Association of BMI with Follicular stimulating Hormone and with sperm parameters in males.

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Abstract
Objective: To ascertain association of raised BMI with Follicular Stimulating Hormone levels and with sperm parameters among infertile males.
Methods: A cross sectional study was conducted from june 2010 to May 2011. Sample size was 300 males (147 infertile and 153 healthy fertile control subjects) aged 30 and 60 years, selected from the Jinnah Postgraduate Medical Center (JPMC), Aziz Medical Center and Abbasi Shaheed Hospital, Karachi. All the subjects were grouped to BMI criteria (Ascian pacific region for WHO). They were selected by purposive sampling after a detailed medical history and physical examination. Semen analysis was done and blood samples were collected for serum FSH levels. Data was analyzed via SPSS 17.0 by using Analysis of Variances (ANOVA) and independent t test to compare the means and to evaluate the significant association with in the group.
Results: Strong positive correlation between FSH and BMI (r = 0.596, p<0.05) .Raised BMI appear to have an association with male infertility due to reduction in sperm count in males.
Conclusion: Adiposity is confirmed as risk factor for male infertility in local population.
Key words: BMI, Male Infertility, FSH, Azoospermia, Oligospermia.

I. Introduction:

Approximately 10–15% of couples are affected by infertility due to various physio-pathologic problems which come into consideration when the couple fails to conceive after unprotected coitus for more than one year. A male factor can be diagnosed in approximately 50% of them. The main causes of this disorder for men are associated with various factors, among them are genetic, physiopathologic and anatomopathologic abnormalities, intense and prolonged physical exercises, aging, drugs, and even excessive time of sexual abstinence (1). The epidemiological definition of infecundity recommended by the World Health Organization (2) is incapability to conceive within two years of exposure to pregnancy. It is important to note that the period of exposure used is often one-year in clinical studies whereas five years in demographic studies. Demographic languages describe "fertility" in terms of quantity of offspring rather than the physiological ability to reproduce and therefore, "infecundity" is sometimes used in preference to "infertility". From the formation of male gamete to fertilization, many physiological processes namely spermatogenesis (including spermiogenesis), ejaculation into the vagina, transport of sperm through the female genital tract, capacitation, egg penetration, and decondensation of the head are involved. All of these processes are necessary for the fertilization and early embryonic development. These complicated processes require balanced autocrine, paracrine, and juxtacrine signaling events between different male reproductive functions and optimum hormonal milieu. Failure of these signaling chain of events leads to male infertility (3). Unfortunately, after decades of extensive research, we are still unable to fully understand the regulation of the complex mechanisms underlying the etiology of male infertility at the molecular level, hence, unable to properly diagnose the cause of infertility for many patients. This is exactly the reason why nearly one quarter of all patients with male infertility is labeled as idiopathic. It is, now, presently believed that most of the diagnosis which are marked as idiopathic may have a genetic etiology (3). About 20% cases of male infertility, which cannot be associated with any other cause, the idea of genetic alterations as being a cause behind it, is being thoroughly scrutinized (4,5).

The most primitive bear witness of understanding the problem of infertility goes back to Egyptians, nearly thousand of years ago. Endocrine function of testicles was first documented by Berthold (1803-1861).Bernard and Zondek and Ashheim are well known for the discovery of FSH and LH. The advancement in technology coincides with the discovery of new product which results in rapid progress in field of reproductive biology. Infertility is defined as inability of couple to achieve pregnancy after one year of unprotected intercourse.(6). Male factors alone constitute 40% - 50% of all infertile study group of infertility (7) and they

contribute to another 30% in combination with female factors. Known etiologies of male infertility include cryptorchidism, testicular torsion or trauma, varicocele, seminal tract infections, antisperm antibodies, Hypogonadotropic hypogonadism, gonadal dysgenesis, environmental factors, and obstruction of the reproductive channels and can not neglect genetic factors as well.(8,9) .Most causes of male infertility results in defective spermatogenesis (9).Obesity is proposed for addition to this list obesity is a well recognized risk factor for female infertility (10).

The dilemma of infertility has its societal impacts on effected couples in terms of mental stress and depression (11). The prevalence of infertility in Pakistan is 21.9 % (12). In conservative South Asian culture, a child brings prestige and security to a woman(13) and the desire of having a child is much more intense in Asian part of the world (14).

Similar to women a sex hormone imbalance may affect reproduction in men, and excess weight can affect male hormone levels (15). Obese males usually express a characteristic hormonal profile described as "hyperestrogenic hypogonadotropic hypogonadism"(15).In fact, both total and free blood T levels are shown to be decreased in obese men. Total body fat has been associated with low levels of total and free T It appears that central obesity, in particular, is associated with a decrease in circulating androgen levels. The decrease in androgen levels is proportional to the degree of obesity(17).In obese males, adrenal androgens are diminished as well. A significantly reduced T to estradiol ratio has been observed among overweight or obese men. Body mass index (BMI) has been demonstrated to affect female fertility; however, little information is available on the impact of BMI on male fertility or semen parameters. Body mass index is associated with alterations in sperm parameters in several reports (18).Previously investigating factors associated with semen quality among couples who visited an assisted reproduction clinic, the prevalence of obesity among men with infertility was 3 times greater than among male partners of couples with idiopathic or female factors(19).Kort et al described the relationship between sperm parameters and BMI in over weight subjects (20).Hinz et al. by his study concluded that obese men presenting having BMI greater than 25 kg/m2 shows defective quality of sperm. (21).

Tsai et al (2004) has concluded that low sex hormone binding globulin and T levels, together with low gonadotropins, are frequently observed in diabetic men with severe obesity (22).Outcomes of assisted fertilization greatly influence by excessive body fat in female (23).Conclusion of various studies has also been shown that also female reproductive health is influence by BMI (24).

Prevalence and Geographical Distribution of primary and secondary   infertility
The prevalence and causation of infertility vary from area to area and it can be due to male and female factor or both (25).
Problem of infertility is the same in Pakistan as in the rest of the world but the desire of having a child is much more intense in this part of the world (26).
According to WHO-DHS comparative reports (2004) one in four married women of reproductive age in most developing countries are infertile because of primary or secondary causes (27).
Male infertility can occur due to variety of causes and qualitative abnormalities in semen analysis. The causes are divided into four categories ˙Infertility affects about 8% to 15% of the world's population and in about half of the infertile study group men are either the single cause of, or contribute to, the couple's infertility (28).
➢ The female factor
➢ The male factor
➢ Combined factor
➢ Unexplained Infertility
In 20-25% of infertile study group the problem is due to the male partner and in 30-40% the problem is predominantly female, in approximately 30% of infertile study group abnormalities were found in both partners, and in 15% no specific factor could be identified (29). Common causes of infertility vary from area to area which include anatomopathologic and physiopathologic anomalies like hormonal abnormalities,genital infections and varicocele(30).
Hypogonadotropic hypogonadism which is a failure of the pituitary gland to produce adequate amount of FSH and LH, can lead to decreased sperm counts and a state of infertility (32). Normal serum concentration of PRL have been shown to exert permissive roles in the male reproductive tract but excessive serum PRL concentration is correlated with infertility, hypogonadism, impotence and galactorrhea (30). The changes in LH and FSH may be the reason that causes the dysfunction of spermatogenesis and sperm maturation in patients with idiopathic azoospermia and oligospermia (32).

The high levels of PRL affect the normal functioning of the testes and indirectly responsible for defective spermatogenesis. (33) Hyperprolactenemia in men is responsible for lack of libido. The rise in the level of PRL may be due to several factors including the use of drugs such as anti-depressants, tranquilizers and anti-vomiting agents. Another cause of high PRL is the presence of small tumors in the pituitary gland which can be detected by CT scan or MRI. Normally treatment with bromocriptine restores normal levels of PRL. In

case the tumor in the pituitary gland is large, surgical removal of the growth is carried out. PRL is determined in subject with galactorrhea and androgen deficiency and loss of libido (34) High levels of PRL interfere with the production of FSH and LH which in turn will affect the testicular function. In men high PRL concentration causes decrease in sexual impotence. The treatment is usually carried out by the administration of bromocriptine .When the tumor in the pituitary gland is large and not responding to medical treatment, surgical removal of the tumor is the only treatment hyperprolactenemia can cause both reproductive and sexual dysfunction(35). Testes produce T which plays a significant role in the process of spermatogenesis. If the quantity of this hormone is low then the process of spermatogenesis is bound to be defective. Decrease intra testicular concentrations of T or other local factors are important for spermatogenesis(36)

Approximately 6mg/d of T is produced by the Leydig cells in a normal young person. T is bound to SHBG and human serum albumin (HAS). The secretion of T is pulsatile hence the serum concentrations may fluctuate between 0.3 to 1.2μg/dl. T is converted into dihydroT (DHT) by 5 α- reductase, which is nearly three times as potent but has a tight binding to plasma proteins. T can either be aromatized to estradiol which occurs in the brain, breast, liver and other tissues. As there is large mass of adipose tissue, aromatization became an important step that causes extra glandular synthesis of estrogen in the body. Approximately 20% of estradiol is produced by the aromatase activity of leydig cell itself (37,38). For proper functioning of reproductive organs an appropriate milieu of male reproductive hormones must exist for the normal sperm production and survival (39). **FSH and LH** hormones are secreted from the pituitary gland which receives signals from the hypothalamus and which in turn receives signals from the testes in the form of male hormone and if this signal is lacking the pituitary gland will be geared up in producing more FSH and LH to stimulate the cells in the testes. If these two hormones reach a level which is much higher than the normal level , testes will have stop functioning which is described as testicular failure (40).

Sperm production depends on the concerted action of FSH and LH on the testis. The action of LH is mediated through production of T by the Leydig cells. Since male germ cells possess neither FSH nor androgen receptor, the action of FSH and T occurs through sertoli cells. Although precise function of two hormone remain elusive the existing evidence suggest that both hormone stimulate spermatogenesis.If the levels of FSH and LH are low, the pituitary gland is not producing enough hormone due to some more complex problems. This situation is amenable to treatment by stimulating the hypothalamus or by injecting the same hormones. Isolated FSH deficiency is extremely uncommon, where as abnormal FSH level is rarely seen with normal spermatogenesis (41)

Various genetic syndrome having obesity and hormonal disturbance along with infertility.**Prader-Willi syndrome is an** inherited disorder which is associated with adult onset diabetes mellitus, hypogonadism, hypomentia, hypotonia at birth and obesity.Low FSH and low LH levels are present in blood and in urine. Defect on chromosome 16 is noticed in 15% of patient(42).Being overweight is also associated with reproductive dysfunctions. A cluster of metabolic abnormalities, including a tendency to accumulate abdominal fat, as well as resistance to and hyper-secretion of insulin and increased risk of developing type-2 diabetes in later life (43). Therefore, the levels of sex hormone binding globulin might be important only as a marker of altered hormone profiles in obese infertile men(44).

BMI and Sexual dysfunction:

Erectile dysfunction has been reported to be more common among men with high BMI(45) Published studies show that there is a link between erectile dysfunction and obesity and metabolic syndrome and some studies have also identified it as an early marker of cardiovascular disease in overweight individuals. Thus, increased sexual difficulties experienced by the overweight male can contribute to their fertility problems(46).

Study Objectives:
To determine the association between BMI (body-mass index) and serum FSH levels in male patients.
To Assertain the association between BMI (body-mass index) and sperm parameters in male patients
To correlate the levels of serum FSH with infertility in male patients.

II. Methadology:

Research Design:
It was a case control study.
Settings:
The study was carried on the patients attending outpatient department (OPD) of Aziz Medical Hospital and Jinnah Post Graduate Medical Center (JPMC) from June 2010 to May 2011. Total no 147infertile study group were included in the study and 153 healthy fertile male were also included for better comparison and categorized as control fertile group (**Control**: Healthy normospermic males)

3.3 Inclusion Criteria and Exclusion Criteria:

- The study is based on samples collected from 147 infertile study group with semen analysis result during the period of Jan 2010 to march 2011. The inclusion criteria for control health male is age between 20 – 50 years having children's and for cases was Idiopathic infertile male patients having age between 20 -50 years. Only patients having normal karyotyping were included in the study.
- The exclusion criteria included secondary infertility which includes infertility due to:
  a. Obstructive Azoospermia, testicular insults (torsion, trauma), Cryptorchidism
  b. Infections (mumps, orchitis, epididymitis)
  c. Pelvic surgery or hernia repair
  d. Patients with diabetic neuropathy
  e. Patients taking anabolic steroids, non-steroidal anti inflammatory drugs (NSAIDS), cimetidine and spironolactone (which may affect spermatogenesis); sulfasalazine and nitrofurantion that affect sperm motility therapy for at least 74 days.
- Patients with psychiatric disorder were also excluded.

Study Duration:
The duration of study was one year.

Sample Size:
147 infertile male and 153 healthy fertile males .

Sampling Techniques:
Sampling technique was non probability, purposive sampling.

Population of Interest (Human Subject /Material Etc.)
This research involves human subjects, a detailed informed consent was taken before participation of individual & confidentiality of study subjects was maintained and this research did not include factors which can harm any human being.

Methodology:
Semen analysis was carried out on azoospermic and oligospermic men with an abstinence of 5 days according to WHO criteria. Patients were also subjected to hormonal assessment which comprises of serum FSH levels.

Semen analysis:
Semen analysis was used to assess the quantity and quality of spermatozoa and seminal fluid (47)After taking detailed history, the process of semen collection was verbally explained to the patients.

Semen Sample Collection:
  a. The semen was obtained in a room separate from the main laboratory.
  b. The sample was collected in a sterile wide mouthed plastic container which was marked with the name of the patient, registration code, the collecting time and date.
  c. Sexual abstinence of minimum 1-3 days but not exceeding seven days was required before sample collection. The sample was examined within two hours after collection.(48,49)

Human Semen:
Semen is a fluid ejaculated at the time of orgasm (50).
The analysis of the collected semen followed the protocol explained in WHO laboratory manual for the examination and processing of human semen (48).

There are two steps in semen analysis:
1. Macroscopic evaluation includes liquefaction time (above 60 min. treat with bromelin), viscosity, appearance (smell, color), volume (quantity) and pH.
2. Microscopic evaluation include aggregation of Spermatozoa, agglutination of Spermatozoa, sperm motility, concentration of sperms and total sperm number, morphology of spermatozoa, cells other than spermatozoa and sperm vitality.
3. **Hormonal Analysis:**
4. Hormone analysis was done by enzyme linked immunosorbent assay (ELISA)(51)
5. **Storage and application:**
6. The temperature of the reagent was brought to room temperature before the usage and kept in refrigerator
7. **Collection of Blood Sample:**
8. After obtaining consent of patient and normal subject, 10 ml blood was drawn from each subject by venepuncture using plastic disposable syringe under aseptic measures in morning between 9am to 2 pm.
9. The blood was collected in a test tube or gel tube and allowed to clot and centrifuged   for separation of serum within an hour of blood collection. After centrifugation   serum was stored in deep freezer at -20°C for subsequent hormonal analysis. Samples were analyzed in the batches of hundred to omit in between

analytical variation. Serum PRL , T and FSH were measured by ELISA using kit supplied by DSI, Italy. (52)

Determination of FSH:
The quantitative determination of follicle stimulating hormone concentration in human serum by a microplate immunoenzymometric assay supplied by Monobind Inc. USA. (53)
Ethical Consideration:
The study was approved from Institutional Review Board (IRB), the Board of Advanced studies and Research (BASR) and funding committee of DUHS.
Data Collection procedure:
Detailed questionnaire was used as data gathering instrument. Patients from JPMC and Abbasi Shaheed Medical hospital, from the period of January 2010 to march 2011 were included in this research.
Data Management and Statistical Analysis:
The data was analyzed using Statistical Package for social sciences (SPSS) version 16.0, the analysis carried out was descriptive and independent t test to find significance of mean value of lab parameter and discriminate functions to differentiate between azoospermia and oligospermia. Anova and person correlation coefficient was used to compare the association between BMI and reproductive hormone.

III. Results:

The study was carried out on 300 subjects which included 153 control healthy fertile male and 147 primary infertile male with mean age 33.30 ± 7.18 and 36.7 ± 6.03 and mean BMI 26.7 ± 4.3 and 24.3 ± 3.7 respectively (Table1). Study sample were collected from infertility clinics of Aziz medical Hospital Karachi and Jinnah post graduate medical hospital Karachi. Statistical analyses were performed using the Statistical Package for the Social Sciences version 16. Mean values were reported together with +/- standard deviation (SD).All categorical variables were presented by frequencies and percentages. Mean differences between groups were tested statistically using the two sample independent t test. A p- value< 0.05 was considered statistically significant.

Age Distribution:
Among study group 38.1% of infertile has < 30 year of age, 44.2 were between 30-39 and 15.6% were 40-49 and 2% were > 50 years of age. Whereas seem in healthy fertile group 67.3% were between 30-39, 28.8% were 40-49, and 4.6% were > 50 years of age.

Sperm Characteristics:
The mean value of sperm count of infertile males 41.2 ± 41.4 millions/ml with 95%C.I (48.0,34.5) and fertile group mean sperm count was 112 ± 23.9 millions/ml with 95%C.I (116.0,108.4) and it is also shown that there is significant difference in sperm count between infertile male and healthy fertile group.
The common age group in infertile study group and controls were 20-40 years followed by mean age of infertile study group is $30.30\pm$ at 95%C.I (34.53,32.18)where as in control healthy males mean age is 36.7 ± 6.3 at 95%C.I (37.68,35.7)years as shown in Table 15.
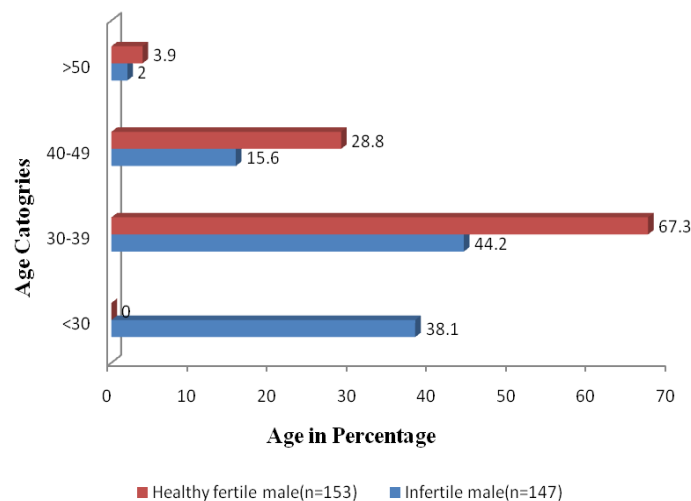
Figure.1 Shows distribution of study subjects (Infertile and fertile group) according to their age.

Table: 1 Descriptive statistics of fertile and infertile study subjects

VARIABLES	p value (< 0.0.5)	Infertile subjects			Fertile study subjects		
		Mean ± S.D	95% Confidence Interval		Mean ± S.D	95% Confidence Interval	
			Lower	Upper		Lower	Upper
Age(years)	0.448	33.35±7.18	32.18	34.53	36.7±6.03	35.7	37.68
Weight(kg)	< 0.01	75.4±12.6	73.4	77.5	70.5±12.5	68.05	72.0
Height(m^2)	0.962	1.67±0.07	1.66	1.68	1.7±0.7	1.68	1.71
FBS	< 0.01	99.7±34.3	94.1	105.3	91.8±11.3	89.6	94.1
BMI	< 0.01	26.7±4.3	26.0	27.5	24.3±3.7	23.6	25.1
FSH(mIU/ml)	< 0.01	20.7±1.0	19.0	20.7	8.5±4.1	7.8	9.1
Sperm Count	< 0.01	41.2±41.46	34.5	48.0	112±23.9	108.4	116.0

Table;2 Biophysical And Endocrine Profiles Of Studied (Infertile)Group With Their Mean (SD) And 95% Confidence Interval

Parameters	Mean(SD)	95% CI
Age(years)	33.30±7.2	(32.1,34.48)
BMI kg/m^2	26.7±4.3	(26.0,27.5)
FSH (mIU/ml)	20.7±1.0	(19.0,20.7)

The mean age of the studied group was 26.7±4.3 years with 32.1years as the lower limit and 34.48 years as the upper limit of 95% confidence interval. Body mass index was also calculated after height and weight measurement, which was observed as 26.7±4.3 kg/m2 with 26.0 as the lower limit and 27.5 as the upper limit of 95% of confidence interval. 20.7± 1.0mIU/m was discovered as the mean value for follicular stimulating hormone and had 19.0 mIU/ml as the lower limit and 20.7 mIU/ml as the upper limit of 95% confidence interval. Mean PRL hormone value was illustrated as 16.9±2.1mIU/ml with 13.5as the lower limit and 20.4 as the upper limit of 95% confidence interval.(Table:2
We divided the studied group of Infertile male into three categories of serum FSH. 25.2% of infertile study group had normal range that is 1.0-14mIU/ml. 37% falls in the category ranging between 1-14mIU/ml. 72.8% of patients had very high FSH that is above 28mIU/ml. Two persons have less than 1.0mgIU/ml.(Table :3)

Table: 3 Frequency Distribution Of FSH In Our Study Population

Follicle stimulating hormone (FSH) mgIU/ml	n=147	Percentage %
Less than 1.0 mgIU/ml	3	2.0
1.0-14.0 mgIU/ml	37	25.2
Above 14.0 mgIU/ml	107	72.8

There is significant difference between reproductive hormones levels of infertile male and health control group. Mean value of FSH in case/infertile male was 20.7 ± 1.0 at 95% confidence interval. There as in control mean FSH is 8.5 ± 4.1. (Table.15)
Body Mass Index was also evaluated during the study. Amongst the total infertile subjects, the mean BMI was found to be 26.7 kg/m^2 with a standard deviation of 4.3 kg/m^2. In healthy fertile subjects, the mean BMI was found to be 24.3kg/m^2 with a standard deviation of 3.7 kg/m^2.The minimum BMI observed in infertile subjects was 15.5 kg/m^2 and in fertile subjects 15.0 kg/m^2 while the maximum was found to be 37.88 kg/m^2 in infertile male subjects and 34.0 kg/m^2 in healthy fertile subjects. The mean BMI was found to differ significantly between the two groups (p < 0.01).
According to the Asian Pacific criteria for BMI, only 29% infertile males and 35.3% fertile males fall in to the normal BMI category. Approximately 78.2% (n=115) infertile men were found to have BMI greater

22.9 thus putting them to a greater health risk. Out of this 78.2%, 12.2% (n=18) patients were classified as over-weight, 40.1% (n=59) as obese type I and 25.9% (n=38) as obese type II. On the other hand, only 62.2% men from the fertile group were found to have BMI greater than 22.9. 22.9% (n=35) were over-weight, 26.8% (n=41) were classified as obese type I, and 10.5% (n=16) were obese type II. 2% (n=3) cases and 4.6% (n=7) controls were observed to have BMI lesser than 18.5. The two groups were found to have statistically significant difference among each other (< 0.01) (Table :4).

Table: 4 Showing frequency and percentage of study groups lying in the different groups of BMI according to the new BMI criteria for Asia Pacific

Body Mass Index (kg/m²)	Infertile males (n = 147)		Fertile healthy males (n = 153)		p value
	Frequency (n=147)		Percentage (%)	Frequency (n=153)	
Under-weight (<18.5)	3		2.0	7	
Normal (18.5 – 22.9)	29		19.7	54	
Overweight (23.0 – 24.9)	18		12.2	35	0.000
Obese I (25.0 – 29.9)	59		40.1	41	
Obese II (≥30)	38		25.9	16	

A comparison between the various parameters determining the quality of sperms was done between the infertile and fertile men during the study shown by Table:5

The mean volume of sperm observed in the fertile group of males (n=153) was found to be 3.43 ml with a standard deviation of 1.08 ml. The infertile group of males (n=147) showed a mean volume of 1.22 ml with a standard deviation of 0.421ml. There was a significant difference observed in the mean volume of sperm in the fertile and the infertile group of individuals (p < 0.01).

On an average, about 56.46 (x 10^6/ml) live sperms were found during the semen analysis of fertile group with a standard deviation of 26.32 (x 10^6/ml). The mean live sperm count in infertile group was found to be 2.47 (x 10^6/ml) with a standard deviation of 5.03 (x 10^6/ml). A significant difference was seen in the total number of live sperm count seen in the fertile and the infertile group (p < 0.01).

Another parameter to determine the sperm vitality of individuals was the pH of the semen. The mean pH found in the group of fertile men was 7.65 with a standard deviation of 0.28. The mean pH observed amongst the infertile males was found to be 7.76 with a standard deviation of 0.59. There was a significant difference seen in the pH of the semen of the fertile and the infertile males (p < 0.01).

The morphology of the sperm is also an important parameter that contributes towards male infertility. It was observed that in the fertile group of individuals, a mean of 53.46% were morphologically normal sperms with a standard deviation of 21.16% while in the infertile group, a mean of 12.33% morphologically normal sperms were found during the semen analysis with a standard deviation of 17.63 %. There was a significant difference observed in the percentage of morphologically normal sperms found in the fertile and the infertile group of men (p < 0.01).

It was observed that the mean percentage value of the motile sperms present in fertile men was 56.11% with a standard deviation of 7.46% while in infertile group; the mean value was 14.42% with a standard deviation of 23.38%. Out of these, 36.00% sperms with a standard deviation of 8.45% were found to have rapid linear progression among the fertile men while in infertile group only 9.82% sperms with a standard deviation of 15.88% were found to have a rapid linear progression. 20.03% sperms with a standard deviation of 9.91% were found to have slow/non-linear progression among the fertile men while in infertile group 6.51% sperms with a standard deviation of 10.27% were found to have a slow/non-linear progression. 44.11% sperms with a standard deviation of 7.24% were non-progressive among the fertile men while in infertile group 16.74% sperms with a standard deviation of 25.04% were non-progressive. There was a significant difference observed in the percentage of motile sperms, rapid progression, slow/non-linear progression and non-progression found in the fertile and the infertile group of men (p < 0.01).

Table: 5 Showing comparison between sperm parameters among infertile men and fertile

Parameter	Fertile group (n=147) Mean ± SD	Infertile Group (n=153) Mean ± SD	p value
Volume (ml)	3.43 ± 1.08	1.22 ± 0.41	<0.01
pH	7.65 ± 0.28	7.76 ± 0.59	0.044
Live Sperm Count 10⁶/ml	56.46 ± 26.32	2.47 ± 5.03	<0.01
Morphology			<0.01
Normal form (%)	53.46 ± 21.16	12.33 ± 17.63	<0.01
Motility			
% motile sperm	56.11 ± 7.46	14.42 ± 23.38	<0.01
Rapid linear progression (%)	36.00 ± 8.45	9.82 ±15.88	<0.01

Slow/non-linear progression (%)	20.03± 9.91	6.51 ±10.27	<0.01
Non-progressive (%)	44.11 ± 7.24	16.74 ± 25.04	<0.01

Coefficient Correlation:
   As attempt has made to investigate the linear association between the continuous variables by applying the standardized co-relation co-efficient statically method. In this study the different seminal and sperm characteristic as well as hormones total 7 variable were co-efficiently co-related. The results obtained from the application of such an approach are presented in Table6.

There is a strong significant correlation between BMI and serum FSH level. Strong positive correlation between FSH and BMI (r = 0.596, p<0.05).

Sperm concentration found to be significantly lower in men with a BMI greater than 25 as compared to men with BMI less than 25.

A significant negative relationship for sperm count, although a lower sperm concentration was only statically significant in obese men compared with normal weight men (P<0.05). No association was found for semen volume and sperm morphology. A significant negative correlation was found for sperm motility (r = -0.231, p = 0.05).

Table: 6 Correlation Between BMI With Different Variable Studied

	BMI	Sperm motility	sperm count	FSH
BMI	1	0.138[*]	-0.250[*]	0.596[*]
Sperm motility		1	-0.231[*]	0.277[*]
sperm count			1	-0.486[*]
FSH				1

*. Correlation is significant at the 0.05 level

IV. Discussion

   Male infertility has become a very common problem and causes severe mental anguish and anxiety to sufferers. It is difficult to assess prevalence of infertility in developing countries. The perceptual experience of infertility in Pakistan is almost same as in different parts of the world, except the fact that infertile males are reluctant to report for evaluation in our country. Demographic health surveys data provided by WHO (2004) shows that female primary infertility   prevalence in Nepal is 6%, in Korea 2%, in Indonesia 7 % ,India 3%, Bangladesh 4% and Pakistan is 4% However the prevalence of male infertility the in Pakistan is 7%.Rahim et al in Pakistan has concluded in their study (151) that 50% cases of infertility are due to male factor. The frequency of infertility was 12%, due to male factors is 45%, 41.64% by female, unexplained in 7.50% and both were involved in 5.70% of infertile study group. Among various causes of male infertility, obesity is one of the leading factor(91) but the relationship of BMI with spermatogenesis and reproductive hormone is certainly not straightforward.

BMI As Marker of Body Fat:
   Various studies has included BMI ,as marker of body fat (54) although waist hip ratio is a better predictor of reproductive outcome (55) as BMI does not distinguish between android body fat distribution and gynaecoid fatty distribution. BMI is not a perfect measure of body fat and can be reliable as surrogate predictor of body fat, its validity and thresholds for excessive body fat or obesity have been questioned (56) but it has been recently used to measure or estimate body fat and obesity(57). Deurenberg in Netherlands concluded BMI as a sensitive body fat predictor by using a age and gender specific formula (58).In France, Wittemer et al, used BMI to measure an outcome of IVF procedure in female and concluded mean that ratio of follicle-stimulating hormone–luteinizing hormone increased significantly in accordance to BMI levels (58).

Another study conducted by Jorge et al in Boston, Massachusetts determined the relationship of BMI with DNA integrity and semen quality (59).In 2007 by systematic review Maheshwari   et al concluded that obesity is a major reproductive health problem and raised BMI were associated with decline in conception rate (54).

Association between Serum FSH and BMI:
   In our study mean values of FSH was 8.5 mIU/m for healthy control group 20.75 mIU/ml for infertile mal.Higher levels of FSH in obese males are in accordance with various studies conducted worldwide. Wu FCW et al (60) found twenty one azoospermic male with raised FSH serum level and concluded that increase in oestradiol (46±6 ± 12±6 pg/ml) is due to excess stimulation of sertoli cells by GnRH stimulation. A case study

conducted by Mara Y Roth   et al in 2008 presented a case of 29-year-old infertile male with morbid obesity, gynecomastia, hypogonadism and low testicular volume. His BMI was 54.5 kg/m2 and his sperm concentration was almost at undetectable levels. Case study typically explains the dispute in diagnosing, managing and treatment of   male infertility with obesity is the primary causative factor. Treatment with an aromatase inhibitor, anastrozole, led to normalization of the patient's testosterone, luteinizing hormone and follicle-stimulating hormone levels, suppression of serum estradiol levels, and normalization of spermatogenesis and fertility(61).

Recently in 2011 Sarwat noticed in her study (62) that excessive body fat are expected to cause alteration in male reproductive hormonal profile.In her study male subjects with BMI > 20 have higher serum levels of Leptin and showed a statistical significant positive correlation with LH (p < 0.01) and FSH (p < 0.002) and a significant negative correlation with T (p < 0.001),There study also pointed out that BMI appears to have significant association with serum leptin levels.

Negative correlation of BMI with T (r = −0.38), FSH (r = −0.22), and inhibin B levels (r = −0.21) has been reported by Pauli (206), no correlation of BMI or skin fold thickness with semen parameters (sperm density, volume, motility, or morphology)was found. Inhibin B level correlated significantly with sperm motility (r = 0.23). Men with paternity had lower BMIs (28.0 kg/m2 vs. 31.6 kg/m2) and lower skin fold thickness (24.7 mm vs. 34.1 mm) as compare to infertile men.Similar results were found in Danish population by Tina Kold Jensen. Serum T, sex hormone-binding globulin (SHBG), and inhibin B all showed decreased trend with increasing trend of BMI. Serum FSH was higher among slim men with a BMI <20 kg/m2 had a reduction in sperm concentration and total sperm count of 28.1% (95% confidence interval [CI] 8.3%–47.9%) and 36.4% (95% CI 14.6%–58.3%), respectively, and men with a BMI >25 kg/m2 had a reduction in sperm concentration and total sperm count of 21.6% (95% CI 4.0%–39.4%) and 23.9% (95% CI 4.7%–43.2%), respectively, compared to men with BMI between 20–25 kg/m2. Semen volume and percentage of motile spermatozoa were not affected by BMI(63).

Association between BMI and spermatogenesis:

Semen is composed of secretions of seminal vesicles, prostate glands, cowper's glands and sperms. During emission, contraction of the vas deferens coincides with contraction of the muscular coats of the two accessory sex glands and results into mixing of the sperms with all the components of semen (64) .

The secretion from the seminal vesicles comprises approximately 60% of the seminal volume. These glands are the primary source of fructose. The seminal vesicles also secrete semenogelins, which induce coagulation of semen after ejaculation. The alkaline secretion of the prostate, which makes up about 30% of the volume, is high in citrate, zinc, spermine and acid phosphatase. PSA is a serine protease that liquefies coagulated semen after a few minutes. The predominant buffers in semen are phosphate and bicarbonate. A third accessory gland, the bulbourethral gland ,empty into the penile urethra in response to sexual excitement prior to emission and ejaculation. This secretion is high in mucus, which lubricates, cleanses and buffers the urethra (64)

Various studies proved a strong positive association between BMI and defective spermatogenesis In obese males with BMI ≥35 kg/m^2 sperm with high DNA damage were significantly more numerous in obese men than in normal-weight men. No association was found between Body mass index and sperm concentration, motility, or morphology. The ejaculate volume of semen showed decreased trend with increasing BMI levels(64).

Also concluded by various studies that raised BMI play crucial role on regulation of HPT axis suggest that impaired sperm production as a result of unopposed feed back by hypothalamus on testicular Leydig cells. Data also shows no link between BMI and steroid hormone but our study is in agreement with report of decrease T level.

We did not found statically significant link between sperm concentration sperm, morphology or sperm mortality among various BMI categories. Although semen volume was lower in male having BMI > 30 relative to control group with normal BMI (29/25). In addition the sperm concentration was dramatically lower in study group having BMI > 35. In addition, the study provides sufficient rationale for a complete analytical profile of the infertile male in order to accurately diagnose the cause of infertility and to propose proper therapy. Finally, the study demonstrates that the Pakistani infertile male (who are highly disturbed emotionally) are reluctant to undergo fertility assessment.

Limitations:
1. Because of the ethical issue males resist to accept their stigma of infertility
2. Family studies could not be performed.
3. DNA studies for mutation were not performed

5.3 Weakness/strength:
1. Only male from the age 20 – 45 age were included in the study
2. We cannot rule out internal anatomical deformity.

V.   Conclusion :

Hence we concluded that the BMI > 30 is associated with   altered reproductive hormone levels in obese males.

Recommendation :

We recommend that BMI can be used as an indicator for obesity and use to measure the risk not only for obesity but for reproductive health measure.

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