Inhibin B and Anti-Müllerian Hormone as predictors of sperm retrieval through an FNA or TESE procedure

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Agenda for Inhibin B and AMH

1. Production: Sertoli cells
2. Structure and function
3. The role of basal levels
4. The role of stimulated levels
5. The role in prediction of sperm retrieval
6. Conclusions
Production: Sertoli cells
Sertoli cell functions

1. **Supportive** and **trophic** functions for the cells of the seminiferous epithelium
2. **Transport** of mature spermatids towards the lumen of seminiferous tubules
3. **Secretion** of Androgen Binding Protein (ABP)
4. **Interaction** with Leydig cells
5. **Production of substances** with endocrine or paracrine action for spermatogenesis control
Leydig - Sertoli cell interaction

Sertoli cell products

- **Transport proteins and enzymes**
  - Androgen Binding Protein (ABP)
  - Transferrin / Ceruloplasmin
  - Plasminogen Activator (PA)

- **Growth factors**
  - TGF-α
  - TGF-β
  - IGF-1
  - IL-1

- **Hormones**
  - Inhibin B
  - Anti-Müllerian Hormone (AMH)
Leydig cell products

- **Endocrine action**
  - Testosterone

- **Neuro-endocrine action**
  - Serotonin
  - IGF-I
  - TGF-β
  - PDGF
  - Substance P
  - Atrial natriuretic peptide type C
  - Enzymes for catecholamine synthesis
  - Neuropeptides and receptors
  - Cell adhesion molecules
  - Elements of NO / cGMP system
  - Elements of renin / angiotensin system
  - Proteins of neurofilaments
Inhibin B and AMH: Structure and Function
Classic hormone function

Hypothalamus: Releasing hormone GnRH

Pituitary: Gonadotropins LH and FSH

Testes: LH → Testosterone

FSH + Testosterone → Sperm production

Target tissue of testosterone: e.g. muscle, bone, skin
# Inhibin family

<table>
<thead>
<tr>
<th>Class</th>
<th>Activity</th>
<th>Complex</th>
<th>Dimer subunits</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
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<tr>
<td>Inhibin</td>
<td>inhibits FSH secretion</td>
<td>Inhibin A</td>
<td>α</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibin B</td>
<td>α</td>
</tr>
<tr>
<td>Activin</td>
<td>stimulates FSH secretion</td>
<td>Activin A</td>
<td>β&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Activin AB</td>
<td>β&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Activin B</td>
<td>β&lt;sub&gt;B&lt;/sub&gt;</td>
</tr>
</tbody>
</table>
Inhibin family

Inhibin A

\[ \alpha \]
\[ \beta_A \]

Activin A

\[ \beta_A \]
\[ \beta_A \]

Activin AB

\[ \beta_A \]
\[ \beta_B \]

Activin B

\[ \beta_B \]
\[ \beta_B \]

Inhibin B

\[ \alpha \]
\[ \beta_B \]
Inhibin B function

Pituitary

FSH

Sertoli cells

Testes

Inhibin B
Inhibin B biosynthesis

Andersson A-M. Bailliere Clin Endocrinol Metab 14:389, 2000
Teixeira J et al.
AMH actions in males

- Differentiation of urogenital ridge to **testis**
- Regulation of transabdominal **testicular descent**
- Inhibition of **aromatase action in Sertoli cells**
- Inhibition of **differentiation of precursor forms to Leydig cells**
- Inhibition of **steroidogenesis in Leydig cells**
- Higher concentration of AMH in semen as compared to plasma after puberty may reflect its role in **spermatogenesis** as well as **sperm motility**

The role of basal levels of Inhibin B and AMH
Serum inhibin-B and FSH as predictors of the presence of sperm in testicular Fine Needle Aspirate in men with azoospermia


Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Thessaloniki, Greece
The aim of this study was to determine basal serum Inhibin B levels in fertile and subfertile men of various etiologies.
Men studied

- **Subfertility group** (n=67)
  - Age 32.8 ± 0.6 years
  - Basal Inhibin B estimation

- **Control group** (n=29)
  - Age 30.3 ± 5.1 years
  - Basal Inhibin B estimation
Subfertile men diagnosis

- INOA: 31
- varicocele: 14
- cryptorchidism: 10

Rare causes:
- Kallmann syndrome: 3
- Klinefelter syndrome: 2
- Congenital aplasia: 2
- Testicular dysgenesis: 1
- 46,XX male: 1
- Sickle cell anemia: 1
- Vanishing testes: 1
- Testicular feminization: 1

INOA: Idiopathic Non-Obstructive Azoospermia
Results

Controls: $115.73 \pm 10.0$ pg/ml
Subfertile: $55.8 \pm 6.3$ pg/ml (1)

Varicocele: $84.0 \pm 15.8$ pg/ml (1)
INOA: $50.1 \pm 7.2$ pg/ml (2)
Cryptorchidism: $28.1 \pm 3.6$ pg/ml (2)

(1) Mann-Whitney U, $p = 0.03$
(2) Mann-Whitney U, $p < 0.001$
ORIGINAL ARTICLE

Serum anti-Müllerian hormone levels differentiate control from subfertile men but not men with different causes of subfertility

DIMITRIOS G. GOULIS, PASCHALIA K. ILIADOU, CHRISTOS TSAMETIS, SPYRIDON GEROU, BASIL C. TARLATZIS, IOANNIS N. BONTIS, & IOANNIS PAPADIMAS

Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Thessaloniki, Greece

(Received 11 July 2007; revised 7 September 2007; accepted 10 September 2007)
<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>INOA</th>
<th>INOD</th>
<th>Cryptorchidism</th>
<th>Varicocele</th>
<th>p Value</th>
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<tbody>
<tr>
<td>n</td>
<td>31</td>
<td>26</td>
<td>17</td>
<td>10</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Left testis volume (ml)</td>
<td>25 (4)</td>
<td>12 (13)a</td>
<td>15 (12)a</td>
<td>14 (7)a</td>
<td>24 (8)b,c,d</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Right testis volume (ml)</td>
<td>25 (4)</td>
<td>11 (14)a</td>
<td>12 (11)a</td>
<td>13 (9)a</td>
<td>25 (8)b,c,d</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hormonal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH (mU/ml)</td>
<td>3.0 (2)</td>
<td>8.7 (6)a</td>
<td>6.1 (3)a</td>
<td>7.3 (4)a</td>
<td>4.2 (3)b,d</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FSH (mU/ml)</td>
<td>2.8 (3)</td>
<td>22.1 (13)a</td>
<td>12.0 (14)a,b</td>
<td>17.5 (18)a</td>
<td>6.5 (8)b,c,d</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>7.6 (3)</td>
<td>9.0 (5)</td>
<td>7.5 (9)</td>
<td>12.0 (5)</td>
<td>8.6 (14)</td>
<td>0.370</td>
</tr>
<tr>
<td>Total testosterone (ng/dl)</td>
<td>592 (440)</td>
<td>323 (204)a</td>
<td>408 (162)b</td>
<td>362 (222)</td>
<td>331 (296)</td>
<td>0.003</td>
</tr>
<tr>
<td>Inh-B (pg/ml)</td>
<td>103 (90)</td>
<td>22 (25)a</td>
<td>59 (35)a</td>
<td>25 (14)b</td>
<td>70 (51)b,c,d</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>11.6 (7.7)</td>
<td>5.5 (4.8)a</td>
<td>4.5 (4.4)a</td>
<td>5.3 (4.8)a</td>
<td>4.9 (2.9)a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spermiogram</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>4.1 (1.7)</td>
<td>3.3 (2.2)</td>
<td>3.7 (2.7)</td>
<td>3.3 (1.7)</td>
<td>3.9 (2.0)</td>
<td>0.080</td>
</tr>
<tr>
<td>Concentration (×10⁶/ml)</td>
<td>45.0 (12.3)</td>
<td>N/A</td>
<td>4.0 (7.7)a</td>
<td>0.1 (2.4)a&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.3 (8.4)b,c,d</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Motility at first hour (%) (categories ‘a’ and ‘b’)</td>
<td>58.5 (28)</td>
<td>N/A</td>
<td>10.0 (15)a</td>
<td>0.0 (10)a</td>
<td>11.0 (25)b,c,d</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Morphology (% normal)</td>
<td>22.0 (19)</td>
<td>N/A</td>
<td>11.0 (34)a</td>
<td>0.0 (10)a</td>
<td>3.0 (25)a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sperm index*</td>
<td>22.8 (9.3)</td>
<td>N/A</td>
<td>0.0 (1.2)a</td>
<td>0.0 (0.1)a</td>
<td>0.1 (4.3)a</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

INOA, idiopathic non-obstructive azoospermia; INOD, idiopathic non-obstructive dyspermia; LH, luteinizing hormone; FSH, follicle-stimulating hormone; Inh-B, inhibin B; AMH, anti-Müllerian hormone; N/A, not applicable; data are given as median (interquartile range); p value refers to comparison among all groups (Kruskal–Wallis test); p < 0.05 vs. acontrol, bINOA, cINOD, dCryptorchidism (Mann–Whitney U test); *sperm index was calculated as: [ejaculation volume (ml)] × [concentration (10⁶)] × [motility at first hour (%)] × [normal morphology (%)]/10⁴.
Correlation

- **Positive**
  - AMH vs. testicular volume
    
    \( r = 0.456, \ p < 0.05 \)
  - AMH vs. Inhibin B
    
    \( r = 0.528, \ p < 0.05 \)

- **Negative**
  - AMH vs. FSH
    
    \( r = -0.378, \ p < 0.05 \)
  - AMH vs. LH
    
    \( r = -0.451, \ p < 0.05 \)
Conclusion - 1

- Serum Inhibin B and AMH levels seem to constitute important diagnostic parameters in male subfertility, as they reflect Sertoli cell status.
The role of stimulated levels of Inhibin B and AMH
Stimulated Inhibin B levels

**EFSERT**

- **Exogenous FSH SErtoli Reserve Test**
  - Serum Inhibin B and AMH levels before and 24 h and 48 h after administration of 300 IU hrFSH subcutaneously

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**Diagram:**
- Arrows indicating 300 IU hrFSH sc at 0 h, 24 h, and 48 h
Aim of the study

- The aim of this study was to determine stimulated serum Inhibin B and AMH levels in fertile and subfertile men.
<table>
<thead>
<tr>
<th><strong>Men studied</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subfertility group</strong> (n=64)</td>
<td></td>
</tr>
<tr>
<td>Age 31.5 ± 0.6 years</td>
<td></td>
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<tr>
<td>Basal and stimulated Inhibin B and AMH levels</td>
<td></td>
</tr>
<tr>
<td><strong>Control group</strong> (n=12)</td>
<td></td>
</tr>
<tr>
<td>Age 31.3 ± 4.5 years</td>
<td></td>
</tr>
<tr>
<td>Basal and stimulated Inhibin B and AMH levels</td>
<td></td>
</tr>
</tbody>
</table>
Results

Tsametis Ch et al, 2009 (submitted for publication)
Results

Tsametis Ch et al, 2009 (submitted for publication)
Conclusion - 2

- Stimulated levels of serum Inhibin B and AMH levels do not add clinically relevant information in subfertile men compared to basal levels of these hormones.
Inhibin B and AMH as predictors of sperm retrieval
Diagnostic approach

Investigations

Clinical  Hormonal  Imaging  Sperm  Genetic  Cytology

Sperm  Cytology  Clinical

Classification

Diagnoses
Sperm retrieval

Testicular Sperm Extraction (TESE)  Testicular FNA
Testicular FNA classification

1. Normal
2. Hypospermatogenesis, mild
3. Hypospermatogenesis, severe
4. Maturation arrest, incomplete
5. Maturation arrest, complete
6. SCOS, incomplete
7. SCOS, complete
Normal spermatogenensis

TESE

FNA
Sertoli Cell-Only Syndrome

TESE

FNA
Serum inhibin-B and FSH as predictors of the presence of sperm in testicular Fine Needle Aspirate in men with azoospermia


Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Thessaloniki, Greece
The aim of this study was to correlate serum Inhibin B levels in subfertile men with cytological testicular findings.
Men studied

- **Subfertility group** (n=67)
  - Age 32.8 ± 0.6 years
  - Basal Inhibin B estimation
  - Testicular FNA
Results

FNA classification
1. normal
2. hypo-, mild
3. hypo-, severe
4. arrest, incomplete
5. arrest, complete
6. SCOS, incomplete
7. SCOS, complete

Kendall’s tau-b, 
\( r = -0.25, p = 0.04 \)
Results

Sperm retrieval in FNA
AUC Inhibin B: 0.725
AUC Inhibin B / FSH: 0.716
AUC FSH: 0.663

Inhibin B
threshold: 54 pg/ml
sensitivity: 59%
specificity: 86%
Results

- Dependent variable
  - FNA findings

- Independent variables

<table>
<thead>
<tr>
<th></th>
<th>$R^2$</th>
<th>$\rho$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>excluded</td>
<td>0.912</td>
</tr>
<tr>
<td>Testicular volume</td>
<td>excluded</td>
<td>0.812</td>
</tr>
<tr>
<td>FSH</td>
<td>excluded</td>
<td>0.671</td>
</tr>
<tr>
<td>Total testosterone</td>
<td>excluded</td>
<td>0.838</td>
</tr>
<tr>
<td>Inhibin B</td>
<td>0.308</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Serum inhibin B and antimüllerian hormone are not superior to follicle-stimulating hormone as predictors of the presence of sperm in testicular fine-needle aspiration in men with azoospermia

Dimitrios G. Goulis, M.D., Ph.D., Christos Tsametis, M.D., Paschalia K. Iliadou, M.D., Paris Polychronou, M.D., Ph.D., Persefoni-Dimitra Kantartzi, M.D., M.Sc., Basil C. Tarlatzis, M.D., Ph.D., Ioannis N. Bontis, M.D., Ph.D., and Ioannis Papadimas, M.D., Ph.D.

Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Thessaloniki, Greece
Figure 1

Scatter diagram and regression line of (A) FSH, (B) INHB, and (C) AMH levels and (D) volume of the larger testis versus FNA diagnoses (1: normal spermatogenesis; 2: hypospermatogenesis; 3: spermatogenesis arrest, incomplete; 4: spermatogenesis arrest, complete; 5: SCOS, incomplete; 6: SCOS, complete).

ROC curves

AUC
1/ FSH: 0.714
Inhibin B: 0.630
AMH: 0.565
Testicular volume: 0.693
ROC curves

AUC: 0.736
AUC: 0.667
Conclusion - 3

- Serum Inhibin B and AMH levels correlate with testicular cytology but are not superior to FSH as predictors of the presence of sperm in FNA in men with azoospermia.
Meta-analysis

- Predictors of sperm retrieval in TESE
  - Inhibin B, serum
  - Inhibin B, semen
  - AMH, serum
  - AMH, semen

Toulis K, Iliadou PK et al, 2009 (submitted for publication)
Meta-analysis characteristics

- Number of studies: 8
- Number of patients: 587
- Time interval: 1999 - 2008
- Sperm retrieval method: TESE

Toulis K, Iliadou PK et al, 2009 (submitted for publication)
Meta-analysis

Toulis K, Iliadou PK et al, 2009 (submitted for publication)

Sensitivity: 67%
Specificity: 84%
Toulis K, Iliadou PK et al, 2009 (submitted for publication)
Toulis K, Iliadou PK et al, 2009 (submitted for publication)

Prevalence Heterogeneity
Uniform Prior Distribution = [0.00 - 1.00]
Unconditional NPV = 0.65 [0.56 - 0.73]
Unconditional PPV = 0.72 [0.63 - 0.81]

Positive Test Result
LR+ = 4.11 [1.44 - 11.71]

Negative Test Result
LR- = 0.40 [0.27 - 0.60]
Toulis K, Iliadou PK et al, 2009 (submitted for publication)
Conclusions
Conclusion - 1

- Serum Inhibin B and AMH levels seem to constitute important diagnostic parameters in male subfertility, as they reflect Sertoli cell status.
Conclusion - 2

- Stimulated levels of serum Inhibin B and AMH levels do not add clinically relevant information in subfertile men compared to basal levels of these hormones.
Serum Inhibin B and AMH levels correlate with testicular cytology but are not superior to FSH as predictors of the presence of sperm in TESE / FNA in men with azoospermia.
Leydig cells

Peritubular cells

Androgens

Sertoli cells

Germ cells

LH

FSH

Androgens

P-Mod-S

TGF-β

TGF-β

Papadimas J et al.
Male Reproductive Endocrinology, 1993
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J. Papadimas
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Ch. Tsametis
P.K. Iliadou
P.-D. Kantartzi
K. Toulis
P. Polychronou
Th. Mikos
D. Tsitlakidis