Implications of blood type for ovarian reserve

Edward J. Nejat1,2, Sangita Jindal1,2, Dara Berger2, Erkan Buyuk1,2, Maria Lalioti3, and Lubna Pal3,*

1Obstetrics & Gynecology and Women’s Health, Division of Reproductive Endocrinology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY 10461, USA 2Montefiore’s Institute for Reproductive Medicine and Health, Hartsdale, NY 10530, USA 3Department of Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, 333 Cedar Street, FMB329, New Haven, CT 06520, USA

*Correspondence address. Tel: +1-203-785-6161; Fax: +1-203-785-7134; E-mail: lubna.pal@yale.edu

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BACKGROUND: We explored the relevance of blood type to ovarian reserve, as reflected by early follicular phase FSH levels.

METHODS: For this cross-sectional observational study, early follicular phase serum levels of FSH (mIU/ml) and estradiol (E2, pg/ml), and information on blood type (A, B, AB and O) and patient age were procured for female patients. ≤45 years age (n = 544), who were undergoing fertility evaluation at one of two tertiary care facilities. Serum FSH > 10 mIU/ml was taken to reflect diminished ovarian reserve (DOR). Data distribution for FSH and age was analyzed and non-parametric tests used for comparisons across blood groups. Multivariable logistic regression analyses determined the relationship between elevated FSH and respective blood types after adjusting for age and study site.

RESULTS: Prevalence of blood types according to order of frequency was: O (45%), A (35%), B (16%) and AB (5%). After adjusting for age and study site, patients with blood type O were twice as likely to exhibit FSH > 10 mIU/ml compared with those with A or AB blood types [odds ratio (OR) 2.36, 95% confidence interval (CI) 1.27–4.41; P = 0.007], and three times as likely to manifest FSH > 12m IU/ml (OR 3.48, 95% CI 1.46–7.32, P = 0.004). The B blood group antigen failed to exhibit any relationship with ovarian reserve as reflected by baseline FSH (P > 0.05).

CONCLUSIONS: The A blood group antigen appears to be protective for ovarian reserve, whereas blood type O appears to be associated with DOR, in a relationship that is independent of advancing age. Further studies are needed to establish causality and identify the underlying mechanisms for the association.

Key words: infertility / diminished ovarian reserve / blood type / FSH

Introduction

The concept ‘ovarian reserve’ reflects the quantity, and possibly the quality, of residual oocytes available for procreation (Navot et al., 1987; Scott et al., 1989; Toner et al., 1991). While numerous surrogate markers are recognized to reflect ovarian reserve, female age remains the strongest predictor of reproductive success in couples undergoing fertility treatment (Rosenwaks et al., 1995; Scott et al., 1995; Sharara et al., 1998; Levi et al., 2001). Despite accruing evidence identifying anti-Müllerian hormone and antral follicle count as sensitive prognostic markers of ovarian reserve, early follicular phase serum level of FSH appears to be the most commonly utilized parameter for assessment of ovarian reserve in clinical practice (Broekmans et al., 2006). A serum FSH level >10 mIU/ml is a commonly utilized threshold to identify women at risk for suboptimal quantitative response to ovarian stimulation and poor reproductive success, an entity alluded to as diminished ovarian reserve (DOR; Greenseid et al., 2009; Thum et al., 2009; Hurwitz et al., 2010). Our prior work has focused on elucidating mechanisms that may underlie DOR as well as assessing broader implications of DOR (Greenseid et al., 2009).

Of interest is a recent report relating blood group antigens and ovarian hyperstimulation syndrome (OHSS; Binder et al., 2008a,b). The authors observed that OHSS was more likely during the course of ovarian stimulation in infertile women with blood type A compared to those with blood type O. Bellver et al. (2010), however, failed to observe a similar relationship between blood type and likelihood of OHSS in their series. Historically, researchers have not found an association between ABO blood group and fertility, although decreased reproductive success in women exhibiting ABO incompatibility with their male partners has been reported (Behrman et al., 1960; Chakravartti and Chakravartti, 1978; Satyanarayana et al., 1978). We herein explore the relationship between ABO blood type and ovarian reserve. Our findings suggest that blood type O may relate to an increased likelihood for DOR, and in contrast, the A antigen may be protective for ovarian reserve.
Materials and Methods

Patients attending the Yale Fertility Center, New Haven, CT, USA (site 1) and Montefiore’s Institute for Reproductive Medicine and Health, Hartsdale, NY, USA (site 2) for infertility-related evaluation and care were included in this study. Patients had screening blood tests at local branches of Quest Diagnostics, USA, between January 2007 and December 2008. The study was approved by the Institutional Review Boards of Yale University School of Medicine and Montefiore Medical Center. Data for women ≥45 years of age who had baseline ovarian reserve testing performed at the local Quest Diagnostics during the study period were procured. The laboratory provided information on patient age, serum FSH (mIU/ml) and estradiol (E2, pg/ml) levels and blood type (A, B, AB or O). Serum FSH was analyzed by the reference laboratory using commercial immunosassays [Immulite, Siemens, Deerfield, IL, USA; intra-assay coefficient of variation (CV) 2.9% and inter-assay CV 4.1% for FSH]. FSH > 10 mIU/ml was taken to reflect DOR as per clinical practice guidelines for each participating study site. Given that information on cycle day of blood testing was not available, the analyses were restricted to FSH values with concomitant E2 level < 80 pg/ml to ensure that the data reflected early follicular phase hormone levels.

Statistical analysis

The distribution of continuous data was evaluated by Shapiro–Wilk normality test. Given the skewed distribution for FSH, non-parametric tests were employed to assess the relationship between FSH and ABO blood types (Kruskal–Wallis rank test and Mann–Whitney U-test). Relationships between categorical variables (DOR and blood type) were assessed using $\chi^2$ test. Multivariable logistic regression analyses determined the relationship between elevated FSH with blood type O, and with A antigen bearing blood types (A or AB) after adjusting for patient age, study site and blood type B. Sensitivity analyses utilized higher FSH thresholds (> 12 IU/ml) to explore the association between DOR and blood type. Goodness of fit for the respective models was assessed using Hosmer–Lemeshow test (Archer and Lemeshow, 2006). The likelihood of association between DOR and various evaluated parameters is reported as odds ratios (ORs) ± 95% confidence interval (95% CI), STATICA IC 10 (StataCorp, College Station, TX, USA) was used for statistical analyses and two-tailed $P < 0.05$ was considered to be statistically significant.

Results

Continuous data are presented as median (inter-quartile range, IQR), and categorical data are presented as percentage (%). Informative data were available for 544 women ages ≥45 (median age 35, IQR 30–39). Prevalence of blood types according to the order of frequency is presented in Table I. Participants’ ages, ovarian reserve parameters and representation of blood types O, A and AB were comparable between the two study sites ($P > 0.20$); blood type B, however, was more commonly encountered in participants enrolled at study site 2 (18 versus 13%, $P = 0.07$).

A relationship between blood types O and blood groups bearing the A antigen (blood types A or AB) with ovarian reserve parameters was observed (Table II). Univariate analyses identified a significantly higher representation of blood type O among patients with FSH > 10 mIU/ml (OR 2.14, 95% CI 1.22–3.80, $P = 0.004$); this association was further magnified for those with FSH > 12 mIU/ml (OR 2.44, 95% CI 1.22–5.04, $P = 0.006$). Conversely, a significantly higher representation of the A antigen (blood types A or AB) was observed among those with normal ovarian reserve, i.e. FSH ≤ 10 mIU/ml, compared with those with DOR (41 versus 26%, $P = 0.02$).

After adjusting for age, blood type B and study site, patients with blood type O were twice as likely to exhibit FSH > 10 mIU/ml compared with those with A bearing blood types (either A or AB; Table III) and three times as likely to manifest FSH > 12 mIU/ml (OR 3.48, 95% CI 1.46–7.32, $P = 0.004$). Goodness of fit for the adjusted logistic regression models was 0.74 and 0.78 for FSH thresholds of 10 and 12, respectively. Conversely, women with the A blood group antigen (blood types A or AB) were significantly less likely to manifest FSH > 10 mIU/ml compared with those with blood type O (Table III). The B blood group antigen failed to exhibit any relationship with ovarian reserve as reflected by baseline FSH (either taken as a continuous variable or categorized based on specified thresholds, $P > 0.05$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n = 544</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35 (30–39)</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.8 (4.5–7.5)</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>31 (31–46)*</td>
</tr>
<tr>
<td>FSH &gt; 10 mIU/ml</td>
<td>65 (12%)</td>
</tr>
<tr>
<td>FSH &gt; 12 mIU/ml</td>
<td>43 (8%)</td>
</tr>
</tbody>
</table>

Continuous data are presented as median (IQR) and categorical data are presented as n (%). *While serum E2 levels were assessed for all participants for eligibility, descriptive data on serum E2 levels are available for data collected at site 2. **FSH > 10 mIU/ml is reflective of DOR. Sensitivity analyses utilized FSH cutoff of 12 mIU/ml.

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Age (years)</th>
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<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>205 (43%)</td>
<td>B</td>
<td>77 (16%)</td>
</tr>
<tr>
<td>A and AB</td>
<td>197 (41%)</td>
<td>AB</td>
<td>25 (4.59%)</td>
</tr>
<tr>
<td>B</td>
<td>85 (15.63%)</td>
<td>AB</td>
<td>25 (4.59%)</td>
</tr>
<tr>
<td>A</td>
<td>189 (34.74%)</td>
<td>A</td>
<td>173 (36%)</td>
</tr>
<tr>
<td>O</td>
<td>205 (43%)</td>
<td>O</td>
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</tr>
<tr>
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</tr>
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</table>

Table I Population characteristics from site 1 and site 2.

Table II Blood type distribution and participant characteristics according to ovarian reserve status.
Our findings relate blood group antigens with biochemical evidence of DOR in women seeking fertility treatment. We demonstrate that blood type O relates to biochemical evidence of DOR, a relationship that is independent of age. Women with blood type O were observed to be twice as likely to have elevated baseline FSH levels compared with those with blood types A or AB. We additionally observed that the A blood group antigen (blood types A or AB) was associated with a reduced likelihood for DOR, an association independent of age. Our findings relating blood type in infertile women to ovarian reserve correspond with a study by Binder et al., who reported OHSS was more likely to be observed during the course of ovarian stimulation in infertile women with blood type A. Our observation that the A antigen relates to reduced likelihood for DOR is thus in agreement with Binder et al., given that the authors describe blood type A as being prognostic of a robust ovarian response, i.e. a parameter considered as diametrically opposite to DOR.

The ABO gene locus has three main allelic forms: A, B and O. The H antigen is an essential precursor to the ABO blood group antigens. The gene products of the A and B alleles are glycosyltransferases that catalyze the transfer of carbohydrates to the H antigen, forming the A and B antigens, respectively (Yamamoto et al., 1990; Palcic et al., 2001). The A allele encodes for a glycosyltransferase (A transferase) that catalyzes the transfer of N-acetylgalactosamine to the H antigen, producing the A antigen. Similarly, the B allele encodes for a glycosyltransferase (B transferase) that catalyzes the transfer of galactose to the H antigen, producing the B antigen. The O allele has a single base deletion, 258-guanine, in the coding region close to the N terminus of the protein. The deletion shifts the reading frame, resulting in translation of an entirely different protein. The product of the O allele is an enzymatically inactive protein leaving the H antigen unchanged on the red blood cells of those with blood type O (Yamamoto et al., 1990). Though traditionally considered red blood cell antigens, the A and B antigens can be found on the cell membranes of a variety of cell types, including epithelial cells (Mandel et al., 1990). It is unknown whether the H antigen is present in ovarian cells.

Considering the protective effect blood group A transferase appears to exhibit against DOR, one may speculate that this enzyme may be relevant to processes of gamete accrual and/or attrition, and the absence of A transferase activity, as in the case of blood type O, may be detrimental to these processes. Several proteins crucial for follicle development and maturation, such as FSH-receptor (FSH-R) and LH-receptor (LH-R) are heavily glycosylated proteins. Of interest in this context are the observed decreased rates of ovulation in mice with oocyte-specific deletion of a glycosyltransferase, N-acetylgalcosaminyltransferase 1 (Williams and Stanley, 2009).

Another possible explanation for the observed relationship between blood type and ovarian reserve may include genetic inheritance. Specific genes (known or predicted from the genome sequence) relevant to ovarian reserve may be linked with the ABO gene, which is located on chromosome 9 (9q34). Nearby unknown variants close to ABO and associated with ovarian function could theoretically explain the observed correlation with blood type. For example, if alleles or genes related to ovarian reserve are inherited with ABO, this may potentially explain how blood type may predict ovarian reserve, as suggested in our study. Notable is a single candidate gene, NR5A1, which is in proximity to the ABO locus and recognized as relevant to ovarian reserve (Lourenco et al., 2009). However, the recombination distance between NR5A1 and ABO loci (NR5A1 is located 9 Mb and 14.7 cM proximal to the ABO locus, Genome Reference Consortium Assembly GRCh37/hg19) allows for a relatively high probability of recombination between NR5A1 and blood type genes (14% chance of recombination per meiosis), rendering linkage disequilibrium as an unlikely phenomenon for explaining the observed relationship of FSH level with blood type O. An alternative explanation for the observed associations may lie within DNA variation in haplotype that can alter the folding and stability of a protein and thereby allow certain allele combinations to be more commonly inherited together (Clark, 2004; Schaid, 2004). Haplotype variations are impacted by ethnicities however, and hence ABO blood types and ovarian reserve need to be evaluated within specific populations for informative linkage and recombination analyses.

Our study design limits exploration of parameters that may relate to elevated FSH, such as smoking history, prior pelvic surgery or fragile X permutation carriage (El-Nemr et al., 1998; Wittenberger et al., 2007; Padhy et al., 2010). Given the study population, being exclusively comprised of infertile women, our observations may not be generalized to the female population at large. Racial variations in blood type are recognized (Mourant et al., 1976; Garratty et al., 2004) as are racial variations in ovarian reserve parameters (Randolph et al., 2004). Although we did not collect information regarding the race and ethnicity for the study population, we have attempted to at least partly address this latter concern by including patients from two study sites, thereby allowing a broader ethnic and racial representation. The lacking information relating to the exact timing of serum sampling in relation to the menstrual cycle also introduces a potential for bias resulting from spurious ‘normalization’ of FSH values for samples collected beyond the early follicular phase of the cycle. However, we have attempted to minimize such an occurrence by restricting analyses to FSH data where the concomitant E2 level was <80 pg/ml. Given the known inter-cycle variability in early follicular phase FSH, our strategy to diagnose DOR based on a single FSH value may have yielded ‘false reassurance’ regarding ovarian reserve status in some participants. Ovarian reserve parameters are recognized to be influenced by the body mass (Freeman et al., 2007; Binder et al., 2008a) and

### Table III Multivariable logistic regression analyses demonstrating association between blood type and DOR.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.19 (1.12–1.17)</td>
<td>1.19 (1.12–1.27)</td>
</tr>
<tr>
<td>Blood type O&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.24 (1.26–3.6)</td>
<td>2.36 (1.27–4.41)</td>
</tr>
<tr>
<td>A antigen bearing blood type&lt;sup&gt;b&lt;/sup&gt; (A or AB)</td>
<td>0.51 (0.28–0.91)</td>
<td>0.42 (0.23–0.79)</td>
</tr>
</tbody>
</table>

Outcome: FSH > 10 mIU/ml.
<sup>a</sup>Adjustment variables: age, blood type and study site.
<sup>b</sup>A antigen bearing blood types (A or AB) taken as reference group.
this parameter is similarly unavailable for the study population. Despite the identified limitations, the relatively large sample size, the adjusted analytic approach and the consistency in our observations to findings reported by Binder et al., add credence to our work.

In summary, our findings suggest that in subfertile women, the A blood group antigen is protective against DOR while blood type O relates to an increased likelihood of DOR. While the exact mechanisms that tie blood type to ovarian reserve are unclear at this juncture, the possibilities that glycosyltransferases may play an important role in ovarian function, or that the presence of a gene that co-segregates with blood group antigens and may be associated with DOR, are both enticing and constitute an area of further investigation.

**Authors’ roles**

L.P., S.J. and E.N. were involved in all steps of this submission, from hypothesis generation to perusal of existing literature to study design, collection and interpretation of data, writing of and revision of the manuscript. Statistical analyses were performed by L.P. with valuable input from E.B.; E.B. participated in data analyses and interpretation, and in the writing and review of the manuscript. D.B. and M.L. made critical contributions towards data interpretation, perusal of existing literature in the context of findings, and to preparation of the manuscript and revisions. The first submission as well as the revised version has been reviewed by each of the contributors and the revised submission has been approved by all co-authors.

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**References**


