

SIMULTANEOUS ANALYSIS OF THE THREE HORMONES INVOLVED IN SPERMATOGENESIS AND THEIR INTERRELATION RATIOS

MUHAMMAD SHOAIB KHAN, IRSHAD ALI*, FAHEEM TAHIR**
AND GUL MAJID KHAN***

Pakistan Medical Research Centre, Hayatabad Postgraduate Medical Complex, Peshawar, Pakistan

**Department of Chemistry, Gomal University, Dera Ismail Khan, N.W.F.P., Pakistan*

***Department of Reproductive Physiology/Health, National Institute of Health, Islamabad, Pakistan*

****Drug Delivery Research Group, Department of Pharmaceutics, Faculty of Pharmacy,
Gomal University, Dera Ismail Khan (NWFP) Pakistan*

ABSTRACT

The Objectives of this non-interventional descriptive study was to determine the variation in serum levels of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Testosterone along with their ratios, among men having abnormal semen and their possible etiological role in male infertility. The study was carried out in the Reproductive Physiology Department of Public Health Laboratories Division, and National Institute of health (NIH), Islamabad during January 2004 to December 2005. Two hundred fifty married men who had presented with a complaint of infertility and who had an abnormal seminal profile on the basis of their prior semen analysis were included in the study. Subjects were classified as azoospermic (50), oligozoospermic (75), asthenozoospermic (50) and normozoospermic (75). In addition (50) normal male subjects, who were known to have fathered children, were included as controls. LH, FSH and Testosterone levels were determined in serum by using Enzyme Immunoassay (EIA), using state-of-art Elecsys-2010 fully automatic Immunology analyzer by Roche Diagnostics (USA). The FSH and LH level indicated inverse/negative correlation to sperm concentration in semen, while decreased Testosterone levels were associated with depleted sperm concentration. The findings indicate that not only the altered/disturbed concentrations of gonadotrophins and androgenic hormones are responsible for male sub-fertility but also the disturbances in gonadotrophic: androgenic hormones ratios lead to infertility since these all hormones act synergistically.

Keywords: Spermatogenic hormones; serum level variations; inter-relation ratio; abnormal semen; infertility in Pakistan males.

INTRODUCTION

Infertility is a common disorder and nearly one out of every six to eight couples suffers from it at any given time. Infertility among couples in their respective age is more common than hypertension, diabetes, heart diseases and even the common flu (Khan *et al.*, 2005; Zaidi *et al.*, 2004). Globally, approximately 10-15% couples seek medical help for the problem of infertility. In 20-25% cases the problems are attributable to the male partner, while, 30-40% represent female factor. Approximately 30% of cases are associated with both partners and in 15% no specific factor can be identified (WHO, 1997). According to recent investigation, the proportion of couples seeking medial care was, on average, 56.1% (range 42-76.3%) in more developed countries and 51.2% (range 27-74.1%) in less developed countries. The proportion of people actually receiving care was substantially less, 22.4%. Based on these estimates and on the current world population, 72.4 million women are currently infertile; of these, 40.5 million are currently seeking infertility medical care (Boivin *et al.*, 2007). In Pakistan where potency is considered a proof of normal

fertility, it is usually the wife who bears the blame and is often maltreated for the offence of not bearing children. The husband, who should also be investigated simultaneously either never submits himself for evaluation or is investigated only at the end (Akhtar and Akhtar, 1991). In general infertility is defined as the failure to conceive after 1 year of regular un-protective intercourse with the same partner. However, the term "Infertility" implies a definitive inability to conceive. Therefore, couples who do not conceive in >1 year should be regarded as sub-fertile. According to these definitions, approximately 14% of the couples are sub fertile (Martin, 1991) To be more exact the term sub-fertile means a male who failed to conceive after I year of regular unprotected intercourse with the same partner and who had a sperm count of less than 20 million/ml (Wong *et al.*, 2000).

Hormones and their role which affect the spermatogenesis are testosterone, secreted by leydig cells located in the interstitial of the testis, is essential for growth and division of germinal cells in forming spermatozoa. Luteinizing hormone, which is secreted by anterior pituitary gland, stimulates the leydig cells to secret

***Corresponding author: Tel: 0092-966-750353; Mobile: 0092-3009091688; e-mail: drgulmajeed@yahoo.com

Table 1: Mean (\pm SEM) serum LH, FSH and testosterone levels

Group	LH (mIU/ml)	FSH (mIU/ml)	Testo (nmol/L)
Azoospermia	13.85 \pm 2.33 ^a	22.92 \pm 4.15 ^c	11.86 \pm 0.70 ^b
Oligozoospermia	10.92 \pm 1.22 ^d	16.82 \pm 3.79 ^a	11.88 \pm 1.06 ^a
Asthenozoospermia	3.92 \pm 1.17 ^b	3.22 \pm 0.61 ^b	16.24 \pm 2.05 ^d
Normozoospermia	7.24 \pm 1.02 ^d	5.53 \pm 0.52 ^d	17.29 \pm 1.02 ^d
Proven fathers (control)	7.44 \pm 0.71	6.75 \pm 1.06	15.88 \pm 1.15

d = Non-significant ($p > 0.05$); a = Significant ($p < 0.05$); b = Significant ($p < 0.01$); c = Significant ($p < 0.001$)
 Normal levels of Testosterone = 5.2-22.9 nmol/L.; Normal level of LH = 2-12 mIU/ml.; Normal levels of FSH = 1-12 mIU/ml.

Table 2: Ratio of various hormones involved in spermatogenesis

Group	Ratio of LH/FSH	Ratio of FSH/LH	Ratio of LH/Testo	Ratio of Testo/LH	Ratio of FSH/Testo	Ratio of Testo/FSH
Azoospermia	0.77 \pm 0.08 ^c	1.84 \pm 0.22 ^c	1.17 \pm 0.28 ^d	0.86 \pm 2.70 ^d	1.93 \pm 5.95 ^d	0.52 \pm 0.17 ^c
Oligozoospermia	1.78 \pm 0.41 ^d	1.47 \pm 0.18 ^a	0.92 \pm 0.28 ^d	1.08 \pm 0.17 ^b	1.42 \pm 3.57 ^d	0.71 \pm 0.28 ^c
Asthenozoospermia	1.51 \pm 0.46 ^d	1.28 \pm 0.28 ^d	0.24 \pm 0.10 ^d	4.14 \pm 10.4 ^d	0.20 \pm 0.30 ^d	5.04 \pm 3.38 ^d
Normozoospermia	1.55 \pm 0.16 ^d	0.85 \pm 0.10 ^d	0.42 \pm 0.08 ^d	2.39 \pm 0.48 ^d	0.35 \pm 1.12 ^d	2.82 \pm 0.90 ^d
Proven fathers	1.67 \pm 0.22	0.94 \pm 0.12	0.49 \pm 0.28	2.05 \pm 0.33	1.042 \pm 0.53	4.09 \pm 0.57

d = Non-significant ($p > 0.05$); a = Significant ($p < 0.05$); b = Significant ($p < 0.01$); c = Significant ($p < 0.001$)

testosterone. Follicle stimulating hormone which is secreted by anterior pituitary gland, stimulate sertoli cells, without this stimulation conversion of spermatids to spermatozoa will not occur (Martin, 2001).

The successful and complete male germ cell development is dependent on the balanced endocrine interplay of hypothalamus, pituitary and the testis. Gonadotropin releasing hormone (GnRH) secreted by the hypothalamus elicits the release of gonadotrophins i.e. follicle stimulating hormone (FSH) and lutenizing hormone (LH) from the pituitary (DeKrester, 1979). FSH binds with receptors in the Sertoli cells and stimulates spermatogenesis, while LH stimulates the production of testosterone in Leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and stimulates spermatogenesis (O'Donnell *et al.*, 1994). The failure of pituitary to secrete FSH and LH will result in disruption of testicular function leading to infertility. Testosterone, estradiol and inhibin control the secretion of gonadotrophins through feed back mechanism (Weinbauer and Nieschlag, 1995).

Status of the male infertility can be assessed through simple semen examination, followed by hormonal profile if required (Martin, 2001). Almost 40% of the total male infertility is mainly because of azoospermia (Rehan, 1991). Absence of spermatozoa in the semen ejaculate is called "azoospermia", count less than 20 million/ml

"Oligospermia" and density of 20 million/ml but motility of less than 50% is called "asthenospermia" (Guyton, 1981). Male infertility is associated with a reduction in the quantity of functional sperms (Amelar, 1966). Decrease in sperm density, eventually leading to azoospermia, has been found to be associated with raised FSH, LH and normal or low testosterone level (Evers-Johannes, 2002).

Hormonal disturbances result in almost 1/6 married couples not having any progeny, and the male partner being responsible in nearly half of such cases (Merino and Carranza-Lira, 1995). Determination of concentration of LH and Testosterone is essential for the evaluation of pituitary gonadal axis and in the evaluation of infertility (Turner and Bagnara, 1976).

Studies on determination of ratios of various hormones are rare (Khan *et al.*, 2005). The present study, although preliminary in nature, aims to identify the association of the spermogram with the ratios of the gonadotrophic hormone and androgenic hormone, to see the flipped condition in various groups of sub fertility, after comparing with proven fathers.

MATERIALS AND METHODS

Subjects

A total of 250 randomly selected male subjects with a history of infertility for the duration of more than 1 year

were included. These patients were referred from the clinics of gynecologists, urologists, and general practitioners from different parts of the country, for semen and hormone analysis. The study was carried out at Reproductive Physiology/Health, Public Health Laboratory Division, National Institute of Health Islamabad. A total of fifty (50) cases of normal subjects belonging to the same socioeconomic status were selected as a control group. The last pregnancy was observed 1-3 years back in the control group. Clinical examinations of all the subjects were carried out and information regarding age, health problems, duration of marriage, history of infertility in the family and parity was recorded. The ages of the patients were ranging from 20-55 years; however, the maximum number of patients were in the age of 25-40 years, and duration of infertility was between 1-20 years. A detailed history was taken, in order to determine any correlation of the problem of infertility faced by couples and the status of the male partner.

Semen analysis

The semen of the Subjects was obtained and analyzed according to WHO recommended procedure (WHO (1987). and categorized as normozoospermic, azoospermic, oligozoospermic and asthenozoospermic.

Hormonal analysis

A 5-10ml fresh blood sample was collected from anterior *cubital vein* of each subject under aseptic condition for hormone analysis, which was transferred to a clean plain labeled tube, allowed to clot, then centrifuged at 6000 rpm for 5 minutes in Hittech centrifuge at room temp, and clear serum was separated and kept at -20°C till the time to conduct assay. FSH, LH and Testosterone assessment was carried out using electrochemiluminescence technology by fully automatic and state of the art equipment Elecsys-2010 analyzer (Fahie-Wilson, 2000).

Quality control of assays

Samples representing the normal and pathological levels of the analytes were used for quality control assays. Results $\pm 2SD$ of the target value were considered acceptable. Only the batches with all controls being within permissible range were accepted.

STATISTICAL ANALYSIS

Data were analyzed statistically, by application of Student's-t test, as described by Steel and Torrie (Steel and Torrie, 1960).

RESULTS

The results of analysis of the hormones and their respective ratios among all sub-groups are presented in tables 1 and 2.

Azoospermic group

It can be seen that in the case of azoospermia, both LH and FSH exhibited significant increases, with the increase in FSH being more profound ($p < 0.001$). Testosterone levels showed a significant ($p < 0.01$) decrease. The ratios of LH/T, FSH/LH, and FSH/Testosterone showed an increase when compared with other groups.

Oligozoospermic group

In conditions of oligozoospermia as well, both LH and FSH again exhibited significant increases, with the increase in FSH being more profound ($p < 0.001$), while testosterone levels were showing a significant ($p < 0.05$) decrease, but however the increase in FSH/LH, and decrease in testosterone, was not more profound, as observed in case of azoospermia. Due to increased LH and decreased Testosterone levels, oligozoospermic subjects exhibited significantly ($p < 0.05$) lower ratios of Testosterone/LH. The ratio of LH/Testosterone, FSH/LH and FSH/Testosterone were increased when compared with proven fathers group.

Asthenozoospermic group

In cases of asthenozoospermia, both LH and FSH exhibited a significant decrease, ($p < 0.05$), while testosterone levels decreased non-significantly ($p > 0.05$). Due to decreased levels of LH and FSH, asthenozoospermic subjects exhibited non-significant ($p > 0.05$) variation in ratios of Testosterone/LH, LH/Testosterone, FSH/LH and FSH/ Testosterone, when compared with proven fathers group.

Normozoospermic group

Among normozoospermic cases, the LH, FSH and testosterone levels exhibited non-significant differences, ($p > 0.05$). Normozoospermic subjects exhibited non-significant ($p > 0.05$) variations in ratios of Testosterone/LH, LH/Testosterone, FSH/LH and FSH/ Testosterone, when compared with proven fathers group.

DISCUSSION

FSH, LH and testosterone are prime regulators of germ cell development. The quantitative production of spermatozoa generally requires the presence of FSH, LH and Testosterone. FSH acts directly on the seminiferous tubules, whereas Luteinizing hormone stimulates spermatogenesis indirectly via testosterone (Anderson *et al.*, 1997).

On the basis of the present study, it is concluded that azoospermic and oligozoospermic subjects are accompanied by a significant rise in FSH levels, and is associated with male sub-fertility. The measurement of FSH in serum may, therefore, be used with advantage in the diagnosis of spermatogenic dysfunction and also to differentiate between secretory and excretory

azoospermia (Subhan *et al.*, 2000; Garcia-Diez *et al.*, 1983; Mann and Lutwak-Mann 1981).

Not only the high levels of FSH and LH were observed, but low level of FSH and LH were also recorded in our study. It has been found that low levels of FSH are also associated with male infertility, as FSH is necessary for sperm maturation (Weinbauer *et al.*, 1997). Low levels of LH, and hence subsequently testosterone, are also found in patients with normal spermatogenic maturation arrest (Weinbauer *et al.*, 1998).

FSH, LH and testosterone evaluation is useful in the management of male infertility (Zabul *et al.*, 1994). De Krester *et al.* (1974) reported elevated levels of serum FSH, and LH with increasing severity of seminiferous epithelial damage. In the present study, elevated levels of LH were observed in azoospermic and oligozoospermic males when compared with the levels in proven fathers, as well as normospermic men. Our results are in agreement with earlier studies (Zabul *et al.*, 1994; Sulthan *et al.*, 1985; Subhan *et al.*, 1995b; Sheikh *et al.*, 2005), which showed gonadotrophic elevation in infertile males. In various studies elevated levels of LH in oligozoospermic and azoospermic males, when compared to normal fertile men, were also documented (Hopkinson *et al.*, 1977; Merino *et al.*, 1980), which is in agreement with our studies.

In the present study, the mean serum testosterone levels in both fertile and infertile men were within permissible levels. Similar observation has been recorded in earlier studies (Smith *et al.*, 1985; Nistal *et al.*, 1990; Turek *et al.*, 1995), who have reported normal level of testosterone in infertile men with Sertoli cell syndrome when compared with control group, but in contrast to another study, where decreased level of testosterone was observed in infertile males (Jones, 1987) as sometimes there is a loss of germinal epithelium, but Leydig cells of testes remain intact. This condition is usually reflected by normal plasma testosterone level.

In the present study, serum concentration of Testosterone was although within the limit, but was on lower side. In a number of related studies, low levels of serum testosterone have been shown to be associated with infertility. It has been demonstrated that low levels of serum LH and testosterone are found in men with oligoasthenozoospermia (Reyes-Fuentes *et al.*, 1997). It has been reported, that decreased secretions of LH and Testosterone in oligozoospermic men are due to prolonged half life of LH, reduced bio-active LH secretory burst amplitude, lower immunoactive ratio for LH burst amplitude, reduced bioactive/immunoactive ratio in the mass of LH secreted per burst and decreased coordinated release of bioactive LH and testosterone (Reyes-Fuentes *et al.*, 1996). The suppression of

testosterone secretion in infertile men could be due to deficiency of hypothalamic Gn RH, resulting in the impairment of Gonadotropin secretion from pituitary. The deficiency of LH and FSH prevents the gonads from either producing sperms or sufficient quality of testosterone (Nieschlag, 1997).

In our investigations we found a small group of subjects in infertile patients with high testosterone concentrations. It has been reported that testicular tumors prior to puberty produced high concentrations of testosterone leading to precocious puberty. After puberty, they tend to produce more estrogens resulting in feminization with characteristic symptoms of gynecomastia, impotence and testicular tumors. In those conditions, hypothalamic/pituitary function is reduced by estrogen via feedback, and atrophy of the testicular tissue surrounding the tumor and of the contra lateral testis may result. Spermatogenesis ceases, and oligozoospermia or azoospermia is found (Nieschlag, 1997). The significant rise in serum LH level in oligozoospermic and azoospermic males in the study, are in contrast with the findings of Saeed *et al.*, (1994), whereas, Kuku *et al.* (1988) observed elevated LH levels in 26.5% subjects and low level in 5% of infertile males, which is an agreement to our study.

In the study under discussion, different combinations were observed with respect to LH, FSH and Testosterone levels, as FSH and LH levels were high in case of azoospermia and oligozoospermia, while Testosterone levels showed significant decrease in these groups, and the variation in these levels affected the ratios. It can be seen that in the case of azoospermia and oligozoospermia, testosterone exhibited significant decreases, while the increase in case of asthenozoospermia as well as normozoospermia was not significant. Significant differences in LH levels were also observed in case of azoospermia ($p < 0.05$) and asthenozoospermia ($p < 0.01$), being elevated in cases of azoospermia and decreased in cases of asthenozoospermia. Similarly it can be seen that in the case of azoospermia, both LH and FSH exhibited significant increases, with the increase in FSH being more profound ($p < 0.001$). Significant ($p < 0.01$), differences in FSH levels were also observed in case of oligozoospermia and asthenozoospermia.

The LH/T ratios were observed as non-significant ($p > 0.05$) in all groups in comparisons to proven fathers. Due to increased LH and decreased testosterone levels, significantly ($p < 0.01$) lower ratios of Testosterone/LH were observed in cases of oligozoospermia. The FSH/LH ratio was (1.84 ± 0.22) in case of azoospermia and decreased to 0.85 ± 0.10 in case of normozoospermia. Even in conditions of oligozoospermia and asthenozoospermia, the FSH/LH ratios were well above 1.00. Due to increase in FSH levels, the FSH/LH ratios exhibited significant

increases in azoospermic ($p < 0.001$) and oligozoospermic ($p < 0.01$) subjects.

Similarly, the ratio of FSH/Testosterone, among all sub groups, was highest (1.93 ± 5.95) in case of azoospermia and decreased to 0.35 ± 1.12 in case of normozoospermia. A high ratio was also recorded in Oligozoospermia, as the FSH/Testosterone ratios were well above 1.00. Elevation of FSH levels, and significant decrease in Testosterone, brought about significant increases in Testosterone/FSH ratios in azoospermic ($p < 0.001$) and oligozoospermic ($p < 0.001$) subjects.

All the subjects were normal individuals and their chromosome analysis did not present any evidence for abnormal karyotypes. In addition, there is no family history of infertility in the cases studied and hence the infertility in these cases might be attributed to hormonal causes. The overall results clearly indicate significant increase in gonadotrophins (FSH and LH) in azoospermia and oligozoospermia. Although there was significant decrease in the testosterone levels in infertile males when compared with the fertile controls, the increase in the levels of gonadotrophin might have disrupted the spermatogenic process leading to the decline in the sperm count and infertility.

The overall results clearly indicate significant increase of LH, in case of depleted sperm concentrations, and significant decrease in cases of asthenozoospermia. Although there was a significant decrease in the testosterone levels in azoospermic and oligozoospermic males when compared with the fertile controls, the increase in the levels of LH might have disrupted the spermatogenic process leading to the decline in the sperm count and infertility.

The study suggests that estimation of serum levels of LH and FSH and their ratios, specially the FSH/LH ratio, could be used as tool to investigate the nature of neuroendocrine cause of infertility, as both these hormones act together. The study also suggests that elevated levels of LH and low level of testosterone are responsible for decreased function of Leydig cells and imparting diminished secondary sexual characteristics in infertile patients. Therefore it is important to say that estimation of serum levels of LH and Testosterone and their ratios, specially the LH/Testosterone ratio, could be used as tool to investigate the nature of neuroendocrine cause of infertility, as both these hormones act in association.

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