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, Dhuyaa Shyan Al-Jameef, Bassim Mohammed Alaasam
1Department of Basic Sciences, Faculty of Dentistry, University of Kufa, Al-Najaf Al-Ashraf, Iraq
2Department of Pathology, Faculty of Medicine, Jabir Ibn Hayyan Medical University, Al-Najaf Al-Asraf, Iraq
3Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Kufa, Al-Najaf Al-Asraf, Iraq

Corresponding author:
Thu-Alfeqar Razzaq Tweij, E mail: thualfeqar.tweij@uokufa.edu.iq

ABSTRACT
Infertility is one of the most important problems among married which known by the inability of couples to conceive within 12 months of unprotected sexual intercourse [1]. 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 viridans, Urea plasma urealyticum, and Staphylococcus aureus. Out of all sample of bacteriological study 26(45%) and 19(63.33%) showed no growth for infertile and fertile men respectively.

Keywords: Male 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: thualfeqar.tweji@uokufa.edu.iq

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INTRODUCTION
Infertility is one of the most important problems among married which known by inability of couples to conceive within 12 months of unprotected 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 (STDs) [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 pregonadal 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 interstitial and serum testosterone concentrations to improve spermatogenesis [24]. This replacement hormonal therapy is the only off-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 spermatid phase. Because of its biophysiological 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 slightylessrecommended 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 ºC 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.

<table>
<thead>
<tr>
<th>Blood Groups</th>
<th>Infertile men</th>
<th>Fertile men</th>
<th>Statistic Test p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Rh+</td>
<td>62(19.5%)</td>
<td>49(18.1%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Rh-</td>
<td>27(8.6%)</td>
<td>27(10.2%)</td>
<td></td>
</tr>
<tr>
<td>Rh+ Sum</td>
<td>9(3%)</td>
<td>6(3%)</td>
<td></td>
</tr>
<tr>
<td>Rh- Sum</td>
<td>8(4%)</td>
<td>7(4%)</td>
<td></td>
</tr>
<tr>
<td>B Rh+</td>
<td>58(18.3%)</td>
<td>44(16.6%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Rh-</td>
<td>18(6.0%)</td>
<td>22(8.2%)</td>
<td></td>
</tr>
<tr>
<td>Rh+ Sum</td>
<td>6(2%)</td>
<td>7(2%)</td>
<td></td>
</tr>
<tr>
<td>Rh- Sum</td>
<td>7(4%)</td>
<td>9(4%)</td>
<td></td>
</tr>
<tr>
<td>AB Rh+</td>
<td>44(13.8%)</td>
<td>29(10.8%)</td>
<td>0.31</td>
</tr>
<tr>
<td>Rh-</td>
<td>12(4.0%)</td>
<td>12(4.3%)</td>
<td></td>
</tr>
<tr>
<td>Rh+ Sum</td>
<td>9(3%)</td>
<td>9(3%)</td>
<td></td>
</tr>
<tr>
<td>Rh- Sum</td>
<td>8(4%)</td>
<td>8(4%)</td>
<td></td>
</tr>
<tr>
<td>O Rh+</td>
<td>153(48.2%)</td>
<td>91(33.1%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Rh-</td>
<td>30(9.6%)</td>
<td>50(18.0%)</td>
<td></td>
</tr>
<tr>
<td>Rh+ Sum</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Rh- Sum</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>317(100%)</td>
<td>150(100%)</td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Type</th>
<th>Clomiphene</th>
<th>Human chorionic Gonadotropin</th>
<th>Follitropin Beta</th>
<th>Clomiphene + Human chorionic Gonadotropin</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response a</td>
<td>18(72%)</td>
<td>13(52%)</td>
<td>8(32%)</td>
<td>15(60%)</td>
<td>0.00</td>
</tr>
<tr>
<td>Not response</td>
<td>7(28%)</td>
<td>12(48%)</td>
<td>17(68%)</td>
<td>10(40%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Total</td>
<td>25(100%)</td>
<td>25(100%)</td>
<td>25(100%)</td>
<td>25(100%)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

a Response mean there is significant increasing in sperm motility percentage 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.

<table>
<thead>
<tr>
<th>Type</th>
<th>Clomiphene</th>
<th>Human chorionic Gonadotropin</th>
<th>Follitropin Beta</th>
<th>Clomiphene + Human chorionic Gonadotropin</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response a</td>
<td>11(44%)</td>
<td>6(24%)</td>
<td>9(36%)</td>
<td>16(64%)</td>
<td>0.0</td>
</tr>
<tr>
<td>Not response</td>
<td>14(56%)</td>
<td>19(76%)</td>
<td>16(64%)</td>
<td>9(36%)</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>25(100%)</td>
<td>25(100%)</td>
<td>25(100%)</td>
<td>25(100%)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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(8.3%). Other infectious agent is Urea plasma urealyticum and Streptococcus viridans with 4(6.67%). The lowest levels of bacterial infections are Klebsiella pneumonia and Enterococcal facialis 2(3.33) and 1(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 viridans, 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

<table>
<thead>
<tr>
<th>Type of bacterial isolates</th>
<th>Number of bacterial isolates in fertile men(%)</th>
<th>Number of bacterial isolates in infertile men(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus epidermidis</td>
<td>9(15)</td>
<td>4(13.33)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5(8.33)</td>
<td>2(6.67)</td>
</tr>
<tr>
<td>Urea plasma urealyticum</td>
<td>4(6.67)</td>
<td>2(6.76)</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>5(8.33)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3(5)</td>
<td>1(3.33)</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>2(3.33)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Streptococcus viridans</td>
<td>4(6.67)</td>
<td>2(6.67)</td>
</tr>
<tr>
<td>Enterococcal facialis</td>
<td>1(1.67)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>No growth</td>
<td>27(45)</td>
<td>19(63.33)</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>30</td>
</tr>
</tbody>
</table>

**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 least [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 whilepersons 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 Cliqupime, human chorionic Gonadotropin, Follitropin-β and Cliqupime 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 Cliqupime 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 Cliqupime has effect on sperm count more than motility %. Wang et al. [42] reported that six (75%) of eight of the infertile men treated by Cliqupime 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 Cliqupime 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 (Cliqupime 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 Cliqupime 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 azoospermic 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


