International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Effects of Paternal Age and Cigarette Smoking on Human Semen Parameters: A Retrospective Study on Infertile Couples

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Abstract: ***The purpose of this study was to estimate and analyze the causes of male infertility in Fertility and IVF center in Maternity Teaching Hospital in Hawler city-Iraq, and to determine the effect of paternal age and smoking habits on basic semen parameters (count x10/ml, motility, and normal morphology). This study was carried out retrospectively over two years period extended from Jan. 2011 to Dec. 2012 on 1055 infertile couples attended Fertility and IVF center of Maternity Teaching Hospital in Hawler city Capital of Kurdistan-Iraq, among them 368(34.8%) were male factor infertility among 368 men, 142 (13.45%) were azoospermic, 687 (65.2%) were female factor infertility with unknown causes. The largest male diagnostic category was men with unknown cause (43%). Beyond this, varicocele (17.2%), then male accessory gland infection (12.9%) after that obstructive (12.6%). However, ejaculatory dysfunction, congenital abnormality, chromosomal abnormality, and male descended testes were very infrequent. Men were divided into two groups according to smoking habit, 198 of them were smoker and 230 of them were nonsmoker. The obtained mean of semen parameters were lower in nonsmoker than smoker and this difference was not significant statistically. 1036 men were separated into seven groups according to their ages. The result showed that the mean and SD of all three semen parameters for men age ≥50 lower than other age groups and this difference was statistically significant for total motility by LSD test with P= 0.012, and for normal morphology 0.014. so in conclusion the influence of paternal age on semen parameters was significant.***

Keywords: Male, Infertility, semen parameters, motility, count

1. Introduction

Infertility is the inability of a sexually active, non contracepting couple to achieve pregnancy in one year (WHO, 2000). About 15% of couples do not achieve pregnancy within 1 year; almost 50% of them do so spontaneously in the second year of unprotected intercourse, and another 14% in the third year. Ultimately < 5% remains childless (Te Veld, Pearson 2002). No cause of infertility can be found using routine diagnostic work-up in 10-15% of couples. A male contribution to infertility is found in 45-50% of the remaining cases (WHO, 2000). While infertility is relatively common, it is very difficult indeed to establish the relative contribution of the male partner, given the profound difficulties which exist in the accurate diagnosis of male infertility. Almost studies that have attempted to evaluate the aetiology of infertility have used the conventional criteria of semen quality, promulgated by WHO 1992 and 2000). Although of great importance, these criteria are of limited diagnostic value (Irvine and Atiken, 1994), and a significant proportion of men with normal conventional criteria of semen quality will be infertile because of defects in sperm function (Aitkan et al, 1991) while a significant number of men with abnormal semen quality will have normal sperm function (Aitkan et al, 1985). In general aetiology of male infertility includes: congenital genital abnormality and spermatogenesis disorder, infections, immunologic and idiopathic, infertility is often multifactorial, couples whose fertility evaluation identifies no abnormalities but who are unable to conceive are said to have idiopathic.

Male reproductive function does not cease abruptly as in women, but become fundamentally changed with age (Sartorelli et al, 2001). Decline in semen parameters, such as volume, concentration, motility and morphology, has been observed in men of increasing age (Aboulghar et al 2007, Kuhnert and Nieschlag, 2004), morphologic changes in the testes could induce a quantitative and qualitative decrease in spermatogenesis (Kuhnert and Nieschlag, 2004) due to a decline in blood concentration with advancing age (Schubert and Jockenhovel, 2005). Some investigators have associated a decline in pregnancy rates with advancing paternal age in couples in which the women is younger (Mathieu et al, 1995, Klonoff-Cohen and Natarajan, 2004). On the other hand, Spandorfer et al(1998) suggested that the pregnancy is not affected by male age, Molina et al, (2010) and Omran et al, (2013) revealed that there was a significant reduction sperm density, motility, percentage of morphologically normal spermatozoa.

Despite worldwide anti-smoking campaigns, cigarette smoking is very common, the highest prevalence of smoking is observed in young adult males during their reproductive period (46% smokers between 20 and 39 years) (Langgassner, 1999). Smoking may impact on fertility, In meta-analysis study of (Vine, 1996) showed a mean reduction in sperm concentration of 13%, a mean reduction of sperm motility of 10%, and a mean reduction of morphologically normal sperm of 3% was reported in smokers, also in the study of (Lewin et al 1991) reported a statistically significant difference in sperm concentration. While in the study of (Trummer et al, 2002) there were no differences observed with respect to conventional semen parameters between smokers and non- smokers as reported

in a study of (Zinaman et al, 2000). Because of lacking of data on male infertility in Hawler- Iraq so the main aim of this study was to estimate and analyze the causes of male infertility in Fertility and IVF center in Maternity Teaching Hospital in Hawler city-Iraq and to determine the effect of paternal age and smoking habits on basic semen parameters (count x10/ml, motility, and normal morphology)

2. Materials and Methods

2.1 Population and Setting

This study was performed retrospectively in Fertility and IVF center in Maternity Teaching Hospital in Hawler city-Iraq between (January 2011- December 2012).A total of 1057 infertile couples attended to the center were included , the average age of infertile men was (33.1±7.3) years. Investigations such as SFA, urine analysis, FSH, LH, testosterone, beta HCG (Elecsys, 2010-Hitachi), ultrasonogrphy (Philips) were performed in Hawler central laboratories, while for chromosomal studies the samples were sent to laboratories outside the country.

Seminal fluid analysis was carried out for each patient; analyses were done in the andrology room according to (WHO, 1992) criteria and morphological evaluation was examined according to Kruger et al., 1986 strict criteria. Before sample collection patient were informed about the relevance of abstinence time (3-7) days and about importance of collecting the complete ejaculate and not using any soap during collection.

2.2 Sample Collection and Assessment

Samples were obtained by masturbation in asterile sample container, in the first five minutes sample container was labeled with name of patient and patients file number, patient's name, the time of ejaculation were recorded on the sample record form. Therafter, samples were inspected for color, opalescent and presence of mucous streaks, if the any sample didn't liquefied completely then the specimen was backed into the incubator for some minutes up to (60min). In this study, samples with moderate to very high viscosity a known volume of saline was added to samples with careful mixing with a wide bore pipette. Then the viscosity of the samples was assessed by estimation of how fast the sample ran out of pipette. A pipette filled (5 ml pipette) with semen and let the semen flowed back into the container if the droplets from (threads) that were more than 2 cm long, noted increased viscosity on the sample record form.

Examination of the samples: The volumes of whole ejaculates were assessed by sample container. examining a well mixed, undiluted preparation of liquefied semen on a glass slide, for each sample 6ul of well mixed semen placed on a clean microscopic slide and a clean cover glass placed on top (18x18mm, t 1 ½) then the slide examined by Olympus microscope (40x objectives) to determine the appropriate dilution and appropriate chambers to use. This is usually the wet preparation, when there were only very few spermatozoa per field of vision then number of motile and non-motile sperm were calculated, if non motile spermatozoa or only a few spermatozoa were found in the samples, the samples then were centrifuged and the pellet were examined under microscope (40 x objectives, microscope), if motile or immotile spermatozoa identified over a whole area of a cover slip then number of spermatozoa weather they were motile or not noted on the sample report form. Sperm aggregation: was determined in ten randomly chosen fields. Other cell debris (RBC, epithelial cells, round cells and bacteria) were assessed in several fields.

The number of spermatozoa was counted by Makler counting chamber. Semen was well mixed with diluents (0.9% normal saline), dilution was done according to (WHO, 1992), the chamber loaded with semen, in any strip of 10 squares of the grid indicated their concentration in millions/ml The total sperm number (10^6/ejaculate) was the products of ejaculate volume and sperm concentration. The total number of spermatozoa counted was divided by the factors given. Duplicate counting done for each sample then the average between two counts was taken for detection of random errors in sampling and counting chamber. (total sperm count less than $39x10^6$regarded as oligozospermia (WHO, 2010)).

2.3 Assessment of Sperm Motility

The assessment of sperm motility was performed immediately (not more than 20-30 min) after collection to avoid temperature drop or dehydration of the prepared sample. 6ul well mixed undiluted semen (+37c˚) was delivered onto a clean slide and covered with a (18x18mm, t 1 ½) cover slip. Five fields were taken for assessment of sperm motility within each field: all rapidly progressive; slowly progressive; non-progressive and non-motile spermatozoa were counted as percentage. Duplicate counting was used for each sample, then the average were taken, on the monitor screen in a circular field a grid with a squares corresponding to 25x25um in the specimen was attached to facilitate estimation of velocity 10 x phase contrast and corresponding condenser phase ring was used. (Total motility less than 40%, progressive motility less than 32% was regarded as asthenozospermia (WHO, 2010)). Type of CASA machine was Hamilton Throne Biosciences Version 12 Ceros.

2.4 Morphological Characteristic of Spermatozoa Assessment

Sperm morphology was assessed according to Tygerberg criteria (Kruger et al, 1986.) In brief; a droplet of the semen sample was measured on a glass-slide, air-dried, fixed and stained with spermac stain according to manufacturer's instruction (FertiPro N.V., Aalter-Lofenhulle, Belgium). For each semen sample, at least 100 spermatozoa were examined microscopically at X100 magnification. Occasionally when an extremely low sperm count was found the number was reduced to 50. To avoid variation in the interpretation of sperm morphology, all assessments were made by one specially trained laboratory technician.

In addition this study was done with collaboration of Fertility and IVF center of Maternity Teaching Hospital- Erbil- Iraq, approved by local committee of College of Medicine-

Hawler Medical University, and was funded by Hawler Medical University.

3. Results

This study was carried out retrospectively over two years period extended from Jan. 2011 to Dec. 2012 on 1055 infertile couples attended Fertility and IVF center of Maternity Teaching Hospital in Hawler city Capital of Kurdistan-Iraq, among them 368(34.8%) were male factor infertility. Among 368 men, 142 (13.45%) were azoospermic, 687 (65.2%) were female factor infertility with unknown causes. Out of 1055, 770 (72.8%) were primary infertility and 285(27.2%) were secondary infertility.

Male infertility causes and percentage of distribution in 1055 patients are shown in Table 1. The result showed that the largest male diagnostic category was men with unknown cause (43%). Beyond this, varicocele (17.2%) then male accessory gland infection (12.9%) after that obstructive (12.6%). However, ejaculatory dysfunction, congenital abnormality, chromosomal abnormality, and maldescended testes were very infrequent.

The frequency distribution for the sperm counts/ ml of 1055 infertile couples are shown in Figure 1. 13.46% were azoospermic, 9.19% had sperm count <10x10^6/ml, 4.45% had (10-14x10^6/ml), 11.85% had (15-25x10^6/ml), 14.79% had (26-40x10^6), the highest group 22.27% had (41-70x10^6/ml),18.20% had (70-100x10^6/ml), 5.31% had (101-150x10^6/ml) and only 0.47% had (>150x10^6/ml).

The frequency distribution of percentage of motile sperm per ejaculate of 1055 infertile couple are shown in Figure 2. Similar to sperm count 15.36% of them had no motile sperm in their ejaculate, 5% had only <10% motile sperm in their ejaculate, 4% had 10-20 % motile sperm, 4.3% had 21-39%, the highest group 28.72% had 40-50% then 22.27% had 51-60 , 17.44% had 61-70% ,and only 2.75% had >70 motile sperm in their ejaculate.

The frequency distributions of percentage of normal morphology of sperm of 1055 men are shown in Figure 3. 13.84% of them had no normal morphologic sperm in their semen, 18.48% had <4 % normal sperm, 12.89% had 4-9% normal sperm, 13.18% had 10-14% normal sperm, 28.34% had 15-20% normal sperm and 13.27% had >20% normal sperm.

The semen parameters (count x10^6/ml, total motility% and normal morphology %) of men of 1055 infertile couples distributed according to the smoking habit are illustrated in Table 2. In this study, men were divided into two groups according to smoking habit 198 of them were smoker and 230 of them were nonsmoker. The obtained mean of semen parameters was lower in nonsmoker than smoker this difference was not significant statistically.

In Table(3, 1036 men were separated into seven groups according to their ages, 102 of them were under 25 years, 244 of them 25-29, 294 of them 30-34, 202 of them 35-39, 119 of them 40-44, 42 of them 45-49, and 33 of them were ≥ 50 years old. The result showed that the mean and SD of all three semen parameters for men age ≥50 were lower than other age groups and this difference was statistically significant for total motility by LSD test with P= 0.012, and for normal morphology 0.014.

Table 1: Illustrates the male infertility causes and associated factors with their percentage of distribution in 1055 patients

Diagnosis	non selected patients%	Azoospermic
	(n=368)	patients % (n=142)
Idiopathic	43	9.2
Varicocele	17	3.3
Infection	12.9	2.7
Hypogonadotrohpic hypogonadism	4.2	1.3
Obstruction	12.6	8.2
Maldescended testis	1.9	0.8
Drugs	2.3	
Systemic disease (diabetic)	2.6	1.4
Sexual or ejaculatory dysfunction	0.3	
Chromosomal abnormality	1.6	1.6
Congenital testicular abnormalities	1.6	0.8

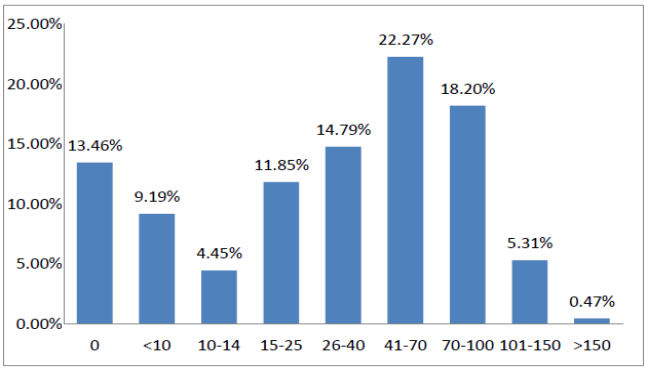

Figure 1: Shows the distribution of sperm count x10^6/ml. of 1055 men of infertile couples

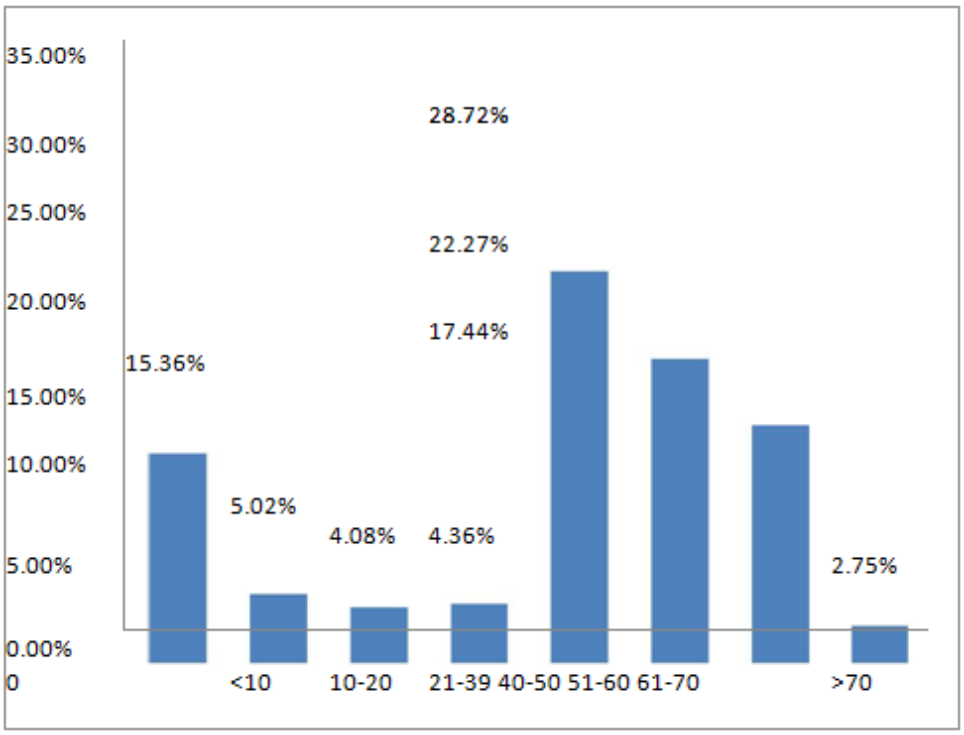

Figure 2: Shows the distribution of percentage of total motile sperm per ejaculate of 1055 men of infertile couples

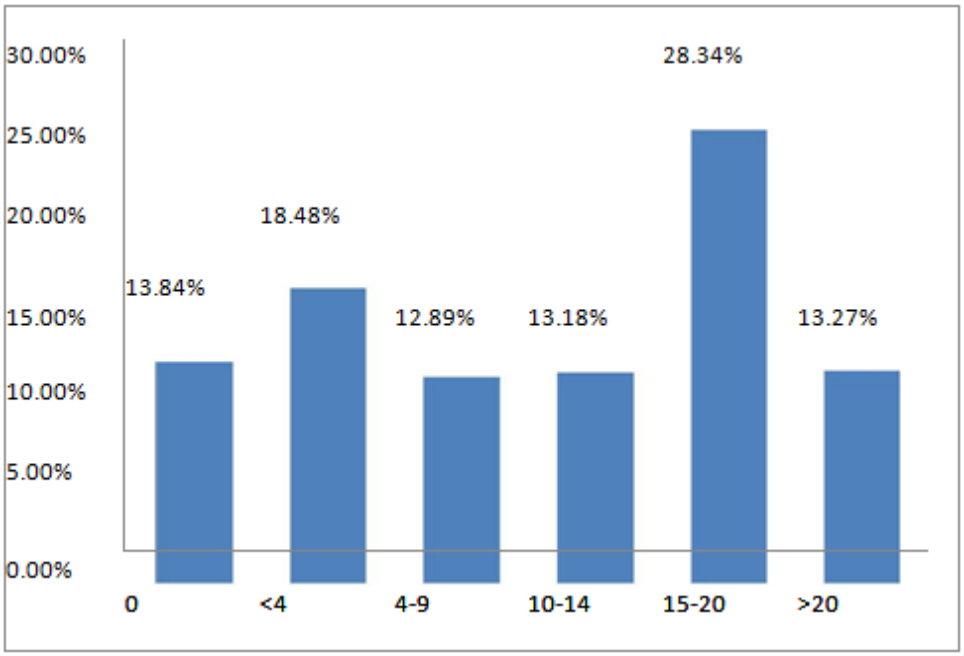

Figure 3: Shows the distribution of percentage of normal sperms of 1055 men of infertile couples.

Table 2: Illustrates the semen parameters (count x10^6/ml, total motility% and normal morphology %) of men of 1055 infertile couples distributed according to their smoking habit.

	Smoking	N	Mean	SD	P
Sperm count	Smoker	198	32.713	36.187	0.364
	Non-smoker	230	29.942	31.05	
Motility	Smoker	198	31.468	26.535	0.827
	Non-smoker	230	30.913	25.938	
Normal Percentage	Smoker	198	7.524	8.695	0.172
	Non-smoker	230	6.426	7.91	

Table 3: Illustrates the semen parameters of 1036 men of infertile couples distributed according to their age groups.

	Age(Years)	*N*	*Mean*	*SD*	*P*	*Significance*
Sperm	A) < 25	102	44.725	36.861	0.479	NS
	B) 25-29	244	48.346	42.008		
	C) 30-34	294	42.705	35.08		
	D) 35-39	202	43.288	35.013		
	E) 40-44	119	43.246	38.517		
	F) 45-49	42	51.029	48.286		
	G) 50	33	38.5	38.785		
	Total	1036	44.612	38.067		
Motility	A) < 25	102	43.397	25.953	0.012	
	B) 25-29	244	45.6	22.791		A X G
	C) 30-34	294	42.245	24.807		B X E
	D) 35-39	202	41.754	25.055		B X G
	E) 40-44	119	37.913	24.555		C X G
	F) 45-49	42	41.562	25.602		D X G
	G) 50	33	30.33	24.713		F X G
	Total	1036	42.148	24.636		
Normal	A) < 25	102	11.568	9.172	0.014	
	B) 25-29	244	11.076	9.494		A X G
	C) 30-34	294	10.971	9.689		B X G
	D) 35-39	202	10.893	10.377		C X G
	E) 40-44	119	9.796	8.929		D X G
	F) 45-49	42	10.771	9.641		F X G
	G) 50	33	6.303	7.16		
	Total	1036	10.748	9.595		

4. Discussion

One of the most important and underappreciated reproductive health problems in developing countries is the high rate of infertility and childlessness (Bergstrom, 1992; Leke et al, 1993). Only a limited number of papers report on the prevalence of infertility in developing countries (Ombelet et al, 2008). It is difficult to determine the proportion of male and female factors. It is likely, however, that approximately one-third of couple infertility is due to the male alone, one-third to the female alone, and one-third to both partners (Niederberger et al., 2003). Therefore in this study male infertility causes and associated factors and percentage of distribution of their semen parameters (sperm count, total motility and normal morphology) in 1055 patients were assessed as well as the effects of male age and smoking on semen parameters was determined.

The risk factors of male infertility differ from one country to another; therefore, it is important that any developing country determines the most influential factors in their population (Bayasgalan et al, 2004). The level and patterns of infertility are also significantly different among various countries and regions (WHO, 1999). In this study among 1055 infertile couples 368 (34.8%) were male factor infertility. This finding is in agreement with Nigerian studies which shown a prevalence of male infertility in 26-43% of cases (Adeniji et al, 2003; Ikechebelu et al, 2003; Olantunji and Sule-Odu, 2003), but they are higher than result of study by (Fathalla et al 1990) which was 20%; 22%. In this study, 142(13.45) were azoospermic this was in agreement with study by (Nieschlag and Behre, 2001; Abdalla, 2011) which was 9.1%,11.2%, and 11% respectively, higher than result of Irvine (1998) which was 4.2%. In current study the largest group was men with unknown causes 43% of non-selected and 9.2% of azoospermic if compared with (Nieschlag and Behre, 2001) which was 30% and 13.3 respectively, near to result of studies by (de Kretser, 1997) which was 40-50% and52.9% respectively, and lower than result of studies by (Lipshultz and Howards, 1997) which was 25.4% and 22.7% respectively. Another investigator (Comhaire et al, 1987) resulted that the largest single male diagnostic category was men with seminal abnormalities of unknown cause (25.3%). The second high group was those with varicocele (17.2% for unselected and 8.7% for azoospermic patients. These results are more or less similar to study by (Nieschlag and Behre, 2001)which was 14.8% and 10.9% respectively and study of (Philippov et al, 1998) which was 10.9%, and lower than study by (Greenberg et al, 1978; Lipshultz and Howards, 1997) which was 37.4% and 42.2% respectively.

Another observation relating to this study is the influence of men cigarette smoking habit on sperm parameters as shown in Table 2. The obtained mean of semen parameters were lower in nonsmoker than smoker this difference was not significant statistically. This study is agreed by the study of (Trummer et al, 2002) there were no differences observed with respect to conventional semen parameters between smokers and non-smokers, On the other hand, in meta-analysis study of (Vine, 1996) showed a mean reduction in sperm concentration of 13%, a mean reduction of sperm motility of 10%, and a mean reduction of morphologically normal sperm of 3% was reported in smokers, also in the study of (Lewin et al 1991) reported a statistically significant difference in sperm concentration, and Ferreira Braga et al, 2012 reported that the sperm motility was negatively influenced by smoking habit.

Some studies have suggested a negative trend in fertility with advanced male age (Kidd et al, 2001; de La Rochebrochard et al, 2006; Ferreira et al 2010). In this study the result showed that the mean and SD of all three semen parameters for men age ≥50 lower than other age groups and this

difference was statistically significant for total motility by LSD test with P= 0.012, and for normal morphology 0.014. This result agreed with the study of (Molina et al, 2010 and Omran et al, 2013)

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