Journal of Education For Pure Science- University of Thi-Qar
Vol.9, No.1 (March 2019)
Website: jceps.utq.edu.iq *Email: jceps@eps.utq.edu.iq*

DOI: http://doi.org/10.32792/utq.jceps.09.01.11

The Relationship Between The Genotypes Frequency For The PRM1 Gene And Some Of Risk Factors In Male Infertile Of Thi Qar Province

Taleb K. Odah[1], Hassan R. Mubark[1], Enas S. Jawad[2]

[1] Collage of Education for Pure Sciences, University of Thi Qar, Thi Qar, Iraq.
[2]Collage of Medicine, University of Thi Qar, Thi Qar, Iraq.

Abstruct:

This study was designed to study the Protamine /1 gene role and it is contribution to the risk of male Infertile in Infertility Center at AL-Hussein Teaching Hospital Thi Qar Province. Highly Percentage of primary infertility (76.5%) compared with secondary infertility (23.5%).Current study noticed significant different between male infertility and homozygous mutant (AA) when OR=1.684 and Heterozygous mutant (CA) when OR=5.150 .The prevalence of the frequency of Homogenous mutant for PRM1 gene is higher among urban (46%) than in rural areas compared with the prevalence of the frequency of heterozygous mutant for PRM1 gene (20%) is among urban than in rural areas. patients who have Homozygous mutant for PRM1 gene with a family history had a higher risk (40%) compared to those without a family history it noticed significant different more than one and half times (OR=1.625) . The results showed that the significant difference between patients and healthy controls when genotype AA (OR= 1.684) is more than one and a half times , otherwise the genotype CA showed a high significant difference between the two groups patients and control five times (OR= 5.150), Current study showed no correlation between genotypes in people smoking and the risk of male infertility in genotype AA where there were not significant differences (OR= 0.882) , While genotype CA showed a significant difference by more than one and a half times (OR=1.803). Current study showed a correlation between genotype of PRM1 gene for the patients ages more than 40 years and the risk of male infertility increased more than two times (OR=1.625).

Key words: Male infertility, PRM1 gene, Primary infertile, Secondary infertile.

Introduction

Male Infertility (MI) affects about 10-15% of couples with a desire to have children (Behre *et al.*,2010), and represents a major clinical problem that affects married couples both medically and physiologically when they fail to achieve conception even after one year of regular unprotected intercourse(WHO,2010). According to World Health Organization ,infertility is the inability of the sexually unprotected ,active couple to achieve pregnancy in one year (WHO,2010) .The proportion of infertility is about 25% is related to male factors. Cause of infertility in 25% of infertile men with abnormal semen is still unknown (Lacono *et al*., 2016).

Many environmental and physiological factors affect male infertility(Bedgoli *et al*.,2011).Environmental factors can contribute to infertility to some extent, but genetic factors also play pivotal role in etiology of male infertility .Mutation in protamine genes have been reported to be the main cause abnormal of spermatogenesis and defect in imprinting and induce sperm chromatin damage and DNA breaks(Iguchi *et al.*,2006;Dindic *et al*.,2010).The nucleoprotein gene PRM1 is closely linked with other genes such as PRM2 and TNP2 in stretch of DNA ,13-15Kb long on human chromosome16p13.3 that are categorized in protamine gene family(Oliva *et al*., 2016).

potamines are the major DNA binding proteins in the sperm nucleus that cause DNA condensation and packaging in spermatozoa by histones replacement during spermatogenesis.(Keime *et al*., 1992;Tanaka *et al.*,2003).Mutation in protamine genes has been reported to be associated with sperm penetration dysfunction, failure of embryonic development and DNA sperm damage(Kempisy *et al*.,2007).These sperm DNA damage and induction of apoptotic pathway induce sperm count and motility(Cho *et al*.,2003). protamine defect proteins have led to abnormal condensation of sperm chromatin, increased sperm DNA strand break and immobility of spermatozoa that can lead to male infertility(MCAdham *et al*.,2017). Male infertility classified into two types, Type 1 infertility is Primary infertility formerly known as type 1. It occurs by failure of couple to conceive and carry a pregnancy to a live birth after 12 months of regular intercourse without the using of contraception (Kumar *et al*., 2015). Type 2 infertility is known as secondary infertility, its occurs when men can achieve conception in a fertile women at first, but then after one year from child birth, failure to achieve conception (Jorgensen *et al*., 2016).

The aim of study is Identify the role of one of nucleoprotein gene PRM1polymorphisms in the male infertility, as well as the relationship between the genotypes distribution frequency for this gene (PRM1) and some risk factors such as, smoking, Age, residential areas, and family history.

Materials and Methods

Samples Collection

A total of 300 blood samples were collected by 200samples from the Infertility Unit of AL-Hussein Teaching Hospital in Thi Qar Governorate for people with type1 Primary infertility and type 2 secondary infertility. A 100 blood samples of healthy people at blood bank including (teachers, students, and others). (3-5ml) of venous blood was taken from the healthy and patients groups. Both blood samples were placed in ependorft tubes(EDTA tube) anticoagulant and preserved at temperature 20-°C .A form of information questioner about the patient and healthy groups including (age, smoking, infertility duration, obesity ,endocrine disturbed, type of infertility , and family history).

DNA Extraction

DNA extraction from patients and healthy samples included several steps based on the leaflet attached to Kit DNA Extraction manufactured by Gene aid (Korean origin).

Polymerase Chain Reaction (PCR)

PCR technique was used to amplify PRM1 gene according to the method of (Arand *et al*., 1996) the following materials were used. The sequence of the primers shown in table (1). The reaction method was perform with a (20µl) reaction mixture as shown in Table (2) based on the leaflet attached to Bioneer Green Master Mix .After completing all the additives, mix the sample with Vortex, then transfer the samples to thermocycler and fill the device according to the program as shown in table(3) .

Table (1) Primers of PRM1 gene.

Genes		Primers sequences	Length	T_M	T_A
PRM1	F R	5-CCCCTGGCATCTATAACAGGCCGC-3 5-TCAAGAACAAGGAGAGAAGAGTGG-3	24 24	60 61	56 57

T_M=Melting Temperature, T_A=Annealing Temperature, R=Reverse, F=Forward

The working method was performed with a(20µl) reaction mixture as shown in the following table(2). Primer design was obtained from past researches

Table (2) represents the chemicals for the reaction mixture and it is sizes.

Chemicals	Volume
Master Mix	lµ5
Primer Forward	lµ 1
Primer Reverse	lµ 1
DNA	µl 5
D.W.	µl 8
Total volume	µl 20

After completing all the additives, the samples were mixed with Vortex for half minunite .the samples were then placed in a thermal cycler which was operated according to the following program:

Table (3) PCR condition for amplification of PRM1 gene.

No. of Stages	Steps	Temperature	Time	No. of Cycle
1	denaturation Inition	C °94	1 min	1
2	Denaturation	C °94	Sec. 30	32
3	Annealing	C°66	min. 1	
4	Extension	C °72	. 1min	
5	Final extension	C °72	10 min	1

PCR products detection

(Electrophoresis) itself used to detect (DNA), But with DNA Marker using, concentration of agarose (%1.2) A dissolve it in 60 mL of TBE precipitate to become the final concentration (2%) After detection by UV, the results were recorded as follows: The appearance of the band at the 557 bp means the presence of the PRM1 gene after comparison with DNA Marker (Arand, *et al.*, 1996).

PCR- RFLP products detection

Restrictions Fragments Lengths Polymorphisms (RFLP): Technique was used to restricted genes such as PRM1 via *BSTU1* Restriction Enzyme from Bioneer company (Korean origin) . After completing all the additives, *BSTU1* has been recognized nucleotides sequences of gene as following.

Recognition Sequence site: 5-CG↓CG -3 GC↑GC-5 3-

RFLP) (Table (4) represents the chemicals for the reaction mixture and it is sizes to restricted PRM1 gene via BSTU1.

Material	Volume
PCR reaction mixture	µl 10
RE 10x buffer	µl 2
Sterile, deionized water	µl 34
(Restriction Enzyme)BSTU1	µl 1
Total	µl 50

Then incubated the components at 37C for 15 minunits, Noticed that *BSTU1* Restriction Enzyme has done from1-3 hours ,but the best results were obtained at one hours ,then loaded via electrophoresis using the same loading stainand agarose (2%).

Results

Population study.

The results of the current study showed that 200 samples were the patients during period of studies starting from (October/2016 to December/2017).The percentage of Primary infertility were 76.5%, while secondary infertility were23.5% compared with control group. Table 5

Table (5) Distribution of Male infertility and Control sample

		N	%
Male Infertile /200	Primary Infertile	153	76.5
	Secondary Infertile	47	23.5
Control/100	Primary Infertile	Nil	0%
	Secondary Infertile	Nil	0%

Table (6) Relationship between infertile groups and study demographic characteristics (Resident areas)

Locally groups by (dwellers)		Primary Infertile	Secondary Infertile
Urban	N	99	34
	%	49.5%	17%
Rural	N	54	13
	%	27%	6.5%
Total	N	153	47
	%	76.5.0%	23.5.0%
Chi-Square X2=0.285	Value	df	
	0.593	1	

The results of the current study showed that more than half cases (66.5%) i.e.49.5%primary and 17% secondary were found in the urban dwellers compared with the percentage of rural dwellers(33.5%) i.e.27%primary and 6.5% secondary.Also both percentage of primary and secondary were increased in urban compared with rural dwellers. however, results analysis shown no significant different at (P≤0.05).

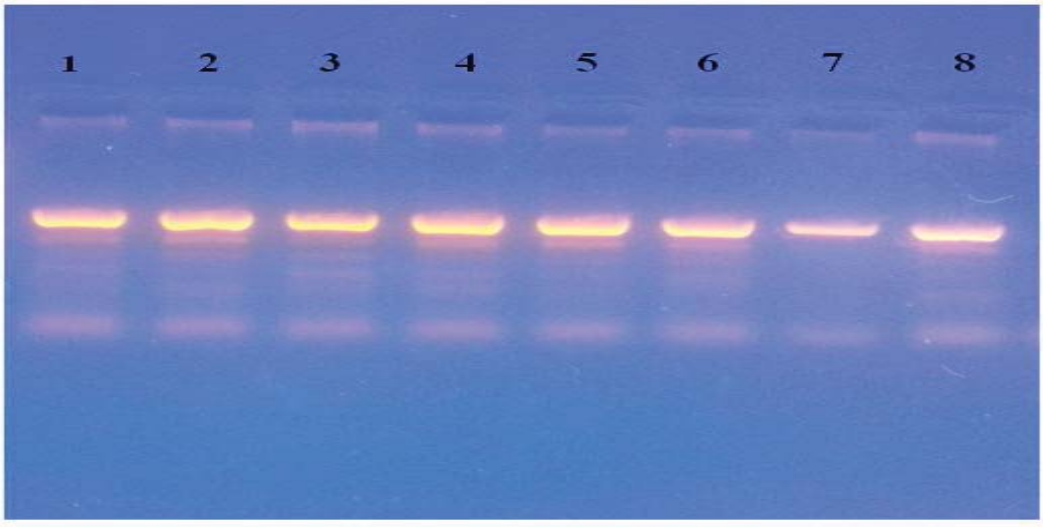
Table (7) Relationship between infertile groups and study demographic characteristics (Occupation).

Occupation groups by(Jobs)	Primary Infertile		Secondary Infertile	
	N	%	N	%
Bakers	34	17%	5	2.5%
Drivers	25	12.5%	12	6%
Military	24	12%	8	4%
Post officers	24	12%	4	2%
Farmers	20	10%	6	3%
Workers	26	13%	12	6%
Total	153	76.5%	47	23.5%
Chi- Square=0.883 df=5 P.Value = 0.971				

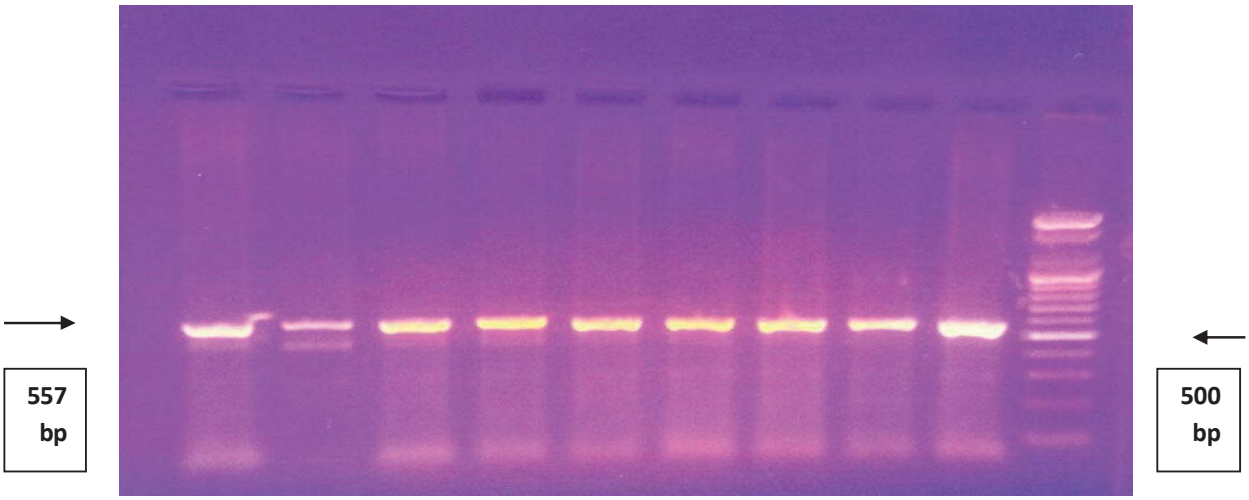
The results of the current study referred to relationship between occupation and infertile, groups. The highly percentage were at bakers (19.5%) i.e17%primary and 2.5% secondary, followed by drivers (18.5%) i.e.125%primary and 6% secondary, while percentage decreased at farmers (13%)i.e(10+3%) The highly percentage of secondary were (6%), while the highly percentage of primary were (17%). results analysis shown no significant different at ($P \leq 0.05$).

Molecular genetics study.

After DNA Extraction, DNA samples from the control and patient group were detected by electrophoresis using loading stain (Bromophenol blue stain)and Ethidium bromide (0.5 µl) was added to agarose gel(concentration0.08%) (Sam brook *et al*., 2001).

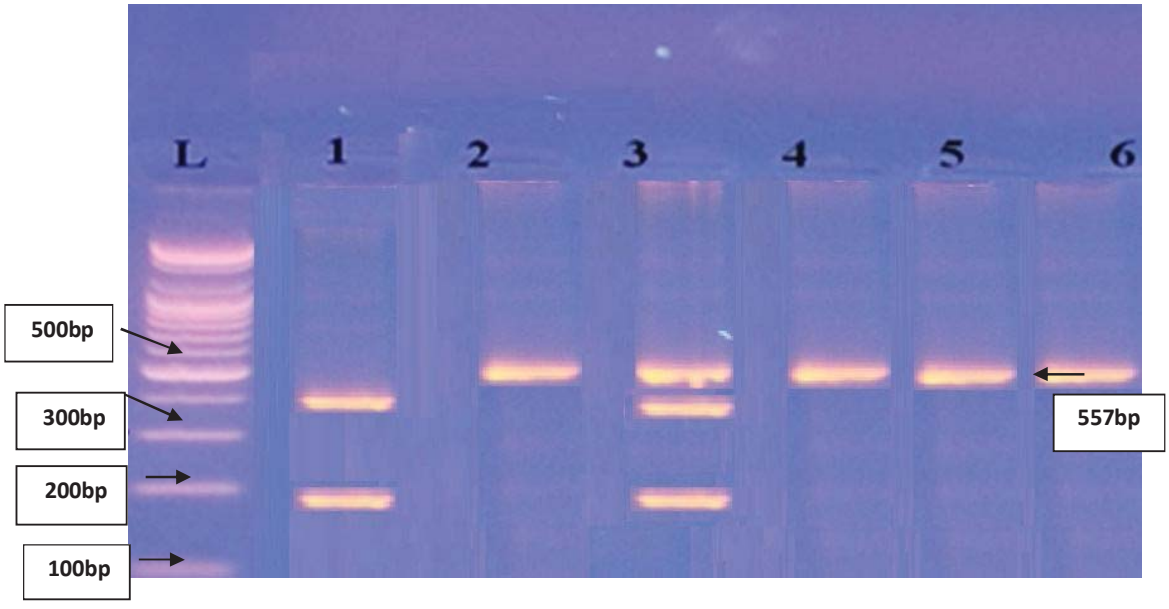


Picture (1) electrophoresis of extracted DNA products on Agarose gel at concentration of 0.08%. (1-4 DNA patients' samples 5-8 DNA control s' samples)



Picture (2) electrophoresis of PCR –products of PRM1 gene on Agarose gel at concentration of 2%.

The results of PCR-RFLP from the control and patients groups were detected by electrophoresis as following. when BSTU1 Restriction Enzyme has restricted PRM1 after comparison with DNA Marker (**Eshkoor *et al.*, 2014)**: using the same concentration of agarose gel 2%.



Picture (3) electrophoresis of PCR-RFLP products on Agarose gel at concentration of 2%.

L: Lader DNA(100-2000 bp)
Lane 1 : Wild Genotype (196,361)
Lane 3: Heterozygous mutant (196 ,557,361)
Lane 2,4,5,6: Homozygous mutant (557)

genotypes Frequency of PRM1 gene samples of patients and healthy controls.

The results of the current study showed that the presence of a correlation between the genotypes of the PRM1 gene and the incidence of male infertility, as the results showed the significant difference between patients and healthy controls when genotype AA (OR= 1.684) is more than one and a half times, while the genotype CA show a high significant difference between the two groups patients and control five times (OR= 5.150) Table (8).

Table (8) Genotypes Distribution of PRM1 gene samples of patients and healthy controls.

Geno type	Control	%	Pateints	%	OR	95% CI
CC	70	70%	106	53%	1.0	
AA	26	26 %	68	34%	0.638	0.670-4.230
AC	4	4%	26	13%	0.833	0.633-41.888

Genotypes Distribution of PRM1 gene samples according to residential areas.

Table(9) showed, no correlation between the genotypes of PRM1 gene and residential areas to incidence the risk of developing Male Infertility, as showed no significant differences between patients in urban and rural areas when there is a genotype AA (OR=0.638) , Also the genotype CA show no significant difference (OR= 0.833) .

Table (9): Genotypes Distribution of PRM1 gene according to residential areas.

Geno type	Rural	%	Urban	%	OR	95% CI
CC	88	44%	22	11%	1.0	-
AA	36	23%	18	9%	0.638	0.231-1.763
AC	20	10%	6	3%	0.833	0.195-3.551

Genotypes Distribution of PRM1gene patient samples according to smoking.

The results of the current study show correlation between genotypes in people smoking and the risk male infertility in genotype CA where there were significant differences (OR= 1.3239) While, genotype AA show no significant difference between smoking and non smoking (OR=0.0282) as seen in table (10).

Table (10) Genotypes Distribution of PRM1 gene of patients according to the smoking.

Geno type	Smoking	%	Nonsmoking	%	OR	95% CI
CC	4	2%	142	71%	1.0	-
AA	2	1%	2	1%	0.0282	0.0013- 0.6302
AC	4	2%	46	23%	1.3239	0.0432-2.4313

Genotypes Distribution of PRM1 gene in patient samples according to family history.

The results of the current study, showed a correlation between patients with Male infertility who have a family history and genotype AA and significant difference more than once and a half (OR= 1.625), While genotype CA does not appear any significant difference (OR= 1.51), as seen in table (11).

Table (11) Genotypes Distribution of PRM1 gene of patients according to the family history.

Genotype	Family history	%	Non – history	%	OR	95% CI
CC	40	20%	80	40%	1.0	
AA	12	6%	56	28%	1.625	0.624-4.231
AC	4	2%	8	4%	1.51	0.396- 5.811

PRM1gene patient samples according to Ages.

The results of the current study show the risk Male infertility was increased more than twice times for men who have age more than 40 years(OR=2. 105) in genotype AA, the genotype CA shows no significant difference(OR=1.368) .Table (12) .

Table (12) Genotypes Distribution of PRM1 gene of patients according to the ages.

Genotype	Olders more than 40	%	Younger less than 40	%	OR	95% CI
CC	88	44%	76	38%	1.0	
AA	46	23%	20	10%	2.105	0.211– 21.008
AC	20	10%	52	26%	1.368	0.34-5.506

Discussion

Infertility is one of the most common diseases in almost all countries(Agrawal *et al*.,2014). The number of people with infertility is increasing due to population growth, urbanization, aging, obesity and mobile phone radiation exposure (Takeda *et al*., 2017). Genetics factors has been shown to play a major role in causing male infertility of two types type 1 primary infertility and type 2 secondary infertility. also play pivotal role in etiology of male infertility(Mohamed *et a*l.,2011) .Mutation in protamine genes have been reported to cause abnormal spermatogenesis and defect in imprinting and induce sperm chromatin damage and DNA breaks(Cho *et al* .,2001; Miyagawa *et al* .,2005; Iguchi *et al* .,2006;Ickioka *et al* .,2015).

The results of the present study as showed a table (6) the percentage of patients in urban areas was 66.5%, while those living in rural areas reached 33.5%. There was no significant difference between the urban and rural areas (P=0.593). This is may be because urban dwellers are less active than rural dwellers, and rural dwellers are less check to specialized centers and less to eat fast food ,also urban dwellers has been exposed to many risk factors such as mobile phone radiation, smoking and eating fast food which enriched within fats than rural dwellers (Cho *et al.*, 2001) .This result agree with Hadi,(2015)

The results of the current study showed that there were significant differences in Occupation, with the highest percentage of bakers (19.5%) and workers (19%) that's agreed with results of (Miyagawa *et al.*, 2011) it's showed (19%) bakers (18%) workers, As well as the study by (Jensen et al., 2013) where the percentage of bakers with infertility was 20.4%, while workers were 37.6%.study of (Dindic *et al*., 2010), recorded the percentage of bakers is (21.5%) and workers (17.5%).

This study differed from a study conducted by (Ickioka *et al*., 2015) which showed that there was significant difference between the frequency of PRM1 genotype and incidence of male infertility when compared patients and control groups It also differed from the study conducted by (Corzett *et al.*, 2015;Mahdi,2016), which showed that there was no significant difference between the family history ,while

this study showed significant between patients who has family history and homozygous mutant(AA) as the OR=1.625 was also different from the study conducted by(Salamlan,. *et al* ,. 2013) Which showed that there was no effect of smoking difference among patients with infertility where. (OR=0.3239). The main factors that make older patients higher than younger are related with several another factors such as Gonadotropic hormones (FSH,LH &Test) which decreased with ages progressive (Tanaka *et al*., 2013).

References

1. **Arand VW, Emery BR, Lui L, Carrell DT(1996).** Protamine levels vary between individual sperm cells of infertile human males and correlate with viability and DNA integrity. *J Androl*. ;27:890–898.
2. **Agarwal A, Makker K and Zinc R. (2014) .** Spermatogenesis: An overview: an update. *Am J Reprod Immunol* 59**:**2-11.
3. **Bedgoli M, Reston S, Mike C.(2011).** Effect of environmental factors on DNA packaging in mouse spermatids Synthesis of protamine variant and four transition proteins. *Exp Cell Res*. ;150:298–308.
4. **Behre, HM. ; Nieschlag, E.(2010).**Testosterone substitution in aging man. *Urologe*.; 39:421-424.
5. **Cho C, Willis WD, Goulding EH, Jung-Ha H, Choi YC, Hecht NB(2001).** Haplo insufficiency of protamine-1 or -2 causes infertility in mice. *Nature Genetics*. ;28:82–86.
6. **Cho Ch, Jung-Ha H, Willis WD, Goulding EH, Stein P, Xu Z, Adman JR,Steven,l (2003).** Protamine 2 deficiency lead to sperm DNA damage and embryo death in mice. *Biol Reprod*. ;69:211–217.
7. **Corzett M, Mazrimas J, Balhorn R(2015).** Protamine 1; protamine 2 stoichiometry in the sperm of eutherian mammals. *Mol Reprod Dev*. ;61:519–527.
8. **Dindic, B. ; Sokolovic, D. ; Krstic, D. ; Petkovic, D. ; Jovanovic,J. and Muratovic, M. (2010) .** Biochemical and genetics effects of mobile phone exposure on rat hepatocytes and brain . *Acta . Medica . Medianae* .49 (1) Pp : 37- 42 .
9. **Eshkoor,S,A.;Ismail,P.and Rahman,S.A.(2014) .** Low frequency protamine 1 gene transversions C102G and C2107G do not correlate with male infertility. *J Med Genet*. ;43:382–384.
10. **Hadi ,E.,Muslim.(2015).** Study of protamin1 polymorphisms of Oligoazoospermia Patients.Msc.Thesis. Baghdad university .
11. **Ichioka, JI.; Adinma JI, Orie EF, Ikegwuonu SO. (2015).** Obstructive azoospermia associated with chronic sinopulmonary infection and situs inversus totalis. *J Uorology* Nov; 23 (1): 204-206.
12. **Iguchi N, Yang S, Lamb DJ, Hecht NB(2006).** An SNP in protamine 1: a possible genetic cause of male infertility? *J Med Genet* . ;43:382–384.
13. **Jensen TK, Jørgensen N, Punab M, Haugen TB, Suominen J, Zilaitiene B, Horte A, Andersen AG, et al.(2013) .**Association of in uteroexposure to maternal smoking with reduced semen quality and testissize in adulthood: a cross-sectional study of 1,770 young men fromthe general population in five European countries. *Am J Epidemiol*. Jan; 1159 (1): 49-58.
14. **Jørgensen N, Andersen AG, Eustache F, Irvine DS, Suominen J,Petersen JH, Andersen AN, Auger J et al. (2016).** Regional differences insemen quality in Europe. *Hum Reprod*. May; 16 (5): 1012-1019.
15. **Keime S, Heitland K, Kumm S(1992).** Characterization of four genes encoding basic proteins of the porcine spermatid nucleus and close linkage of three of them. *Biol Chem Hoppe-Seyler*. ;373:261–270.

16. **Kempisty B, Depa-Martynow M, Lianeri M, Jedrzejczak P, Darul-Wasowicz A, Jagodzinski PP(2007).** Evaluation of protamines 1 and 2 transcript contents in spermatozoa from asthenozoospermic men. *Folia Histochemica et cytobiologica.* ;45:109–113.
17. **Kichine E, Msaidie S, Bokilo AD, Ducoumeau A, Navarro A, Levy N, et al(2008).** Low frequency protamine 1 gene transversions C102G and C2107G do not correlate with male infertility. *J Med Genet*. ;43:382–384.
18. **Kumar N,Kant S,Raj H.(2015)**.Tends of male factors infertility ,an important cause of infertility :A review of literature .*J.Hum reprod Sci* 123:191-196.
19. **Luconi, M.; Forti, G. and Baldi, E.(2016):** Pathophysiology of sperm motility. Front Biosci.11:1433 - 1447.
20. **MC Adham I, Nayernia K, Burkhardt-Gottges E, Topaloglu O, Dixkens Ch, F Holstein A, et al (2017).** Terazoospermia in mice lacking the transition protein 2 (TNP2) *Mol Hum Reprod*. ;7:513–520.
21. **Mhadi ,O.,Adnan.(2016).** Molecular screening study for some variants of mitDNA of Asthenozoospermia.PhD.Thesis.Baghdad university..
22. **Miyagawa Y, Nishimura H, Tsujimura A, Matsuoka Y, Matsumiya K, Okuyama A, et al(2005).** Single-nucleotide polymorphisms and mutation analyses of the TNP1 and TNP2 genes of fertile and infertile human male populations. *J Andrology*. ;26:779–786.
23. **Miyagawa Y, Tsujimura A, Matsumiya K, Takao T, Tohda A, Koga M, Takeyama M, Fujioka H, Takada S, Koide T and Okuyama A .(2011).** Outcome of gonadotropin therapy for male hypogonadotropic hypogonadism at university affiliated male infertility centers: a 30-yearretrospective study. *J Urol* 173:2072-2075.
24. **Mohamed, F.A. ; Ahmed,A.A. ; El-Kafoury, B.M. and Lasheen, N.N. (2011) .** Study of the cardiovascular effects of exposure to electromagnetic field **.**
25. **Oliva R. Protamines and male infertility(2016).***Hum Reprod Update*.;12:417–435.
26. **Salamlan A, Ghaedi K, Razavi Sh, Tavalaee M, Tanhael S, Tavalaee M, et al(2013).** Single nucleotide polymorphism analysis of protamine genes in infertile men. Int J Fertil Steril. ;2:13–18.
27. **Sambrook J, Fritsch E, Maniatis T(2001).** Isolation of DNA from mammalian cells Molecular cloning. New York, USA: *Cold spring Harbour Press*;.7:2-12.
28. **Takeda R.;Ueda M.(2017).**Pitutary-gonadal function in male patients with myotonic dystrophy-serum luteinizing, folicle stimulstion and testesteronr hormone levels and hitolgical dammage of the testtis..*Acta Endocrinol*.;9:69-73.
29. **Tanaka H, Miyagawa Y, Tsujimura A, Matsumiya K, Okuyama A, Nishimune Y(2013).** Single nucleotide polymorphisms in the protamine-1 and -2 genes of fertile and infertile human male populations. *Mol Hum Reprod*. ;9:69–73.
30. **WHO. (2010) .**Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male. *Cambridge University Press*.