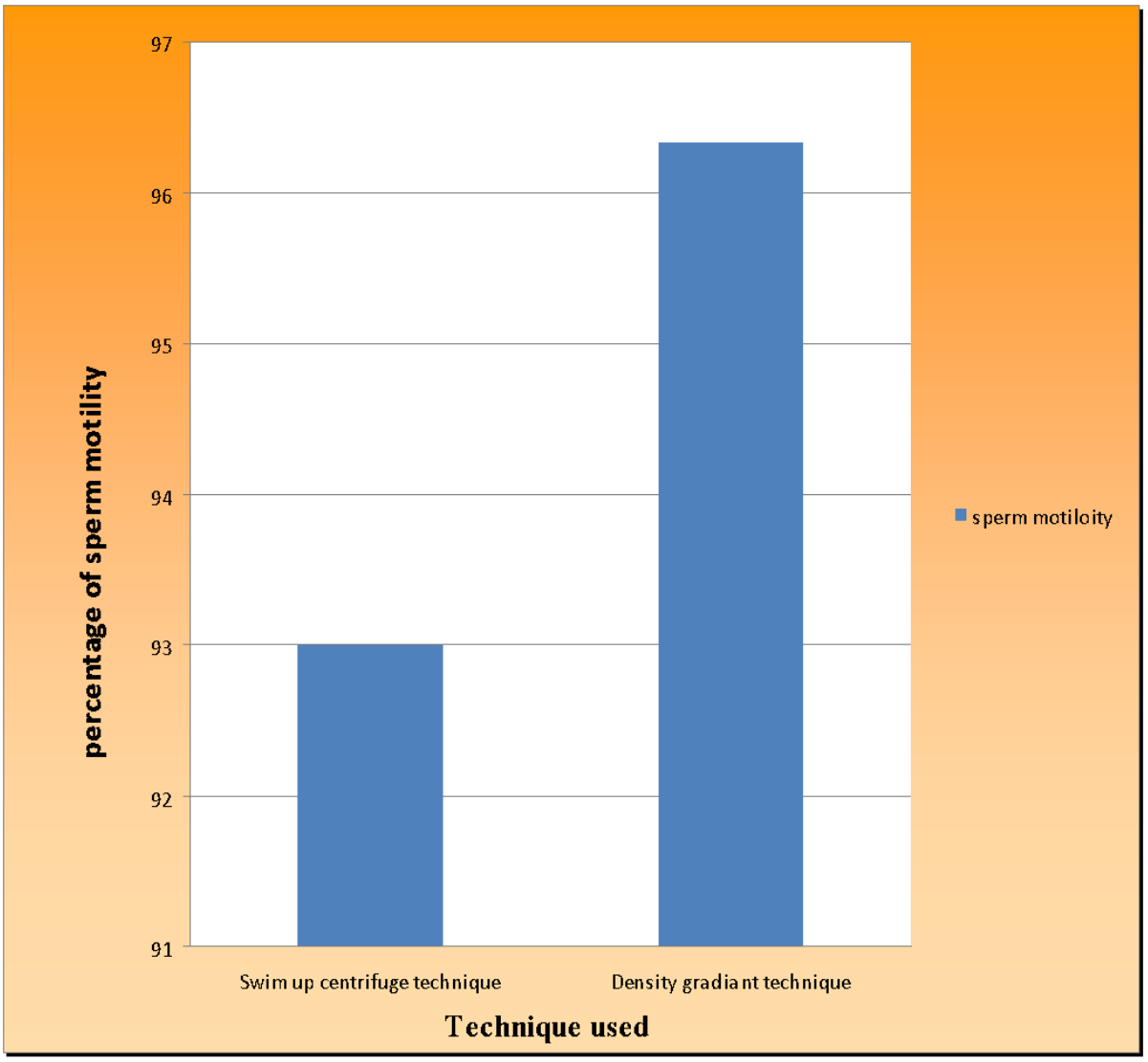



**Figure 3: Percentage of post-activation sperm motility in positive IUI groups using different techniques.**

**Number of positive IUI outcome couples using centrifugation swim up technique= 7. Number of positive IUI outcome couples using density gradient technique= 9.**

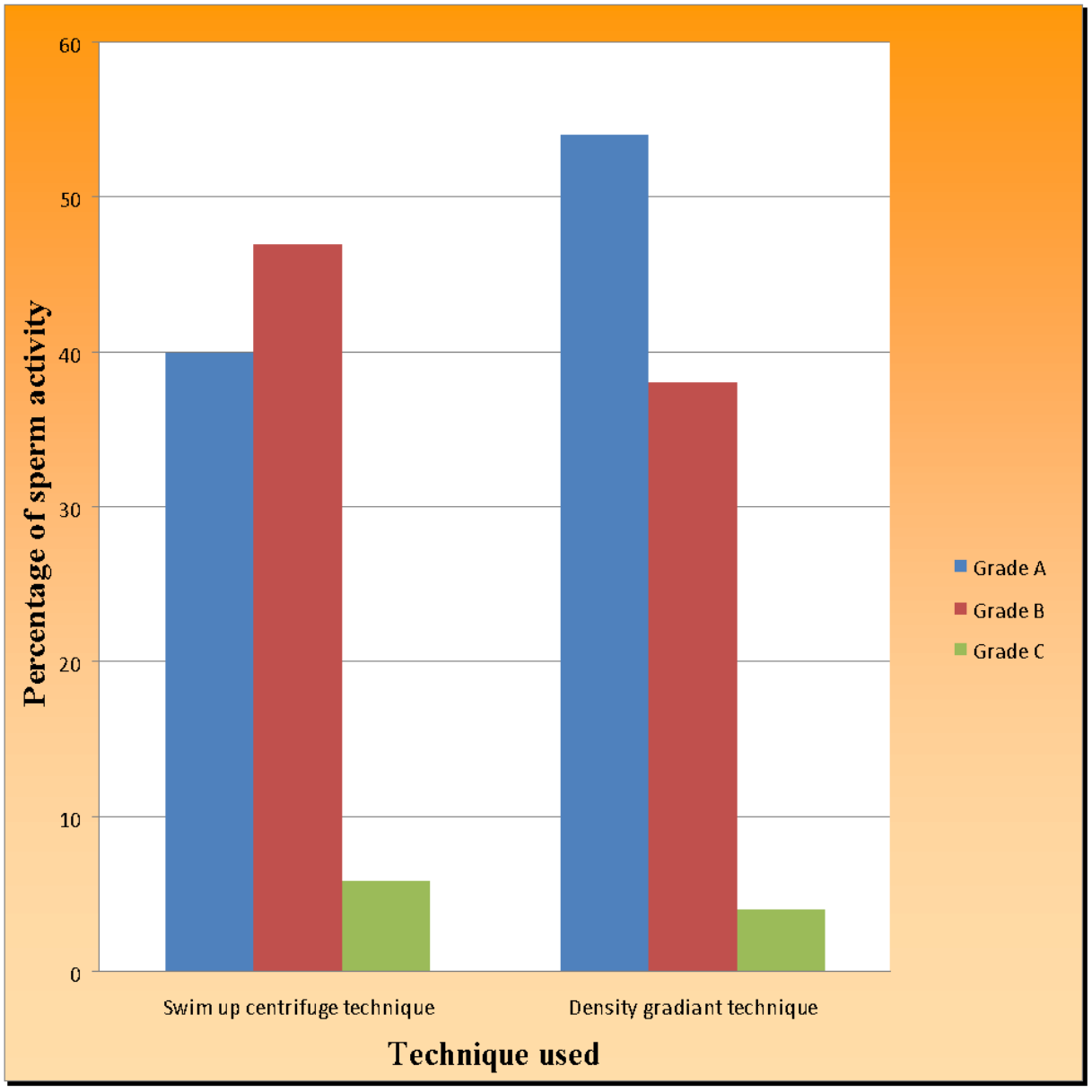



**Figure 4: Percentages of post-activation sperm activity grades A, B, C in positive IUI groups using two techniques.**

**Number of positive IUI outcome couples using centrifugation swim up technique= 7.7**
**Number of positive IUI outcome couples using density gradient technique= 9.**

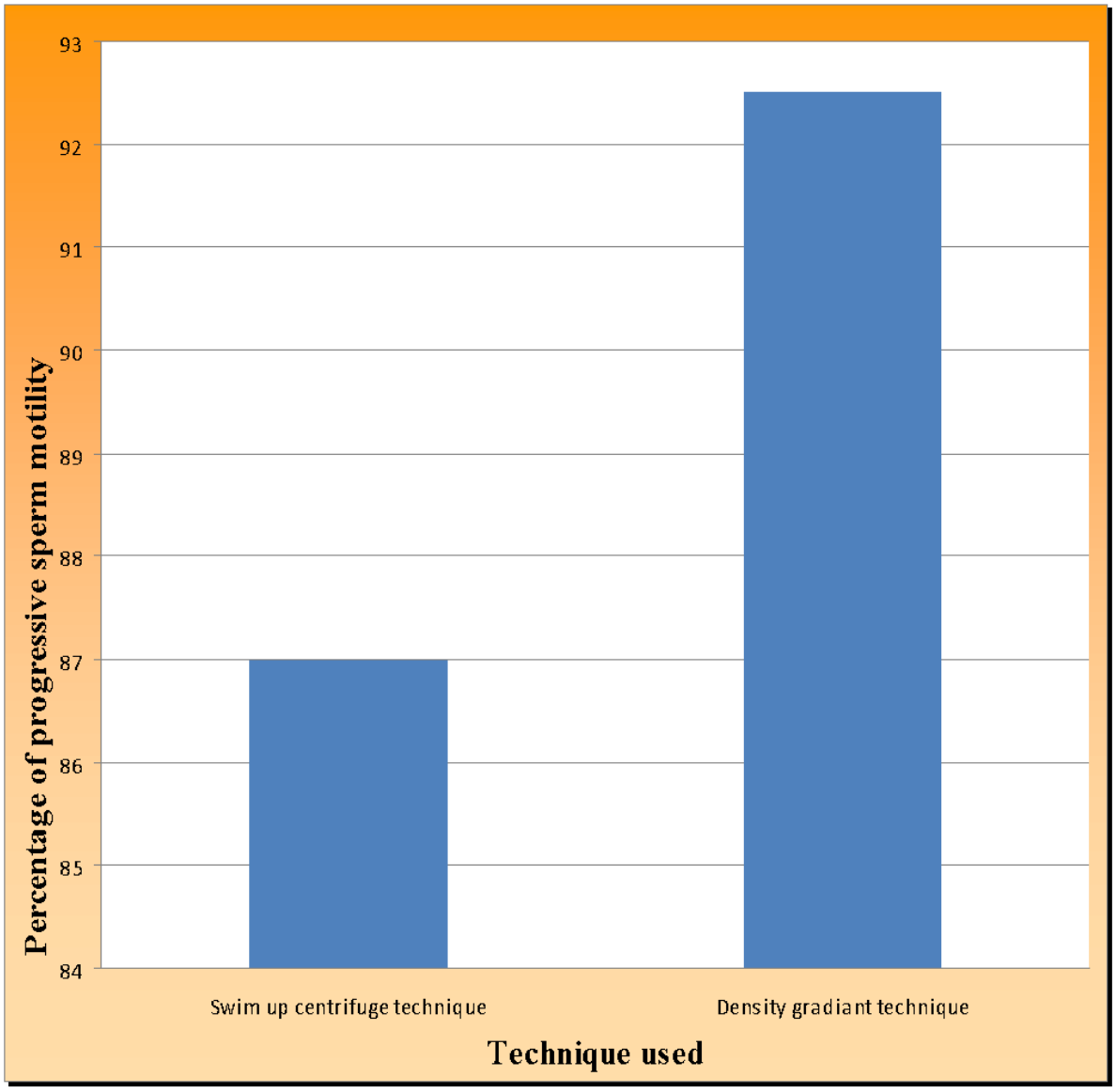



**Figure 5: Percentage of post-activation progressive sperm motility grades (A and B) in positive IUI groups using two techniques.**

**Number of positive IUI outcome couples using centrifugation swim up technique= 7.**

**Number of positive IUI outcome couples using density gradient technique= 9.**

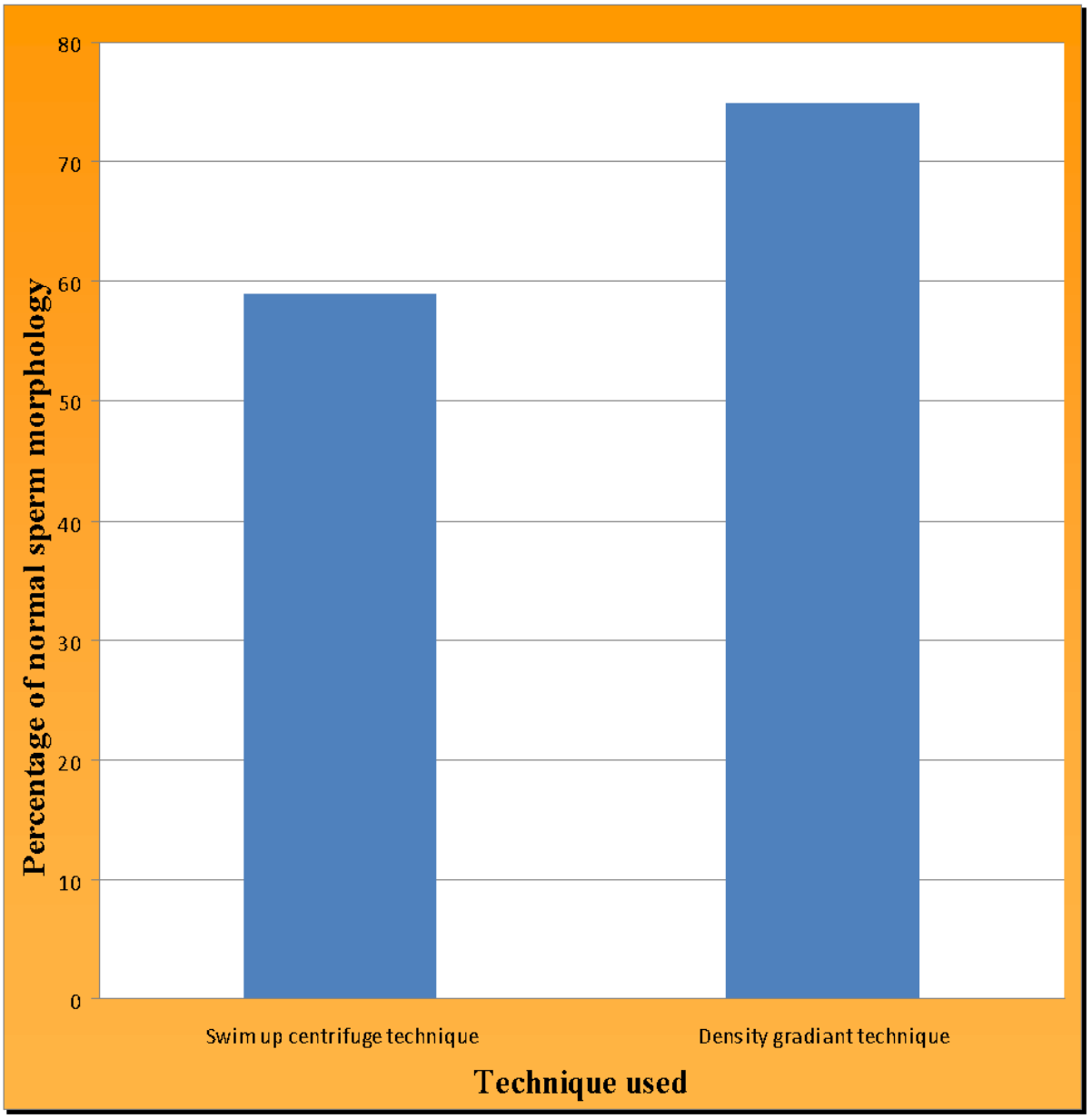



**Figure 6: Percentage of post-activation normal sperm morphology in positive IUI groups using different techniques.**

## DISCUSSION

Artificial insemination (AI) it is the least complex of the assisted reproductive technologies and is often tried first in uncomplicated cases of infertility (Mortimer, 1994 b). The intrauterine insemination (IUI) is the process whereby prepared sperm are injected directly into the uterine cavity in order to bypass the cervix and place the sperm closer to the egg (Acosta et al., 1992). It is used in couples with unexplained infertility may have problems with egg quality, tubal function, or sperm function that are difficult to diagnose and/or treat. Fertility drugs and IUI have been used in couples with unexplained infertility with some success (ASRM, 2010).

The present study showed that the highest percentage of positive IUI outcome (13.8%) was among the group used density gradient technique in sperm preparation, when sperm parameters were with significantly higher percentages of progressive sperm motility grades (A and B), grade (A) progressive sperm motility, also total sperm motility and morphology than the IUI outcome in the group of patients used the centrifugation swim-up technique. It is of sure that the spermatozoa selected by density gradient technique enhanced sperm penetration results in the zona free hamster egg by sperm penetration assay (Mahadevan and

Andtrounson, 1984).

It is preferable to perform IUI if 5 million total motile spermatozoa can be used per insemination, particularly if sperm morphology is normal or only slightly abnormal (Sergio, 2000).

Pregnancy results for IUI in male infertility are higher with concomitant ovarian stimulation. Such regimens include use of clomiphene citrate, a combination of and gonadotropins, or gonadotropins alone (Campana*et al*., 1996).

In this study IUI was performed for 135 infertile couples, positive IUI outcome was in 16 couples with percentage of (11.85%).Although the overall clinical pregnancyrate per cycle is relatively low for couples with male infertilityundergoing IUI, the cumulative pregnancy rate (3 cycles of insemination) may vary from 35% (in 30- year-old women) to 10% (in 39-year-old women) ( Morshedi*et al*., 1999).

Intrauterine insemination involves the preparation of a semen sample in the laboratory, sperm preparation involves either washing (in a capacitating medium) or washing and separation of the motile fraction (typically using a gradient centrifugation method), the latter will result in a better selection of functional cells at the price of a decreased total number of spermatozoa (Baldi*et al.*, 1996). Gradient separation is recommended in the presence of round cells, antisperm antibodies, or both, and when a low recovery of motile sperm is anticipated (Sergio, 2000).

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