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Original Research Article

Multifactorial determinants of infertility: a case control study integrating clinical, hormonal, nutritional, genetic and lifestyle factors in 664 couples

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ABSTRACT

Background: Infertility is a multifactorial reproductive disorder influenced by male, female, genetic, infectious, nutritional and lifestyle factors. Despite advances in reproductive medicine, studies integrating multiple determinants of infertility with comprehensive evaluation remain limited. The present study aimed to evaluate the multifactorial determinants associated with infertility among couples attending a tertiary fertility centre.

Methods: This hospital-based case–control study included 412 infertile couples and 252 fertile couples. Participants underwent detailed clinical and laboratory evaluation. This included complete blood counts, hormonal profiling (follicle-stimulating hormone, luteinizing hormone, anti-Müllerian hormone, prolactin, thyroid-stimulating hormone, estradiol and progesterone), semen analysis, infectious disease screening, nutritional assessment (vitamin D, vitamin B12 and ferritin), imaging studies and selected genetic investigations. Associations between infertility risk factors were analysed using the chi-square test. Odds ratios with 95% confidence intervals were calculated and a p-value<0.05 was considered statistically significant.

Results: Among infertile couples, primary infertility was observed in 77.2% and secondary infertility in 22.8%. Male factors included abnormal semen parameters in 30.6% of cases. Smoking (odds ratio 3.8, 95% confidence interval 2.2–6.4, p<0.001) and increased sperm DNA fragmentation (odds ratio 4.5, 95% confidence interval 2.0–9.7, p<0.001) were significantly associated with infertility. Among female partners, low ovarian reserve (anti-Müllerian hormone <1.1 ng/mL) was observed in 174 women (42.2%) and was significantly associated with age ≥30 years. Additional contributing factors included endometriosis (18.9%), tubal blockage (14.6%), thyroid dysfunction (9.2%) and hyperprolactinemia (6.3%). Nutritional deficiencies were common, particularly vitamin B12 deficiency (70.9%), vitamin D deficiency (45.4%) and iron deficiency (41.0%).

Conclusions: Infertility is a complex condition resulting from multiple interacting determinants including male reproductive factors, diminished ovarian reserve, gynecological disorders, nutritional deficiencies and lifestyle influences. Comprehensive evaluation of both partners is essential for early identification of modifiable risk factors and improved infertility management.

Keywords: Anti-Müllerian hormone, Infertility, Lifestyle factors, Ovarian reserve, Reproductive health, Vitamin B12 deficiency

INTRODUCTION

Infertility is defined as the failure to achieve a pregnancy after 12 months of regular unprotected intercourse and affects approximately 10–15% of couples worldwide.[1,2] It represents a significant reproductive health issue with important psychological, social and economic consequences. Despite advances in assisted reproductive technologies, identifying the exact cause remains challenging due to its multifactorial nature.[3] Both male and female factors contribute substantially to infertility. Male-related causes account for nearly one-third of cases and are commonly associated with abnormalities in sperm parameters and genetic integrity.[4,5] Lifestyle factors such as smoking, environmental exposures and poor dietary habits further negatively influence semen quality.[6,7] Female infertility is frequently linked to ovulatory dysfunction, diminished ovarian reserve, tubal abnormalities and endocrine disorders.[8] Anti-Müllerian hormone (AMH) serves as an important marker of ovarian reserve.[9] Conditions such as endometriosis and tubal blockage can further impair fertility.[10]

Recent evidence highlights the role of nutritional and lifestyle factors in reproductive health. Micronutrient deficiencies, including vitamin D, vitamin B12 and iron, have been associated with impaired hormonal balance and poor reproductive outcomes.[11,12] Lifestyle-related factors such as delayed marriage and irregular habits may also contribute.[13] However, most studies have evaluated these determinants in isolation and the combined and interactive effects remain inadequately explored. Infertility is increasingly recognized as a condition arising from complex interactions between biological, nutritional and environmental factors.[14,15]

Therefore, the present study aimed to evaluate multifactorial determinants of infertility by integrating clinical, hormonal, nutritional, genetic and lifestyle factors, with a particular focus on interaction effects and risk prediction.

METHODS

This hospital-based case–control study was conducted at a tertiary fertility care center, Merryland Women's Hospital, IVF Center and ART Bank, Ahmedabad, Gujarat, India, from February 2019 to November 2025. The study included couples attending the infertility clinic for evaluation and management during the study period. As the study involved retrospective analysis of anonymized clinical data, the requirement for individual informed consent was waived by the institutional ethics committee.

A total of 412 infertile couples were included as cases, while 252 fertile couples who had conceived naturally and had at least one live birth were included as controls. Infertility was defined as the inability to achieve pregnancy after 12 months of regular unprotected sexual intercourse, in accordance with the definition provided by the World Health Organization.[14] Couples were included if they were diagnosed with primary or secondary infertility and attended the infertility clinic for evaluation. Women of reproductive age who underwent comprehensive clinical, hormonal and imaging assessment were eligible. Fertile couples with at least one naturally conceived child were included as controls. Couples were excluded if clinical or laboratory data were incomplete, if they had not undergone adequate evaluation or if prior infertility treatment records were unavailable.

Clinical and laboratory evaluation

All couples underwent routine clinical and laboratory evaluation as part of infertility assessment. Baseline investigations included complete blood count, blood group and Rh typing, random blood sugar, serum creatinine, liver function tests and screening for infectious diseases including human immunodeficiency virus, hepatitis B surface antigen, hepatitis C virus and syphilis (VDRL test).

Hormonal assessment

Hormonal evaluation in female partners was performed during the early follicular phase (day 2–5 of the menstrual cycle) whenever applicable. Serum levels of follicle-stimulating hormone, luteinizing hormone, anti-Müllerian hormone, estradiol, progesterone, prolactin and thyroid-stimulating hormone were measured using standard laboratory methods.

Reduced ovarian reserve was defined as anti-Müllerian hormone level <1.1 ng/ml, based on commonly accepted clinical thresholds used in reproductive medicine.[16]

Nutritional assessment

Nutritional status was evaluated by measuring serum vitamin D, vitamin B12 and ferritin levels. Deficiency status was determined according to the laboratory reference ranges.

Male factor evaluation

Male partners underwent semen analysis according to the World Health Organization laboratory guidelines for examination of human semen.[14] Semen parameters evaluated included volume, sperm concentration, motility and morphology. In selected cases, sperm DNA fragmentation testing was performed.

Imaging and gynecological assessment

Female reproductive evaluation included ultrasonography of the abdomen and pelvis, transvaginal sonography and ovulation monitoring. Hysterosalpingography was performed where indicated to assess tubal patency and uterine cavity abnormalities. Gynecological conditions such as endometriosis, ovarian cysts and uterine abnormalities were recorded.

Genetic and advanced investigations

In selected couples with suspected genetic abnormalities or recurrent implantation failure, additional investigations such as karyotyping, chromosomal microarray analysis and other advanced reproductive tests were performed as clinically indicated.

Statistical analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS) version 25.0. Continuous variables were expressed as mean±standard deviation, while categorical variables were presented as frequency and percentage. Associations between variables were analysed using the chi-square test or Fisher's exact test where appropriate. Odds ratios with 95% confidence intervals were calculated to assess the strength of associations. A p value<0.05 was considered statistically significant.

RESULTS

A total of 664 couples were included in the analysis, consisting of 412 infertile couples (cases) and 252 fertile couples (controls). Baseline demographic characteristics of the study population are summarized in Table 1. The study evaluated male and female reproductive parameters, nutritional status and lifestyle factors associated with infertility. A comparison of the major infertility-related variables between infertile and fertile couples is presented in Table 2. Several reproductive, nutritional and lifestyle factors were significantly associated with infertility. Detailed comparisons between infertile and fertile couples are presented in Table 2 and Figure 1.

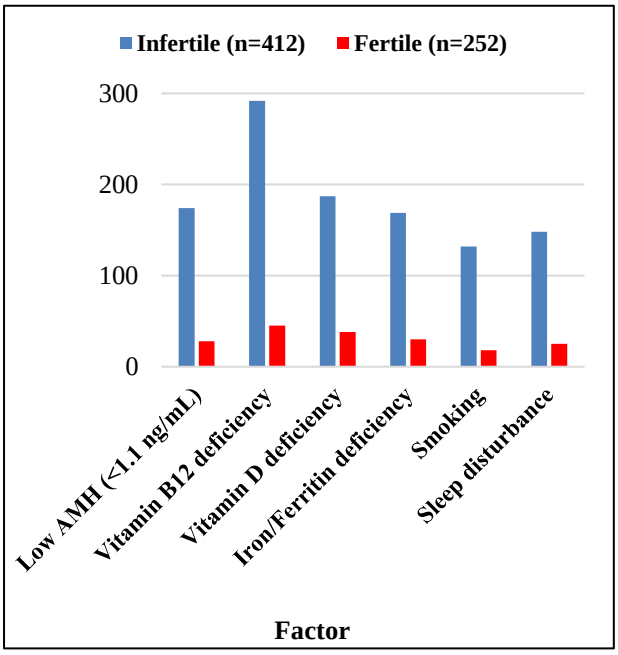
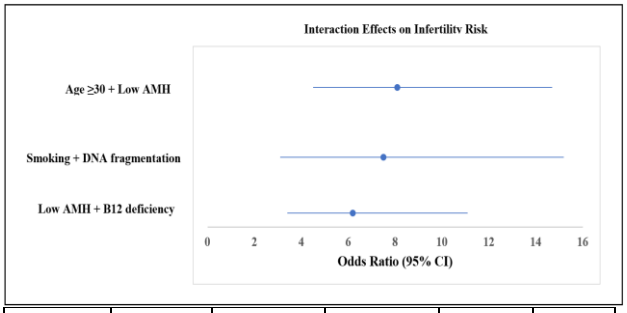


Figure 1: Comparison of selected reproductive, nutritional and lifestyle factors between infertile and fertile couples.



OR (X-Values)	Y-Values	Lower CI	Upper CI	Minus Error	Plus Error
8.1	1	4.5	14.7	3.6	6.6
7.5	2	3.1	15.2	4.4	7.7
6.2	3	3.4	11.1	2.8	4.9

Y-Values	Label
1	Age ≥30 + low AMH
2	Smoking + DNA fragmentation
3	Low AMH + B12 deficiency

Figure 2: Forest plot demonstrating interaction effects between reproductive and lifestyle factors on infertility risk. Combined exposures such as low AMH with vitamin B12 deficiency and smoking with sperm DNA fragmentation show markedly elevated odds ratios.

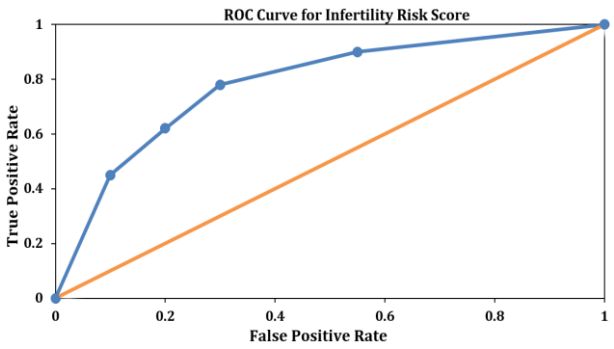


Figure 3: Receiver operating characteristic (ROC) curve for the proposed infertility risk score model showing discrimination between infertile and fertile couples.

To evaluate potential synergistic effects between reproductive and lifestyle variables, an interaction model using logistic regression was constructed. Significant interaction effects were observed. Women with both low AMH and vitamin B12 deficiency demonstrated substantially increased infertility risk compared with those with either factor alone. Similarly, the combination of smoking and high sperm DNA fragmentation was strongly associated with infertility among male partners. In addition, the interaction between age≥30 years and reduced ovarian reserve suggested accelerated ovarian aging and a higher probability of infertility. The interaction effects are summarized in table 3 and the magnitude of these interactions is visualized in the forest plot shown in Figure 2.

Multivariate logistic regression analysis

To identify independent predictors of infertility, a multivariate logistic regression analysis was performed including demographic, reproductive, nutritional and lifestyle variables. Reduced ovarian reserve (low AMH), abnormal semen parameters, tubal blockage and endometriosis were among the strongest independent predictors of infertility. Nutritional deficiencies, particularly vitamin B12 deficiency and lifestyle factors such as smoking also remained significantly associated with infertility after adjustment for potential confounders. The results of the multivariate analysis are presented in Table 4.

Development of a clinical infertility risk score

Based on the variables identified in the multivariate regression model, a composite infertility risk score was developed using clinically relevant predictors including female age, ovarian reserve status, vitamin B12 deficiency, smoking exposure and semen parameters. Higher scores were associated with progressively greater infertility probability. Individuals with scores ≥6 demonstrated the highest likelihood of infertility, indicating potential utility of this scoring system for early clinical risk stratification. The diagnostic performance of the model is illustrated in Figure 3.

Table 1: Baseline demographic characteristics.

Variable	Infertile couples (n=412)	Fertile couples (n=252)	P value
Female age (in years)	30.8±4.6	27.2±3.8	<0.001
Male age (in years)	33.5±5.2	30.1±4.4	<0.001
Duration of infertility (in years)	4.2±2.1	–	–
BMI (female)	26.1±3.4	24.3±2.8	0.002
Primary infertility (%)	77.2%	–	–
Secondary infertility (%)	22.8%	–	–

Table 2: Comparison of major infertility-related factors between infertile and fertile couples.

Factor	Infertile couples (n=412)	Fertile couples (n=252)	Odds ratio (95% CI)	P value
Male factors				
Abnormal semen parameters	126 (30.6%)	25 (9.9%)	4.2 (2.6–6.9)	<0.001
Smoking	92 (22.3%)	18 (7.1%)	3.8 (2.2–6.4)	<0.001
High sperm DNA fragmentation	47 (11.4%)	7 (2.8%)	4.5 (2.0–9.7)	<0.001
Female factors				
Low AMH (<1.1 ng/ml)	174 (42.2%)	32 (12.7%)	5.0 (3.2–7.8)	<0.001
Thyroid abnormality	38 (9.2%)	12 (4.8%)	2.0 (1.0–4.0)	0.045
Prolactin abnormality	26 (6.3%)	6 (2.4%)	2.7 (1.0–7.0)	0.043
Tubal blockage	60 (14.6%)	8 (3.2%)	5.1 (2.3–11.2)	<0.001
Endometriosis	78 (18.9%)	4 (1.6%)	14.2 (5.0–40.2)	<0.001
Nutritional deficiencies				
Vitamin D deficiency	187 (45.4%)	72 (28.5%)	2.1 (1.5–3.0)	<0.001
Vitamin B12 deficiency	292 (70.9%)	96 (38.1%)	4.1 (3.0–5.6)	<0.001
Iron/ferritin deficiency	169 (41.0%)	55 (21.8%)	2.5 (1.7–3.6)	<0.001
Lifestyle factors				
Delayed family planning	210 (51.0%)	48 (19.0%)	4.4 (3.0–6.4)	<0.001
Poor diet	145 (35.2%)	30 (11.9%)	3.9 (2.5–6.0)	<0.001
Sleep disturbance	110 (26.7%)	14 (5.5%)	6.0 (3.2–11.1)	<0.001

(CI=confidence interval).

Table 3: Interaction effects on infertility risk.

Interaction factor	Odds ratio (95% CI)	P value	Interpretation
Low AMH+Vitamin B12 deficiency	6.2 (3.4–11.1)	<0.001	Synergistic impairment of ovarian function
Smoking+high sperm DNA fragmentation	7.5 (3.1–15.2)	<0.001	Strong male factor infertility
Age ≥30+low AMH	8.1 (4.5–14.7)	<0.001	Accelerated ovarian aging

(CI=confidence interval).

Table 4: Multivariate logistic regression analysis of infertility predictors.

Variable	Adjusted odds ratio (AOR)	95% CI	P value
Age ≥30 years	2.6	1.8–3.9	<0.001
Low AMH (<1.1 ng/ml)	4.8	3.1–7.4	<0.001
Abnormal semen parameters	3.7	2.3–5.9	<0.001
Vitamin B12 deficiency	2.1	1.4–3.0	<0.001
Vitamin D deficiency	1.5	1.0–2.3	0.041
Iron/ferritin deficiency	1.7	1.1–2.5	0.019
Smoking	2.9	1.7–4.8	<0.001
Endometriosis	3.5	1.9–6.3	<0.001
Tubal blockage	4.1	2.0–8.4	<0.001
Thyroid dysfunction	1.8	1.0–3.3	0.047

(CI=confidence interval).

Cluster analysis of infertility phenotypes

Cluster analysis was performed to explore potential subtypes of infertility within the study population. Three distinct infertility phenotypes were identified. The first cluster represented an ovarian aging phenotype, characterized by increased female age, reduced ovarian reserve markers and endometriosis.

The second cluster represented a nutritional-metabolic phenotype, characterized by micronutrient deficiencies and endocrine abnormalities. The third cluster corresponded to a male factor phenotype, defined by abnormal semen parameters, smoking exposure and elevated sperm DNA fragmentation. These findings suggest that infertility arises from multiple interacting biological pathways rather than a single isolated cause.

DISCUSSION

Infertility is increasingly recognized as a multifactorial disorder resulting from complex interactions between biological, environmental and lifestyle-related factors. In the present study, we provide an integrated evaluation of clinical, hormonal, nutritional and behavioral determinants, highlighting not only individual risk factors but also their combined effects on reproductive outcomes. Unlike traditional approaches that assess isolated variables, our findings emphasize the importance of a multidimensional framework in understanding infertility risk. These findings reinforce the need for a paradigm shift from isolated risk assessment to an integrated, systems-based approach in infertility evaluation and management.[3,14,15]

Advancing female age showed a strong association with infertility, consistent with the known decline in ovarian reserve and oocyte quality after 30 years of age.[18,19] Low AMH levels remained an important predictor and may be useful in clinical decision-making and patient counseling.[16,17] Male-related factors also played a significant role. Abnormal semen parameters and increased sperm DNA fragmentation were strongly associated with infertility, emphasizing the importance of evaluating the male partner.[4,5] These findings align with evidence highlighting the role of oxidative stress and environmental exposures in male infertility.[21]

Among female factors, endometriosis and tubal blockage were important contributors, as they interfere with normal reproductive processes including gamete transport, fertilization and implantation.[10] An important observation in this study was the high prevalence of nutritional deficiencies, particularly vitamin B12, vitamin D and iron deficiency. These deficiencies were independently associated with infertility, suggesting their role in hormonal regulation and reproductive function.[23] Lifestyle factors such as smoking further increased infertility risk. Smoking is known to induce oxidative stress and negatively affect both ovarian function and sperm quality.[24,25] Interaction analysis demonstrated that combined risk factors had a greater impact than individual factors alone. For example, low AMH combined with vitamin B12 deficiency and smoking combined with sperm DNA fragmentation showed markedly increased odds of infertility. A key novel contribution of this study is the identification of a nutritional–reproductive axis, where micronutrient deficiencies interact with hormonal and clinical factors to influence fertility outcomes. These findings suggest a synergistic rather than additive effect and highlight the importance of integrating nutritional assessment into reproductive evaluation. The predictive model developed in this study may have practical clinical utility in identifying high-risk individuals and guiding early intervention strategies.

Recent global guidelines emphasize that infertility should be evaluated as a multifactorial condition rather than a single-cause disorder. Recommendations support an integrated approach combining clinical, laboratory and lifestyle factors.[20] Evidence from recent studies further highlights the role of ovarian reserve, oxidative stress and systemic influences on reproductive outcomes.[3,21,22] From a clinical perspective, these findings support a shift toward a multidimensional and individualized risk assessment approach. Incorporating nutritional screening, lifestyle modification and combined risk profiling into routine

infertility workup may improve early detection and enable targeted interventions.

This study has certain limitations. Being a hospital-based case–control study, the findings may not be generalizable to the broader population. Some advanced genetic investigations were performed only in selected cases, which may limit the comprehensive evaluation of genetic factors. Additionally, lifestyle factors were partly self-reported and may be subject to recall bias. Despite these limitations, the study provides a comprehensive assessment of multifactorial determinants of infertility using an integrated clinical and laboratory approach.

CONCLUSION

Infertility is a multifactorial condition influenced by age, ovarian reserve, male reproductive parameters, gynecological disorders, nutritional deficiencies and lifestyle behaviors. This study highlights advanced maternal age, low AMH, abnormal semen parameters, endometriosis and tubal blockage as significant determinants. Modifiable factors, particularly micronutrient deficiencies and smoking, also contribute substantially to infertility risk.

The findings underscore the importance of comprehensive evaluation of both partners and support the development of predictive frameworks integrating clinical, biochemical and lifestyle variables. Early identification and correction of modifiable risks may improve reproductive outcomes, guide counselling and inform timely referral for specialized care. Future studies with larger and more diverse populations are warranted to validate these results and refine predictive models for evidence-based infertility management.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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