Journal of Education for Pure Science- University of Thi-Qar
Vol.9, No.2 (June, 2019)
Website: jceps.utq.edu.iq Email: jceps@eps.utq.edu.iq

DOI: http://doi.org/10.32792/utq.jceps.09.02.01

Physiological Study of the Gonadotropic Hormones Role on The Male Infertility in Thi-Qar Province

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Received 17/09/2018, Accepted 06/11/2018, Published 02/06/2019

Abstract:

This study was designed to Identify the role of hormones profile such as Gonadotropic hormonesof male infertile in Thi-Qar Province through the assessing of gonadotropic hormones which is responsible for infertility occurring. A 200 blood serum samples were collected from the patients at the Infertility Unit who directly deal with infertility and other 100 blood serum samples collected too from healthy people. The hormones were Evaluated from the two serum sets and tested hormones by technology of using a sandwich immunodetection method. Results noticed that the highest percentage (11%) of serum hormones low levels of Gonadotropic hormones (Test., LH. &FSH.) for Oligozoospermia and Azoospermia equality, therefore it was observed significant difference between hormones levels of infertile groups compared with control at ($p \leq 0.05$).

Keywords: Gonadotropic hormone, FSH, LH, Infertility.

Introduction:

Infertility is a major modification in structural and functional properties of male reproductive organs during adult life involve or which results from an imbalance in the production of gonadotropic hormones such as Luteinizing hormone LH, Follicle stimulation hormone FSH and testosteron. as well as Prolactine hormone unbalanced (Endocrine disruptors) Which affect on the production of FSH that stimulated sertoli cell to produce spermatozoon and their nutrition therefore Sertoli cells called some time mother cells or nurse cells, also LH. hormone stimulate Leydig cell (Interstitial cells) to produce testosterone hormone in testes. while Testosterone hormone which majority produced in testes nearly 95% and other ratio 5% from Adrenal cortex is responsible for many functions such as embryonic development through differentiation growth of male germ cells in testes besides testes descend normally from abdomen into scrotum at the post phase of embryonic development ,play critical role in spermatogenesis, stimulation protein synthesis, signals of sex climax and finally accessory sex properties appearance, so continuous disorders may lead to functional changes in the cells of the body, especially male productive cells, causing the emergence of

complications of infertility either primary or secondary male infertile affecting the sex male organs and Pititutary gland-hypothalamus system and may lead to sterility (Frier, *et al*.,2013).

There are other clinical and physical symptoms cause to hormones unbalanced, such as atrophy of testes, Cryptorchidism (Testicular maldescent) Varicocele, acquired testicular damage, congenital abnormalities, Iatrogenic cases, sexual or ejaculatory dysfunction and endocrine disruptors increased male infertile, Infertility is classified into two types, Primary and secondary infertility (Frier, *et al*.,2013).

Approximately 85-90% of infertility are characterized by an increase in Prolactine, low FSH and high levels of LH Type 2 secondary infertility become highly increase in the 21st century, an autosomal metabolic disease involving complex interactions between multiple genes, pathways and environmental factors, has been characterized by insufficient levels of hormones production and the irregular balance of Pititutary gland-hypothalamus system (Agrawal *et al*.,2016).

The identification and description of the gonadotropic hormones in addition to Prolactine hormone scene become "important" for the development of targeted therapies and preventive measures (Tomass *et al*., 2014).

The aim of study is Identify the role of these gonadotropic hormones (FSH, LH, &Prolactin.) in male infertile. In addition to identify infertility risk factors such as obesity, smoking, mobile phone radiation etc. and their contribution of male infertile occurrence.

Materials and Methods:

Samples Collection

A total of 300 blood samples were collected by 200 samples from the Infertility Unit and Hormones Unit of AL-Hussein Learning Hospital in Th iQar Governorate for people with type1 Primary infertility and type 2 secondary infertility. 100 blood samples of healthy people (teaching, students, and others). Three to five ml of venous blood was taken from the patients and healthy groups. Blood samples were placed in container tubes called Jell tubes to separate serum and plasma from other blood components and preserved at temperature 20-$^{\circ}$C .A form of information questioner about the patient and healthy groups including (age, smoking, infertility duration, mobile phone radiation, obesity, endocrine disturbed , type of infertility, incidence of stress and family history).

Hormones Estimation

Hormones Estimation from patients and healthy blood serum samples included several steps based on the leaflet attached to Kit Hormones Estimation manufactured by boditech ((Korean origin) :

Intended used

Ichroma LH ,FSH is a fluorescence immunoassay (FIA) for the quantitative determination of luteinizing hormone (LH) and follicle stimulation hormone (FSH) in human serum which is useful as an aid in management and monitoring of determination of evaluating fertility issues, function of reproductive organs (ovaries or testicular),or detection of the ovulation hormones, .Estimation technique was used to according to the method of (Beastall *et al.,* 1987). The following materials were used shown in Table (1).

Table (1) represents the Materials supplied for the reaction mixture.

Chemicals	Volume
Blood serum	150 uL
Detection buffer	25uL
Sample mixing tube	75uL
Displacing reagent	8uL

The Functional properties of the components shown in Table (2). The reaction method was performed with a 150 µl of serum react with detection buffer sample mixture must be used immediately ,then transfer 75 µL from sample mixture and load it into sample well on cartridge of ichroma to scan the sample insert cartridge into cartridge holder of instrument for ichroma test press select button to star scanning process.

Table (2) represents the Components and Functional properties for the reaction mixture

Components	**Functional properties**
Serum hormones	Has antigen sample bind to antibody in buffer, forming antigen-antibody complexes
Cartridge	Has a test strip, membrane has anti human LH, at test line while rabbit IgG at control line
Displacing reagent	Has anti KLH, fluorescence conjugate
Detection buffer	Has anti human serum hormones fluorescence conjugate, bovine serum albumin

The working method was performed with a 75 µL reaction mixture by using a sandwich immunodetection method the detector antibody in buffer bind to antigen in sample forming antigen-antibody complexes and migrates onto nitrocellulose matrix bee captured by other immobilized antibody on test strips. More antigen in sample form antigen-antibody complexes and lead to stronger intensity of fluorescence signal on detector antibody which is processes by instrument for ichroma test to show serum hormones concentration(Test,FSH,LH&PROL). After completing all the additives, samples were mixed by shaking 10 times. then placed in the ichroma that was operated to record results from reader.

Statistical analysis: By using Chi-sequare for all samplesm via spss programe(Scefler,1980).

Results and Discussion:

Table: 3.Comparison of serum hormone level between patients and control group

Serum H./200	Cases	Infertile Parameters (200)												P-value
		Oligo/31	Azoo/27	Terato/10	OA/36	OAT/28	Nor/14	AS/38	Crp/8	Leauko/2	AS/2	Nec/4	C/100	
Test	Normal	4.50	1.00	2.50	6.50	6.00	6.00	7.50	2.00	1.00	0.00	0.00	100.00	0.004
	Down	11.00	11.00	1.00	6.00	7.00	1.00	6.00	1.00	0.00	1.00	2.00	100.00	
	Up	0.00	1.50	1.50	5.50	1.00	0.00	5.50	1.00	0.00	0.00	0.00	100.00	
FSH	Normal	2.00	11.00	2.50	6.00	6.50	5.00	6.50	2.50	1.00	0.00	0.00	100.00	0.004
	Down	11.00	1.00	2.00	5.50	7.50	1.00	8.00	1.50	0.00	1.00	2.00	100.00	
	Up	2.50	1.50	0.50	6.50	0.00	1.00	4.50	0.00	0.00	0.00	0.00	100.00	
LH	Normal	2.00	0.50	2.00	6.00	6.00	5.50	7.50	3.00	1.00	0.00	0.00	100.00	0.004
	Down	11.00	2.00	2.50	5.00	7.00	1.00	7.50	0.50	0.00	1.00	2.00	100.00	
	Up	2.50	11.00	0.50	7.00	1.00	7.50	4.00	0.50	0.00	0.00	0.00	100.00	
Prol	Normal	0.00	1.00	2.00	6.00	5.00	6.00	6.00	0.50	1.00	0.00	0.00	100.00	0.010
	Down	2.00	4.00	1.00	2.00	2.00	1.50	4.00	2.00	0.00	0.00	0.00	100.00	
	Up	13.50	8.50	2.00	10.00	7.00	6.50	9.00	1.50	0.00	1.00	2.00	100.00	

Estimation of serum hormones profile.

Estimation of serum hormones of patients and control groups.

Results shown that comparison of these hormones level between patients groups and control at table (3). The highly percentage of Testosterone hormone low level(decrease) was at Oligzoospermia and Azoospermia (11%)since testosterone was necessary for spermatogenesis, low of testosterone mean that decrease of sperm (oligo)and this agreed with Agrawal *et al.*(2016) .Results appeared different significant of Testosterone hormone level(decrease) between infertile parameters and control at ($p \leq 0.05$) .Similarly for Follicle stimulation hormone and luteinized hormone levels at ($p \leq 0.05$).Also the low of hormones(FSH) mean that decrease in the numbers of Sertoli cells which responsible for sperm formation as well as nutrient and nourishment of spermatozoa. Otherwise low of LH mean that decrease in testosterone synthesis On the contrary of Prolactine hormone that increased highly percentage up level (13.5%) for Azoospermia . Hormones help spermiation of armature spermatids to free itself from the sertoli cell and enter the lumen of the tubules as spermatozoon. Hormones also play role in spermiogenesis that processes which include differentiation of spermatids into spermatozoa Agrawal ,and McGill,(2014).And this study has been agreed with recent study as shown at Table (3).

Table (4) Comparison of serum hormone between Primary and secondary infertility

Serum Hormones /200	Cases	Primary infertility		Secondary infertility		*P*- value
		153	76.5	47	23.5	
		No.	%	No.	%	
Test	Normal	60	30.00	17	8.50	0.030
	Down	77	38.50	11	11.50	
	Up	16	8.00	7	3.50	
FSH	Normal	50	25.00	15	7.50	0.012
	Down	88	44.00	20	10.00	
	Up	15	7.50	12	6.00	
LH	Normal	67	33.50	16	8.00	0.057
	Down	70	35.00	17	8.50	
	Up	16	8.00	14	7.00	
Prol	Normal	30	15.00	12	6.00	0.020
	Down	29	14.50	6	3.00	
	Up	94	47.00	29	14.50	

Estimation of serum hormones of primary and secondary infertility.

In this study, most infertile men had primary infertility (76.5%) and (23.5%) had secondary infertility. The Increased number of primary infertility over secondary seminal characteristics and serum hormones by infertility status infertility indicates that congenital abnormalities or severe impairment of sperm production with endocrine disturbance are more likely to be found. On the other hand, men with secondary infertility had a better chance for future fertility because Varicocele, exposure to certain risk factors, or accidents are the reason beyond decreased fertility among secondary infertile men. In Northern Nigeria primary infertility (96%) was higher compared with recent results (76.5. %) and only 4% of cases had secondary male infertility. While in Egypt, 70.7% of couples had primary infertility and29.3% had secondary infertility. However, in Thailand primary infertility was lower than our findings (61.8%) while 35.6% of couples had secondary infertility.

Results shown the comparison of these hormones level between patients groups Primary and secondary infertility at table (4). The highly percentage of Testosteron hormone low level was at primary infertility (38.5%) compared with secondary infertility (11.5%).Results appeared different significant(increase) of Testosterone hormone level between Primary infertility and Secondary infertility at ($p \leq 0.05$) .While the FSH hormone low level was so highly percentage (increase) for primary infertility (44%) compared with secondary infertility (10%) Results shown different significant of FSH hormone level between Primary infertility and Secondary infertility at ($p \leq 0.05$). Also result appeared equality of LH to FSH in lowering and upping. On the contrary of Prolactine hormone that increased highly percentage up level (47%) for primary compared with secondary infertility (14.5%) table (4). therefore results have been shown different significant of Prolactine hormone level(Increase) between Primary infertility and Secondary infertility at ($p \leq 0.05$).

Table: 5. Relationship between serum hormones level and infertile causes

Serum H./200	Cases	Infertile causes(200)											*p*-value
		Unexplained (34)	Varicocele (40	H.D(30)	Idiopathic /22	S.D/8	Iatrogenic(8	(A.A)19	(C.A)7	(T.D)23	(Azoo)9	C/100	
Test	Normal	5.00	7.00	1.00	6.50	2.00	0.50	4.50	1.00	6.50	1.50	100.00	0.002
	Down	11.00	12.00	10.00	4.00	1.50	2.00	3.00	1.50	3.50	2.00	0.00	
	Up	1.00	1.00	4.00	0.50	0.50	1.50	2.00	1.00	1.50	1.00	0.00	
FSH	Normal	2.00	9.00	1.50	6.00	2.00	1.00	4.00	1.00	6.00	2.00	100.00	0.004
	Down	8.00	9.00	11.00	3.50	1.00	2.00	2.50	1.50	4.00	2.00	0.00	
	Up	7.00	2.00	2.50	1.50	1.00	1.00	3.00	1.00	1.50	0.50	0.00	
LH	Normal	3.00	7.50	2.00	5.00	2.50	1.00	4.00	1.50	6.50	1.50	100.00	0.010
	Down	8.00	8.50	12.00	4.00	1.00	1.50	3.00	1.50	4.50	2.00	0.00	
	Up	6.00	4.00	1.00	2.00	0.50	1.50	2.50	0.50	0.50	1.00	0.00	
Prol	Normal	3.50	5.00	2.50	1.50	0.50	0.50	3.00	1.00	4.00	1.50	100.00	0.020
	Down	2.50	4.00	3.50	2.00	1.00	0.50	3.00	1.00	3.00	1.00	0.00	
	Up	11.00	11.00	9.00	7.50	2.50	3.00	3.50	1.50	4.50	2.00	0.00	

Relationship between serum hormones level and infertile causes for patient and control groups.

According to the infertile causes and hormones level, result has been noticed significant difference increase between infertile groups and control at ($p \leq 0.05$). Highest percentage of low level for testosterone was at Varicocele. Twelve percent (24/200) of the infertile men were found to have varicocele.The commonest cause of hypogonadism and the chief causes if oligoazoospermia in men. Similarly in the current study Varicocele was the most observed physiological among the infertile men (12%). Varicocele is a dilation of the testicular veins pampiniform plexus of the spermatic cord that holds up a man testicular (Agrawal,2016).These man have abnormal testicular and their testicular like a bag of worms ,mainly impaired Leydig cell function has been reported to be associated with Varicocele (Wang *et al*.,2010). The presence of Varicocele in testicular lead to increase temperature of scrotum,and therefore affect on somniferous tubules function and sperm motility(Data *et al*.,2017. and explained (11%) subsequently. Un explained occurs when the reason for fertility is not clear with normal semen analysis and partner evalution,the infertility is termed unexplained rarely patients with normal semen analysis have sperm that do not function in a manner necessary for fertility .the result agreed with Good *et al*.(2016) when the unexplained play role in fertility occurrence ,While don't agreed with Jamal *et al*.(2015) that considered unexplained less role in fertility . While highest percentage low level (11%).for FSH at hormonal disturbance, and (12%) for LH hormone. Disorders of sperm production may results from either diseases that affect on the testis which called primary hypogonadism or from disorders of the pituitary or hypothalamus which called secondary hypogonadism Matsumoto, (2012). In men with primary hypogonadism the gonadotropin levels are increased (hypergonadotropic hypogonadism), while in men with secondary hypogonadism gonadotropin levels are low or low to normal (hypogonadotrophic hypogonadism).

Measurement of FSH concentration is necessary to distinguish between hypergonadotropic and normo-or hypogonadotrophic hypogonadism Matsumoto,(2012).

The hypothalamus-pituitary endocrine systems regulate the hormonal events that required to the normal testicular function. Hypothalamus stimulated the pituitary gonadotropins which are: Luteinizing Hormone (LH) stimulate the production of testosterone, and Follicle-Stimulating Hormone (FSH) which stimulate

the production of somniferous fluid Normal levels of LH and FSH are necessary for maintenance of spermatogenesis. Disorders of the pituitary or hypothalamus will cause inadequate Gonadotropins stimulation of the testis and that will lead to problems with fertility Matsumoto, (2012). On the contrary of Prolactine that increased up level to (9%).

Table(6) Correlation of Sperm County, BMI and hormones levels

parameters	SC	T	FSH	LH	Prol	BMI
SC	1.000	-0.140	-0.517b	-0.551b	-0.581b	-0.045
T	0.232a	0.385*	-0.108	-0.087	0.521	-0.260**
FSH	-0.517 [b]	-0.016	1.000	0.574**	0.513	-0.012
LH	-0.551b	-0.169	0.574**	1.000	-613	-0.049
PROL	-0.551b	-0.169	-0.652	0.555	1.000	0.650
IBM	-0.045	-0.082	-0.012	-0.049	0.444	1.000

Effect of risk factors on parameters associated with male infertility.

Effect of body mass index (BMI)

Body mass index (BMI) in this study was calculated after recording the height and weight of the patients when their detailed history was recorded at the time of semen collection. A strong correlation between all the reproductive sex hormones and the sperm count was observed Table (6). In the present study, no association between BMI and sperm parameters was observed. Overweight (BMI >25) is one of the recent epidemiological factors that are believed on the rise in developing countries (Ogden *et al.,* 2016). Hence, BMI may adversely affect the reproductive health in both men and women at a younger age (Must *et al*., 2013). Increase in obesity is a worldwide problem, especially in people who practice sedentary lifestyle. The excess body weight not only results in chronic disease but is also shown to have increased risk of reproductive problems (Must *et al.,* 2013). So far, several studies have shown that women with excess body weight are more likely to have fertility problems (Jensen *et al.,* 2014), however the correlation between weight and infertility is not well demonstrated in men,because many of men with highly body mass index were fertile and some of men with lowely body mass index were infertile. Similar to earlier reports in women, the current study showed that testosterone, FSH and LH have a strong role in impaired fertility. Excess weight or altered BMI over or below the normal range can affect male hormone levels (Jensen *et al*., 2014, Fejes *et al*., 2016). The current study showed significantly reduced testosterone levels among overweight or obese men (BMI>25) when compared with men with lower BMI similar to earlier findings (Fejes *et al*., 2016). Infertile men with higher BMI have also exhibited altered sperm parameters (Jensen *et al*., 2014). There is sparse population-based data on the effect of men's body mass on a couple's fertility (Sallmen *et al*., 2016; Ramlau-Hansen *et al*., 2017). Men with BMI of 32–34 have been reported to have twice the risk of infertility compared to men with BMI of 20–22 (Sallmen *et al*., 2006). Another study by Ramlau-Hansen *et al*. (2007) showed increased infertility among men with excess weight compared to men with normal weight. In the present study, no association between BMI and sperm parameters was observed, however, a strong negative correlation (r=-0.260) between testosterone levels and BMI was observed in the current study .Table (6), which is similar to findings by Mac Donald *et al* (2013) but not with FSH and LH levels. Increased BMI over the normal range may result in hypogonadism, increased scrotal temperatures,

impaired spermatogenesis, 119decreased sperm concentration, decreased motility, and increased sperm DNA damage (Kasturi *et al*., 2012). Similar to present findings, testosterone levels were reported to be lower in obese groups (Allan *et al*., 2010). However, a recent study also confirmed low levels of testosterone in
obese men (Dhindsa *et al*., 2010).

However, a strong negative correlation (r=-0.260) between testosterone levels and BMI was observed in the current study (Table4. 4),while there were correlation between serum hormone Prolactine and body mass index(r=0.650).

The possible mechanisms that may explain low testosterone levels in the obese men is increase in adipose tissue mass which results in increase daromatase activity that results in the conversion of testosterone into estradiol (Giagulli *et al*., 2010). This could lead to the suppression of hypothalamic gonadotropin releasing hormone (GnRH) and secretion of pituitary gonadotropin. Ultimately, the above mentioned changes may result in the reduction of both testosterone secretion by Leydig cells and spermatogenesis in the seminiferous tubules (Jensen *et al*., 2014). These findings are in accordance to the recent studies, which showed that BMI has strong negative correlation with testosterone levels but not with sperm parameters (MacDonald *et al*., 2013). Therefore, reduction of weight may be advised in men with increased BMI and low testosterone levels to increase their semen quality.

Table: 7 .Relationship between serum hormones level and Obesity (Kg).

Serum /200	Cases	Obesity (Kg)														p-ue
		55-64		65-74		75-86			87-96	97-106		107-116		117-126		
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Test	Normal	8	4.00	7	3.50	11	5.50	17	8.50	10	5.00	8	4.00	4	2.00	
	Down	4	2.00	5	2.50	5	2.50	11	5.50	16	8.00	32	16.00	11	5.50	0.018
	Up	5	2.50	7	3.50	7	3.50	5	2.50	6	3.00	4	2.00	5	2.50	
FSH	Normal	7	3.50	8	4.00	12	6.00	16	8.00	9	4.50	9	4.50	3	1.50	
	Down	4	2.00	7	3.50	6	3.00	9	4.50	15	7.50	31	15.50	12	6.00	0.021
	Up	6	3.00	2	1.00	5	2.50	8	4.00	8	4.00	4	2.00	7	3.50	
LH	Normal	9	4.50	7	3.50	11	5.50	15	7.50	11	5.50	30	15.00	4	4.00	
	Down	3	1.50	4	2.00	6	3.00	12	6.00	14	7.00	19	9.50	12	6.00	0.040
	Up	5	2.50	6	3.00	6	3.00	6	3.00	7	3.50	4	2.00	4	2.00	
Prol	Normal	9	4.50	2	1.00	5	2.50	4	2.00	6	3.00	6	3.00	4	2.00	
	Down	4	2.00	6	3.00	5	2.50	3	1.50	4	2.00	5	2.50	3	1.50	0.034
	Up	4	2.00	9	4.50	13	6.50	26	13.00	22	11.00	33	15.50	13	6.50	

Relationship between serum hormone profile and Obesity.

Results have been observed the relationship between serum testosterone hormone that appeared a percentage of down level (16%) and obesity (significant different at p≤0.05) for patients with highest weight period (107-116kg) compared with lowest weight period (55-64kg), similarly FSH. and LH. hormones have been shown hormone that appeared a percentage of down level(16%) (significant different at p≤0.05)for patients with highest weight period(107-116kg) compared with lowest weight period(55-64kg) .Adiposity serum Prolactin hormone that appeared a percentage of up level(15.5%) for

patients with highest weight period(107-116kg) compared with lowest weight period(55-64kg).So there were significant different at ($p \leq 0.05$).

Table (8) Comparison of semen parameters between smokers and non-smokers

Semen parameters	Non-smokers (82)	Moderate Smokers(14)	Heavy Smokers (30)	A-p value	B-p value	C-p value
pH	7.77±1.32	8.02±0.23	8.09±0.23	0.151	0.486	0.040
Volume	7.12±1.67	4.11±2.14	3.06±1.0	0.122	0.108	0.804
Sc /M/ml	28.9(3.5,40.70)	23.4(3.57,31.10)	31.85(6.30-71.0)	0.315	0.241	0.484
SpM %	10(0-30)	10(5-16.2)	20(0-40)	0.5047	0.575	0.269
NspM%	35(0-30)	40(10-56.2)	30(20-50)	0.649	0.634	0.072

A- P value between non-smokers and moderate smokers, B- P value between moderate smokers and heavy smokers , C- P value between non-smokers and heavy smokers, Values are expressed as median (interquartile range), SpM- Normal Sperm Morphology.

Effect of smoking on male infertility

Out of 150 cases, 82 were non-smokers, 14 were mild smokers (less than 3 cigarettes per day), 30 were heavy smokers (more than 3 cigarettes per day) and in 24 cases, no clear history of smoking was known or these men quit smoking after some time. All the three groups were compared for their seminal fluids, and hormone parameters. The average seminal pH of heavy smokers were found to be significantly ($P \leq 0.05$) higher compared to non-smokers .Table(8).

However, a non-significant increase in pH was observed in moderate smokers compared to non-smokers. Though, no significant association was found in the seminal volume between the groups, decreased seminal volume was observed in the heavy smokers compared to non-smokers. Decreased seminal volume in smokers was the only parameter associated with smoking in a study reported by Pasqualotto *et al* (2016). Smoking is one of the factors that tend to adversely affect male fertility through a host of mechanisms. Not only in men, but it also has theability to affect pregnancy in women also. It has been reported that men who smoke regularly have reduced sperm parameters and increased number of spermatozoa with abnormal morphology (Colagar *et al*., 2017).

However, some studies have shown association between smoking and reduced sperm parameters (Gaur *et al*., 2010). It has also been reported that smoking had a significant impact on semen quality (Wang *et al*., 2015).

In studies conducted on fertile smokers, the semen volume was found to be reduced compared to fertile non-smokers and was also proportional to the number of cigarettes (Pasqualotto *et al*., 2016).

Similar to studies by Kazim *et al* (2010) found no association between smoking and the sperm parameters were observed in the present study. Since, this study did not have any strict inclusion criteria to ascertain

the effect of smoking on fertility parameters, detailed studies with large number of cases with smoking history may provide valuable information on the effect of smoking on male fertility.

Table (9) Comparison of semen parameters between smokers and non-smokers

parameters Semen	Non-smokers 82	Moderate Smokers(14)	Heavy Smokers 30	A	B	C
pH	7.77±1.32	8.02±0.23	8.09±0.23	0.151	0.486	0.040
Volume	7.12±1.67	4.11±2.14	3.06±1.0	0.122	0.108	0.804
Sc /M/ml	28.9(3.5,40.70)	23.4(3.57,31.10)	31.85(6.30-71.0)	0.315	0.241	0.484
SpM %	10(0-30)	10(5-16.2)	20(0-40)	0.5047	0.575	0.269
NspM%	35(0-30)	40(10-56.2)	30(20-50)	0.649	0.634	0.072

A- P value between non-smokers and moderate smokers, B- P value between moderate smokers and heavy smokers , C- P value between non-smokers and heavy smokers, Values are expressed as median (interquartile range), SpM- Normal Sperm Morphology.

Smoking also increases leukocytes in the ejaculates, leading to increased levels of seminal ROS (Saleh *et al*., 2012). Apart from these observations, decreased ascorbic acid levels in the seminal plasma of smokers have also been reported (Mostafa *et al.,* 2016). prolactin levels in smokers compared to non-smokers were observed, suggesting the influence of smoking on dopaminergic tonus (Allan *et al*., 1984 and 1985, Halmenschlage *et al*., 2009). Since dopamine has been shown to inhibit prolactin secretion, the current results suggest that smoking has animportant dopaminergic influence on the central nervous system. Similar to studies reported in women, decreased prolactin also play an important role in male infertility.

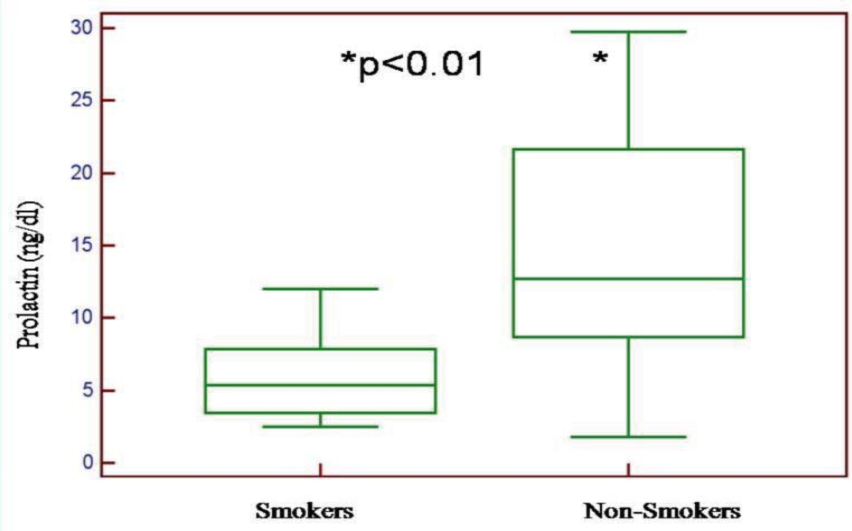
Prolactin secretion is not only controlled by dopamine (which blocks prolactin) but also by serotonin (which triggers prolactin release) and thyroid-producing hormone (which also triggers prolactin). Seminal volume and serum prolactin levels may have an important role in smoking-associated impaired fertility.

Table (10) Comparison of serum hormone profiles between smokers and non-smokers.

Serum hormones	Smokers	Non-Smokers	P.Value
T/(ng/dl)	4.07(2.10-7.17)	4.65(93.41-6.82)	0.493
FSH/(mIU/ml)	66(3.3-20.21)	5.22(3.22-12.20)	0.538
LH/(mlU/ml)	5.64(3.77-13.18)	4.95(3.11-10.42)	0.336
PROL/(ng/dl)	5.36(3.4500-7.8375)	12.68(8.7100-21.6225)	0.008*

Serum reproductive hormones are important predictors of spermatogenesis in men. Since data of serum reproductive hormones were available only in less number of cases, moderate and heavy smokers were

grouped into smokers to compare their serum hormone levels. No differences in the testosterone, FSH and LH levels between smokers and non-smokers were observed. However, serum prolactin level was found to be significantly lower in smokers compared to non-smokers (Figure. 1).



Figure(1) Comparison of serum prolactin levels between smokers and non- smokers infertile men.

Table (11) Comparison of semen parameters between cell phone users and non users in infertile men

parameters Semen	Group I/66 Non user	GroupII/18 Moderate/1-3years	GroupIII/45 >3 years	A	B	C
PH	7.96±0.46	8.16±0.46	7.62±1.71	0.183	0.080	0.197
Volume	3.27±1.64	3.48±1.77	3.13±1.60	0.538	0.536	0.661
Sc /M/ml	22(3.67,43.35)	19.2(1.06,63.2)	14.6(5.5,37.1)	0.692	0.727	0.407
SpM %	10(0,40)	20(0, 32.5)	6(0,20)	0.671	0.256	0.047
NspM	31(15,52.60)	50(27.560)	25(10,50)	0.585	0.290	0.337

Group I- Non users, Group II- Users of duration from 1-3 years, Group III- Users of duration >3 years. Values are expressed as median (interquartile range) A- p value between non users - and moderate users, B- p value between moderate users and heavy users , C- p value between non- users and heavy users.

Effect of cell phone radiation on male infertility

In the current study, to understand the effect of cell phone exposure of fertility parameters, the following groups were categories from the 150 infertile cases:Cell phone non –users were counted to 66(males who did use cellular phone),18 males were using cellular phone sporadically for the period of 1-3 years, and 45

males who were using regularly for the period of more than 3 years.All the users were exposed to a GSM mobile at frequency 900-1800MHz.

Though no significant association between the duration of exposure and any of the parameters were observed, non-significant association of cell phone usage and deterioration of sperm quality was observed table (11).The median of the sperm count in the group exposed to longer duration on cell phone was found be lower compared to the non-users and users for a lesser duration . the median sperm count of lesser duration group was lower compared to median sperm count of cell phone non-users. However, significantly at ($P \leq 0.05$) lower sperm motility in longer duration exposed group compared to non-exposed group was also observed. The median percent normal sperm morphology was compromised in both the exposed group compared to the non-users, but found to be non-significant at ($P \leq 0.05$).

Decreased semen quality in cell phone exposed group are similar to the findings reported earlier (Agarwal *et al*., 2016)**.**

Mobile technology has been observed as the one of the fastest evolution in recent years worldwide. The effect of electromagnetic radiation (EMR) emitted by the cell phones is equally an important issue in fast developing countries. Earlier studies on the effect of EMR on human health revealed controversial results, as some studies showed no effect of EMR on human health (Agrawal *et al* .,2014).EMF occurred disturbance of leydig cells to steroids formation and defect in the epithelial germ ability for spermatids differentiation to mature sperms . The EMR exposures were included several abnormalities of sperms shapes in head,mid piece and tail that increase in patients compared with normal sperm ,otherwise reason may be due to the affectation of testicular tissue by EMR, since spermatogenesis depended on testicular safety ,because it is considered safety main position for spermatozoa production, as Bushra *et al*. (2011) noticed EMR affection on testicular structure during evolution period, also Said,(2018)and Agrawal *et al*.(2013) explained that EMR occurred disruptive reason of spermatogenesis and it is maturation due to the free radicals formation especially Reactive oxygen species(ROS)which act as induction of Apoptosis in the germs cells leading to decrease of total count sperms .

Perhaps other reason which leads to total count sperms decreasing and abnormalities increasing was due to low protein kinase activity caused by EMR. Protein kinase regulated biological processes in the leydig cells which responsible of testosterone synthesis, therefore any decrease in this enzyme mean decrease in testosterone which lead to Sertoli cells damage. This damage impaired function such as nourishment and nutrieient of spermatozoa, the final result was reduction of spermatogenesis. There are many probability reasons to explained total count sperms decreasing and abnormalities increasing such as low of zinc percentage that caused damage to the reproductive system due to decrease some minerals like zinc and manganese, and that agreed with Caputa *et al*.(2015) that reported to low zinc level in the testes mean testicular damage. (Hales *et al*., 2015). whereas recent studies showed that prolonged exposure to mobile radiation causes brain tumour and various health problems (Young *et al*., 2013). Similarly the concern over the uses of mobile phone by males and carrying it in the trouser (pant) pocket is of serious concern with regard to their reproductive health, and subsequently their fertility.

A recent *in vitro* study by Agarwal *et al* (2009) exposing semen to the cell phones on talk mode, showed significant decline in sperm quality and increased ROS production. No effect on sperm DNA has been observed in that study. This may be due to short duration of exposure of the cells to EMR.

Though controversial results have been reported in animal models, recently, it has been shown that EMR emitted by mobile phones may have a significant effect on male fertility (Agarwal *et al*., 2013). Though

various mechanisms explain the protection of radiation-induced damage in the human body, detailed animal studies are required to find the effect of mobile radiation on testicular cells and spermatogenesis (Jena *et al*., 2015).

In an attempt to study the effect of cell phone radiation on patients serum reproductive hormones, the two exposed groups were combined into a single group due to the less number of cases evaluated for serum reproductive hormones. Though no significant association of serum reproductive hormone levels between the cell phone users and non-users was observed in the current study, non-significant decrease in prolactin and LH levels among the cell phone users compared to non-users were found Table (12).

Table (12) Comparison of serum hormone profiles between cell phone non-users and users.

S.H	Non-users (25)	Users (33)	P.Value
Testosterone/ng/dl	5.85 ± 2.82	5.78 ± 2.35	0.926
FSH/mIU.ml	6.94 ± 6.57	6.97 ± 5.92	0.984
L.H/mIU/ml	5.61 ± 4.51	4.98 ± 2.82	0.560
Prolactine/ng/dI	13.69 ± 7.97	9.90 ± 7.03	0.242

Only few studies are available on this aspect which was conducted on healthy volunteers that did not show any alteration in reproductive and pituitary hormones after exposure to cell phones (Djeridane *et al*., 2011). Since cell phone radiation affects brain, which controls the secretion of pituitary hormones, studies in this aspect are necessary to obtain more information about the effect of cell phone radiation on hormone parameters However, using large sample size with strict inclusion criteria may provide useful information on the effect of cell phone usage on male reproductive parameters. Small sample size using cell phone and other uncorrected factors are the limitation in the current study. Since India is one of the largest populated countries with a huge number of cell phone users, a large cohort study may provide useful information about the effect of cell phone on male reproductive parameters.

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