Sys Rev Pharm 2020; 11(1): 326-331
A multifaceted review journal in the field of pharmacy

Interrelationship between ABO Blood Group and Bacterial Infection with Men Infertility and "Comparison Effect of Clomiphene, Human Chorionic Gonadotropin, Follitropin-B, and Clomiphene Plus Human Chorionic Gonadotropin on Sperm Parameters" in Middle Euphrates Area of Iraq

Thu-Alfeqar Razzaq Tweij[1], Dheyaa Shnan Al-Jameel[2], Bassim Mohammed Alaasam[3]
[1]Department of Basic Sciences, Faculty of Dentistry, University of Kufa, Al-Najaf Al-Ashraf, Iraq
[2]Department of Pathology, Faculty of Medicine, Jabir Ibn Hayyan Medical University, Al-Najaf Al-Ashraf, Iraq
[3]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Kufa, Al-Najaf Al-Ashraf, Iraq
Corresponding author:
Thu-Alfeqar Razzaq Tweij, E mail: thualfeqarr.tweij@uokufa.edu.iq

Article History: *Submitted: 20.10.2019* *Revised: 24.12.2019* *Accepted: 12.01.2020*

ABSTRACT
Infertility is one of the most important problems among married which known by the inability of couples to conceive within 12 months of un-protective sexual intercourse. It affects 20% of married, the male factors constitute about 50-60% of all reasons of infertility. There are several medications used in the treatment of male infertility whether hormonal and non- hormonal therapy. The study aims to investigate if there is a relationship between blood group and infertility in men and between selected drug and response in another word makes a comparison between fourth therapies. Overall distribution of ABO blood groups of infertile men was O (46.57%), A (20.28%), B (18.29%), and AB (14.86%). There is a significant relationship between type of drug and response that result from variation in significant increasing in both sperm concentration and sperm motility percent. The result of bacteriological study indicate the prevalence of *Staphylococcus epidermidis* 9(15%) then *Staphylococcus aureus* and *Neisseria gonorrhoeae* with 5(8.33%) for each in the semen of infertile men while fertile men infection record 4(13.33) for *Staphylococcus epidermidis* then 2(6.67) for each of *Streptococcus viridians*, *Urea plasma urealyticum*, and *Staphylococcus aureus*. Out of all sample of bacteriological study 27(45%) and 19(63.33%) showed no growth for infertile and fertile men respectively.
The present study disclosed that the predominance of infertility in the blood group O is more than in other groups. There is a strong relationship between type of therapy and response. Clomiphene alone or in combination with human chorionic Gonadotropin induced highest response.

Keywords: Mal infertility, Blood groups, Clomiphene, Human chorionic Gonadotropin, Follitropin-β, Sperm concentration, Sperm motility.

Correspondence:
Thu – Alfeqar Razzaq Tweji
Department of Basic Sciences, University of Kufa,
Al – Najaf Al – Najaf, Iraq
E-mail: thualfeqarr.tweji@uokufa.iq

DOI: 10.5530/srp.2020.1.42

INTRODUCTION
Infertility is one of the most important problems among married which known by inability of couples to conceive within 12 months of un-protective sexual intercourse [1]. It affects 20% of married, the male factors constitute about 50-60% of all reasons of infertility [2]. Numerous of male infertility causes have been attributed into; congenital and acquired reproductive abnormalities, venereal tract infections, genetically and chromosomal oddities, varicocele, exposure to the gonad toxins, and endocrine disturbances [3]. First person who put forward ABO blood groups was Karl Landsteiner in 1901 [4]. Surface antigen of RBC, leucocytes, and platelets all are included in the incompatibility test of blood transfusion [5]. Up to now the international society of blood groups (ISBT) reported that the registered blood group systems are 38 and there are 45 genes which responsible for these blood group systems where they identified and sequenced and polymorphisms with these antigens are well known [6]. Antigens of blood groups are associated with blood transfusion, organ transplantation, anthropology, and ancestral relations [7]. Many studies supports the relationship between male infertility and sexually transmitted diseases (STIs) [8-10], the most common bacteria that affect the sperm functions are *Staphylococcus epidermidis, Mycoplasma genitalium, Escherichia coli, Chlamydia trachoma is, Neisseria gonorrhoeae, Staphylococcus aureus, Urea plasma urealyticum* [11-13]. Leukocytes count in the sample of semen is mostly associated with presence of bacterial pathogen, where there is increasing level of leukocytospermia which is mostly related to bacteriospermia [14], the medical therapy of male infertility either specific or nonspecific. The specific therapies are utilized in certain etiologies, like i) hypogonadotropic hypogonadism, ii) male accessory gland infection, iii) retrograde ejaculation, iv) positive antisperm antibody. While nonspecific treatments are both hormonal and no hormonal therapies, suggested for the treatment of patients [15]. Clomiphene is a synthetic, nonsteroidal medication, structurally is like to diethylstilbestrol. Although it predominant acts as antiestrogen but it also has a mild estrogenic impact [16]. Clomiphene is most widely prescribed drug for male infertility. It has been used in the treatment of unexplained infertility, oligospermia, asthenospermic, hypogonadism, and unconstructive ozoospermic [17]. Clomiphene is a selective estrogen receptor modifier (SERM) which blocks negative feedback at hypothalamus and pituitary level. This indirectly increases the liberation of the luteinizing hormone (LH) and the follicle stimulating hormone (FSH) by the anterior pituitary.

Enhancing LH and FSH will increases testosterone production and spermatogenesis, respectively [18]. Clomiphene has successfully been given as an off-label treatment for male hypogonadism and infertility [19-21].Recent data has appeared that Clomiphene causes moderate increases in the LH, FSH, and the sperm counts in individuals with pregerminal hypo-fertility and also those with unexplained infertility [18]. It also prompts endogenous androgen production. So, it can be utilized as an alternative to testosterone in male with hypogonadism [22]. The common side effects of the Clomiphene involve headache, nausea, dizziness, hot flush, visual disturbance, fluid retention, weight gain. Clomiphene may cause endocrine disorders [23]. Human chorionic Gonadotropin (HCG), is a luteinizing hormone analogue, has the ability to stimulate leydig cells to generate further of testosterone. HCG can be obtaining from urine, in addition to other recombinant sources. Exogenous HCG thus enhances intratesticular and serum testosterone concentrations to improve spermatogenesis [24]. This replacement hormonal therapy is the only on-label pharmaceutical which use in treatment of male infertility. In women, HCG is utilized during infertility treatment for trigger final follicular ripeness and ovulation, in addition to luteal phase support [25]. One modern study appeared that hypogonadal men whose treated only by HCG experienced an raise in their serum testosterone about 250 ng/dL with an passable improvement of hypogonadal symptoms [26]. Furthermore, HCG acts directly on the testis, exciting a prompt response relative to indirect drugs such as Clomiphene that relies on synthesis of LH by pituitary gland. Regrettably, its route of administration (subcutaneous), comparatively short half-life (33 hrs) as well as the price prevent it from applied for most hypogonadal individuals as primary therapy [27]. Follicle stimulating hormone plays important role in spermatogenesis. During fetal and neonatal phases, the FSH activates Sertoli cells proliferation. In the pubertal stage, it affects the mitotic activity of the spermatogonia in addition to encourages cellular differentiation, unto arrival at the round spermatic phase. Because of its bio-physiological function in spermatogenesis, different attempts have been done to treat idiopathic oligozoospermic men by FSH [28]. FSH is a glycoprotein composing of 2 chains, one with 92 amino acids constitute the alpha chain and the second has 111 amino acids forming the β chain, that are coupled via a noncovalent bond [29]. Previously, the urinary human menopausal Gonadotropin is the only source of FSH, such highly purified urinary FSH, having only little LH bioactivity and immunoreactivity [30]. Currently, recombinant human FSH preparations (rFSH) have been used in reproductive medicine, although it is still discussed if they are more efficient and safer than urinary FSH [31].Recombinant FSH preparations are produced via Chinese hamster ovarian cells and have several advantages as it is available in limitless amount, highly purified, highly specific activity as well as proven higher efficacy [30].Two rFSH preparations are now available for clinical use in stimulating spermatogenesis, follitropin-α and follitropin-β. Follitropin-β is somewhat more effective than follitropin-α. Follitropin-β was first marketed with lower dosages, in conformity with the slightly lesser recommended doses [32].

MATERIALS AND METHODS

The study executed on 350 infertile men of those who are reviewing the fertility center in AL-Sader medical city in An Najaf city along with 225 fertile men during the period from January 2018 to September 2019, the age between 22- 40 years. The subjects participated in this study are belong to Middle Euphrates area of Iraq. From each person 2±0.5 mL of blood were drawn from antecubital vein by a sterile disposable syringe, then transferred instantaneously to a glass tube containing anticoagulant ethylene diamine tetra-acetic acid. The blood grouping (ABO) in addition to Rhesus factors (Rh) were done via antigen antibody agglutination test [33]. Seminal fluid analysis was done for each infertile male where the specimen was collected into disposable clean, dry, germ-free plastic containers via masturbation following an abstinence time 3-5 days, was analyzed within one hour of collection. After letting as a minimum 30 min for liquefaction, the seminal analysis was performed to assess the sperm parameters like concentration and motility percentage, each semen's container labeled with the requisite information including the name and age, number of file, abstinence duration and time of specimen collection. Macro- and microscopic examinations were carried out according to WHO methodology as mentioned with details in WHO manual [34]. Several classes of medications were used to treat infertile men (100 patients) from who attended the fertility center. A quarter of them (n = 25) were administered Clomiphene for infertility, The second quarter treated with human chorionic Gonadotropin, the third with Follitropin beta and the last with combination of Clomiphene + human chorionic Gonadotropin. After three months of treatment another seminal fluid analysis was down. Total of 60 infertile and 30 fertile men submitted to bacterial infections study where samples of semen collected in screw-capped container. The semen sample stored at 2-8 °C maximum for 24 hours, then transported to microbiology lab for culture and identifications. A volume of 0.01 ml of semen sample cultured on nutrient agar, blood agar, chocolate agar, and other selective media, all isolates were diagnosed to the species level using the traditional morphological and biochemical tests [35,36]. The data of 575 male of both fertile and infertile were collected then statistically analyzed through chi secure by using SPSS program version 21.

RESULTS

The ratio of fertile to infertile men was 1:1.55. Blood groups distribution among the infertile men in this study appeared that the O blood group have the maximum frequency (46.57%), in the second degree, blood group A (20.28%) followed by B (18.29%), while AB was the lowest one (14.86%).In fertile men, distribution of ABO groups showed similar sequencing, the groups O (40.89%), blood group A (23.11%), group B (22.22%) and group AB (13.78%) to that showed in infertile men. Although the men with blood group O exist more

susceptible to infertility and those with AB are the lowest but there are no significant relationship between the blood group and the infertility as shown in table-1.

Table-1: Normal distribution of fertile and infertile men among blood group.

Blood Groups	Infertile men			Fertile men			Statistic Test P-value
	Rh+	Rh-	Sum	Rh+	Rh-	Sum	
A	62(19.56%)	9(27.27%)	71(20.28%)	49(24.14%)	3(13.63%)	52(23.11%)	0.442
B	58(18.30%)	6(18.18%)	64(18.29%)	44(21.67%)	6(27.27%)	50(22.22%)	
AB	44(13.88%)	8(24.24%)	52(14.86%)	29(14.29%)	2(9.10%)	31(13.78%)	
O	153(48.26%)	10(30.30%)	163(46.57%)	81(39.90%)	11(50.00%)	92(40.89%)	
Total	317(100%)	33(100%)	350(100%)	203(100%)	22(100%)	225(100%)	

The current study displayed that there is a significant correlation between class of therapy and increasing of sperm concentration in infertile men (Table-2).

Table-2: Distribution of sperm concentration response in different groups.

Type	Clomiphene	Human chorionic Gonadotropin	Follitropin beta	Clomiphene + Human chorionic Gonadotropin	p-value
Response [a]	18(72%)	13(52%)	8(32%)	15(60%)	0.036
Not response	7(28%)	12(48%)	17(68%)	10(40%)	
Total	25(100)	25(100)	25(100)	25(100)	

[a] Response mean there is significant increasing in sperm concentration when compare between before and after treatment. The variation of the response in sperm motility percentage among groups in table-3 appears a significant relationship between the response and type of treatment.

Table-3: Distribution of sperm motility percentage response among groups.

Type	Clomiphene	Human chorionic Gonadotropin	Follitropin beta	Clomiphene + Human chorionic Gonadotropin	p-value
Response [a]	11(44%)	6(24%)	9(36%)	16(64%)	0.034
Not response	14(56%)	19(76)	16(64%)	9(36%)	
Total	25(100)	25(100%)	25(100%)	25(100%)	

[a] Response mean there is significant increasing in sperm motility percentage when compare between before and after treatment.

Out of 60 infertile men submitted to bacterial infection study 27(45%) of isolates showed no growth while Staphylococcus epidermidis was the most infectious type of bacteria 9(15%) then both Staphylococcus aureus and Neisseria gonorrhoeae with 5(8.33%). Other infectious agent is Urea plasma urealyticum and Streptococcus viridians with 4(6.67%). The lowest levels of bacterial infections are Klebsiella pneumonia and Enterococcal facials 2(3.33) and1 (1.67) respectively. Otherwise fertile men 30 samples showed about 19(63.33) with no growth, Staphylococcus epidermidis is the most bacterial isolate 4(13.33%) then Streptococcus viridians, Urea plasma urealyticum, and Staphylococcus aureus all with 2(6.67%) and only 1(3.33%) for Escherichia coli.

Table-4: Distribution of bacterial isolate between fertile and infertile men

Type of bacterial isolates	Number of bacterial isolates in infertile men(%)	Number of bacterial isolates in fertile men(%)
Staphylococcus epidermidis	9(15)	4(13.33)
Staphylococcus aureus	5(8.33)	2(6.67)
Urea plasma urealyticum	4(6.67)	2(6.76)
Neisseria gonorrhoeae	5(8.33)	0(0.0)
Escherichia coli	3(5)	1(3.33)
Klebsiella pneumonia	2(3.33)	0(0.0)
Streptococcus viridians	4(6.67)	2(6.67)
Enterococcal faecalis	1(1.67)	0(0.0)
No growth	27(45)	19(63.33)
Total	60	30

DISCUSSION

The childbearing is one of important goals of marriage because of its role in the continuity of life. Therefore, infertility is one of the problems that may encounter the married couples which may lead to painful psychological and social problems, hence the importance of infertility treatment for both genders. In this regard, several of studies were conducted, including the current one, which dealt with many of drugs that are used for treatment of infertility and made a comparison to find the best. Several of previous studies reported association of the blood group with many of diseases including that individual with blood group A is more susceptible to myocardial infarction and angina and those with blood group O are lowest [37].A study found that the incidence of hypertension was more in blood group B, less in blood group A, while the group AB appeared the lowest incidence of hypertension [38]. Other study showed the subjects having blood group O possess the lowest risk of type 2 diabetes mellitus while persons with blood group B possess the highest risk [39]. The current study showed that blood group AB individuals have lowest risk of infertility while blood group Omen were highest risk followed by blood group A and then B. According to Abdollahi et al. [40] men in group O is more susceptible

to infertility, followed by group A, B and AB respectively. The distribution of the blood groups of infertile men appeared the maximum frequency in the blood group O, pursue by blood groups A then B. The AB blood group was the lowest one [41]. In the present study, noticing an overall improvement in the sperm count, and motility percentage in groups that received Clomiphene, human chorionic Gonadotropin, Follitropin-β and Clomiphene plus human chorionic Gonadotropin. This improvement appeared with different degree among groups, i.e. presence significant relationship between type of treatment and degree of enhancement of sperm parameters. The results showed that Clomiphene produces highest percentage response in sperm concentration (72% (18 patients)) while the response in motility % occurs by less percentage (44% (11 patients)). That is mean Clomiphene has effect on sperm count more than motility %. Wang et al. [42] reported that six (75%) of eight of the infertile men treated by Clomiphene have baseline sperm concentrations >10 x 10^6 mL^{-1}. Pretreatment analysis showed that the patients suffered from marked oligospermia (<5 x 10^6 mL^{-1}). The results of Moradi et al. [43] revealed that Clomiphene possess considerable effect on sperm concentration, morphology, and motility (P = .01), but not much influence on the semen volume (P > .05).The collected impact estimates demonstrated that estrogen antagonists use (Clomiphene or tamoxifen) was linked with a statistically significant elevation of pregnancy rate in comparison with controls (P = 0.0004). Significant rise in sperm count (P = 0.001) and motility percent (P = 0.03) were also noted [44]. From p-value can conclude that the drugs have effect on sperm concentration more than motility. Also the current study shows that the human chorionic Gonadotropin exerts beneficial effect on sperm count higher than that on sperm motility %. Where it increases sperm count significantly in 52% of patients and motility % in 24%. Study of Dubin et al. [45] demonstrated that adjuvant hCG gives better seminal parameters and increase pregnancy rate when compare with varicocelectomy alone. Sperm counts of patients who have treated with injection of hCG, 4,000 units IM twice weekly for ten weeks are < 10 million/ml. A 55% improvement in seminal fluid quality and a 45% pregnancy rate was observed. A total of seventeen Oligospermia patients were treated with 5000 unit of hCG once per week for 4 month. Monthly, serum testosterone level and semen count were analyzed. Progressive raise of sperm count was observed in 4 months [46]. In this study, the patients whose received Follitropin-β showed significantly improve in both sperm concentration and motility % in approximately similar percentage 32% (8 patients) and 36 (9 patients) respectively. That is mean Follitropin-β has a balance effect on the studied sperm parameters and it considered the least effectiveness among other drugs. Casamonti et al. [47] evaluated two of sperm parameters: total sperm number (TSN) and the total motile sperm count (TMSC). The study found statistically significant amelioration of TSN and TMSC following three months of treatment. Other study reported that treatment with r-hFSH (Follitropin-β) at a dose of 100 unit produce a significant raise in sperm concentration [48]. The study conducted by Palombaet al.[49] showed that administration of 150 unit of FSH 3 time a weekly for three months resulted in a significant enhancement in sperm count (p = 0.047), motility percent (p = 0.041) and morphology (p = 0.023). The present study showed that combination of Clomiphene and human chorionic Gonadotropin induced good improvement of sperm concentration in 60% of patients (15/25) and motility percent in 64% of patients (16/25). Kohn et al [50] studied spermatogenesis recovery by HCG and Clomiphene or tamoxifen in men with infertility associated with testosterone use. Seventy percent of the 66 patients were able to achieve the total motile sperm count of greater than 5 million after twelve month in the study. Hussein et al [51] showed that the non-obstructive ozoospermic men who did not respond for Clomiphene as monotherapy and were managed by addition of hCG and/or hMG. 38/62 patients (61%) (Clomiphene + HCG) ultimately have improvement of sperm production. They did observe that these men had about doubled sperm retrieval rate when compared with control group. Several studies indicate the dominance of sexually transmitted bacteria and its role in infertility [52,53]. The result of this study presents the prevalence of Staphylococcus epidermidis, Staphylococcus aureus, and Streptococcus viridans in both fertile and infertile men that agree with other study which hint that the most isolated bacteria in the culture of prostatic secretion of infertile men is Staphylococci and E. coli [54]. Ureaplasma urealyticum is one of dominant isolated bacteria from the semen of infertile men and with Ureaplasma parvum is more common isolated [55,56]. Isolation of Klebsiella spp. shows the role of this genus of bacteria with wide range of virulence factor [57]. Spermatogenesis deterioration, sperm functions impairment, and seminal tract obstruction is mostly resulted by microbial infections [58]. About 27(45%) of samples from infertile men showed no growth that may be indicating for viral infection or other causes like Sperm dysfunction and sperm defects, teratozoospermia, asthenozoospermia, and other causes like genetic including single gene mutations and chromosomal aberrations [59-61].

References

[1]. World Health Organization. Cambridge University Press; Cambridge: 2000. WHO Manual for the Standardised Investigation and Diagnosis of the Infertile Couple.

[2]. RingJD, LwinAA, and KöhlerTS. Current medical management of endocrine-relatedmale infertility. Asian Journal of Andrology. 2016;18:357–363.

[3]. Tournaye H. Male factor infertility and ART. Asian J Androl. 2012;14:103–108.

[4]. Schwarz HP, and Dorner F. Karl Landsteiner and his major contributions to haematology. British Journal of Haematology. 2003;121(4):556-565.

[5]. Valsami S, Dimitroulis D, Gialeraki A, Chimonidou M, and Politou M. Current trends in platelet transfusions practice: The role of ABO-RhD and human leukocyte antigen incompatibility. Asian journal of transfusion science. 2015;9(2):117.

[6]. ISBT International Society of Blood Groups, https://www.isbtweb.org/ Accessed 3 December, 2019.

[7]. Khurshid B, Naz M, Hassan M, and Mabood SF. Frequency of ABO and Rh (D) blood groups in district Swabi NWFP (Pakistan). J Sci Tech Univ. Peshawar. 1992;16:5-6.

[8]. Çalışkan T, Koçak İ, Kırdar S, Serter M, Okyay P, and Gültekin B. Human papillomavirus and Chlamydia trachomatis in semen samples of asymptomatic fertile and infertile men: prevalence and relation between semen parameters and IL-18 levels. Turkish Journal of Urology. 2010;36(2):143-148.

[9]. Pellati D, Mylonakis I, Bertoloni G, Fiore C, Andrisani A, Ambrosini G, et al. Genital tract infections and infertility. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2008;140(1):3-11.

[10]. Ochsendorf FR. Sexually transmitted infections: impact on male fertility. Andrologia. 2008;40(2):72-75.

[11]. Abusarah EA, Awwad ZM, Charvalos E, and Shehabi AA. Molecular detection of potential sexually transmitted pathogens in semen and urine specimens of infertile and fertile males. Diagnostic microbiology and infectious disease. 2013;77(4):283-286.

[12]. Merino G, Carranza-Lira S, Murrieta S, Rodriguez L, Cuevas E, and Moran C. Bacterial infection and semen characteristics in infertile men. Archives of andrology. 1995;35(1):43-47.

[13]. Comhaire F, Verschraegen G, and Vermeulen L. Diagnosis of accessory gland infection and its possible role in male infertility. International Journal of Andrology. 1980;3(1-6):32-45.

[14]. Gdoura R, Kchaou W, Znazen A, Chakroun N, Fourati M, Ammar-Keskes L, et al. Screening for bacterial pathogens in semen samples from infertile men with and without leukocytospermia. Andrologia. 2008;40(4):209-218.

[15]. Santi D, Granata ARM, and Simoni M. FSH treatment of male idiopathic infertility improves pregnancy rate: a meta-analysis. Endocrine Connections. 2015;4:R46–R58.

[16]. ElSheikh MG, Hosny MB, Elshenoufy A, Elghamrawi H, Fayad A, and Abdelrahman S. Combination of vitamin E and clomiphene citrate in treating patients with idiopathic oligoasthenozoospermia: A prospective, randomized trial. Andrology. 2015;3(5):864-867.

[17]. Roth LW, Ryan AR, and Meacham RB. Clomiphene citrate in the management of male infertility. Seminars in Reproductive Medicine. 2013;31(4):245-250.

[18]. Chehab M, Madala A, and Trussell JC. On-label and off-label drugs used in the treatmentof male infertility. FertilSteril. 2015;103:595–604.

[19]. Kaminetsky J, Werner M, Fontenot G, and Wiehle RD. Oral enclomiphene citrate stimulates the endogenous production of testosterone and sperm counts in men with low testosterone: comparison with testosterone gel. J Sex Med. 2013;10:1628– 1635.

[20]. Katz DJ, Nabulsi O, Tal R, and Mulhall JP. Outcomes of clomiphene citrate treatment in young hypogonadal men. BJU International. 2012;110:573– 578.

[21]. Wiehle RD, Fontenot GK, Wike J, Hsu K, Nydell J, and Lipshultz L. Enclomiphene citrate stimulates testosterone production while preventing oligospermia: a randomized phase II clinical trial comparing topical testosterone. FertilSteril. 2014;102:720–727.

[22]. Shabsigh A, Kang Y, Shabsign R, Gonzalez M, Liberson G, Fisch H, et al. Clomiphene citrate effects on testosterone/estrogen ratio in male hypogonadism. J Sex Med. 2005;2(5):716–721.

[23]. Viola MI, Meyer D, and Kruger T. Association between clomiphene citrate and visual disturbances with special emphasis on central retinal vein occlusion: a review. GynecolObstet Invest. 2011;71:73–76.

[24]. Kim ED, Crosnoe L, Bar-Chama N, Khera M, and Lipshultz LI. The treatment of hypogonadism in men of reproductive age. FertilSteril. 2013;99:718–724.

[25]. Lunenfeld B, Bilger W, Longobardi S, Alam V, D'Hooghe T and Sunkara SK. The Development of Gonadotropins for Clinical Use in the Treatment of Infertility.Front. Endocrinol. 2019;10:429.

[26]. Habous M, Giona S, Tealab A, Aziz M, Williamson B, Nassar M, et al. Clomiphene citrate and human chorionic gonadotropin are both effective in restoring testosterone in hypogonadism: a short-course randomized study. BJU International. 2018;122:889–897.

[27]. Coviello AD, Matsumoto AM, Bremner WJ, Herbst KL, Amory JK, Anawalt BD, et al. Low-dose human chorionic gonadotropin maintains intratesticular testosterone in normal men with testosterone-induced gonadotropin suppression. J Clin Endocrinol Metab. 2005;90:2595–2602.

[28]. ForestaC, SeliceR, FerlinA, ArslanP, and Garolla A.Hormonal treatment of male infertility: FSH. Reproductive BioMedicine Online. 2007;15(6):666-672.

[29]. Balkan M, Gedik A, Akkoc H, Izci Ay O, Erdal ME, Isi H, et al. FSHR single nucleotide polymorphism frequencies in proven fathers and infertile men in Southeast Turkey. Biomed Res Int. 2010;2010:640318.

[30]. Craenmehr E, Bontje PM, Hoomans E, Voortman G, and Mannaerts BMJL. Follitropin-β administered by pen device has superior local tolerance compared with follitropin-α administered by conventional syringe. Reproductive BioMedicine Online. 2001;3:(3):185–189 .

[31]. Sinisi D, Esposito G, Bellastella L, Maione V, Palumbo L, Gandini F, et al. Efficacy of recombinant human follicle stimulating hormone at low doses in inducing spermatogenesis and fertility in hypogonadotropic hypogonadism. J. Endocrinol. Invest. 2010;33:618-623.

[32]. Orvieto R, Nahum R, Rabinson J, Ashkenazi J, Anteby EY, and Meltcer S. Follitropin-alpha (Gonal-F) versus follitropin-beta (Puregon) in controlled ovarian hyperstimulation for in vitro fertilization: is there any difference? Fertility and Sterility. 2009;91(4 Suppl):1522-1525.

[33]. Khan MS, Farooq N, Qamar N, Tahir F, Subhan F, Kazi BM, et al. Trend of blood groups and Rh Factor in the twin cities of Rawalpindi and Islamabad. J Pak Med Assoc. 2006;56(7):299-302.

[34]. World health Organization (WHO): WHO laboratory manual for the examination of humansemen and sperm cervical mucus interaction. 4th ed.; New York; Cambridge University Press;1999;4-59.

[35]. George MG, Julia AB, and Timothy GL. (2004) Taxonomic outline of the prokaryotes Bergey's manual of systemic bacteriology. 2nded, Springer, p114.

[36]. Washington W, Allen S,Janda W,Koneman E,Procop G,Schreckenberger P, Woods G, and

Koneman's (2006). Color Atlas and Textbook of Diagnostic Microbiology, 6th edition. Lippincott Williams and Wilkins. PP 200-259.
[37]. Akhund IA, Alvi IA, Ansari AK, Mughal MA, and AKhund AA. A study of relationship of ABO blood groups with myocardial infarction and angina pectoris. J Ayub Med Coll Abbottabad. 2001;13(4):25-26.
[38]. El-Sayed MIK, and Amin HK. ABO blood groups in correlation with hyperlipidemia, diabetes mellitus type II, and essential hypertension. Asian J Pharm Clin Res. 2015;8:236–243.
[39]. Fagherazzi G, Gusto G, Clavel-Chapelon F, Balkau B, and Bonnet F. ABO and Rhesus blood groups and risk of type 2 diabetes: evidence from the large E3N cohort study. Diabetologia. 2015;58:519–522.
[40]. Abdollahi E, Tavasolian F, Ghasemi N, Vakili M, and Amini A. The effect of parental ABO blood group on fetal surveillance. Iran J PedHematolOncol. 2013;3(4):154-158.
[41]. Prasad B, Lalit A, and Sharma NC. Distribution of ABO blood group among fertile and infertile males in central India: a pilot study. Int J Med Sci Public Health. 2015;4(12):1708-1710.
[42]. Wang C, Chan CW, Wong KK, and Yeung KK. Comparison of the effectiveness of placebo, clomiphene citrate, mesterolone, pentoxifylline, and testosterone reound therapy for the treatment of idiopathic oligospermia. FertilSteril. 1983;40(3):358–365.
[43]. Moradi M, Moradi A, Alemi M, Ahmadnia H,Abdi H, Ahmadi A, et al. Safety and efficacy of clomiphene citrate and L-carnitine in idiopathic male infertility. Urol J. 2010;7(3):188–193.
[44]. Chua ME,EscusaKG, Luna S, TapiaLC,Dofitas B, and Morales M.Revisiting oestrogen antagonists (clomiphene or tamoxifen) as medical empiric therapy for idiopathic male infertility: a meta-analysis. Andrology. 2013;1(5):749-757.
[45]. Dubin L, and Amelar R. Philadelphia: Saunders; 1977. The varicocele and infertility.
[46]. Mushtaq M,Jafri S, Sheikh A, Ahmad S,Deeba F,and Salam K.. Human chorionic gonadotropin (hCG): A treatment of oligospermia. Pakistan Journal of Medical Sciences. 2007;23(6):840-846.
[47]. Casamonti E, Vinci S, Serra E, Fino MG, Brilli S, LottiF, et al. Short-term FSH treatment and sperm maturation: a prospective study in idiopathic infertile men. Andrology. 2017;5:414–422.
[48]. Foresta C, Bettella A, Merico M, Garolla A, Ferlin A, and Rossato M. Use of recombinant human follicle-stimulating hormone in the treatment of male factor infertility. FertilSteril. 2002;77(2):238–244.
[49]. Palomba S, Falbo A, Espinola S, Rocca M, Capasso S, Cappiello F, et al. Effects of highly purified follicle-stimulating hormone on sperm DNA damage in men with male idiopathic subfertility:a pilot study.J Endocrinol Invest. 2011;34(10):747–752.
[50]. Kohn TP, Louis MR, Pickett SM, Lindgren MC, Kohn JR, Pastuszak AW, et al. Age and duration of testosterone therapy predict time to return of sperm count after human chorionic gonadotropin therapy. FertilSteril. 2017;107:351–357.
[51]. Hussein A, Ozgok Y, Ross L, Rao P andNiederberger C. Optimization of spermatogenesis-regulatinghormones in patients with non-obstructiveazoospermia and its impact on sperm retrieval:a multicentre study. BJU International. 2012;111:E110–E114.
[52]. Abusarah EA, Awwad ZM, Charvalos E, and Shehabi AA. Molecular detection of potential sexually transmitted pathogens in semen and urine specimens of infertile and fertile males. Diagnostic microbiology and infectious disease. 2013;77(4):283-6.
[53]. Shehabi AA, Awwad ZM, Al-Ramahi M, Charvalos E, and Abu-Qatouseh LF. Detection of Mycoplasma genitalium and Trichomonas vaginalis infections in general Jordanian patients. Am J Infect Dis. 2009;5(1):7-10.
[54].Giamarellou H, Tympanidis K, Bitos NA, Leonidas E, andDaikos GK. Infertility and chronic prostatitis. Andrologia. 1984;16(5):417-22.
[55].Zeighami H, Peerayeh SN, Yazdi RS, and Sorouri R. Prevalence of Ureaplasma urealyticum and Ureaplasma parvum in semen of infertile and healthy men. International journal of STD & AIDS. 2009;20(6):387-390.
[56]. Knox CL, Allan JA, Allan JM, Edirisinghe WR, Stenzel D, Lawrence FA, et al. Ureaplasma parvum and Ureaplasma urealyticum are detected in semen after washing before assisted reproductive technology procedures. Fertility and sterility. 20031;80(4):921-929.
[57].Ibadin OK, and Ibeh IN. Bacteriospermia and sperm quality in infertile male patient at University of Benin Teaching Hospital, Benin City, Nigeria. Malaysian Journal of Microbiology. 2008;4(2):65-67.
[58]. Keck C, Gerber-Schäfer C, Clad A, Wilhelm C, and Breckwoldt M. Seminal tract infections: impact on male fertility and treatment options. Human Reproduction Update. 1998;4(6):891-903.
[59]. Hull MG, Glazener CM, Kelly NJ, Conway DI, Foster PA, Hinton RA, et al. Population study of causes, treatment, and outcome of infertility. Br Med J (Clin Res Ed). 1985;291(6510):1693-1697.
[60]. Thonneau P, Marchand S, Tallec A, Ferial ML, Ducot B, Lansac J, et al. Incidence and main causes of infertility in a resident population (1850 000) of three French regions (1988–1989). Human reproduction. 1991;6(6):811-816.
[61].Ferlin A, Arredi B, and Foresta C. Genetic causes of male infertility. Reproductive toxicology. 2006;22(2):133-141.