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POTENTIAL ROLE OF A BIOMARKER REPERTOIRE IN FORTIFYING THE MANAGEMENT OF MALE INFERTILITY

Clinical Biochemistry

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ABSTRACT

Infertility constitutes a global health dilemma, affecting approximately 10–15% of all couples. Although semen analysis constitutes the backbone of the diagnosis of male infertility, the contribution of a plethora of hormonal and nutritional factors cannot be ignored, particularly in the setting of normal semen analysis results. The current study sought to determine how serum levels of Testosterone, Estrogen, Prolactin, Thyroid Stimulating Hormone (TSH), free Thyroxine (fT4), folate, and homocysteine differed between male infertility patients and age-matched controls, as well as their effect on sperm parameters in infertile men. Seventy diagnosed cases of male infertility and 70 age-matched controls were recruited after acquiring informed consent from each subject. Blood samples were collected and analyzed for the above hormones and nutritional parameters by using a chemiluminescence-based automated platform. Serum levels of prolactin ($P<0.0001$), testosterone ($P<0.0001$), Estrogen ($P<0.0001$), TSH ($P<0.0001$) and homocysteine ($P<0.0001$) were significantly higher, while serum levels of fT4 ($P<0.0001$) and Folate ($P = 0.0015$) were significantly lower in infertile males vis-a-vis age-matched controls. Moreover, a significant positive correlation of serum Estrogen levels with sperm count ($r = 0.3300$; $p = 0.01$) and a significant negative correlation of serum Prolactin with sperm motility ($r = -0.5017$; $p<0.0001$) were observed in cases of male infertility. Besides routine semen analysis, various hormonal and nutritional factors can aid in cementing the diagnosis of male infertility.

Summary:

- Semen analysis constitutes the backbone of the diagnosis of male infertility.
- However, the contribution of hormonal and nutritional factors cannot be ignored.
- Blood samples were analyzed for hormones and nutritional parameters.
- Various factors aid in cementing the diagnosis of infertility, besides routine semen analysis.
- Correction of these factors may potentially help to improve fertility status in men.
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KEYWORDS

INTRODUCTION

Infertility, widely considered to be a global health dilemma, is in fact a multi-factorial disorder, affecting approximately 10–15% of all couples (Zegers-Hochschild et al., 2009). Infertility is a disease of the reproductive system described by the inability to obtain pregnancy clinically, after 12 months or more of frequent unprotected intercourse, according to WHO monitoring of Assisted Reproductive Technology (ART) (Zegers-Hochschild et al., 2009). There are yet no reliable figures for the global prevalence of infertility, although it is estimated that globally, nearly 72.4 million couples experience fertility problems (Boivin, Bunting, Collins, & Nygren, 2007), with the WHO estimating that there are at least 60–80 million afflicted couples ("Infecundity, Infertility, and Childlessness in Developing Countries," 2004). Prevalence varies across regions of the world and it was reported that 40% of infertility cases are attributable to men, 40% of women and 20% involving both sexes. In India, the malefactor constitutes approximately 23% (Kumar & Singh, 2015).

One of the most important aspects of reproductive health is the interaction between several hormones and nutrients. Perturbations in the serum levels of these hormones may hinder spermatogenesis and cause infertility in males. Male infertility is commonly due to deficiencies in semen, and semen quality is used as a surrogate measure of male fecundity. Although lacking in perfection, semen analysis still constitutes the cornerstone of the investigation of male infertility (Barratt, 2007). Testing for serum hormones is required in the setting of abnormal semen analysis or any established hormonal disorder (De Kretser & Baker, 1999). Two components of semen – spermatozoa and seminal fluid, are of particular interest in this regard; one being the number of spermatozoa in the semen sample; and the other, the volume of the seminal fluid produced. Sperm motility, defined as the percentage of sperm that shows signs of movement, and sperm morphology, defined as the percentage of sperm that appears to have a normal cellular structure, are also of significance while assessing male fertility status (Patel, Leong, & Ramasamy, 2018). The reference ranges for semen characteristics defined by the WHO laboratory manual are given in [Table 1] ("WHO laboratory manual for the Examination and processing of human semen.," 2010).

Table 1: Defined lower reference limits (WHO laboratory manual; 2010) for the examination, analyses and processing of human semen.

S. No.	Semen characteristic	Lower reference limit
1.	Volume, mL	1.5
2.	Sperm concentration, 106 mL-1	39
3.	Total sperm number, 106	15
4.	Total motility (PR+NP), %	40
5.	Progressive motility (PR), %	32
6.	Vitality (live spermatozoa), %	58
7.	Sperm morphology (normal forms), %	4
8.	pH	≥7.2
9.	Seminal fructose, µmol per ejaculate	≥13

PR, progressive motility; NP, non-progressive motility.

Men with aberrant sperm morphology, poor motility, or with low concentrations of sperm in at least one sample of two sperm examinations obtained 1 and 4 weeks apart are deemed to have male factor fertility issues, according to WHO reference values (Toragall MM, 2019).

The most severe of these are reduced concentrations of sperm (oligozoospermia), reduced motility of sperm (asthenozoospermia),

and aberrant morphology of sperm (teratozoospermia) (Iwamoto, Nozawa, & Yoshiike, 2007). Thus, analysis of semen is a straightforward procedure that evaluates the generation and maturation of sperm as well as the sperm's interaction with the seminal fluid. Additionally, it offers information not only on the production of sperm (count) but also on the quality of sperm (motility, morphology) (Butt & Akram, 2013; Fisch, 2008). Testosterone, a steroid hormone, is necessary for the development and maintenance of secondary sexual characteristics (Dohle, Smit, & Weber, 2003). Testosterone also functions in initiation and maintenance of spermatogenesis. It activates genes in Sertoli cells, which promote differentiation of spermatogonial (Mehta, Jones, & Josephs, 2008). Leydig cells secrete high levels of intratesticular testosterone, necessary for spermatogenesis (Mehta et al., 2008).

Estradiol, the predominant form of estrogen, also plays a crucial role in male sexual function. Estradiol in men is essential for many functions including modulating libido, erectile function, and spermatogenesis (Nerenz RD, 2018). Prolactin inhibits the pulsatile GnRH secretion and consequently also inhibits the pulsatile release of follicle stimulating hormone (FSH), leutenizing hormone (LH) and testosterone (Dabbous & Atkin, 2018). This inhibition results in marked effects on spermatogenesis ranging from alteration in sperm quality to complete spermatogenic arrest (Dabbous & Atkin, 2018). Thyroid gland, is now being recognized as playing a pivotal role in male reproductive functions. Hypothyroidism may result in depletion of sex hormone binding globulin (SHBG) levels, as well as levels of total serum testosterone, LH and FSH. Prolonged pre- pubertal hypothyroidism is known to hamper differentiation of Sertoli & Leydig cells into mature forms, thereby negatively impacting spermatogenesis (Freedman DB, 2018).

Folate also plays an essential role in DNA synthesis, methylation reactions and protein synthesis. Folate deficiency is also seen to be associated with indicators of reduced male fertility, namely low concentration of folate in seminal plasma is associated with lowered sperm counts and increased sperm DNA damage in humans (K. Singh & Jaiswal, 2013). Dietary or genetically determined folate deficiency may lead to mild hyperhomocysteinemia, which is often associated with various pathological conditions. Prolonged exposure to homocysteine has been linked to an increase in the expression of proinflammatory mediators such as proinflammatory cytokines (IL-6, etc.), an altered bioavailability of nitric oxide, an increase in the production of reactive oxygen species (ROS), an increase in cell death, and aberrant methylation of genes (Forges et al., 2007). Inflammatory cytokines, induced by hyperhomocysteinemia, have seen to be associated with impaired sperm parameters and male infertility, such as in penile erection, spermatogenesis, and dynamics of the blood–testis barrier, sperm motility, capacitation, acrosome reaction and fertilization (Forges et al., 2007).

In India, the prevalence of infertility in men is increasing steadily (Radhakrishnan Deventhiran, 2017). The incidence cannot be ignored, therefore, the present study has been undertaken to comprehensively evaluate and see effect of serum levels of Testosterone, Estrogen, Prolactin, Thyroid Stimulating Hormone (TSH), free Thyroxine (fT4), Folate and Homocysteine on male reproductive system and also determine their effect on semen parameters in male infertility cases.

MATERIALS AND METHODS

Study Design and Subjects

Case-control research was conducted from January 2019 to June 2020. Following approval from the Institutional Ethics Committee, R.G.Kar Medical College & Hospital, Kolkata, West Bengal's Department of Obstetrics and Gynaecology, 60 infertile males as cases and 60 age-matched fertile males as controls were recruited. A description of the study's purpose and scope was provided to all patients, followed by a signed informed consent form. Male patients with infertility who met the WHO's criterion of infertility after a year of unprotected sexual relations with the same partner were eligible for the study, as were infertile males between the ages of 20 and 50. A pre-designed history proforma sheet was completed for each patient, including information on family history, the length of the marriage, any health-related issues, and the risk behaviours of the participants. Males with congenital disorders such as ectopic testis, single testis, or cryptorchidism, a history of erectile dysfunction or premature ejaculation, varicocele, testicular torsion, previous or current infections such as mumps, tuberculosis, or HIV positivity, diabetes, chronic liver or kidney disease, or any malignancy, and a history of smoking or chronic alcoholism were excluded from this study.

Semen analysis was conducted after a 3–7 day period of abstinence. After their semen quality was checked, the patients were divided into a number of groups based on WHO criteria. The case group consisted of individuals with oligozoospermia (n=47), asthenozoospermia (n=21) and oligoasthenozoospermia (n = 32). Control subjects were chosen based on proven paternity during the previous two to three years.

Estimation of serum hormones and nutritional biomarkers

Samples of blood have been taken from all participants in both the case and control groups to determine serum biomarker levels. The levels of Prolactin, Testosterone, Estrogen, TSH, fT4, Folate, and Homocysteine were determined in serum using the Chemiluminescence immunoassay method on the ADVIA CENTAUR XP immunoassay system. Reference ranges for normal Prolactin, Testosterone, Estrogen, TSH, fT4, Folate, and Homocysteine levels for men were 2.0-18.0 ng mL^{-1}, 50-210 ng dL^{-1}, 0.0-39.8 pg mL^{-1}, 0.35-6.15 µIU mL^{-1}, 0.8-2.0 ng dL^{-1}, 2.0-20.0 ng mL^{-1} and 4.0-15.0 µmol L^{-1} respectively.

Statistical analysis

The data gathered were tabulated and statistical analysis was done with the help of software graph pad prism (version 5.0.1). To evaluate the difference between cases and controls, we used parametric test Unpaired t-test if the data was following Gaussian distribution and Mann Whitney U test if data were not following Gaussian distribution. D'Agostino& Pearson omnibus normality test was done to test the normality. Any P value <0.05 was considered statistically significant. Pearson r or Spearman r rank-order correlation coefficients, whichever applicable, were used to test correlations between the hormonal and nutritional parameters and sperm count and motility.

RESULTS

In our study, most of the infertile cases (60%) were observed in the age group of 21-30 years followed by 31-40 years (38%), and 41-50 years (2%). Furthermore, 63% of the controls were from the 21–30 year old age bracket, while 35% of the controls were from the 31–40 year old age bracket [Figure 1a]. In our study 47% of cases were oligozoospermia, 21% of cases were asthenozoospermic while 32% of cases in the study were oligoasthenozoospermia [Figure 1b].

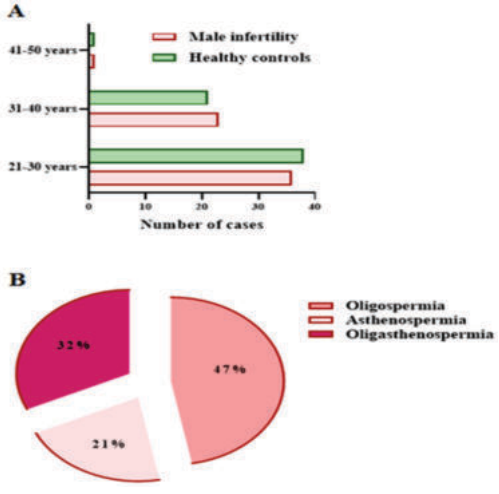

Figure 1: A Distribution of cases of male infertility and controls according to age in years. 36 cases of male infertility were in the age group of 21-30 years, 23 cases were in 31-40 years while 1 case was in the age group of 41-50 years. In the control group, 38 belonged to the age group of 21-30 years, 31 were in the age group of 31-40 years while 1 control was in the age group of 41-50 years. **B** A pie chart depicting the distribution of sperm count and motility defects among cases of male infertility.

Comparison of different levels of serum hormonal and nutritional biomarkers in cases of male infertility and controls

We compared the mean (±SD) levels of several hormones between patients and controls. Serum Prolactin levels (ng mL^{-1}) were found to be significantly higher in cases in comparison to controls (P<0.0001) [Figure 2a; Table 2].

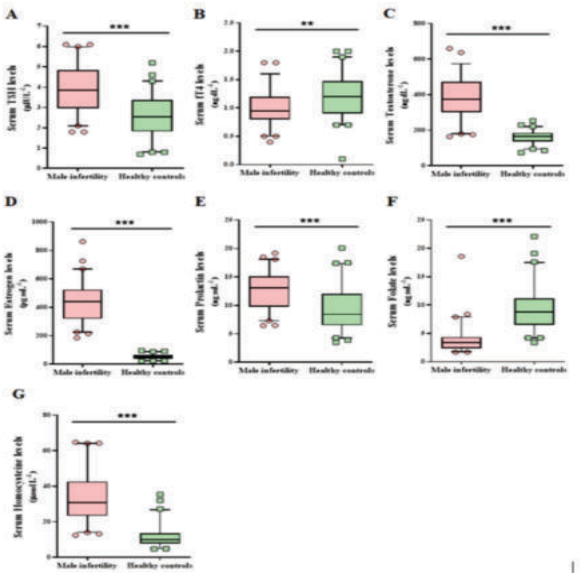

Figure 2. Box whiskers plot depicting the comparison of serum biomarker levels, **A** Serum TSH levels (µIU L^{-1}); **B** Serum fT4 levels (ng dL^{-1}); **C** Serum Testosterone levels (ng dL^{-1}); **D** Serum Estrogen levels (pg mL^{-1}); **E** Serum Prolactin levels (ng mL^{-1}); **F** Serum Folate levels (ng mL^{-1}); **G** Serum Homocysteine levels (µmol L^{-1}**),** among male infertility cases with respective age-matched controls. Data represents distribution of each parameters (CI 95%) in terms of Range (min-max) and Mean ±SD (Table 2), Unpaired t-test was applied, a *P* value ≤ 0.05 was considered significant. N=60; *P < 0.05, ***P < 0.001, ****P < 0.0001.

Table 2. Comparative levels of different serum biomarkers amongst the Male infertility cases and healthy controls (age-matched).

S. No.	Serum biomarkers	Male infertility (Cases)		Healthy Control (age-matched)		
		(N = 60)		(N = 60)		
		Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD	P value
1	Serum Prolactin levels (ng mL-1)	13.10 (6.47-19.19)	12.87 ± 3.261	8.45 (3.50-20.10)	9.467 ± 3.837	P<0.0001*
2	Serum Testosterone levels (ng dL-1)	376.0 (165.0-660.0)	392.7 ± 115.5	165.00 (74.0-254.0)	162.9 ± 37.2	P<0.0001*
3	Serum Estrogen levels (pg mL-1)	441.0 (186.0-863.0)	436.0 ± 138.0	52.50 (22.00-96.00)	53.2 ± 19.02	P<0.0001*
4	Serum TSH levels (µIU mL-1)	3.85 (1.80-6.10)	3.993 ± 1.116	2.55 (0.70-5.20)	2.577 ± 0.1302	P<0.0001*
5	Serum fT4 levels (ng dL-1)	0.95 (0.40-1.80)	1.007 ± 0.313	1.20 (0.10-2.00)	1.212 ± 0.373	P=0.0015*
6	Serum Folate levels (ng mL-1)	3.375 (1.70-18.60)	3.882 ± 2.538	8.80 (3.40-22.10)	9.35 ± 3.880	P<0.0001*
7	Serum Homocysteine (µmol L-1)	30.85 (12.50-64.80)	34.32 ± 14.35	10.10 (4.80-35.50)	11.57 ± 5.84	P<0.0001*

N = No of enrolled subjects; SD, Standard Deviation; TSH, Thyroid Stimulating Hormone; fT4, free Thyroxine; * shows result data was statistically significant.

Serum Testosterone levels (ng dL^{-1}) and serum Estrogen levels (pg mL^{-1}) were also significantly higher in cases of male infertility compared to age-matched controls (*P*<0.0001) [Figure 2b & 2c; Table 2]. Serum levels of Thyroid hormones, TSH (µIU mL^{-1}) was significantly higher (*P*<0.0001), while free Thyroxine levels (ng dL^{-1}) were significantly lower (*P*<0.0015) in cases of male infertility compared to controls [Figure 2d and 2e respectively; Table 2].

In the case of nutritional biomarkers, a significantly lower level of folate was observed in cases compared to controls (ng mL^{-1}; *P*<0.0001) [Figure 2f; Table 2], while serum Homocysteine levels were significantly higher in cases of male infertility compared to age-matched controls (µmol L^{-1}; *P*<0.0001) [Figure 2g; Table 2].

Correlation between serum biomarkers and sperm count of male infertility cases

In our present study, Correlation of serum parameters with sperm count in male infertility cases was seen. Pearson's correlation test revealed a strong positive association between blood Estrogen levels and sperm count in the case group (*r* = 0.3300; *P* = 0.01) [Figure 3a].

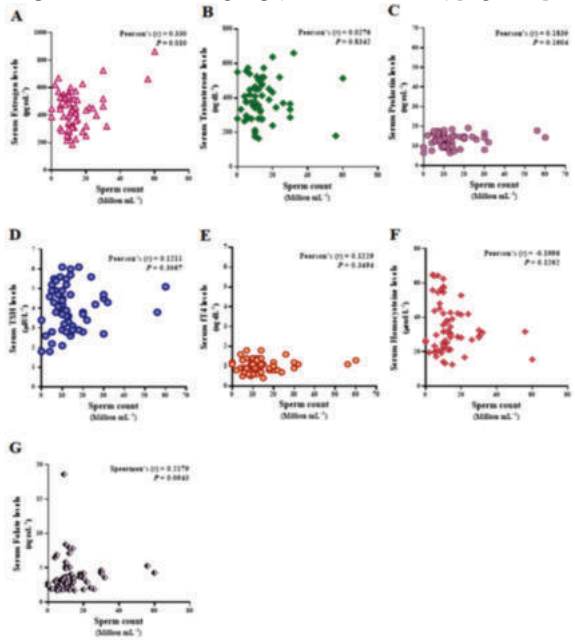

Figure 3. Scatter diagram depicting the Correlation between serum biomarkers levels and sperm count of male infertility cases, **A** Serum estrogen with sperm count; **B** Serum testosterone with sperm count; **C** Serum prolactin with sperm count; **D** Serum TSH with sperm count; **E** Serum fT4 with sperm count; **F** Serum Homocysteine with sperm count; **G** Serum folate with sperm count. Pearson's and/or Spearman's correlation coefficient shows the extent of correlation(s). N=60; *r* value Pearson's and/or Spearman's correlation coefficient (CI 95%); a *P* value ≤ 0.05 was considered significant.

While other hormonal and nutritional biomarkers serum Testosterone (*r*= 0.02760; *P*= 0.8342)[Figure 3b], Prolactin (*r* = 0.1835; *P* = 0.1604) [Figure 3c], TSH (*r*= 0.1211; *P*= 0.3567) [Figure 3d], fT4 (*r*= 0.1229; *P* = 0.3494) [Figure 3e] and Homocysteine (*r* = -0.1996 ; *P* = 0.1262) [Figure 3f] showed no significant correlation with sperm count of cases of male infertility. The Spearman Rank Order Correlation test showed no significant correlation between serum Folate levels (*r*=0.2179; *P* = 0.0493) [Figure 3g] and sperm count in cases.

Correlation between serum biomarkers and sperm motility of male infertility case

The Correlation of serum parameters with sperm motility in male infertility cases was also seen. In case group, Pearson's correlation test showed a significant negative correlation of serum Prolactin sperm motility (*r* = -0.5017 ; *P*<0.0001) [Figure 4a], while other biomarkers serum Testosterone (*r*= -0.05838; *P* = 0.6577) [Figure 4b], Estrogen (*r*= -0.07361; *P*=0.5762) [Figure 4c], TSH (*r* = -0.2243; *P*=0.0849) [Figure 4d] , fT4 (*r* = -0.08995; *P* = 0.4943) [Figure 4e] , Folate (*r* = -0.1586; *P* = 0.2262) [Figure 4f] and Homocysteine (*r* = -0.1240, *P* = 0.3452) [Figure 4g] showed no significant correlation with sperm motility of cases of male infertility.

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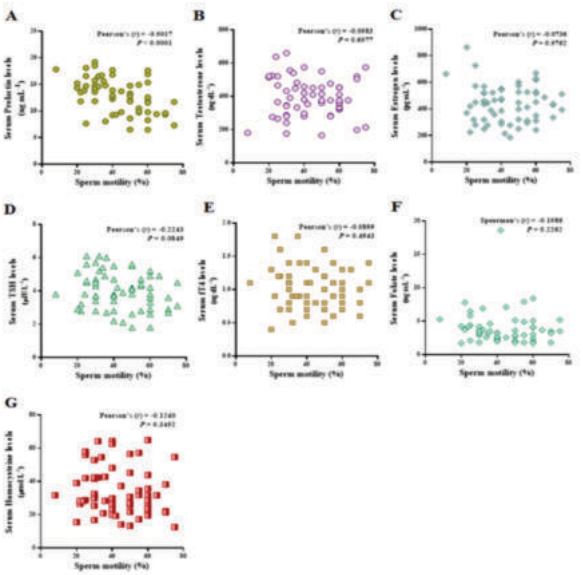

Figure 4. Scatter plots for the correlation between serum biomarkers levels and sperm motility percentage in male infertility cases, **A** Serum prolactin with sperm motility; **B** Serum testosterone with sperm motility; **C** Serum estrogen with sperm motility; **D** Serum TSH with sperm motility; **E** Serum fT4 with sperm motility; **F** Serum folate with sperm motility; **G** Serum Homocysteine with sperm motility. Pearson's and/or Spearman's correlation coefficient shows the extent of correlation(s). N=60; *r* value, Pearson's and/or Spearman's correlation coefficient (CI 95%); a *P* value ≤ 0.05 was considered significant.

DISCUSSION

Male infertility is mostly caused by anomalies in the semen parameters and quality, which are used to assess optimal spermatogenesis (Iwamoto et al., 2007). Reproductive hormones play pivotal role in the process of spermatogenesis. Hence alterations in their levels can result in abnormal spermatogenesis and consequent infertility. In cases of male infertility, endocrinological examination is critical for effective diagnosis and therapy. There were a large number of participants in our research who were between the ages of 21 and 30 years old (60%). This was in parlance with a study conducted by Bhale et al. (2013), where majority of the infertile patients belonged to the age group of 25-30 years (Gangwar et al., 2020). As previously reported by Najar et al. (2010), the current study indicated that the majority of infertile patients had oligozoospermia (47%), followed by oligoasthenozoospermia (32%), and asthenozoospermia (21%) (A., 2010).

Our present study showed abnormal levels of serum prolactin in cases of male infertility compared to age matched controls (Mean ± SD = 12.87 ± 3.261 vs Mean ± SD = 9.467 ± 3.837). In a study by Dabboos et al., it was concluded that amongst the different components of the hypothalmo-pituitary-gonadal (HPG) axis, Prolactin plays an important role in the pathophysiology of the male infertility by potentially altering testicular function and semen production (Dabbous & Atkin, 2018). Hyperprolactinemia has also been linked to a state of hypogonadism and a reduction in semen quality. Study also showed that treatment of hyperprolactinemia leads to an improvement in reproductive function, health and well-being (Dabbous & Atkin, 2018).

Singh et al., concluded that hyperprolactinemia also directly influences spermatogenesis and steroidogenesis by acting on prolactin receptors present in Sertoli cells and Leydig cells in testes, and produces primary hypogonadism and infertility (P. Singh, Singh, Cugati, & Singh, 2011). On the other hand in one of the studies Gangwar.Pk et al. 2020 observed Pearson's correlation analysis in the overall infertile patients, an inverse correlation between serum PRL level and sperm motility. Some sources, however, claim that hyperprolactenemia is a very uncommon cause of male infertility (Gangwar et al., 2020).

Increased PRL levels disturb the hypothalamic feedback system, further impairing the pulsatile release of GnRH and consequently lowering the pulsatile release of LH, FSH, and testosterone, which is a significant cause of impaired spermatogenesis, poor sperm motility, and poor quality (P. Singh et al., 2011).

In the present study we have measured endogenous testosterone levels on selected subjects with no prior history of any drug intake or any surgical conditions. In the present study serum testosterone levels were significantly higher in cases of male infertility compared to controls (mean±SD= 392.7 ± 115.5 vs mean±SD= 162.9 ± 37.2). Till date, not many studies have been done to demonstrate effects of raised endogenous testosterone levels on male fertility. Since testosterone elevation always has severe indirect effects, it is suggested that in order to encounter the type of infertility this hormone causes, special efforts should be made to pinpoint the disturbance that actually is the cause of elevation in this hormone for the particular patient.

In present study serum estrogen levels were also significantly higher in cases compared to controls (Mean±SD= 436.0 ± 138.0 vs Mean±SD= 53.2 ± 19.02). A significant and positive correlation was seen between serum estrogen levels and sperm counts (p=0.01) of male infertility cases, while no correlation found between sperm motility and estrogen levels (p=0.5762). As in the study male infertility cases were seen to have raised serum Testosterone levels, which might be a possible cause of increased levels of serum Estrogen, as the main source of peripheral estradiol is the peripheral aromatization of testosterone. Salama and Blgozah's 2020 investigation into low, normal, and high Estrogen levels in male infertility patients anticipated a very significant correlation between testosterone and estradiol. It observed that testosterone levels were significantly higher in the high estradiol group than in the normal or low groups. Physiological levels of estrogens are essential for sperm production as their high level or absence leads to spermatogenesis disruption, although their exact role in the pathophysiology of spermatogenesis is not yet fully understood. The same study also reported that morbid alterations in estradiol level be it high or low could be associated with disturbed spermatogenesis. They discovered that elevated Estrogen levels may impede the function of Leydig cells. Additionally, it may affect testosterone production and pituitary gonadotropin release (Salama & Blgozah, 2020). A study by Manici et al., showed that anti-estrogen drugs like Tamoxifen seem to have a strong effect on sperm count and concentration in eugonadal patients (Mancini et al., 2013).

Thyroid hormones have been extensively examined in the past several decades for their potential role in male reproduction. They are regarded as critical regulators of male reproductive activities and are involved in the formation of the male gonadal plexus. As a result, our next significant hormonal parameter to investigate was thyroid hormones. We noticed that serum TSH levels were within the normal range in both cases and controls, but were slightly elevated in instances of male infertility when compared to controls (Mean ± SD= 3.993 ± 1.116 vs Mean ± SD= 2.577 ± 0.1302), whereas male infertility patients had somewhat lower serum fT4 levels than controls (Mean ± SD= 1.007 ± 0.3135 vs Mean ± SD= 1.212 ± 0.3738).

In our research, statistical analysis revealed that male infertility cases had considerably lower blood folate levels (Mean ± SD= 3.882 ± 2.538 vs Mean ± SD= 9.35 ± 3.880) than age matched controls, however serum homocysteine levels were significantly higher in the male infertility cases group than controls (Mean ± SD= 34.32 ± 14.35 vs Mean ± SD= 11.57 ± 5.84). There was no significant link between serum folate and homocysteine levels in male infertility patients and their sperm parameters (p=0.0943 & p=0.1262 for sperm count and p=0.2262 & p=0.3452 for motility, respectively). As a result, the current study's case group demonstrated folate deficiency in addition to hyperhomocysteinemia.

A 2007 research by Ebisch.I.M.W et al. shown that folate plays a significant impact in spermatogenesis by correlating folate levels with other dietary factors (Ebisch, Thomas, Peters, Braat, & Steegers-Theunissen, 2007).

According to a study conducted by Kang.L et al., 2017 oligozoospermic males had significantly higher serum homocysteine levels than controls (Liu KS, 2017).

Folic acid treatment for six months dramatically improved the semen parameters and raised the sperm concentration of subfertile males, according to randomised clinical studies (Ebisch, Pierik, FH, Thomas,

& Steegers-Theunissen, 2006). However, the precise processes behind the detrimental impact of folate deficiency or elevated homocysteine on sperm quality and count need additional investigation in order to understand the nutritional indicators' true significance for male fertility and subsequent pregnancy outcomes.

In conclusions, male infertility is a major public health problem that deserves attention on a worldwide scale. The current research demonstrates that, in addition to semen analysis, the simultaneous assessment of a panel of hormones and nutritional markers is critical for the diagnosis and subsequent management of such individuals.

The Future Perspective would be; as science marches ahead mere counting of spermatozoa and checking for motility and abnormal morpholgy cannot remain the sole means of establishing the diagnosis of infertility. The role of the endocrine system (particularly androgens, estrogens, pituitary and thyroid hormones) and nutritional factors, in the form of direct or surrogate biomarkers, will not only help immensely in strengthening the diagnosis, but can also offer tremendous insight into correction of the problem. Modulating the release of hormones such as stress hormones or those which affect nutritional mileau such as glycemic status, can go towards a long way in improving spermatogenesis as well as sperm quality. Such biomarkers can well provide the much coveted ounce of preventionin the near future, rather than the pound of cure.

Author's Contributions

Shilpa Barmecha and **Srabani Biswas** performed the literature search, research methodology, and drafting of the manuscript. **Debdutta Ghosh** provided aid in patient recruitment and intellectual inputs for the study design. **Sharmistha Choudhuri** contributed to the conceptualization of study design, gaining ethical approval, data analysis, Writing - review & editing, and critical assessment of the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest:

The authors declare no conflicts of interest regarding the publication of this article.

Ethics approval

The Institutional Ethics Committee (IEC) of R. G. Kar Medical College and Hospital, Kolkata, West Bengal (INDIA), approved this study (REC number: RKC/464)

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

The authors affirm that human research participants provided informed consent for publication.

Availability of data and material

The data underlying this article cannot be shared publicly due to patient data privacy protection required by the ethics approval. The data will be shared at reasonable request to the corresponding author.

REFERENCES

1. A., A. N. (2010). Prevalence and pattern of endocrinological abnormalities in oligospermic and azoospermic patients. *Journal of the Faculty of Medicine Baghdad, 52*(4), 402-404. doi: https://doi.org/10.32007/jfacmedbagdad.524941
2. Barratt, C. L. (2007). Semen analysis is the cornerstone of investigation for male infertility. [Review]. *Practitioner, 251*(1690), 8-10, 12, 15-17.
3. Boivin, J., Bunting, L., Collins, J. A., & Nygren, K. G. (2007). International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. [Research Support, Non-U.S. Gov't]. *Hum Reprod, 22*(6), 1506-1512. doi: 10.1093/humrep/dem046
4. Butt, F., & Akram, N. (2013). Semen analysis parameters: experiences and insight into male infertility at a tertiary care hospital in Punjab. *J Pak Med Assoc, 63*(5), 558-562.
5. Dabbous, Z., & Atkin, S. L. (2018). Hyperprolactinaemia in male infertility: Clinical case scenarios. *Arab J Urol, 16*(1), 44-52. doi: 10.1016/j.aju.2017.10.002
6. De Kretser, D. M., & Baker, H. W. (1999). Infertility in men: recent advances and continuing controversies. [Research Support, Non-U.S. Gov't
7. Review]. *J Clin Endocrinol Metab, 84*(10), 3443-3450. doi: 10.1210/jcem.84.10.6101
8. Dohle, G. R., Smit, M., & Weber, R. F. (2003). Androgens and male fertility. [Review]. *World J Urol, 21*(5), 341-345. doi: 10.1007/s00345-003-0365-9
9. Ebisch, I. M., Pierik, F. H., FH, D. E. J., Thomas, C. M., & Steegers-Theunissen, R. P. (2006). Does folic acid and zinc sulphate intervention affect endocrine parameters and sperm characteristics in men? [Randomized Controlled Trial Research Support, Non-U.S. Gov't]. *Int J Androl, 29*(2), 339-345. doi: 10.1111/j.1365-2605.2005.00598.x
10. Ebisch, I. M., Thomas, C. M., Peters, W. H., Braat, D. D., & Steegers-Theunissen, R. P. (2007). The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. [Review]. *Hum Reprod Update, 13*(2), 163-174. doi: 10.1093/humupd/dml054
11. Fisch, H. (2008). Declining worldwide sperm counts: disproving a myth. [Review]. *Urol Clin North Am, 35*(2), 137-146, vii. doi: 10.1016/j.ucl.2008.01.001
12. Forges, T., Monnier-Barbarino, P., Alberto, J. M., Gueant-Rodriguez, R. M., Daval, J. L., & Gueant, J. L. (2007). Impact of folate and homocysteine metabolism on human reproductive health. [Review]. *Hum Reprod Update, 13*(3), 225-238. doi: 10.1093/humupd/dml063
13. Freedman DB, H. D., Marshall WJ, and Ellervik C. (2018). Thyroid Disorders. Tietz Textbook of Clinical Chemistry And Molecular Diagnostics. (6th ed., pp. 1572-1580). Boston USA: Elsevier.
14. Gangwar, P. K., Sankhwar, S. N., Pant, S., Krishna, A., Singh, B. P., Mahdi, A. A., & Singh, R. (2020). Increased Gonadotropins and prolactin are linked to infertility in males. *Bioinformation, 16*(2), 176-182. doi: 10.6026/97320630016176
15. . Infecundity, Infertility, and Childlessness in Developing Countries. (2004) *ORC Macro and the World Health Organization* (DHS Comparative Report ed.). Calverton, Maryland, USA.
16. Iwamoto, T., Nozawa, S., & Yoshiike, M. (2007). Semen quality of Asian men. [Review]. *Reprod Med Biol, 6*(4), 185-193. doi: 10.1111/j.1447-0578.2007.00184.x
17. Kumar, N., & Singh, A. K. (2015). Trends of male factor infertility, an important cause of infertility: A review of literature. [Review]. *J Hum Reprod Sci, 8*(4), 191-196. doi: 10.4103/0974-1208.170370
18. Liu KS, P. F., Chen YJ, Mao XD. . (2017). The influence of sperm DNA damage and semen homocysteine on male infertility. *Reprod Dev Med, 1*, 228-232. doi: 10. 4103/2096-2924.224910
19. Mancini, A., Raimondo, S., Persano, M., Di Segni, C., Cammarano, M., Gadotti, G., . . . Meucci, E. (2013). Estrogens as antioxidant modulators in human fertility. [Review]. *Int J Endocrinol, 2013*, 607939. doi: 10.1155/2013/607939
20. Mehta, P. H., Jones, A. C., & Josephs, R. A. (2008). The social endocrinology of dominance: basal testosterone predicts cortisol changes and behavior following victory and defeat. [Research Support, Non-U.S. Gov't
21. Research Support, U.S. Gov't, Non-P.H.S.]. *J Pers Soc Psychol, 94*(6), 1078-1093. doi: 10.1037/0022-3514.94.6.1078
22. Nerenz RD, J. E., and Gronowski AM. (2018). *Reproductive Endocrinology and Related Disorders. Tietz Textbook of Clinical Chemistry And Molecular Diagnostics.* (6th ed.). Boston USA: Elsevier.
23. Patel, A. S., Leong, J. Y., & Ramasamy, R. (2018). Prediction of male infertility by the World Health Organization laboratory manual for assessment of semen analysis: A systematic review. [Review]. *Arab J Urol, 16*(1), 96-102. doi: 10.1016/j.aju.2017.10.005
24. Radhakrishnan Deventhiran, K. R., Nagamurugan Nandakumar. (2017). Prevalence of Male Infertility in India: Studies on the Effects of Gonadotropin Releasing Hormones. *Asian Journal of Pharmaceutical and Clinical Research, 10*(8), 208-210. doi: https://doi.org/10.22159/ajpcr.2017.v10i8.18254
25. Salama, N., & Blgozah, S. (2020). Serum estradiol levels in infertile men with non-obstructive azoospermia. *Ther Adv Reprod Health, 14*, 2633494120928342. doi: 10.1177/2633494120928342
26. Singh, K., & Jaiswal, D. (2013). One-carbon metabolism, spermatogenesis, and male infertility. [Research Support, Non-U.S. Gov't Review]. *Reprod Sci, 20*(6), 622-630. doi: 10.1177/1933719112459232
27. Singh, P., Singh, M., Cugati, G., & Singh, A. K. (2011). Hyperprolactinemia: An often missed cause of male infertility. *J Hum Reprod Sci, 4*(2), 102-103. doi: 10.4103/0974-1208.86094
28. Toragall MM, S. S., Kadadevaru GG, Hiremath MB. (2019). Association of demographic and lifestyle factors with semen quality of men with fertility problems attending infertility center in North Karnataka. *Indian J Med Spec, 10*, 79-83. doi: 10.4103/INJMS.INJMS_3_19
29. WHO laboratory manual for the Examination and processing of human semen. (2010) (Fifth edition, chapter 2-2.3, 13-15. ed., pp. 13-15).
30. Zegers-Hochschild, F., Adamson, G. D., de Mouzon, J., Ishihara, O., Mansour, R., Nygren, K., . . . Vanderpoel, S. (2009). International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. [Dictionary]. *Fertil Steril, 92*(5), 1520-1524. doi: 10.1016/j.fertnstert.2009.09.009