



# Inhibin B and FSH as markers of Sertoli cell function in impaired spermatogenesis

Inhibina B i FSH jako markery funkcji komórek Sertoliego w przypadkach upośledzonej spermatogenezy

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## Abstract

**Introduction:** The relationships between inhibin B, FSH and sperm count have never been fully elucidated. Our aim was to search for associations between serum concentrations of inhibin B/FSH and the impairment of spermatogenesis.

**Material and methods:** In an observational study, we compared sperm count, serum levels of inhibin B and FSH in men with oligozoospermia (n = 46) and in normozoospermic, fertile controls (n = 38).

**Results:** Concentration of FSH was  $10.27 \pm 11.24$  IU/L in the oligozoospermic and  $3.84 \pm 2.76$  IU/L in the normospermic group ( $p < 0.01$ ). Although the concentration of inhibin B was higher in the oligozoospermic group ( $424 \pm 443$  v.  $297 \pm 219$  pg/mL), the difference was statistically insignificant.

**Conclusions:** Our results suggest that there is a tendency toward increased serum inhibin B levels in subjects with altered sperm count and increased serum FSH. (*Pol J Endocrinol* 2010; 61 (6): 695–698)

**Key words:** inhibin B, FSH, sperm count, Sertoli cell, idiopathic oligozoospermia

## Streszczenie

**Wstęp:** Związki między inhibiną B, FSH i obrazem nasienia nie zostały dotąd w pełni wyjaśnione. Celem pracy było poszukiwanie zależności między stężeniem inhibiny B i FSH a zaburzeniami spermatogenezy.

**Materiał i metody:** W badaniu o charakterze obserwacyjnym oceniano spermogramy, osoczowe stężenie inhibiny B i FSH u pacjentów z oligozoospermia (n = 46) i w grupie mężczyzn normospermicznych, płodnych (n = 38).

**Wyniki:** Stężenie FSH wynosiło  $10,27 \pm 11,24$  jm./l w grupie oligozoospermii i  $3,84 \pm 2,76$  jm./l w grupie normospermii ( $p < 0,01$ ). Nie stwierdzono istotnej statystycznie różnicy pomiędzy stężeniem inhibiny B, które wyniosło w grupie normospermii i oligozoospermii odpowiednio:  $297 \pm 219$  i  $424 \pm 443$  pg/ml (ns).

**Wnioski:** Uzyskane wyniki sugerują, że stężenie inhibiny B może być podwyższone u pacjentów z zaburzeniami spermatogenezy i podwyższonym stężeniem FSH. (*Endokrynol Pol* 2010; 61 (6): 695–698)

**Słowa kluczowe:** inhibina B, FSH, spermogram, komórki Sertoliego, idiopatyczna oligozoospermia

## Introduction

Inhibin B belongs to the family of transforming growth factors. It is produced by Sertoli cells and is thought to be a marker of their function. Inhibin B influences interactions between Sertoli cells and germ cells and shows a negative feedback-loop with FSH [1–4]. Serum concentrations of inhibin B and FSH closely correlate with the functional status of Sertoli cells [5, 6].

Clinically, FSH is elevated in some but not all subjects with severe disturbances of spermatogenesis. On the other hand, a number of cases with minor sperm count alterations present considerably increased FSH.

In an observational study, we compared the serum concentrations of inhibin B and FSH together with the sperm count in men with idiopathic oligozoospermia and also in normozoospermic, fertile controls.



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**Table I.** Serum concentrations of FSH and inhibin B in normospermic and oligozoospermic men (mean  $\pm$  SD)**Tabela I.** Osoczowe stężenia FSH i inhibiny B u mężczyzn normospermicznych i oligozoospermicznych (średnia arytmetyczna  $\pm$  odchylenie standardowe)

	Normospermia (n = 38)	Oligozoospermia (n = 46)	p value*
FSH [IU/L]	3.84 $\pm$ 2.76	10.27 $\pm$ 11.24	< 0.01
Inhibin B [pg/mL]	297 $\pm$ 219	424 $\pm$ 443	ns

\*Mann-Whitney U test; ns — non-significant

## Material and methods

The protocol of the present study was approved by the Bioethics Committee at Wrocław Medical University.

Eighty-four men were enrolled for the investigation (aged 26.75  $\pm$  2.38). They were consecutive patients referred to the outpatient andrological clinic because of marital infertility. They had no history of andrological disturbances and did not present any pathology on andrological examination. Subjects with infections, chronic diseases, abnormalities in the ultrasound imaging of the testes or who had used hormonal therapies within the previous six months were excluded.

In each subject, two spermograms were acquired with a time interval of 2–4 weeks (with 2–5 days sexual abstinence before the examination). Sperm analysis was performed manually according to the WHO criteria (1999). Among the studied men, 46 had idiopathic oligozoospermia (sperm concentration < 20 mln/mL) and 38 were considered as fertile controls (normal semen analysis with fathered children).

Considering FSH plasma levels, the studied subjects were divided into three groups: normozoospermia & FSH < 11 IU/L, oligozoospermia & FSH < 11 IU/L (n = 30) and oligozoospermia & FSH > 11 IU/L (n = 16).

We also evaluated the associations between FSH and inhibin B in the three groups based upon sperm concentration: severe oligozoospermia (1–5 mln/mL), mild oligozoospermia (5–20 mln/mL) and normozoospermia (> 20 mln/mL).

Blood specimens were obtained between 8.00 and 9.00 a.m. from the ulnar vein. Serum was stored at –20°C until use. FSH was determined by the IRMA method (Coat-A-Count, DPC, Los Angeles, CA, USA) and inhibin B by the ELISA method (Diagnostic Systems Laboratories, USA). The inter- and intra-assay variability coefficients were respectively 5.7–5.0% and 3.8% for FSH, and 7.6–6.2% and 3.5–5.6% for inhibin B. The normal range for FSH in men aged 20–50 is 2.0–11.0 IU/L.

Data was analysed using StatSoft's STATISTICA (data analysis software system), version 6.0. Pearson and Spearman correlation coefficients were used for data evaluation, and comparisons between groups were assessed using the Mann-Whitney U test.

**Table II.** FSH and inhibin B concentrations in subjects with: severe oligozoospermia (< 5 mln/mL), moderate oligozoospermia (> 5–20 mln/mL) and normospermia (> 20 mln/mL). Data presented as mean  $\pm$  SD. Differences among groups were statistically insignificant

**Tabela II.** Stężenia FSH i inhibiny B u mężczyzn z: ciężką oligozoospermią (< 5 mln/ml), umiarkowaną oligozoospermią (> 5–20 mln/ml) i normospermią (> 20 mln/ml). Dane przedstawione jako średnia arytmetyczna  $\pm$  odchylenie standardowe. Różnice pomiędzy grupami nie osiągnęły istotności statystycznej

Groups	FSH [IU/L]	Inhibin B [pg/mL]
Severe oligozoospermia (n = 25)	13.90 $\pm$ 13.06	377 $\pm$ 356
Moderate oligozoospermia (n = 21)	4.59 $\pm$ 2.61	493 $\pm$ 552
Normospermia (n = 38)	3.84 $\pm$ 2.76	297 $\pm$ 219

## Results

Mean plasma FSH in men with normal sperm count was 3.84  $\pm$  2.76 IU/L and in men with oligozoospermia 10.27  $\pm$  11.25 IU/L. The difference between two groups was statistically significant (p < 0.01).

Mean plasma inhibin B was 297  $\pm$  219 pg/mL in normozoospermic subjects and 424  $\pm$  443 pg/mL in men with oligozoospermia (Table I).

In a further analysis, we used sperm count to distinguish two subgroups among oligozoospermic subjects. FSH in men with sperm concentration < 5 mln/mL was 13.90  $\pm$  13.09 IU/L, while in subjects with sperm count between 5 and 20 mln/mL it was 4.59  $\pm$  2.61 IU/L (ns). We did not observe any difference of FSH level among subjects with normozoospermia, moderate and severe oligozoospermia.

Distinct subgroups of men with oligozoospermia did not differ as to mean concentration of inhibin B. In moderately oligozoospermic men, it was 493  $\pm$  552 pg/mL while in severely oligozoospermic subjects, it was 377  $\pm$  356 pg/mL. In normozoospermic volunteers the level of inhibin B was 297  $\pm$  356 pg/mL (Table II).

We have not found any differences of inhibin B levels among groups distinguished upon FSH concentration.

**Table III.** *Inhibin B serum concentration in subgroups distinguished upon combined sperm concentration and FSH level. Data presented as mean  $\pm$  SD. Differences among groups were statistically insignificant*

**Tabela III.** *Osoczowe stężenie inhibiny B w podgrupach wyodrębnionych na podstawie liczby plemników i stężenia FSH. Dane przedstawione jako średnia arytmetyczna  $\pm$   $\pm$  odchylenie standardowe. Różnice pomiędzy grupami nie osiągnęły istotności statystycznej*

Groups	Inhibin B [pg/mL]
Normospermia & FSH < 11 IU/L (n = 38)	297 $\pm$ 219
Oligozoospermia & FSH < 11 IU/L (n = 30)	453 $\pm$ 492
Oligozoospermia & FSH $\geq$ 11 IU/L (n = 16)	352 $\pm$ 296

Though oligozoospermic men with FSH < 11 IU/L had markedly higher inhibin B level than oligozoospermic men with FSH  $\geq$  11 IU/L, the observed difference was statistically insignificant (Table III).

## Discussion

In spite of our growing knowledge as to the function of male gonads [7–9], clinical assessment of specific cases often turns out to be difficult. Routine seminal and hormonal evaluations give only an approximate picture of the pathologies underlying the disturbances of spermatogenesis. Thus in many cases alterations of spermatogenesis are classified as idiopathic. Evaluation of inhibin B level has raised some interest because of its negative correlation with FSH and due to its influence on interactions between Sertoli and germ cells [10].

Although the clinical value of inhibin has been questioned [11, 12], most authors agree as to the presence of a close relationship between concentrations of inhibin B in serum and the status of spermatogenesis [13–17].

De Kretser et al. evaluated serum concentrations of inhibin and gonadotropins in 39 normal men and 127 infertile subjects. They found no correlation between inhibin and the level of testicular damage or FSH concentration [11]. Kolb et al. observed that concentrations of inhibin B in men with testicular or primary germ cell failure is lower than in fertile controls. It was also decreased in patients with idiopathic hypogonadotropic hypogonadism. In one case of Kallmann syndrome, inhibin B was undetectable [6].

In another study, Foresta et al. showed that men with Sertoli cell only syndrome (SCOS), severe hypospermatogenesis and spermatogonial/spermatocytic arrest had lower levels of inhibin B than men with obstructive azoospermia and spermatocytic arrest or healthy controls. Nevertheless, they pointed to the fact that a considerable subgroup of subjects with SCOS had normal

levels of inhibin B [12]. Yet other authors showed that infertile men had lower concentrations of inhibin B and higher concentrations of FSH than healthy men from the general population [18].

Our study found no statistically significant differences of inhibin B between men with normospermia and men with testicular impairment. Inhibin B did not differ among groups distinguished upon FSH level, either. However, we noticed a tendency toward a higher concentration of inhibin B in men with altered spermatogenesis. This was not evident as concentration of inhibin B was higher in men with oligozoospermia and normal FSH, and lower in men with oligozoospermia and increased FSH.

Our results agree with suggestions that not only Sertoli cells, but also germ cells, affect the level of inhibin B. It is worth mentioning that  $\beta$  subunits of inhibin B are found in germ cells (pachytene spermatocytes and early spermatides), while both  $\alpha$  and  $\beta$  subunits are present in Sertoli cells [11, 19, 20]. These observations lead to a hypothesis that inhibin B consists of two subunits: a subunit deriving from Sertoli cells, and  $\beta$  from germ cells. Thus a lack of germ cells could result in the production of biologically inactive  $\alpha$  chains, something in fact that has been observed in some men with azoospermia (SCOS, radiation-induced testicular damage) with accompanying increased levels of FSH.

Increased inhibin B and normal FSH should confirm the proper function of the loop inhibin B-FSH and an efficient secretory function of Sertoli cells. In our study, it was true for the group with sperm concentration between 5–20 mln/mL. Increased concentration of inhibin B in these subjects went along with mild, idiopathic alterations of spermatogenesis.

Subjects with decreased inhibin B and increased FSH may present a damage at first steps of spermatogenesis. This rationale is consistent with the results presented by Foresta et al. [21]. They compared a group of men with DAZ gene deletion to three groups of men with mild, moderate and severe alterations of spermatogenesis. Patients with DAZ gene deletion had increased FSH and normal inhibin B. Such hormonal constellation suggested normal function of Sertoli cells and appropriate interactions between Sertoli and germ cells. Men with severe testiculopathies had increased FSH, decreased inhibin B and did not respond to FSH treatment. This could be explained by the damage to both Sertoli and germ cells. The same authors made a hypothesis that Sertoli cells could be at their maximal functional capability in men with idiopathic disturbances (who had increased FSH and normal inhibin B) [21, 22].

Leifke et al. presented data on significantly higher inhibin B plasma levels in men with focal SCOS compared to complete SCOS. They suggested that secretion

of inhibin B may depend not only on Sertoli cells, but also on the spermatogenic activity within seminiferous tubules [23]. Similarly to patients with SCOS, subjects with bilateral atrophy of testes had elevated levels of FSH.

In line with previous reports, our results point to a possible association between alterations of spermatogenesis and serum levels of inhibin B and FSH. However, these relations are not evident when only routine analyses (such as FSH and sperm count) are considered.

We have not found statistically significant differences of inhibin B serum levels between normozoospermic and oligozoospermic men.

## Conclusions

Our results suggest that serum inhibin B may be elevated in subjects with altered sperm count and increased serum FSH.

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