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ABO Blood grouping and Rhesus factor: Association with ovarian reserve and the outcomes after in-vitro fertilization

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Abstract

Background: The evidence currently available fair to suggest a relation between ABO grouping and women infertility such as antral follicles count, recurring miscarriage and live delivery. ABO antigens system was linked with some reproductive disorders, like endometriosis and ovarian cancer. Still, the link between blood grouping and infertility has been a topic of debate, even though some scholars support an absent association in various populations. Ovarian reserve (OR) denotes the potential reproductive capacity as a function of the quality and quantity of residual female oocytes. Around 10% of infertile women reveals reduced OR. Several revisions have described associations between the ABO groups and OR, and displayed that O blood group is more possible to have reduced OR. There is a deficient or debatable literature inspecting associations of ABO blood group with the OR among infertile females. To illuminate the relationship between ABO blood antigens and OR, the authors plan this study. **Results:** Mean age of patients was 34.1 ± 2.8year and most of patients were presented with primary infertility. Blood group O represent 35.4% of patients, blood group A and B were rather equal, while blood group AB was the least (8.6%). Majority of patients (91.9%) presented with Rh^{+ve}. No significant differences between the means of study variables according to blood groups. A significant association between blood grouping with the classes of FSH and AFCs was detected. Non-significant association was detected between the ABO groups and AMH levels. There was non-significant association between Rh and all other study variables. There was insignificant difference in the association of FSH among any two blood groups. **Materials and methods:** This was a retrospective, single-center study. It included 1063 women with mean age of 34.1 ± 2.8years. The study included females with unexplained infertility and submitted to controlled ovarian stimulation, gonadotropin triggering, oocyte recovery, cultured embryo, and transferred embryo. The following data were obtained from each women: age, infertility

duration, AFCs, ABO blood type, Rhesus (Rh) factor, serum FSH and AMH levels, parity, detailed gravity, birth outcomes, and history of abortion. Antral follicle counting (AFC) was calculated on the third cycle day using vaginal ultrasonography. The statistics were finalised through SPSS version 25 for Windows. **Conclusion:** The present study concluded that blood groups and Rh factor associated with the reduced OR. The outcomes of pregnancy after IVF are not influenced by blood types. Excessive focusing on blood groups is not essential during management of infertile females and assisted reproductive techniques.

Keywords
ABO blood groups, ovarian reserve (OR), in vitro fertilization, rhesus factor, DOR.

ABO human system is the glycoproteins or glycolipids symbol of the ABO group antigens represented on the erythrocytes surface and several other cells and tissues. It plays principal role in transfusion reaction, and in the progress of various pathologies (Bao et al., 2021; Zhao et al., 2021). On the basis of the evidence currently available, it seems fair to suggest a relation between ABO grouping and women infertility such as antral follicles count, recurring miscarriage and live delivery (Bao et al., 2021).

The ABO antigens reveals an imperative role in body immunology and organ transplantation (Zhao et al., 2021). Other hematogenous and lymphatic malignancy like lymphoma (Ghazi, Al-Taee, & Al-Hindy, 2020) and leukemia had also been associated with ABO (Tavasolian et al., 2014; Vadivelu et al., 2004). As well, malignant tumors of breast tissue that invade adjacent tissues and subsequent blood and lymphatic spread like breast carcinoma (Burhan et al., 2020) is another pattern of blood group association (Meo et al., 2017). In recent times, researches have described that ABO antigens system was linked with some reproductive disorders, like endometriosis and ovarian cancer (Zhao et al., 2021).

It had been proposed that incompatible ABO antigens were a likely immune source of infertility, in which couples with un-explained infertility had a higher frequency of incompatible ABO blood group (Schwimmer, Ustay, & Behrman, 1967). Still, the link between ABO groups and female infertility has been a topic of debate, even though some scholars support an absent association in various populations (Bao et al., 2021).

Ovarian reserve (OR) denotes the potential reproductive capacity as a function of the quality and quantity of residual female oocytes (Deng et al., 2017). Around 10% of infertile women reveals reduced OR. Lately, alterations in the social milieu, standard of living, and extended reproductivity of women have resulted in gradual rises in their infertility (Wiener-Megnazi, Auslender, & Dirnfeld, 2012). Females with reduced OR gradually develops "premature ovarian failure" that subsidizes atherosclerotic disease, osteoporosis, and other clinical issues (Shuster et al., 2010). These disorders have serious influences on maternal reproductive wellbeing and life quality that in turn, induce a burden on the couples and society.

Several revisions have described an association between the ABO groups and OR, and displayed that O blood group is more possible to have reduced OR (Nejat et al., 2011). However other scholars reported that antigen B are more expected to have reduced OR (Lin et al., 2014), and later reports no relation of blood ABO groups with OR or ovarian response (Awartani et al., 2016; Deng et al., 2017).

Despite the aforesaid outcomes, there is a deficient or debatable literature inspecting the precise association of blood groups with the OR among infertile females. To illuminate the associations between ABO antigens and OR, the authors plan this study.

Materials and methods:

This study was a retrospective, single-center. It included 1063 women with mean age of 34.1 ± 2.8years, who were attending "Teba Center". This study was one of a series of researches covering infertility among both sexes in Middle-Euphrates, Iraq (Al-Bdairi, Al-Hindy, & Al-Shalah, 2021). The study included anovulatory females and/or eumenorrheic females with unexplained infertility during the period from 2015 – 2020. In the center they were submitted to controlled ovarian stimulation, gonadotropin triggering, oocyte recovery, cultured embryo, and transferred embryo completed following a proven protocols (Fang et al., 2020).

The following data were obtained from each women: age, infertility duration, AFCs, blood grouping, and Rhesus (Rh) factor, parity, detailed gravity, birth outcomes, and history of abortion.

Women with history of ovarian surgery, chemotherapy, severe autoimmune or chronic disease, and those lacking complete records were excepted rom the study.

Antral follicle counting

Ultrasonic pelvic examination was performed with a vaginal transducer (7.5 MHz), using a "Samsung WS80 A elite 2017 (Korea)", and by the same gynecologist at the early menstrual cycle (follicular phase). Antral follicle counting (AFC) was calculated on the third cycle day, as the amount of antral follicles measured 2-10mm in diameter. A serum FSH level ⩾10 mIU/ml is considered as a threshold for diminished ovarian reserve (Greenseid et al., 2009). The term diminished ovarian reserve was defined in this study as day-3 AFCs ⩽5 and level of FSH >10 mIU/ml (Kan et al., 2019). Accordingly, there were two classes in this study based of the levels of FSH and AFC.

Laboratory analyses

Venous blood sampling was drained on the 3rd day of the mens for ABO groups, Rh, and biochemical analysis of FSH (mIU/ml) and AMH (pg/ml) from all applicants. Women were subdivided into groups according to ABO typing and Rh factor. Then various study parameters were compared among the groups as the primary outcome measures.

Ethical consideration

The current work was in accordance with the Declaration of Helsinki. Written informed consent were gotten from all applicants, and was permitted by the ethical committee of health institution.

Data Analysis

All the statistics was finalised through SPSS version 25 for Windows. Clear-cut variables appeared as frequencies/percentages. Constant variables appeared as (Means ± SD). When compare means between two groups, Student t-test was applied. Otherwise, Mann-Whitney Test was indicated when variable not exhibit normal distribution. ANOVA test was applied to associate means between blood groups, while Kruskal-Wallis test indicated when variable not exhibit normal distribution. Pearson chi-square were used to find any associations between the categorical variables. A significant *p*-value of less than 0.05 was selected in this study.

Results

Table 1 shows the basal characteristics of the studied variables. Mean age of patients was 34.1 ± 2.8year and majority of patients 658 (61.9%) were presented with primary infertility. Blood group O represent 35.4% of patients, blood group A and B represent 29.3% and 26.8%, while blood group AB was the least (8.6%). Majority of patients (91.9%) presented with Rh^{+ve}.

Majority of patients were presented with G0 and P0 (89.3% and 92.3%), respectively. Only 58 (5.5%) had history of abortion, which was for the first time in 60.35. Pregnancy occurs in (45.9%) of patients and majority of them (53.9%) end with full term baby.

Table 1: Basal characteristics of the study variables among the studied women (1063)

Study variables	Number	%
Age (years)	(34.13 ± 2.8)	(30.0-39.0)
Type of infertility		
Primary	658	61.9%
Secondary	405	38.1%
Blood groups		
O	376	35.4%
A	285	26.8%
B	311	29.3%
A	91	8.6%
Rh factor		
Rh + ve	977	91.9%
Rh - ve	86,	8.1%
AFC	137	12.8%
< 5	926	87.2%
⩾ 5		
Gravidity		
G0	949	89.3%
G1-G2	73	6.8%
G3-G4	27	2.5%
G5-G6	14	1.4%
Parity		
P0	981	92.3%
P1-P2	56	5.2%
P3-P4	21	2.0%
P5-P6	5	0.5%
Pregnancy		
Positive	488	45.9%
Negative	575	54.1%
Pregnancy Outcome		
Term baby	263	53.9%
Preterm baby	59	12.1%
Ectopic pregnancy	9	1.8%
Abortion	157	32.2%
Total	488	100.0%
Abortion		
Yes	58	5.5%
No	1005	94.5%
Frequency of abortion		
Once	35	60.3%
Twice	11	19.0%
Three times	7	12.1%
Four times	5	8.6%

The Distribution of women patients according to their blood groups and rhesus factors (N=1063) is shown in table-2.

Table 2: Distribution of female patients according to Blood group and RH (N=1063)

Blood group and Rh	Number	%
Blood group O (N-376)		
O^+	357	94.9%
O^-	19	5.1%
Blood group B (N-311)		
B^+	286	91.9%
B^-	25	8.1%
Blood group A (N-285)		
A^+	251	88.1%
A^-	34	11.9%
Blood group AB (N-91)		
AB^+	83	91.2%
AB^-	8	8.8%

Table 3 revealed no significant differences between the means of study variables according to blood group.

Table 3: The mean differences of age, FSH and AMH according to blood group

Study variables	Blood groups				Significance
	O (N=376)	A (N=285)	B (N=311)	AB (N=91)	
Age (years)	33.98 ± 2.82	34.00 ± 2.83	34.37 ± 2.72	34.35 ± 2.68	0.2
FSH (mIU/ml)	7.85 ± 2.68	7.77 ± 2.96	7.51 ± 2.40	8.28 ± 2.99	0.1
AMH (pg/ml)	2.90 (3.50)	3.12 (4.56)	3.30 (4.42)	2.63 (3.22)	0.2

Table-4 shows the association between ABO grouping and study variables (pregnancy, antral follicle counts and AMH (<1.5 pg/ml)). There was a significant association between blood group and FSH classes (FSH > 10 and FSH < 10 mIU/ml). There were significant associations between ABO group and the two classes of antral follicle count (AFC > 5 and AFC < 5). As well, there were significant associations between blood groups and AFC when comparing women with diminished AFC (AFC < 5 & FSH > 10mlU/ml) to those with normal OR (AFC > 5 & FSH < 10mlU/ml). Non-significant associations were observed between the blood ABO groups and AMH levels.

Table 3: The association between ABO blood group and pregnancy, antral follicle counts and AMH

Study variables	Blood group				P-value
	O (N=376)	A (N=285)	B (N=311)	AB (N=91)	
Pregnancy					
Positive	183 (48.7)	131 (46.0)	130 (41.8)	44 (48.4)	
Negative	193 (51.3)	154 (54.0)	181 (58.2)	47 (51.6)	0.3
FSH					
> 10mlIU/ml	47 (12.6)	48 (17.0)	36 (11.6)	18 (20.2)	
< 10mIU/ml	327 (87.4)	235 (83.0)	275 (88.4)	71 (79.8)	
Total	374 (100.0)	283 (100%)	311 (100%)	89 (100%)	0.04
AFC (N-1063)					
< 5	41 (10.9)	36 (12.6)	40 (12.9)	20 (22.0)	
⩾ 5	335 (89.1)	249 (87.4)	271 (87.1)	71 (78)	0.03
Diminished OR groups					
AFC < 5 & FSH > 10mlU	14 (4.5)	20 (8.4)	16 (6.0)	9 (12.9)	
AFC > 5 & FSH < 10mIU	300 (95.5)	219 (91.6)	251 (94.0)	61 (87.1)	
Total	314 (100.0)	239 (100.0)	276 (100.0)	70 (100.0)	0.04
AMH (N-1040)					
<1.5 pg/ml	77 (20.9)	66 (23.7)	82 (26.8)	25 (28.7)	
⩾ 1.5 pg/ml	291 (79.1)	213 (76.3)	224 (73.2)	62 (71.3)	
Total	368 (100.0)	279 (100.0)	306 (100.0)	87 (100.0)	0.2

Table-4 shows the association between Rh factor with pregnancy, AFC, AMH, and FSH. There was no significant association between Rh and all other study variables. As well, there was no association of blood groups with AFC and FSH when comparing women with diminished AFC (AFC < 5 & FSH > 10mlU/ml) to those with normal OR (AFC > 5 & FSH < 10mlU/ml).

Table-4: Association between Rh factor with pregnancy and other study markers)

Study variables	Rh factor		Odds ratio	95% CI	P-value
	RH $^{+ve}$	RH $^{-ve}$			
Pregnancy					
Positive	445 (45.5)	43 (50.0)			
Negative	532 (54.5)	43 (50.0)			
Total	987 (100%)	86 (100%)	1.098	0.716-1.201	0.4
FSH (mIU/ml)					
>10	136 (14.0)	13 (15.3)			
⩽ 10	836 (86.0)	72 (84.7)			
Total	972 (100.0)	85 (100.0)	0.901	0.486-1.671	0.7

Study variables	Rh factor		Odds ratio	95% CI	P-value
	RH $^{+ve}$	RH $^{-ve}$			
AFC					
< 5	128 (13.1)	9 (10.5)			
>5	849 (86.9)	77 (89.5)			
Total	977 (100.0)	86 (100.0)	1.29	0.631-2.637	0.5
Diminished OR groups					
AFC < 5 & FSH > 10mIU/ml	56 (6.8)	3 (4.3)			
AFC > 5 & FSH < 10Miu/ml	765 (93.2)	66 (95.7)			
Total	821 (100.0)	69 (100.0)	1.61	0.491-5.285	0.6
AMH (pg/ml)					
< 1.5	230 (24.1)	20 (23.5)			
⩾ 1.5	725 (75.9)	65 (76.5)			
Total	955 (100.0)	85 (100.0)	0.998	0.911-3.021	0.9

Table 5 shows the association between blood groups and FSH [>10 and HSH < 10 (mIU/ml)]. There were non-significant differences in the association of FSH among any two blood ABO groups.

Table 5: Association between blood group and FSH levels (>10 mIU/ml and <10 mIU/ml)

FSH (mIU/ml)	Blood group		Odds ratio	95% CI	P-value
	A (N=283)	O (N=374)			
>10	48 (17.0)	47 (12.6)	1.421	0.919-2.197	0.1
⩽ 10	235 (83.0)	327 (87.4)			
	AB (N=89)	**O (N=374)**			
>10	18 (20.2)	47 (12.6)	1.764	0.967-3.217	0.06
⩽ 10	71 (79.8)	327 (87.4)			
	B (N=311)	**O (N=374)**			
>10	36 (11.6)	47 (12.6)	0.911	0.573-1.447	0.7
⩽ 10	275 (88.4)	327 (87.4)			
	B (N=311)	**A (N=283)**			
>10	36 (11.6)	48 (17.0)	0.641	0.402-1.021	0.06
⩽ 10	275 (88.4)	235 (83.0)			

Discussion

To our best awareness, this was the largest cohort investigates the association of ABO blood groups, Rh factor with OR and IVF outcomes in Iraq. On the basis of the evidence presented by this single-center retrospective observational study, it seems fair to claim that blood groups (but not Rh factor) are associated with the OR evaluated by AFC, FSH levels. However, the current study exhibited that blood groups and Rh factor are not predictive of the IVF outcomes.

There has been an inconclusive debate about whether OR is differ among the blood types in women or not. There is an association between ABO grouping and incidence of diminished OR in women. Women with O group less expected to have diminished OR, while those with blood group B or AB were more expected to have diminished OR. Blood group A was not associated with diminished OR (Lin et al., 2014). Nejat et al. (2011) concluded that group A blood antigen is a protective for OR, however blood group O was

related with diminished OR (Nejat et al., 2011).

As a rebuttal to this opinion, it might be claimed that more than few scholars have suggested no association between blood types and OR. In recent times, no differences in the rate of diminished OR between ABO groups were observed by two Chinese systematic review and meta-analyses five years apart (Zhao et al., 2021) and (Deng et al., 2017). Turkish article published three years ago revealed that women' blood group have no any impact on OR. Moreover, neither ABO group nor Rh-factor have an influence on the predictive IVF results (Kan et al., 2019). Awartani et al. (2016) revealed a non-significant association between blood groups and OR or IVF outcomes in women attended the "King Faisal Specialist Hospital and Research Center", Saudi Arabia (Awartani et al., 2016).

The specialists may investigate whether these inconsistent outcomes were a result of deviations in population size, race, design of these studies, ethnicity, RO biomarker, or whether they were only a statistical finding.

In this study, the frequency of blood groups was analogous to that reported in a prior Iraqi (Al Mahfooth, 2019) and Turkish study (Sengül et al., 2014). As well a larger earlier Saudi cohort included more than 57000 patients, revealed frequency of blood groups similar to the outcomes of this study (Bashwari et al., 2001). Two decades late, other Saudi study confirm the same findings (Awartani et al., 2016). Thus, these records further support the former data in various countries about the lack of association between ABO groups and infertility. Timberlake et al. also published similar outcomes, and found that neither OR nor the AECs recovered in the IVF cycle were linked with ABO groups (Timberlake et al., 2013).

The chromosome 9q34 coding the gene of ABO blood group and comprises three alleles: A, B, and O alleles (Yazer, 2005). Both A and B (but not O) alleles encode transferase enzyme that catalyzes transferring of sugars to H antigen, in that way forming A or B red blood cell antigens, while the H antigen of O allele remains unaffected on the red blood cell (Palcic, Seto, & Hindsgaul, 2001). The likely explanations of blood group association with OR were suggested as following:

(1) The glycosylated polypeptide receptors of FSH and LH are vital for ovary follicular maturation. The bioactivities of the two hormones could be changed by the effect of transferase enzyme encoded by the O allele (Palcic et al., 2001). As well, transferase could alter the bioactivity and half-life of LH (Zhao et al., 2021).

(2) Genes related with ovarian biology situated adjacent to the ABO locus, like nuclear receptor 5A1 (Lourenço et al., 2009). As well, "transforming growth factor beta (TGF-β)" that is a pleiotropic cytokines with multicellular functions (Dleikh et al., 2020; Fouad Shareef Dleikh, 2020; Mousa, Al Saffar, & Al-Hindy, 2020), the genes encoding the TGF-β receptor is also situated near the ABO locus (Lourenço et al., 2009). Hence, such genes besides ABO genes might recombine and be inherited genetically along with ABO blood groups (Zhao et al., 2021).

(3) Lastly, other inherent factors like polymorphism of FSH receptors (Huang, Cao, & Shi, 2019) and the "fragile X mental retardation-1 gene" (Peprah, 2014) are linked with high FSH levels and ovarian insufficiency. Current wide association genome studies have detected around twenty loci linked with menopause (Wood & Rajkovic, 2013).

(4) If there is a diminished OR caused by any disordered gene and is associated with the locus of blood ABO group, it is expected to be younger than this locus. Hence, in case of mutation, though it can be dispersed in some blood subgroups, it will not be distributed on entire carriers of group O antigen. Consequently, every relationship between ABO groups and OR can be ignored.

Normal OR is expected to show a great response to hyper-ovulation that may develop to ovarian hyperstimulation syndrome (OHSS) (Binder, Flegel, Emran, Müller, Cupisti, et al., 2008). Women with group A are more prone to progress to early-onset-OHSS, while blood group O develop lower rate of early-onset OHSS. This finding caused by the 25% lower blood concentrations of factor VIII and Von Willebrand factor in blood O group compared with the group A persons (O'Donnell & Laffan, 2001).

Vascular endothelium dysfunction rises the capillary permeability and cause changes in the hemo-physiology (Soares et al., 2008). Based on reports, in various clinical conditions like OHSS, Von Willebrand factor concentrations are raised associated with endothelial cell dysfunction (Zhao et al., 2021).

Concerning the link between the blood types and gestational outcomes, the preceding publications have showed that some ABO types has less chance to complete a positive gestation. As stated, the blood types is the chief contributor of Von Willebrand factor and factor VIII blood concentrations. As well, the hemoglobin and factor VIII values rise in persons with non-O blood types (Schleef et al., 2005). Of late, one scholar anticipated a "ADAMTS13-VWF pathway" to has a crucial contribution in normal gestation and pathophysiology of pre eclampsia (Xiao et al., 2017). Several revisions have as well exposed that some polymorphisms of ABO genes are related with high concentrations of some immunomediators (Qi et al., 2010), which are linked with initial embryo and later placental implanting (Staun-Ram & Shalev, 2005). Hence, it is rational to consider that the inflammatory background related with certain ABO types may disturb the embryo implantation and/or growth during IVF (Zhao et al., 2021).

In our study, no latent racial variations in our outcomes as all women were Iraqis, from the same geographic zone. The demographical variables were comparable between blood ABO types. Mean ages, BMI measures, duration of infertility between ABO types were analogous. ABO group and Rh subgrouping ratios between FSH ⩽10 mIU/mL and FSH >10 mIU/mL classes were nearby. AMH was evaluated to indicate diminished OR in a < 1.5 pg/ml was considered DOR (Binder, Flegel, Emran, Müller, Dittrich, et al., 2008), which revealed non-significant differences in the OR among blood groups and Rh factor.

A recording of several parameters were strength points in this study besides a good sample size, that evidently impact ovarian function, like timing of blood assays, type of infertility, measurement of AMH, and exclusion criteria that including: previous cytotoxic or radiotherapy, any ovarian surgery, any ovarian diseases (endometriosis, infections). These collectively give a strong statistical power to this study and decrease the influence of the confounders, which were not overcome by several prior studies. ABO groups may or may not be related with some traits of female reproductive physiology. This study involved only infertile patients, restricting the validity of its outcomes to the general population. The study was single-center, which was the second potential limitation of similar studies. Nevertheless, additional studies are clearly desired to ratify this association and to clarify the principal pathways. The evidence obtainable so far is not satisfactory to label ABO groups as a risk factor for OR in clinical field.

In conclusion

The existing study concluded that ABO groups and Rh factor are associated with the reduced OR. The outcomes of pregnancy after IVF are not influenced by blood types. Excessive focusing on blood groups is not essential during management of infertile women and assisted reproductive techniques. Additional well-designed prospective researches are desirable to ratify the associations between ABO grouping and/or Rh factor and OR in women.

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