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ASSISTED REPRODUCTION TECHNOLOGIES

Non-O blood group and outcomes of in vitro fertilization

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Abstract

Purpose Retrospective and cross-sectional studies suggested that non-O blood group may be associated with failures of in vitro fertilization (IVF), but data remain controversial. The aim of this observational cohort study was to prospectively evaluate the effect of non-O blood type on clinical outcomes of IVF.

Methods Women < 40 years who underwent IVF and had ABO blood type recorded as part of the routine workup were eligible. The primary study outcome was live birth. Secondary outcomes included spontaneous abortion, positive pregnancy test, and clinical pregnancy.

Results A total of 497 women with a mean age of 34.6 (standard deviation 3.2) years were included. The mean number of embryos transferred was 2.3 (standard deviation 0.6). The most common ABO blood types were O ($n = 213$, 42.9%) and A ($n = 203$, 40.8%), while 63 (12.7%) and 18 (3.6%) women had the B and AB blood types, respectively. Differences in live birth (21.8 vs. 24.3%, odds ratio [OR] 1.17; 95% confidence intervals [CI], 0.76 to 1.78), positive pregnancy test (37.9 vs. 36.6%, OR 0.96; 95% CI, 0.66 to 1.38), clinical pregnancy (35.1 vs. 33.8%, OR 0.95; 95% CI, 0.66 to 1.39), and spontaneous abortion (12.3 vs. 9.2%, OR 0.72; 95% CI, 0.41 to 1.29) between women with O and non-O blood type were not statistically significant.

Conclusions In a prospective cohort study, we confirmed the lack of a significant association between non-O blood type and clinical outcomes of IVF. Further studies are needed to clarify whether non-O blood group has any prognostic relevance in women undergoing IVF.

Keywords ABO blood type · Thrombophilia · Assisted reproductive techniques

Introduction

Inherited and acquired thrombophilia represent well-known risk factors for venous and arterial thrombosis [1, 2]. The prothrombotic tendency associated with thrombophilia may predispose to thrombosis of vessels at the maternal–fetal interface causing implantation or placentation failures [3]. A number of studies suggested an effect of thrombophilia on the clinical outcomes of assisted reproductive techniques such as in vitro fertilization (IVF), but data remains controversial [4].

Genome-wide association studies repeatedly reported an association between ABO blood type and thrombosis with individuals of A or B blood type experiencing an increased risk of thrombotic events compared with individuals with the O blood type [3–12]. Given the higher thrombotic risk associated with the non-O blood type and its high prevalence in the general population, several authors have proposed that ABO blood type should be included in the genetic screening for thrombophilia [11, 12]. The increased thrombotic tendency among individuals with non-O blood type may be partly explained by the reduced clearance of von Willebrand factor

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resulting in higher plasma levels of von Willebrand factor and/or factor VIII compared to individuals with O blood type [13–15]. Recent observations have suggested that these effects could be relevant in normal pregnancy and in the pathogenesis of preeclampsia [16].

A number of studies early in the 1960s hypothesized that ABO blood type could have an effect on ovarian reserve, ovarian hyperstimulation syndrome, and recurrent miscarriage and represent an important determinant of female infertility [17, 18]. However, cross-sectional investigations evaluating the influence of ABO blood type on ovarian reserve generated conflicting findings [19–22]. In addition, while a retrospective analysis suggested a potential effect of blood type on the odds of live birth [23], these findings were not confirmed in another more recent retrospective study that did not observe a significant association between ABO blood type and live-birth rate [24].

The aim of the present study was to prospectively evaluate the potential association of ABO blood type and clinical outcomes in a cohort of women undergoing IVF.

Materials and methods

Study population

Prospective observational cohort study of women < 40 years who were scheduled for IVF at our University Center of Reproductive Medicine, Ortona General Hospital, Chieti, Italy, and had the ABO blood type recorded as part of the routine workup. Patients were excluded if there was an indication for anticoagulant treatment, embryo transfer was not performed, or patient did not provide informed consent. All women followed a standardized protocol of controlled ovarian stimulation using a combined regimen of recombinant hCG and GnRH antagonist. All patients underwent follicle growth monitoring, ovum pick-up, and intracytoplasmic sperm injections (ICSI) as described in a previous independent cohort [25]. Pre-implantation genetic screening was not performed. All patients included underwent embryo transfer 72–76 h after ovum pick-up. A maximum of three embryos were transferred according to national guidelines. We recorded information on demographics, comorbidities, personal obstetric history, causes of infertility, prior IVF attempts, and concomitant treatments. The local institutional review board approved the study, and all participating women signed a written informed consent before study procedures. The study is registered in clinicaltrial.gov (NCT02407730).

Study outcomes

The primary study outcome was live birth. Secondary outcomes included spontaneous abortion and clinical pregnancy. In addition, we recorded pregnancy test results and pregnancy-related complications which included ectopic pregnancy, preeclampsia, placental abruption, and intrauterine growth restriction. Pregnancy test was performed by measuring β-human chorionic gonadotropin (β-hCG) 14 days after embryo transfer. Clinical pregnancy was defined as pregnancy diagnosed initially by serum β-hCG levels with evidence of one or more gestational sacs on the transvaginal ultrasound at 6 weeks of gestation. Biochemical pregnancy was diagnosed by an initial rise followed by a decrease of β-hCG values with no ultrasonographic signs of intrauterine or extrauterine pregnancy. Spontaneous abortion was defined as fetal demise before 20 weeks of gestation. The study considered only outcomes of fresh non-donor embryo transfers.

Statistical considerations

Continuous variables were reported as mean (± standard deviations) or median (range), and categorical variables as number (percentages). We assessed differences between O blood type and non-O blood type by chi-square test, Student *t* test or Mann–Whitney *U* test as appropriate. The association between non-O blood type and study outcomes was evaluated in univariate regression analysis reporting odds ratio (OR) and the relative 95% confidence intervals (CIs). Other variables considered in the analysis were age, smoking, body mass index, cardiovascular disease, indication for IVF, number of previous cycles, history of abortion, and previous pregnancies. We performed multivariate logistic regression analysis with backward elimination at a significance level of 0.1. Explorative subgroup analysis was conducted for the primary study outcome in women with idiopathic infertility. In the absence of adequate information on the incidence of the primary outcome according to non-O blood type, a formal sample size calculation was not feasible at the time the study was designed. *P* values of 0.05 (two tailed) were considered significant. All analyses were conducted using IBM SPSS version 19 (SSPS Inc., Chicago, IL, USA).

Results

A total of 497 women with a mean age of 34.6 (± 3.2) years were included in the present analysis. The mean number of embryos transferred was 2.36 (standard deviation 0.60). Overall, 115 women (23.6%) delivered 143 live children for a live birth of 29% (143/497). One hundred and eighty-four women (37.0%) had a positive pregnancy test, 14 had a biochemical pregnancy (2.8%), 170 (34.2%) achieved a clinical pregnancy, and 52 women (9.3%) experienced spontaneous abortion, which occurred in 43 patients (82.7%) within the first 10 weeks of gestation (Table 2). The most common ABO blood types were the O blood type ($n = 213$, 42.9%) and A blood type ($n = 203$, 40.8%), while 63 (12.7%) and

18 (3.6%) women had B and AB blood type, respectively. Age, baseline, and procedural characteristics were well balanced between women with non-O and O blood types (Table 1).

Table 2 describes IVF and pregnancy outcomes according to O blood and non-O blood groups. A total of 69 women (24.3%) with a non-O blood group delivered 88 live children for a live birth of 31% (88/284). One hundred and four women (36.6%) had a positive pregnancy test, eight a biochemical pregnancy (2.8%), and 96 (33.8%) a clinical pregnancy. In the group with O blood type, 46 (21.8%) delivered a live born corresponding to a live birth of 26% (55/211). Eighty women (37.6%) obtained a positive pregnancy test, six patients had a biochemical pregnancy (2.8%), and 74 (34.7%) a clinical

Table 1 Baseline characteristics of study population

Characteristic	All *N* = 497	O blood group *N* = 213	Non-O blood group *N* = 284	*P* value
Age, years	34.6 (3.2)	34.6 (3.3)	34.5 (3.2)	0.696
Body mass index, kg/m^2	23.1 (4.1)	23.1 (4.2)	23.2 (4.0)	0.695
Smoking				0.975
Current	76 (15.3)	32 (15.0)	44 (15.5)	
Previous	11 (2.2)	5 (2.3)	6 (2.1)	
Previous venous thromboembolism	–	–	–	–
Family history positive for venous thromboembolism	4 (0.8)	2 (0.9)	2 (0.7)	0.772
Indication for IVF				0.157
Ovulatory	36 (7.2)	14 (6.6)	22 (7.7)	
Tubaric	107 (21.5)	36 (16.9)	71 (25.0)	
Endometriosis	41 (8.2)	23 (10.8)	18 (6.3)	
Male	134 (27.0)	62 (29.1)	72 (25.4)	
Idiopathic	106 (21.3)	49 (23.0)	57 (20.1)	
Uterus	11 (2.2)	3 (1.4)	8 (2.8)	
Recurrent abortion	2 (0.4)	–	2 (0.7)	
Multiple	60 (12.1)	26 (12.2)	34 (12.0)	
Previous IVF cycles				0.702
0	306 (61.6)	130 (61.0)	176 (62.0)	
1	106 (21.3)	50 (23.5)	56 (19.7)	
2	49 (9.9)	19 (8.9)	30 (10.6)	
3	26 (5.2)	12 (5.6)	14 (4.9)	
4	6 (1.2)	2 (0.9)	4 (1.4)	
5	2 (0.4)	–	2 (0.7)	
Previous pregnancies				
Spontaneous	47 (9.5)	16 (7.5)	31 (10.9)	0.205
Following ICSI	43 (8.7)	21 (9.9)	22 (7.7)	0.407
Previous ectopic pregnancy	29 (5.8)	9 (4.2)	20 (7.1)	0.338
Previous abortion				
Before week 12	22 (4.4)	10 (4.7)	12 (4.3)	0.393
After week 12	5 (1.0)	2 (0.9)	3 (1.1)	0.899
Polycystic ovarian syndrome	16 (3.2)	5 (2.3)	11 (3.9)	0.344
Total embryos transferred	2.3 (0.6)	2.4 (0.6)	2.4 (0.5)	0.148
Grade A embryos				0.299
0	29 (5.8)	10 (4.7)	19 (6.7)	
1	63 (12.7)	21 (9.9)	42 (14.8)	
2	284 (57.1)	126 (59.2)	158 (55.6)	
3	120 (24.1)	56 (26.3)	64 (22.5)	
4	1 (0.2)	–	1 (0.4)	
Grade B embryos				0.290
0	375 (75.5)	166 (77.9)	209 (73.6)	
1	85 (17.1)	34 (16.0)	51 (18.0)	
2	33 (6.6)	13 (6.1)	20 (7.0)	
3	4 (0.8)	–	4 (1.4)	
Grade C embryos				0.192
0	483 (97.2)	205 (96.2)	278 (97.9)	
1	11 (2.2)	7 (3.3)	4 (1.4)	
2	–	–	–	
3	1 (0.2)	1 (0.5)	–	

Data are reported as number of patients (%), unless otherwise indicated. *ICSI* intracytoplasmic sperm injections, *IVF* in vitro fertilization

Table 2 IVF and pregnancy outcomes according to blood group

Outcome	0 blood group $N = 213$	Non-O blood group $N = 284$	All $N = 497$
Deliveries	46 (21.6)	69 (24.3)	115 (23.6)
Singleton	38 (18.0)	54 (19.0)	92 (18.5)
Twin	7 (3.3)	14 (4.9)	21 (4.2)
Triplet	1 (0.5)	2 (0.7)	3 (0.6)
Abortion	26 (12.2)	26 (9.2)	52 (9.3)
≤ Week 10	22 (10.4)	21 (7.4)	43 (8.6)
Pregnancy test			
Negative	133 (62.4)	180 (63.4)	313 (63.0)
Positive	80 (37.6)	104 (36.6)	184 (37.0)
Clinical pregnancy	74 (34.7)	96 (33.8)	170 (34.2)
Singleton	62 (29.4)	76 (26.8)	138 (27.8)
twin	10 (4.7)	17 (6.0)	27 (5.4)
triplet	2 (0.9)	3 (1.1)	5 (1.0)
Pregnancy complications			
Preeclampsia	0	5 (1.8)	5 (1.0)
Intrauterine growth restriction	4 (1.9)	2 (0.7)	6 (1.2)
Placental abruption	1 (0.5)	1 (0.4)	2 (0.4)
Ectopic pregnancy	3 (1.4)	3 (1.1)	6 (1.2)
Venous thromboembolism	0	0	0
Lost to follow-up	2 (0.9)	0	2 (0.4)

Data are reported as number of patients (%)

pregnancy. Preeclampsia developed in five women with a non-O blood type (1.8%) and none of those with O blood type ($p = 0.052$). Intrauterine growth restriction and placental abruption occurred in similar proportions in the two groups (0.7 vs. 1.9% and 0.4 vs. 0.5%; $p = 0.235$ and $p = 0.837$, respectively).

In univariate analyses, there were no statistically significant associations between non-O blood group and clinical outcomes (Table 3). The estimates pointed in the direction of a positive association between non-O blood group and live birth, and an inverse association between the non-O blood group and abortion. Similar findings were found in women with idiopathic infertility with wide confidence intervals around the point estimates. When pooling current data with those from previous retrospective series [23, 24], the summary estimate showed no significant effect of non-O blood group on the risk of live birth (OR 1.03; 95% CI, 0.90 to 1.19), with no between study heterogeneity (Fig. 1).

In multivariate logistic regression analysis with backward elimination, previous pregnancy after ICSI was associated with live birth (OR 2.49; 95% CI, 1.18 to 5.26), positive pregnancy test (OR 2.56; 95% CI, 1.29 to 5.07), and clinical pregnancy (OR 2.70; 95% CI, 1.36 to 5.36). The number of previous IVF cycles was inversely correlated with these outcomes (OR for live birth, 0.69; 95% CI, 0.53 to 0.91; OR for positive pregnancy test, 0.79;95% CI, 0.64 to 0.98; OR for clinical pregnancy 0.78; 95% CI, 0.63 to 0.97). Higher body

Table 3 Association between blood group and clinical outcomes of in vitro fertilization

	All women			Women with idiopathic infertility		
	0 blood group $N = 213$	Non-O blood group $N = 284$	OR (95% CI)	0 blood group $N = 49$	Non-O blood group $N = 57$	OR (95% CI)
Live birth	46 (21.6%)	69 (24.3%)	1.17 (0.76 to 1.78)	11 (22.4%)	18 (31.6%)	1.59 (0.67 to 3.82)
Abortion	26 (12.2%)	26 (9.2%)	0.72 (0.41 to 1.29)	3 (6.1%)	5 (8.8%)	1.47 (0.33 to 6.51)
Clinical pregnancy	74 (34.7%)	96 (33.8%)	0.95 (0.66 to 1.39)	15 (30.6%)	23 (40.3%)	1.53 (0.68 to 3.43)
Positive pregnancy test	80 (37.6%)	104(36.6%)	0.96 (0.66 to 1.38)	15 (30.6%)	23 (40.3%)	1.53 (0.68 to 3.43)

Results from univariate analyses, depicting the odds on the event in the non-O blood group relative to the odds of the event in the O-blood group phenotype. *CI* confidence intervals, *OR* odds ratio

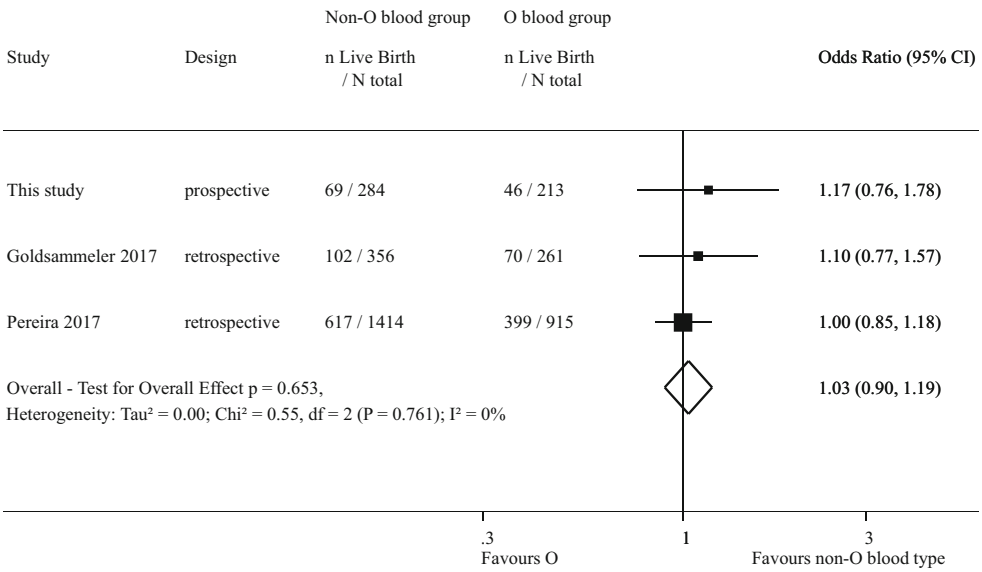

Fig. 1 Forest plot of differences in live birth among women with non-O blood type and O blood type in three cohort studies. Weights are from random-effects meta-analysis. Live birth event is expressed as odds ratio

mass index predicted a higher risk of spontaneous abortion (OR 1.10; 95%CI, 1.03 to 1.16). There was no significant association between age, smoking, cardiovascular disease, indication for IVF, history of abortion, or previous pregnancies with IVF outcomes (data not shown).

Discussion

In a prospective cohort of women undergoing IVF, we did not find a significant association between non-O blood group and clinical outcomes. Women with non-O blood type had rates of live birth, abortion, clinical pregnancy, and positive pregnancy test comparable to those of women with the O blood type.

In 1960, Behrman and colleagues suggested that ABO blood group incompatibility could be an immunologic cause of infertility [26]. However, data on the potential effect of ABO blood group on ovarian reserve have been conflicting [19–22]. Since the initial observations by Jick and colleagues in 1969, a number of studies and two meta-analyses consistently reported a higher risk of thrombosis in individuals with non-O blood phenotype [5–12]. As hypothesized for other inherited thrombophilia, non-O blood type could increase the risk of thrombosis of the vessels at the maternal-fetal interface with potential negative consequences for implantation or development of the placenta. Two recent retrospective studies evaluated the effect of ABO blood type on live birth in women undergoing IVF [23, 24]. In a series of 626 women at their first or repeated IVF attempt, Goldsammler and colleagues suggested that blood type B was associated with an increased likelihood of live birth [23]. These observations were not confirmed in a larger retrospective cohort of 2329 women < 40 years undergoing their first IVF cycle with single embryo transfer [24]. The current study provides valuable prospective confirmation of the lack of effect of non-O blood type on IVF outcomes. Pooling our results on live birth with those from earlier retrospective studies by random effects meta-analysis showed no association of non-O blood type with live birth in an overall population of 3443 women (Fig. 1). All secondary study outcomes occurred in similar proportions among women with O blood type and non-O blood type. The estimates of the associations of non-O blood type with abortion in women with idiopathic infertility were contra-intuitive and may represent a chance finding in light of the small sample size.

ABO blood group is a major determinant of von Willebrand factor and factor VIII plasma levels, which are both found increased in non-O blood type individuals [13–15]. Recently, Xiao and colleagues suggested that the ADAMTS13–von Willebrand factor pathway may influence normal placental development and be implicated in the pathogenesis of preeclampsia [16]. We observed five cases of preeclampsia in women with non-O blood type versus none in women with O-blood type, although this difference was not statistically significant. Larger studies with higher number of events are warranted to confirm these observations.

The current study has some limitations that need to be acknowledged. Although it represents the largest prospective cohort study evaluating the association between non-O blood group and IVF outcomes published so far, the difference in event rates between blood types was small and we lacked statistical power to detect relevant associations. We estimated that 6224 women should be included to detect a difference in event rates as small as the observed 3% with 80% power and a two-sided alpha of 5%, assuming a blood group type O prevalence of 40%. Our study remains a good source to inform future meta-analyses, once additional prospective studies are available and can be pooled to increase statistical power. Another potential limitation is the inclusion of a genetically

homogenous cohort, which may limit the applicability of these findings to other ethnic backgrounds. To avoid the confounding effect of age, we included women younger than 40 years and our findings, therefore, may not apply to older women in whom the role of blood group type, if any, remains unclear.

In summary, we found no significant associations between non-O blood type and clinical outcomes of IVF. Further studies are warranted to clarify whether non-O blood group holds any prognostic value in women undergoing IVF.

Authors' contribution Conception and design (MDN, EP); acquisition of data (MDN, AP, GMT, MDG); analysis and interpretation of data (MDN, AWSR, AP, GMT, MDG, EP); drafting the article or revising it critically for important intellectual content (MDN, AWSR, AP, GMT, MDG, EP); final approval of the version to be published (MDN, AWSR, AP, GMT, MDG, EP).

Compliance with ethical standards

Conflict of interest MDN has received personal fees from Daiichi-Sankyo and Bayer outside the submitted work. All other authors declare that they have no conflict of interest.

Ethical approval All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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