SPERM ABNORMALITIES AND HORMONAL CORRELATES AMONG INFERTILE MEN IN GUWAHATI, ASSAM, INDIA

Ankita Bhattacharjee[1], Sandip Mukherjee[2], Arijit Chakraborty[3]

ABSTRACT

OBJECTIVE: The present study aimed to evaluate the spectrum of sperm abnormalities among infertile men in Guwahati, Assam, and to examine their association with key reproductive hormones. Specifically, it sought to correlate semen parameters with serum levels of follicle-stimulating hormone, luteinizing hormone, testosterone, and prolactin, in order to elucidate hormonal influences on male infertility in this regional population.

METHODS: This cross-sectional study evaluated semen samples from 185 male between January - December 2022 aged 21–48 years attending infertility clinics for clinical evaluation of infertility in Guwahati, Assam, India. Semen analysis and characterization of infertility, and hormonal profiles (Estrogen, FSH, LH, Prolactin, free testosterone and total testosterone level) were gathered from the hospital records. Statistical analysis was conducted using Graph Pad Prism software, with $P < 0.05$ regarded as statistically significant.

RESULTS: Increased estrogen along with reduced free testosterone levels were observed in the case of severe OAT (Oligoasthenoteratozoospermia), azoospermia, ED (erectile dysfunction), asthenozoospermia, oligo-astheno, oligozoospermia, occasional dead sperm, occasional dead and motile subgroups in comparison to the normozoospermia, clearly indicating an altered testosterone/estrogen ratio. In the same way, the total testosterone level was abruptly elevated in severe OAT, azoospermia, ED and occasional dead group in comparison to the normozoospermia groups. Elevated FSH and reduced LH levels were observed in almost all the groups, together with variable prolactin concentrations indicative of heterogeneous hormonal profiles in the different infertility subgroups.

CONCLUSION: This study highlights distinct endocrine alterations across all infertile sub-groups including changes in the testosterone-estrogen ratio, gonadotrophin secretion and prolactin levels. The observed hormonal variations reflect the heterogeneous endocrine profiles of male infertility and support the use of comprehensive hormonal assessment as part of the clinical evaluation of infertile men. Further longitudinal studies are warranted to determine the temporal and causal relationships between these hormonal changes and infertility in those populations.

KEYWORDS: Mmale-infertility, Northeast-India, hormone, spermatogenesis, testosterone

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INTRODUCTION

Infertility is a global issue and 15% of married couples worldwide suffer from infertility[1-3]. It was previously reported that 30 million (approx.) couples in India struggle with infertility. Both men and women experience infertility, but in developing nations, women are typically held accountable. However, new improvements reveal the equal role (40-50%) of male partners in overall infertility situations. In India, the incidence is approximately 23%, while in the general population, the male factor accounts for 20–40% of the 15-20% prevalence of infertility [4-6]. Recent evidence suggests a possible decline in sperm count and overall semen quality among certain groups of Indian men, as indicated by earlier meta-analyses. However, newer large-scale regional studies report no significant temporal decline, highlighting substantial geographic and population variability. These mixed findings underscore the need for standardized, long-term national surveillance of male reproductive health.

Hormonal imbalances and oxidative stress are closely associated with modifiable lifestyle factors that negatively impact spermatogenesis and semen quality. These factors include elevated body mass index (BMI), tobacco and alcohol use, poor dietary patterns, physical inactivity, and psychological stress[7-9]. In fact, according to Pandey et al., male reproductive health may be affected by geographic and environmental factors that change the quality of semen[10]. A study involving 500 infertile men attending private infertility clinics in Guwahati, Assam, reported a higher frequency of Y chromosome (Yq) microdeletions among individuals with abnormal semen parameters. This observation was attributed to the testis-specific nature of many genes located within the Yq region, which play a crucial role in spermatogenesis[11]. Along the same vein, a study conducted by Juneja et al. in north eastern India demonstrated that abnormal semen quality is a major factor contributing to infertility in couples[12] . Therefore, considering the distinct socio-cultural, ethnic, environmental, and nutritional characteristics of the Northeast region, there remains a significant need for extensive, region-specific investigations to elucidate the determinants of male infertility and inform evidence-based reproductive health interventions.

Endocrinal variables also play a vital function in the development and maintenance of reproductive processes. It is widely documented that normal male fertility is represented by the entire development of male germ cells and proper spermatogenesis, which occurs due to the balanced endocrine interaction of hypothalamic-pituitary-gonadal (HPG) axis[3]. According to a preliminary World Health Organisation multi-center research, oligozoospermia or azoospermia afflicted 45% of infertile males[13,14]. To determine the likelihood of hormonal imbalances or abnormalities, the semen analysis is first evaluated, and if it is discovered to be abnormal (if sperm concentration is <15 million per milliliter), the sample is then subjected to additional examination[15]. It is advised that people with such aberrant sperm counts undergo a blood hormonal test because these tests have been shown to be accurate and reliable.

Men who are infertile often exhibit hormonal abnormalities, such as changed levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estrogen, and prolactin. These endocrine markers are linked to DNA fragmentation and sperm chromatin instability in addition to reflecting testicular function[16,17]. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone are the primary endocrine regulators of spermatogenesis and germ cell development. Alterations in the circulating levels of these hormones have frequently been associated with impaired spermatogenesis and male infertility. In a study involving 96 infertile men, serum concentrations of FSH, LH, and testosterone were evaluated in patients with diverse infertility phenotypes, including azoospermia (n = 35), oligozoospermia (n = 35), varicocele (n = 11), and histopathological abnormalities such as hypospermatogenesis, spermatid arrest, and Sertoli

cell-only syndrome (n = 15). The findings highlighted the importance of hormonal assessment in understanding the endocrine alterations associated with different causes of male infertility[18]. Along the same vein, elevated prolactin levels have a direct impact on the sexual system via the central nervous system and can hinder androgen production[19]. For individuals with high prolactin levels has low sex drive[20]. Some cross sectional and longitudinal studies also showed that low prolactin levels in male associated with sexual dysfunction including psychogenic erectile and ejaculatory dysfunctions[21]. Similarly, estrogen affects the central nervous system during foetal and perinatal life by modifying the growth of certain brain regions dedicated to regulating male sexual behavior, including gender identity, the development of sexual orientation, and the evolution of typical adult male sexual behaviours[22]. Infertility in mice is caused by the loss of function of either the aromatase enzyme or the estrogen receptor alpha. Estrogens can operate on various reproductive cells and the reproductive tract at many levels in men[23,24].

Although numerous global studies have examined the hormonal determinants of male infertility, very few have explored alterations in serum hormone profiles across different subtypes of male infertility within the North-East Indian population. Moreover, evidence linking variations in serum LH, FSH, testosterone, estrogen, and prolactin levels with distinct infertility patterns remains limited and inconsistent. To address this gap, the present study investigates these hormonal differences and their correlations among infertile men from North-East India providing novel insights elucidating region-specific factors in idiopathic male infertility.

METHODS

Participants

This retrospective cross-sectional study included 185 infertile men aged 21–48 years who attended a renowned infertility clinic in Guwahati, Assam, India, between January 2022 and December 2022. Clinical and laboratory records were retrospectively reviewed, and only participants with complete demographic, semen analysis, and hormonal profile data were included in the final analysis, while records with incomplete or missing data were excluded. The purpose of the study was informed to the participants and written consents were obtained. Information regarding primary or secondary infertility was not consistently available in the archived clinical records and, therefore, was not included in the analysis. The study protocol was reviewed and approved by the Institutional Human Ethics Committee (IHEC), The Assam Royal Global University, Guwahati, Assam, India (Approval No. RGU/IHEC/I/AC/Proposal/AC-01/2021–22; approved on 9th September 2021). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. As this was a retrospective record-based study, only anonymized clinical and laboratory data were used for analysis to maintain participant confidentiality.

Semen analysis

The participants were asked to obtain the semen samples by masturbation in a sterile polypropylene container after 2-7 days of sexual abstinence. Processing of the samples was initiated with 30-40 minutes of ejaculation. The semen samples were allowed to liquify at 37°C in the incubator until examination. Afterwards, analysis of semen samples was performed according to WHO recommended guidelines according to the World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen (5th Edition, 2010)[25]. Counting of the sperm was determined by the use of the Makler counting chamber, one sample per patient.

Hormonal assays

Venous blood samples were collected between 08:00 and 10:00 h as part of the routine clinical evaluation of infertility. Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, free testosterone, estradiol, and prolactin were measured using a

chemiluminescent immunoassay (CLIA) in the same clinical laboratory according to the manufacturer's instructions. Hormone concentrations were interpreted using the laboratory-specific reference ranges applicable during the study period. All hormonal analyses were performed under standardized laboratory conditions following the laboratory's standard operating procedures, with routine internal quality control and quality assurance measures implemented to ensure the accuracy, precision, and reliability of the test results while minimizing inter-assay variability.

Statistical analysis:

All statistical parameters were estimated using GraphPad Prism statistical software. Levels were considered as Mean ± SD. Data were checked for normality by the Shapiro-Wilk test and one-way ANOVA was performed followed by Tukey's multiple comparison test. Differences were considered significant if P<0.05.

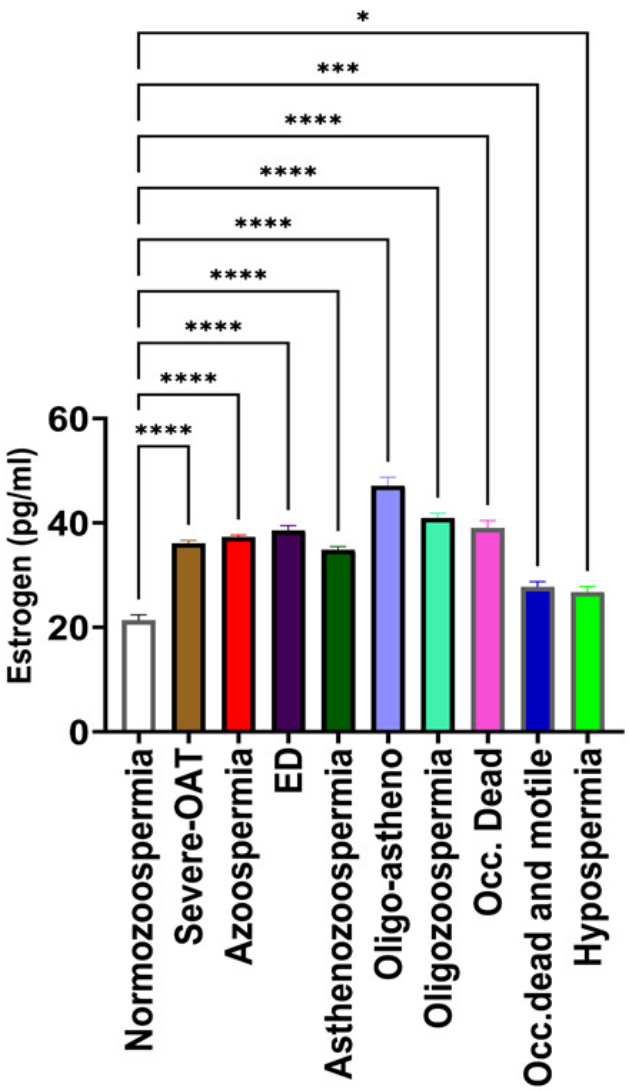

Figure 1. Comparative analysis of circulating estrogen levels between infertile men. Data are presented as mean ± standard deviation (SD). P-values represent comparisons with the normozoospermic reference group using one-way ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant. # Severe OAT - Severe oligoasthenoteratozoospermia, ED - Erectile dysfunction, Occ. Dead - Occasional dead, Occ. dead and motile - Occasional dead and motile.

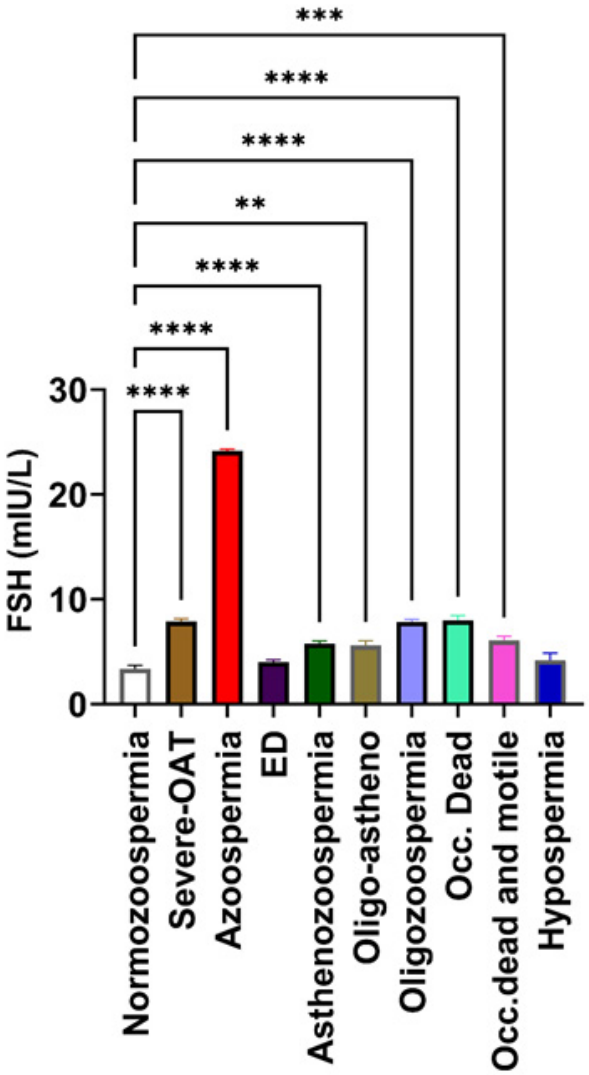

Figure 2. Comparative analysis of serum follicle-stimulating hormone (FSH) levels between infertile men. Data are presented as mean ± standard deviation (SD). P-values represent comparisons with the normozoospermic reference group using one-way ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant. # Severe OAT - Severe oligoasthenoteratozoospermia, ED - Erectile dysfunction, Occ. Dead - Occasional dead, Occ. dead and motile - Occasional dead and motile.

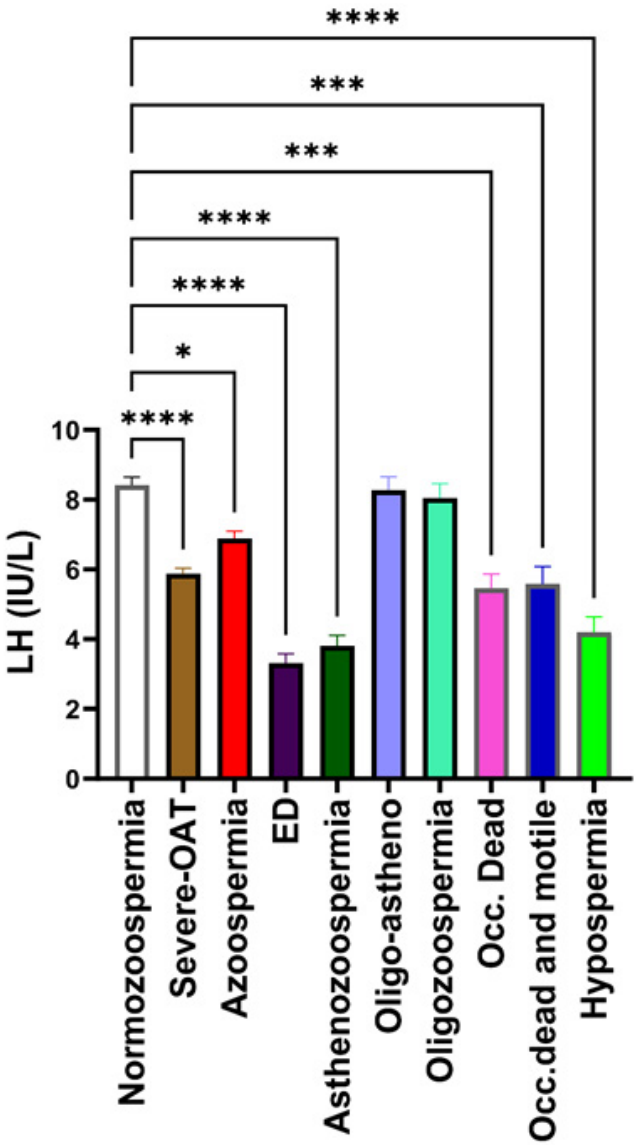

Figure 3. Comparative analysis of luteinizing hormone (LH) levels between infertile men. Data are presented as mean ± standard deviation (SD). P-values represent comparisons with the normozoospermic reference group using one-way ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant. # Severe OAT - Severe oligoasthenoteratozoospermia, ED - Erectile dysfunction, Occ. Dead - Occasional dead, Occ. dead and motile - Occasional dead and motile.

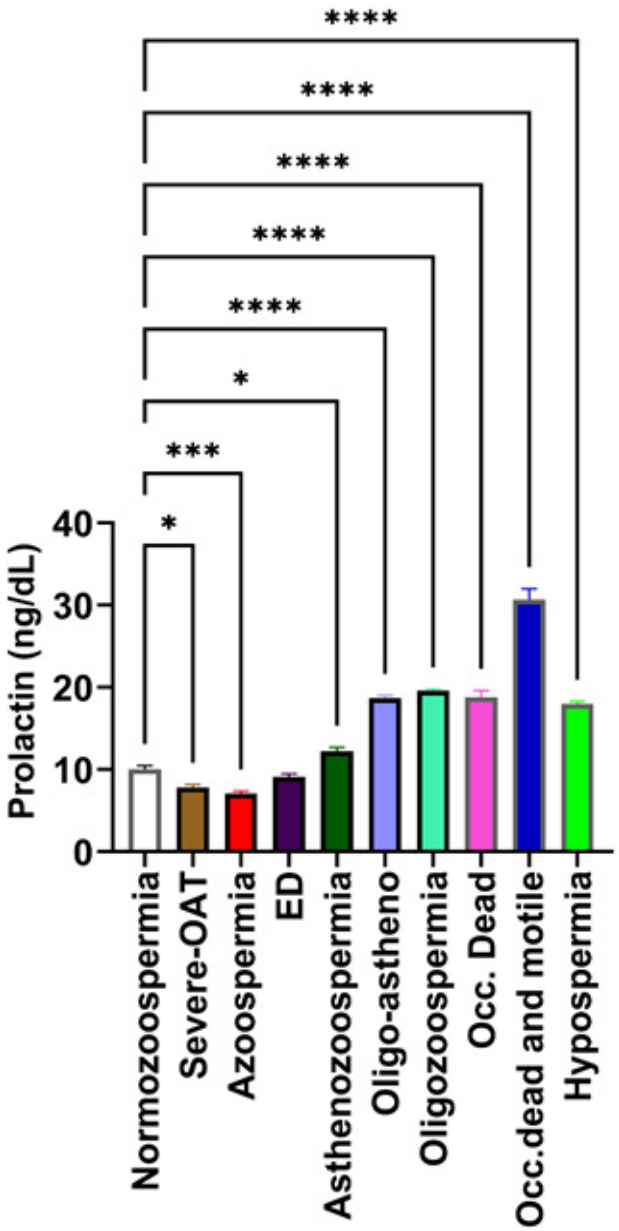

Figure 4. Comparative analysis of prolactin levels between infertile men. Data are presented as mean ± standard deviation (SD). P-values represent comparisons with the normozoospermic reference group using one-way ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant. # Severe OAT - Severe oligoasthenoteratozoospermia, ED - Erectile dysfunction, Occ. Dead - Occasional dead, Occ. dead and motile - Occasional dead and motile.

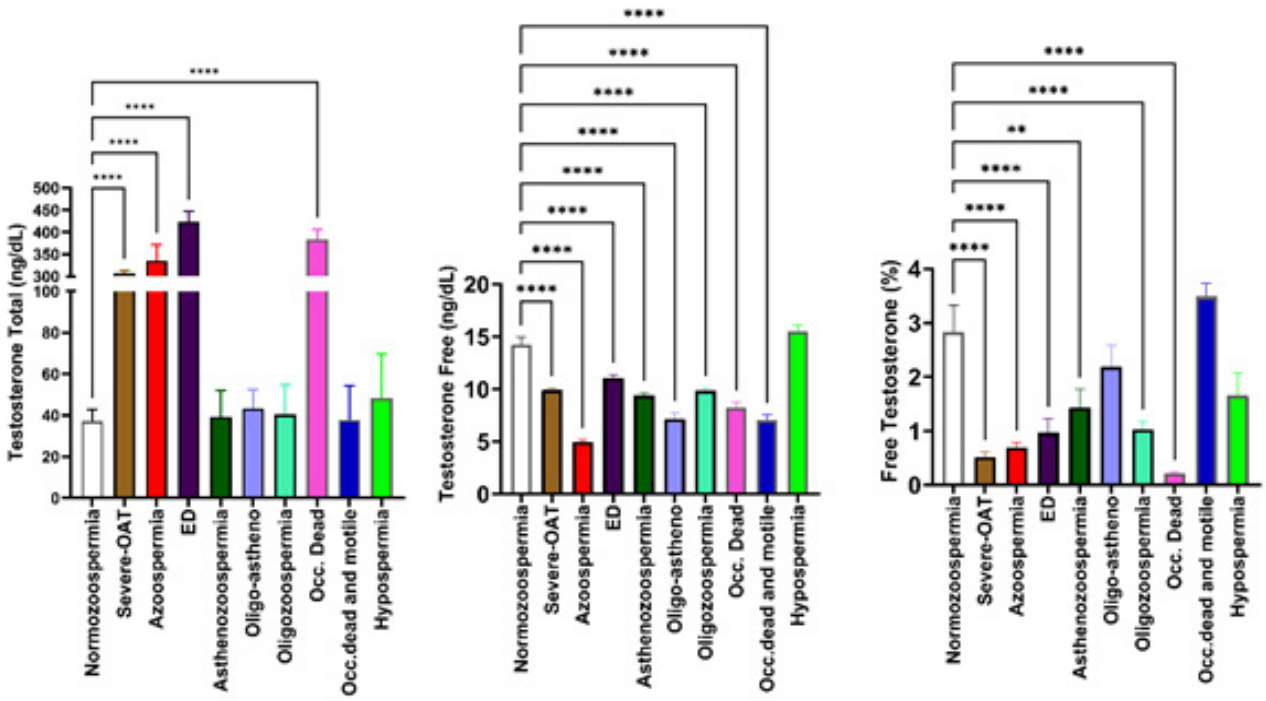

Figure 5. Comparative analysis of serum testosterone total, free testosterone, and percentage of free testosterone levels between infertile men. Data are presented as mean ± standard deviation (SD). P-values represent comparisons with the normozoospermic reference group using one-way ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant. # Severe OAT - Severe oligoasthenoteratozoospermia, ED - Erectile dysfunction, Occ. Dead - Occasional dead, Occ. dead and motile - Occasional dead and motile.

RESULTS

For proportional representation of infertility phenotypes, the cohort of 185 infertile men was stratified into ten subgroups based on WHO semen analysis criteria and commonly reported clinic-based prevalence patterns. The categories included asthenozoospermia (n = 35), oligo-asthenozoospermia (n = 9), oligozoospermia (n = 20), normozoospermia (n = 9), azoospermia (n = 39), severe oligoasthenoteratozoospermia (OAT) (n = 26), erectile dysfunction (n = 23), occasional dead spermatozoa (n = 7), occasional dead and motile spermatozoa (n = 10), and hypospermia (n = 7). This proportional stratification was adopted to enable comparative analysis of semen characteristics and hormonal profiles across clinically relevant infertility subtypes while maintaining consistency with WHO-based diagnostic classifications.

Hormonal Analysis

In this study, the figure 1 showed increased estrogen level (pg/ml) in the case of severe OAT (P≤0.0001), azoospermia (P≤0.0001), ED (P≤0.0001), asthenozoospermia (P≤0.0001), oligo-astheno (P≤0.0001), oligozoospermia (P≤0.0001), occasional dead (P≤0.0001), occasional dead and motile (P≤0.001) and hypospermia (P≤0.05) groups in comparison to the normozoospermia.

The FSH (mIU/L) level showed significantly elevated in the case of severe OAT (P≤0.0001), azoospermia (P≤0.0001), asthenozoospermia (P≤0.0001), oligo-astheno (P≤0.01), oligozoospermia (P≤0.0001), occasional dead (P≤0.0001), occasional dead and motile (P≤0.001) groups in comparison to the normozoospermia according to the figure 2 though, ED and hypospermia groups showed no significant. But, in the case of the LH (IU/L) hormone, severe OAT (P≤0.0001) azoospermia (P≤0.05), ED (P≤0.0001), asthenozoospermia (P≤0.0001), occasional dead and motile (P≤0.001), occasional dead (P≤0.001) and hypospermia (P≤0.0001) groups were found to be reduced in figure 3 in comparison to the normozoospermia group. Oligo-astheno and oligozoospermia groups were not significant in the case of LH hormone.

Prolactin (ng/dl) levels were slightly lower in the case of the severe OAT (P≤0.05), azoospermia (P≤0.001) and ED (though not significant) but the elevated prolactin levels were observed in the case of asthenozoospermia (P≤0.05), oligo-astheno (P≤0.0001), oligozoospermia (P≤0.0001), occasional dead (P≤0.0001) and hypospermia (P≤0.0001) groups in comparison to the normozoospermia. So the altered expression of prolactin was observed in figure 4.

Figure 5 clearly indicate that the decreased percentage (%) of free testosterone level in the case of the severe OAT (P≤0.0001), azoospermia (P≤0.0001), ED (P≤0.0001), asthenozoospermia (P≤0.01), oligozoospermia (P≤0.0001), occasional dead (P≤0.0001) groups though the occasional dead and motile group and hypospermia groups were found not significant. Similarly, the amount of free testosterone (ng/dl) levels was significantly reduced (P≤0.0001) in almost all the groups in comparison to the normozoospermia except hypospermia. Along the same vein, it was observed that the total testosterone level (ng/dl) was abruptly elevated in the case of severe OAT (P≤0.0001), azoospermia (P≤0.0001), ED (P≤0.0001) and occasional dead group (P≤0.0001) in comparison to the normozoospermia group. Though the level of total testosterone in asthenozoospermia, oligo-astheno, oligozoospermia, occasional dead and motile and hypospermia groups were found not significant.

Table 1: Comparison of serum hormone profiles among infertile men with different semen abnormalities and the normozoospermic reference group.

	Estrogen (pg/ml)	FSH (mIU/L)	LH (IU/L)	Prolactin (ng/dL)	Total Testosterone level (ng/dL)	Free Testosterone (ng/dL)	Free Testosterone level (%)
Normozoospermia	21.35±2.76	3.36±0.90	8.41±0.66	10.02±1.18	37.08±0.66	14.23±2.03	2.83±1.29
Severe-	3P<0.0001	7.86±1.44	5.86±0.78	7.78±1.40	307.31±6.12	9.88±1.00	0.51±0.45
oligoastheno-	P <0.0001	P<0.0001	P<0.0001	P=0.0425	P<0.0001	P<0.0001	P<0.0001
teratozoospermia							
(OAT)	37.31±2.74	24.12±1.39	6.89±1.22	7.07±1.55	335.25±36.03	4.92±1.35	0.69±0.61
Azoospermia	P<0.0001	P<0.0001	P=0.0425	P=0.0008	P<0.0001	P<0.0001	P<0.0001
	38.55±4.65	4.00±1.05	3.31±1.27	9.10±2.02	424.00±22.39	11.05±1.42	0.96±1.25
Erectile	P<0.0001	P=0.681	P<0.0001	P=0.6896	P<0.0001	P<0.0001	P<0.0001
dysfunction	34.85±2.51	5.77±1.00	3.80±1.20	12.20±2.05	39.29±1.05	9.37±1.44	1.43±1.29
Asthenozoospermia	P<0.0001	P<0.0001	P<0.0001	P=0.0379	P>0.9999	P<0.0001	P=0.0036
	47.13±4.52	5.59±1.16	8.27±0.93	18.65±1.30	43.34±1.84	7.16±1.53	2.18±1.15
	P<0.0001	P= 0.0037	P>0.9999	P<0.0001	P>0.9999	P<0.0001	P=0.5141
Oligo-astheno	40.93±4.03	7.83±1.10	8.04±1.77	19.57±1.24	40.39±1.14	9.82±1.14	1.02±0.66
Oligozoospermia	P<0.0001	P<0.0001	P=0.9993	P<0.0001	P>0.9999	P<0.0001	P<0.0001
	38.99±3.55	7.99±1.11	5.46±0.99	18.79±1.95	383.74±21.15	8.21±1.26	0.21±0.06
Occasional Dead	P<0.0001	P<0.0001	P=0.0003	P<0.0001	P<0.0001	P<0.0001	P<0.0001
	27.74± 3.01	6.08±1.07	5.58±1.31	30.63±4.50	37.80±1.07	7.05±1.41	3.48±0.74
Occasional Dead	P=0.0009	P=0.0002	P=0.0003	P<0.0001	P>0.9999	P<0.0001	P=0.4824
and motile	26.75 ±2.57	4.18±1.77	4.19±1.08	17.98±0.72	48.24±1.03	15.49±1.52	1.65±1.00
Hypospermia	P= 0.0164	P= 0.6524	P<0.0001	P<0.0001	P>0.9999	P=0.3292	P=0.0733

Values are presented as mean ± SD. P-values represent comparisons with the normozoospermic reference group using one-way ANOVA followed by Tukey's multiple comparison test. $P < 0.05$ was considered statistically significant.

DISCUSSION

Altered hormonal states, whether excessive or insufficient, produce distinct reproductive traits in rodents and humans, thereby providing valuable evidence on the endocrine mechanisms governing male fertility. Clinical observations in men with congenital or acquired hormonal imbalances reveal distinct infertility biological traits beyond classical androgen deficiency. These findings collectively challenge the long-held view that androgens are the sole or predominant regulators of male reproductive capacity, highlighting a complex, multi-hormonal network essential for normal male fertility. In the present hospital-based study, semen samples were obtained from patients undergoing evaluation and treatment for infertility, providing a clinically relevant cohort for examining these hormonal variations. This approach allows for a more precise assessment of how deviations in LH, FSH, testosterone, estrogen, and prolactin correlate with specific forms and subtypes of male infertility. Every stage of spermatogenesis in the testes is influenced by estrogen, beginning with the HPA, Leydig, Sertoli, and germ cells, and ending with the ductal epithelium, epididymis, and mature sperm. Additionally, lower semen characteristics have been linked to an aberrant testosterone/estrogen ratio of less than 10. Male infertility was found to be substantially linked with elevated serum estradiol levels[26]. Estrogen concentrations in this study were markedly elevated in severe oligoasthenozoospermia and oligozoospermia, with intermediate increase observed in azoospermia, erectile dysfunction, and asthenozoospermia groups, whereas comparatively lower levels were noted in hypospermia and men with a high proportion of dead and immotile sperm. These findings suggest a phenotype-specific dysregulation of estrogen homeostasis in male infertility. Elevated estrogen in oligozoospermic and oligoteratoasthenozoospermic men may reflect increased peripheral aromatization of testosterone to estradiol or impaired clearance, leading to suppression of the HPT axis and consequent defects in spermatogenesis[27,28]. Excess estrogen is known to exert negative feedback on gonadotropin secretion and to disrupt Sertoli cell function, thereby compromising sperm production and motility[29]. So the results in our study of significantly high estrogen levels in each case of abnormal semen parameters are also well in line with earlier reports highlighting the critical role of balanced estrogen signaling in the regulation of spermatogenesis and sperm quality in infertile men.

Determining testosterone hormonal assessment is essential to comprehending the pathophysiology of infertility and identifying spermatogenic malfunction. Compared to total testosterone, free testosterone (and its percentage) is thought to be a better indicator of male infertility since it accurately represents the "active" hormone that is accessible to tissues[30]. Low free testosterone and high total testosterone are linked to infertility, which may be caused by increased Sex Hormone-Binding Globulin (SHBG) binding more testosterone, rendering it inaccessible to the body and perhaps interfering with sperm formation. This imbalance may indicate problems with spermatogenesis, or the generation of sperm[31]. Rather than decreased Leydig cell steroidogenesis, low free testosterone in infertile males with normal or increased total testosterone indicates a disruption in androgen bioavailability and downstream signalling[30,32,33]. As a crucial gatekeeper of circulating testosterone, SHBG elevation limits intra-testicular androgen activity independent of total testosterone levels by lowering the readily diffusible fraction available to reach the seminiferous tubules[34,35]. Crucially, testosterone and FSH work together to sustain the metabolic function of Sertoli cells and the maturation of germ cells; this hormonal interaction is disrupted by the reduction of androgen signalling, which results in poor spermatogenic development even when gonadotropin stimulation is maintained[33]. Furthermore, through estrogen-mediated feedback and receptor-level dysregulation, increased peripheral aromatisation of testosterone to estradiol and modified androgen receptor sensitivity may

further impede effective intratesticular androgen signalling[36,32]. Excess aromatase, which changes testosterone into estrogen, is frequently the cause of high estrogen levels, which set off a negative feedback loop in the pituitary and hypothalamus. This lowers the amount of natural testosterone (produced by the testes) by suppressing the secretion of luteinizing hormone (LH)[37]. When taken as a whole, these mechanisms may cause functional hypogonadism at the testicular level, which increases the risk of both quantitative and qualitative abnormalities in semen. Our findings are consistent with previous research, and low free testosterone level in most of the groups (Severe-OAT, azoospermia, ED, asthenozoospermia, oligo-asthenozoospermia, oligozoospermic, occasionally dead, and occasionally dead and motile); clearly indicate that high estrogen level and possibly SHBG played an important role in the alteration of those hormonal levels. Along the same vein increased total testosterone level was observed in the case of the severe-OAT, azoospermia, ED and occasional dead sperm groups. Furthermore, exogenous testosterone or anabolic steroid use, rare tumors of the adrenal glands or testes (like Leydig cell tumors), estrogen conversion imbalances can also enhance total testosterone level[38,39]. These mechanisms remain plausible explanations; however, the present study was not designed to ascertain their contribution, and the precise underlying cause requires further investigation.

The current cohort's serum FSH profile shows a distinct gradation that corresponds to the degree of spermatogenic impairment across semen morphologies. The lowest FSH levels were seen in normozoospermia, which demonstrated normal Sertoli cell function and maintained inhibin B-mediated negative feedback. On the other hand, azoospermic and severe oligoasthenoteratozoospermic groups showed a considerable and statistically significant increase in FSH, which is consistent with severe damage to seminiferous tubules and Sertoli cell failure, where loss of inhibin B causes uncontrolled pituitary FSH release. A partial disruption of spermatogenesis with varied Sertoli cell dysfunction is suggested by intermediate elevations in FSH among oligozoospermic, oligo-asthenozoospermic, asthenozoospermic, and sperm vitality-compromised phenotypes. Reduced inhibin B secretion eliminates negative feedback on pituitary FSH synthesis, and elevated FSH is linked with poor spermatogenesis and Sertoli cell dysfunction[40,41]. Furthermore, differential regulation of the HPA axis is highlighted by the diverse and non-uniform endocrine pattern revealed by the LH profile among semen phenotypes, which contrasts with the FSH response.

The present findings reveal a distinct and phenotype-specific alteration in serum LH levels across different categories of male infertility when compared with the normozoospermic group. LH concentrations were significantly reduced in men with erectile dysfunction and asthenozoospermia, with further suppression observed in hypospermia and in individuals exhibiting a high proportion of dead and immotile sperm, suggesting compromised HPT axis signaling or impaired Leydig cell responsiveness in these subgroups. In contrast, oligoasthenozoospermic and oligozoospermic men demonstrated relatively preserved or elevated LH levels, which may reflect a compensatory pituitary response to suboptimal intratesticular testosterone production or reduced androgen bioavailability[42]. It is well-known that GnRH pulsatility and feedback from testosterone and estradiol derived from leydig cells are the main factors regulating LH secretion, relatively preserved or dysregulated leydig cell steroidogenesis may maintain enough sex-steroid feedback to suppress LH despite impaired seminiferous tubule function. Additionally, because LH synthesis is more sensitive to variations in pulse frequency and amplitude than FSH, which is also influenced by intrapituitary activin–follistatin signalling, changes in GnRH pulse dynamics disproportionately impact LH secretion in those infertile groups[43,44]. So the results in our study are also well in line with earlier reports suggesting that

LH and FSH play a critical role in the alterations of sperm parameters. All of these results point to a hormonal pattern seen in some non-obstructive and mixed aetiologies of male infertility that the observed gonadotropin profile reflects predominant Sertoli cell failure with relative retention of Leydig cell feedback mechanisms.

Hyperprolactinemia, a disorder characterised by high prolactin levels in men, can have detrimental consequences on fertility. Male infertility may be exacerbated by hyperprolactinemia, which has been linked to lower sperm concentration, motility, and morphology[45]. Elevated prolactin levels can further impair fertility by causing oxidative stress and DNA damage in sperm. Pituitary tumours (prolactinomas), drugs, stress, and specific medical disorders are some of the causes of hyperprolactinemia[46]. Managing male infertility linked to hyperprolactinemia requires determining and treating the underlying cause of increased prolactin levels[47]. Our data clearly showed a significantly elevated level of prolactin in most of the subgroups (asthenozoospermia, oligoasthenozoospermia, oligozoospermic) in comparison to the normozoospermia group which is in accordance with some previous investigations. Contradictorily, few references assumed that hyperprolactinemia was an infrequent cause of male infertility[48]. The elevated level of prolactin modifies the feedback mechanism on the hypothalamus, further suppress the pulsatile nature of GnRH secretion, thereby diminishing the pulsatile discharge of LH, FSH and testosterone, which is a vital point or reason of reduced spermatogenesis, unusual sperm motility and quality[48-50]. Low prolactin (hypoprolactinemia) has been discovered to be positively connected with sperm concentration and sperm motility, implying that lower prolactin levels are associated with inferior semen quality, which can cause severe oligozoospermia, asthenozoospermia, hypofunction of seminal vesicles and hypoandrogenism[51]. In fact, low prolactin levels have been associated with metabolic syndrome and erectile dysfunction[52]. Similarly, in our study, prolactin levels were low in case of severe-OAT and erectile dysfunction subgroups. Prolactin levels in azoospermic males are typically normal or raised (hyperprolactinemia) rather than low (hypoprolactinemia), according to previous study[48]. In this study, prolactin level was low in the case of the azoospermia group, which clearly indicates that some other factors were involved in lowering the hormonal level of the males of the North-East region of India. Some studies suggested that low prolactin levels have been associated with decreased serotoninergic tone in the CNS because it is one of the main releasers of prolactin at the hypothalamic level[53-55]. Along the same vein, decreased serotoninergic activity is associated with anxiety and mood disturbances[56,57], which ultimately results in ejaculatory dysfunctions[58,59] and can also create a worse metabolic profile in them[15]. So, all those reasons probably or possibly indicate that hypoprolactinemia could be a factor in male infertility. In the end, the cause of varying serum prolactin level results remains ambiguous. Further research is required to validate those results.

This hospital-based cross-sectional study has several limitations. As it included only men attending infertility clinics, the findings may be subject to referral and selection bias and are not generalizable to the broader male population. The use of retrospective hospital records limited standardization of semen analysis and hormone assays, while single-time-point hormone measurements did not account for physiological variation. The absence of a fertile control group and data on SHBG, inhibin B, genetic, lifestyle, and environmental factors limited causal inference. Furthermore, the relatively small subgroup sample sizes reduced statistical power and generalizability.

Conclusion

This study demonstrates substantial endocrine dysregulation underlying diverse sperm abnormalities in infertile men, characterized by altered testosterone–estrogen balance, disrupted gonadotropin secretion, and prolactin abnormalities. Reduced free testosterone despite elevated total

testosterone in some subgroups suggests impaired androgen bioavailability, potentially related to sex hormone–binding globulin. Elevated FSH with suppressed LH across most infertility phenotypes indicates primary testicular dysfunction and impaired spermatogenesis. These findings highlight male infertility as a multifactorial endocrine disorder rather than an isolated semen abnormality. Future multicenter longitudinal studies incorporating SHBG, inhibin B, hormonal ratios, and genetic, metabolic, and environmental risk factors are needed to clarify underlying mechanisms and support personalized diagnosis and management.

Conflict of Interest statement:
None declared

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