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Original Article

Diagnostic Markers in Specific Subsets of Infertile Males: Exploring the Significance of Fructose, Seminal histone Deacetylase, and Serum Reproductive Hormones

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Abstract

Objective: The objective of the study was to evaluate the diagnostic markers in specific subsets of infertile males and exploring the significance of fructose, seminal histone deacetylase, and serum reproductive hormones.

Methods: This investigation encompassed 80 infertile males and an equal number of fertile males (used as controls). Anthropometric measurements such as weight and height were taken to compute the weight-to-height ratio (BMI). Blood from all participants was collected through venipuncture, undergone coagulation, followed by centrifugation to isolate serum for analysis. This analysis focused on the concentrations of follicle stimulating hormone (FSH), testosterone, and luteinizing hormone (LH). Additionally, spermatozoa samples were collected via sexually self-stimulation to assess spermatozoa quality, activity of histone deacetylase and levels of seminal fructose.

Results: Comparisons between different subgroups of infertile men yielded the following outcomes: body mass index, height and weight were similar across the groups; semen volume and the hormones FSH and LH were significantly higher than expected, higher levels of sugar (fructose) and specific enzyme activity (HDAC) in semen, but lower sperm count, movement (motility), and male hormone (testosterone) levels. Furthermore, a strong and positive correlation was identified between sugar (fructose) and specific enzyme activity (HDAC). The study did not find a strong or meaningful relationship between HDAC activity and sperm motion, unlike the significant negative associations observed with sperm count and testosterone.

Conclusion: This study suggests a promising new way to diagnose male infertility. By checking levels of sugar (fructose) and specific enzyme activity (HDAC) in semen, alongside the usual tests, doctors could catch problems they might miss otherwise. This could help men get the treatment they need sooner and improve their chances of having a family.

Keywords: Infertility, Biomarkers, Semen Analysis, Spermatozoa Abnormalities, Reproductive Tract Infections diagnosis

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Introduction

Engaging in consistent, ungaurded sexual intercourse for a duration of one year or longer without success-fully achieving conception can result in various adverse consequences, including psychological and social consequences, strained marriages, divorce, multiple marriages, and significant burdens.[1] Men facing infertility may encounter problems associated with sperm cell formation, concentration (such as azoospermia or oligospermia), or the transport of sperm cells, often attributable to an inadequate supply of macronutrients essential for energy provision. This classification helps in investigating the potential root causes of infertility and devising appropriate treatment approaches.[b] Addressing these underlying issues and finding solutions is highly valued when positive results are achieved.

In today's world, couples are increasingly exploring advanced techniques like IVF (in-vitro fertilization), ICSI (intracytoplasmic sperm injection), and IUI (intrauterine insemination) to overcome fertility challenges.

When it comes to addressing male infertility, the choice of the most suitable assisted reproductive method depends on identifying the precise underlying cause through physiological or pathologically related biochemical markers.[3,4] The release of biochemical compounds into semen by the prostate, seminal vesicles, and epididymis provides valuable insights into the operational condition of these organs. Concentrations of these compounds can serve as predictive factors for diagnosing and understanding the causes of male infertility. Three important clues for healthy sperm production are:

Acid phosphatase: Tells us how active the prostate is, which helps sperm move.

Fructose: A sugar from the seminal vesicles that nourishes sperm.

Zinc and carnitine: These minerals come from the epididymis, where sperm mature and gain swimming power.

Other markers involve measurements of plasma gonadotropin and testosterone levels, lipid composition, and MDA (malondialdehyde) concentrations.[4] Moreover, discussions continue regarding the plasma levels of reproductive hormones (such as LH, FSH, and testosterone) and seminal attributes (including sperm motility, quality, and fructose levels) in specific groups of males facing infertility. Data on fertility markers like X, Y, and Z in infertile males is conflicting. Some studies report elevated plasma and seminal levels, while others observe decreases or no significant changes. Although these markers can assist in diagnosing certain cases of unexplained infertility, there is ongoing exploration of alternative markers that could be integrated into clinical practices to manage infertility in men.[5,6]

Recently, the significance of histone deacetylases (HDACs) in metabolism has come to light due to their involvement in glucose regulation through histone modification. This involvement in glucose regulation could potentially influence seminal fructose concentration, given that glucose acts as a precursor to fructose. Seminal fluid contains a notable concentration of alkaline phosphatase, which breaks down various phosphorylated fructose derivatives like 1,6-diphosphofructose, 6-phosphofructose, and I-phosphofructose into phosphoric acid and free fructose. Sperm movement is critically dependent on energy derived from fructose oxidation in seminal fluid, occurring via oxidative phosphorylation and glycolysis.[7]

This study aims to evaluate the combined utility of seminal histone deacetylase (HDAC) activity, semen parameters, and plasma reproductive hormone levels as potential markers for differentiating subcategories of male infertility.

Methods

This study employed a case-control, cross-sectional design, calculating the necessary sample size based on Araoye's formula (2004) and assuming an 11.1% prevalence rate of infertility.[8,9] A group of 160 adult males participated, split into two categories: the test group consisted of 80 individuals experiencing infertility, and the control group included 80 fertile men. Data collection was accomplished through the use of a questionnaire administered to the participants. The study included male participants having primary and/or secondary infertility, as well as fertile men who willingly consented to participate as controls. We excluded the individuals with infertility or fertility issues related to drug usage (e.g., cimetidine, cocaine), chronic alcoholism, smoking, obesity, prior vasectomy, and those who did not provide consent.

Physical Examination: An extensive physical assessment was carried out, with particular attention to aspects like gynecomastia, external genitalia conditions (penile curvature and hypospadias) and hair distribution. Additionally, the presence of surgical scars was noted. The primary objective of this examination was to exclude the possibility of any injuries affecting the blood supply to the testicles and vas deferens. Furthermore, the scrotum underwent manual palpation to evaluate the presence, size, texture, the occurrence of testicular masses or irregularity, and potential amplification of the epididymis.

Anthropometric Data: Using a weighing machine and a measuring tape, participants' weight and height were recorded. Weight measurements were taken after participants removed bulky clothing, shoes, and emptied their pockets. They were instructed to stand at the center of the bathroom scale. Heights were measured while participants stood upright on a stable, flat surface, without wearing shoes, heavy clothing, or hair accessories. Participants were directed to position themselves against a wall, ensuring that their head, buttocks, back, and heels all touched the surface, with their feet positioned properly. Participants had their height measured using a vertical tape measure. They stood still, looking straight ahead, and their height was rounded to the nearest meter. Body Mass Index (BMI) was then calculated using the specified formula: BMI = Weight / Height^2 (kg/m^2).

Blood Sampling: To gather venous blood samples, aseptic procedures were followed by extracting blood from the antecubital vein of each participant. Each participant provided ten milliliters (10mL) of blood, which was subsequently transferred into a clean, unlabeled tube. Following clot formation, blood was centrifuged at room temperature at 5000 rpm for 5 minutes. The resulting supernatant (serum) was isolated and aliquoted for storage at -20°C until further analysis.

Germ cell Sampling: Samples were procured from participants (individuals facing male infertility) through self-stimulation. Participants were required to abstain from sexual activity for 5 to 7 days before collection. The collection was done in the laboratory to maintain consistent temperature and minimize the interval for collection and ultimately examination. The researchers collected semen in a specially designed container to keep the sperm healthy. They kept the container at a comfortable temperature (30°C) and then counted the sperm using a special tool following international guidelines.

Biochemical Parameter Analysis: Serum levels of TRT (testosterone), FSH (follicle-stimulating hormone), and LH (luteinizing hormone) were assessed using ELISA kits (Abnova). To measure the activity of an enzyme called histone deacetylase (HDAC) in semen, the researchers used a special test kit. They also checked the level of sugar (fructose) in the semen, as this can be an indicator of sperm health.

SPSS version 21 was used for statistical analysis. It encompassed the utilization of basic measurement (descriptive statistics) and bar chart visualizations to elucidate and depict variables. They also compared the average values (means) between two groups using a special test (independent t-test) and among multiple groups using another test (one-way ANOVA). They considered results statistically significant if they had a less than 5% chance of being due to chance ($p<0.05$).

Results

The results of the current study revealed the mean age of both the groups as 38.0±4.0 in normal group and 39.6±2.2, 40.8±6.2 and 41.4±2.8 in subjects sub-groups. The anthropometric characteristics analysis of the patients indicated not any momentous distinction ($p>0.05$) in terms of height, weight and BMI, when comparing sub-groups of infertile males to the control group. The anthropometric data is displayed in table I.

Among the infertile group, 10.0% exhibited normospermic characteristics, 15.0% were azoospermic, and 75.0% were oligospermic. The results are depicted in Figure 1.

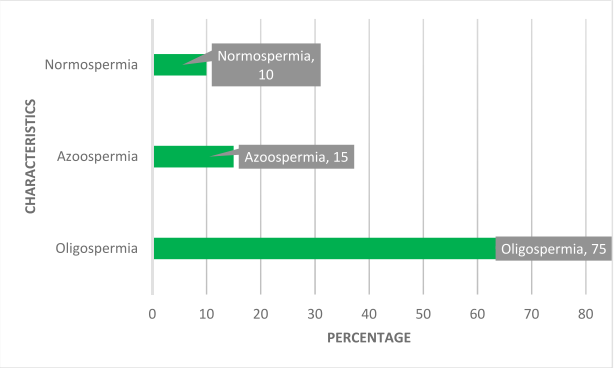

Figure 1: *Pattern of Infertility Among Subject Group*

The examination of spermatozoa characteristics in the subjects unveiled a noteworthy elevation ($p<0.05$) in semen quantity among azoospermic and oligospermic barren subjects. Furthermore, there was a substantial decline ($p<0.05$) in sperm count observed among oligospermic participants, whereas no significant reduction ($p>0.05$) in sperm motility was observed in oligospermic infertile men when compared to the control group. The results are detailed in Table 2.

Table 1: *Anthropometric characteristics of the study groups*

Parameters	Control (n=80)	Infertile subjects (n=80)			*P* value
		Normospermia (n=8)	Azoospermia (n=12)	Oligospermia (n=60)	
Age (Years)	38.0±4.0	39.6±2.2	40.8±6.2	41.4±2.8	0.772
Weight (Kgs)	62.6±8.8	68.2±3.3	72.0±2.6	71.9±6.4	0.520
Height (Inches)	62.2±2.8	64.0±2.2	63.2±1.4	64.0±2.3	0.001*
BMI (Kg/m^2)	25.0±3.6	23.2±4.0	25.4±2.1	24.8±4.4	0.004*

*The values are presented as the mean ± standard deviation (mean ± SD), and * indicate statistical significance at a p-value less than 0.05.*

Table 2: *Spermatozoa traits exhibited by both the control group and specific subgroups of males with infertility*

Parameters	Control (n=80)	Infertile subjects (n=80)			*P* value
		Normospermia (n=8)	Azoospermia (n=12)	Oligospermia (n=60)	
Vol (mL)	1.76±0.21	1.26±0.20	2.26±0.42	2.44±0.18	0.010*
Count (x10^6)	20.2±2.18	17.3±2.23	0.0±0.0	10.91±2.24	0.000*
Motility (%)	56.3±8.8	52.0±6.22	0.0±0.00	54.10±7.43	0.771

*The values are presented as the mean ± standard deviation (mean ± SD), and * indicate statistical significance at a p-value less than 0.05.*

The study on sexual and reproductive hormones (SRH) revealed a significant increase ($p<0.05$) in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels in the blood, along with a notable decrease ($p<0.05$) in testosterone among men facing oligospermia and azoospermia compared to both normospermic infertile men and the control group (as detailed in Table 3).

Table 3: *Serum levels of reproductive hormones in both the control group and distinct subgroups of males with infertility*

Parameters	Control (n=80)	Infertile subjects (n=80)			*P* value
		Normospermia (n=8)	Azoospermia (n=12)	Oligospermia (n=60)	
LH (IU/L)	8.30±1.08	6.10±1.00	10.0±1.42	9.0±1.32	0.020
FSH (IU/L)	8.22±1.18	6.13±1.23	7.20±1.80	6.10±1.24	0.000
Testosterone (IU/L)	14.23±4.8	10.0±4.22	7.0±4.10	4.10±4.13	0.000

*The values are presented as the mean ± standard deviation (mean ± SD), and * indicate statistical significance at a p-value less than 0.05.*

Upon analyzing important biochemical factors, it was observed that there was a significant rise ($p<0.05$) in fructose levels and HDAC activities in the semen of infertile males with oligospermia and azoospermia, as compared to the control group. The summary of these findings is given in Table 4.

Table 4: *Comparison of fructose and HDAC among the study groups*

Parameters	Control (n=80)	Infertile subjects (n=80)			*p* value
		Normospermia (n=8)	Azoospermia (n=12)	Oligospermia (n=60)	
Fructose (mg/mL)	4.30±0.02	4.44±0.00	4.60±0.12	4.32±0.11	0.000
HDAC (ng/mL)	2.82±0.28	2.0±.02	2.78±0.80	3.20±0.20	0.000

*The values are presented as the mean ± standard deviation (mean ± SD), and * indicate statistical significance at a p-value less than 0.05.*

The correlation (r=-0.089, p=0.498) between spermatozoa motility and HDAC actions is outlined in table 5.

Table 5: *The Pearson correlation analysis examined the relationship between spermatozoa characteristics and plasma parameters in both the control group and various subgroups of infertile males.*

	BMI	Vol	Count	Motility	FSH	LH	Testosterone	Fructose	HDAC
BMI	1								
Vol	003	1							
***p* value**	0.820								
Count	034	-332	1						
***P* value**	0.661	0.001*							
Motility	054	180	-0687	1					
***P* value**	0.610	0.114	0.660						
FSH	-070	378	-726	067	1				
***P* value**	0.434	0.001*	0.001*	0.064					
LH	-028	238	-354	-056	210	1			
***P* value**	0.772	0.044	0.002*	0.080	0.664				
HDAC	106	-270	776	-080	-580	-178	1		
***P* value**	0.338	0.030	0.001*	0.664	0.000	0.111			
Testosterone	022	-182	434	-218	-408	-004	488	1	
***P* value**	0.724	0.114	0.000	0.067	0.002	0.787	0.006		
Fructose	118	-366	802	030	-622	0.178	498	812	1
***P* value**	0.339	0.002*	0.000	0.646	0.000	0.110	0.000	0.000	

*The values are presented as the mean ± standard deviation (mean ± SD), and * indicate statistical significance at a p-value less than 0.05.*

Discussion

The features of the participant's anthropometry revealed no statistically momentous variances ($p > 0.05$) in terms of height, weight, and BMI when comparing barren males to the control group. BMI is an indicator of overall fat contents and susceptibility to weight-related health conditions. In our study, the average BMI values were 25.0±3.6, 23.2±4.0, 25.4±2.1, and 24.8±4.4 kg/m² for the control, normospermic, oligospermic, and azoospermic groups, respectively (see Table 1). These results indicate that none of the participants met the criteria for obesity, which is typically defined as a BMI above 30 kg/m²[14]. Therefore, any observed differences in the infertile men cannot be attributed to obesity.

The analysis of sperm count results revealed that within the group of infertile men, 10% exhibited normospermic characteristics, 15.0% had azoospermic conditions, and 75.0% displayed oligospermic conditions, as depicted in Figure 1. These percentages closely mirror the findings of Olooto et al., who reported similar proportions[15]. This consistency underscores the prevalence of azoospermia as the most common form of male infertility, backing and contrasting the observations of other researchers respectively[16,17].

Common indicators of semen fertility encompass factors such as sperm concentration (ranging from 12 to 16 million), germinal volume (between 1.4 and 1.7 mL), fructose levels (equal to or greater than 13mmol per ejaculate), and advanced motility (typically between 31% and 34%), among other parameters. Infertile males were categorized into three groups: normozoospermic (with a sperm concentration of 15 billion/mL or more), oligozoospermic (with a sperm concentration of less than 15 billion/mL), and azoospermic (having no sperm present).[10]

The examination of sperm characteristics indicated significant alterations ($p<0.05$) in semen quantity for both azoospermic infertile and oligospermic males. Oligospermic infertile men also displayed a noteworthy decrease ($p<0.05$) in sperm count and a less pronounced reduction ($p>0.05$) in sperm motility when compared to the control group (see Table 2). For men with azoospermia, normal ejaculated quantity (1.4-1.7mL), potential issues such as epididymal/vassal blockage or abnormalities in spermatogenesis should be considered. Notably, the reflection that fructose quantity and semen volume fall in the standard range suggests the likelihood of non-obstructive azoospermia in the premeditated barren men, although it's worth noting that these basis can also be inferior in cases of "obstructive azoospermia".

Spermatogenesis, the intricate process of sperm production, is meticulously orchestrated by the hypothalamic-pituitary-testicular axis. This axis releases key hormones like GnRH, LH, FSH, and testosterone, guiding and sustaining spermatogenesis through meiotic division, spermatid development, inhibin B production, and ultimately, mature spermatozoa formation. The findings of this study revealed a significant increase ($p<0.05$) in LH and FSH serum levels, along with a notable decrease ($p<0.05$) in serum testosterone levels among infertile men with oligospermia and azoospermia, as compared to the control group (as shown in Table 3). These results align with previous research (references [18-20]) but in contrast with the findings reported by researchers[21].

An increase in hormonal levels acts as a signal indicating infertility linked to testicular failure in the patients being studied. Still, it's crucial to highlight that raised levels in adults having zero sperm count don't completely rule out the likelihood of impediment and fecundity challenges. Some men with NOA have reported instances of localized normal spermatogenesis[22,23]. Declining testosterone levels serve as a signal to the body, stimulating the hypothalamic-pituitary-gonadal (HPG) axis. This triggers the hypothalamus to release gonadotropin-releasing hormone (GnRH), which prompts the pituitary gland to secrete gonadotropins (luteinizing hormone, LH, and follicle-stimulating hormone, FSH). In turn, these hormones activate certain cells (Sertoli and Leydig) within the gonads, leading to increased testosterone production and release. This rise in testosterone then suppresses further GnRH and gonadotropin secretion, completing the feedback loop.

The effectual crusade of sperm cells through the vaginal passage to reach the fallopian tube after sexual intercourse is influenced by their speed, which must be at least 25μm/s for rapid progression[24]. To achieve this velocity, sperm cells require energy in the form of ATP, which is produced within the cells through the oxidation of various substrates such as fructose, glucose, sorbitol, lactate, or pyruvate. This energy production occurs through processes like glycolysis and oxidative phosphorylation.[7]

The vaginal environment contains microorganisms that traditionally compete with sperm cells for the utilization of glucose. However, the utilization of fructose eliminates this competition, ensuring an adequate supply of ATP since bacteria usually prefer glucose. In a comparative analysis between the control group and infertile groups, it was evident that seminal fructose levels were significantly lower (with a p-value of less than 0.05) in the control group. Among men facing infertility, those with azoospermia demonstrated the highest levels of fructose, followed by those with oligospermia, and then the normospermic infertile group, as outlined in Table 4. This discovery extends the findings of Zahoor et al. and Nguyen et al.[25,26] by providing further evidence that the decline in fructose concentration directly links to its

crucial role in fueling sperm movement. Consequently, higher sperm concentration and motility are linked to lower fructose levels in semen, suggesting increased sugar consumption for energy by active sperm.

Both the glucose and fructose are interconvertible metabolites crucial for energy generation through certain pathway (glycolysis). The participation of histone deacetylases (HDACs) in regulating glucose balance suggests their significant role in metabolic processes. These findings highlight the intricate relationship between sperm movement, energy generation, and metabolic processes in the context of semen composition and fertility.

Both control and azoospermic infertile men exhibited significantly elevated Seminal HDAC activities compared to the other infertile groups ($p<0.05$). A clear relationship was observed between the concentration of fructose in the seminal fluid and HDAC activities. Pearson correlation analysis conducted on fertile and various infertile subgroups found a robust positive association between spermicide histone deacetylase (HDAC) activity and fructose concentration in seminal plasma, highlighting a statistically significant link between these two factors. Additionally, there was a weak and statistically insignificant inverse correlation between sperm motility and HDAC activities.

Conclusion

While body composition assessments are valuable, male infertility remains a multifaceted issue influenced by diverse factors beyond adiposity. Fluctuations in hormones, hypothalamic-pituitary-gonadal axis function, genomic susceptibility, and germinal composition all contribute. Integrating germinal HDAC activity's evaluation as a marker of sperm health into male infertility assessments could expand diagnostic and therapeutic options.

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