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ORIGINAL RESEARCH

Causal Effects of Immune Cells on Reproductive Ill-Health, Including Abnormal Spermatozoa, Polycystic Ovary Syndrome and Spontaneous Abortion: Mendelian Randomization Analyses

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Background: Accumulative prior studies have demonstrated that immune inflammation profoundly influences reproductive disorders of mesodermal origin. However, little is known about the causal relationship between immune factors and diseases of the reproductive system.

Methods: Thorough two-sample Mendelian randomization (MR) analyses were conducted to determine the causal effects of 731 immune traits on reproductive ill-health, including abnormal spermatozoa (AS), polycystic ovary syndrome (PCOS), and spontaneous abortion (SA). Causal links were decrypted using genome-wide association study (GWAS) data. Sensitivity analyses were performed to assess the strength, heterogeneity, and horizontal pleiotropy of the results.

Results: For AS, 34 causal relationships were identified, with BAFF-R, CD20, and CD27 in the B-cell panel having protective effects against AS. A crucial causative connection between *CD11c+ CD62L− monocyte%monocyte* (cDC panel) and AS pathogenesis was also revealed. For PCOS, 40 causal effects were established, with CD20, CD24, and CD27 in the B-cell panel playing different roles in PCOS. *CD4 on CM CD4+* (maturation stages of the T-cell panel) significantly increased the risk of PCOS. For SA, 33 causative associations were determined, and a protective effect of *CCR2 (C-C chemokine receptor type 2) on CD14+ CD16+ monocytes* (monocyte panel) in SA was particularly noted. The diverse functions of the CD28, CD39, and CD25 molecules in the Treg cell panel in SA were also observed.

Conclusion: This study comprehensively evaluated the causal impact of immune traits on reproductive illnesses, stressing the complex and important role of immunogenic factors in pathogenesis and highlighting a novel direction for clinical work.

Keywords: Mendelian randomization, immunity, causal inference, abnormal spermatozoa, polycystic ovary syndrome, spontaneous abortion

Introduction

The prevalence of infertility is 10–15%, with male factors accounting for approximately 40%. Reproductive disorders have emerged as an issue of concern throughout the world.[1,2] The current downward trend in global fertility, with projections of a decline in the world population after a possible peak of 9.7 billion in 2064 to a world population of 8.8 billion by 2100, might have dramatic negative implications for social advancement.[3] The mesodermal origin of the reproductive system, including the testes, endometrium, and ovaries, is crucial for the formation and development of the embryo, and corresponding reproductive disorders, such as abnormal spermatozoa (AS), polycystic ovary syndrome (PCOS) and spontaneous abortion (SA), cause distress and confusion in countless families.[4,5] High-quality sperm is essential for the penetration and activation of oocytes.

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However, approximately 2% of infertile males have abnormal sperm parameters.[6] Given that sperm morphology is linked to sperm motility, the ability of sperm to penetrate the cervical mucus, and the capacity of sperm to enter the oocyte zona pellucida, increases in the rates of sperm deformity may postpone natural conception and increase the likelihood of spontaneous miscarriage.[7] Therefore, it is critical to investigate the pathogenesis of AS to improve clinical practice. Infertility in women is predominantly caused by PCOS, which afflicts 6–12% of women of reproductive age worldwide.[8,9] In addition to having typical clinical manifestations encompassing hyperandrogenaemia, ovulatory dysfunction, and polycystic ovarian morphology, PCOS patients suffer from a wide range of endocrine metabolic dysfunctions, such as insulin resistance, as well as the accompanying potential risk for type 2 diabetes mellitus and cardiovascular disease, all of which are detrimental to physical and mental health.[10–12] Despite recent advances suggesting that PCOS is a complex disorder influenced by genetic and environmental factors, its aetiology and underlying biological processes remain unclear. SA, generally referred to as miscarriage, is defined as the loss of a nonviable intrauterine pregnancy before 20 weeks of gestation and affects approximately 10–20% of pregnancies, with the majority of SA (~80%) occurring before 13 weeks of gestation.[4,13–15] Successful embryo implantation and gestational processes rely on a high-quality endometrium to afford the embryo the opportunity to attach, invade, and develop, highlighting that both the endometrium and embryo quality play pivotal roles in the outcome of embryo implantation.[16] The decidualized endometrium functions as a biosensor of embryo quality and, if disrupted, can lead to the implantation of embryos destined for miscarriage.[17] A decreased number of p16-positive senescent cells in the endometrium has been recognized as a biomarker of miscarriage.[18] Further research aiming to achieve a more thorough understanding of the causative mechanisms of miscarriages is needed, as this topic is highly important for preventing miscarriages.

In recent years, with the introduction of the "reproductive immune microenvironment (RIM)", research on the underlying connection between the immune and reproductive systems has been in the spotlight.[19] Increasing evidence suggests that the immune microenvironment is strongly associated with reproductive disorders.[20–22] As the core sites for the production and growth of germ cells and fertilized oocytes, the testes, ovaries and uterus together constitute a specialized "reproductive microenvironment with immune privilege".[23–26] Nevertheless, multiple potential threats may have negligible effects on the RIM, resulting in immune infertility afflicting couples, for which a great deal of research is underway.[19] Testicular macrophages promote spermatogenesis via colony-stimulating factor 1[27] and steer embryonic testicular development by secreting growth factors and cytokines.[28] Thus, testicular macrophages are recognized as potential targets for male infertility treatment.[20] The immune response of the ovary is an exciting and emerging research area that is essential for understanding the complexity of ovary-related diseases.[22] As early as 1978, the hypothesis that immune cells play a pivotal regulatory role in ovarian cycle regulation and follicle number selection was proposed.[29] Immune cells are pivotal in follicle development and oestrogen synthesis, ranging from phagocytosis and antigen presentation to the secretion of proteases, cytokines, chemokines, and growth factors, among others.[22,30,31] In addition, studies have revealed the importance of immune cells in endometrial tolerance and early placental development.[32–34] Altered numbers or dysfunctions of uterine immune cell populations, especially natural killer cells and regulatory T cells, have also been demonstrated to be strongly involved in miscarriage.[32] Decidual NK cells, which are involved in vascular remodelling and contribute to trophoblast invasion, are also implicated in miscarriages and other pregnancy failures when suppressed.[35] To date, investigations into the effects of immunological factors on the pathogenesis of reproductive health problems have not been widespread or sufficiently thorough. More refined and holistic studies are urgently needed to explore the potential causal relationships between immunologic factors and reproductive disorders. Exploration of the risk or protective factors involved in these diseases from an immune perspective can yield information for infertility diagnosis, and potential therapeutic strategies such as treatment to suppress immune cells may be of clinical utility.

Mendelian randomization (MR) is a unique tactic of genetic epidemiology research designed to assess the causal effects of exposures on clinical outcomes in specific diseases.[36,37] This strategy employs genetic variants strongly linked to certain exposures as instrumental variables (IVs), offering reliable and impartial estimations of genotypic determinations during conception.[38] To perform MR analyses, three essential assumptions must be satisfied: first, the genetic variance should be closely related to the exposure; second, the genetic variance should not be susceptible to other confounding variables; and third, genetic IVs can influence the outcome only via the exposure.[39,40] With this credible approach, investigators can more precisely and holistically probe the underlying causal influences of multiple factors on the disease, laying the groundwork for future clinical therapeutic strategies.[41] Over the past decade, MR has been broadly used to deduce causality with publicly available genome-wide association study (GWAS) data.[42–44]

Herein, we performed a comprehensive two-sample Mendelian randomization analysis to evaluate the cause-and-effect relationships between immune traits and reproductive ill-health, including AS, PCOS, and SA. This study is the first to reveal a new underlying causality between the immune system and reproductive ill health, which has not been identified in previous studies, paving the way for the discovery of immunogenic factors in the etiopathogenesis of reproductive ill health.

Materials and Methods

Study Design

A comprehensive two-sample MR study was conducted to investigate the causal links between 731 immune traits and reproductive ill-health. Figure 1A depicts the study design alongside three indispensable MR assumptions: (1) genetic instruments are linked to the exposures, (2) genetic variants are unrelated to any confounding factors, and (3) genetic instruments influence outcomes only via risk variables.[45] A graphical representation of the study design is presented in Figure 1B.

GWAS Data Sources for Reproductive Ill-Health Status

Summary statistics for AS were obtained from the FinnGen Consortium R7 release data (https://r7.finngen.fi/). With a total of 1913 cases and 293,878 controls, GWAS data with the "abnormal spermatozoa" phenotype were retrieved. Statistics for PCOS and SA were obtained from the FinnGen Consortium R9 release data (https://r9.finngen.fi/). For PCOS, the specific phenotype code was categorized as "R9_E4_PCOS", and a total of 1424 cases and 200,581 controls were derived. For SA, the corresponding phenotype code used in this study was "O15_ABORT_SPONTAN", with 16,906 cases and 149,622 controls.

Immunity-Wide GWAS Data Sources

To evaluate the causal relationships between immune metrics and reproductive ill-health, we employed the most extensive GWAS data from peripheral blood immunophenotyping to date, which utilized raw genetic immune profiles from 3757 Europeans. In total, 731 immunophenotypes were identified, covering absolute cell (AC) counts (n = 118), median fluorescence intensities (MFI, n = 389, indicating surface antigen levels), morphological parameters (MP, n = 32, including forward and side scatter, reflecting cell volume, intracellular complexity, and cell-surface texture), and relative cell counts (RC, n = 192). The GWAS summary statistics of 731 immune traits are available in the GWAS Catalog (accession numbers from GCST0001391 to GCST0002121).[46] Specifically, the MFI, AC, and RC metrics encompassed B cells, cDCs, mature stages of T cells, monocytes, myeloid cells, TBNK (T cells, B cells, and natural killer cells), and Treg panels, whereas the MP feature incorporated the cDC and TBNK panels.

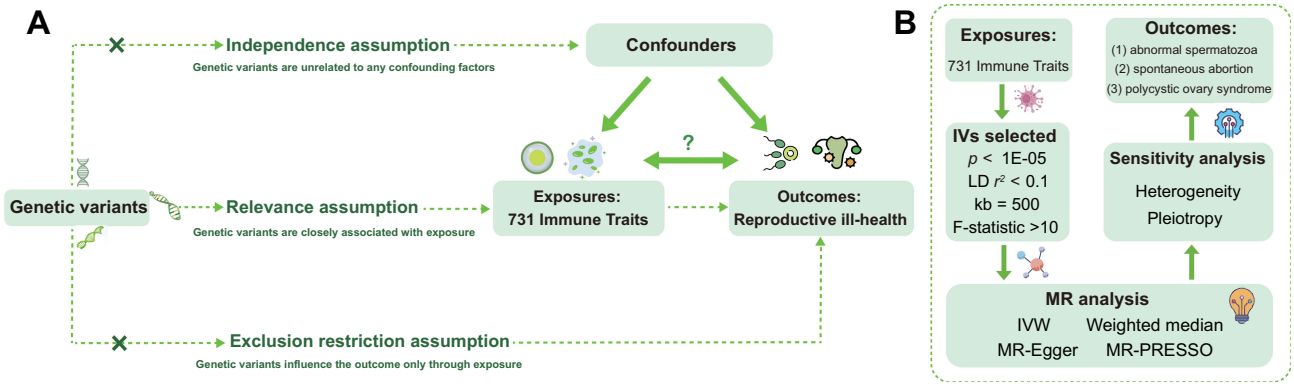

Figure 1 Overview of the study design. (**A**) Schematic representation of the three core assumptions underlying MR analysis: Assumption 1: The correlation hypothesis: genetic variation is directly related to exposure; Assumption 2: The Independence hypothesis: there is no connection between the genetic variant and the possible confounders between exposure and outcome; Assumption 3: The exclusion hypothesis: the genetic variation will not affect the outcome by means other than exposure. (**B**) Flowchart outlining the overall study design.

Abbreviations: MR, Mendelian randomization; IVs, instrumental variables; IVW, inverse-variance weighted; MR-PRESSO, MR pleiotropy residual sum and outlier.

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Selection of Instrumental Variables (IVs)

A rigorous screening procedure was applied to the IVs to ensure the precision and credibility of the analyses. Drawing from published studies,[46] we set a significance level threshold of 1 × 10–5 for IVs pertaining to each immune trait. This predetermined criterion continued to dictate the significance of the differences in the results for reproductive system problems. Pruning of single nucleotide polymorphisms (SNPs) was conducted via the clumping procedure of PLINK software (version v1.90), and we established a linkage disequilibrium (LD) r^2 threshold below 0.1 within a 500-kb distance. As a reference panel, the 1000 Genomes Project was utilized to calculate LD r^2 values.[47] To mitigate the risk of biased discoveries due to improperly chosen IVs, specific palindromic SNPs were excluded. To avert latent instrumental bias arising from weak instruments, the F-statistic (β^2_exposure/SE^2_exposure) was computed to appraise the potency of IVs, and F-statistics exceeding 10 indicated that IVs were sufficient for reliable MR analysis.[48,49] These criteria not only adhered to the recommendations of previous studies but also ensured the dependability and accuracy of the IVs employed in this study.

Assessment of Causal Effects and Sensitivity Analysis

To investigate the causal effects of immune traits on reproductive disorders, we utilized several reliable MR methods built on distinctive assumptions. Primary analysis relied on the inverse-variance weighted (IVW) method,[50] whereas the weighted median,[51] MR–Egger,[52] and MR pleiotropy residual sum and outlier (MR-PRESSO)[53] were employed to validate the robustness of the IVW results.

Thorough and methodical sensitivity analyses were also conducted to eliminate underlying violations of the MR assumptions. Heterogeneity was estimated using Cochran's Q statistic, where a p value less than 0.05 suggested significant heterogeneity.[54] In cases where the null hypothesis was rejected, implying possible heterogeneity within IVs, we applied the random-effect IVW method as an alternative to the fixed-effect IVW approach.[50] Horizontal pleiotropy and potential bias due to invalid IVs were examined using MR-Egger intercept analysis.[55] MR-PRESSO outlier tests were employed to investigate the possibility of horizontal pleiotropy introducing bias into the MR results. We detected genetic variations in the outliers using MR-PRESSO and reevaluated the impact estimates after their removal.[53] The 'leave-one-out' (LOO) method validated the dependability and reliability of the results by the iteratively removing each SNP and subsequently performing MR analyses. LOO visually confirmed whether a single SNP could drive the predominant causation.[52] Scatter plots revealed that the outcomes were unaffected by outliers. Funnel plots illustrated the robustness of causal inferences, with no evident heterogeneity. Additionally, we adopted MR Steiger directionality tests to determine the accuracy of the deduced causal direction.[56]

A strong causal relationship between immune traits and reproductive disorders could be established in this study only when the following criteria were met: 1) The IVW method indicated a significant association with a p-value <0.05; 2) The estimates derived from the IVW method were consistent with those from the other three MR methods; 3) Cochran's Q test, MR-Egger intercept analysis, and MR-PRESSO global test showed no significant results ($p > 0.05$); 4) The MR-Steiger directionality test confirmed a correct causal direction; 5) The MR estimates were not significantly influenced by a single SNP in the LOO analysis, ensuring the robustness of the findings. Only immune traits that met all these criteria could be considered as potential protective or risk factors for reproductive disorders.

Statistical Analysis

All MR analyses were performed using R software (version 4.2.0). Packages, including *MendelianRandomization* and *MR-PRESSO*, were used. A comprehensive array of methods and tests has established a logical framework for estimating the causal effects between immune traits and reproductive ill health. The criteria for establishing a causal relationship have been detailed in the preceding sections. Statistical significance was defined as a two-tailed p-value <0.05, in all sensitivity analysis tests.

Results

Exploration of the Causal Effects of Immunophenotypes on AS

To assess the causal effects of immunophenotypes on AS, a two-sample MR analysis was applied. Among the 731 immunophenotypes, 34 immune traits were found to be significantly causally associated with AS, 13 of which were distributed in the B-cell panel (Figure 2). Except for *IgD+ CD38dim AC* (B-cell panel, odds ratio [OR]: 1.036; 95% confidence interval [CI]:

Immune Traits	Panels	SNPs	OR (95%CI)	P value
Absolute Count				
IgD+ CD38dim AC	B cell	28	1.036(1.001,1.072)	0.042
CD8br AC	TBNK	30	0.936(0.893,0.980)	0.005
DP (CD4+CD8+) AC	TBNK	18	0.861(0.772,0.961)	0.008
CD8dim AC	TBNK	29	0.938(0.882,0.997)	0.039
T cell AC	TBNK	22	0.873(0.796,0.958)	0.004
HLA DR+ T cell AC	TBNK	38	0.951(0.905,1.000)	0.048
CD28+ CD45RA+ CD8br AC	Treg	63	0.986(0.973,0.999)	0.040
Median Fluorescence Intensities				
BAFF−R on CD24+ CD27+	B cell	113	0.968(0.937,0.999)	0.044
BAFF−R on IgD− CD24−	B cell	106	0.965(0.932,0.999)	0.044
CD20 on IgD+ CD38−	B cell	31	0.921(0.860,0.986)	0.018
CD20 on memory B cell	B cell	44	0.933(0.875,0.995)	0.035
CD20 on unsw mem	B cell	35	0.933(0.877,0.994)	0.031
CD27 on IgD− CD38−	B cell	43	0.929(0.870,0.992)	0.029
CD27 on IgD− CD38dim	B cell	56	0.944(0.904,0.986)	0.010
CD27 on memory B cell	B cell	41	0.950(0.909,0.993)	0.023
CD27 on unsw mem	B cell	48	0.938(0.883,0.997)	0.039
CD27 on sw mem	B cell	58	0.926(0.872,0.984)	0.012
CD62L on CD62L+ myeloid DC	cDC	18	1.112(1.006,1.229)	0.038
CD28 on CD45RA− CD4 not Treg	Treg	23	1.102(1.008,1.204)	0.033
CD25 on CD45RA− CD4 not Treg	Treg	28	0.930(0.880,0.983)	0.010
HLA DR on CD14− CD16+ monocyte	Monocyte	46	1.083(1.021,1.148)	0.008
CCR2 on CD14− CD16−	Monocyte	15	0.899(0.820,0.986)	0.024
CD16 on CD14+ CD16+ monocyte	Monocyte	47	1.075(1.013,1.141)	0.017
CD39 on CD39+ CD4+	Treg	81	1.060(1.012,1.111)	0.014
CD45 on CD33br HLA DR+ CD14−	Myeloid cell	19	1.093(1.001,1.194)	0.047
CD45 on CD33br HLA DR+ CD14dim	Myeloid cell	16	0.916(0.851,0.986)	0.019
HLA DR on HLA DR+ CD8br	TBNK	21	1.137(1.022,1.265)	0.018
Relative count				
PB/PC %B cell	B cell	28	0.917(0.858,0.980)	0.010
IgD− CD38− %B cell	B cell	13	0.857(0.744,0.988)	0.033
CD11c+ monocyte %monocyte	cDC	22	1.111(1.016,1.214)	0.021
CD11c+ CD62L− monocyte %monocyte	**cDC**	**17**	**1.228(1.094,1.378)**	**0.0005**
CD62L− plasmacytoid DC %DC	cDC	23	1.101(1.021,1.188)	0.013
HLA DR+ CD4+ %lymphocyte	TBNK	22	0.867(0.770,0.976)	0.018
CD28− CD127− CD25++ CD8br %T cell	Treg	18	0.917(0.849,0.992)	0.030

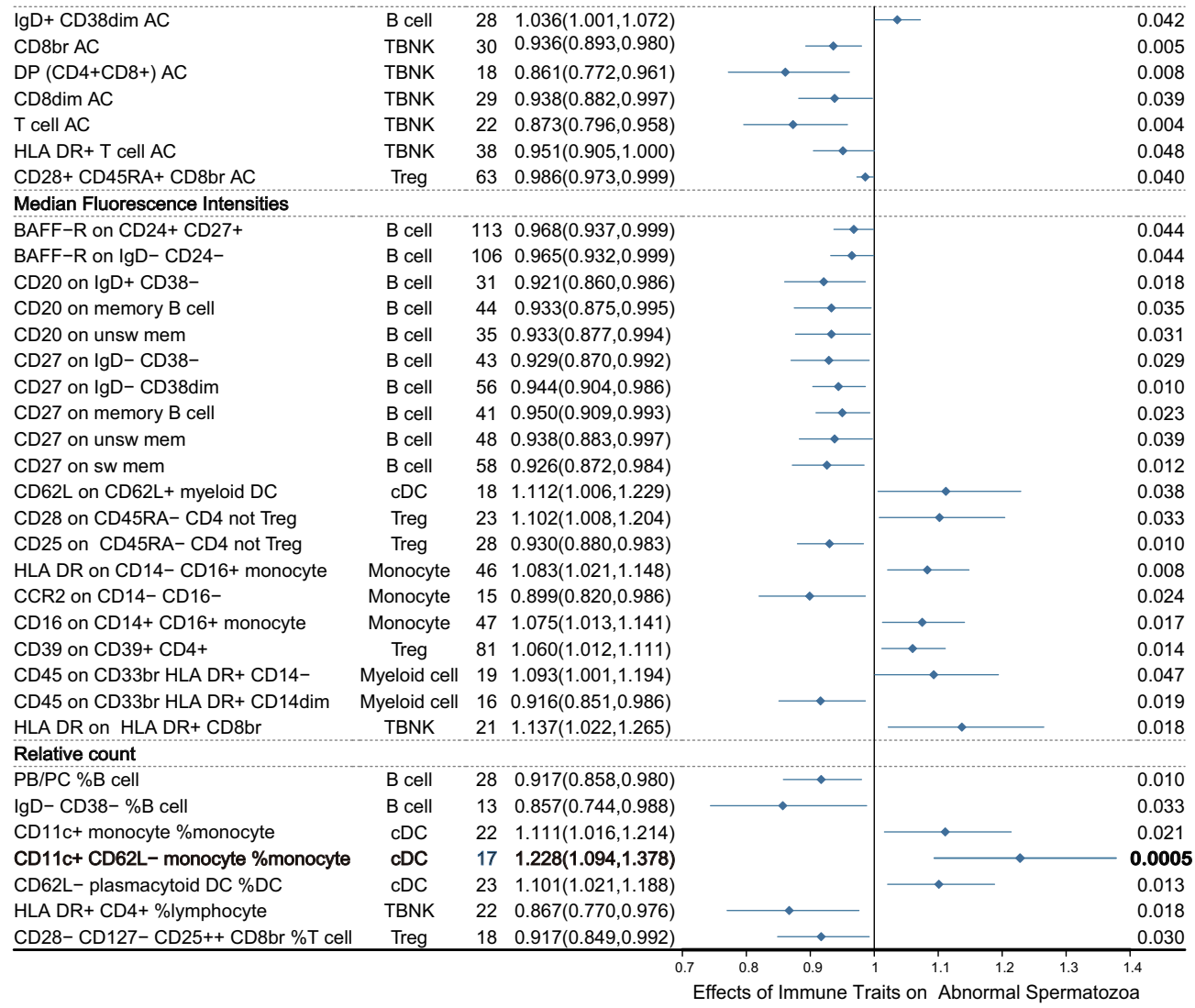

Figure 2 Causal associations between immune traits and AS: Results of MR analyses using the IVW method.
Abbreviations: AS, abnormal spermatozoa; MR, Mendelian randomization; IVW, inverse-variance weighted; OR, odds ratio.

1.001–1.072; P_{IVW} =0.042), which increased the risk of AS onset, the remaining 12 immune signatures of the B-cell panel had protective effects against AS. BAFF-R, CD20, and CD27 were highlighted in the B-cell panel, and the associated immunophenotypes were causally linked to a reduced risk of AS development, including *BAFF−R on CD24+ CD27+ cells* (B-cell panel, OR: 0.968; 95% CI: 0.937–0.999; P_{IVW} =0.044), *CD20 on IgD+ CD38−* (B-cell panel, OR: 0.921; 95% CI: 0.860–0.986; P_{IVW} =0.018), and *CD27 on IgD− CD38−* (B-cell panel, OR: 0. 929; 95% CI: 0. 870–0. 992; P_{IVW} =0.029). Notably, the causal relationship between *CD11c+ CD62L- monocyte%monocyte* and AS was the most significant (cDC panel, P_{IVW} =0.0005). With 17 SNPs as proxy predictors, an elevated *CD11c+ CD62L- monocyte%monocyte* was strongly correlated with a substantial increase in AS risk (OR: 1.228; 95% CI: 1.094–1.378). Similarly, other immune traits in the cDC panel, including *CD11c+ monocyte%monocyte* (cDC panel, OR: 1.111; 95% CI: 1.016–1.214; P_{IVW} =0.021) and *CD62L− plasmacytoid DC%DC* (cDC panel, OR: 1.101; 95% CI: 1.021–1.214; P_{IVW} =0.188), were also found to contribute to the increased risk of AS development.

Although the IVW technique is fairly effective at determining whether an exposure causes a complicated disease, it may be influenced by weak instrumental biases. To mitigate these biases, we employed multiple MR analytical strategies to determine the reliability of the 34 causal relationships deduced by the IVW method. Causality estimates consistent

with the IVW approach were also derived from the three other MR methods, namely, MR–Egger, weighted median, and MR-PRESSO (Table S1). Interestingly, *CD11c+ CD62L- monocyte%monocyte*, the most salient immunophenotype identified by the IVW approach, also passed some additional sensitivity tests, showing a strong cause-and-effect relationship with AS risk ($OR_{weighted\text{-}median}$: 1.24, 95% $CI_{weighted\text{-}median}$: 1.04–1.47, $P_{weighted\text{-}median}$ = 0.016; $OR_{MR\text{-}PRESSO}$: 1.23, 95% $CI_{MR\text{-}PRESSO}$: 1.07–1.40, $P_{MR\text{-}PRESSO}$ = 0.0025), indicating that more in-depth studies are needed (Figure S1A and Table S1). The LOO method demonstrated the absence of major SNPs in IVs that might significantly impact the results after culling, thus increasing the confidence in causality (Figure S1B). The funnel plots showed no signs of horizontal pleiotropy or heterogeneity, further supporting the dependability of the causal effect of *CD11c+ CD62L- monocyte%monocyte* on AS (Figure S1C).

Subsequently, a range of sensitivity analyses was conducted to test the plausibility of the results. For the 34 immune features mined using IVW, Cochran's Q test revealed no heterogeneity among the different IVs (Table S2). MR–Egger's intercept test and MR-PRESSO global tests showed no potential effects of level-multiplicity. MR Steiger directionality tests affirmed the precision of the causal effects' directionality between immune features and AS (Table S3).

Investigation of Causal Relationships Between Immunophenotypes and PCOS

Among the 731 immunophenotypes, 40 immune signatures were causally associated with PCOS (Figure 3). The maturation stages of the T cell panel were highlighted for causality in relation to the increased risk of PCOS onset,

Immune Traits	Panels	SNPs	OR (95%CI)	P value
Absolute Count				
Memory B cell AC	B cell	17	0.942(0.890,0.997)	0.040
CD24+ CD27+ AC	B cell	27	0.947(0.896,1.000)	0.050
CD20− AC	B cell	24	0.937(0.883,0.995)	0.033
Mo MDSC AC	Myeloid cell	29	0.936(0.877,1.000)	0.048
CD28− DN (CD4−CD8−) AC	Treg	34	1.117(1.031,1.211)	0.007
DC AC	cDC	27	1.114(1.010,1.228)	0.031
Median Fluorescence Intensities				
CD19 on IgD+ CD24+	B cell	39	1.055(1.008,1.105)	0.023
CD20 on CD20− CD38−	B cell	20	1.162(1.038,1.301)	0.009
CD20 on IgD− CD38br	B cell	14	1.073(1.006,1.145)	0.033
CD24 on IgD+ CD24+	B cell	27	1.057(1.005,1.112)	0.033
CD24 on IgD− CD38−	B cell	31	1.058(1.001,1.119)	0.045
CD27 on CD20−	B cell	15	0.925(0.861,0.994)	0.035
CD27 on CD20− CD38−	B cell	22	0.890(0.803,0.986)	0.027
CD27 on IgD− CD38−	B cell	44	0.924(0.860,0.993)	0.032
CD27 on memory B cell	B cell	42	0.928(0.883,0.975)	0.003
CD38 on CD20−	B cell	22	0.852(0.734,0.989)	0.035
HVEM on EM CD8br	Maturation stages of T cell	19	1.096(1.018,1.180)	0.015
CCR7 on naive CD8br	Maturation stages of T cell	25	1.097(1.005,1.197)	0.038
CD4 on CM CD4 +	**Maturation stages of T cell**	**20**	**1.171(1.059,1.294)**	**0.002**
CX3CR1 on CD14+ CD16+ monocyte	Monocyte	42	0.937(0.880,0.997)	0.038
PDL−1 on CD14− CD16+ monocyte	Monocyte	27	0.917(0.862,0.976)	0.007
CD66b on CD66b++ myeloid cell	Myeloid cell	26	1.095(1.022,1.174)	0.010
HLA DR on CD33dim HLA DR+ CD11b−	Myeloid cell	39	0.936(0.882,0.994)	0.030
CD16−CD56 on NKT	TBNK	46	1.088(1.013,1.170)	0.021
CD45 on lymphocyte	TBNK	24	0.894(0.816,0.979)	0.016
CD28 on CD39+ CD4+	Treg	41	0.931(0.872,0.994)	0.033
CD28 on CD39+ resting Treg	Treg	23	1.065(1.012,1.121)	0.015
CD25 on activated Treg	Treg	19	1.167(1.016,1.340)	0.029
CD4 on CD39+ activated Treg	Treg	32	1.087(1.004,1.177)	0.039
CD86 on myeloid DC	cDC	22	1.134(1.019,1.261)	0.021
Relative Count				
IgD− CD27− %B cell	B cell	22	1.125(1.001,1.264)	0.048
Memory B cell %lymphocyte	B cell	29	0.936(0.888,0.986)	0.013
Naive−mature B cell %lymphocyte	B cell	22	0.865(0.756,0.988)	0.033
CD20− %lymphocyte	B cell	16	0.919(0.852,0.991)	0.029
CM CD8br %T cell	Maturation stages of T cell	24	0.841(0.746,0.948)	0.005
CD14+ CD16− monocyte %monocyte	Monocyte	30	1.049(1.001,1.099)	0.045
CD8dim %leukocyte	TBNK	23	0.898(0.820,0.985)	0.022
HLA DR+ CD8br %lymphocyte	TBNK	43	0.927(0.863,0.995)	0.035
CD28− DN (CD4−CD8−) %T cell	Treg	24	1.139(1.039,1.249)	0.005
CD62L− DC %DC	cDC	38	1.073(1.005,1.145)	0.034

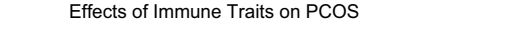

Figure 3 Causal associations between immune traits and PCOS: Results of MR analyses using the IVW method.
Abbreviations: PCOS, polycystic ovary syndrome; MR, Mendelian randomization; IVW, inverse-variance weighted; OR, odds ratio.

with *CD4 on CM CD4+* presenting the most significant causal link to PCOS (maturation stages of T cell panel, OR: 1.171; 95% CI: 1.059–1.294; P_{IVW} = 0.002). Similarly, *HVEM on EM CD8br* (maturation stages of T cell panel, OR: 1.096; 95% CI: 1.018–1.180; P_{IVW} = 0.015) and of *CCR7 expression on naive CD8b* (maturation stages of T cell panel, OR: 1.097; 95% CI: 1.005–1.197; P_{IVW} = 0.038) were identified as potential risk factors for PCOS pathogenesis. Notably, complex correlations between B cells and PCOS (11 protective associations and 6 risk associations in causality) were detected, and the CD20, CD24, and CD27 molecules in the B cell panel were stressed. In particular, the protective role of CD27 in PCOS was brought to attention, comprising *CD27 on CD20−* (B-cell panel, OR: 0.925; 95% CI: 0.861–0.994; P_{IVW} = 0.035), *CD27 on CD20− CD38−* (B-cell panel, OR: 0.890; 95% CI: 0.803–0.986; P_{IVW} = 0.027), among other instances. Furthermore, immune features of the cDC panel, such as *DC AC* (cDC panel, OR: 1.114; 95% CI: 1.010–1.228; P_{IVW} = 0.031), *CD86 on myeloid DC* (cDC panel, OR: 1.134; 95% CI: 1.019–1.261; P_{IVW} = 0. 021), *and CD62L− DC%DC* (cDC panel, OR: 1.073; 95% CI: 1.005–1.145; P_{IVW} = 0.034) increased the risk of PCOS. Monocytes containing *CX3CR1 on CD14+ CD16+ monocyte* (monocyte panel, OR: 0.937; 95% CI: 0.880–0.997; P_{IVW} = 0.038) and *PDL−1 on CD14− CD16+ monocyte* (monocyte panel, OR: 0.917; 95% CI: 0.862–0.976; P_{IVW} = 0.007) were detected as protective immunogenic factors in PCOS patients.

Congruent causality estimates for the 40 causal effects were also supported by the MR–Egger, weighted median, and MR-PRESSO analyses (Table S4). The prominent risk-predictive role of *CD4 on CM CD4+* for PCOS was evidenced by additional tests (maturation stages of T cell panel, $OR_{MR\text{-}PRESSO}$: 1.17, 95% $CI_{MR\text{-}PRESSO}$: 1.08–1.27, $P_{MR\text{-}PRESSO}$ = 0.00013) (Figure S2A and Table S4), and there were no possible outliers in the IVs according to the LOO approach (Figure S2B). Funnel plots further indicated the reliability of this causality and no heterogeneity (Figure S2C). In addition, no signs of heterogeneity were found in our study using the Cochran's Q test (Table S5). MR–Egger's intercept and MR-PRESSO global tests confirmed that our MR analyses were not potentially affected by horizontal pleiotropy. These 40 immunophenotypes were also examined using MR Steiger directionality tests, which verified the directional stability of the immune signatures in patients with PCOS (Table S6).

Examination of Causal Links Between Immunophenotypes and SA Incidence Status

In this study, we identified notable causal effects involving 33 immune signatures among 731 immunophenotypes in individuals with SA (Figure 4). *CCR2 (C-C chemokine receptor type 2) on CD14+ CD16+ monocyte*, a possible protective factor, had the most significant causal relationship with SA (monocyte panel, OR: 0.975; 95% CI: 0.962–0.989; P_{IVW} = 0.004). The CD28 and CD39 molecules in the Treg cell panel were also found to reduce the risk of developing SA, as did *CD28+ DN (CD4−CD8−)%DN* (Treg panel, OR: 0.974; 95% CI: 0.950–0.999; P_{IVW} = 0.040), *CD39+ CD8br%T cell* (Treg panel, OR: 0.985; 95% CI: 0.971–0.999; P_{IVW} = 0.036), and *CD39+ CD8br%CD8br* (Treg panel, OR: 0. 985; 95% CI: 0.972–0.999; P_{IVW} = 0.036). In contrast, CD25 molecules on Treg cells increased the risk of developing SA, encompassing *CD25++ CD8br%T cell* (Treg panel, OR: 1.046; 95% CI: 1.008–1.085; P_{IVW} = 0.017), *CD28− CD25++ CD8br%T cell* (Treg panel, OR: 1.072; 95% CI: 1.004–1.0145; P_{IVW} = 0.039), and *CD28− CD127− CD25++ CD8br%T cell* (Treg panel, OR: 1.035; 95% CI: 1.007–1.064; P_{IVW} = 0.014). Similarly, CD8 molecules on the TBNK panel, such as *CD8br NKT%T cell* (TBNK panel, OR: 1.039; 95% CI: 1.001–1.079; P_{IVW} = 0.043) and *CD8br NKT%lymphocyte* (TBNK panel, OR: 1.045; 95% CI: 1.008–1.084; P_{IVW} = 0.016), were also revealed as potential risk factors for SA. Intriguingly, the complicated patterns observed between the B-cell panel and SA population drew special attention, especially for CD25 molecules, which performed different roles in distinctive immune functions. For example, *CD25 on IgD+ CD38br* increased the risk of SA development (B-cell panel, OR: 1.039; 95% CI: 1.000–1.080; P_{IVW} = 0.048), whereas *CD25 on IgD− CD38dim* had a protective effect on SA.

The estimated analysis of the 33 causal interactions inferred from the IVW method received corroborative support from the MR–Egger, weighted median, and MR-PRESSO approaches (Table S7). The significant protective impact of *CCR2 on CD14+ CD16+ monocyte* on SA was further established through additional assessments (monocyte panel, $OR_{MR\text{-}Egger}$: 0.97, 95% $CI_{MR\text{-}Egger}$: 0.96–0.99, $P_{MR\text{-}Egger}$ = 0.013; $OR_{weighted\ median}$: 0.98, 95% $CI_{weighted\ median}$: 0.96–1.00, $P_{weighted\ median}$ = 0.032; $OR_{MR\text{-}PRESSO}$: 0.98, 95% $CI_{MR\text{-}PRESSO}$: 0.96–0.99, $P_{MR\text{-}PRESSO}$ = 0.0029) (Figure S3A and Table S7), and no significant outliers were found among IVs via LOO analysis (Figure S3B). According to the funnel plot, there was no indication of horizontal pleiotropy or heterogeneity, which further confirmed the credibility of the causal relationship

Immune Traits	Panels	SNPs	OR (95%CI)	P value
Absolute Count				
Secreting Treg AC	Treg	29	0.989(0.980,0.999)	0.034
CD4+ AC	TBNK	23	0.973(0.949,0.997)	0.027
CD8br NKT AC	TBNK	26	1.041(1.003,1.081)	0.036
CD28+ CD45RA− CD8br AC	Treg	39	0.990(0.980,1.000)	0.050
Median Fluorescence Intensities				
CD25 on CD24+ CD27+	B cell	29	1.009(1.000,1.018)	0.043
CD25 on IgD+ CD38br	B cell	17	1.039(1.000,1.080)	0.048
CD25 on IgD− CD38dim	B cell	25	0.971(0.944,0.998)	0.035
CD25 on transitional	B cell	23	1.038(1.005,1.072)	0.023
CD3 on CM CD8br	Maturation stages of T cell	31	1.028(1.004,1.053)	0.022
HVEM on naive CD4+	Maturation stages of T cell	16	1.025(1.002,1.049)	0.032
CD86 on CD62L+ myeloid DC	cDC	22	1.026(1.001,1.053)	0.045
CD127 on CD8br	Treg	24	0.957(0.926,0.989)	0.009
CCR2 on CD14+ CD16+ monocyte	**Monocyte**	**40**	**0.975(0.962,0.989)**	**0.0004**
CX3CR1 on CD14− CD16+ monocyte	Monocyte	22	0.972(0.947,0.997)	0.030
CD80 on granulocyte	cDC	34	0.977(0.957,0.998)	0.036
CD45 on lymphocyte	Myeloid cell	20	1.020(1.002,1.037)	0.028
Relative Count				
Memory B cell %B cell	B cell	27	1.028(1.002,1.056)	0.038
CD62L− DC %DC	cDC	38	1.027(1.007,1.047)	0.008
Im MDSC %CD33dim HLA DR− CD66b−	Myeloid cell	19	0.976(0.955,0.998)	0.035
CM CD4+ %T cell	Maturation stages of T cell	24	1.037(1.007,1.069)	0.015
CD8br NKT %T cell	TBNK	27	1.039(1.001,1.079)	0.043
CD8br NKT %lymphocyte	TBNK	28	1.045(1.008,1.084)	0.016
NK %CD3− lymphocyte	TBNK	31	0.983(0.968,0.998)	0.029
HLA DR+ NK %CD3− lymphocyte	TBNK	44	0.971(0.949,0.993)	0.011
CD28− DN (CD4−CD8−) %DN	Treg	29	1.027(1.001,1.053)	0.040
CD28+ DN (CD4−CD8−) %DN	Treg	29	0.974(0.950,0.999)	0.040
CD28− CD8dim %CD8dim	Treg	16	1.012(1.002,1.023)	0.017
CD39+ CD8br %T cell	Treg	106	0.985(0.971,0.999)	0.036
CD39+ CD8br %CD8br	Treg	100	0.985(0.972,0.999)	0.032
CD28− CD127− CD25++ CD8br %T cell	Treg	17	1.035(1.007,1.064)	0.014
CD28− CD25++ CD8br %T cell	Treg	8	1.072(1.004,1.145)	0.039
CD25++ CD8br %T cell	Treg	18	1.046(1.008,1.085)	0.017
CD28− DN (CD4−CD8−) %T cell	Treg	24	1.035(1.006,1.064)	0.018

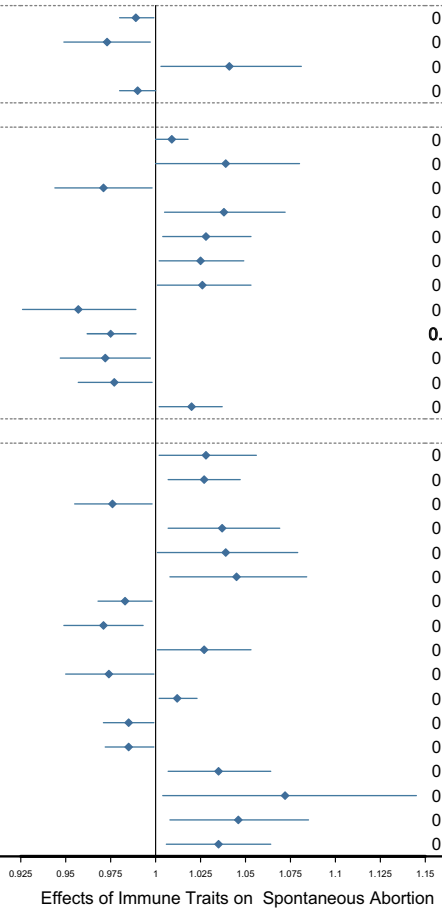

Figure 4 Causal associations between immune traits and SA: Results of MR analyses using the IVW method.
Abbreviations: SA, spontaneous abortion; MR, Mendelian randomization; IVW, inverse-variance weighted; OR, odds ratio.

between *CCR2 on CD14+ CD16+ monocyte* and SA (Figure S3C). Moreover, our investigation showed the absence of heterogeneity in the 33 causal effects, as evidenced by the results of Cochran's Q statistic test (Table S8).

The MR–Egger regression and MR-PRESSO global tests corroborated the absence of pleiotropy in our MR evaluations. The causal directions of the links between immune markers and SA were scrutinized and confirmed by MR-Steiger tests (Table S9).

Discussion

Utilizing a two-sample MR analysis approach, this study pioneers the comprehensive and impartial establishment of causal links between 731 immunophenotypes and reproductive health concerns, including AS, PCOS, and SA. Drawing on GWAS data at the gene level, this study investigated the intricate involvement of the immune system in the evolution of reproductive disorders. For AS, 34 causal links were established, notably, between the protective effects of the BAFF-R and CD27 molecules in the B-cell panel against AS. A pivotal causative link between *CD11c+ CD62L− monocyte% monocyte* (cDC panel) and AS pathogenesis was also identified. Regarding PCOS, our study confirmed 40 causal effects and underscored the vital roles of the CD20 and CD24 molecules in the B-cell panel in PCOS pathogenesis. *CD4 on CM CD4+* (maturation stages of the T-cell panel) was found to significantly increase the risk of PCOS onset. Specifically, 33 causative associations were identified for SA, and the protective effect of *CCR2 on CD14+ CD16+ monocyte* (monocyte panel) on SA was elucidated. Our study also strongly emphasized the varied roles of the CD39, CD28, and CD25

molecules in the Treg cell panel in relation to SA. This study provides essential insights for further investigations of reproductive ill-health mechanisms, highlighting the pivotal role of the immune system in AS, PCOS, and SA.

An array of immune features significant for AS were identified in our study, particularly the protective causal relationship between BAFF-R and CD27 molecules in the B-cell panel. BAFF-R, a component of the tumor necrosis factor receptor (TNFR) family, is encoded by the TNFRSF13C gene located at chromosomal region 22q13. Its mRNA is translated into a transmembrane protein expressed on the surface of all Ig+ B cells.[57,58] As a critical receptor for B-cell survival, BAFF-R may exert its protective effects against sperm abnormalities by regulating B-cell homeostasis in the testicular immune microenvironment. Previous studies have emphasized the essential role of BAFF-R signaling in maintaining the survival and function of mature B cells.[59] Upon binding to BAFF, BAFF-R activates the PI3K/AKT signaling pathway, which is crucial for protein synthesis, metabolic adaptation, and cell survival.[60] BAFF-R and its ligands also create independent homeostatic niches for B cell subsets,[61,62] which are vital for maintaining B-cell homeostasis, particularly in complex immune environments like the testis. The specific mechanisms by which BAFF-R may lower the risk of AS should be explored in future experimental studies. Additionally, CD27, a key molecule guiding B cells toward plasma cell differentiation,[63] has drawn attention for its potential role in reducing the risk of AS. Prior studies in chronic myelogenous leukemia have shown that CD27 interaction with CD70 activates Wnt target genes by enhancing the nuclear localization of active β-catenin and TNIK, promoting the proliferation and differentiation of leukemia stem cells.[64] This suggests that CD27 may reduce the risk of AS by influencing similar processes in spermatogenesis, which involves the proliferation and differentiation of spermatogonia. Moreover, the mTOR signaling pathway plays a crucial role in regulating sperm quality, with mTOR activity suppressed in populations of highly viable spermatozoa.[65] High CD27 expression has been reported to be closely linked to reduced mTOR activity.[66] Therefore, it is speculated that CD27 may indirectly influence spermatogenesis by interacting with signaling pathways such as mTOR, affecting processes like cell proliferation, differentiation, and metabolism. Given the critical roles of BAFF-R and CD27 in B-cell function, the lack of extensive research on their roles in AS underscores the urgent need for further studies on these molecules as a potential protective factor in AS therapy.

PCOS is characterized by a multifaceted pathophysiology, with chronic low-grade inflammation being predominant, intertwining immune and metabolic imbalances.[67] This study investigated the pathogenesis of PCOS from a causative perspective, focusing particularly on the complex role of *CD4 on CM CD4+* (maturation stages of the T-cell panel), as well as CD20 and CD24 on the B-cell panel, in increasing the risk of PCOS. Previous studies reported a significantly higher frequency of *CD4(+) CD28(null) T cells* in PCOS patients.[68] This study was the first to identify a causal link between *CD4 on CM CD4+ T cells* and an increased risk of PCOS. Abnormal activation of CD4+ T cells and alterations in the number of central memory T cells (T_{CM}) have been documented to be associated with the development of several immune-related diseases.[69,70] Given the immune dysregulation and chronic inflammation in PCOS, along with the complex roles of CD4+ T_{CM} cells in immune regulation,[70–72] it is suggested that CD4+ T_{CM} may increase PCOS risk by disrupting immune balance, thereby affecting ovarian function and endocrine metabolism. In addition to T-cell dysfunction, B-cell abnormalities also play a crucial role in immune regulation in PCOS. CD20, due to its aberrant high expression in many diseases, has become an important therapeutic target in various conditions, such as B-cell lymphomas. CD20 induces cytosolic calcium flux through interactions with the B-cell receptor and activates related signaling pathways. Its dysregulation is closely associated with immune-related diseases.[73–76] This suggests that CD20 on B cells may influence the inflammatory microenvironment of PCOS through similar mechanisms, by regulating inflammatory signaling pathways and affecting the production and release of inflammatory factors, thereby contributing to the pathological progression of PCOS. Moreover, CD24 expression is closely linked to B-cell energy metabolism and intracellular signaling pathways,[77] and can enhance DNA damage-induced apoptosis by regulating the NF-κB signaling pathway.[78] CD24 has been identified as a potential biomarker in diseases such as ovarian cancer, where it is associated with disease development, invasion, and metastasis.[79] Changes in CD24 expression are closely related to ovulation, as it participates in regulating prostaglandin synthesis and transport, making it a target for the treatment of ovulatory disorders.[80] This indicates that CD24 may play a similar role in the pathological process of PCOS, and further investigation is needed to determine its precise function in this context.

The aetiology of SA may be closely associated with abnormal inflammation.[81] This study highlighted several key immune factors that influence the risk of SA, including CD39, CD28, CD25, and CCR2. Notably, the protective association of CD39 with SA revealed in this study demonstrates conserved characteristics across different species. Mouse model studies showed that CD39 activity was crucial for preventing miscarriage in antiphospholipid syndrome (APS)-induced abortion models.[82] In CD39-overexpressing mice, abortion induced by antiphospholipid antibodies (aPL-ab) was prevented, and there was a reduction in trophoblast TF expression, C3d deposition, lipid peroxidation, and TNF-α expression. These findings suggested that CD39 might prevent miscarriage by regulating inflammatory and coagulation-related mechanisms. These findings are in agreement with human data, further confirming the protective role of CD39. Although the GWAS data in our study were derived from a European population, cross-population comparisons revealed similar findings in Asian populations, further indicating the key role of CD39 in maintaining immune balance at the maternal-fetal interface and preventing miscarriage.[83] Recent studies on recurrent SA in Asian populations show reduced CD39+ cells in the decidua, possibly due to TGF-β-mTOR-HIF-1α pathway downregulation. The conserved nature of these findings across species and populations suggests that CD39-mediated immune regulation may be an evolutionarily conserved mechanism for maintaining pregnancy in mammals, further reinforcing the universal significance and relevance of our MR study.

In addition to CD39, CD28 expressed on Treg also plays critical roles in regulating immune responses, which influences the risk of SA. CD28, a homodimeric stimulatory cell surface receptor of the Ig superfamily,[84] is essential for the activation of helper T cells, and its persistence is required for helper T cell polarization in response to infection.[85] Prior studies have demonstrated that the second co-stimulatory signal provided by CD28 in the matrix plays a significant role in the embryo implantation process in mice.[86] This suggests that CD28 might also be involved in pregnancy-related processes, which is consistent with our findings indicating its role in reducing the risk of SA. In contrast, high expression of CD25 on CD8br Treg cells was disclosed to be significantly associated with an increased risk of SA. Overexpression of CD25, the alpha chain of the IL-2 receptor, increases the receptor's affinity for IL-2, which may lead to excessive consumption of IL-2.[87] This reduction in local IL-2 concentrations can impair the activation and function of other immune cells, such as CD8+ and CD4+ T cells, even causing their functional exhaustion.[88,89] Meanwhile, high CD25 expression may enhance IL-2 signaling, increasing pro-inflammatory cytokine secretion such as interferon-gamma, thereby triggering an inflammatory response.[90] These immune alterations, collectively, may contribute to the increased risk of SA. Furthermore, this study provides the first evidence of a protective causal association between *CCR2 on CD14 + CD16+ monocytes* and SA. Earlier research has demonstrated that CD16+ monocytes play a crucial role in steady-state immune surveillance,[91] and that CCR2+ monocytes are significantly involved in the repair of cerebrovascular damage caused by chronic social defeat stress.[92] These findings suggest that *CCR2 on CD14+ CD16+monocytes* may reduce the risk of SA by modulating immune homeostasis and tissue repair processes.

This study performed elaborate MR analyses of 731 immunophenotypes, drawn from the latest GWAS cohort data. We uncovered causative links between immune traits and several reproductive ill-health conditions, namely, AS, PCOS and SA, which opens new avenues for identifying the underlying immunogenic mechanisms in these reproductive disorders. Nonetheless, there are certain limitations to our study. First, the significant causal relationships identified require further experimental validation. Second, the absence of available detailed personal data hindered a further stratified analysis of the population. Finally, as this study relied on European data, our findings cannot be applied to other ethnicities, thus constraining the generalizability of our results.

Conclusion

In summary, our study applied the MR approach to delineate the causal relationships between 731 immunophenotypes and reproductive health concerns, including AS, PCOS, and SA. We elucidated the intricate interplay between the immune system and reproductive diseases and filled a crucial void in related fields, with a focus on specific immunophenotypes. These findings expand the understanding of immune mechanisms and provide valuable insights for targeted prevention, early diagnosis, and the development of tailored therapeutic strategies for reproductive health issues.

Data Sharing Statement

Publicly available datasets were analyzed in this study. Summary statistics for abnormal spermatozoa were obtained from the FinnGen consortium R7 release data (https://r7.finngen.fi/). Statistics on polycystic ovary syndrome and spontaneous abortion were obtained from the FinnGen consortium R9 release data (https://r9.finngen.fi/). GWAS summary statistics for 731 immune traits could be publicly available in the GWAS Catalog (https://www.ebi.ac.uk/gwas/, accession numbers from GCST0001391 to GCST0002121).

Ethics Approval Statement

This study is based on publicly available, de-identified summary-level genetic data obtained from previous genome-wide association studies (GWAS), in which ethical approval and informed consent were obtained by the original authors. According to item 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (dated February 18, 2023, China), our study does not involve direct interaction with human participants, biological samples, or identifiable personal data; thus, it is exempt from ethical review by our Institutional Review Board (IRB).

Consent for Publication

All authors read and approved the final version of the manuscript for publication.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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