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Association of ABO blood groups and ART outcomes among subfertile South Asian women: a retrospective cohort study

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Abstract

Background: Blood groups are expressed on the surface of the red cells, and their association with the ovarian reserve and pregnancy outcomes has been an area of interest. The aim of the current study is to study the association of blood groups with live birth rates among South Asian women undergoing assisted reproductive technology treatment. It is a retrospective cohort analysis of women undergoing assisted reproductive technology (ART) at Christian Medical College and Hospital, India, between January 2007 and June 2017. All women ≤ 40 years undergoing the first ART cycle with fresh embryo transfer were included and stratified into four groups (A, B, AB, and O) based on the blood group type. The ART outcomes were analyzed among the groups.

Results: A total of 2524 women underwent fresh embryo transfer cycles during the study period, among whom 2079 women were analyzed. There was no statistically significant difference in the live birth rates for women with blood group B (odds ratio, OR 0.96, confidence interval, CI, 95% 0.74–1.24), blood group AB (OR 0.89, 95% CI 0.58–1.35), and blood group O (OR 0.87, 95% CI 0.68–1.12) with blood group A as the reference. After adjusting for important confounders, there was no statistically significant difference in the live birth rates for women with blood group B, AB, and O in comparison with blood group A.

Conclusion: The current study showed no association of blood groups with the ART treatment outcomes in South Asian women.

Keywords: ART outcomes, Blood groups, Live birth rates, Pregnancy outcomes, South Asian women

Background

The uptake of assisted reproductive technology (ART) treatment has risen steadily over the past three decades [1]. This is reflected in an increase in the number of ART cycles being performed worldwide with currently over eight million children born and over 2.5 million treatment cycles being performed every year [2]. However, over the years, live birth rate (LBR) following fresh autologous ART cycles has remained low at around 22 to 25% per initiated cycle [3]. Across the world, the clinical researchers have made considerable efforts to identify the various factors affecting the ART success which may help in prognostication and improve the treatment outcomes. Among those, one of the less studied factors is the association of blood groups with ART outcomes. Earlier studies have suggested a link between blood groups and reproductive function, and therefore, it is of relevance in fertility treatment [4–6].

The ABO blood type system is a characterization of the human blood group antigens that are expressed on the surface of red blood cells as well as other human cell

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types including epithelium. The alleles or genes related to ovarian reserve are inherited with ABO, and the blood type may probably have an association with ovarian reserve [4]. Blood group A transferase appears to protect against diminished ovarian reserve (DOR) whereas the absence of A transferase activity in blood type O may be detrimental to the ovarian reserve [7]. Blood type polymorphisms may affect the expression of immune mediators which play a role in embryo implantation as well as subsequent placentation [8, 9]. Therefore, the immunological or inflammatory mediators associated with certain ABO blood types could affect the early implantation and subsequent growth of embryos during in vitro fertilization (IVF) and hence can affect treatment outcomes [10, 11].

A number of studies have analyzed a relationship between ABO blood type and infertility with conflicting results. Few studies have suggested an association between ABO blood group type and ovarian reserve which is one of the important predictors of ART outcomes [4, 12, 13]. Nejat et al., found that blood group O was associated with DOR, while blood group A is protective. Deng et al., in their systematic review, noted no association between blood groups and ovarian reserve [4, 13]. However, Pereira et al., looked further at the association between blood group type and LBR in ART and found no significant association [14], while Goldslammer et al., found that blood group B was associated with increased likelihood of live birth [5].

Furthermore, it has been suggested that South Asian ethnicity is linked with poorer outcomes following ART compared with Caucasian women [15]. Therefore, we planned a study to explore the association between ABO blood group and ART treatment outcomes in our population.

Materials and methods

Study population

The current study was a retrospective cohort analysis of women underwent ART at Christian Medical College, India, between January 2007 and June 2017. The study protocol was approved by the Institutional Review Board (IRB No 11737). The study was conducted in accordance with the principles laid down in the declaration of Helsinki. Only data from those women who allowed the use of anonymous data for retrospective studies and gave written informed consent were included in the current study. The details of ART and other clinical data were obtained from the department's electronic medical records. All women ≤ 40 years undergoing first autologous ART cycle with fresh embryo transfer were included. The frozen embryo transfer cycles, cycles canceled before oocyte retrieval, and the women undergoing repeat IVF cycles during the same time period were excluded from the analysis. Each participant was included only once in the analysis.

The blood groups of the women were obtained from the hospital records, the women were divided into four groups (A, B, O, and AB groups), and the outcomes were assessed according to the groups. Both Rh-positive and Rh-negative groups were included among the ABO groups.

ART protocol

Ovarian stimulation, ovulation trigger, oocyte retrieval, embryo culture, and embryo transfer were carried out based on established protocols and department policy. Gonadotropin dose was individualized according to the age, body mass index (BMI), ovarian reserve, and previous cycle response. The commonly used ART protocols were gonadotropin-releasing hormone (GnRH) antagonist (flexible), long agonist (luteal phase), short/flare, and ultralong protocols. In the flexible antagonist protocol, the antagonist (Ganirelix, Ferring pharmaceuticals, USA or Cetrorelix, Merck Serono, Netherlands) was administered from the day when the lead follicle reached a diameter of 12–13 mm size. In the long GnRH agonist protocol, GnRH agonist (Lupride acetate, Sun pharmaceuticals, India) was started from the luteal phase of the previous cycle and was continued until the day of trigger. In the ultralong protocol, downregulation was achieved using between two to three doses of GnRH depot preparation (Lupride depot 3.75 mg, Sun pharmaceuticals, India). In the agonist flare protocol, the agonist was initiated on the first day of the cycle and continued until the day of trigger. Controlled ovarian hyperstimulation was started using recombinant gonadotrophin (Recagon, Merck Sharp &Dohme, New Jersey, USA, and Gonal-F, Merck Serono, Switzerland), and follicular monitoring was done by transvaginal ultrasonography (TVS), when three lead follicles reached ≥ 17 mm diameter final oocyte maturation was triggered with urinary 5000 IU (Koragon Ferring pharmaceuticals, USA; Pregnyl—Organon, India) or recombinant human chorionic gonadotropin (hCG) 250mcg (Ovitrelle -Merck Serono, Switzerland) or GnRH agonist trigger 2 mg subcutaneously (Leuprolide Acetate, Luprolin 4, Intas). Oocyte retrieval was performed 35–36 h after the trigger under ultrasound guidance, and based on the semen sample and the couple's reproductive history, fertilization was carried out with either conventional in vitro insemination or intracytoplasmic sperm injection (ICSI) according to the existing lab protocols. The embryo transfer was done at either cleavage stage or blastocyst stage, and between one to three embryos were transferred depending upon the age, previous unsuccessful attempts, and day of embryo transfer.

Luteal support was initiated on the day of oocyte retrieval with progesterone vaginal suppository 400 mg (Naturogest, Zydus healthcare limited, India) twice daily and parenteral progesterone (Gestone, Ferring pharmaceuticals, Switzerland) 100 mg twice weekly, until the pregnancy test (18 days after oocyte retrieval). The pregnancy outcomes were followed up through hospital records, e-mails, and telephonic communication.

Outcomes

The primary outcome was "live birth," defined as a fetus showing any sign of life, beyond 22 completed weeks of gestational age. The live birth rate was expressed per embryo transfer. The secondary outcomes were clinical pregnancy rate, defined as pregnancy (diagnosed by ultrasonographic visualization of one or more gestational sacs) expressed per embryo transfer. In addition to intrauterine pregnancy, it includes a clinically documented ectopic pregnancy. Multiple pregnancy rate is defined as more than one gestational sac on ultrasonography, expressed per clinical pregnancy. The "miscarriage" was defined as the spontaneous loss of a pregnancy before 22 completed weeks of gestational age, expressed as miscarriage per clinical pregnancy [16].

Statistical analysis

Sample size calculation was not done as it was a retrospective study. Data was summarized using mean ± standard deviation (SD)/median (inter quartile range, IQR) for continuous variables depending on normality. Categorical variables were presented as frequency and percentage. Comparisons between more than two groups for continuous and categorical outcome variables were analyzed using one-way analysis of variance (ANOVA) or Kruskal–Wallis test and chi-square test or Fisher's exact test, respectively. Multivariable analysis was done by binary logistic regression analysis by entering clinically important variables associated with live births and the results were expressed as odds ratio (OR) with 95% confidence interval (CI). A two-sided *P* value of less than 0.05 was considered statistically significant. Statistical analysis was done with SPSS (Ver. 21.0, IBM, USA) and STATA IC version 16.

Results

Baseline and ART characteristics

A total of 2524 women underwent fresh embryo transfer cycles during the study period at Christian Medical College and Hospital, India, from January 2007 to December 2017, and among those, 2079 women were eligible and analyzed (Fig. 1). The mean age of the overall cohort was 31.9 ± 4.4 years. The most common cause for infertility was male factor (33.5%), and GnRH antagonist protocol was the most commonly used (49.1%). The distribution of the women based on blood group is as follows: 23% (486/2079) had a blood group A, 32% (673/2079) women were with blood group B, 6.5% (136/2079) had a blood group AB, and 38% (784/2079) had blood group O.

The baseline clinical characteristics of the study participants are summarized in Table 1. No statistically significant difference was observed in the distribution of age, BMI, IVF protocol, or indication between the blood groups A, B, AB, and O groups as shown in Table 1. The ART cycle treatment and response findings are

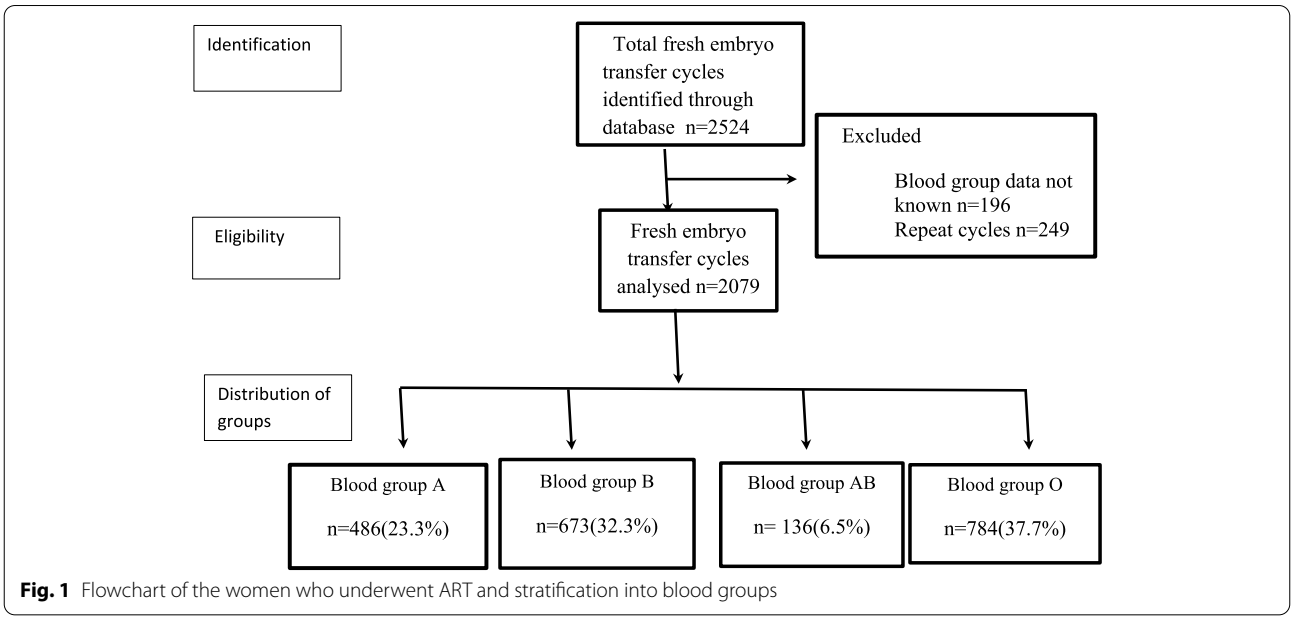

Fig. 1 Flowchart of the women who underwent ART and stratification into blood groups

Table 1 Baseline characteristics of the study groups

	A blood group *n* = 486	B blood group *n* = 673	AB blood group *n* = 136	O blood group *n* = 784	Total *n* = 2079	*P* value*
Age (years)[a]	31.9 ± 4.4	31.9 ± 4.5	31.6 ± 3.8	31.8 ± 4.5	31.9 ± 4.4	0.860
BMI (kg/m^2)[a]	25.1 ± 4.1	25.3 ± 4.2	25.9 ± 4.0	25.5 ± 4.1	25.4 ± 4.1	0.216
Infertility *n* (%)						
Primary	366 (75.3)	487 (72.4)	111 (81.6)	582 (74.2)	1546 (74.4)	0.145
Secondary	120 (24.7)	186 (27.6)	25 (18.4)	202 (25.8)	533 (25.6)	
Protocol, *n* (%)						
Antagonist	253 (52.1)	318 (47.3)	71 (52.2)	380 (48.5)	1022 (49.1)	0.378
Long	160 (32.9)	240 (35.7)	45 (33.1)	299 (38.1)	744 (35.8)	
Long depot	40 (8.2)	55 (8.2)	12 (8.8)	49 (6.3)	156 (7.5)	
Short	33 (6.8)	60 (8.9)	8 (5.9)	56 (7.1)	157 (7.6)	
Indication, *n* (%)						
Tubal	87 (17.9)	113 (16.8)	26 (19.1)	128 (16.3)	354 (17.0)	0.206
Ovulation disorder	59 (12.1)	67 (10.0)	15 (11.0)	63 (8.0)	204 (9.8)	
Endometriosis	61 (12.6)	6 1(9.1)	12 (8.8)	66 (8.4)	200 (9.6)	
Male factor	144 (29.6)	227 (33.7)	43 (31.6)	283 (36.1)	697 (33.5)	
Unexplained	39 (8.0)	70 (10.4)	10 (7.4)	73 (9.3)	192 (9.2)	
Combination	96 (19.8)	135 (20.1)	30 (22.1)	171 (21.8)	432 (20.8)	

Data expressed as number of women (percentage) for categorical variables

* One-way ANOVA for continuous variables and chi-square test for categorical variables

[a] Mean ± standard deviation

summarized in Table 2. There was no significant difference in the median gonadotropin dose and duration of stimulation among the four blood groups. The majority of embryos were transferred at the cleavage stage (83.2%) (Table 2). The ovarian response among the study groups based on the number of oocytes retrieved was analyzed, and no significant difference was noted between the study groups (Table 2).

Outcomes

The ART outcomes among the various study groups are summarized in Table 3. The overall clinical pregnancy

Table 2 ART characteristics of the study groups

	A blood group *n* = 486	B blood group *n* = 673	AB blood group *n* = 136	O blood group *n* = 784	Total *n* = 2079	*P* value*
Total Gn dose (IU)[a]	2200 (1500–3000)	2250 (1500–3300)	2100 (1587–2862)	2100 (1500–3000)	2200 (1500–3000)	0.284
Duration of stimulation (days)[a]	10 (9–11)	10 (9–12)	10 (9–11)	10 (9–11)	10 (9–11)	0.641
No of oocytes retrieved[a]	6 (3–10)	6 (4–10)	7 (4–10)	7 (4–10)	6 (4–10)	0.176
No of embryos transferred[a]	2 (2–3)	2 (2–3)	2 (2–3)	2 (2–3)	2 (2–3)	0.881
Stage of embryo transferred						
Cleavage, *n* (%)	412 (84.8)	559 (83.1)	643 (82.0)	116 (85.3)	1730 (83.2)	0.555
Blastocyst, *n* (%)	74 (15.2)	114 (16.9)	20 (14.7)	141 (18.0)	349 (16.8)	
Ovarian response, *n* (%)						
> 9 oocytes retrieved	125 (25.7)	195 (29.0)	39 (28.7)	218 (27.8)	577 (27.8)	0.375
< 4 oocytes retrieved	123 (25.3)	137 (20.4)	29 (21.3)	156 (19.9)	445 (21.4)	
4–9 oocytes retrieved	238 (49.0)	341 (50.7)	68 (50.0)	410 (52.3)	1057 (50.8)	

Data expressed as number of women (percentage) for categorical variables

Interquartile range (IQR): (25th percentile, 75th percentile)

* Kruskal–Wallis test for non-normally distributed continuous variables and chi- square test for categorical variables

[a] Median (interquartile range) for non-normally distributed continuous variables

Table 3 ART outcome characteristics

	A blood group *n* = 486	B blood group *n* = 673	AB blood group *n* = 136	O blood group *n* = 784	Total *n* = 2079	*P** value
Implantation rate (%)	29.2 [322/1099]	26.7 [406/1515]	27.7 [84/303]	24.8 [435/1750]	26.7 [1247/4667]	0.881
Clinical pregnancy rate, *n* (%)[a]	229/486 (47.1)	304/673 (45.1)	62/136 (45.5)	340/784 (43.3)	935/2079 (45.0)	0.673
Miscarriage rate, *n* (%)[b]	30/194 (15.4)	35/259 (13.5)	10/50 (20.0)	44/286 (15.5)	119/789 (15.0)	0.686
Multiple pregnancy rate, *n* (%)	91 (39.7)	98 (32.2)	20 (32.2)	105 (30.8)	314 (33.5)	0.014
Live birth rate *n* (%)[b]	161/451 (35.6)	219/628 (34.8)	41/124 (33.0)	239/730 (32.7)	660/1933 (34.1)	0.720

Data expressed as number of women (percentage) for categorical variables

* Chi-square test

[a] Clinical pregnancies 935 = 660 live births, 8 still births, 19 ectopic pregnancies, 119 miscarriages, and 146 lost to follow-up

[b] Analyzed for whom the outcome data was available (excluded *n* = 146 who were lost to follow up—A = 35, B = 45, AB = 12, O = 54)

rate of the study cohort was 935/2079 (45%), and live birth rate was 660/2079 (32%). The live birth rates stratified by the blood group types A, B, AB, and O were 161/451 (35.6%), 219/628 (34.8%), 41/124 (33.0%), and 239/730 (32.7%), respectively. The clinical pregnancy rates in blood group A 229/486 (47.1%), blood group B 304/673 (45.1%), blood group AB 62/136 (45.5%), and blood group O 340/784 (43.3%) did not show any significant difference among the groups. Similarly, the miscarriage rates were not statistically significant among the blood groups. However, the multiple pregnancy rates were significantly higher in blood group A (40%, 91/486), compared with blood group B (32%, 98/673), blood group AB (32%, 20/136), and blood group O (31%, 105/784).

Logistic regression analysis was done to observe the association of blood groups with live births with the blood group A as the reference group. The unadjusted odds of live birth for women with blood group B (odds ratio, OR 0.96, 95% confidence interval, CI 0.74–1.24), blood group AB (OR 0.89, 95% CI 0.58–1.35), and blood group O (OR 0.87, 95% CI 0.68–1.12) was not significantly different (Table 4). After adjusting for important confounders (age, BMI, indication for ART, number of oocytes retrieved, number of embryos transferred, stage of embryo transferred and cycle number), no significant association was observed between the live birth rate and the blood group B (adjusted odds ratio. aOR, 95% CI 0.94(0.72–1.22), blood group AB (aOR 0.82, 95% CI 0.64–1.07), and blood group O (aOR 0.85, 95% CI 0.55–1.32) with blood group A being the reference.

Discussion

The current retrospective study assessed the ART treatment outcomes of 2079 South Asian women. The current study results did not demonstrate any association between the various blood groups and LBR following fresh ART. No significant association was observed between blood groups and other ART outcomes such as clinical pregnancy, implantation, and miscarriage rate. However, the multiple pregnancy rate was significantly higher in women with blood group A compared with the other groups.

There are limited number of studies exploring the association the blood groups and ART outcomes, and all the published studies were from European and North American continent. The current study findings are similar to the retrospective study of 2329 cycles by Periera et al. (2017), which showed no significant association of blood groups with LBR in ART cycles following single blastocyst transfer. Though the findings are similar to

Table 4 Binary logistic regression analysis based on live birth rate

	No live birth (*n* = 1273)	Live birth (*n* = 660)	Unadjusted OR (95% CI)	*P* value	Adjusted OR[a] (95%CI)	*P* value
Blood groups						
A blood group	290 (22.8%)	161 (24.4%)	Reference			
B blood group	409 (32.1%)	219 (33.2%)	0.964 (0.749–1.242)	0.779	0.946 (0.727–1.229)	0.676
AB blood group	83 (6.5%)	41 (6.2%)	0.890 (0.584–1.355)	0.297	0.829 (0.641–1.072)	0.153
O blood group	491 (38.6%)	239 (36.2%)	0.877 (0.685–1.122)	0.586	0.856 (0.555–1.321)	0.482

[a] Age, BMI, POSEIDON groups, indication for ART, number of mature oocytes retrieved, number of embryos transferred, and stage of embryo transferred were adjusted in multivariate analysis

the current study, they selected only normoresponders and included only women undergoing single blastocyst transfer [14]. Another retrospective study by Goldslammer et al., which included 626 women, observed a higher odds of live birth following fresh transfer in the women with blood group B when compared with blood group A (aOR 2.2, 1.15–4.11) [5]. These findings are in contrast to the current study, which may be due to the smaller sample size of the study and different ethnicity compared with our population. A recent systematic review and meta-analysis (2021) by Zhao et al., which included four studies, found no difference in the LBR and CPR among the various blood groups. There were no significant differences in the miscarriage rates and ovarian reserve among the various blood groups, and these findings are similar to the current study results [17]. The association of blood group type with ovarian reserve was studied in many studies previously, and the evidence was conflicting. A cross-sectional observational study showed that blood group A antigen appears to be protective for ovarian reserve, whereas blood group O appears to be associated with DOR, independent of advancing age based on the FSH values [4]. However, a systematic review in 2018 by Deng et al. has shown no association between the blood groups and ovarian reserve [13]. In a study by Nigel Periera which evaluated the ovarian response in women with diminished ovarian reserve, the authors did not find any difference in the ovarian response [18]. The current study also did not find any difference in oocyte yield among women with different blood groups, indirectly indicating minimal or no impact of blood group on ovarian reserve.

The strength of the current study is the large sample size. The current study included less studied population of South Asian women to explore association of blood group and ART outcome. Because ethnicity also plays a role in the blood group distribution, the current study contributes to the knowledge in this particular ethnic population. The limitation of the study is that it is a retrospective study, and although we have adjusted for many potential confounding factors in our analysis, the effect of some unknown confounders cannot be ruled out. We did not report the cumulative birth rate which is also a limitation. The cycle cancelation rate prior to oocyte pick up was not reported in our study which is another drawback.

Conclusion

The current study showed no association between the blood groups and ART treatment outcomes among sub fertile South Asian women. Overall, the study findings can be used to design more prospective studies with a large sample size comparing the cumulative live birth rates among various blood groups that could help to elucidate the association.

Abbreviations

aOR: Adjusted odds ratio; CI: Confidence interval; CPR: Clinical pregnancy rate; DOR: Diminished ovarian reserve; LBR: Live birth rate; OR: Odds ratio.

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Authors' contributions

MSK conceptualized the study design and revised the manuscript. KR analyzed and interpreted the data. PJ was a major contributor to the acquisition of data, analysis, interpretation of data, and drafting manuscript. CP and ATK critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board (IRB No 11737). Only data from those women who allowed the use of anonymous data for retrospective studies and gave written informed consent were included in the current study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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