Original Article

Exploring the link between sperm DNA fragmentation index and recurrent abortions in infertility centers

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ABSTRACT

This research endeavors to explore the correlation between the sperm DNA fragmentation index (DFI) and recurrent miscarriage in women seeking fertility treatment. 81 participants who experienced recurrent miscarriages from March 2020 to March 2022 and had their sperm DFI measured were selected from infertility centers in Ahvaz, Iran, and compared to the control group. Sperm volume, count, motility, and morphology were assessed. Sperm DNA fragmentation was examined using the TUNEL method and flow cytometry. Demographic information from patient records and the results of TSH, Anti-TPO, FSH, and AMH tests were collected and evaluated. The average age of males and females in the two groups with a history of miscarriage and those without showed a significant difference ($p<0.05$). Furthermore, this study found a higher prevalence of irregular menstrual cycles in the group with a history of miscarriage compared to the group without it ($p<0.05$). Furthermore, no significant differences were observed between the BMI of males and females, alcohol consumption, smoking habits, and drug usage in the two groups. Regarding laboratory variables, TSH and DFI levels were higher in the group with a history of miscarriage, while sperm counts, progressive motility, and morphology were higher in the group without miscarriage ($p<0.05$). However, the two groups had no significant differences in Anti-TPO, FSH, and AMH levels. Investigating paternal factors such as sperm counts, volume, motility, morphology, and DFI is critical in assessing recurrent miscarriages in women. These factors can contribute to diagnostic improvements.

Keywords: DNA fragmentation , semen analysis, recurrent abortion, paternal age, maternal age, menstural cycle.

Introduction

In numerous societies, fertility is esteemed. The yearning for offspring is a fundamental human instinct [1]. Failure in conception can lead to emotional turmoil. Childbirth solidifies a woman's identity, as they often associate their biological, psychological, and social triumphs with their ability to bear a child. A sense of inadequacy may arise if this ability is absent. Childlessness can occur due to several factors, including abortion [2].

Approximately 50 to 60% of pregnancies are terminated in the initial weeks following conception. This often goes unnoticed by the expectant mothers, who mistake the bleeding from the abortion for a regular menstrual cycle. A noticeable end to pregnancy occurs in 12 to 15% of cases [3]. Infertility and recurrent miscarriages pose significant individual and societal challenges, leading to profound distress or even tragedy for the families affected. These circumstances can subject individuals to a variety of psychological stresses. Impulsive behavior, diffuse anger, stress, feelings of helplessness, worthlessness, and inadequacy, anxiety, worry, particularly during prolonged and unsuccessful treatments, negative self-perceptions, concerns about sexual attractiveness, feelings of rejection, intense fear, and diminished self-esteem are among the psychological issues reported by researchers [4]. Following such life events, families often seek answers to questions about the cause of the abortion or fetal death, the origins of complications, their role in these complications, genetic and hereditary factors, repeated abortions, and how to ensure health in subsequent pregnancies - answers that are often elusive. Recurrent miscarriage is losing a fetus in three or more consecutive pregnancies before the 20th week of gestation [5].

Abortions post the 20th week are termed premature births. They typically have distinct causes compared to early pregnancy abortions. Recurrent miscarriage is a multifactorial disease, implicating numerous factors [6]. These include uterine structural disorders, hormonal imbalances, chromosomal abnormalities, polycystic ovary syndrome, autoimmune diseases, and coagulation disorders. Genetic factors, including polymorphisms, also play a role. Interestingly, the cause of at least 50% of recurrent abortions remains undetermined [7].

World Health Organization statistics reveal that approximately 40% of infertility and recurrent miscarriages are attributed to male fertility disorders. The condition of sperm chromatin structure serves as a crucial determinant of seminal fluid quality, thereby predicting male fertility [8]. Contemporary research indicates that men can also contribute to frequent miscarriages due to various factors, encompassing genetic elements, semen and sperm-related issues, and even the man's age. Problems associated with sperm include sperm aneuploidy, nuclear chromatin decondensation, and factors leading to sperm DNA fragmentation. Genes implicated in recurrent abortions, categorized as factors contributing to male infertility, comprise mitochondrial genes, DNA polymerase gamma (POLG), Deleted in Azoospermia (DAZ), Transition Protein (TNP), and Endothelial Nitric Oxide Synthase (eNOS) [9].

Research indicates a correlation between sperm quality and the health of sperm DNA. Lower-quality sperm often presents more issues. Individuals with defective sperm or unexplained infertility are potential candidates for assisted reproductive techniques. However, DNA damage in these men's sperm can lead to treatment failure [10]. Therefore, assessing the fragility of sperm DNA is crucial before selecting an appropriate treatment method. In many instances, if DNA damage is detected, sperm quality can be enhanced through suitable treatment. The SDFA (Sperm DNA Fragmentation Assay) test evaluates sperm DNA health. This reliable test aids in identifying individuals potentially at risk of an unhealthy pregnancy. Understanding the health of sperm DNA is instrumental in the clinical diagnosis and treatment of male infertility, significantly contributing to the success of their treatment strategies [11].

This current research aims to ascertain the correlation between the index of sperm DNA fragmentation and repeated miscarriages in women seeking help from infertility clinics in Ahvaz, Iran.

Materials and Methods

This study is experimental research approved as a thesis with an Ethical code number of "IR.AJUMS.HGOLESTAN.REC.1401.021" in Ahvaz Jundishapur of Medical Sciences, Ahwaz, Iran.

Following the necessary approvals from the Research Council and the Ethics Committee, this study was conducted on a comprehensive population of women with a history of recurrent abortion who sought care at infertility centers in Ahvaz. Employing a census sampling method, all women experiencing recurrent abortion from March 2020 to March 2022 and seeking care at Ahvaz infertility centers were included. Patients without recurrent abortion but with recorded sperm DNA fragmentation index in their medical records were selected as the control group for investigation.

Information regarding age, body mass index (BMI), alcohol consumption, smoking status, medication use, and menstrual status (regular or irregular) was collected through a checklist. Laboratory data, including Thyroid-Stimulating Hormone (TSH), Antithyroid Peroxidase Antibodies (Anti TPO), Follicle-Stimulating Hormone (FSH), and Anti-Müllerian Hormone (AMH) levels, were extracted from patients' medical records and evaluated.

The inclusion criteria encompassed all women with a history of two or more miscarriages and aged below 35 years. The exclusion criteria consisted of the presence of uterine septum or uterine anomalies such as Asherman syndrome, women aged 35 or older, women with cervical insufficiency, women with a history of antiphospholipid syndrome (APS), men with obstructive and non-obstructive azoospermia, and individuals who declined to participate in the study.

Measurement of DNA Fragmentation Index (DFI)

Initially, sperm samples were collected in sterile plastic containers and incubated at 37 degrees Celsius. Subsequently, using disposable syringes, the sample volume was measured. Immediately after liquefaction, a drop of well-mixed semen was placed between a slide and a coverslip and examined under a microscope at 100x magnification. The total sperm count was determined by using a Neubauer chamber. Sperm motility (total, slow, rapid, and immotile) and visual sperm morphology were also assessed using a microscope. Furthermore, thin smears were prepared on two slides from each patient. After air drying and fixation using the Papanicolaou method, staining was performed, and the morphological analysis was carried out under 1000x magnification. In this study, sperm morphology was categorized into four groups based on the World Health Organization classification: normal, abnormalities in the head, tail, and midpiece.

Sperm DNA fragmentation was examined using the TUNEL method and analyzed via flow cytometry. The interpretation of the percentage of sperm containing damaged DNA was reported in four statistical categories with fertility likelihood. DFI was categorized as follows: DFI $\leq$ 15%: Excellent to good sperm DNA integrity, DFI > 15% and $\leq$ 25%: Good to fair sperm DNA integrity, DFI > 25% and $\leq$ 50%: Fair to poor sperm DNA integrity, DFI $\geq$ 50%: Very poor sperm DNA integrity.

Statistical Analysis

The data were collected and analyzed using SPSS software version 22, employing descriptive statistics for qualitative data (utilizing frequencies and percentages) and quantitative data (mean $\pm$ standard deviation). The Chi-squared test was

employed to investigate the association between nominal variables, while the Kolmogorov-Smirnov test was used to assess the normality of quantitative data distributions. Independent t-tests, non-parametric Mann-Whitney U tests, and Kruskal-Wallis tests were applied to compare quantitative variables. P-values less than 0.05 were considered significant.

Results and Discussion

In this investigation, 81 participants (50%) with a history of miscarriage and 81 (50%) without a history of miscarriage were analyzed. Subsequently, the miscarriage cohort underwent meticulous analysis, resulting in its subdivision into three discrete categories: miscarriage during the initial trimester (n=67, 41.4%), miscarriage in the second trimester (n=5, 3.1%), and concurrent fetal loss in both trimesters (n=9, 5.5%)—as comprehensively detailed in **Table 1**.

Table 1. The frequency of individuals in the study groups.

Variable	N (%)
Without abortion	81(50)
With abortion	81 (50)
History of abortion in the first trimester of pregnancy	67 (41.4)
History of abortion in the second trimester of pregnancy	5 (3.1)
History of abortion in both trimesters	9 (5.5)

The average age of males in the examined groups, distinguished by the presence or absence of prior miscarriages, is 36.68 and 38.68, respectively, revealing a statistically significant disparity between the two groups ($p<0.05$). Correspondingly, the mean age for female participants in the same groups, with and without a history of miscarriage, stands at 30.70 and 31.90, respectively, demonstrating a significant difference ($p<0.05$). In the group without a history of miscarriage, 70 subjects (87.5%) exhibited regular menstrual cycles, while 10 (12.5%) reported irregular menses. Conversely, in the cohort with a history of miscarriage, 60 individuals (74.1%) experienced regular menstruation, whereas 21 (25.9%) encountered irregular menses, underscoring a substantial variance between the two categories ($p<0.05$ Analysis of **Table 2** reveals that, there is no significant difference between BMI in men and women, alcohol consumption, smoking status, and medication use in the two groups under investigation ($p> 0.05$) **(Table 2)**.

The average TSH concentrations in the two groups, with and without prior miscarriages, are 2.14 and 2.82, respectively, evidencing a statistically significant distinction ($p<0.05$). Moreover, the DFI in the two groups, without a history of miscarriage and with a history of miscarriage, is 15.78 and 25.94, respectively. Sperm quantification in these groups is 51,407,894.74 and 25,782,051.28, respectively. Progressive motility percentages manifest as 42.96 and 24.85, while sperm morphology indices exhibit values of 2.48 and 1.05 in the two groups. The observed discrepancies achieve statistical significance by applying the Mann-Whitney non-parametric test ($p<0.05$). Consequently, TSH and DFI concentrations are notably elevated in the group with a history of miscarriage compared to their counterparts without such a history. Correspondingly, sperm count, progressive motility, and sperm morphology present higher values in the group lacking a history of miscarriage. However, no significant differences emerge between the two groups concerning Anti-TPO, FSH, and AMH ($p>0.05$) **(Table 2)**.

Table 2. The correlation of demographic and laboratory variables in two groups: women with a history of abortion (Group B) and women without a history of abortion (Group A).

	Variable	Group A	Group B	p-value
Demographic variables	Men's age [a]	38.68 ± 5.36	36.68 ± 5.56	0.033*
	Women's age [a]	31.90 ± 3.07	30.70 ± 3.61	0.038*
	Men's BMI [a]	26.66 ± 2.48	26.71 ± 3.10	0.855
	Women's BMI [a]	27.50 ± 4.04	27.03 ± 3.48	0.423
	Alcohol consumption [b]	No: 78(96.3) Yes: 3(3.7)	No: 80(98.8) Yes: 1(1.2)	0.620
	Smoking [b]	No: 59(72.8) Yes: 22(27.2)	No: 60(74.1) Yes: 21(25.9)	0.859
	Medication use [b]	No: 81(100.0) Yes: 0(0.0)	No: 80(98.8) Yes: 1(1.2)	1.000
	Menstruation [b]	Regular: 70(87.5) Irregular: 10(12.5)	Regular: 60(74.1) Irregular: 21(25.9)	0.031*
Laboratory variables	TSH [c]	2.14 ± 1.26	2.82 ± 1.83	0.006*
	Anti-TPO [c]	19.00 ± 45.06	44.41 ± 95.57	0.144
	FSH [c]	6.40 ± 2.32	7.17 ± 3.70	0.367
	AMH [c]	2.43 ± 1.88	2.82 ± 2.86	0.562
	DFI [c]	15.78 ± 2.59	25.94 ± 6.19	<0.0001*
	Sperm count [c]	51407894.74 ± 12055624.006	25782051.28 ± 12497945.219	<0.0001*
	Progressive movement [c]	42.96 ± 4.66	24.85 ± 4.69	<0.0001*

	Sperm Morphology [c]	2.48 ± 0.77	1.05 ± 0.27	<0.0001*

***: Statistically significant**
a: based on mean ± standard deviation; Independent T-test
b: based on number (%); Chi-square test
c: Based on mean ± standard deviation; Nonparametric Mann-Whitney test

In this investigation, individuals with a history of miscarriage underwent further subdivision into three distinct subgroups. A comparison was made between demographic variables in the group lacking a history of miscarriage against the three subgroups marked by a history of miscarriage **(Table 3)**. Upon scrutinizing these comparisons, no statistically significant differences were found in all the studied variables, encompassing the age of men and women, BMI of both genders, alcohol consumption, smoking status, medication use, and menstrual regularity, within the study groups (p>0.05). Additionally, no significant differences were observed among the examined groups regarding Anti-TPO, FSH, and AMH variables (p>0.05). However, significant differences were evident among the studied groups in terms of the variables TSH, DFI, sperm count, Progressive motility, and morphology (p<0.05) **(Table 3)**.

Table 3. Correlation of demographic and laboratory variables in the group without a history of abortion (A) and subgroups of women with a history of abortion

	Variable	Group A	Abortion in the first trimester	Abortion in the second trimester	Abortion in both trimesters	p-value
Demographic variables	Men's age [a]	38.68 ± 5.36	36.55 ± 5.65	35.80 ± 6.57	38.11 ± 4.54	0.151
	Women's age [a]	31.90 ± 3.07	30.73 ± 3.74	30.20 ± 1.64	30.78 ± 3.59	0.224
	Men's BMI [a]	26.66 ± 2.48	26.69 ± 3.18	24.69 ± 2.82	28.05 ± 2.09	0.194
	Women's BMI [a]	27.50 ± 4.04	27.26 ± 3.53	23.91 ± 2.44	27.04 ± 2.98	0.229
	Alcohol consumption [b]	No: 78(96.3) Yes: 3(3.7)	No: 66(98.5) Yes: 1(1.5)	No: 5(100.0) Yes: 0(0.0)	No: 9(100.0) Yes: 0(0.0)	0.769
	Smoking [b]	No: 59(72.8) Yes: 22(27.2)	No: 48(71.6) Yes: 19(28.4)	No: 4(80) Yes: 1(20)	No: 8(88.9) Yes: 1(11.1)	0.720
	Medication use [b]	No: 81(100.0) Yes: 0(0.0)	No: 66(98.5) Yes: 1(1.5)	No: 5(100.0) Yes: 0(0.0)	No: 9(100.0) Yes: 0(0.0)	0.699
	Menstruation [b]	Regular: 70(87.5) Irregular: 10(12.5)	Regular: 50(74.6) Irregular: 17(25.4)	Regular: 3(60.0) Irregular: 2(40.0)	Regular: 7(77.8) Irregular: 2(22.2)	0.145
Laboratory variables	TSH [a]	2.14 ± 1.26	2.64 ± 1.60	3.38 ± 1.48	3.80 ± 3.07	0.022*
	Anti-TPO [a]	19.00 ± 45.06	39.28 ± 82.76	10.02 ± 11.31	99.99 ± 171.72	0.444
	FSH [a]	6.40 ± 2.32	7.27 ± 3.65	6.03 ± 3.27	7.14 ± 4.52	0.576
	AMH [a]	2.43 ± 1.88	2.79 ± 2.55	5.39 ± 6.54	1.60 ± 0.86	0.221
	DFI [a]	15.78 ± 2.59	26.06 ± 6.23	22.20 ± 2.58	27.22 ± 7.04	<0.0001*
	Sperm count [a]	51197530.86 ± 12467377.18	24268656.72 ± 7093706.32	26800000.00 ± 9679876.03	32888888.89 ± 32048574.24	<0.0001*
	Progressive movement [a]	42.92 ± 4.62	25.11 ± 4.30	24.40 ± 5.17	22.00 ± 6.96	<0.0001*
	Sperm Morphology [a]	2.14 ± 1.26	2.64 ± 1.60	3.38 ± 1.48	3.80 ± 3.07	0.022*

***: Statistically significant**
a: based on mean ± standard deviation; Kruskal-Wallis test
b: based on number (%); Chi-square test

This study aimed to explore the relationship between sperm DFI and recurrent abortion in women seeking infertility treatment at Ahvaz County infertility centers from March 2020 to March 2022.

Within the group marked by a history of recurrent abortion in this investigation, the incidence rates of fetal loss within the first and second trimesters were 41.4% (n=67) and 3.1% (n=5), respectively. Additionally, 5.5% (n=9) experienced pregnancy loss across both trimesters. Consequently, it can be inferred that most individuals with a history of abortion experienced it during the first trimester of gestation. Notably, chromosomal abnormalities emerge as the foremost causative factor for fetal loss in the first trimester, spontaneously identified in 50% to 85% of pregnancy tissue samples following miscarriage [12]. It is estimated that 26% of all pregnancies result in fetal loss. Furthermore, 80% of pregnancy losses occur during the first trimester, wherein the risk diminishes post the 12th week of pregnancy [13].

In this study, it was discerned that the mean age of both males and females within the group marked by a recurrent abortion history was notably inferior compared to those lacking such a reproductive history (p<0.05). Nevertheless, in contrast to the present study's outcomes, it is worth noting that parental age could potentially be linked to fetal loss. Specifically, SDF levels exhibit an incremental pattern with advancing age, starting in young adulthood and doubling between 20 and 60 [14, 15]. This relationship is predominantly attributed to increased oxidative stress, chromatin damage in sperm, and irregular apoptosis, which also increases with age [16]. A multicenter European study in 2002 revealed an increased risk of fetal loss in couples where

women were over 35 years old and men were over 40, compared to couples with varying age ranges [17]. A plausible rationale for the observed phenomena may stem from elevated levels of sperm DNA damage in individuals of advanced age. This propensity is postulated to arise from the accrual of double-strand DNA breaks within the sperm of older males [18]. Furthermore, the likelihood of producing offspring with aneuploid structures increases in older men, given the heightened incidence of chromosomal aberrations in their sperm. Additionally, the age-related decline in egg quality underscores the diminished inherent capacity for repairing sperm DNA damage within the eggs of older females [19]. However, in the present study, the group without a history of fetal loss had a higher average age, which does not align with the findings from the studies mentioned above.

The mean BMI for males and females within the two groups failed to manifest a statistically significant difference ($p>0.05$). Consequently, it can be inferred that BMI did not exert a discernible influence on the incidence of miscarriage within the purview of this study. Contrary to these findings, other research posits that individuals classified as obese tend to exhibit heightened levels of oxidative stress and SDF in comparison to those with average or overweight BMI status [20-22]. Mechanisms linking obesity to altered sperm function and diminished fertility potential involve factors such as elevated testicular temperature, hormonal imbalances, and chronic systemic inflammation. Furthermore, substantial evidence indicates that weight loss interventions have yielded notable improvements in SDF levels and overall fertility outcomes [23, 24]. However, in the context of the current investigation, a meaningful association between BMI and the two cohorts of men and women was not discerned, contrasting with the abovementioned findings. An analysis conducted in 2008, comprising initial studies on infertile populations, revealed a significant increase in the rate of fetal loss when comparing women with a BMI equal to or surpassing 25 kg/m² to those with a BMI below 25 kg/m² [25]. This inclination toward an elevated risk of fetal loss is observed even in women with a history of pregnancy loss. It is important to note that a statistically significant increase in the risk of fetal loss was observed solely in obese women (BMI ≥ 30 kg/m²) [26]. Furthermore, logistic regression analysis demonstrated that, beyond maternal age, an increase in BMI is the most crucial factor in predicting fetal loss in women with a history of pregnancy loss. A systematic review encompassing five retrospective studies, one prospective study, and approximately 30,000 women examined the relationship between fetal loss and obesity (BMI ≥ 28 or 30 kg/m²) following conception. This study revealed a significant association between obesity and fetal loss in sporadic pregnancies and those with a history of pregnancy loss [27]. This warrants further research to evaluate the impact of reducing BMI.

The two groups, one with a history of miscarriage and the other without, did not exhibit statistically significant differences in alcohol consumption, tobacco use, and medication intake ($p>0.05$). This suggests that these factors had no discernible impact on miscarriage rates in this study. Notably, tobacco use during pregnancy carries heightened significance as the effects of smoking on the health of pregnant women and their fetuses are of particular concern. These effects include reduced fetal size, increased risk of stillbirth, higher perinatal mortality rates, miscarriage likelihood, elevated risk of preterm birth, early lung aging, and the development of chronic obstructive pulmonary diseases [28, 29]. Given these repercussions, it is crucial to address this issue, as jeopardizing the health of both mother and fetus can have adverse effects on family and societal well-being, with consequences affecting psychological, social, economic, and communal aspects. The harmful effects of maternal smoking can extend damage to future generations in any society. Tobacco use can detrimentally impact DNA integrity in males through the influence of metabolites such as nicotine, cadmium, lead, and benzo[a]pyrene [30-32]. Furthermore, alcohol consumption has been implicated in adverse effects on SDF by amplifying DNA damage and apoptosis [33, 34]. Elevated rates of fetal loss have been documented in women with alcohol consumption, although the precise direct effects of alcohol and the secondary consequences of alcohol dependence, such as cirrhosis, remain ambiguous. Abel *et al*. noted that blood alcohol levels exceeding 200 mg/dl can induce spontaneous fetal loss [35]. However, the relationship between moderate alcohol consumption and involuntary fetal loss is not well-defined. Several studies support this association, exemplified by the investigation conducted by Harlap and Shiono, which posits that the risk of fetal loss is discernible only in women who consistently and regularly partake in alcohol consumption while absent in those who consume alcohol sporadically [36]. In the Anokute study, a dose-response relationship between alcohol consumption and fetal loss was observed [37]. On the other hand, the Parazzini study refutes the connection between alcohol and fetal loss [38]. The precise mechanism by which alcohol exerts its detrimental effects on the fetus remains unclear. Alcohol can pass through the placental barrier and reach the same plasma levels as in the mother's bloodstream. Alcohol likely has direct toxicity, but one of its metabolites, acetaldehyde, is teratogenic and accumulates in the fetus [39].

The present study observed a significant difference between the two groups in menstruation regularity (regular and irregular). Irregular menstruation was more prevalent in the group with a history of miscarriage compared to the group without ($p<0.05$). Therefore, the findings suggest that irregular menstrual cycles are more prevalent in the group with a history of miscarriage compared to the group without a history of miscarriage. A successful pregnancy begins with the implantation of a healthy embryo in a receptive uterine environment. It's important to note that disruptions in the early stages of the implantation process can lead to fetal loss. To prepare for implantation, the endometrium undergoes reconstruction, regulated by the ovarian hormones estradiol and progesterone during the menstrual cycle. This reconstruction peaks during a specific window where the embryo can be implanted. This implantation window is limited to the mid-secretory phase of the menstrual cycle when the uterine epithelium allows for the attachment of the embryonic trophoblast cells (the outer cells of the

blastocyst). In contrast, in other phases of the menstrual cycle, trophoblast cells and various types of endometrial epithelial cells do not adhere, potentially resulting in irregular menstrual cycles that can lead to fetal loss [40].

In the current study, no significant differences were observed between the two groups regarding Anti-TPO, FSH, and AMH variables ($p>0.05$). However, significant differences were found in TSH, DFI, sperm count, progressive motility, and morphology ($p<0.05$). Specifically, TSH and DFI levels were higher in the group with a history of miscarriage. At the same time, sperm count, progressive motility, and morphology were higher in the group without such history. Furthermore, among the subgroups with a history of miscarriage, the DFI level was higher in the subset with miscarriage in the first trimester compared to the other subgroups. The lowest sperm count was also observed in the subset with miscarriage in the first trimester. Elevated levels of SDF have been reported in men with testicular cancer and other malignancies. This suggests a secondary association with changes in endocrine glands or oxidative stress (OS) in these diseases [41-43]. In the present study, only a significant difference was observed between the two groups regarding TSH levels, while Anti-TPO and FSH did not exhibit substantial differences. Thyroid dysfunction, with or without thyroid autoimmunity, is significantly common in fertile women of childbearing age. Studies indicate that thyroid disorders and the presence of thyroid antibodies may be associated with infertility and pregnancy loss in women, whether they are euthyroid with thyroid antibodies or have elevated levels of TSH [44]. Based on a meta-analysis of 38 different studies, the presence of Anti-TPO increases the risk of fetal miscarriage with an odds ratio of 3.73 (95% confidence interval (CI) 1.8 to 7.6). Additionally, it elevates the risk of pregnancy loss with an odds ratio of 2.3 (95% CI 1.5 to 3.5) [45]. Another study, which included pregnant women without thyroid antibodies, showed that having TSH levels within the normal range but above 2.5 mIU/L in the first trimester nearly doubles the risk of fetal miscarriage [46]. However, the true significance of thyroid dysfunction and its impact on improving outcomes in individuals with a history of pregnancy loss remains uncertain.

Sperm DNA integrity is a fundamental determinant of natural fertilization and embryonic development. However, sperm with DNA damage retain their potential to fertilize an egg [47]. In Robinson's meta-analysis, a significant association between high levels of sperm DNA damage and fetal miscarriage was also demonstrated [48], aligning with the present study's findings. It has been observed that the level of DNA damage in ejaculated sperm is significantly higher than that in testicular sperm, suggesting that the most substantial DNA damage occurs post-testicular exit [49]. Therefore, individuals with a positive and high level of sperm DNA damage may still have the potential to conceive a healthy infant.

Sperm DNA damage can result from six primary mechanisms, including apoptosis during sperm production, alterations in chromatin structure during sperm production, fragmentation of DNA segments due to the influence of reactive oxygen species in the sperm transport pathway from the testes, fragmentation of DNA segments caused by endonucleases, DNA damage due to radiotherapy and chemotherapy treatments, and DNA damage from environmental factors such as smoking and air pollution [50]. In women experiencing recurrent miscarriages, a significant reduction in the frequency of abnormal karyotypes in fetuses is observed concerning their history of previous miscarriages. This observation suggests that in this population, other factors, including possibly sperm DNA damage, might also play a role [51].

Conclusion

The significance of reproductive health is undeniable, influencing the experiences of numerous individuals and couples. It is crucial to acknowledge that single factors do not determine fertility and recurrent miscarriages but rather emerge from the complex interplay of genetics, lifestyle choices, and environmental factors. The study's findings underscore the significance of examining paternal factors, including sperm count, volume, motility, morphology, and sperm DNA fragmentation index, in investigating recurrent miscarriages in women. These factors can contribute to diagnostic improvements.

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Ethics statement:

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