CORRELATION OF SPERM PARAMETERS WITH AGE, DURATION OF INFERTILITY AND IUI OUTCOME

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ABSTRACT

Background: Infertility is failure to achieve pregnancy after one year of un protected intercourse. In approximately 40% of infertile couples, the male partner is either the sole or a contributing cause of infertility.

A severe defect of spermatogenesis including reduced sperm count, motility and many sperm with abnormal morphology, will probably always be relevant. However, mild defects may only become relevant as the age and the duration of infertility increases.

Objectives: Evaluate the relationship of age and duration of infertility with sperm parameters and correlate them with IUI outcome.

Patients, materials and methods: One hundred thirty-five infertile couples were participated in this study where the male partners subjected to *in vitro* sperm preparation and activation, while the female partners were normal and had ovarian stimulation program. *In vitro* sperm activation was performed using two different methods, the centrifugation swim-up technique and density gradient technique with. The mean ages of infertile males were 35.614±0.539 years with a range from 22 to 52 and the mean duration of infertility was 6.113±3.75 years with a range from 1 to 20 years. Both groups were subjected to IUI.

Results: Lowest sperm concentration and motility with age group (> 50) years. However; best results in this study regarding total sperm motility was with duration of infertility (3-4) years and the lowest total sperm motility with duration (>10) years. Regarding IUI, the highest IUI outcome was with age group (30-39) years and with duration of infertility (<2) years (25%).

Conclusion: This study concluded that sperm parameters and IUI outcome inversely correlated with duration of infertility. Also better sperm parameters regarding concentration, motility, morphology and IUI outcome was with young age group. Since the sperm parameters and IUI outcome decline with age and with longer duration of infertility as shown by the results of this study so better to interfere with IUI in couples with duration of infertility less than two years.

Keywords: sperm parameters, age, duration of infertility, IUI.

INTRODUCTION:

Of couples planning a pregnancy up to 50% conceive in the first cycle and in the remainder, the percentage who conceive in each successive month declines as the proportion of sub fertile couples left continuing to try increases. Approximately 85% conceive a first pregnancy by 6 to 12 months (Joffe, 2000). The 6- to 12- month period used to define infertility means that it afflicts approximately 15% of couples (Gnoth *et al*., 2005). Infertility is thus common and the male contribution is substantial (Holden *et al*; 2005).

For couples over age 35 it is generally recommended that infertility testing begins after 6 months of unsuccessful attempts at conception (Williams and Elam, 2007; ASRM, 2008). In some cases, an evaluation may be warranted prior to one year if there is a known male infertility risk factor such as bilateral cryptorchidism or known female risk factor (AUA, 2010).

Although diagnostic problems make it difficult to establish the extent of a male partner's contribution with certainty, a number of studies suggest that male problems represent the most common single defined cause of infertility (Irvine, 1998). Therefore, standard semen analysis has long been the primary laboratory test of male infertility and including sperm concentration, motility, and morphology (Mackenna, 1995).

A variety of sperm tests and semen parameters have been developed with the hope of clarifying whether or not a man could impregnate his partner (Saibel and Zilberstain, 2005).

Problems with the production and maturation of sperm are the most common causes of male infertility. Sperm may be immature, abnormally shaped, or unable to move properly. But, normal sperm may be produced in abnormally low numbers (oligozoospermia) or seemingly not at all (azoospermia) (ASRM, 2010). For this semen analysis comprises a set of descriptive measurements of spermatozoa and seminal fluid parameters that help to estimate semen quality (Campana,et al., 1995).

In semen analysis the volume of the semen is measured, as well as the number of sperm in the sample. How well the sperm move is also assessed. This is the most common type of fertility testing (Gaur et al., 2007).

Treatment options for male infertility now include a large number of surgical and nonsurgical urological procedures and medical-pharmacological interventions, as well as low complexity and advanced assisted reproductive technologies (ARTs) (Sergio, 2000). In many situations, however, intrauterine inseminations are performed with success (Francavilla *et al.,* 2009).

Success rates, or pregnancy rates for artificial insemination may be very misleading, since many factors including the age and health of the recipient have to be included to give a meaningful answer, e.g. definition of success and calculation of the total population (Hurd *et al.*, 1993). Generally, it is 10 to 15% per menstrual cycle using ICI, and 15-20% per cycle for IUI. In IUI, about 60 to 70% have achieved pregnancy after 6 cycles (Bhattacharya *et al.,* 2008).

However, a study in 2002 suggested IUI as a reasonable first option for many women under age 43. It is less expensive and has less risk for multiple births than the more advanced assisted reproductive technologies (ART), such as *in vitro* fertilization. Although IVF procedures are more effective per cycle, couples tend to be able to afford more IUI cycles, so the pregnancy rates over time are very similar (Min *et al*., 2004).

Success rates have increased in all age groups (although they are still considerably lower in older than in younger women). Chances for ART success also are greater among women who do not have uterine abnormalities and have had previous successful pregnancies (Halliday, 2007).

MATERIALS AND METHOD:

One hundred and thirty-five infertile couples were participated in this study where the male partners needed sperm preparation and activation *in* vitro, while the female partners were normal and subjected to ovarian

stimulation program. The husband's ages were 35.614 ±0.539 years with a range from 22 to 52 years while the wife's ages were 31.451±0.64 with a range from 19 to 48 years. The duration of infertility was 6.113±3.75 with a range from 1 to 20 years.

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 culturee media weree used in this study: Global All Grad Solution, Global medium, Clomiphen citrate (clomid) tablets (50) 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 and sterile disposable Petri-dish by masturbation mostly or coitus interruptus in a private and quite room adjacent to the semen analysis laboratory. Furthermore; the container must be 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 so that to allow liquefaction. The liquefied semen is then carefully mixed for few seconds, and then the specimen was examined by macroscopic and microscopic examinations. The standard form of WHO (1992) is used to record the results of seminal fluid analysis.

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 semen samples were prepared for *in vitro* sperm activation and IUI.

The male partners were grouped in to four subgroups according to their age, and in to six sub groups according to the duration of infertility. Sperm parameters was assessed according to different sub groups.

Each group results were correlated to IUI outcome.

Statistical analysis:

Sciences Statistical analysis was performed using the Statistical Package for Social (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) to compare between pre-and post-activation for sperm parameters. Independent sample t-test for tables with mean and standard error of mean (S.E.M) to compare between Global media and ALL Grad solution for sperm parameters. As well as ANOVA test was applied to compare among mean groups of this study with regard to the age. P-value < 0.05 was used as a level of statistically significance.

RESULTS:

Depending on WHO recommended criteria, the macroscopic and microscopic parameters of seminal fluid analysis

for the subjects involved in this study are shown in table (1). Most parameters of SFA within normal ranges according to the criteria of WHO (1999), except progressive sperm motility (%) which was less than that of normal limit of WHO, while sperm agglutination (%), and round cell count were more than the WHO (1999) recommended criteria. The results of the present study indicated for the age groups of infertile males that the largest group was within age group (30-39) years (50.4%) where the number was 68. The second degree was occupied by the infertile males within age group (40-49) years (29.6%) and their number was 40 patients. This was fallowed by males of age group (≤29) years (17.8%), their number was 24. The smallest division were with in age group ≥50 years (no. 3; 2.2%) as it presented in figure (1).

Table1: Parameters of seminal fluid analysis for infertile males* involved in the present study compared with (1999) recommended values

Parameters		Seminal fluid analysis	Normal values according to criteria of WHO (1999)
Semen volume		2.905± 0.082	2-5 ml
Liquefaction time		31.185± 0.561	≤60 minutes
Sperm concentration		60.326±1.926	≥ 20 X 10^6 sperm / Ml
Sperm motility%		60.222± 1.217	≥ 50 %
Percentage of sperm grade activity	Grade A	3.519± 0.574	≥ 50% with progressive motility (a+b) or ≥ 25% with rapid progressive motility (grade A) within 60minutes of ejaculation
	Grade B	36.585± 1.111	
	Grade C	20.415± 0.851	
	Grade D	39.259± 1.206	
Progressive sperm motility %		40.141± 1.345	Grade A+ Grade B≥ 50%
Normal sperm morphology %		35.326± 0.863	≥ 30 %
Sperm agglutination %		14.206± 0.865	< 10 %
Round cells count		9.622± 0.870	Less than one million/ ml

Data are Mean±SEM.
SEM= Standard error of mean.
*** Number of infertile males= 135.**

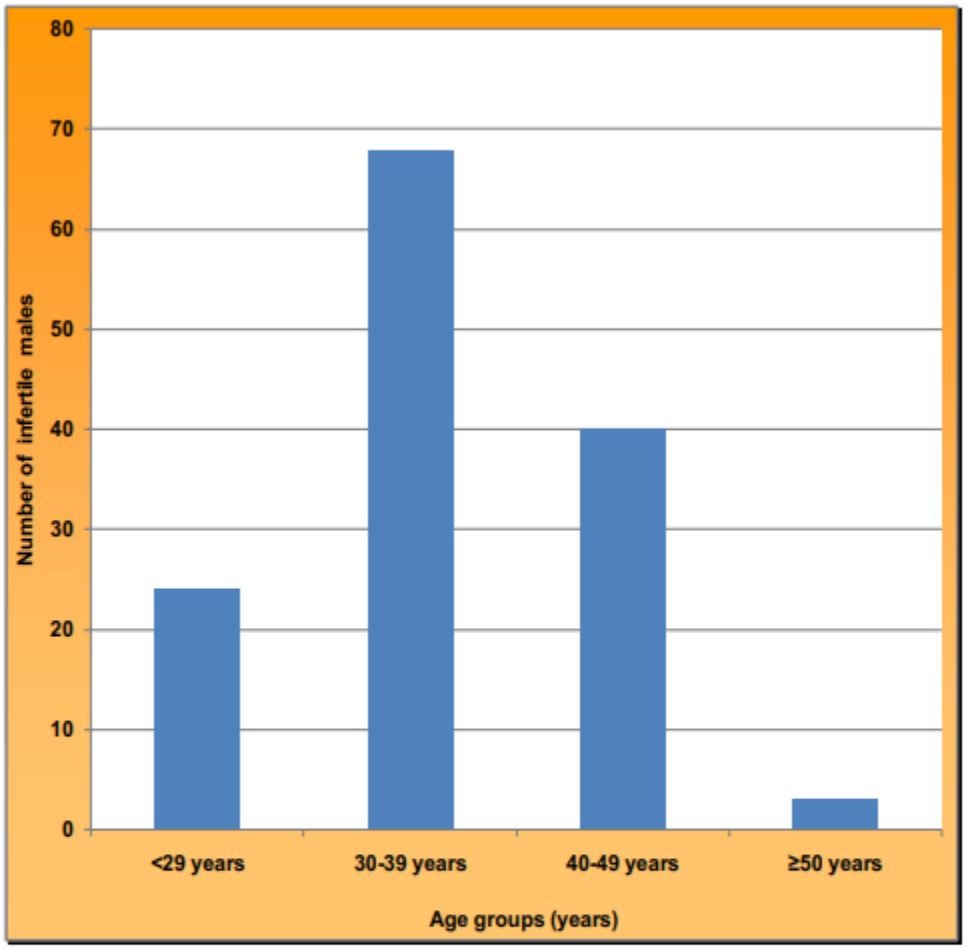

Figure1: Number of infertile males*classified according to age group.

Total number of infertile males= 135.

The parameters of SFA for infertile males classified according to age group as it shown in table (2) indicated that the highest sperm concentration (million/mL) was within age group (40-49) years (63.700±3.579) fallowed by age group (30-39) years (60.397±2.851), while the lowest concentration was within age group (≥50) years (50.667±16.746). However, regarding sperm motility best results were obtained within age group (≤29) (62.167±2.579) fallowed by age group (40-49) years (61.125±2.378), but the lowest sperm motility percentage

was within age group (≥50) years (55.000±10.408). Furthermore, the best results regarding progressive sperm motility grade (A) were noticed within age group (40-49) years (4.250±1.208) and the lowest values were in the age group (≥50) years (2.333±0.332) and age group (30-39) (2.941±0.750) respectively.

However, regarding progressive sperm grade (B) the best results were in age group (≤29) years (40.667±1.948) and the lowest values were in age group (≥50) years (23.000±12.583).

The overall percentage of sperm progressive motility (grade A+B) best results were in age group (≤ 29) years (43.583±2.392) and the second best results were in age group (40-49) years (40.875±2.506), while the lowest results were in age group (≥50) years (25.333±4.410). For the percentages of normal sperm morphology as well as agglutination the results of the present study showed they were reduced as much as increased in the age of the infertile subjects recorded in this study. Finally the count of round cells, highest values were among age group (30-39) years (10.044±0.972) compared with the lowest results in age group (≥50) years (3.000±1.528).

Numbers of infertile males classified according to the duration of infertility were observed in the figure (2). As shown the largest group of infertile males was with duration of infertility (5-6) years with count of (37) patients, and the second largest division was duration of infertility (7-8) years with 26 infertile males.

While, the smallest group with count of (13) infertile males was with duration of infertility (9-10) years.

Table (3) shows the parameters of SFA classified according the duration of infertility. It was observed that the best results of total sperm motility percent (66.385±2.208) and progressive sperm motility percentage (grades A+B) (47.038±2.524) were with duration of infertility (3-4) years, while the lowest values of total sperm motility percent were with duration of infertility (≥10) years (56.211±3.600) and that of progressive sperm motility percentage with duration of infertility (7-8) years (36.087±2.965). However; for the percentage of normal sperm morphology the highest values were with duration of infertility (9-10) years and the lowest with duration of infertility (3-4) years (38.846±3.260 and 32.696±1.743; respectively). Furthermore, the percentages of sperm agglutination highest values were with duration of infertility (≤2) years (20.533±2.952) while the lowest values were with (7-8) group (12.808±1.963). As for the counts of round cells the highest values were in group of (9-10) years.

Table 2: Parameters of SFA for infertile males classified according to age group.

Parameters		Age groups				*P*-value
		<29 years	30-39 years	40-49 years	≥50 years	
		No.= 24	No.= 68	No.= 40	No.= 3	
Semen volume		2.875 ±0.176	2.812 ±0.121	3.113 ±0.149	2.500 ±0.289	0.378
Liquefaction time		33.750 ±1.571	31.309 ±0.660	29.275 ±1.121	33.333 ±3.333	0.056
Sperm concentration		55.708 ±3.606	60.397 ±2.851	63.700 ±3.579	50.667 ±16.746	0.481
Sperm motility (%)		62.167 ±2.579	58.794 ±1.709	61.125 ±2.378	55.000 ±10.408	0.652
Sperm grade activity (%)	Motility A	3.333 ±1.028	2.941 ±0.750	4.250 ±1.208	2.333 ±0.332	0.466
	Motility B	40.667 ±1.948	35.044 ±1.747	36.125 ±1.712	23.000 ±12.583	0.196
	Motility C	19.000 ±1.531	20.515 ±1.284	20.375 ±1.391	30.000 ±12.583	0.348
	Motility D	37.000 ±2.396	41.059 ±1.736	37.875 ±2.306	45.000 ±10.408	0.496
Progressive motility (%)		43.583 ±2.392	37.912 ±2.015	40.875 ±2.506	25.333 ±4.410	0.190
Sperm morphology (%)		36.875 ±2.967	35.559 ±1.072	34.525 ±1.392	28.333 ±4.410	0.512
Sperm agglutination (%)		15.652 ±2.657	15.164 ±1.249	11.842 ±1.106	11.667 ±6.009	0.326
round cell count		8.958 ±3.153	10.044 ±0.972	9.800 ±1.544	3.000 ±1.528	0.681

Data are mean ± SEM.*
Total number of infertile males = 135.

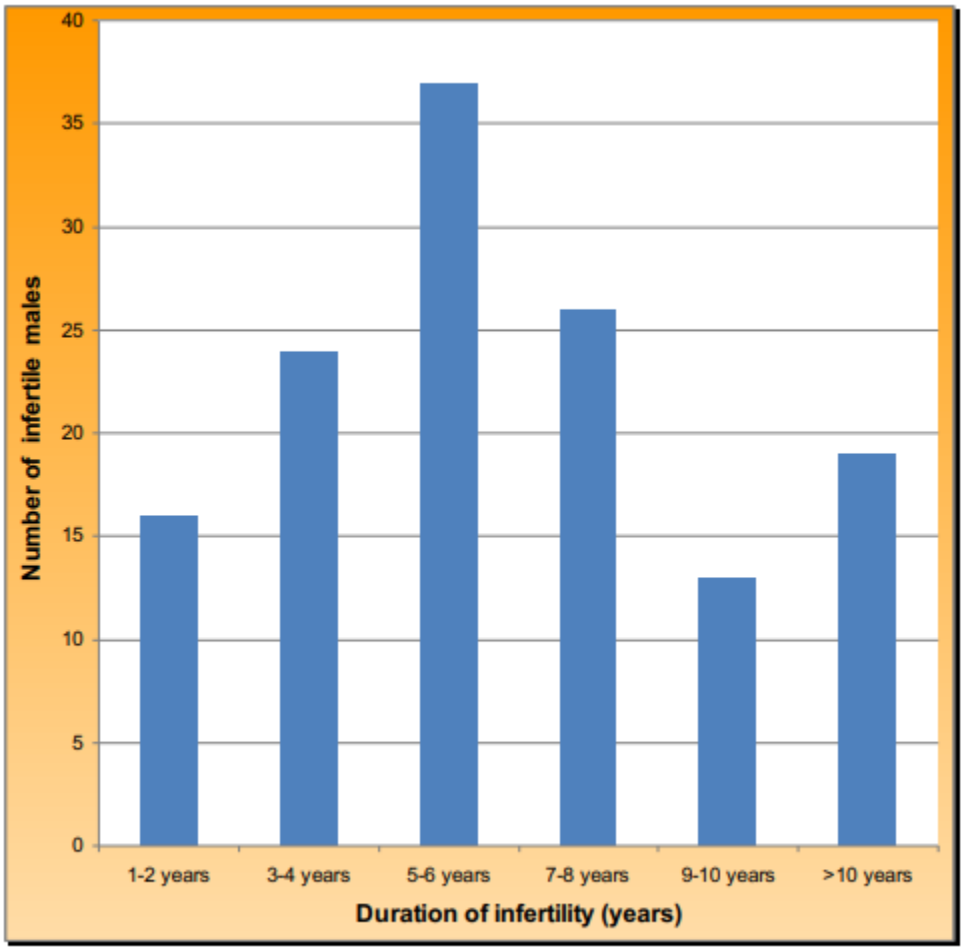

Figure 2: Number of infertile males* classified according to duration of infertility.

*** Total number of infertile males= 135.**

Table 3: Parameters of SFA for infertile males classified according to duration of infertility.

Parameters		≤2 years	3-4 years	5-6 years	7-8 years	9-10 years	>10 years	*p* value
		No.= 16	No.= 24	No.=37	No.= 26	No.= 14	No.=18	
Semen volume		2.733 ±0.296	3.022 ±0.210	3.117 ±0.168	2.981 ±0.145	2.769 ±0.251	2.500 ±0.209	0.272
Liquefaction time		32.000 ±1.069	31.522 ±1.063	31.389 ±0.707	32.231 ±1.891	30.769 ±0.769	28.789 ±2.214	0.625
Sperm concentration		64.800 ±8.190	61.304 ±4.480	59.306 ±3.896	59.577 ±3.765	59.385 ±4.036	62.526 ±5.380	0.972
sperm motility (%)		61.333 ±4.939	66.385 ±2.208	60.583 ±2.186	.04358 ±2.409	57.385 ±4.061	56.211 ±3.600	0.118
Sperm grade activity (%)	Grade A	3.333 ±1.517	6.154 ±1.804	3.194 ±0.665	0.435 0.300±	3.923 ±1.206	5.789 ±2.396	0.034
	Grade B	36.667 ±4.043	41.077 ±1.675	36.833 ±2.172	36.304 ±2.634	33.462 ±4.098	33.947 ±2.876	0.435
	Grade C	21.333 ±2.693	19.154 ±1.537	20.417 ±1.535	21.739 ±2.386	20.000 ±2.887	19.368 ±2.734	0.949
	Grade D	39.333 ±4.753	33.231 ±2.299	39.139 ±2.183	41.087 ±2.266	41.538 ±4.251	43.789 ±3.600	0.179
Progressive motility (%)		38.667 ±4.511	47.038 ±2.524	40.028 ±2.339	36.087 ±2.965	37.692 ±5.052	39.474 ±4.474	0.219
Sperm morphology (%)		33.867 ±1.175	32.696 ±1.743	33.944 ±1.957	37.962 ±2.094	38.846 ±3.260	36.053 ±2.044	0.302
Sperm agglutination (%)		20.533 ±2.952	13.864 ±1.859	12.943 ±1.352	12.808 ±1.963	15.923 ±3.990	13.235 ±2.184	0.175
round cell count		7.800 ±0.906	11.696 ±2.306	7.889 ±1.026	9.462 ±1.354	13.231 ±5.861	7.316 ±1.406	0.346

Data are Mean ±SEM

Total number of infertile males = 135

Among the 135 infertile couples participated in this study, positive IUI outcome was achieved in 16 couples. The positive IUI outcome, classified according to age groups, are shown in figure (3), and as it was obvious from that figure, the largest group of couples with positive IUI outcome was in (30-39) years (14.7%), infertile male patients in that age group were (68), from those (10) couples had positive IUI outcome. The second largest group was (≤29) years (12.5%) with a count of (3) couples had positive IUI outcome from (24) infertile patients involved

in that age group, this was fallowed by positive IUI outcome in age group (40-49) years (7.5%), here (3) couples had positive IUI outcome from (40) infertile males present in that age group, while the smallest group was (≥ 10) years when no one had positive IUI outcome from 3 patients involved in that age group.

Figure (4) clarified the positive IUI outcome classified according to the duration of infertility, as it shown in that figure, the group of patients with duration of infertility (≤2) years was with highest percentage of positive IUI outcome (25%) with a count of (4) from (16) patients involved in that group, while the lowest percentage was in the group with duration of infertility (≥10) years when no patient had positive IUI outcome from (19) infertile males involved in that group.

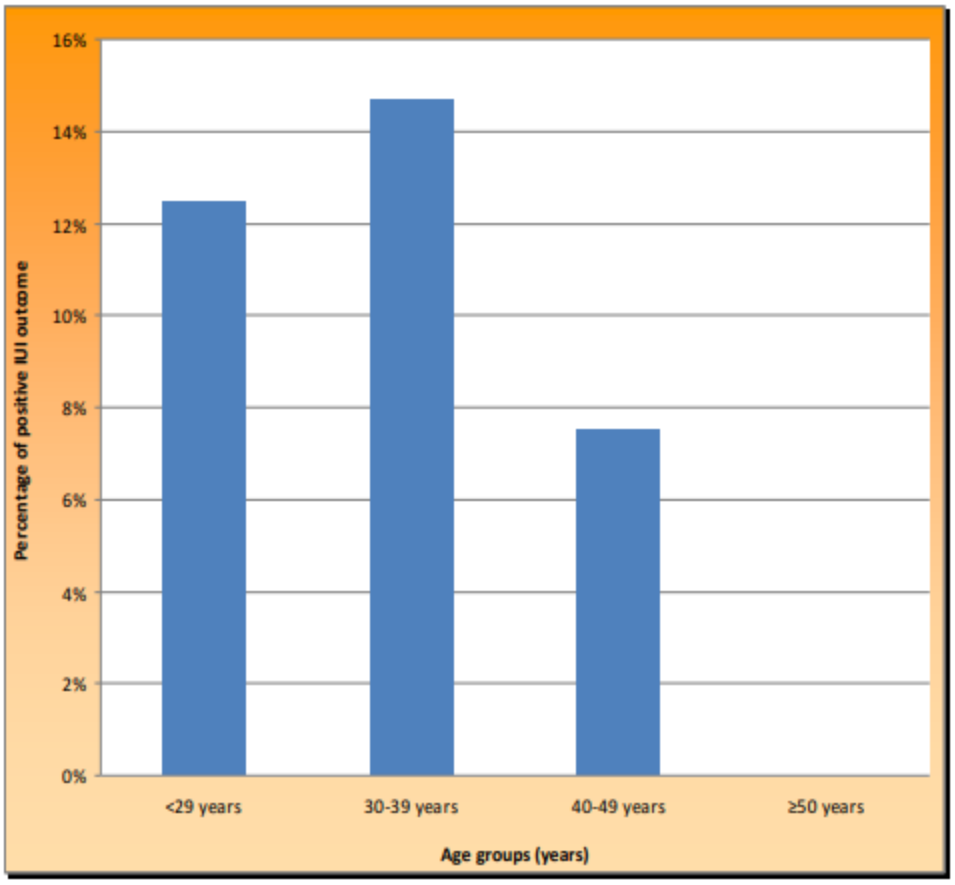

Figure 3: Percentage of positive IUI outcome classified according to age group.

Total number of positive IUI outcome in the study = 16 couples.

(≤29) years age group = 24 Infertile males, 3couples positive IUI (12.5%).

(30-39) years age group= 68 Infertile males, 10 couples positive IUI (14.7%).

(40-49) years age group= 40 Infertile males, 3 couples positive IUI (7.5%).

(≥50) years age group= 3 Infertile males, non with positive IUI (0%).

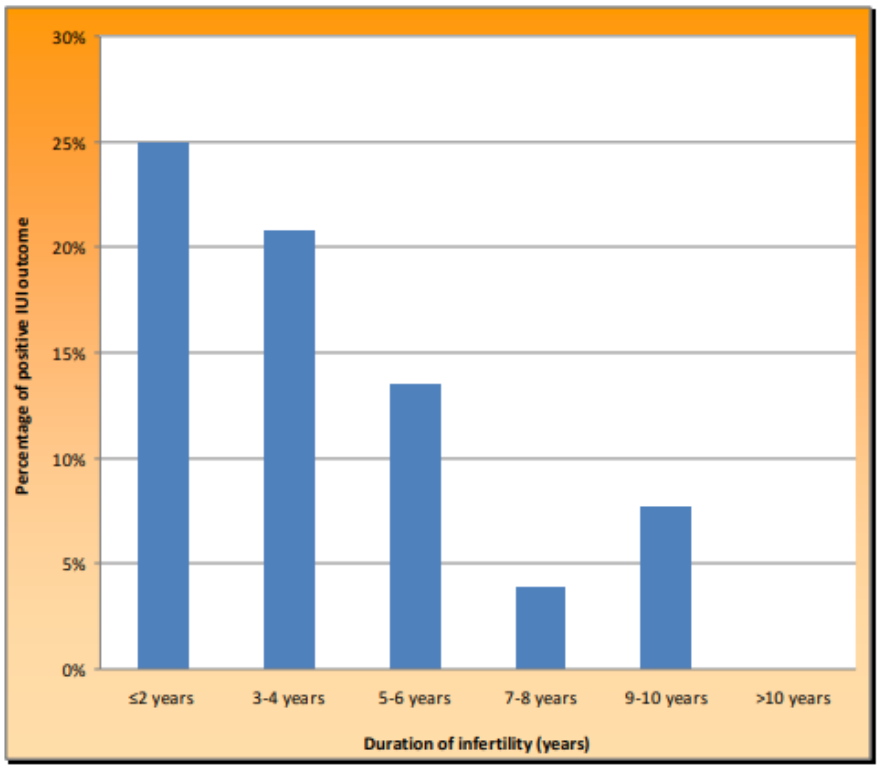

Figure 4: Percentage of positive IUI outcome classified according to duration of infertility.

Total number of positive IUI outcome in the study = 16 couples.
(1-2) years duration of infertility= 16 Infertile males, 4 couples positive IUI (25%).
(3-4) years duration of infertility= 24 Infertile males, 5 couples positive IUI (20.8%).
(5-6) years duration of infertility= 37 Infertile males, 5 couples positive IUI (13.5%).
(7-8) years duration of infertility= 26 Infertile males, 1 couple positive IUI (3.8%).
(9-10) years duration of infertility= 13 Infertile males, 1 couples positive IUI (7.69%).
(≥10) years duration of infertility= 19 Infertile males, non had positive IUI (0%).

DISCUSSION:

As for the major age group of infertile males was according to this study within (30-39) years which constitutes (50.4%) of total number of infertile males involved in this study, while the smallest group (2.2%) was within ≥50 years age. However, this result was in agreement with same result obtained by Al-Ani (2009), and this may explain that the youngest age groups are seeking more medical advices and are more directed to fathering a child in addition they are facing more psychological pressure and stress about their families (WHO,1999). Furthermore, the parameters of SFA for subjects in this study showed that the sperm concentration, percentages of sperm motility, sperm progressive motility, morphology, round cell count were higher in young age groups than in infertile males ≥ 50 years age and the same thing for the percentage of positive IUI outcome patients where the highest percentage was with in (30-39) years age and non with in ≥ 50 years age males. These results were in agreement with the results of many studies done in this Institute (Al- Zubaidy, 2005 and Kouty, 2007). It was assessed that semen quality, frequency of ejaculation, and sperm functions gradually decreases with highly developed age and starts to decrease after 35 years (Serre *et al.,* 1998).

It was clear that younger men have a stronger body defense mechanism than the older men. The normal physiological activity of the reproductive organs gradually decreases with advancing age due to induction irreversible abnormal physiological changes in the testis and ultimately affects the fertilization potential of human spermatozoa (Ford *et al.,* 2000).

In addition, it was mentioned that the male sex hormones and genetic quality for semen sample of infertile couples prepared for IUI procedure decline with progress age leading to an increased risk of genetic defects (Bhasin and Buckwalter, 2001). The spermatozoa from older men have increased incidence of abnormalities and there is some evidence which indicated that children born from older men may have an increased chance of abnormalities (Hargreave,1999). In general, the age is an independent risk factor that is involved in determining a couple's chance of achieving a successful pregnancy (Guerra *et al.,* 1998).

The present study results clarified that the groups of infertile males with history of infertility of (5-6) years duration were the largest groups compared to the other groups that may explain when increment duration of infertility, the patients become less interested in seeking the medical services. That beside the psychological factors especially the depression and hopeless play a major role for those cases (WHO, 1999).

Nevertheless, it was documented that the increments in the duration of infertility results in changes in the testicular supply which cause reduction in the testicular blood supply especially in the older patients and this affects the normal physiology of the testes and epididymis which results in marked elevation of the serum FSH and LH levels (Wolff, 1995).

However, for the SFA parameters of infertile patients, it was found that the male patients with duration of infertility ≥ 10 years were with lowest percentage of sperm motility, and it was found that the highest percentage of positive IUI outcome was with ≤ 2 years duration of infertility while it was non with patients with duration of infertility of > 10 years. A study was done in 2001 to determine the prognostic factors for achieving pregnancy with IUI, the results were best with short duration of infertility, secondary infertility, unexplained infertility, higher number of motile sperms inseminated and double inseminations in a cycle (Van Voorhis *et al*., 2001).

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