Study impact smoking on Sperm Morphology and DNA fragmentation in Iraqi male fertility

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Abstract

Background: Male infertility is issue a prevalent, involves various factors. Sperm quality, particularly influenced by smoking, is crucial for fertility. DNA fragmentation, indicating damage to DNA strands in sperm cells, is gaining importance in male infertility research, especially concerning abnormal sperm morphology.

Aim: To investigate the potential influence of smoking on both sperm morphology and DNA fragmentation in male fertility.

Materials and methods: In this cross-sectional observational study, 83 participants (38 nonsmokers and 45 smokers) with Normozoospermia provided informed consent. Semen samples were collected following at WHO 2021 guidelines. Sperm morphology was evaluated using Kruger Strict Criteria with Hematoxylin stain, and DNA fragmentation index was assessed using Aniline Blue Stain and Sperm Chromatin damage.

Results: The significant correlation signs were especially clear in various parameters, including sperm concentration ($P = 0.000484$), sperm morphology ($P = 0.0001$), as well as specific morphological characteristics such as pin & small head ($P = 0.039$), round head ($P = 0.002$), tapered head ($P = 0.008$), irregular neck ($P = 0.002$), short tail ($P = 0.020$), sperm nuclear maturity ($P = 0.048$), and sperm chromatin dispersion ($P = 0.042$). Notably, no significant correlation was found between smoking and non-smoking individuals with normal sperm morphology and undamaged DNA in sperm.

Conclusion: Show a links between sperm abnormal morphology and DNA damage in smoking and nonsmoking. Additionally, both smokers and nonsmokers with abnormal sperm morphology according to the Tygerberg criteria exhibited a notable rise in DNA damage index as well as appear important to use Kruger strict criteria to detect sperm morphology in routine semen analysis.

Keywords: DNA Fragmentation index, Smokers, Sperm Morphology, Hematoxylin Stain and Kruger Strict Criteria

Introduction

Male infertility is the inability to fertilize as a result of problems in the reproductive system (Mustafa et al., 2019). A factor for male infertility includes; 1: sperm production problems, these are oligospermia, teratozoospermia, asthenozoospermia, azoospermia, and sertoli cell syndrome only (Babakhanzadeh et al., 2020). 2: problems related to the components of sperm cells; quality and motility, and DNA fragmentation or damage (Kolesnikova et al., 2015). 3: Obstructive factors, which are ejaculatory duct obstruction and obstructive azoospermia (Krausz & Cioppi, 2021). 4: Hormonal imbalances are Low testosterone or an imbalance in luteinizing and follicle-stimulating hormones might impact the development of sperm cells (Al-Darawsha, 2023). 5: Genetic factors that include Klinefelter syndrome, chromosomal translocations, Y chromosome micro deletions, and Noonan syndrome (Krausz & Riera-Escamilla, 2018). 6: Varicocele are enlarged veins that raise the testicle's temperature (Sasson & Kashanian, 2020). 7: Infections pertaining to the reproductive system, such as sexually transmitted diseases (Sherrard et al., 2018). 8: Lifestyle factors such as high blood pressure, smoking, alcohol drinking, abusing drugs, and being obese (Durairajanayagam, 2018). 9: Toxins, radiation, and occupational hazards are examples of environmental influences (Krzastek et al., 2020). 10: Medications and medical conditions related to a long-term illness and some drugs that

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interfere with fertility (Leisegang et al., 2021). 11: As a men age, their sensitivity to DNA damage rises and their sperm counts decrease (Condorelli et al., 2020). 12: Higher DNA fragmentation is linked to a higher body mass index, which influences sperm quality (Bellastella et al., 2019).

Smoking and its public health risks associated with various diseases are well known (DeLancey et al., 2018; Salonia et al., 2021). The focus has now switched to how it affects male reproductive health, particularly sperm quality (Qiu et al., 2020). Biomarkers of male infertility include abnormal sperm shape, motility, vitality, and fragmentation in the DNA strand (Boe-Hansen et al., 2018; Ferlin et al., 2022), which can impact the likelihood of pregnancy. Recent data emphasizes smoking's numerous detrimental effects on sperm abnormalities and quality (Omolaoye et al., 2022; Walke et al., 2023), particularly with regard to sperm shape and DNA integrity. It is crucial for reproductive health (Scieszka, 2023) to comprehend the connection between male smokers and alterations in sperm shape and DNA damage since this affects sperm function and a couple's capacity to conceive (Fair et al., 2019; Skakkebaek et al., 2022). Tobacco smoke contains several harmful chemicals that can upset the delicate balance necessary for effective fertilization of semen (Jasper et al., 2021). An increasing corpus of research in the field of reproductive biology indicates that smoking may entwine itself into the process of producing sperm, upsetting the delicate balance of cellular growth (Kallman & Ferorelli, 2024). The integrity of sperm DNA is crucial since it plays a major role in pregnancy (Drevet & Aitken, 2019). However, the insidious effects of smoking, known to cast shadows across various aspects of health, have found their way into the microscopic landscape of genetic material (Kim et al., 2017). DNA strand damage, a delicate measure of the structural safety of the paternal genetic payload, emerges as a focal point in understanding the repercussions of tobacco use on male fertility (Henkel & Leisegang, 2020). Comprehending these connections is essential for individuals facing infertility issues as well as for advocating for increased public knowledge of the extensive effects of tobacco use on human reproduction.

Semen analysis is not a crucial diagnostic tool for assessing various aspects of male reproductive health, aiding in the evaluation of fertility and the identification of potential causes of infertility (Agarwal, Panner Selvam, et al., 2019; Barbărosie et al., 2021). The evaluation of key parameters includes; 1. Sperm concentration refers to the number of sperm in a given volume of semen sample (Feferkorn et al., 2022). 2. Sperm motility evaluates the ability of sperm to move forward effectively, essential for reaching and penetrating the egg (Waberski et al., 2022). 3. Sperm morphology assesses the type of defects in the head, midpiece, and tail with the normal shape of sperm (Teves & Roldan, 2022). 4. Semen volume measures the total fluid semen present in the ejaculate (Baskaran et al., 2021). 5. Semen pH assesses acidity or alkalinity (Lavanya et al., 2022). 6. Semen Liquefaction examines the time between when semen samples were collected and the time of semen samples became more liquid after incubation (Parker, 2020). 7. Sperm viability measures the percentage of live and healthy sperm (Raad et al., 2021). 8. Elevated levels of white blood cells (leukocytes) may signal infection or inflammation, which impact sperm quality and fertility (Morselli et al., 2022). 9. Agglutination refers to sperm clumping, which can interfere with movement and fertilization (Brown et al., 2021).

The World Health Organization definition of a typical sperm lacks a biological basis, leading to controversy over new sperm morphology standards (Esteves, 2022). However, using the KSC, sperm morphology, especially head shape, neck and midpiece abnormalities, and tail issues, is more strongly linked to fertilization rates than sperm count and motility (Del Giudice et al., 2022; Esteves, 2022). The Kruger standards, evaluating these aspects, are more rigorous than WHO guidelines, classifying sperm not meeting these criteria as morphologically abnormal (Chemes, 2018; Czubaszek et al., 2019; Danis & Samplaski, 2019; De Zorzi et al., 2022; Engin-Ustun et al., 2018; Kleshchev et al., 2023; Nordhoff et al., 2023).

Sperm morphology refers to the form or shape of sperm cells, categorized into normal and abnormal morphology (Hook & Fisher, 2020). 1. Normal morphology: well-defined structures in the head, midpiece, and tail, with an oval or elliptical head, straight Midpiece (neck), and uniform tail (flagellum) (Patel, 2023). 2. Abnormal morphology includes head, mid-piece (neck), and tail abnormalities that may affect human fertilization (Brito, 2021). The percentage of normal forms in the total ejaculate indicates abnormal morphology (Perry, 2021). Sperm head abnormalities include: without, pin, tapered, large, small, amorphous, round, double, and pyriform (Pear-shaped) (Panchal and Kupesic-Plasvic, 2018). Neck abnormalities include; the absent, thin, thick, bent, and presence of cytoplasm droplets (de Sousa Barbosa et al., 2019). Flagellum abnormalities may be short, double, bent, coiled, or absent in the tail (Leung et al., 2023).

Sperm DNA breakage testing in an andrology lab is crucial for assessing male fertility and identifying potential causes of infertility (Selvam & Agarwal,

2018). Both DNA fragmentation index and morphology contribute to male infertility, and the relationship is intricate (Kim, 2018). DNA fragmentation involves damage to the genetic material within the sperm head, impacting fertility and leading to issues like reduced embryonic development, higher miscarriage rates, and an increased risk of genetic abnormalities in offspring (Agarwal, Parekh, et al., 2019; Siddique et al., 2011). Direct and indirect methods such as the aniline blue stain and the halo sperm test are used to assess sperm DNA integrity (Al-Darawsha et al., 2023a). Various sperm morphology staining kits, including hematoxylin and eosin stain, giemsa stain, shoor stain, eosin-Nigrosin stain, and rapid Papanicolaou stain, provide essential information for accurate evaluation of male fertility and reproductive health (Aksoy et al., 2012).

While this article explores the potential correlation between male smokers and nonsmokers with DNA breakage and abnormal sperm morphology, it may not definitively establish the causal direction.

The aim of the study was to investigate the relationship between male smokers and nonsmokers concerning abnormal sperm shape and DNA damage. The findings may contribute to selecting preferable sperm for intra-cytoplasmic sperm injection in IVF centers.

Methodology and materials

This study was a cross-sectional observational study conducted in the infertility treatment and artificial insemination unit at Al-Safeer Imam Hussein Surgical Hospital in Karbala City, Iraq. The research aimed to explore the relationship between smokers and non-smokers and assess sperm morphology by the hematoxylin stain method and, nucleoprotein by Aniline Blue Stain, and DNA damage by halo sperm in the context of male infertility. The study participants were adult males (n = 83; they were 38 nonsmokers and 45 smokers) aged between 25 and 40 years who presented with problems related to delayed reproduction infertility (Schneuer et al., 2018).

The following chart illustrates the methodology for sample distribution and the working process.

Participants in the study underwent a semen analysis, revealing varying levels of sperm DNA fragmentation, normal values must be more than 25%, according to the manufacturer. The assessment also included sperm morphology, more than 4%, according to the 6th edition. In the research, both smokers and non-smokers served as controls and met the WHO 2021 criteria for normozoospermia, ensuring consistency in sperm motility (greater than 32%), sperm concentration (exceeding 15 million sperm per milliliter), and sperm morphology based on KSC (Tygerberg criteria) (World Health Organization, 2021).

And the exclusion criteria were individuals suffering from known genetic or medical conditions such as varicocele and cryptorchidism and, those not take chemotherapy and radiotherapy that affect fertility and sperm quality, as those who had A low sperm count, also called oligoospermia, is defined as less than 15 million/ml, according to the last edition of WHO (Oud et al., 2019).

The study involved collecting semen samples from participants using standardized procedures at the Infertility Unit. The hospital provided the necessary facilities and expertise. The research study adhered to ethical guidelines, receiving approval from the Karbala Health Department's ethics committee (reference number 2023172, dated September 25, 2023). The study, conducted from September 2023 to January 2024, upheld ethical principles and participant rights.

Semen analysis

Conducting a semen analysis according to the 2021 World Health Organization guidelines involves standardized steps; these include sample collection through masturbation without lubricants, maintaining a 2—5 days abstinence period, and proper handling for temperature preservation. In the laboratory, the sample undergoes liquefaction at 37 °C, followed by macroscopic and microscopic examination. Sperm concentration is measured using a hemocytometer, aiming for a minimum of 15 million sperm per milliliter for normozoospermia. Sperm motility is assessed through microscopy, with a
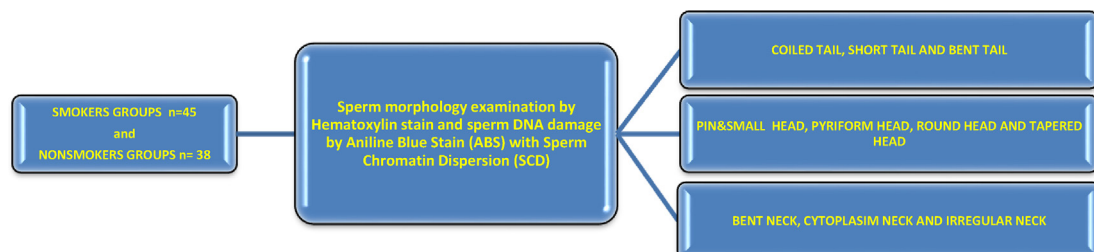
minimum of 32% exhibiting progressive motility. Sperm morphology is scrutinized using Kruger Strict Criteria (KSC), requiring at least 4% normal morphology for compliance with WHO 2021 normozoospermia criteria (Björndahl et al., 2023) (see Fig. 1).

Figures shows sperm morphology (1; normal, 2; tailless, 3; coiled tail, 4; Globozoospermi, 5; headless, 6; short tail, 7; macrocephaly, 8; multiple parts, 9; thin head, 10; pin head, 11; small head, 12; tapered head) (Paoli et al., 2020) (see Table 1).
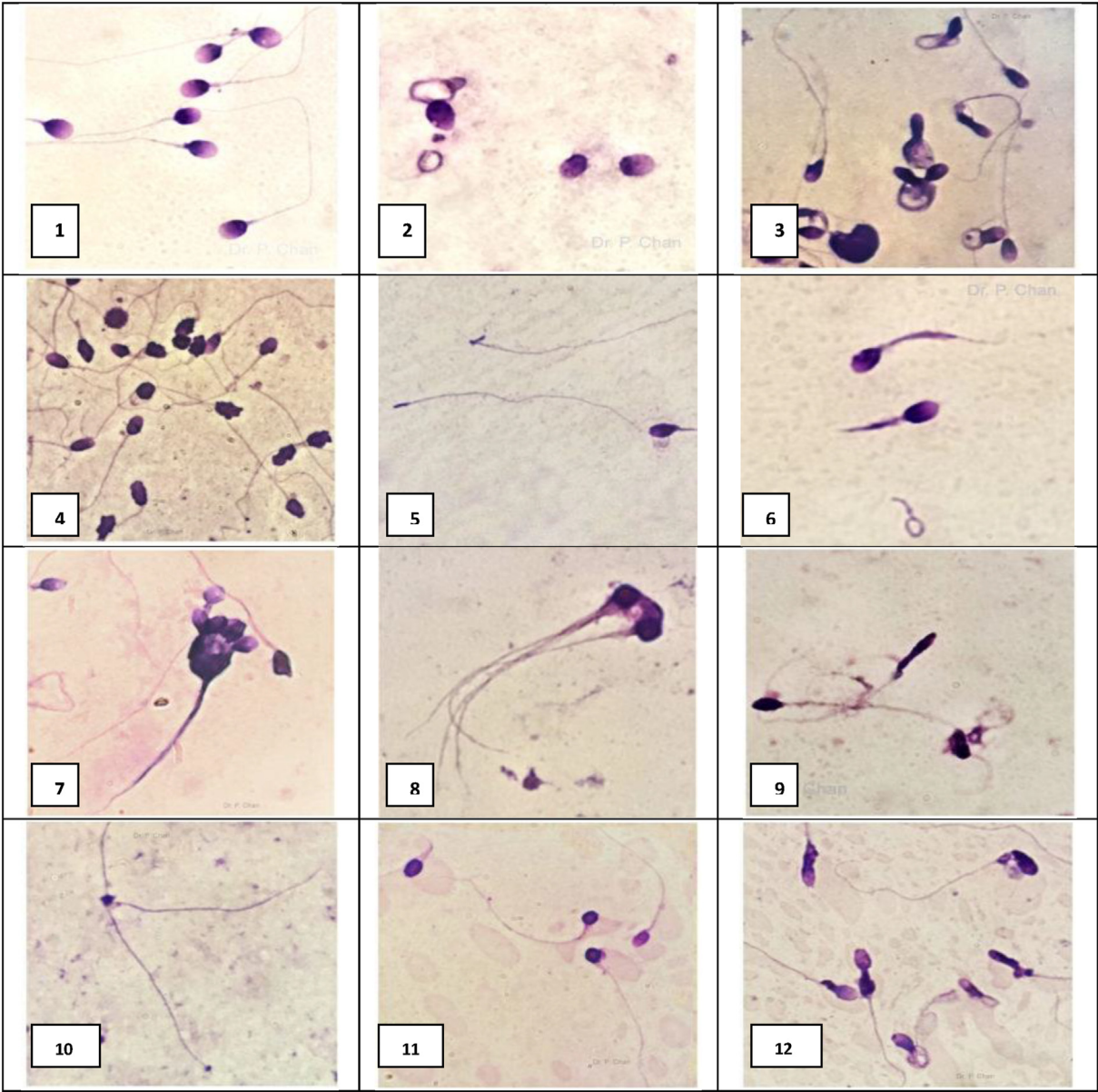

Fig. 1. The images below depict sperm with abnormal sperm shapes as per the KSC.

Table 1. Tygerberg strict criteria for sperm morphology (Franken and Kruger, 2004).

Normal Sperm Morphology;	A normal sperm has an oval head with normal neck and a single long tail.
Abnormal Sperm Morphology;	
Head;	Tapered, thin, small, pin, large, multiple (including double), abnormal base (more or less similar to pyriform), and abnormal or absent acrosome.
Neck;	Bent mid-piece, Thickened mid-piece, Irregular mid-piece, Cytoplasmic droplets.
Tail;	Absent, short, irregular width, coiled, or multiple.

Prepare sperm morphology by hematoxylin stain

Initially, a fresh semen sample is obtained through masturbation, undergoing liquefaction at 37 °C for 15–30 min. A portion is transferred to a sterile container, and microscope slides are prepared by cleaning, spreading semen drops, air drying, fixing in methanol or formalin for 10 min, and air drying again. Hematoxylin staining is applied for 10 min to enhance the visibility sperm. After rinsing, dehydration follows with ethanol solutions (70%, 90%, and 100%) for 2–3 min each. Examination using a light microscope with phase-contrast optics focuses on sperm morphology (Lingappa et al., 2015) (see Fig. 2).

Figures 1: round head, 2: normal shape, 3: cytoplasmic neck, 4: tapered head, 5: pyriform head, 6: normal form, 7: macro head, 8: bent neck, 9: multiple part head, 10: cytoplasmic droplets, 11: pin head, 12: bent tail.

Halo sperm (SCD)

The assessment of effectiveness relies on distinguishing sperm with intact DNA from those with fragmented DNA through a controlled process involving DNA denaturation and nuclear protein extraction. The procedure, outlined in accordance with the manufacturer's protocol, involves dissolving a gel, mixing semen with normal saline, applying the mixture to slides, and subjecting them to denaturation and lysis solutions. The slides undergo concentration solutions, washing, and staining with Wright's solution. Sperm counting under a light microscope at $100\times$ magnifications follows, where those with fragmented DNA lack a halo, while those with intact DNA exhibit a halo without fragmentation (Al-Darawsha et al., 2023a) (see Fig. 3).

In figures 1, 2, 3, 4: normal halo sperm, 5, 6, 7, 8: abnormal halo sperm, 9, 10, 11, 12: abnormal halo sperm with abnormal forms.
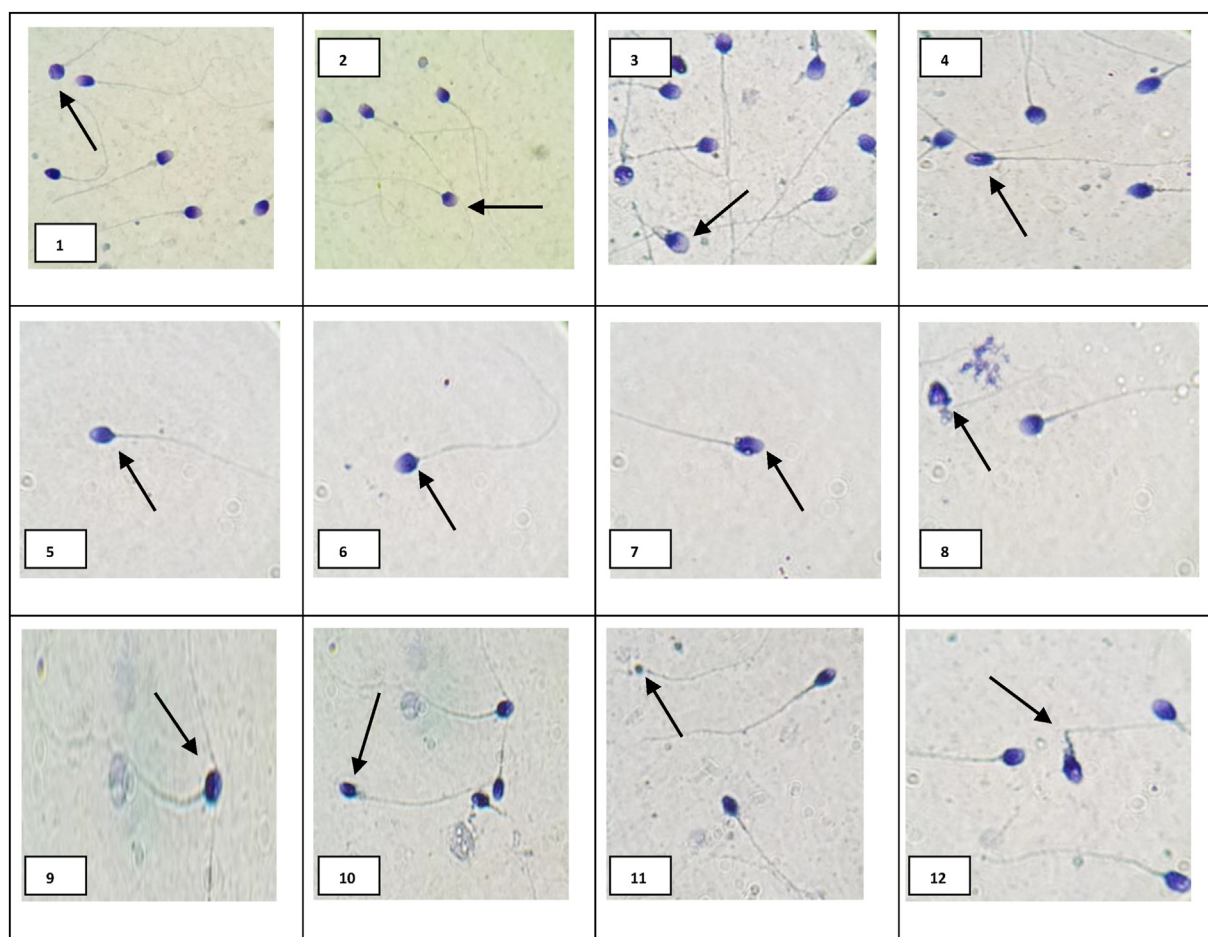

Fig. 2. The image* below displays cleared sperm morphology assessed according to KSC with the stain by hematoxylin under microscope light.
*Pictures taken in infertility treatment and Artificial Insemination Unit of Al-Safeer Imam Hussain Surgical Hospital in Kerbala city-Iraq.
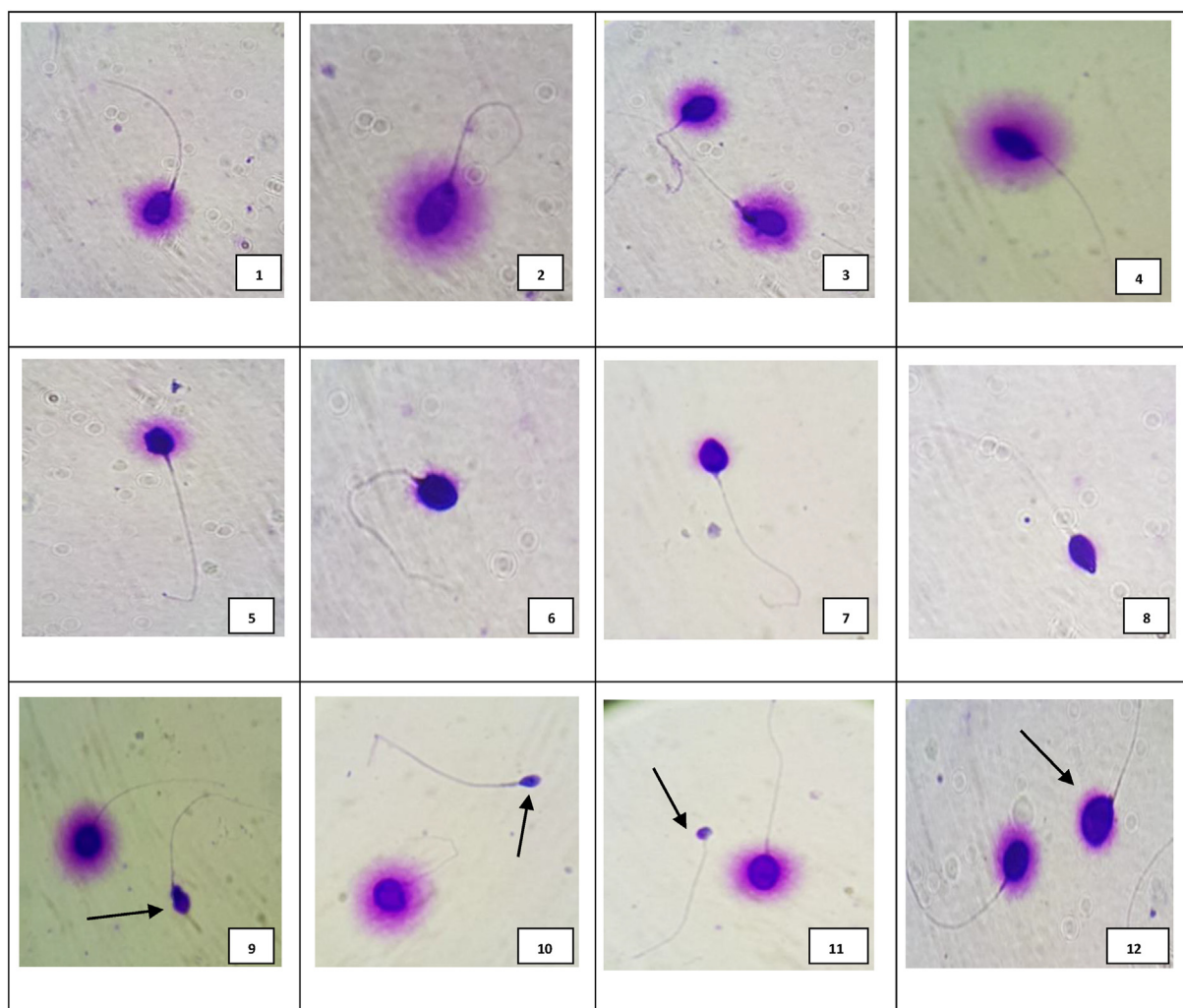
Fig. 3. *The images* below display sperm without halos and sperm with halos under a bright field microscope. *Pictures taken in infertility treatment and Artificial Insemination Unit of Al-Safeer Imam Hussain Surgical Hospital in Kerbala city-Iraq.*

Sperm nucleoprotein damage (ABS)

Mature sperm nucleoprotein, rich in protamine, stains red, while immature nucleoprotein, containing histones, is marked by aniline blue dye affinity for lysine residues, forming a dark blue compound. As per the manufacturer's protocol, the procedure involves liquefying the sample, centrifugation, and mixing with solutions. Sperm wash medium, fixative solution (methanol), Aniline blue stain, elution (HCL), and xanthene stain (xanthenes) and sodium azide, and applying the mixture to slides. Staining, washing, and drying steps follow, culminating in the counting of stained sperm under an oil immersion lens at $100\times$ magnification (Al-Darawsha et al., 2023a) (see Fig. 4).

In images 1, 2, 3, and 4, cutaneously stained heads indicate immature nucleoproteins.

Statistical analysis

Statistical analyses in the study involved the student's t-test for normally distributed variables, Fisher's exact test for percentage comparisons, and the Mann—Whitney U test for non-normally distributed data. The research focused on smokers and non-smokers, evaluating sperm morphology Kruger's strict criteria. Assessments used Sperm Chromatin Dispersion (SCD) and Aniline Blue Staining (ABS). Data analysis includes means, averages, minimums, maximums, standard deviations, and percentages, with Spearman's rank correlation coefficient for exploring relationships. Microsoft Excel 2007 generated tables for data visualization. Statistical Package for the Social Sciences (SPSS) version 26.0, provided by IBM in the United States, conducted all analyses.
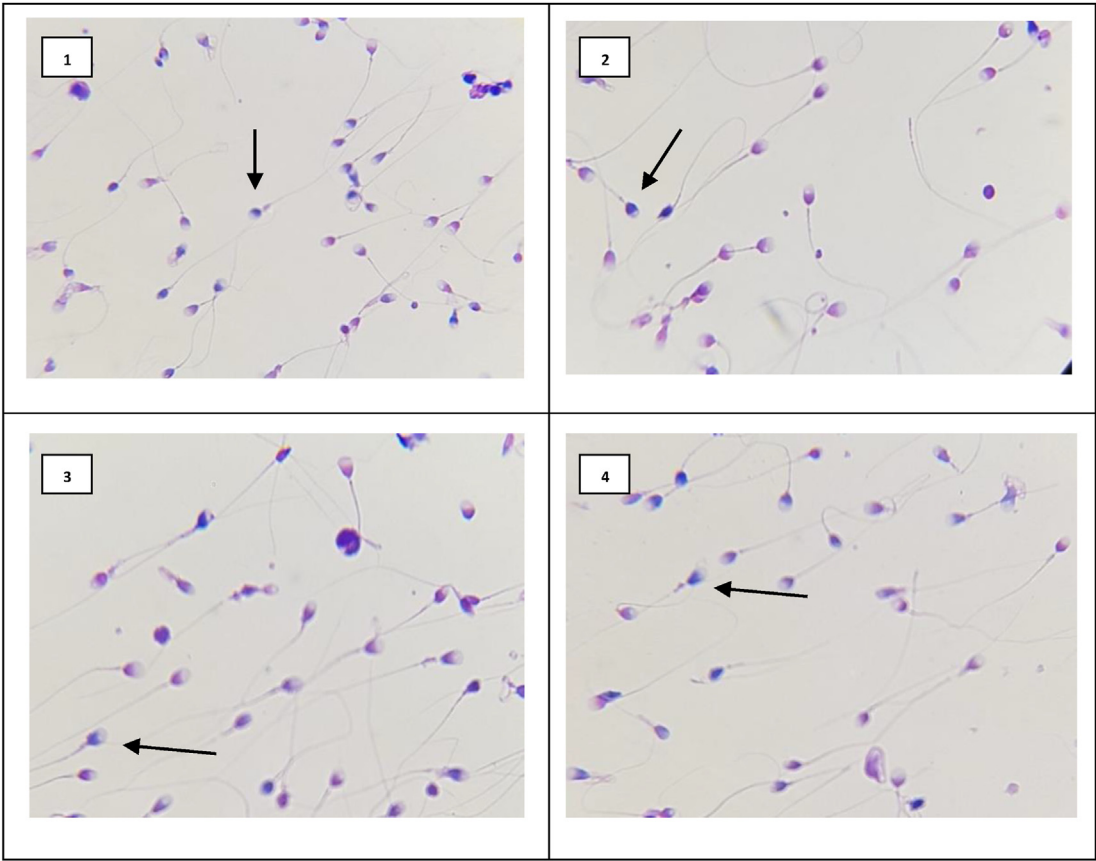
Fig. 4. The images* below depict mature sperm and immature sperm under light microscope. *Pictures taken in infertility treatment and Artificial Insemination Unit of Al-Safeer Imam Hussain Surgical Hospital in Kerbala city-Iraq.

Results

Tables 2 and 3 outline demographic characteristics and semen parameters, distinguishing between smokers and non-smokers. The age range for smokers was 21−49 years, with an average of 35.51 years, while non-smokers had a similar age range, with a slightly lower mean of 34.57 years. Smokers showed a sperm concentration range of 15−52 million per milliliter and an average of 34.31 million, while non-smokers ranged from 15 to 60 million per

Table 2. The demographic data and semen parameters according to smoker's status.

Parameters	Range	Median	Minimum	Maximum	Average	Standard error
Samples	N = 45					
Age (years)	21−49	3.0	21	49	34.61	0.94
Sperm Concentration (million∖ml)	15−52	40.0	15	52	37.31	2.01
Sperm Progressive motility (grad A + B)	5−40	25.0	5	40	24.61	1.42
Sperm Morphology by Hematoxylin stain	0−7	2.0	0	7	2.86	0.34
Head; Pin & Small	0−48	1.5	0	48	8.02	2.29
Pyriform	2−71	46.5	2	71	46.13	2.12
Round	2−21	8.0	2	21	10.02	0.91
Tapered	4−68	10.0	4	68	15.81	2.27
Neck; Bent	0−13	5.0	0	13	6.11	0.53
Cytoplasm	0−18	8.0	0	18	8.29	0.64
Irregular	2−15	8.0	2	15	8.27	0.52
Tail; Short	0−6	2.0	0	6	2.40	0.29
Bent	0−15	4.0	0	15	5.93	0.63
Coiled	0−15	4.0	0	15	5.47	0.58
Aniline Blue Stain % (ABS)	9−52	27	9	52	28.84	1.42
DNA fragmentation index % (DFI)	5−42	19	5	42	20.59	1.48

Table 3. The demographic data and semen parameters according to non-smokers status.

Parameters	Range	Median	Minimum	Maximum	Average	standard error
Samples	N = 38					
Age (years)	21−49	34	21	49	34.57	0.92
Sperm Concentration (million\ml)	15−60	80	15	60	37.43	2.54
Sperm Progressive motility (grad A + B)	5−40	30	5	40	28.78	1.61
Sperm Morphology by Hematoxylin stain	0−9	4	0	9	3.86	0.37
Head; Pin & Small	0−46	5	0	46	15.18	3.01
Pyriform	5−71	52	5	71	47.08	2.94
Round	0−21	6	0	21	10.67	1.24
Tapered	5−81	11	5	81	16.43	2.25
Neck; Bent	0−13	6	0	13	6.89	0.75
Cytoplasm	1−18	8	1	18	7.83	0.69
Irregular	1−15	8	1	15	7.91	0.74
Tail; Short	0−6	1	0	6	2.32	0.33
Bent	2−17	6	2	17	7.02	0.69
Coiled	2−16	6	2	16	6.94	0.61
Aniline Blue Stain % (ABS)	11−46	30	11	46	29.82	1.39
DNA fragmentation index % (DFI)	5−38	22	5	38	20.36	1.36

milliliter with a mean of 37.43 million. Other parameters exhibited statistically significant differences between the two groups, except for sperm concentration. In terms of sperm morphology, smokers ranged from 0 to 7%, while non-smokers ranged from 0 to 9%. Sperm progressive motility ranged from 5 to 40% for both groups. Differences were noted in sperm nucleoprotein damage and DNA fragmentation. Smokers showed absolute nucleoprotein damage ranging from 9 to 52% and sperm DNA fragmentation 5−42%, while non-smokers exhibited ABS 11−46% and SCD 5−38%.

Tables 4 and 5 provide a detailed comparison of semen analysis parameters, focusing on the differentiation between normal and abnormal sperm morphology in both smokers and non-smokers. Significant and significant correlation coefficients were observed in sperm concentration and abnormal sperm morphology, including sperm concentration ($r = 0.04$, P = 0.00048), in sperm morphology ($r = -0.797$, P = 0.0001), and specific morphological characteristics. Notable distinctions were found in parameters of sperm morphology, such as pin and small head, ($r = -0.249$, P = 0.039), round heads, ($r = 0.038$, P = 0.002); tapered heads, ($r = -0.330$, P = 0.008), irregular necks, ($r = 0.038$, p = 0.002), short tails, ($r = P = 0.020$), aniline blue stain ($r = 0.311$, P = 0.048), and halo sperm

Table 4. Comparison parameters normal sperm morphology in smokers and non-smokers.

Parameters	Sig. (2-tailed)	Correlation coefficient	Mean	Standard deviation
Samples	N = 20			
Age (years)	0.033	[a]−0.479	38.25	6.16
Body Mass Index (BMI)	0.191	−0.305	26.35	1.89
Sperm Concentration (million\ml)	0.018	[a]0.615	37.70	8.48
Sperm Progressive motility (grad A + B) %	0.034	[a]−0.477	35.00	3.24
Sperm Morphology % by Hematoxylin stain	0.704	0.090	5.60	1.63
Head; Pin & Small	0.017	[a]−0.528	18.05	18.07
Pyriform	0.256	−0.266	60.30	5.59
Round	0.941	−0.018	16.50	3.34
Tapered	0.369	0.212	23.20	16.02
Neck; Bent	0.020	[b]−0.653	10.90	1.83
Cytoplasm	0.019	[b]0.685	11.40	2.16
Irregular	0.475	−0.169	11.85	2.45
Tail; Short	0.005	[b]0.606	3.80	1.47
Bent	0.379	−0.208	10.60	2.72
Coiled	0.853	0.044	9.05	2.58
Aniline Blue Stain % (ABS)	0.032	[b]0.561	35.95	5.09
Halo sperm % (SCD)	0.028	[b]0.792	27.90	4.63

[a] Correlation is significant at the 0.05 level (2-tailed).
[b] Correlation is significant at the 0.01 level (2-tailed).

Table 5. Comparison parameters abnormal sperm morphology in smokers and non-smokers.

Parameters	Sig. (2-tailed)	Correlation coefficient	Mean	Standard deviation
Samples	N = 63			
Age (years)	0.837	0.026	33.86	4.624
Body Mass Index (BMI)	0.581	0.071	27.20	1.87
Sperm Concentration (million\ml)	0.000484	[b]0.0427	26.33	10.35
Sperm Progressive motility (grad A + B) %	0.124	−0.196	20.95	8.02
Sperm Morphology % by Hematoxylin stain	0.0001	[b]−0.797	1.68	0.89
Head; Pin & Small	0.039	[a]−0.249	1.60	1.64
Pyriform	0.553	0.076	37.95	13.45
Round	0.002	[a]0.390	5.16	2.76
Tapered	0.008	[a]−0.330	9.41	2.53
Neck; Bent	0.059	0.240	3.49	1.94
Cytoplasm	0.119	0.198	5.38	2.76
Irregular	0.002	[a]0.381	5.51	2.48
Tail; Short	0.020	[a]0.294	0.94	0.75
Bent	0.442	−0.099	3.62	1.50
Coiled	0.052	−0.246	3.46	1.07
Aniline Blue Stain % (ABS)	0.048	[a]0.311	23.65	5.80
Halo sperm % (SCD)	0.042	[a]0.290	15.40	5.33

[a] Correlation is significant at the 0.05 level (2-tailed).
[b] Correlation is significant at the 0.01 level (2-tailed).

($r = 0.290$, P = 0.042). Conversely, in the analysis of normal sperm morphology in both smokers and non-smokers, no statistically significant differences were observed.

Discussion

Male infertility is a complex and multifaceted issue affecting millions of couples worldwide (Chaudhuri et al., 2022). Sperm quality is a critical factor in achieving successful conception, and its assessment often involves the evaluation of parameters such as sperm count, motility, and morphology (Tanga et al., 2021).

While these factors are well-recognized markers of male fertility, recent research has shed light on the interplay between two crucial aspects of sperm health; DNA fragmentation and abnormal sperm morphology (Jurkowska et al., 2019). Sperm DNA fragmentation refers to the susceptibility of sperm DNA strands to breakage, mutations, or other damage (Dos Santos Hamilton and Assumpção, 2020). It has gained increasing attention as a potential contributor to male infertility (Cho & Agarwal, 2018). Elevated levels of sperm DNA fragmentation can compromise the genetic integrity of sperm, leading to difficulties in fertilization and an increased risk of miscarriages and genetic abnormalities in offspring (Al-Darawsha et al., 2023b). The exact causes of DNA fragmentation in sperm are still under investigation, but factors such as oxidative stress, infections, and environmental exposures have been implicated (Liu et al., 2021).

In our study upon comparing semen parameters between the smokers and nonsmokers groups, a noteworthy correlation emerged, revealing a substantial association between smoking and non-smoking individuals and abnormalities in sperm morphology and sperm DNA integrity. This connection manifested prominently in multiple dimensions, encompassing sperm concentration, sperm shape, and specific morphological traits such as head abnormalities like pinhead, small round head, pointed head, irregular neck, short tail, as well as factors related to sperm nuclear maturation and sperm chromatin dispersion.

Abnormal sperm morphology involves variations in the size and shape of sperm cells (Sunanda et al., 2018). It is assessed through strict criteria that categorize sperm as normal or abnormal based on their appearance (Dias et al., 2019). While there are many reasons for sperm abnormalities, some of the more common ones include genetics, hormone imbalances, and environmental impacts (Tomaiuolo et al., 2022). Sperm shape abnormalities can also have an impact on fertility by limiting the sperm's capacity to enter and fertilize an egg (Kamiński et al., 2020; Oumaima et al., 2018).

Our research reveals a strong, semen analysis confirms alterations in aberrant sperm morphology, and our research shows a robust, independent relationship between smoking and non-smoking people. Notably, there was a notable increase in sperm DNA damage in both the smoker and non-smoking groups exhibiting aberrant sperm morphology in accordance with the stringent Kruger criteria. These results highlight the complicated effects of smoking on

the health of the male reproductive system and highlight the unique consequences for sperm shape and genetic integrity.

Recent studies have suggested a possible connection between aberrant sperm morphology and sperm DNA fragmentation (Oumaima et al., 2018). Public health and reproductive medicine both greatly benefit from an understanding of the detrimental effects of smoking on sperm quality (Tang et al., 2019), and prior research has shown similar findings, highlighting the detrimental impacts of tobacco smoke on sperm quality measures (De Brucker et al., 2020). The rise in abnormalities seen in sperm among smokers highlights the need to address and discourage tobacco use, particularly among those who are trying to conceive (Swan & Colino, 2022). The assessment of sperm DNA fragmentation and morphology was a crucial component of our investigation. They are extremely significant because they have the potential to create genetic changes in progeny that might have an impact on their health (Scarpato et al., 2020). This result is in line with other studies that suggested smoking causes oxidative stress, which can harm sperm DNA (Nowicka-Bauer & Nixon, 2020). It is critical to recognize that DNA fragmentation plays a significant role in determining the results of conception, which makes its link to smoking extremely concerning (Scarpato et al., 2020).

This study emphasizes the dangers of smoking and how it affects male fertility, offering crucial information to people, policymakers, and healthcare professionals. It could act as a motivator for quitting smoking in order to safeguard the health of male reproductive systems. Nonetheless, there are still problems and gaps in our knowledge, which need for more research. These include understanding causal links, underlying processes, and particular relevant situations. It could be required to modify diagnostic and therapeutic approaches in order to properly incorporate new information in this area.

Recommendation

1. Choosing the appropriate staining techniques is critical for correctly evaluating sperm morphology.
2. The hematoxylin stain approach is especially suggested because it makes sperm head characteristics visible, which is critical for detecting abnormalities and assessing a cell's potential to reproduce.
3. When advanced reproductive procedures, such as intra-cytoplasmic sperm injection or in vitro fertilization, are unavailable, prohibitively costly,

or both, sperm morphology analysis becomes critical.
4. Analyzing sperm morphology frequently offers valuable information about the causes of infertility, which aids in diagnosis and therapy planning.
5. Deviations from the normal shape and content of sperm might indicate underlying concerns that can be addressed through a combination of medicines or lifestyle modifications.
6. Furthermore, encouraging men to quit smoking and live a smoke-free lifestyle has been associated to improved semen qualities, notably sperm morphology.

Conclusion

In summary, understanding the link between smokers and non-smokers with abnormal sperm morphology and sperm DNA fragmentation is an important first step in addressing the male infertility conundrum. Future research must continue to investigate the complex relationships between abnormal sperm morphology and sperm DNA fragmentation. It will be critical to investigate the genetic, epigenetic, and environmental factors that impact this connection. Furthermore, clinical research is required to test the efficacy of emerging therapy methods that consider these aspects.

Ethical consideration

The study was conducted at Al-Safeer Imam Hussein Surgical Hospital in the city of Karbala - Iraq, and approval from the hospital administration was obtained before starting the research.

Funding

Study design and the setting

A cross-sectional study was conducted at Infertility treatment and Artificial Insemination Unit at Al-Safeer Imam Hussein Surgical Hospital in Karbala City - Iraq, from September 2023 to January 2024 at reference number 2023172, dated September 25, 2023.

Consent for publication

Acceptance of publication by author and co-authors.

Availability of data and material

Data were collected in the Infertility treatment and Artificial Insemination Unit at Al-Safeer Imam Hussein Surgical Hospital, Karbala/Iraq.

Conflicts of interest

The author declares non conflicts of interest.

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