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The Influence of Paternal Age on Semen Parameters and Pregnancy Outcome Following Intrauterine Insemination

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Abstract

Introduction: Male factors contribute for 25% to 40% of infertility cases, and semen quality has been commonly regarded as a measure of male fecundity. There is reported significant negative association of semen volume, total sperm count, sperm motility, and morphology with advanced male partner's age. Therefore, combination of the sperm preparation and assisted reproductive treatment were used for a better chance of pregnancy. This study was aimed to evaluate the influence of age on the sperm quality and pregnancy outcome in couple with subfertility undergoing Intrauterine Insemination (IUI).

Material and Methods: This retrospective cross-sectional study investigating 181 cases of IUI cycles from reproductive clinic of two public universities from January 2019 to December 2020. They were categorized into study group (male partner's age 40 years and above) and control group (male partner's age less than 40 years old). Sperm parameter reports were extracted from the patients' records. Clinical pregnancy was the criteria for the IUI success.

Results: The mean age of male and female partners were similar in both groups. Sperm parameters showed an improvement after density gradient centrifugation sperm preparation with significant results in grade 1 and grade 3 sperm motility. There was no significant different in the clinical pregnancy between the groups.

Conclusion: There is an improvement in sperm parameters after density gradient centrifugation and the male age is not significantly affect the pregnancy outcome following IUI. Further study with larger sample is needed to confirm these findings.

Keywords: Density gradient centrifugation; Insemination; Paternal age; Pregnancy outcome; Semen analysis

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Introduction

Lately, the topic of male infertility has been popularly discussed. A man who failed to produce high quality sperm in terms of density, motility and morphology is considered as infertile male. There are increase rates of infertility and adverse pregnancy outcomes seen after the age of 40 years old (independent of female partner's age), thus the male age factor might have some contribution to this matter, even though the spermatogenesis known to continue in elderly men, in contrast to oogenesis [1]. From the literature, contribution to conception by male partner is still occur even after 40 years of sexual maturity [2]. But, degenerative changes in germinal epithelium occur with aging, causing Leydig cells to reduce in quantity and its function, thus affecting the spermatogenesis process due to decrease testosterone level, in which it starts at 30 years of age [1,3,4]. Studies suggest that male partner's age is associated with diminished semen volume, sperm motility and/or sperm morphology. Increasing seminal ROS levels and changes in epididymal and accessory sex gland function may be possible causative factors for the decline in motility with aging. Emergence idea of intrauterine insemination with sperm preparation has shown some improvement in semen parameters but with conflicting data. In this respect, our objective is to established evidence on influence of age on the sperm quality and effect of density gradient centrifugation on pregnancy outcome in infertile couple undergoing Intrauterine Insemination (IUI).

Materials and Methods

This was a retrospective cross-sectional study involving two university hospitals in Malaysia

with fertility services. Completed one hundred and eighty-one IUI cycles from January 1st, 2019 to December 31st, 2020 were included in this study.

Couples who underwent IUI during the study period were enrolled in this study. Only the first IUI cycle of the couples were included in the study.

The inclusion criteria were as follow: Men age between 18 to 70 years old, with semen analysis results either normal or one, or two of the sperm parameters were below the normal value established by the WHO [5]. The semen parameters are the sperm count, sperm motility with characterized into Grade 1 (rapid and slow linear progressive motility), Grade 2 (non-progressive motility) and Grade 3 (immotile spermatozoa). The female partner had regular menstrual cycle, normal pelvic ultrasound findings and at least one fallopian tube patent in hysteroscopy or laparoscopy dye insufflation test.

The exclusion criteria were including men with azoospermia, female partner with endometriosis, polycystic ovarian syndrome, poor ovarian responder, or premature ovarian insufficiency. Incomplete data on pre- and post-processing semen analysis or missing data regarding the pregnancy outcome were also being excluded.

All the data such as age of the couple, duration of infertility, semen analysis parameters before and after sperm processing, and the IUI results were obtained from the patient's records. Pregnancy was defined as positive serum beta Human Chorionic Gonadotropin (b-HCG) taken at 14 days after IUI followed by presence of the fetal heart activity after 6 weeks period of a menorrhea.

The eligible couple were stratified into two groups according to the male partner's age – study group (male partner's age 40 years and above) and control group (male partner's age less than 40 years old). The semen parameters and pregnancy outcome were compared between the groups.

Semen sample collection and preparation for IUI

Semen samples were collected from the male partners following 2 to 5 days of sexual abstinence by masturbation in sterile plastic containers at the clinic. Liquefaction was performed at room temperature for 30 min. The initial analysis of the semen parameters (semen volume, sperm count, and sperm motility) was performed manually according to WHO guidelines (2010) [5].

Subsequently the semen samples were processed using the density gradient centrifugation to remove seminal fluid and enhance sperm quality for IUI. The total volume of semen was divided so that a 15-ml conical tube marked with patient's identification would contain 2 ml of a 90% density lower layer, 2 ml of a 45% density upper layer, and 2 ml of semen upper most layer. A gentle and careful move was required to ensure the layers were not mixed-up at each centrifugal stage. The sample was then centrifuged at 1,500 rpm for 20 min at Room Temperature (RT). After centrifugation, the supernatant was removed and the spermatozoa (pellet) was placed into another 15-ml conical tube, which contained 2.0 ml of sperm rinsing medium and mixed thoroughly; it was then centrifuged at 1,500 rpm for 10 min at room temperature. The supernatant was again removed, and the final pellet was suspended in the same medium solution, obtaining a final volume of 0.5 ml. A 10-µl aliquot was used to perform the analysis of post-processing seminal parameters. The motility of spermatozoa in the sample determined and incubated sample at 37°C until ready for insemination. The Makler counting chamber was used to assess the count and motility and the processed sample in the best possible way. The sample was ensured to maintain its temperature at 37°C by placing it in a test tube warmer before IUI procedure.

Ovulation induction and insemination

Standard protocol was used involving administration of 100 mg clomiphene citrate from the second day of menstruation for five days and injection of human menopausal gonadotropins (75 IU to 150 IU) on day five onwards up to day ten of menstruation, either daily or every other day. When at least one follicle achieved more than 17 mm, intramuscular HCG 10,000 IU was given for final maturation. Single insemination was done at thirty-six hours later with 0.5 mL to 1.0 mL of processed sperm from the fresh semen sample, injected intrauterine using a Gynetics catheter. After the insemination the patient was lying in the supine position for 15 min to 30 min. Oral dydrogesterone 10 mg three times daily was prescribed for the luteal phase support.

Statistical analysis

The Statistical Package for Social Sciences for Windows (SPSS version 22.0) was used to record and analyzed the data. Quantitative variables were compared using Mann-Whitney test and the results were expressed as mean ± Standard Deviation (SD) and percentage. Categorical variables were evaluated using the Chi-square and Fisher's exact test. A *p*-value of less than 0.05 is considered as statistically significant.

Results

A total of 181 of IUI cycles, from January 1st, 2019 and December 31st, 2020, were analyzed. The mean (± Standard Deviation, SD) age of male and female partner included in this analysis were similar for each group. Majority of the indication for the IUI was unexplained infertility (Table 1).

Table 2 showed the results of sperm parameters of the two studied groups. As can be noted, there was no difference of sperm parameters among the male partner with different age groups. There was an improvement in the sperm concentration and Grade 1 sperm motility after the DGC, however not statistically significant. In the study group (male age 40 years and above), there is significant increment in the grade 1 sperm motility and significant reduction of immotile sperm after GDC as illustrated in Table 3. Regarding the successful pregnancy, there was also no significant difference of the semen parameters between the studied group (Table 4). The number of pregnancies was higher in group of male partner less than 40 years old, compared with older age male partner, however, it was not statistically significant (Table 5).

Discussion

Semen fluid analysis is the first step in the evaluation of male infertility. Semen characteristics that being evaluated includes the volume, sperm concentration, sperm motility, and sperm morphology. The standard value of semen analysis that is accepted by the World Health Organization (WHO) 2010 is the volume of 1.5 mL, pH of 7.2, sperm concentration of 20 million/mL or more, total motility (progressive and non-progressive) of 40%, and normal sperm morphology of 4% [6].

There was an association between male age and sperm parameters in the literature. Decrease in sperm quantity except for concentration was more compelling among men aged 30 to 50 years old [7]. A similar

Table 1: Demographic characteristics.

Variables	Male 40 years and above N=31 (100)	Male less than 40 years N=150 (100)	p value
Age, mean ± SD			
Male	43.32 ± 3.15	33.60 ± 3.35	<0.01
Female	38.39 ± 2.79	33.08 ± 3.86	<0.01
Indication of IUI, N (%)			
Anovulation	5 (16.13)	30 (20.00)	
Tubal factor	6 (19.35)	8 (5.33)	
Unexplained infertility	11 (35.48)	68 (45.34)	
Male factor	7 (22.58)	30 (20.00)	
Recurrent miscarriage	1 (3.23)	0 (0)	
Discordant couple	0 (0)	3 (2.00)	
Others (ED, vaginismus)	1 (3.23)	11 (7.33)	

IUI: Intrauterine Insemination; ED: Erectile Dysfunction

Table 2: Sperm parameters of the between two different male age group.

Sperm parameters	Male 40 years and above N=31	Male less than 40 years N=150	p value
Sperm concentration (×10⁶), mean ± SD	73.19 ± 46.64	69.96 ± 52.23	0.75
Motility G1 (%), mean ± SD	41.12 ± 16.74	41.74 ± 21.39	0.86
Motility G2 (%), mean ± SD	17.61 ± 8.28	21.44 ± 11.71	0.85
Motility G3 (%), mean ± SD	41.14 ± 18.56	36.93 ± 18.92	0.26

G1: Grade 1 (progressive rapid and slow linear), G2: Grade 2 (non-progressive linear), G3: Grade 3 (immotile)

Table 3: The effect of Density Gradient Centrifugation (DGC) on sperm parameters of each group.

Semen parameters	Male 40 years and above, N=31			Male less than 40 years, N=150		
	Pre-DGC	Post-DGC	p value	Pre-DGC	Post-DGC	p value
Sperm concentration (×10⁶), mean ± SD	73.19 ± 46.64	78.74 ± 61.41	0.60	69.96 ± 52.23	87.47 ± 70.81	<0.01
Motility G1 (%), mean ± SD	41.12 ± 16.74	77.50 ± 14.41	<0.01	41.74 ± 21.39	77.46 ± 19.89	<0.01
Motility G2 (%), mean ± SD	17.61 ± 8.28	13.11 ± 10.93	0.03	21.44 ± 11.71	13.87 ± 12.60	<0.01
Motility G3 (%), mean ± SD	41.14 ± 18.56	8.92 ± 5.21	<0.01	36.93 ± 18.92	8.64 ± 12.07	<0.01

G1: Grade 1 (progressive rapid and slow linear), G2: Grade 2 (non-progressive linear), G3: Grade 3 (immotile)

Table 4: Semen parameters between the groups with successful pregnancy after Density Gradient Centrifugation (DGC).

Sperm parameters	Male 40 years and above N=3	Male less than 40 years N=6	p value
Sperm concentration (×10⁶), mean ± SD	64.33 ± 59.80	80.47 ± 110.19	0.82
Motility G1, mean ± SD	55.43 ± 30.91	86.35 ± 16.16	0.08
Motility G2, mean ± SD	29.57 ± 28.65	3.6 ± 2.10	0.26
Motility G3, mean ± SD	15.00 ± 3.22	8.4 ± 16.38	0.53

G1: Grade 1 (progressive rapid and slow linear), G2: Grade 2 (non-progressive linear), G3: Grade 3 (immotile)

Table 5: Reproductive outcome of intrauterine insemination procedure between the groups.

Reproductive outcome	Male 40 years and above N=31	Male less than 40 years N=150	p value
Pregnancy	3	6	0.19
No pregnancy	28	144	

study by Stone et al. affirmed that decrease in semen parameter starts at the age of 34 and it postulated to affect the pregnancy status [8]. In conjunction, study in Central India by classified age 36 to 42 as under the 3rd group in a five-age class (21 to 60 years) that is relates with the decline in semen parameters and it is significant when the individual is after the age of 35 [1]. Meanwhile, Gao et al. revealed a significant association between the increasing age and a drop in sperm quantity and quality based on 7-age groups among men aged 25 to 51 [9]. The large dispersion also believed to derive this smaller number of groupings to elucidate age as factors in differentiating the sperm counts. This study showed better sperm motility of the younger male compared to the older male who aged more than 40 years old. Therefore, testing the subjects with different age level may validate the influence of age on semen parameters at certain degree of comparison.

Most of the finding showed an increase of the mean value in the count and motility. The significant difference was obtained in the study mostly in the post DGC for Grade 3 motility (immotile

sperm) suggesting positive effect of DGC on semen motility. Density gradient is the preferred technique for sperm processing for ART as it improved sperm motility and quality [10]. This study also highlighted the higher chance of pregnancy in female with the male partner who aged less than 40 years old, control group (3.3%) compared to female with male partner aged more than 40 years old. It showed that as the age of the couple grows older, the likelihood of pregnancy decreases, and most studies link aging to a drop in sperm parameters [11].

On a separate note, Dong et al. concluded that chances of pregnancy from IUI decreases as semen sample contained lower than 2 million motile sperm [12]. Thus, other studies specified that pregnancy from IUI is more probable with the existing of linear progressive motile among pairings with subfertility male with the higher likelihood of pregnancy from larger counts of post-prepared sperm with greater motility in IUI [13,14]. In addition, Ajayi et al. suggested than men with lower sperm counts had a 4 times higher likelihood to develop progressive sperm motility than men with normal sperm counts [15]. Somehow, Ombelet et al. reviewed that studies should consider association of sperm parameters with other factors that may determine the success of IUI such as female age and period of subfertility. In addition, they iterate that prediction on pregnancy rate is acceptable yet considered as poor in sensitivity as comparison made with standardized limit sperm counts classing. Perhaps this study may output to different significance if sperm counts categorized in to non-parametric groupings for comparison with the nominal measure of pregnancy status. For the time being, this study suggests that IUI has done effectively in ensuring pregnancy from samples with lower sperm counts from pre-processed total or from post-processed non-progressive spermatozoa that originates from men below 40 years old. On the other side, female may conceive offspring in relation with lower counts of motile sperm or higher counts of immotile sperm after the post wash procedures conducted for semen samples of men aged 40 and above [16].

Readings of bHCG level recorded pregnancy outcome either pregnant or not were insignificantly correlated with male age group as a result from IUI denoted from Fisher's Exact Test *p*-value of 0.185. This hypothesis in several manner is consistent with findings from other study with differing fertilization procedures that remarks male age as not a defining factors of pregnancy rates or outcome [17-20]. On the other hand, Tatsumi et al. described age among men does not correlate with pregnancy outcome, yet simulation from IUI revealed women at the age of 38 to 40 years old as the sole factor influencing chances of pregnancy [21].

Conclusion

In conclusion, similar semen parameters and comparable pregnancy outcome was seen among couples with male partner's age below and above 40 years old. There was an improvement in sperm parameters after density gradient centrifugation which may involve in the achievement of pregnancy following IUI. This study is limited to sperm count and motility without analysis of morphology and sperm DNA, therefore further study with these added sperm parameters and larger sample could be considered in the future study.

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