

# Correlation of serum and seminal plasma human growth hormone concentration with semen parameters of normozoospermic and infertile men

Muhammad-Baqir M-R. Fakhrildin

Institute of Embryo Research and Infertility Treatment, Al-Nahrain University

Corresponding author e-mail: art\_bmrfd@yahoo.com

## Abstract:

### Background

Background: Human growth hormone (HGH) is undisputedly a potent hormone with a wide variety of biological effects. The anabolic actions of human growth hormone have made it attractive as a potential agent for catabolic problems in a wide range of clinical conditions.

### Objectives

In the present study, we investigated the possible relationship between levels of HGH in serum (S. HGH) and seminal plasma (Sp. HGH) and semen parameters in normozoospermic (NZS) men and infertile patients.

### Materials and Methods:

In total, 66 infertile patients with azoospermia (AZS; no. 52), asthenozoospermia (AsZS; no. 6) and oligoasthenoteratozoospermia (OATZS; no. 8), and normozoospermic men (NZS; no. 12) were included in this study. From each subject, semen and blood samples were taken to assess seminal plasma and sperm parameters and levels of S. HGH and Sp. HGH; respectively.

### Results

The highest S. HGH was evaluated in AZS patients when compared to NZS and other infertile groups. Significant elevation ( $P < 0.05$ ) in the level of S. HGH was assessed for AZS patients as compared to NZS men. In contrast, the level of Sp. HGH in NZS men was significantly elevated ( $P < 0.05$ ) when compared to AZS patients only. Positive and significant correlations were noticed between levels of Sp. HGH and semen liquefaction time in NZS, AZS and OATZS groups. In NZS men, positive and significant correlation were observed between Sp. HGH and semen volume and negative and significant correlations were detected between Sp. HGH and percentages of sperm motility, progressive motility, immobility and agglutination.

### Conclusion

Our study represents the first study in Iraq showing that the level of HGH in serum and seminal plasma affect male fertility and sperm parameters in normozoospermic males and infertile patients. Further biochemical and molecular studies are recommended to investigate the mode of action of HGH and its receptors in relation to fertility.

**Keywords:** Growth hormone, Male infertility, semen.

## Introduction

Growth hormone (GH), also called somatotropin, is a 22000-Dalton protein secreted from anterior pituitary gland in mammals. Human GH (HGH) is required for normal development during childhood and is involved in the regulation of a variety of anabolic processes (1). GH plays a pivotal role in regulating body growth and development, which is modulated by sex steroids (2). The gene is mainly expressed in the pituitary gland as a protein, which is characterized by an arrangement of four anti

parallel -helices separated by connecting loops (3). The GH receptor contains seven cysteine residues in its extracellular domain (4). From an endocrine vantage, normal physical growth and sexual maturation require time-evolving coordination among the somatotropic, gonadotropic and adipostate systems (5, 6). Additional inter-axis control is accomplished by convergent and divergent actions of the corresponding hormones on the pituitary gland and peripheral target tissues (7, 8). Multiple

neurotransmitter pathways, as well as, a variety of peripheral feedback signals, regulate GH secretion either by acting directly on the anterior pituitary gland and / or by modulating GH-releasing hormone (GHRH) or somatostatin release, or both, from the hypothalamus (9). Infertility means that a couple has failed to achieve a pregnancy within one year of regular, at least three times per month, unprotected intercourse (10). Male infertility is a significant problem affecting 7.5 % of the male population and approximately 40 % of all infertility cases (11). It is considered a male factor when an alteration in the sperm concentration and/or motility and/or morphology could be found in at least one sample of two semen analyses which comply with World Health Organization guidelines (12) collected between 1-4 weeks apart (13). Usually, typical measurements done in a semen analysis include: semen volume, pH, viscosity, liquefaction time, sperm concentration, motility, progressive motility and sperm morphology (14). Male infertility factors involve azoospermia (AZS) which means no sperm cells in the semen ejaculate; as then azoospermia (AsZS) which means a reduction of progressive sperm motility (< 50 %); while reduction in the number of sperm concentration, sperm motility and normal sperm morphology is called oligoasthenteratozoospermia (OATZS) (15). Therefore, in the present study, we studied the possible relationship between levels of HGH in serum (S. HGH) and seminal plasma (Sp. HGH) and semen parameters in normozoospermic (NZS) men and infertile patients.

## Subjects, Materials and Methods

### 1. Subjects:

This study was conducted on 78 men who attended the Institute of Embryo Research and Infertility Treatment at Al-Nahrain University. The criteria which applied for subjects were proper history taking, physical examination and hormone levels profile. In the present study, the grouping of males into either normozoospermic (NZS) men (12 men, Mean age 34.2 years) or infertile patients (66 men, Mean age 35.6 years; mean duration of infertility ~ 5 years) was done according to parameters of WHO (12). Also, infertile patients were subdivided into three groups including Azoospermic (AZS), Asthenozoospermic (AsZS) and Oligoasthenteratozoospermic (OATZS) patients.

### 2. Semen analysis

The semen sample for each male partner was collected after 3-5 days of abstinence by masturbation. The sample was collected in a clean and sterile Petri dish. The container of each male was labeled with his name, age and time of collection. Semen analysis was done according to methodology of WHO manual (12), and involves macroscopic and microscopic examinations.

### 3. Hormonal assay:

Blood samples were collected from all subjects involved in the present work. A volume 3 mL of peripheral blood was collected, by vein puncture into sterile tube. At the same time, seminal plasma free of spermatozoa was prepared from semen sample. The procedures for final preparations of serum and seminal plasma were mentioned in details by Fakhridin (16).

Levels of serum HGH (S. HGH) and seminal plasma (Sp. HGH) were measured using immunoradiometric (IRMA) technique. The sensitivity of HGH was 0.04  $\mu\text{IU/mL}$ , intra- and inter-assay coefficients of variation were 1.5 % and 2.5 %; respectively.

### 4. Statistical analysis:

Computerized statistical analyses were performed using Statistical Package of Social Science (SPSS, version 12) software. Crude data was statistically computed and expressed as mean  $\pm$  standard error of mean (SEM). The results were statistically analyzed using multiple analyses of variance (MANOVA) and multiple correlation and regression tests were used to compare among different means of study groups. Significance level was set at  $P < 0.05$  (17).

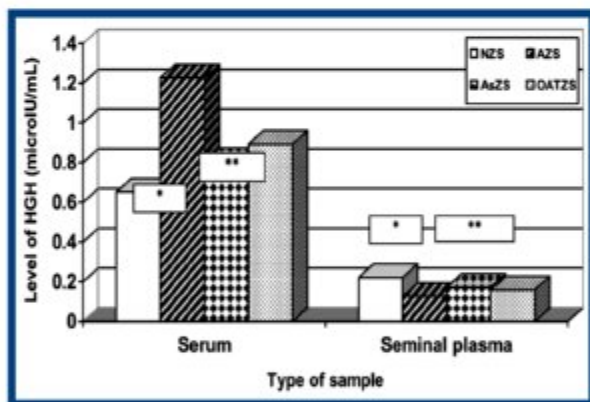
## Results

Figure (1) shows the HGH levels in serum (S. HGH) and seminal plasma (Sp. HGH) of men included in the current study. The highest S. HGH was evaluated in azoospermic (AZS) patients when compared to normozoospermic (NZS) and other infertile groups. Significant elevation ( $P < 0.05$ ) in the level of S. HGH was assessed for AZS patients as compared to NZS men. Meanwhile, the levels of S. HGH among three groups of infertile patients were different non significantly ( $P > 0.05$ ). Also, from the same figure, non significant ( $P > 0.05$ ) differences in the levels of Sp. HGH were reported among three groups of infertile patients. In contrast, the level of Sp. HGH in NZS men was significantly elevated ( $P < 0.05$ ) when compared to AZS patients only.

From table (1), non significant differences ( $P > 0.05$ ) in the body weight was found among the four groups of this study. However, the highest body weight was recorded for AZS groups as compared to other study groups. Correlation study between body weight and different groups of NZS men and infertile patients showed positive and significant correlation ( $r = 0.350$ ;  $P = 0.042$ ) with the level of S. HGH in AZS patients only. Meanwhile, negative and significant correlation ( $r = -0.298$ ;  $P = 0.05$ ) was obtained between body weight and Sp. HGH in AZS patients. Moreover, negative and significant correlation ( $r = -0.826$ ;  $P = 0.43$ ) was demonstrated between body weight and Sp. HGH in AsZS patients. Positive and highly significant correlations were detected between levels of S. HGH and S. FSH and S. LH in

AZS patients ( $r = 0.798$ ;  $P = 0.001$  and  $r = 0.891$ ;  $P = 0.001$ ; respectively) only. On the other hand, non significant correlations were reported between S. HGH and S. FSH, S. LH, S. Testosterone, S. PRL and Sp. HGH in NZS men and infertile patients groups (Table 1).

Correlations between levels of Sp. HGH and parameters of semen analyses in different study groups were presented in the table (2). Positive and significant correlations were reported between Sp. HGH and semen liquefaction time in NZS, AZS and OATZS groups. In NZS men, positive and significant correlation were observed between Sp. HGH and semen volume ( $r = 0.561$ ;  $P = 0.05$ ), and negative and significant correlations between Sp. HGH and percentages of sperm motility, progressive motility, immobility and agglutination. In AsZS patients, negative and significant correlations were assessed between Sp. HGH and semen pH, non-progressive sperm motility (%), total progressive motile sperm/ejaculate and sperm agglutination (%). However, in OATZS patients, strong positive and highly significant correlations were noticed between Sp. HGH and semen pH ( $r = 0.795$ ;  $P = 0.01$ ) and total progressive motile sperm/ejaculate ( $r = 0.803$ ;  $P = 0.01$ ) (Table 2).



**Figure1:** Levels of human growth hormone (HGH) in the serum and seminal plasma of normozoospermic and infertile men#

#: Number of men for each group (NZS= 12; AZS=52; AsZS=6; OATZS=8).

\*: Means significant difference ( $P < 0.05$ ) between NZS group and AZS group.

\*\* : Means non significant difference ( $P > 0.05$ ) among three groups of infertile patients.

(NZS: Normozoospermic group; AZS: Azoospermic group; AsZS: Asthenozoospermic group; OATZS: Oligoasthenoteratozoospermic group).

**Table 1:** Mean and correlation of body weight and levels of serum human growth hormone (HGH) to levels of serum reproductive hormones and levels of HGH in serum and seminal plasma of normozoospermic and

Parameters	NZS men	Groups of infertile patients			
		AZS	AsZS	OATZS	
Body weight (Kg)	65.82 $\pm 2.77$	77.35 $\pm 2.18$	75.12 $\pm 4.83$	72.75 $\pm 3.83$	
Body weight correlation to level of HGH in	Serum; S. HGH	$r = 0.369$ $P = 0.108$	$r = 0.350$ $P = 0.042$	$r = 0.491$ $P = 0.098$	$r = -0.257$ $P = 0.132$
	Seminal plasma; Sp. HGH	$r = 0.136$ $P = 0.674$	$r = -0.298$ $P = 0.05$	$r = -0.826$ $P = 0.043$	$r = 0.553$ $P = 0.155$
S. HGH correlation with reproductive hormones	S. FSH	$r = 0.418$ $P = 0.187$	$r = 0.798$ $P = 0.001$	$r = 0.372$ $P = 0.109$	$r = 0.368$ $P = 0.121$
	S. LH	$r = 0.391$ $P = 0.088$	$r = 0.891$ $P = 0.001$	$r = 0.311$ $P = 0.122$	$r = 0.376$ $P = 0.128$
	S. Testosterone	$r = 0.265$ $P = 0.148$	$r = 0.122$ $P = 0.491$	$r = 0.218$ $P = 0.363$	$r = 0.142$ $P = 0.632$
	S. PRL	$r = -0.346$ $P = 0.137$	$r = -0.137$ $P = 0.454$	$r = -0.227$ $P = 0.186$	$r = -0.167$ $P = 0.841$
	Sp. HGH	$r = 0.402$ $P = 0.095$	$r = -0.043$ $P = 0.809$	$r = -0.462$ $P = 0.093$	$r = 0.138$ $P = 0.681$

\* Number of men for each group (NZS= 12; AZS= 52; AsZS= 6; OATZS= 8).

(NZS: Normozoospermic group; AZS: Azoospermic group; AsZS: Asthenozoospermic group; OATZS: Oligoasthenoteratozoospermic group).

**Table 2:** Correlations between parameters of semen analysis and HGH levels in seminal plasma of normozoospermic and infertile men\*

Semen analysis	NZS	AZS	AsZS	OATZS
	Sp. HGH	Sp. HGH	Sp. HGH	Sp. HGH
Semen liquefaction Time (min.)	$r= 0.733$ $P= 0.007$	$r= 0.298$ $P= 0.05$	$r= 0.228$ $P= 0.664$	$r= 0.689$ $P= 0.05$
Semen volume (mL.)	$r= 0.561$ $P= 0.05$	$r= - 0.084$ $P= 0.554$	$r= 0.115$ $P= 0.829$	$r= 0.041$ $P= 0.923$
Semen pH	$r= - 0.494$ $P= 0.103$	$r= 0.157$ $P= 0.268$	$r= - 0.803$ $P= 0.05$	$r= 0.795$ $P= 0.018$
Sperm concentration ( $\times 10^6$ /mL)	$r= 0.108$ $P= 0.739$	-	$r= - 0.952$ $P= 0.003$	$r= 0.011$ $P= 0.979$
Sperm motility (%)	$r= - 0.676$ $P= 0.016$	-	$r= - 0.430$ $P= 0.395$	$r= 0.217$ $P= 0.606$
Progressive sperm motility (%)	$r= - 0.846$ $P= 0.001$	-	$r= - 0.178$ $P= 0.736$	$r= 0.393$ $P= 0.335$
Non-progressive sperm motility (%)	$r= 0.472$ $P= 0.121$	-	$r= - 0.901$ $P= 0.014$	$r= - 0.256$ $P= 0.540$
Immotile sperm (%)	$r= - 0.676$ $P= 0.016$	-	$r= - 0.430$ $P= 0.395$	$r= 0.217$ $P= 0.606$
Total progressive motile sperm / ejaculate	$r= 0.140$ $P= 0.665$	-	$r= - 0.858$ $P= 0.029$	$r= 0.803$ $P= 0.016$
Normal sperm morphology (%)	$r= - 0.298$ $P= 0.347$	-	$r= 0.475$ $P= 0.341$	$r= 0.374$ $P= 0.361$
Sperm agglutination (%)	$r= 0.622$ $P= 0.031$	-	$r= - 0.803$ $P= 0.05$	$r= - 0.256$ $P= 0.540$

\*: Number of men for each group (NZS= 12; AZS= 52; AsZS= 6; OATZS= 8)

(NZS: Normozoospermic group; AZS: Azoospermic group; AsZS: Asthenozoospermic group; OATZS: Oligoasthenoteratozoospermic group).

## Discussion

The GH/GH receptor interaction ultimately results in alterations of lipid, nitrogen, mineral and carbohydrate metabolisms well as cellular differentiation (18). Human GH has been reported multiple functions and actions on different tissues and metabolism of the body including general growth (19), muscle development, protein synthesis (6,20) and age (21). In addition, and to the best of our knowledge, very little literatures were done on the effects of HGH on fertility, and no literatures on correlation between HGH and parameters of semen analysis. Therefore, in this study, we assessed the level of HGH in serum (S. HGH) and seminal plasma (Sp. HGH), as well as relation to parameters of semen analysis.

In the present work, infertile patients with azoospermia (AZS) had the highest level of S. HGH as compared to the other study groups. Elevation of S. HGH causes several biochemical changes related to metabolism (22). One of the most important metabolic changes is the elevation of blood glucose (23). Also, HGH induces production of insulin-like growth factors (IGFs) from different body tissues (24). IGF has been implicated in the pathophysiology of diabetes mellitus (25). Indeed, diabetes is related to damage in the sperm production and function (26), impotence (27), subfertility (28), infertility (29) or combination of them. HGH regulates growth and body composition through a complex process that combines mitogenic and metabolic actions elaborated through mechanisms dependent and independent of IGF-I (2). However, the lowest HGH secretion rates are observed in older or obese adults, and those with hypothyroidism or type II diabetes mellitus (30). Meanwhile, elevation of S. HGH in AZS patients may be combined with GH receptor deficiency and/or insensitivity and/or antagonism. The clinical phenotype of GH deficiency, but with elevated S. HGH, was originally described by Laron. Laron syndrome represents the primary hereditary form of GH insensitivity (31), or receptor antagonism (32).

The support and complement the previous results, the level of S. HGH in AZS patients only was strongly positive and highly significant correlated with levels of serum gonadotropins (FSH and LH). Therefore, hypersecretion of HGH stimulates subsequent over secretion of gonadotropins which lead to azoospermia. In a previous study performed by Fakhridin (16), levels of serum gonadotropins for azoospermic patients were highly elevated as compared to the other groups of infertile and healthy men. Most studies reported that the elevation of serum gonadotropins is correlated with azoospermia (33, 34). Other observations suggest that testosterone may also modify growth hormone releasing hormone (GHRH) actions on the pituitary gland, via one or more mechanisms activated *in vivo*. Therefore, androgens in the absence of HGH are insufficient in the human to drive the fully normal tempo of clinical pubertal maturation (9). Available data indicate that IGF-I may inhibit HGH secretion by way of combined feedback actions on hypothalamus and

pituitary gland (35).

Elevation of HGH reduces the levels of low density lipoprotein (LDL)-cholesterol and total cholesterol (21). In turn, reduction in the cholesterol and LDL-cholesterol negatively has impact on sperm maturation and capacitation (36). Certainly, epididymal sperm maturation regulates the function of sperm plasma membrane and needs to presence of cholesterol (37). Therefore, sperm capacitation is the first important process for acrosomal reaction and normal fertilization (38).

Also, elevation of S. HGH increases the body weight (39). Therefore, in this study, higher weight was recorded in the AZS group when compared to the other groups of healthy and infertile men. Furthermore, elevation of S. HGH negatively affects prostate volume and stimulates prostate to produce higher level of prostate-specific antigen (S. PSA), which considered the most indicator of prostate cancer risk (40). However, the least level of S. HGH and body weight were registered in the NZS group as compared to three groups of infertile patients. Hintz (41) reported that the HGH abuse causes fertility problems. The reduction in the HGH secretion with healthy aging begins after the pubertal maximum and is correlated with increased percentage total body and visceral fat and proteins, decreased physical fitness, and decreased testosterone concentrations in men (9, 42). While, low HGH concentrations in obesity result from reduced secretion and increased elimination (43). In contrast, deprivation of GH attenuates human phallic growth and reduces adult testicular size (7).

Level of Sp. HGH for the NZS men, in this study, was higher than other groups of infertile patients, and the least level of Sp. HGH was observed in the AZS patients. The occurrence of HGH is related to several factors including presence, structure and type of receptors (4, 8), gene expression and autocrine HGH activity (44) and other factors related to disturbances in the regulation of mechanism of action and axis or pathway of stimulation and production (45), as well as molecular and immunological factors (2). Possibly, in the testes of infertile patients, reduced GH receptor/GH-binding protein expression might be a possible cause for the partial GH insensitivity (3). Reproductive sequelae of HGH excess can also be appraised via such molecular strategies and, conversely, HGH deficiency can be assessed by GH receptor knockout mice (9).

In normozoospermic men of the present study, positive and significant correlation was obtained between Sp. HGH and seminal plasma volume. This result may be represents synergistic actions between Sp. HGH and testosterone on prostate growth. It is known that the functions of male accessory glands under biological action of testosterone (46). Although weak and non significant correlations were observed between S. HGH and serum testosterone and Sp. HGH in NZS men. Several studies reported that the administration of combination of

prepared HGH and testosterone have biological activities higher than administration of each prepared hormone alone (20, 21, 22).

In this work, positive and significant correlations were assessed between Sp. HGH and pH of seminal plasma and total progressive motile sperm within ejaculate in AsZS and OATZS patients. This result is agreement with studies reported that reduction of seminal plasma pH is correlated with decrease in the progressive sperm motility (12). Furthermore, the reduction in the levels of Sp. HGH in AsZS and OATZS patients may be related to reduction in the total antioxidant capacity (TAC) together with increased reactive oxygen species (ROS) were found in the tubula and the seminal plasma of the majority of OATZS patients (47). An increase in the oxidative stress (OS) markers has been observed in the serum, semen, and testicular tissues of infertile patients (48). ROS damages to sperm plasma membrane by peroxidation of their lipid content. Spermatozoa are particularly susceptible to OS-induced damage because of the large polyunsaturated fat content in their membranes (49). OS is also responsible for damage to the DNA structure of the spermatozoa (50).

Our study represents the first study showing that the level of HGH in serum and seminal plasma affect male fertility and sperm parameters in normozoospermic males and infertile patients. Further biochemical and molecular studies are recommended to investigate the mode of action of HGH and its receptors in relation to fertility.

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