

Trans-ethnic GWAS meta-analysis of idiopathic spermatogenic failure highlights the immune-mediated nature of Sertoli cell-only syndrome

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Overall this is a well-executed meta-analysis. There is some evidence of phenotypic heterogeneity, but it is explored appropriately.

Major Points:

1. Table 1 should be a presentation of the cohorts meta-analyzed with their source publications and demographics.
2. The current Table 1 should be Table 2 as results. It has the effect allele but no information on the minor allele frequencies in any of the populations.
3. Nowhere in the text are the minor allele or effect allele frequencies reported. These should be included where ORs and p-values for SNPs are reported.

Minor points:

Page 8, line 181

"On the other hand,"

Reduce the use of transition cliché such as this. Use these phrases in the Discussion section if needed.

Reviewer #2

(Remarks to the Author)

This paper describes a trans-ancestry GWAS meta-analysis of severe spermatogenic failure (SPGF), including data from European and Asian cohorts. Male infertility genetics is an important and understudied topic in reproductive genetics. While the study confirms associations with several loci, the results are constrained by sample size, making it underpowered and limiting post-GWAS analyses. The phenotypes are inconsistent, and because of the lack of histological data in the Asian cohort, trans-ethnic generalizations are limited. The study is scientifically sound, and the manuscript is well-organized and written, but its contribution is limited and, in the absence of larger samples, needs to provide deeper insights to clarify its novelty.

Major Comments

- 1) The title overstates the contribution of the study with the phrases "unravels the genetic landscape" and "trans-ethnic" and should be better aligned with the results. Emphasizing the autoimmune aspect may provide more interest and value than making such a broad and vague statement.
- 2) Please provide more detail on the phenotyping process for the cohorts used and the cohort source (community, reproductive center). While these details are in the parent publications, a brief overview would be helpful. A short discussion of differences in the sample composition would also be helpful, as would probably an acknowledgment of these differences in the limitations.
- 3) The trans-ancestry analyses are limited, and the main benefit of the meta-analysis is increased sample size. While the effort to combine these samples is valuable, the conclusions regarding ancestry are few and should be balanced. Both samples are underpowered, and commenting on genetic factors "across ancestries" may be premature until more variants are identified and can be contrasted. Identifying shared variants is promising but does not provide evidence of consistent genetic effects across ancestries at this time.
- 4) It would be helpful if Figure 1 clarified the ancestry of each cohort. As the methods are currently written, it is unclear how

the European samples may overlap.

5) This study replicated previous associations in the MHC region and SOX5. Potential novel loci, trending towards significance, are under-interpreted. Including a pathway analysis to place THP1 and TAF1B into a broader network could add value to the current analyses. Speculative biological mechanisms based on public resources like GTEx or ENCODE could be of value if presented correctly.

6) Table 1 would benefit from a column indicating locus status (novel, replicated, study number, or some other way to track the results across studies. Clarifying which loci belong to which phenotype would also help.

7) Figure 2 needs to be clarified, and a legend would be beneficial.

8) A growing body of research implicates MHC class II region in immune-mediated pathways related to SPGF, specifically SCO. This aligns with broader evidence of immune dysfunction in infertility, but the manuscript does not elaborate on these associations in a clinical context or explore the parallel in reproductive immune dysfunction in women, including endometriosis. Evaluating the nominal replication of top findings could be a valuable opportunity to investigate this potential biological mechanism.

9) Post-GWAS analyses would provide deeper insights. Methods like MAGMA or DEPICT could add to the study by highlighting connections between loci and underlying biological processes and overlap with related traits. Similarly, LD Score regression could show how these phenotypes are related to each other and other reproductive and immune disorders.

10) A PheWAS of related results could generate insights to put these conditions into a broader context.

Reviewer #3

(Remarks to the Author)

1. The manuscript is well written. The Abstract, Introduction, Discussion, and conclusion are clearly presented.

2. The overall manuscript has been written very logical and scientific manner.

3. Author identified variants on different gene (loci) which may involve to cause idiopathic spermatogenic failure (NOA) in male. This GWAS data used for prediction of Pathogenicity of the variants by using different programs. No study done to check the functional impact of identified variants in NOA infertile males. I suggest performing a functional analysis of these variants to check the functional impact of identified variants in NOA infertile males.

This would be helpful to confirm the role of these variants in male infertility.

4. Before the acceptance, the author may improve the manuscript by incorporating the suggestions in their manuscript.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

My main comments have been addresses.

Minor comments to be updated for acceptance:

1. In Table 2, these variables are shown twice, please combine or annotate why you are listing them twice.

Follicle stimulating hormone (FSH) levels, IU/L

Luteinizing hormone (LH) levels, IU/L

2. In Table 1 list both the reference allele and the effect allele.

Reviewer #2

(Remarks to the Author)

The authors have thoroughly addressed all of my concerns.

Reviewer #3

(Remarks to the Author)

1. I thank the authors for addressing my initial comments.

2. The research article is well written.

3. The author performed a very well genomic and bioinformatics analysis in three ethnic populations. The predicted variants in the GWAS study would build future diagnostic strategies for NOA male infertility.

4. All sections of the manuscript are presented logically and scientifically.

5. Following the revision to the article, I recommend that this manuscript be considered for publication.

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REVIEWER #1:

We appreciate the appropriate comments and suggestions of the reviewer for helping us to provide a more accurate overview of our results.

1) Table 1 should be a presentation of the cohorts meta-analyzed with their source publications and demographics.

RESPONSE: We thank the reviewer for this insightful suggestion. In response, we have introduced a new table that offers a detailed summary of the cohorts included in the trans-ethnic meta-analysis, to the extent allowed by the available data. This table shows information such as source publications, sample sizes, average age, and all available clinical data for each cohort. We believe this addition significantly improves the transparency and clarity of the manuscript, while also providing readers with a more thorough understanding of the data analyzed in this study. We would like to note that this table is presented as "Table 2" due to the format of the journal, which places the methods section at the end of the document.

2) The current Table 1 should be Table 2 as results. It has the effect allele but no information on the minor allele frequencies in any of the populations.

RESPONSE: As mentioned in the previous response, this table remains as "Table 1" because the methods (along with the new table) are placed at the end of the manuscript. In response to the reviewer suggestion, we have updated the table to include additional columns showing the minor allele frequencies (MAFs) for both European and Asian populations. Additionally, we have replaced the term "effect allele" with "reference allele," as we believe it is more appropriate.

3) Nowhere in the text are the minor allele or effect allele frequencies reported. These should be included where ORs and p-values for SNPs are reported.

RESPONSE: As recommended by the reviewer, we have incorporated the MAFs for both Europeans and Asians of the described SNPs into the text in the "Results" section (pages 6 and 7). These frequencies are also presented in the updated Table 1. We would like to note that the MAFs for the control groups align with those reported in publicly available resources, such as the 1KGP3 dataset.

4) Page 8, line 181: "On the other hand," Reduce the use of transition cliché such as this. Use these phrases in the Discussion section if needed.

RESPONSE: We want to thank the reviewer for pointing this out. We have revised the text to replace clichés with more precise language. The sentence previously beginning with "On the other hand" ("Results" section, page 9) has been updated to "*Notably, the G allele of the lead SNP (rs34915133) was found to be completely linked...*". We hope this change enhances the clarity and flow of the paragraph while addressing the reviewer concern.

REVIEWER #2:

We are sincerely grateful for the input provided by the reviewer, which has definitively helped us to improve our manuscript. We hope that the reviewer will consider the modifications performed appropriate.

1) The title overstates the contribution of the study with the phrases “unravels the genetic landscape” and “trans-ethnic” and should be better aligned with the results. Emphasizing the autoimmune aspect may provide more interest and value than making such a broad and vague statement.

RESPONSE: We appreciate the feedback of the reviewer on the title and have revised it to better reflect the results and findings of the study, as suggested. In this regard, we have modified the title by this new one:

“A trans-ethnic GWAS meta-analysis of idiopathic spermatogenic failure highlights the immune-mediated nature of the Sertoli cell-only syndrome”.

We think this title aligns more closely with the results and emphasizes the autoimmune aspect of the findings.

2) Please provide more detail on the phenotyping process for the cohorts used and the cohort source (community, reproductive center). While these details are in the parent publications, a brief overview would be helpful. A short discussion of differences in the sample composition would also be helpful, as would probably an acknowledgment of these differences in the limitations.

RESPONSE: We have carefully considered the suggestion of the reviewer and, to address this, we have included the following brief paragraph about the phenotyping process in the “Methods” section (pages 18 and 19), specifying the inclusion criteria and the clinical analysis performed:

“Patients were included in the study based on comprehensive andrological evaluations, which encompassed a review of medical history, physical examination, semen analysis, hormone profiling (FSH, LH, and testosterone), karyotyping, and Y-chromosome microdeletion screening. To ensure diagnostic accuracy, azoospermia was confirmed by the absence of spermatozoa in two semen samples after high-speed centrifugation. Patients with known causes of male infertility, including cryptorchidism, orchitis, testicular malformations, obstruction of the vas deferens, chromosomal abnormalities, or Y-chromosome microdeletions, were excluded.”

We have also clarified the histological diagnosis of European NOA patients with the following paragraph included in the Methods section (page 18):

“Amongst these NOA patients, 120 also had a histological diagnosis of HS, 98 of MA, and 214 of SCO following testicular biopsy. The biopsy results also allowed their classification based on whether testicular sperm retrieval was successful or unsuccessful (Table 2).”

A new “Table 2” has also been included, summarizing all relevant clinical information for the cohorts, where available.

As recommended by the reviewer, we have specified each cohort source, including the following modified paragraph in the Methods section (page 18):

"[...] were recruited in different public health centers and private fertility clinics from Spain and Portugal, and at the Centre of Reproductive Medicine and Andrology (University Hospital Münster, Germany), respectively. Additionally, 981 Han Chinese men diagnosed with idiopathic NOA were recruited from the Centre of Reproductive Medicine in Nanjing."

Finally, a short discussion about differences in the sample composition has been included as limitation in the last paragraph of the "Discussion" section (page 17), as follows:

"However, the lack of detailed phenotype data in the analyzed Chinese cohort represent an important limitation of this study. While consistent diagnostic criteria were applied for inclusion, differences in sample composition across the study cohorts may have contributed to heterogeneity in the dataset."

3) The trans-ancestry analyses are limited, and the main benefit of the meta-analysis is increased sample size. While the effort to combine these samples is valuable, the conclusions regarding ancestry are few and should be balanced. Both samples are underpowered, and commenting on genetic factors "across ancestries" may be premature until more variants are identified and can be contrasted. Identifying shared variants is promising but does not provide evidence of consistent genetic effects across ancestries at this time.

RESPONSE: Based on the comment of the reviewer, we have expanded the paragraph acknowledging the limitations at the end of the "Discussion" section (page 17), as follows:

"This heterogeneity, along with the limited sample size, weakened the ability to draw definitive conclusions about genetic markers across ancestries. Although the identification of shared variants is promising, it does not yet provide definitive evidence of consistent genetic effects between populations at this time. Establishing large and well-characterized SCO cohorts from Asian populations will be essential to confirm our findings."

4) It would be helpful if Figure 1 clarified the ancestry of each cohort. As the methods are currently written, it is unclear how the European samples may overlap.

RESPONSE: We perfectly understand the reviewer suggestion about the need to improve the labeling of "Figure 1" for better clarity. This figure represents the final results of the three trans-ethnic meta-analyses accordingly with the different European datasets (overall SPGF, NOA, and SCO). To address this, we have updated the figure to clearly indicate that the Manhattan plots correspond to the three distinct meta-analysis comparisons. Additionally, we have revised both the "Methods" section and the legend of "Figure 1" to specify that two European datasets were included, and to clarify that the figure presents the combined results of the overall meta-analyses, as follows:

- "Methods" section, "Statistical analysis of the individual populations" subsection (page 19): [...] including two European cohorts (Iberian and German) and one Chinese cohort.

- “Figure 1” legend (page 25): *Manhattan plot representations of the three trans-ethnic meta-analyses conducted between the two European and the Asian genome-wide association studies of male infertility due to spermatogenic failure (SPGF).*

We believe these changes will improve the clarity and interpretation of the data.

5) This study replicated previous associations in the MHC region and SOX5. Potential novel loci, trending towards significance, are under-interpreted. Including a pathway analysis to place TJP1 and TAF1B into a broader network could add value to the current analyses. Speculative biological mechanisms based on public resources like GTEx or ENCODE could be of value if presented correctly.

RESPONSE: We appreciate the suggestion of the reviewer and agree on the importance of conducting a more detailed evaluation of the functional roles of *TJP1* and *TAF1B* in the context of SCO. To address this, we have taken the following steps:

- We performed a functional enrichment annotation analysis using the same pipeline employed for *SOX5* and the *HLA* region (which involves the use of databases such as GTEx and ENCODE, among others, as recommended), along with a new analysis utilizing the Database for Annotation, Visualization, and Integrated Discovery (DAVID). The results from these analyses have been summarized and provided in the “Supplementary Data 1” (which it now includes all retrieved data from *TJP1* and *TAF1B*) and in a new “Supplementary Data 2” (please note that the previous Supplementary Data files 2 and 3 have been renamed to Supplementary Data 10 and 11, respectively).

- The expression patterns of both genes were more comprehensively investigated using the GTEx dataset, and these findings have been integrated into a new Supplementary Figure 2.

- To place *TJP1* and *TAF1B* within a broader biological network, we conducted a pathway analysis using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING). The resulting protein networks involving *TJP1* and *TAF1B* are included in a new Supplementary Figure 3.

- We analyzed whether the genes encoding members of the *TJP1* and *TAF1B* biological networks retrieved by STRING showed evidence of association with SCO in our dataset. The results have been included in the newly added “Supplementary Data 3”.

All the above mentioned new findings are detailed in the “Results” section (pages 9 and 10), as follow:

The suggestive association haplotypes within the TAF1B and TJP1 regions also included SNPs with strong evidence of functionality (Supplementary Data 1). Both genes were predominantly expressed in testicular tissues (Supplementary Figure 2), and several TAF1B genetic variants were annotated in the GTEx database as eQTL and sQTL in the testis, with highly relevant RDB scores (i.e. 1b and 1f; see Supplementary Data 1).

Further functional annotation analyses using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) and the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) revealed that the proteins encoded by both genes are essential for maintaining

transcriptional and cellular architecture (Supplementary Data 2 and Supplementary Figure 3). TAF1B plays a critical role in RNA polymerase I transcription and rRNA synthesis, with key domains such as the RRN7-type zinc finger facilitating DNA binding and transcriptional regulation. TJP1, on the other hand, is crucial for cell-cell junction assembly, including tight junctions, and supports cytoskeletal organization and cell-cell barrier integrity, with PDZ and SH3 domains driving protein interactions. Interestingly, genetic regions encoding components of the molecular networks linked to TAF1B and TJP1 harbor variations showing some evidence of genetic association with SCO (Supplementary Data 3).

As part of these updates, we have revised the first line of the “In silico characterization of associated variants” subsection in the “Methods” section (page 21), which now reads:

To understand the functional implications of suggested associated variants with NOA, and their proxy variants [...].

Additionally, the following paragraph has been added to the end of the “Methods” section (page 22), accompanied by three new references:

To place the putative NOA genes within a broader biological network, we further explored their potential biological functions using the MAGMA tool integrated into Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA)⁶⁹, as well as DAVID⁷⁰. Additionally, we conducted pathway analyses with STRING⁷¹ to examine protein-protein interaction networks, aiming at identifying potential interaction partners for these genes.

Moreover, the sentence “By performing the first trans-ethnic meta-analysis of GWAS data in SPGF, we have been able to advance our understanding about the genetic component of this condition.” in the first paragraph of the “Discussion” section (page 13) has been revised as follows:

By performing the first trans-ethnic meta-analysis of GWAS data in SPGF and subsequent functional annotation analyses, we have been able to advance our understanding about the genetic component of this condition.

Finally, the following sentences have incorporated to the “Discussion” section:

- page 16 (second paragraph): *This aligns with the results of our in silico analysis, which revealed high expression of TJP1 in testicular tissues.*

- page 16 (last paragraph): *Interestingly, the functional characterization of the TAF1B signal from our meta-analysis based on SCO revealed that several risk variants function as sQTL for this gene in the testis. Given the critical role of TAF1B in rRNA transcription⁵³, these findings suggest that alterations in the transcript isoform ratio may disrupt ribosomal function and cellular metabolism, potentially contributing to the SCO phenotype.*

A new citation has been included: “53. Hahn S. The role of TAFs in RNA polymerase II transcription. *Cell* 95, 579-582 (1998)”.

We believe these additions significantly improve the interpretation of our findings and we also appreciate the guidance of the reviewer in this regard.

6) Table 1 would benefit from a column indicating locus status (novel, replicated, study number, or some other way to track the results across studies. Clarifying which loci belong to which phenotype would also help.

RESPONSE: We thank the reviewer for their valuable suggestion. In response, we have updated “Table 1” to include a new column indicating the *locus* status, as recommended. Regarding the second point, we note that the table already provides information on the phenotypes associated with each *locus*, as detailed in the “Phen” (phenotype) column. We believe this adequately establishes the connection between *loci* and their respective phenotypes.

7) Figure 2 needs to be clarified, and a legend would be beneficial.

RESPONSE: We appreciate the effort of the reviewer to improve the clarity of “Figure 2”. After revisiting the manuscript, we confirm that a detailed legend is already provided for this figure, as shown below:

Figure 2. Enrichment of functional annotations of the human genome for the Sertoli cell-only (SCO)-associated polymorphisms rs34915133 and rs10842262 (highlighted in red) as well as their proxies. The different functional features are represented with specific colors, with color grade correlating with the probability of functional impact for each tested variant (darker colors indicate higher probability). This figure summarized the information provided in Supplementary Table 2. CADD, combined annotation dependent depletion score; ChIP-seq, proteins bound from chromatin immunoprecipitation flowing by sequencing experiments in the testis (using ENCODE data); eQTL, expression quantitative trait locus effects in the testis; GRCh38, genome reference consortium human build 38; sQTL, splicing quantitative trait locus effects in the testis; TFBS, transcription factor binding sites modifications related to transcription factors involved in spermatogenesis based on protein weight matrix data.

However, if there are any specific aspects of “Figure 2” that remain unclear, we would be happy to provide further clarification.

8) A growing body of research implicates MHC class II region in immune-mediated pathways related to SPGF, specifically SCO. This aligns with broader evidence of immune dysfunction in infertility, but the manuscript does not elaborate on these associations in a clinical context or explore the parallel in reproductive immune dysfunction in women, including endometriosis. Evaluating the nominal replication of top findings could be a valuable opportunity to investigate this potential biological mechanism.

RESPONSE: We fully agree with the insightful observations of the reviewer regarding the potential relevant role of immune dysregulation in infertility. In line with their suggestion, we have expanded the discussion to draw parallels between immune-mediated infertility across sexes, particularly through MHC class II-mediated pathways. This addition highlights the shared mechanisms between SCO and female infertility conditions such as endometriosis, emphasizing the critical need for further exploration of this biological pathway. The following paragraph has been incorporated after the second paragraph of the “Discussion” section (page 14):

These observations support the hypothesis that SCO is predominantly an immune-mediated condition of NOA, which may result from the loss of the immune privilege of the testis (a mechanism essential for preventing autoimmune responses against germline cells) ^{11, 30}. Beyond male infertility, parallels can be drawn to inflammatory processes or autoimmune reactions targeting reproductive tissues in women. For instance, ovarian endometriosis (a condition in which endometrial tissue is present in the ovary leading to infertility) is characterized by chronic inflammation and immune activation. Notably, high-precision single-cell RNA sequencing has recently revealed elevated expression of the MHC class II complex in epithelial cells within ectopic endometrial lesions of affected women, thus activating CD4+ T cells and perpetuating chronic inflammation ³¹. The above is consistent with our PheWAS analysis, in which endometriosis and other immune-mediated conditions were linked to the SCO-associated variants. Overall, these similarities highlight shared biological mechanisms underlying immune-mediated infertility across sexes. Future research should aim to comprehensively elucidate these molecular pathways and evaluate their potential for therapeutic intervention.

Two new references have been added:

30. Gong J, Zeng Q, Yu D, Duan YG. T Lymphocytes and Testicular Immunity: A New Insight into Immune Regulation in Testes. *International journal of molecular sciences* 22 (2020).

31. Yan J, et al. Single-cell analysis reveals insights into epithelial abnormalities in ovarian endometriosis. *Cell reports* 43, 113716 (2024).

9) Post-GWAS analyses would provide deeper insights. Methods like MAGMA or DEPICT could add to the study by highlighting connections between loci and underlying biological processes and overlap with related traits. Similarly, LD Score regression could show how these phenotypes are related to each other and other reproductive and immune disorders.

RESPONSE: We thank the reviewer for their valuable suggestions regarding post-GWAS analyses. To improve our study, we have performed an additional functional annotation analysis using MAGMA to explore the connections between the suggested SCO genetic variants and biological functions, pathways, and tissues. The results of this analysis have been summarized in a new “Supplementary Table 2”. Additionally, the following changes have been made to the manuscript:

We added the paragraph below to the “Results” section (page 10):

Finally, a functional enrichment analysis was performed on the grey zone (i.e., signals with P-values < 1E-05) from our trans-ethnic GWAS meta-analysis to further investigate the genetic basis of this condition (Supplementary Table 2). The Multi-marker Analysis of GenoMic Annotation (MAGMA) gene-set analysis identified the “WP_SARSCOV2_B117_VARIANT_ANTAGONISES_INNATE_IMMUNE_ACTIVATION” pathway as the most significantly associated with SCO (P = 1.05E-05, β = 1.21). This pathway also emerged as the top signal in the MAGMA analyses of SPGF and NOA grey zones. However, SCO demonstrated unique enrichment for other immune-related pathways, including the NLRP3 inflammasome complex and MHC class II antigen presentation, highlighting its distinct immunological profile (Supplementary Table 2).

As described in point 5, the following paragraph has been included at the end of the “Methods” section (page 22):

To place the putative NOA genes within a broader biological network, we further explored their potential biological functions using the MAGMA tool integrated into Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA)⁶⁹, as well as DAVID⁷⁰. Additionally, we conducted pathway analyses with STRING⁷¹ to examine protein-protein interaction networks, aiming at identifying potential interaction partners for these genes.

In relation with the LD score regression suggestion, we would like to inform the reviewer that we are currently conducting a separate and comprehensive study aimed at evaluating the genetic overlap between SCO and various immune-mediated conditions using multiple advanced approaches (including LDSR). As this analysis represents a broader investigation beyond the specific scope of the present study, we have not included these results here. However, to address this valuable point, we have added the following paragraph at the end of the “Discussion” section (page 17):

Moreover, additional post-GWAS analyses, such as LD score regression, could provide insights into the heritability and genetic correlations of SCO with related traits, including immune-mediated disorders. While beyond the scope of this study, we are pursuing a complementary analysis to explore these questions, which may further shed light on the immune-related pathways underlying SCO.

We hope the reviewer will appreciate our efforts to delineate the scope of the current work while highlighting future directions.

10) A PheWAS of related results could generate insights to put these conditions into a broader context.

RESPONSE: Again, we thank the reviewer for helping us to enhance the quality of our manuscript with insightful suggestions. We have carried out a PheWAS analysis and the results obtained have been included in 6 new supplementary files (Supplementary Data 4-9). The modifications implemented in the text are detailed below:

- The following new subsection has been added to the “Results” section (pages 10 and 11):

Phenome-Wide Association Study

*A Phenome-Wide Association Study (PheWAS) was conducted to complement the findings from our GWAS, allowing us to investigate the roles of the most relevant risk variants (i.e. those reported in **Table 1**) across a broad spectrum of phenotypes. This approach provided insights into potential pleiotropic effects, where a single genetic variant may influence multiple traits. Several significant traits associated with our male infertility signals also implicated other reproductive system-related issues (**Supplementary Data 4-9**). For example, DMRT1-rs7873478 was linked to “bioavailable testosterone levels” ($P = 0.0043$, $\beta = 0.0084$), and IL17A-rs13206743 was associated with both “sex hormone-binding globulin levels” ($P = 0.0040$, $\beta = 0.0036$) and “benign neoplasm of male genital organs” ($P = 0.0025$, $\beta = 0.2332$) (**Supplementary Data 4-5**). Interestingly, the SCO-risk variants showed associations with traits related to female reproductive system*

dysfunction, including “endometriosis of ovary” for HLA-DQA1/HLA-DRB1-rs34915133 ($P = 0.0046$, $\beta = 0.1031$), “endometriosis of fallopian tube” for SOX5-rs10842262 ($P = 0.0045$, $\beta = -0.3474$), “pain and other conditions associated with female genital organs and menstrual cycle” for TJP1-rs4492992 ($P = 0.0024$, $\beta = 0.0876$), and “ovarian dysfunction” for TAF1B-rs112307255 ($P = 0.0026$, $\beta = 0.1591$) (**Supplementary Data 6-9**).

Apart from reproductive traits, the PheWAS analysis also revealed associations with immune system conditions, especially when considering the SCO variants. Those included “autoimmune diseases” for HLA-DQA1/HLA-DRB1-rs34915133 ($P = 4.06E-67$, $\beta = -0.2198$), “ulcerative colitis” for SOX5-rs10842262 ($P = 0.0041$, $\beta = 0.1760$), “circumscribed scleroderma” for TJP1-rs4492992 ($P = 0.0024$, $\beta = 0.6640$), and “multiple sclerosis” for TAF1B-rs112307255 ($P = 0.0049$, $\beta = 0.0863$) (**Supplementary Data 6-9**).

- This sentence has been included in the “Discussion” section (page 14):

The above is consistent with our PheWAS analysis, in which endometriosis and other immune-mediated conditions were linked to the SCO-associated variants.

- The following paragraph has been placed at the end of the “Methods” section (page 22):

Finally, a PheWAS analysis was conducted to identify pleiotropic effects of the top signals using the tool for that purpose of the Open Targets Genetics platform¹⁵. This approach aimed to uncover the broader implications of the most relevant genetic variants associated with male infertility phenotypes, maximizing the insights gained from our GWAS findings

REVIEWER #3:

1) The manuscript is well written. The Abstract, Introduction, Discussion, and conclusion are clearly presented.

2) The overall manuscript has been written very logical and scientific manner.

3) Author identified variants on different gene (loci) which may involve to cause idiopathic spermatogenic failure (NOA) in male. This GWAS data used for prediction of Pathogenicity of the variants by using different programs. No study done to check the functional impact of identified variants in NOA infertile males.

4) Before the acceptance, the author may improve the manuscript by incorporating the suggestions in the file.

RESPONSE: We sincerely thank the reviewer for their positive feedback on the clarity and structure of our manuscript. We appreciate their recognition of our study and acknowledge the importance of functional validation for the identified variants, as noted by all reviewers. As outlined above, we have performed the suggested analyses and incorporated the recommended revisions to further improve the manuscript.

EDITORIAL COMMENTS:

As requested by the Editor, we have tried to appropriately address all the reviewers' comments. Additionally, we have made the following minor corrections to the text:

- The affiliation of M. Cerván-Martín has been updated.
- Abstract: "*Sertoli cell-only (SCO) syndrome*" has been replaced by "*Sertoli cell-only (SCO) syndrome*".
- Introduction, page 5: "*transethnic*" has been replaced by "*trans-ethnic*".
- Results, page 9: "*R2*" has been replaced by "*R²*".
- Definitions of different acronyms have been added to the legend of Supplementary Table 1, 2 as well as Supplementary Data 1, 3, 10 and 11.

We hope this revised version meets the standards for publication in *Communications Biology*.

REVIEWER #1:

We sincerely appreciate the time and effort of the reviewer in evaluating our manuscript. We have tried to carefully address their final considerations.

1) In Table 2, these variables are shown twice, please combine or annotate why you are listing them twice. Follicle stimulating hormone (FSH) levels, IU/L. Luteinizing hormone (LH) levels, IU/L.

RESPONSE: The hormone values appear twice in the table because they are reported separately for SO and NOA. However, we understand that this may not be immediately clear and could cause confusion. To address this, we have added two lines to the table to visually separate the information for SO and NOA. Additionally, we have specified the corresponding phenotype for each variable by including "in SO" and "in NOA" in each line.

2) In Table 1 list both the reference allele and the effect allele.

RESPONSE: We apologize for any confusion caused by the terminology used for the alleles in table 1. In this table, "Ref" refers to the allele used as the reference in the statistical analyses (that is, the allele for which OR is reported). These alleles are also the "effect alleles", though we decided to label them as "Ref". However, we recognize that this may be unclear, so we have revised our table to improve clarity. We have decided to include also the alternative alleles ("Alt") and specify the following acronyms for better understanding:

"Alt, alternative allele; [...] Ref, reference effect allele used for OR calculation".

REVIEWER #2:

We are very grateful for the positive feedback of the reviewer and for acknowledging our revisions.

REVIEWER #3:

We sincerely appreciate the positive comments and support for our manuscript. We are pleased that the genomic and bioinformatics analyses were found to be well-executed and appropriately presented.

EDITORIAL COMMENTS:

We have provided a more detailed description of one of the funding sources in the 'Funding' section and have added an ethics statement that was previously omitted at the end of the 'Study Population' subsection in the 'Methods' section (page 19).

We would like to thank the editorial board again for their time and consideration. We look forward to the final decision on our manuscript.