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PRACE ORYGINALNE
położnictwo

Inhibin A and B levels in serum and follicular fluids of women with various reproductive failures undergoing *in vitro* fertilization

Poziom inhibiny A i B w surowicy i płynie pęcherzykowym kobiet z niepowodzeniami rozrodu poddanych zapłodnieniu *in vitro*

Katarína Babčová[1,2], Zdenka Ulčová-Gallová[1,3], David Rumpík[4], Zdenka Mičanová[1,3], Katarína Bibková[1,3]

[1] Department of Gynecology and Obstetrics, Medical Faculty of Charles University, Pilsen, Czech Republic
[2] Department of Gynecology and Obstetrics, Regional Hospital T. Bata, Havlíčkovo nábřeží 600, Zlín, 76001, Czech Republic
[3] Genetics, Pilsen, Czech Republic
[4] Centrum of Reproductive Medicine, Zlín, Czech Republic

Abstract

Objective: *Ovarian inhibins (INH) are hormones participating in the regulation of gametogenesis. We monitored inhibin A and B levels in serum (S) and follicular fluids (FF), depending on the type of fertility failure and treatment outcome.*

Material and methods: *We examined INH A and B levels in S and FF of 72 women undergoing ovarian stimulation for in vitro fertilization, including embryo transfer. We took serum samples at the time of egg collection (S1), embryo transfer (S2), and diagnostics of early pregnancy (S3). FF samples were obtained during egg collection. INH A and B levels were measured by ELISA set kit in all media.*

Results: *Healthy women had median of INH A S1 592.02pg/ml, INH A S2 593.58pg/ml, INH A S3 15.17pg/ml and INH B S1 242.46pg/ml, INH B S2 and INH B S3 zero levels. Women with ovarian disorders had significantly lower levels of INH A S1 and INH A S2 ($p<0.05$). Women with polycystic ovaries had significant higher INH B S2 levels ($p<0.05$). No statistically significant differences were found in women with endometriosis. Presence of oocyte in the dominant follicle positively correlated with INH B FF levels ($p<0.05$).*

Conclusions: *We confirmed differences in the levels of inhibins in sera depending on type of fertility failure. Inhibin B better reflected the presence of an oocyte. The potential paracrine role of inhibins needs to be examined to improve preparation for the in vitro fertilization treatment (IVF).*

Key words: **inhibin A and B** / **infertility** / **endometriosis** / **follicular fluid** /

Corresponding author:
Katarína Babčová
Department of Gynecology and Obstetrics, Regional Hospital T. Bata,
Havlíčkovo nábřeží 600, Zlín, 76001, Czech Republic
Tel.: 00420723394353
e-mail: bkata@centrum.sk

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Streszczenie

Cel: *Inhibiny jajnikowe (INH) są hormonami biorącymi udział w regulacji gametogenezy. Oznaczyliśmy poziom inhibiny A i B w surowicy (S) i płynie pęcherzykowym (FF) w zależności od typu niepowodzenia rozrodu i wyniku leczenia.*

Materiał i metoda: *Zbadaliśmy poziom INH A i B w S i FF od 72 kobiet poddanych stymulacji jajników do zapłodnienia in vitro, łącznie z transferem zarodka. Pobrano próbki surowicy w momencie uzyskania komórek jajowych (S1), przeniesienia zarodka (S2) i zdiagnozowania wczesnej ciąży (S3). Próbki FF uzyskiwano podczas pobierania komórek jajowych. Poziom INH A i B był mierzony metodą ELISA.*

Wyniki: *Zdrowe kobiety miały średni poziom INH A S1 592.02pg/ml, INH A S2 593.58pg/ml, INH A S3 15.17pg/ml i INH B S1 242.46pg/ml, a INH B S2 i INH B S3 zero. Kobiety z zaburzeniami funkcji jajników miały istotnie niższe poziomy INH A S1 i INH A S2 ($p<0.05$). Kobiety z jajnikami policystycznymi miały znacząco wyższe poziomy INH B S2 ($p<0.05$). W grupie kobiet z endometriozą nie znaleziono istotnych statystycznie różnic. Obecność oocytów w pęcherzykach dominujących wykazywała dodatnią korelację z poziomem INH B FF ($p<0.05$).*

Wnioski: *Potwierdziliśmy zróżnicowanie poziomów inhibin w surowicy w zależności od typu niepłodności. Inhibina B lepiej odzwierciedla obecność oocytów. Możliwa funkcja parakrynna inhibin wymaga dalszych badań w celu poprawy przygotowania do leczenia przy pomocy zapłodnienia in vitro (IVF).*

Słowa kluczowe: **inhibina A** / **inhibina B** / **niepłodność** / **endometrioza** / / **płyn pęcherzykowy** /

Introduction

Inhibins are heterogeneous glycoproteins composed of two subunits – alpha and beta. The beta type subunit determines the specificity of inhibins. In female gonads, inhibin cells of granulosa and corpus luteum express mRNA for the alpha, beta A, beta B, and follistatin subunits. Internal thecal cells express mRNA for the same proteins, especially for the alpha subunit. Prenatal and small antral follicles express mRNA for beta A and B subunits, and at a lower level, also for the alpha subunit as well as for follistatin. Healthy large oocytes also express mRNA for beta A and B subunits; mRNA for the alpha subunit and follistatin is expressed at an increased level. The expression of mRNA for all proteins decreases in the atretic follicle. Corpus luteum expresses mRNA for subunit alpha, beta A and follistatin, but the expression rates for beta B are low [1, 2]. Human follicular fluid contains inhibin A, inhibin B, activin A, activin B, activin AB, some isoforms of follistatin, and the monomer alpha subunit [3].

Inhibition of production and secretion of FSH in the adenohypophysis is the principal role of inhibins, as mediators of negative feedback [4]. They also participate in gametogenesis by auto/paracrine effects on the ovaries in concert with activin and follistatin as modulators of cell proliferation, tissue response to gonadotropins, production of steroids, and maturation of oocytes. Furthermore, inhibins participate in the process of ovulation and influence the function of the corpus luteum. They participate in the process of selecting the dominant follicle or follicular atresia.

We monitored inhibin A and inhibin B levels in serum and follicular fluid of women undergoing ovarian stimulation for assisted reproductive treatment and investigated their correlations with the type of the reproductive failure and treatment outcome.

Material and methods

We evaluated 72 randomly selected women, aged 22-41 (Ø 30.3) years, with failed fertility. They were treated at the Centre for Reproductive Medicine in Zlín. Average effort of conception was 2.89 (≤0–14) years, with 26 couples undergoing treatment for more than 2 years, 20 couples for more than 4 years, and 47 couples during their first complete IVF cycle. In addition, 5 couples had already completed two or more IVF cycles. The control group consisted of six healthy women and presented only the andrologic factor.

Twenty-one women had positive ovarian factor (OF) with proven hypo- to anovulation; 23 had positive endocrinological factor (EF), which means thyroid disease and hyperprolactinemia in the anamnesis; 11 had evidence of a single or double tubal blockage, or the uterine subseptum (TDF); 25 women had proven endometriosis (E) of the I or II degree, either after medical or surgical treatment, or previously untreated. Also, there were 3 patients with polycystic ovary syndrome (PCOS) in the study group.

The inclusion criteria were completed for IVF. All patients underwent routine anamnestic, hormonal and gynecological examination. In accordance with the currently recommended levels of FSH and LH, conventional stimulation by a long protocol with the use of GnRH agonists and short stimulation protocol with the use of GnRH analogues was used. With controlled maturation of oocytes (administration of 5 000-10 000 IU of hCG), we performed oocyte retrieval (over 15 mm in size) by transvaginal ovarian aspiration under aseptic conditions and general anesthesia on day 12-14 of the induced cycle controlled by ultrasonography.

We recorded the number of mature follicles, volume of the aspirated follicular fluid, number of the collected oocytes, and presence of an oocyte in the biggest follicle from each ovary. The follicular fluid was centrifuged (5.000 rpm/10min) and frozen at -70°C. Peripheral blood was taken at the same time and serum was also frozen. INH A and B levels were determined immediately after oocyte collection (S1) using a commercial ELISA kit (Dynex, Oxford, UK) in pg/ml, at the time of embryo transfer (S2), and evaluation for hCG positivity (S3).

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The results were statistically analyzed by statistical description, non-parametric testes such as Wilcoxon and Kruskal--Wallis test, parametric ANOVA, and Spearman's correlation coefficient. The significance value was set at 5%, p- value of <0.05. Statistical analysis was performed using software Statistica 9.0.

Results

Median values of INH A and B S1, S2, S3 in different groups according to the cause of infertility are presented in Table 1. Women with positive OF had significantly lower levels of INH A S1 and S2 as compared to women without OF (p-value <0.05). Women with EF had significantly higher levels of INH B S3 as compared to women without endocrinopathy (p-value of <0.0001). Women with TDF had similar hormonal status as compared to controls, and the levels of both, INH S nor INH FF were not different, either. There were also no differences in women with endometriosis. Patients with PCOS had significantly higher levels of INH B S2, B FF and lower INH A FF levels as compared to their peers without this syndrome (p-value of <0.05). Positive correlation of serum and follicular levels was observed between INH B S1 and INH B FF, regardless of the type of reproductive disorder (r_s=0.0082). The levels of both inhibins correlated neither with the amount of the follicular fluid, nor with total number of the retrieved oocytes. INH B FF positively correlated with the presence of an oocyte in the dominant follicle (p-value of <0.05) (Figure 1).

In the group consisting of 72 couples, 44 women failed to become pregnant (group G0/N=44). Singleton pregnancy was the result in 17 cases, 9 women expected twins, and 2 women triplets (group G1/N=28). Pregnant women had higher levels of INH A S3 (p-value of <0.0001 (Figure 2). Older women had lower levels of INH B S1 (r_s=0.0382). Age was not the decisive factor in the success of therapy.

Discussion

Based on our findings, it seems safe to conclude that inhibin A and B are produced at different times of a woman's cycle and by several different cell types. Inhibin A is secreted mainly by the corpus luteum and is present in the negative feedback loop to FSH secretion during the luteal-follicular transition. Small antral follicles express more mRNA βB subunits. The expression of mRNA for all proteins is reduced in an atretic follicle. The corpus luteum expresses mRNA for subunit α, βA and follistatin, but only a few βB [2]. These individual patterns of expression raise the possibility that inhibin A and B may be functionally distinct [5]. The main form of inhibin in the follicular phase of the cycle is inhibin B, and in the luteal phase inhibin A (6). We confirmed different concentrations of inhibin A and B in both, serum and follicular fluid. Regardless of the type of reproductive failure, serum level of inhibin A increased slightly from the time of egg collection to the time of embryo transfer, and decreased in early pregnancy. Serum levels of inhibin B reached the highest values at the time of egg collection, followed by a steep decline, and reaching zero or undetectable values at the time of hCG examination. On day 14 after embryo transfer during the first biochemical diagnosis of pregnancy, serum levels of inhibin A were statistically significantly higher for pregnant women (p-value of <0.0001).

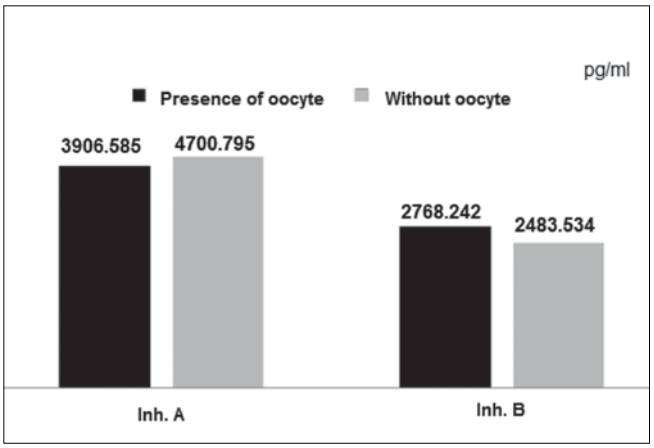

Figure 1. Median concentration of inhibin A and B in follicular fluid according to the presence of an oocyte in the dominant follicle.
A positive correlation with levels of inhibin B FF was found (p- value of 0.05).

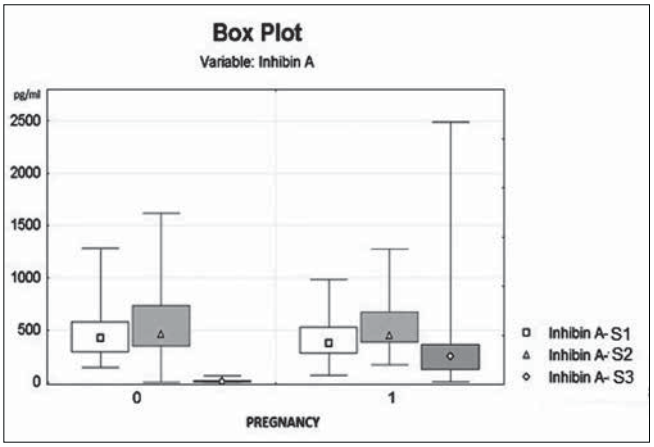

Figure 2. Serum inhibin A concentration at the time of egg collection (□S1), embryo transfer (ΔS2), and hCG examination (◊S3).
Women with negative hCG test (0) had median levels of INH A S1 424.69 pg/ml, INH A S2 458.1 pg/ml, INH A S3 4.12 pg/ml.
Women with positive hCG test (1) had median levels of INH A S 1 375.04pg/ml, INH A S2 449.88 pg/ml, INH A S3 245.0 pg/ml.
Pregnant women had higher levels of INH A S3 (p-value of <0.0001).

Our observations are consistent with the results reported in 1995. Inhibin A is the major circulating form of inhibins in early pregnancy [7].

We also demonstrated that concentrations of inhibin A in the follicular fluid are higher than inhibin B and gradually higher than their concentrations in the serum [8]. There is a positive correlation of inhibin B serum levels at time of oocyte collection with levels of inhibin B in the follicular fluid. Intrafollicular concentrations of inhibin B have been known to increase with follicular diameter [9]. In our study, intrafollicular inhibin B levels correlated with the presence of an oocyte in the dominant follicle. Although the presence of inhibin B in the follicular fluid seems to be a suitable marker of quality of oogenesis [10]; neither follicular fluid inhibin B nor inhibin A predicts pregnancy.

We confirmed that patient age cannot be the marker of the IVF outcome alone. However, we observed significantly lower levels of INH B S1 in older women. It is possible that inhibin B

	S1		S2		S3		FF	
	INH A	INH B	INH A	INH B	INH A	INH B	INH A	INH B
Control group	592.02	242.46	593.58	0	15.17	0	5793.43	2417.61
Ovarian factor	304.12	220.32	363.68	0	11.83	0	3778.17	2530.03
F. of endocrinology	450.27	284.58	466.26	0	11.83	0	3827.97	2549.02
PCOS	261.69	315.35	401.65	116.87	26.3	0	2663.77	4747.23
Endometriosis	412.01	300.02	491.94	0	11.1	0	3888.36	2531.48
TDF	312	245.32	401.65	0	26.3	0	3945.76	2545.9

Table I. Median concentration [pg/ml] of inhibin A and inhibin B in serum (S) and follicular fluid (FF) in groups with various types of fertility failure at the time of egg collection (S1), embryo transfer (S2), and hCG examination (S3). Women with positive OF had significantly lower levels of INH A S1 and S2 as compared to women without OF (p-value of <0.05).
Women with EF had significantly higher levels of INH B S3 as compared to women without endocrinopathy (p-value of <0.0001).
Women with PCOS had significantly higher levels of INH B S2, B FF and lower INH A FF as compared to patients without this syndrome (p-value of <0.05).
There were no significant differences in groups with endometriosis and TDF.

reflects the lower number of preantral follicles in older women, regardless of ovarian hyperstimulation, what is consistent with the findings of previous studies [11].

In our study, there were no significant differences between serum or follicular fluid inhibin A nor inhibin B concentrations in women with and without endometriosis, what is consistent with reports of no differences in concentrations of inhibin A, inhibin B, and activin A in the peritoneal fluid [12], or AMH in the follicular fluid [13] in women with endometriosis. This confirms the assumption about the pathophysiology of endometriosis, i.e. primarily pathology of the endometrial cells. Reduced fertility occurs as a secondary process, through the activation of immune endocrine processes. On the assumption that the intraovarian environment is intact, the ovary of a woman with endometriosis has similar response to stimulation as the ovary of a healthy woman. Similarly, women with morphological causes of sterility, such as tubal or uterine factor, have a similar gametohormonal status as healthy women. We found no differences in inhibin levels of either serum or follicular fluid.

Previous studies determined serum inhibin A and B levels to be significantly increased in women with PCOS 24 hours after acute FSH stimulation [14]. Serum inhibin B concentration remained elevated, with no changes during low-dose gonadotropin ovulation induction [15]. In our study, we observed elevated inhibin B levels in serum at the time of embryo transfer, i.e. after hCG administration and egg collection, as compared to healthy women. It suggests that dissimilarity of inhibin A and B levels in PCOS women is probably caused by prolonged survival of small antral follicles. Significantly lower levels of inhibin A and higher levels of inhibin B in the follicular fluid can reflect damage of the follicular microenvironment in PCOS ovaries.

We noted significantly higher serum inhibin B levels at the time of hCG examination in women with hypothyreosis or hyperprolactinemia. All patients underwent ovarian stimulation after endocrinology treatment and both, thyroidal hormones and prolactin were within the normal range. As far as this finding is concerned, the role of inhibins and other proteins which cooperate in gametogenesis and their participation in the neuroendocrine axis needs to be considered. Hypothyreosis is known to stimulate secretion of prolactin. Higher levels of prolactin cause reduction in ovulation, with luteal insufficiency. Significantly increased concentrations of human prolactin have the capacity to raise insulin-like growth factor IGF-I in serum of patients with hypopituitarism [16]. FSH and IGF-I differentially regulate inhibin A and B secretion in granulosa cells [17]. Serum IGF-I levels have been reported to be down regulated in elderly women and to influence the ovarian function [18]. Based on these findings, paracrine regulation in the ovary needs to be further investigated.

Conclusions

In our extensive study, we proved that inhibin A and B have gradually higher concentrations in the follicular fluid than serum. Also, inhibin A and B maintain the dynamics of their serum concentrations during ovarian stimulation, regardless of the type of fertility failure. We confirmed that serum inhibin A is the major circulating form of inhibins in the early pregnancy. Our results confirmed the role of inhibin A and B in predicting IVF success. Neither of the inhibins was a good predictor of pregnancy rate after IVF treatment. To the best of our knowledge, our results have been the first to show that there are no differences in serum or in follicular fluid levels of both inhibins in women with and without endometriosis. There are some changes in inhibin concentrations in women with PCOS and EDF, which can reflect damage of the follicular microenvironment. We can consider inhibin B in the follicular fluid a suitable marker of quality of oogenesis. A potentially paracrine role of inhibins needs to be examined to improve preparation for IVF.

Authors' contribution:
1. Katarína Babčová – concept, acquisition of data, analysis and interpretation of data, article draft, corresponding author.
2. Zdenka Ulčová-Gallová – concept, analysis and interpretation of data, revised article critically.
3. David Rumpík – acquisition of data.
4. Zdenka Mičanová – acquisition of data.
5. Katarína Bibková – acquisition of data.

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