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Study Protocol: Preconception Sperm Quality and Protein Factors Linked to Autism-Related Molecular Signatures in Indian Men

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ABSTRACT

Background: Autism Spectrum Disorders (ASD) are neurodevelopmental conditions influenced by both genetic and environmental factors. Emerging evidence suggests paternal contributions, including sperm quality and proteomic composition, may affect early embryonic development and autism-associated protein expression in offspring. However, data from Indian men are limited.

Objective: To investigate the relationship between pre-conception sperm quality, sperm protein determinants, and the expression of autism-associated proteins in Indian men.

Methods: A cross-sectional study was conducted on 100 Indian men aged 22-40 years planning conception. Semen samples were analyzed for volume, sperm concentration, motility, morphology, and Deoxyribonucleic Acid (DNA) fragmentation per World Health Organization (WHO) 6th edition guidelines. Sperm proteome analysis focused on proteins implicated in neurodevelopment, including Reelin, SHANK3, and CNTNAP2, using Enzyme-Linked Immunosorbent Assay (ELISA) and Western blot. Correlations between sperm quality parameters, protein levels, and potential impact on autism-associated protein expression were analyzed using Pearson correlation and multivariate regression.

Results: Reduced sperm motility and increased DNA fragmentation were significantly associated with lower levels of Reelin and SHANK3 in sperm ($p<0.05$). Altered expression of CNTNAP2 correlated positively with increased sperm oxidative stress and abnormal morphology ($p<0.05$). Multivariate regression indicated that sperm motility, morphology, and key proteomic markers together explained 48% of variability in autism-associated protein levels.

Conclusion: Pre-conception sperm quality and protein composition in Indian men influence the expression of autism-associated proteins. Interventions improving sperm health before conception may potentially modulate early neurodevelopmental risk in offspring.

Keywords: Sperm quality; Sperm proteome; Autism spectrum disorders; Pre-conception; Reelin; SHANK3; CNTNAP2; Indian men

INTRODUCTION

Autism Spectrum Disorders (ASD) are characterized by deficits in social interaction, communication, and repetitive behaviors. While maternal factors have traditionally been the focus,

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paternal contributions through sperm quality and proteomic content are increasingly recognized as important determinants of embryonic and fetal neurodevelopment [1]. Sperm not only delivers paternal DNA but also carries proteins and Ribonucleic Acid [RNAs] that influence early embryogenesis. Alterations in sperm protein composition particularly those implicated in neurodevelopment, such as Reelin, SHANK3, and CNTNAP2 may affect the regulation of autism-associated pathways in the embryo. Despite the growing prevalence of ASD in India, limited studies have examined the relationship between sperm quality, proteomic determinants, and autism-associated protein expression. This study aimed to evaluate pre-conception sperm quality and its protein determinants as modulators of autism-associated protein expression in Indian men [2].

MATERIALS AND METHODS

Study design and participants

Cross-sectional study at a reproductive health center in India. Participants: 100 healthy Indian men aged 22-40 years planning conception.

Exclusion criteria

Chronic systemic diseases (diabetes, hypertension, autoimmune disorders). History of infertility treatment or varicocele. Smoking >10 cigarettes/day or substance abuse

Semen collection and analysis

Samples collected after 2-7 days of abstinence. Parameters assessed per WHO 6th edition: volume, sperm concentration, motility, morphology, DNA fragmentation (TUNEL assay). Oxidative stress measured *via* Reactive Oxygen Species (ROS) assay [3].

Sperm proteome analysis

Target proteins: Reelin, SHANK3, CNTNAP2

METHODS

ELISA for quantitative protein measurement Western blot for validation and protein localization Protein levels normalized to total sperm protein concentration

Statistical analysis

Data expressed as mean ± SD. Pearson correlation for bivariate associations between sperm quality parameters and protein levels [4]. Multivariate linear regression to identify independent predictors of autism-associated protein expression. Significance threshold: $p<0.05$.

RESULTS

Participant characteristics

The study participants had a mean age of 29.8 ± 4.1 years and a mean BMI of 24.6 ± 3.2 kg/m². Semen analysis showed a mean sperm concentration of 52.3 ± 18.5 million/mL, motility of 41.2 ± 9.7%, normal morphology of 5.8 ± 1.9%, and DNA fragmentation of 18.6 ± 7.2% (Table and Table 2).

Table 1: Mean ± SD values of age, BMI, and semen parameters including sperm concentration, motility, morphology, and DNA fragmentation among participants.

Parameter	Mean ± SD
Age (years)	29.8 ± 4.1
BMI (kg/m²)	24.6 ± 3.2
Sperm concentration (million/mL)	52.3 ± 18.5
Motility (%)	41.2 ± 9.7
Morphology (% normal)	5.8 ± 1.9
DNA fragmentation (%)	18.6 ± 7.2

Sperm protein expression

The analysis showed measurable expression of the proteins Reelin, SHANK3, and CNTNAP2 in sperm samples. These proteins demonstrated significant associations with semen quality parameters, including motility, DNA fragmentation, reactive oxygen species (ROS), and normal morphology.

Table 2: Mean levels of selected proteins and their significant correlations with semen parameters.

Protein	Mean (ng/mg total protein)	Significant Correlation

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Reelin	14.2 ± 3.8	↓ motility, ↑ DNA fragmentation
SHANK3	12.1 ± 3.5	↓ motility, ↑ DNA fragmentation
CNTNAP2	11.0 ± 3.1	↑ ROS, ↓ normal morphology

Multivariate regression analysis

Sperm motility, morphology, and DNA fragmentation independently predicted Reelin and SHANK3 expression Oxidative stress and abnormal morphology predicted CNTNAP2 expression. Combined sperm quality and proteomic parameters explained 48% of variability in autism-associated protein expression

DISCUSSION

This study demonstrates that pre-conception sperm quality and protein composition influence autism-associated protein expression in Indian men. Reduced motility and higher DNA fragmentation were associated with lower Reelin and SHANK3, proteins critical for neuronal migration and synaptic formation. Oxidative stress and abnormal sperm morphology correlated with altered CNTNAP2 expression, which has been implicated in synaptic organization and ASD susceptibility. These findings highlight the importance of optimizing paternal health prior to conception. Interventions targeting sperm quality, including lifestyle modifications, antioxidant supplementation, and management of metabolic health, may reduce risk of neurodevelopmental disorders in offspring.

CONCLUSION

Pre-conception sperm quality and proteomic determinants significantly influence autism-associated protein expression in Indian men. Assessing and improving sperm health before conception may serve as a preventive strategy to support early neurodevelopment and reduce ASD risk in children.

IMPORTANCE

Understanding autism risk factors before conception

Autism Spectrum Disorder (ASD) is influenced by both genetic and environmental factors. Most studies focus on maternal factors during pregnancy, but emerging evidence suggests paternal factors, including sperm quality and molecular composition, can affect the risk of ASD in offspring. Investigating pre-conception sperm allows researchers to identify early biomarkers of potential neurodevelopmental disorders in children.

Sperm quality as a marker of reproductive and neurological health

Sperm is not just a carrier of DNA; it also contains proteins, RNAs, and epigenetic markers that can influence embryonic development. Poor sperm quality-such as low motility, abnormal morphology, or DNA fragmentation may alter the expression of proteins critical for brain development, potentially increasing autism risk.

Proteins as mechanistic links

This study looks at protein determinants in sperm that influence autism-associated protein expression. Identifying these proteins helps to: Understand how paternal biology can directly affect neurodevelopment. Discover potential biomarkers for early risk assessment. Reveal molecular targets for interventions or lifestyle changes in men before conception.

Focus on Indian population

Genetic, lifestyle, and environmental factors can differ by population. Most previous research on ASD risk and paternal factors has been conducted in Western countries. Studying Indian men provides population-specific insights, which is critical for developing culturally and genetically relevant preventive strategies.

Public health implications

If certain sperm proteins are linked to higher autism risk, men could potentially modify risk factors through lifestyle changes (diet, exercise, reducing toxin exposure) before conceiving. Early identification of risk can inform family planning and counseling, potentially reducing the incidence or severity of ASD in children.

Advancing personalized medicine

This research could contribute to personalized reproductive health care, where paternal molecular profiles guide interventions to improve offspring outcomes.

DECLARATIONS

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

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Consent for publication

Not applicable.

Declaration of interest

The authors declare no conflicts of interest related to this work.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

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