ESHRE – Special Group in Andrology

Reproductive Andrology:

linking laboratory to clinical practice

Thessaloniki, 1 - 3 October, 2009

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

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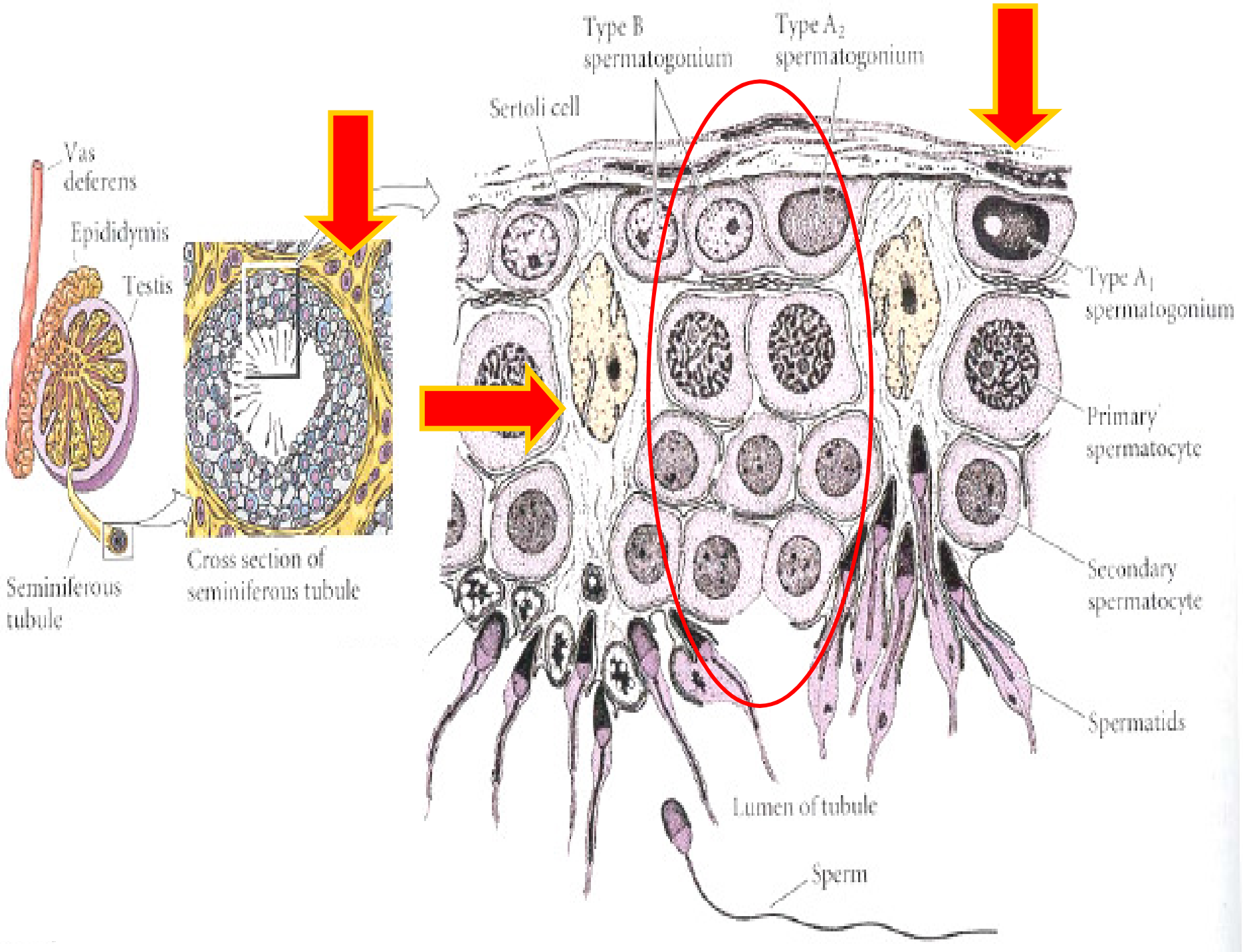
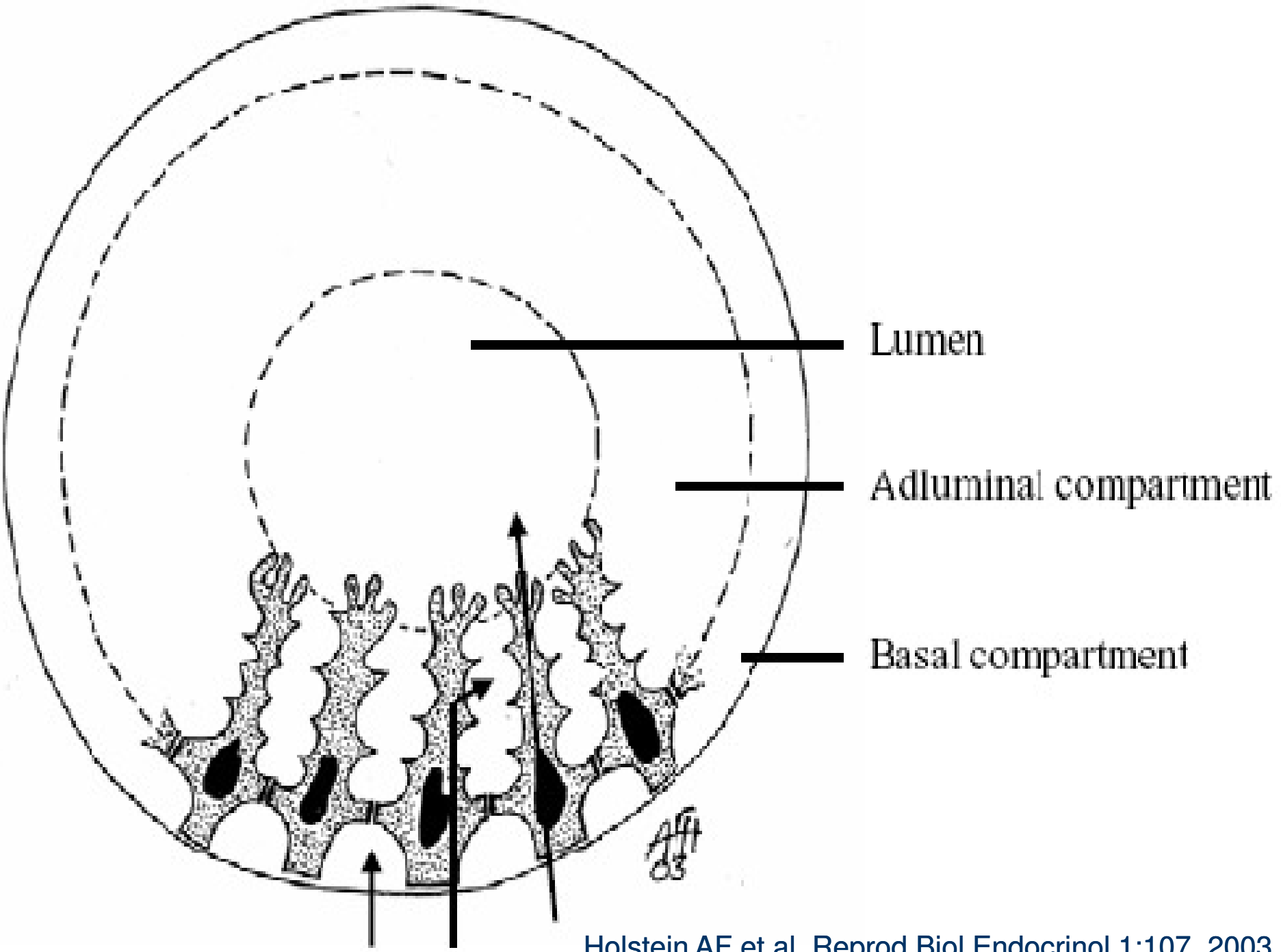
Agenda for Inhibin B and AMH

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

1

Production: Sertoli cells

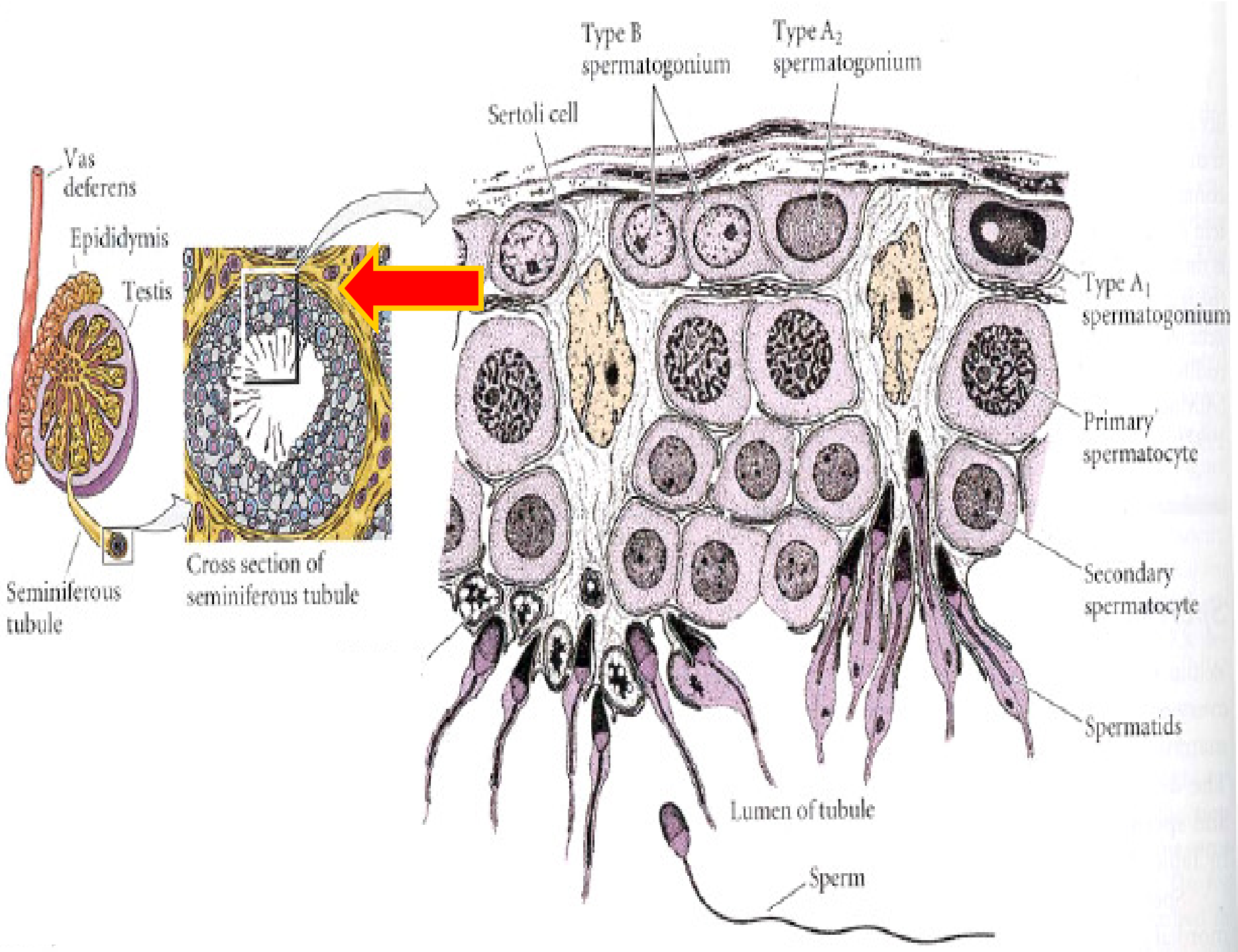

Vas deferens
Epididymis
Testis
Seminiferous tubule
Cross section of seminiferous tubule
Type B spermatogonium
Type A2 spermatogonium
Sertoli cell
Type A1 spermatogonium
Primary spermatocyte
Secondary spermatocyte
Spermatids
Lumen of tubule
Sperm



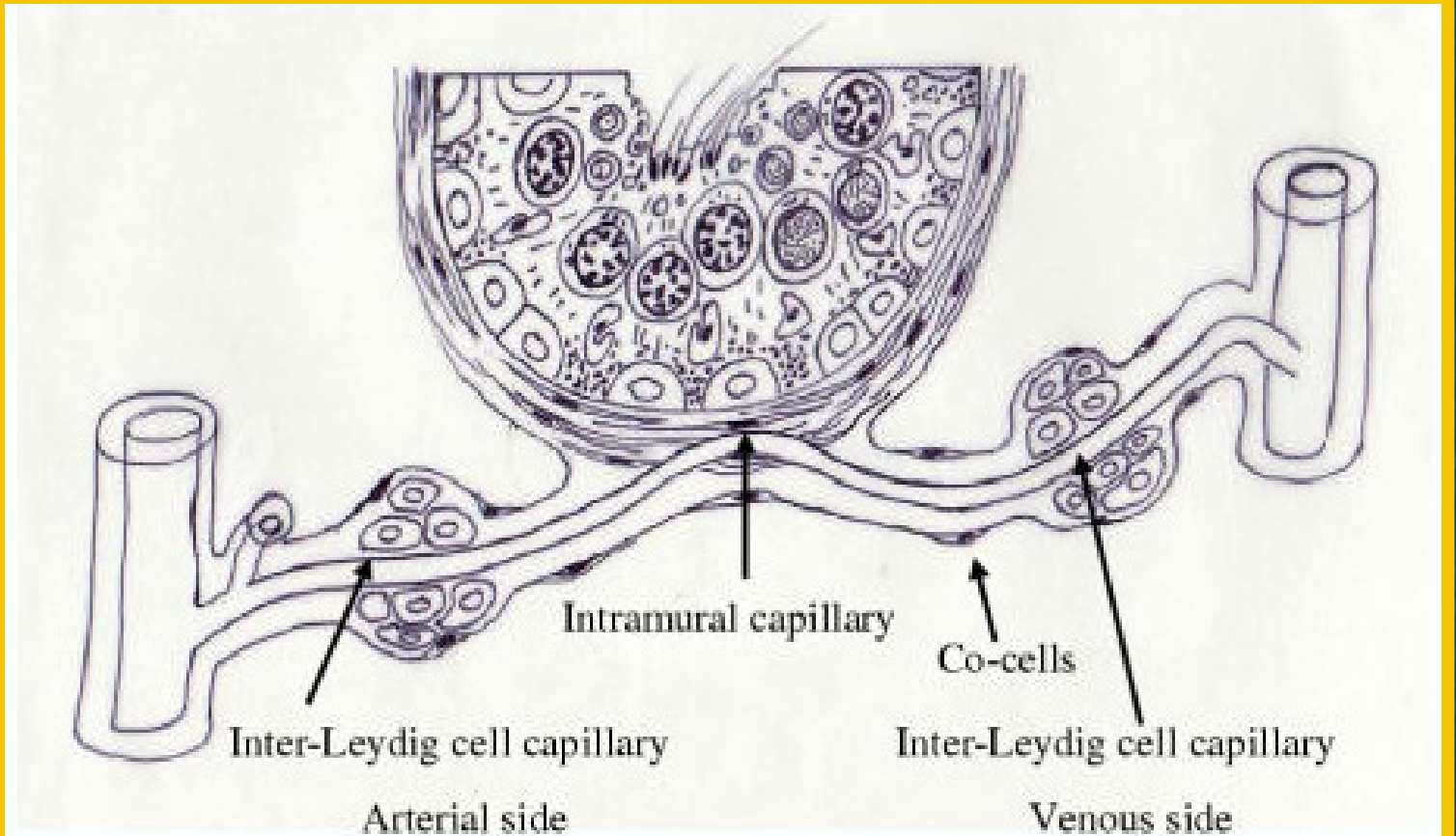
Holstein AF et al. Reprod Biol Endocrinol 1:107, 2003

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


Type B spermatogonium
Type A_2 spermatogonium
Sertoli cell
Vas deferens
Epididymis
Testis
Type A_1 spermatogonium
Primary spermatocyte
Secondary spermatocyte
Spermatids
Seminiferous tubule
Cross section of seminiferous tubule
Lumen of tubule
Sperm

Leydig - Sertoli cell interaction



Holstein AF et al. Reprod Biol Endocrinol 1:107, 2003

Sertoli cell products

- Transport proteins and enzymes
  - Androgen Binding Protein (ABP)
  - Transferrin / Ceruloplasmin
  - Plasminogen Activator (PA)
- Growth factors
  - TGF-α
  - TGF-β
  - IGF-I
  - IL-1
- Hormones
  - Inhibin B
  - Anti-Müllerian Hormone (AMH)

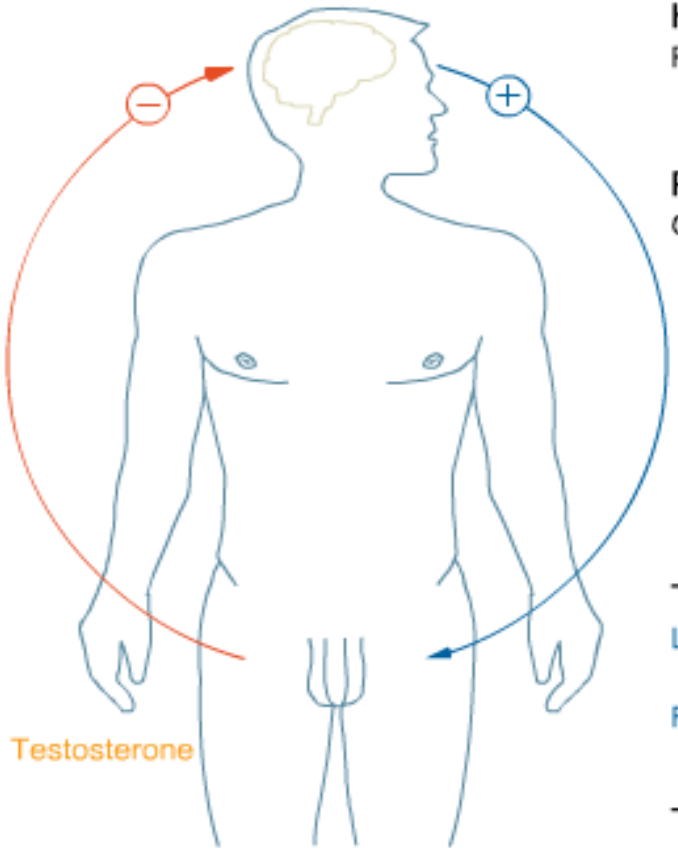
Leydig cell products

- Endocrine action
  - Testosterone
- Neuro-endocrine action
  - Serotonin
  - IGF-I
  - TGF-β
  - PDGF
  - Substance P
  - Atrial nartiuretic 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

2

Inhibin B and AMH: Structure and Function

Classic hormone function



Hypothalamus:
Releasing hormone GnRH

Pituitary:
Gonadotropins LH and FSH

Testes:
LH → Testosterone

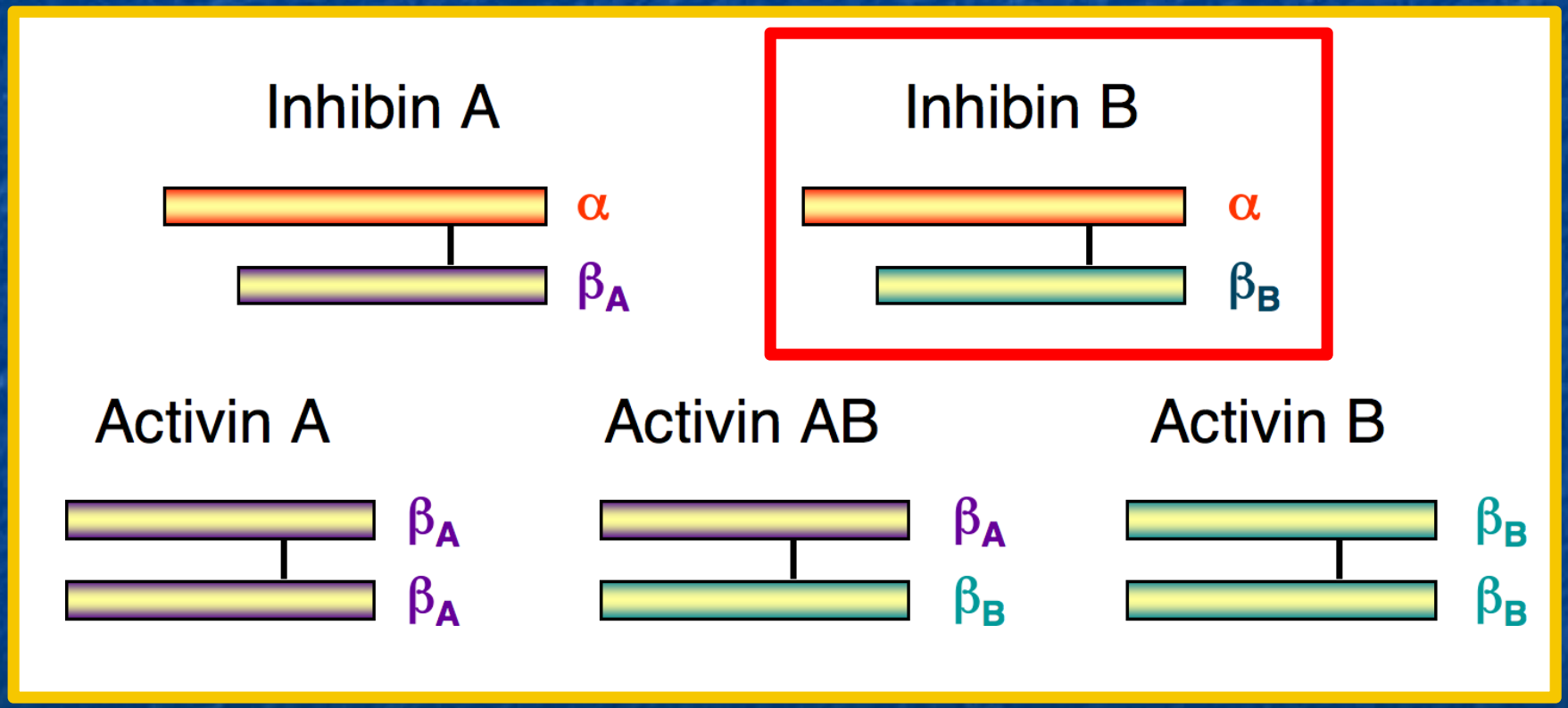
FSH + Testosterone → Sperm production

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

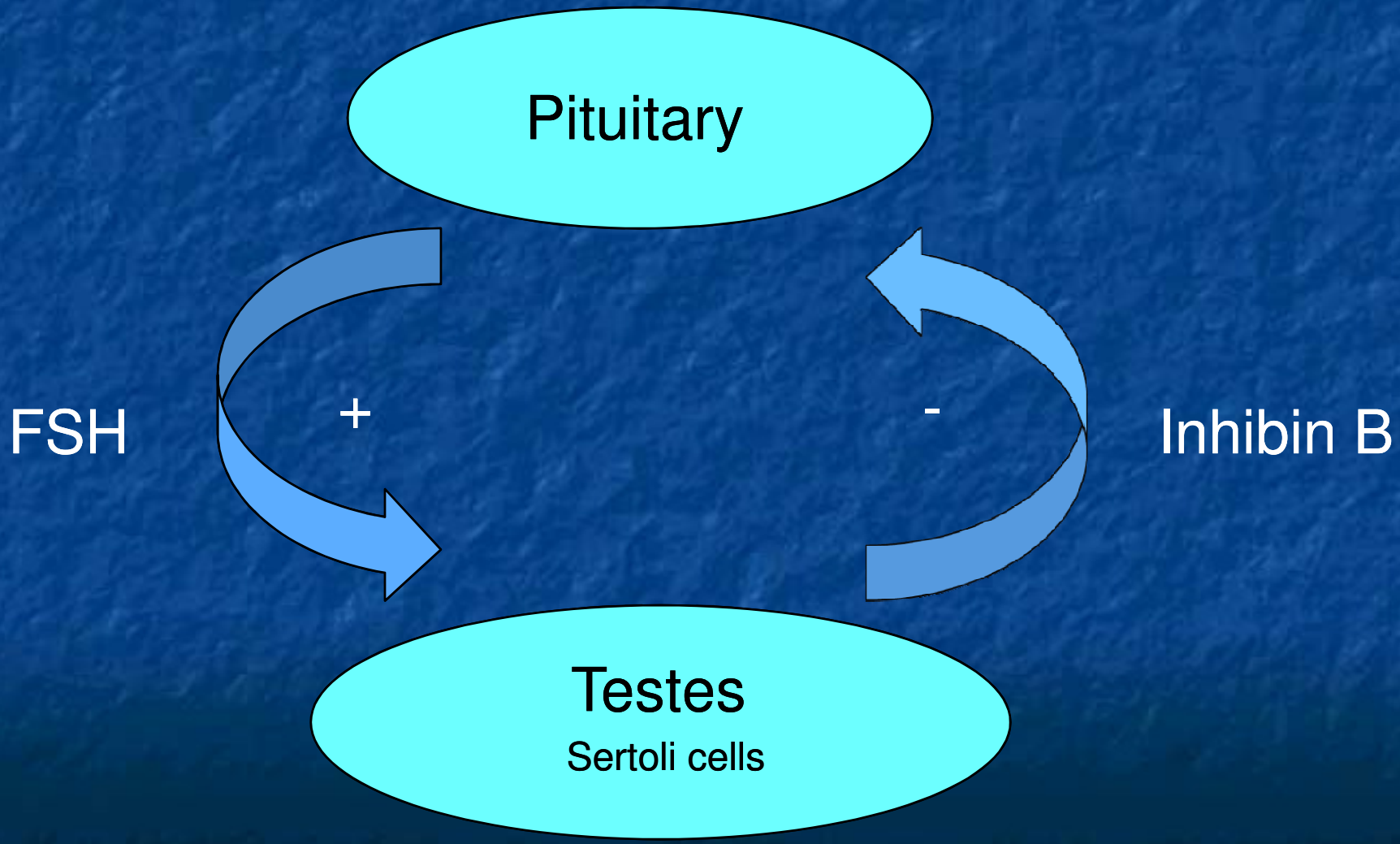
Inhibin family

Class	Activity	Complex	Dimer subunits	
			1	2
Inhibin	inhibits FSH secretion	Inhibin A	α	β_A
		Inhibin B	α	β_B
Activin	stimulates FSH secretion	Activin A	β_A	β_A
		Activin AB	β_A	β_B
		Activin B	β_B	β_B

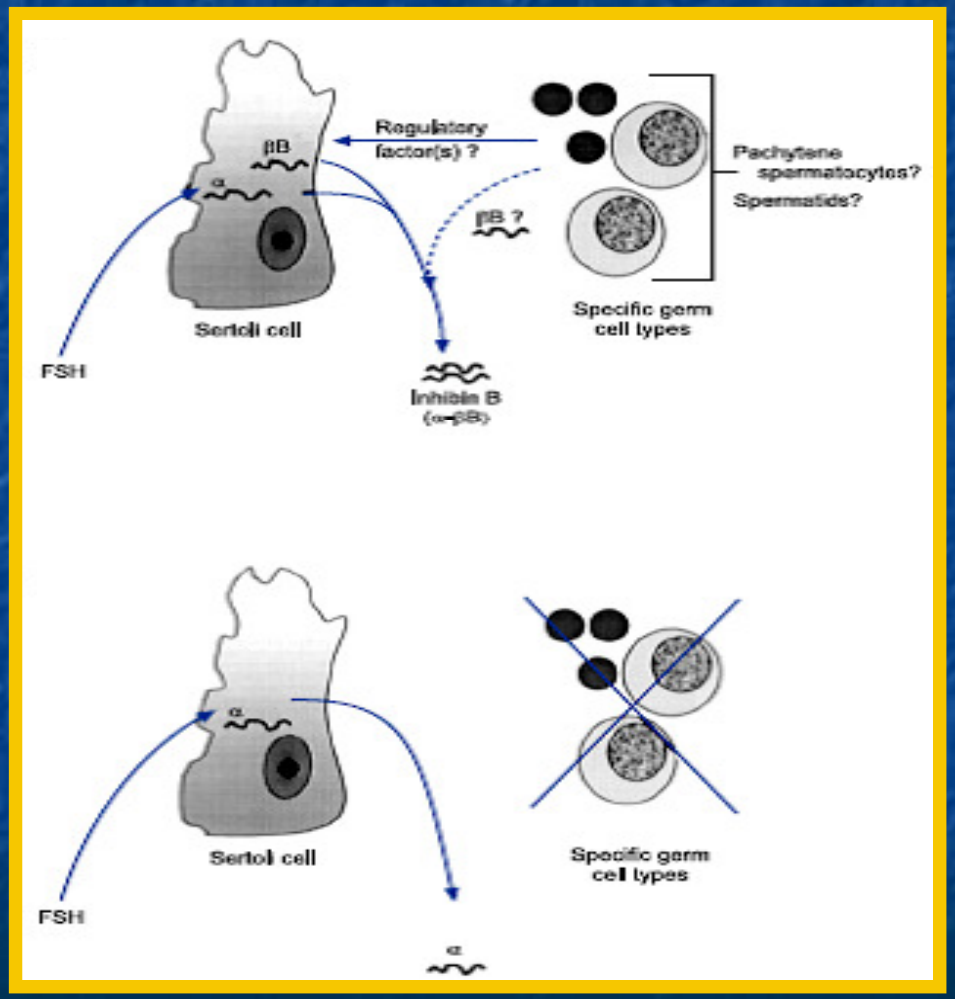
Inhibin family



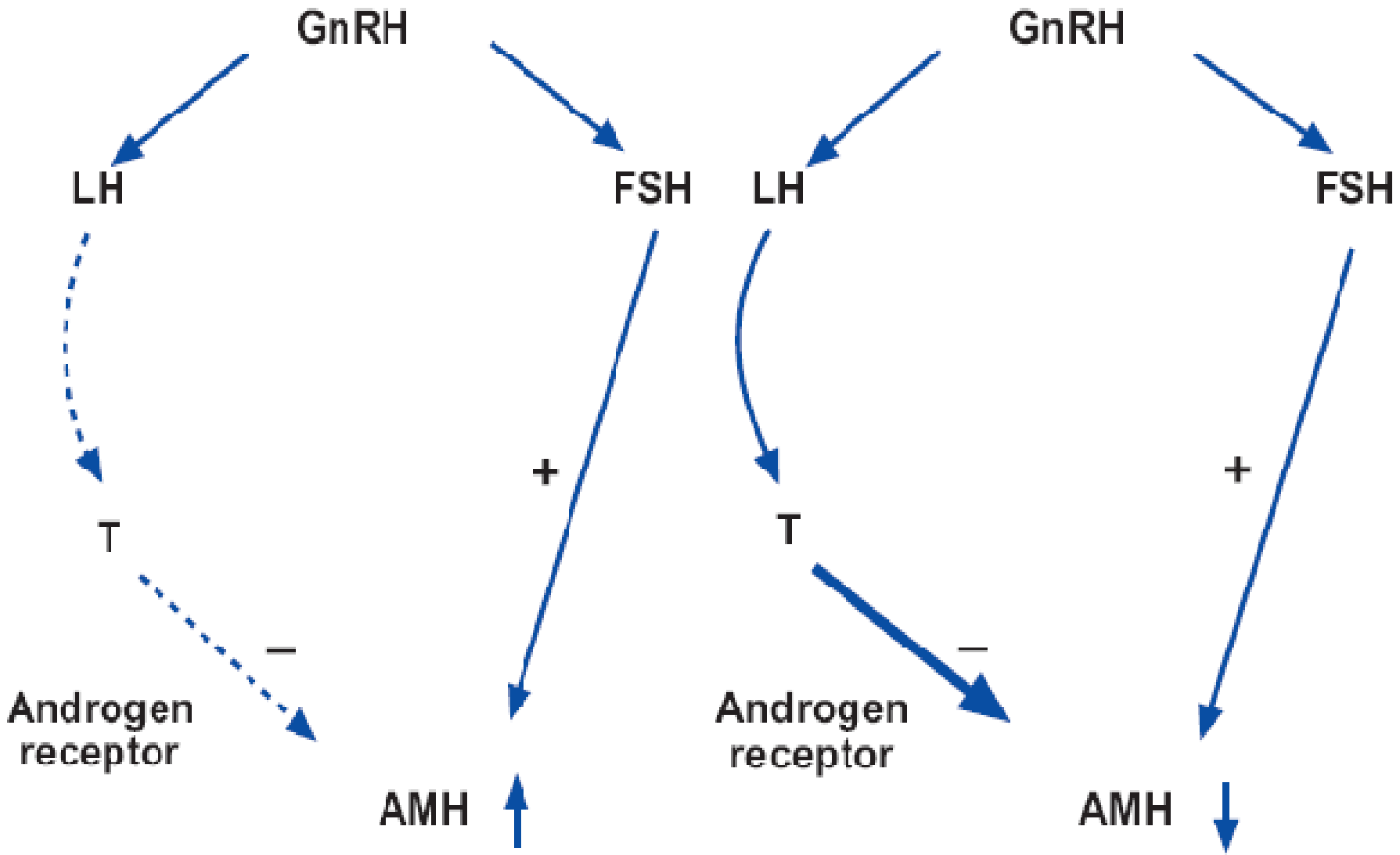
Inhibin B function



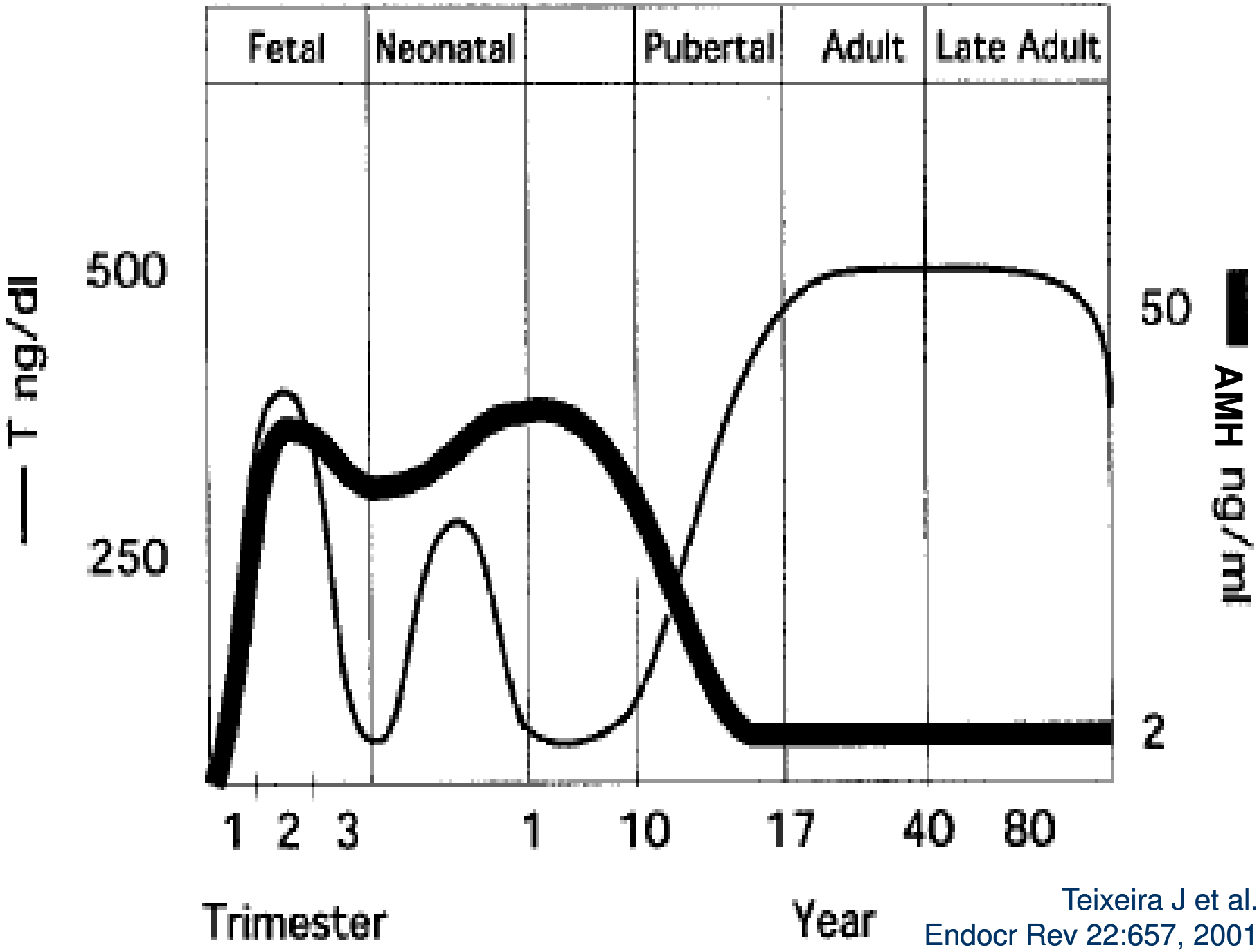
Inhibin B biosynthesis



Andersson A-M. Bailliere Clin Endocrinol Metab 14:389, 2000



Ray R. Bailliere Clin Endocrinol Metab 14:399, 2000



Teixeira J et al.
Endocr Rev 22:657, 2001

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

Teixeira J et al. Endocr Rev 22:657, 2001

3

The role of basal levels of Inhibin B and AMH

HORMONES 2008, 7(2):140-147

Research paper

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

Dimitrios G. Goulis, Paris Polychronou, Themistokis Mikos, Grigorios Grimbizis, Sriridon Gerou, Vassiliki Pavlidou, Athanasios Papanikolaou, Basil C. Tarlatzis, Ioannis N. Bontis, Ioannis Papadimas

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Aim of the study

- 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

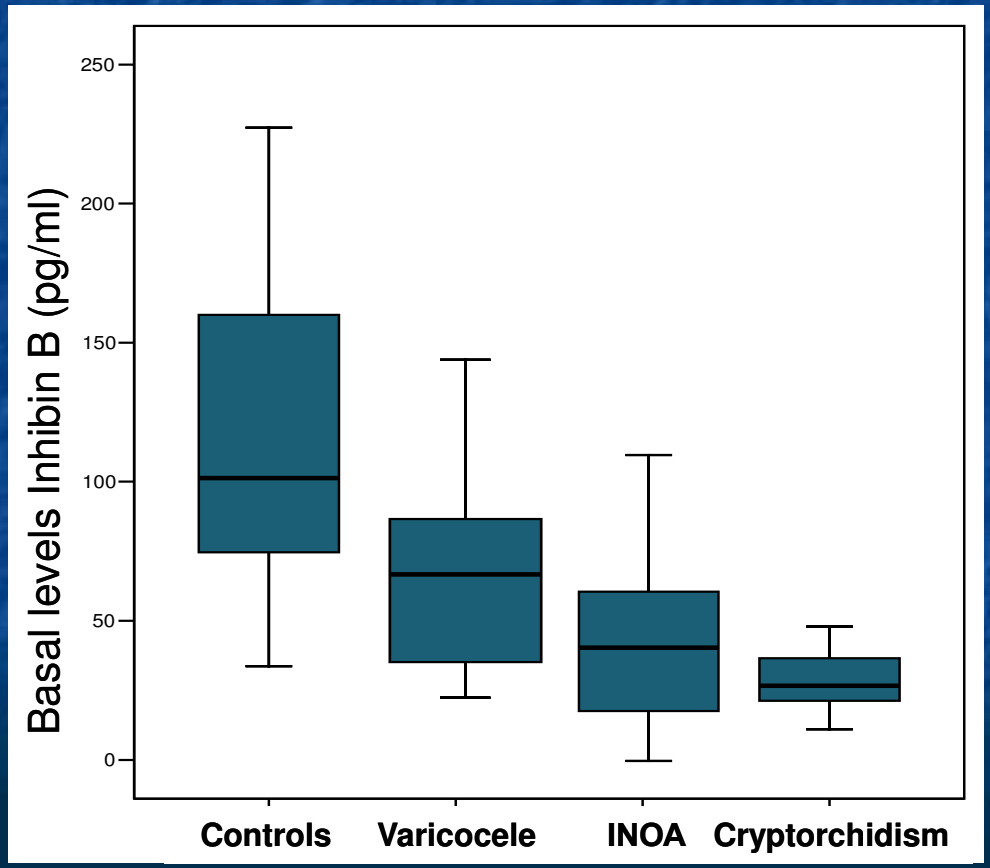
Subfertile men diagnosis

- 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 ± 10.0 pg/ml
Subfertile: 55.8 ± 6.3 pg/ml (1)

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

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

Gynecological Endocrinology, September 2007; 23(9): 1–3

informa
healthcare

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

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(Received 11 July 2007; revised 7 September 2007; accepted 10 September 2007)

Table I. Clinical and laboratory parameters of control and subfertile men.

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

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. [a]control, [b]INOA, [c]INOD, [d]cryptorchidism (Mann–Whitney *U* test); *sperm index was calculated as: [ejaculation volume (ml)] × [concentration (10^6)] × [motility at first hour (%)] × [normal morphology (%)]/10^4.

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 ($r = -0.451$, $p < 0.05$)

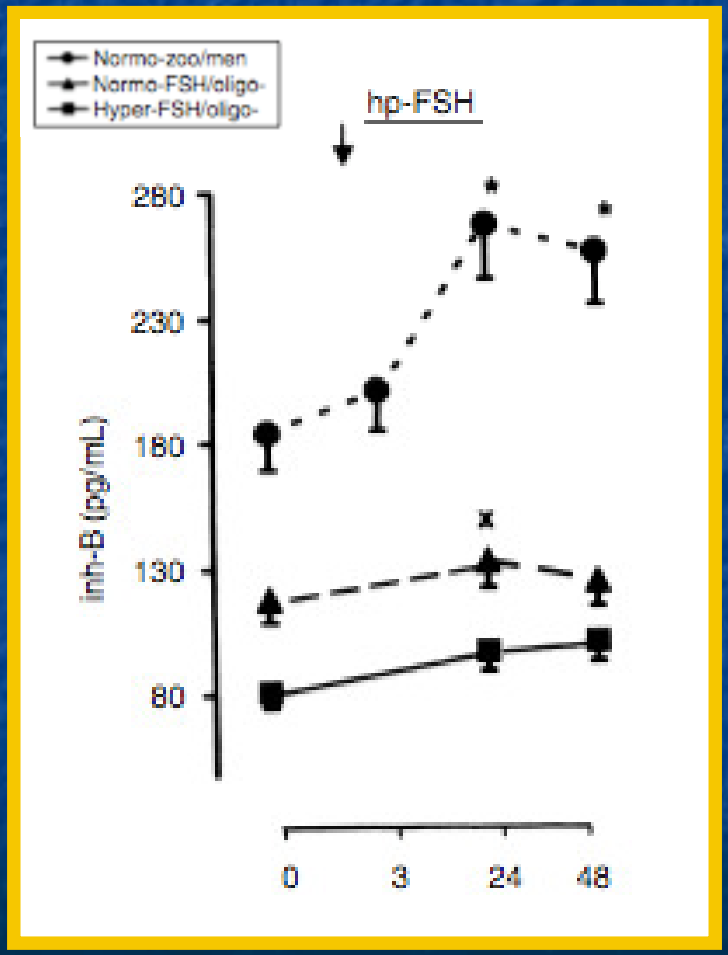
Conclusion - 1

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

4

The role of stimulated levels of Inhibin B and AMH

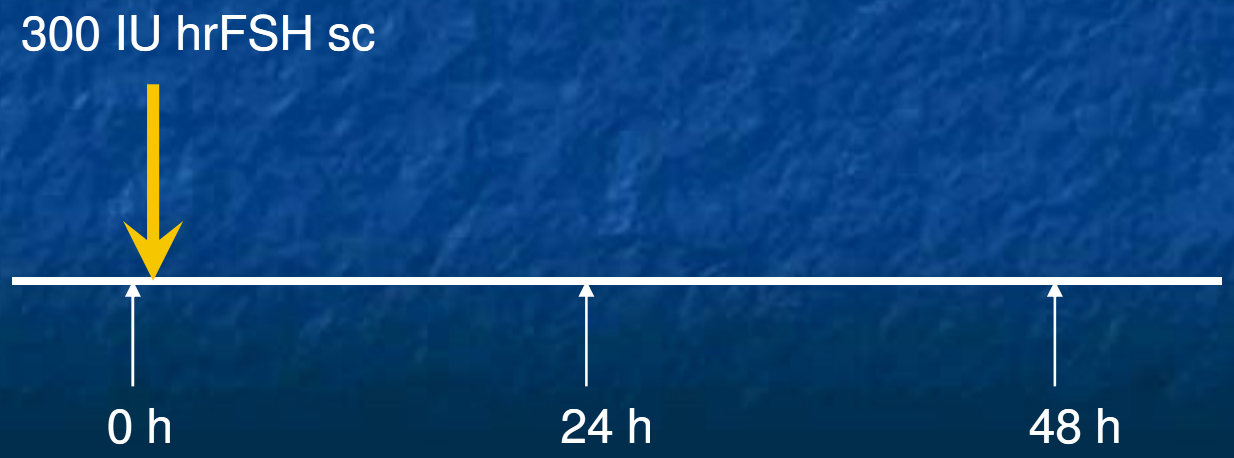
Stimulated Inhibin B levels



Adamopoulos D et al. Int J Androl 26:215, 2003

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



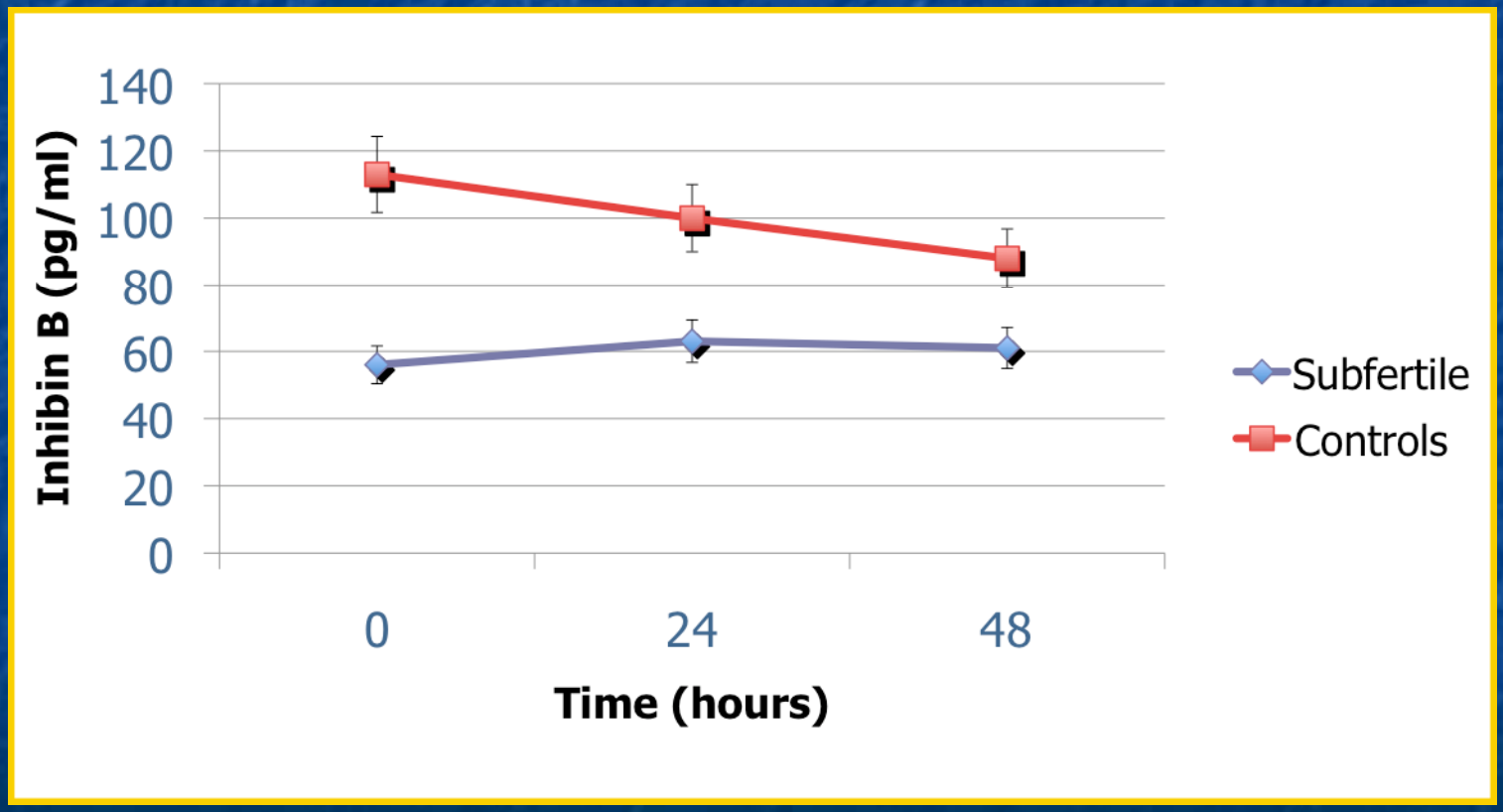
Aim of the study

- The aim of this study was to determine stimulated serum Inhibin B and AMH levels in fertile and subfertile men.

Men studied

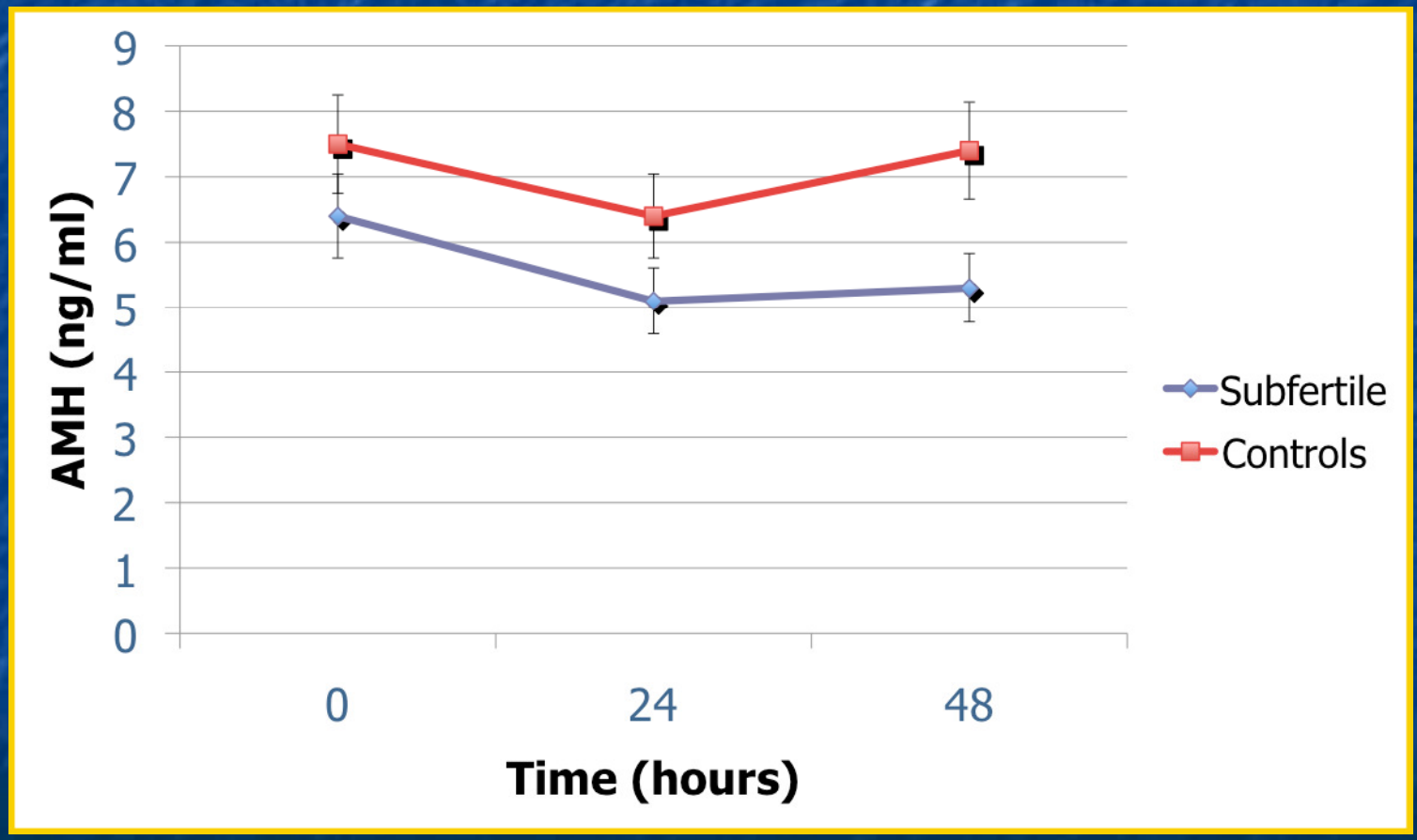
- Subfertility group (n=64)
  - Age 31.5 ± 0.6 years
  - Basal and stimulated Inhibin B and AMH levels
- Control group (n=12)
  - Age 31.3 ± 4.5 years
  - Basal and stimulated Inhibin B and AMH levels

Results



Tsametis Ch et al, 2009 (submitted for publication)

Results



Tsametis Ch et al, 2009 (submitted for publication)

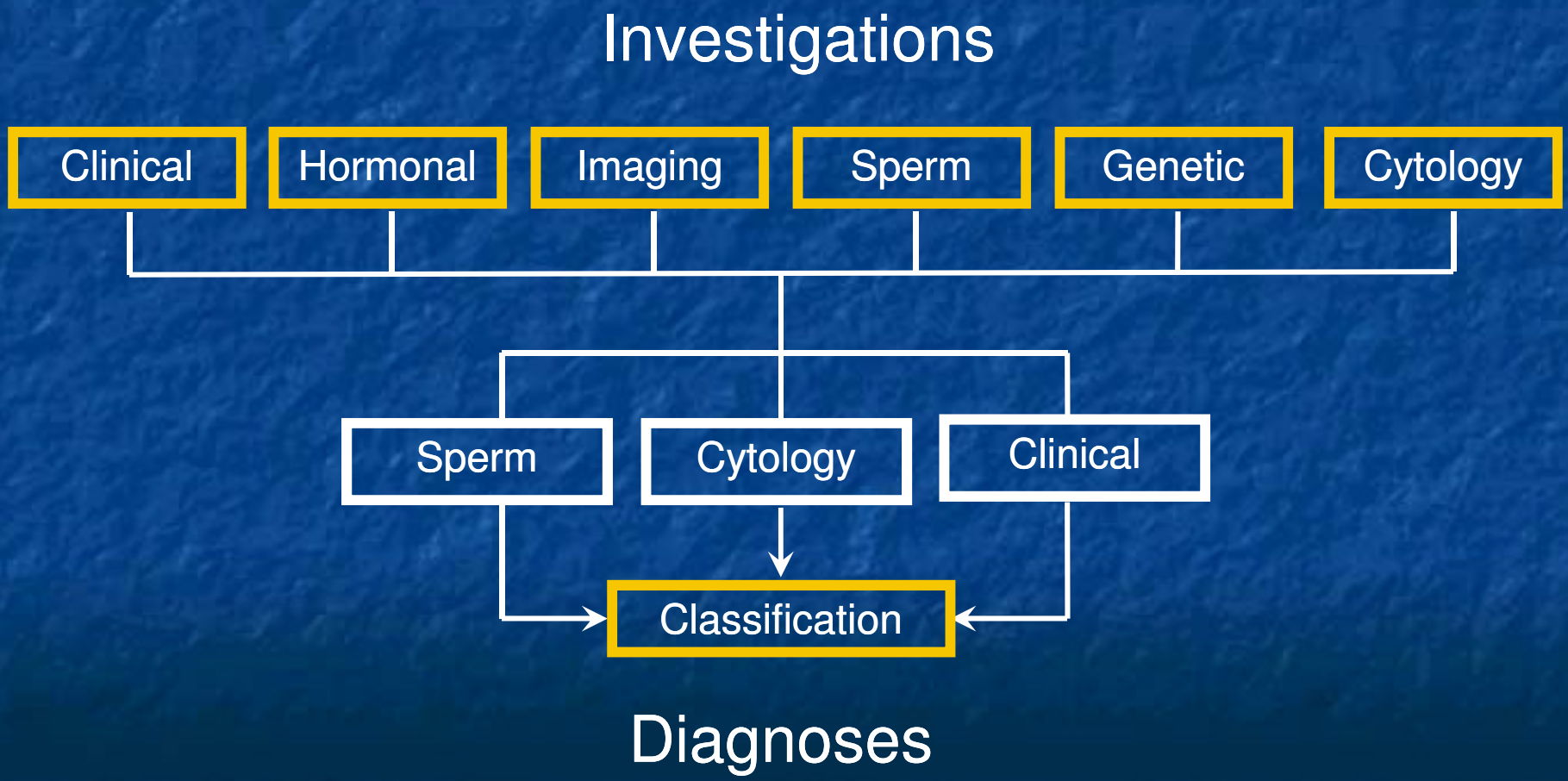
Conclusion - 2

- 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.

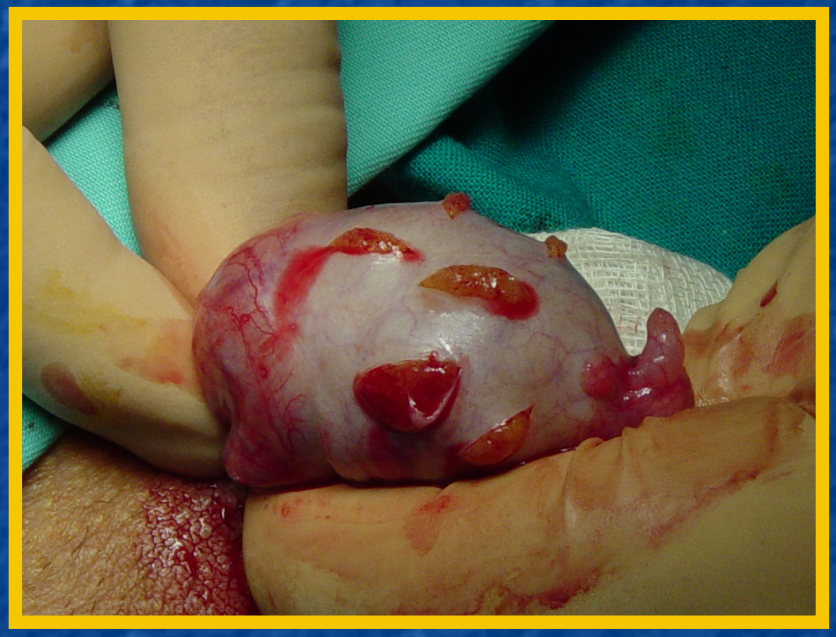
5

Inhibin B and AMH as predictors of sperm retrieval

Diagnostic approach



Sperm retrieval



Testicular Sperm Extraction (TESE)

Testicular FNA

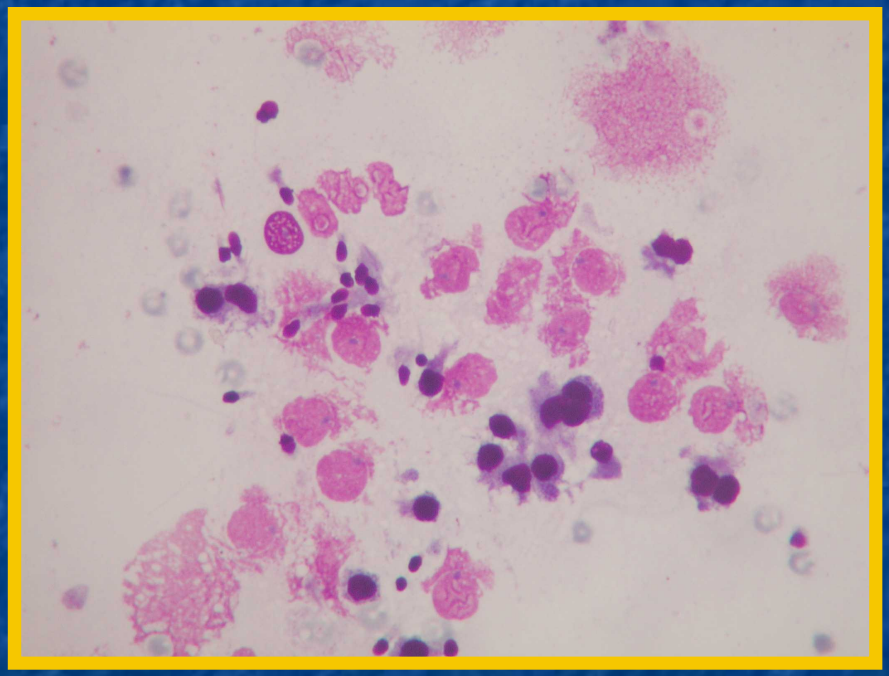
Testicular FNA classification

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

Normal spermatogenesis

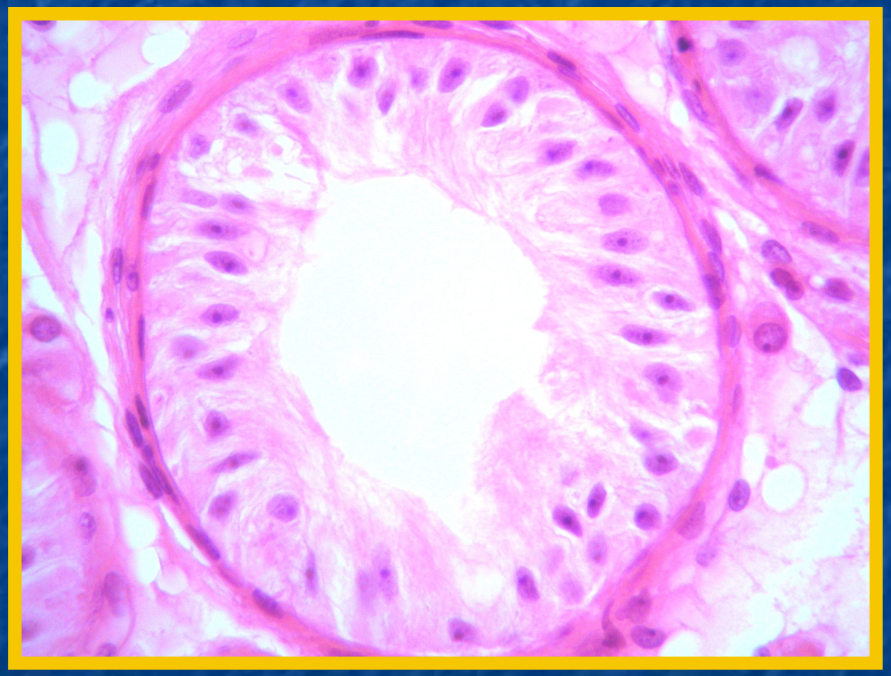
TESE

FNA

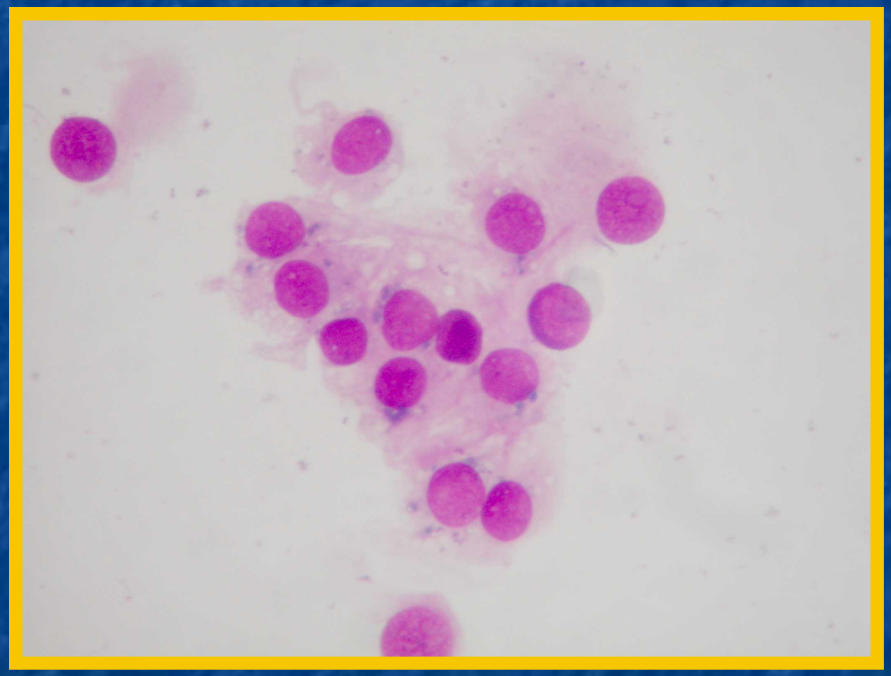


Sertoli Cell-Only Syndrome

TESE



FNA



HORMONES 2008, 7(2):140-147

Research paper

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

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

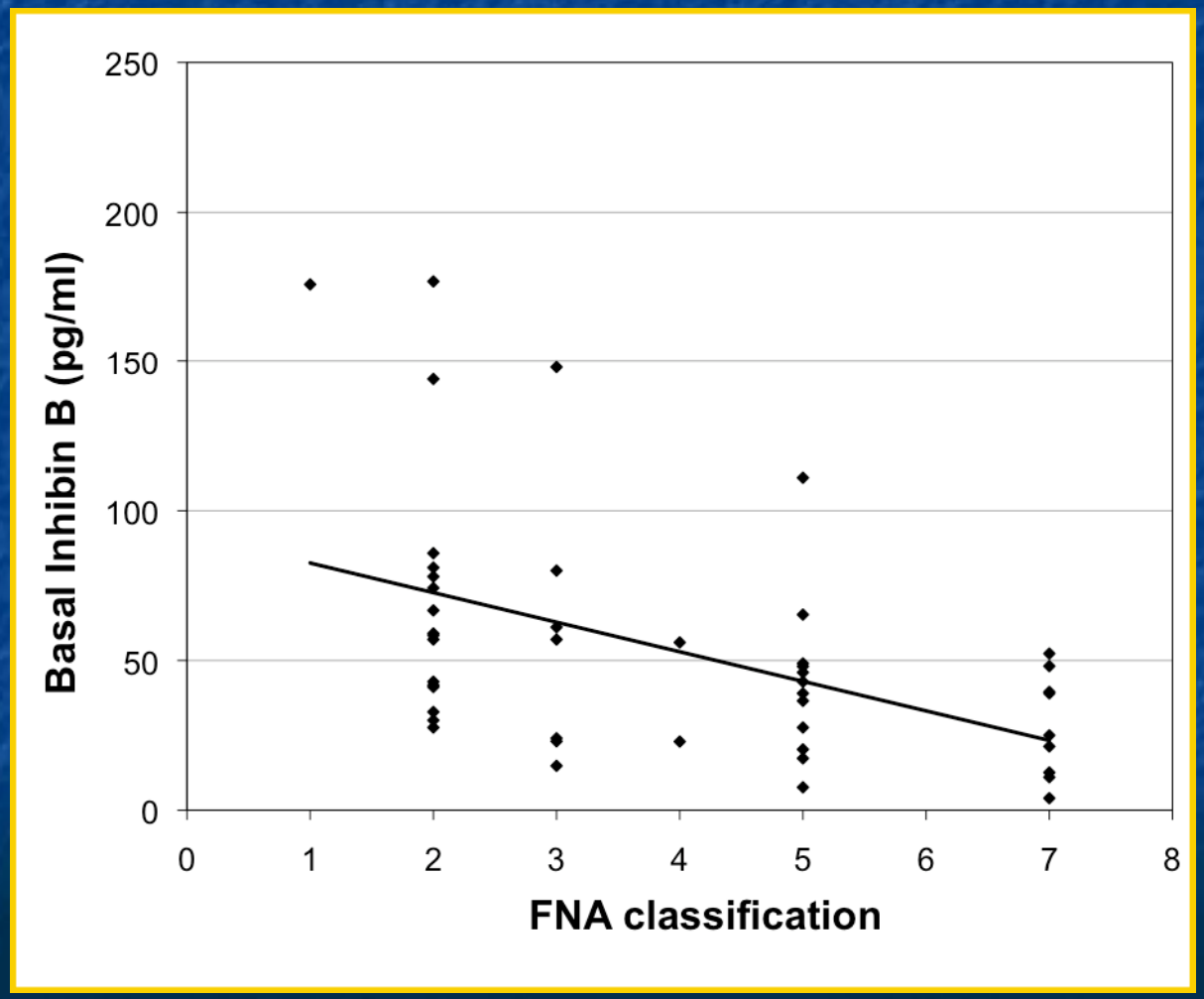
Aim of the study

- 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

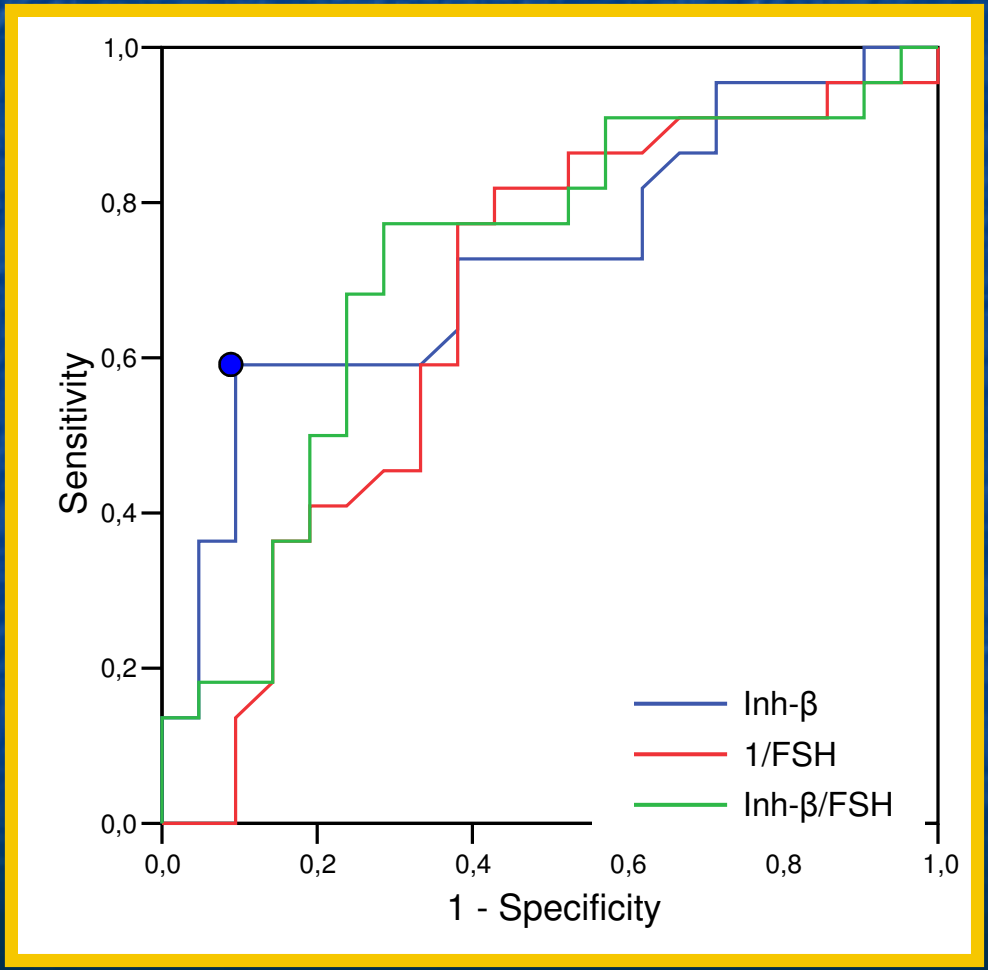


FNA classification

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

	R^2	*p*
Age	excluded	0.912
Testicular volume	excluded	0.812
FSH	excluded	0.671
Total testosterone	excluded	0.838
Inhibin B	0.308	0.001

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.

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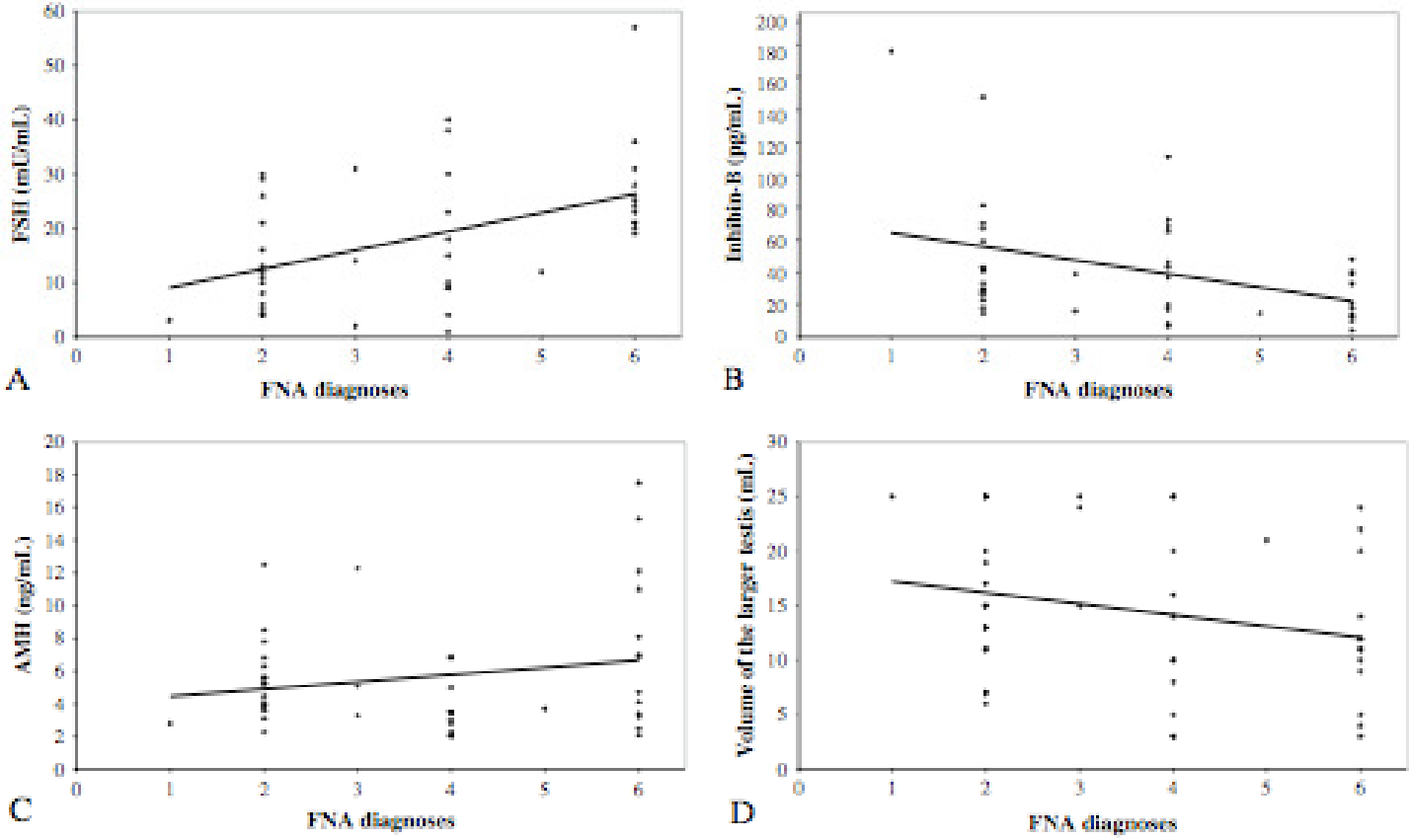
0015-0282/09/$36.00
doi:10.1016/j.fertnstert.2008.01.010

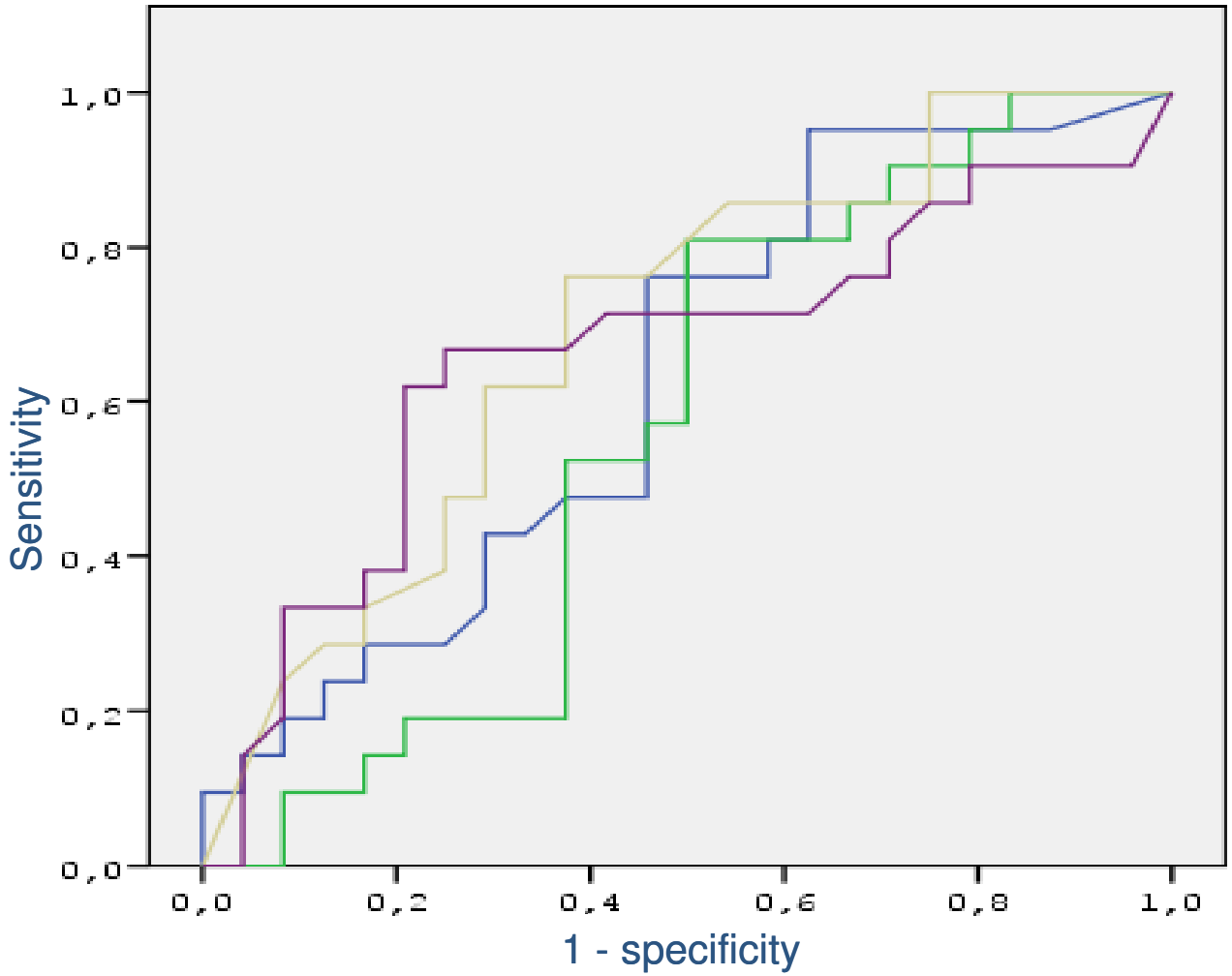
FIGURE 1

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).



Goulis. Serum inhibin B and AMH in testicular FNA. Fertil Steril 2009.

ROC curves

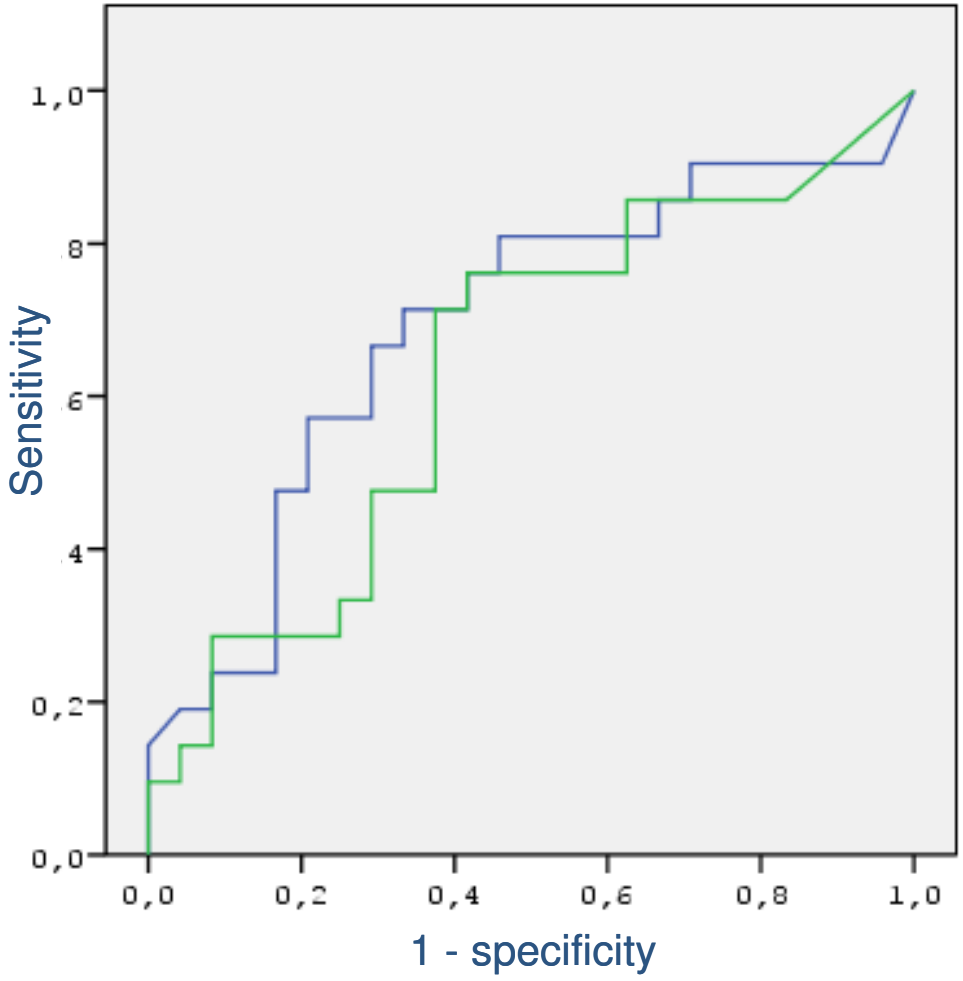


Inhibin B
AMH
Testicular volume
1/ FSH

AUC

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

ROC curves



Testicular volume / FSH

Testicular volume x Inh B x AMH / FSH

AUC:

0,736

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

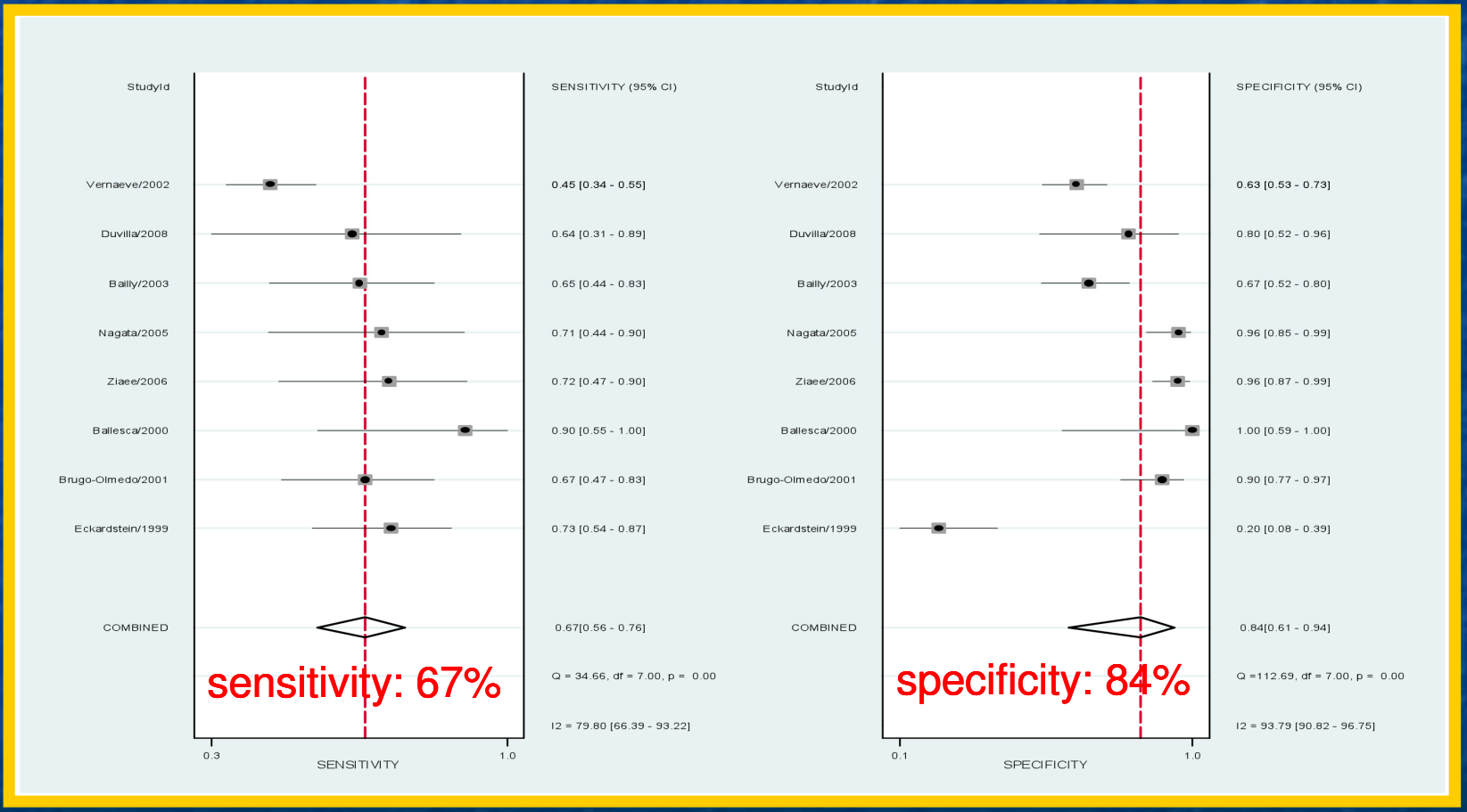
Toulis K, Iliadou PK et al, 2009 (submitted for publication)

Meta-analysis characteristics

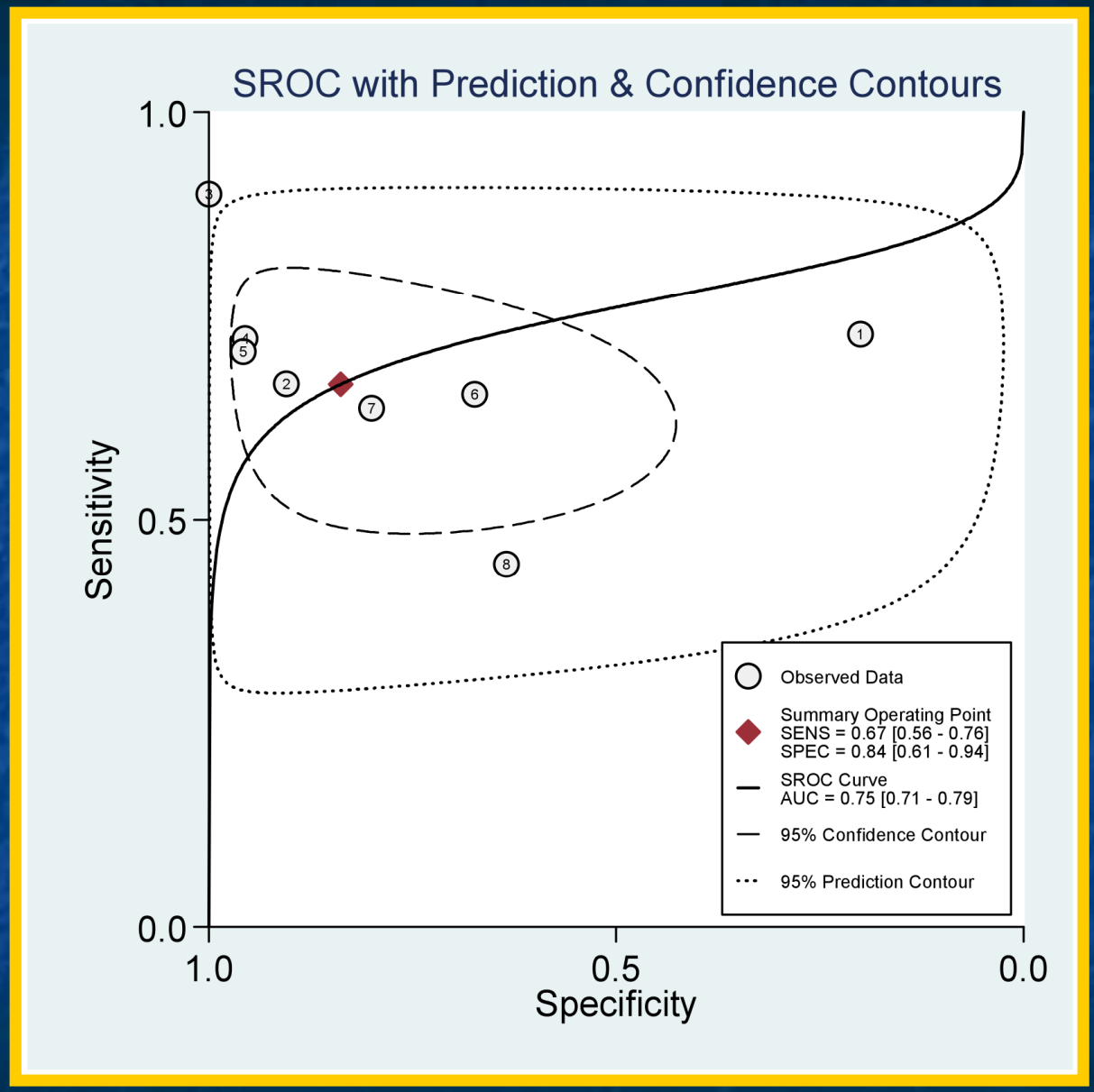
- Number of studies: 8
- Number of patients: 587
- Time interval: 1999 - 2008
- Sperm retrieval method: TESE

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

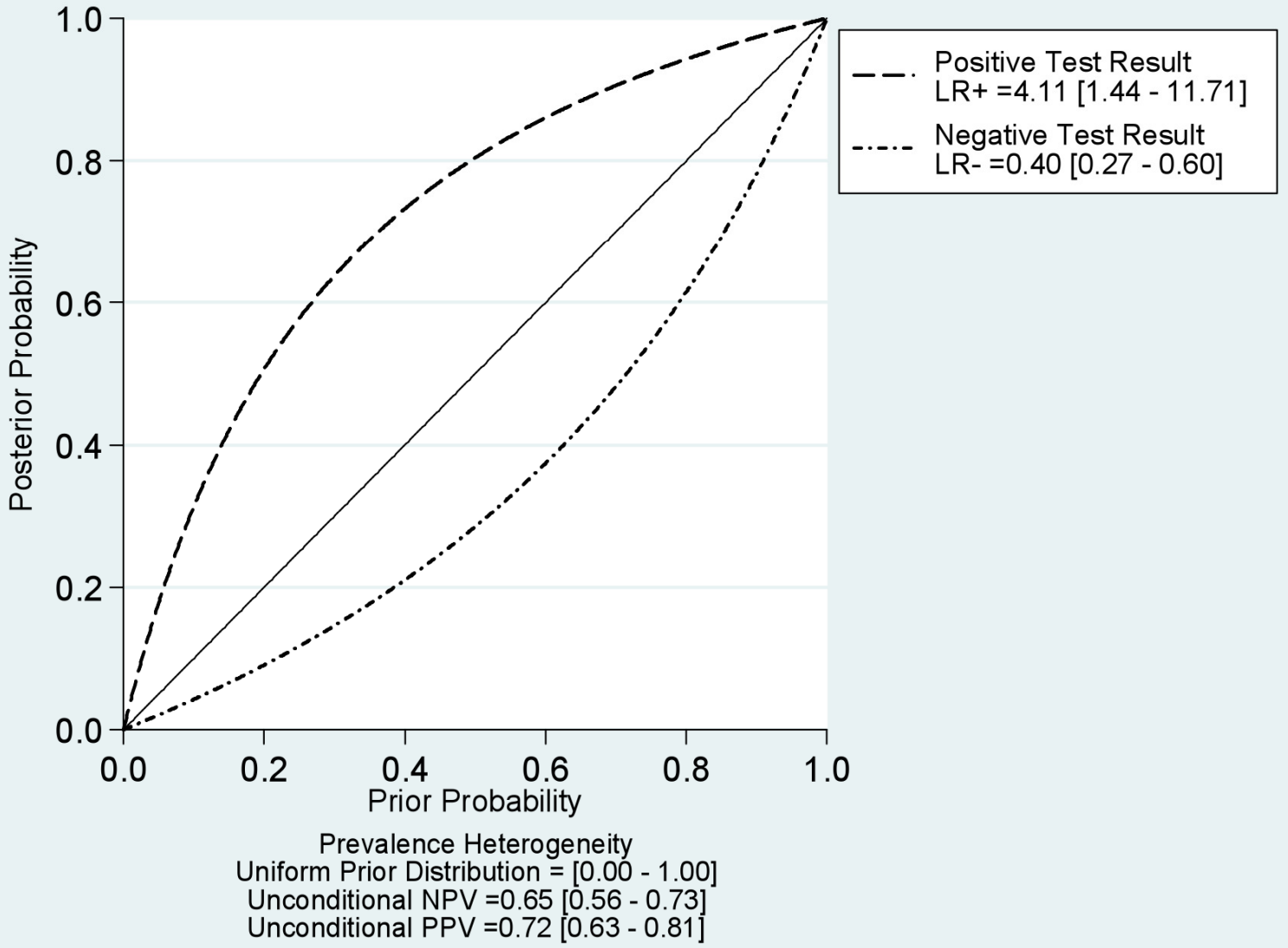
Meta-analysis



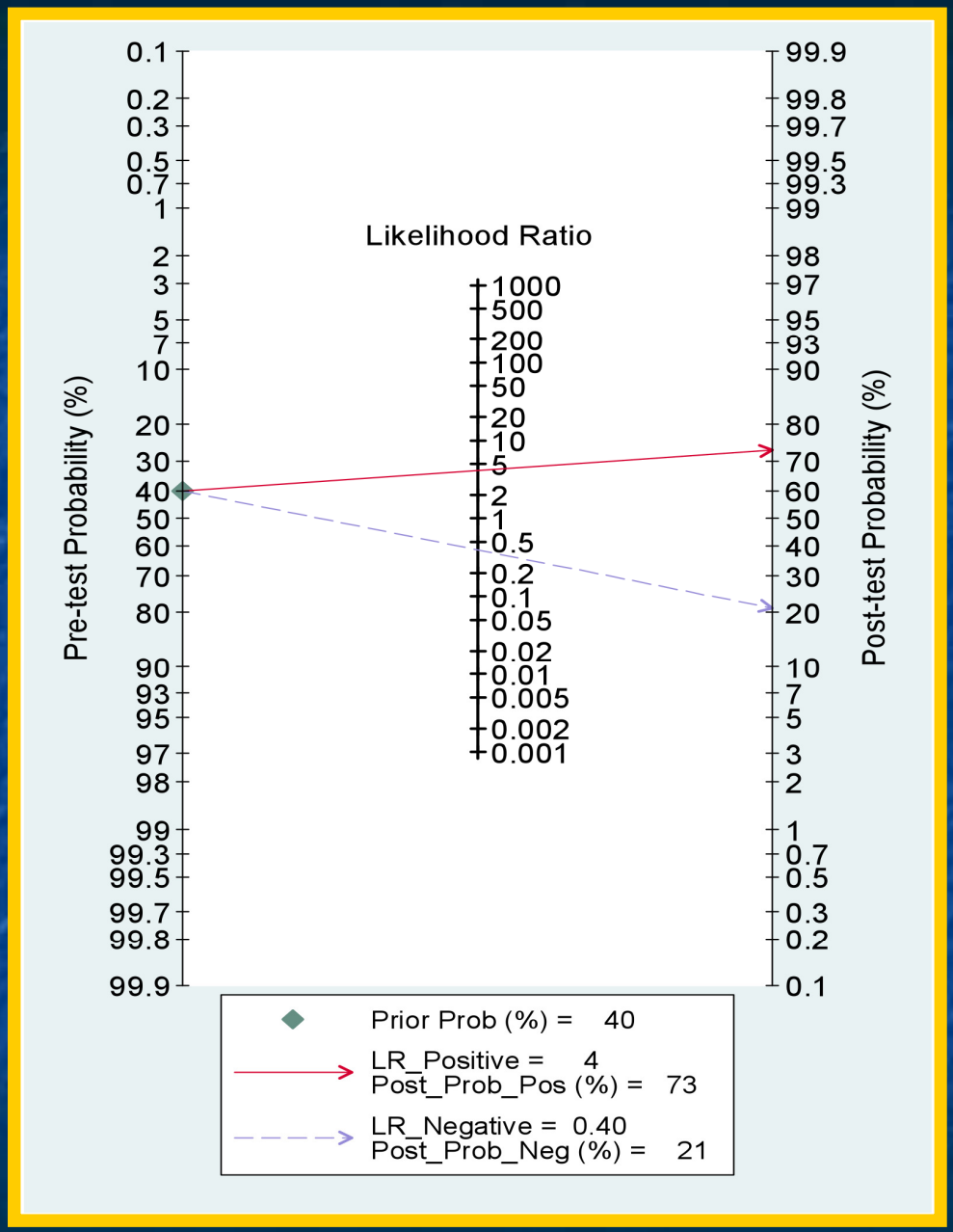
Toulis K, Iliadou PK et al, 2009 (submitted for publication)



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



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



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

6

Conclusions

Conclusion - 1

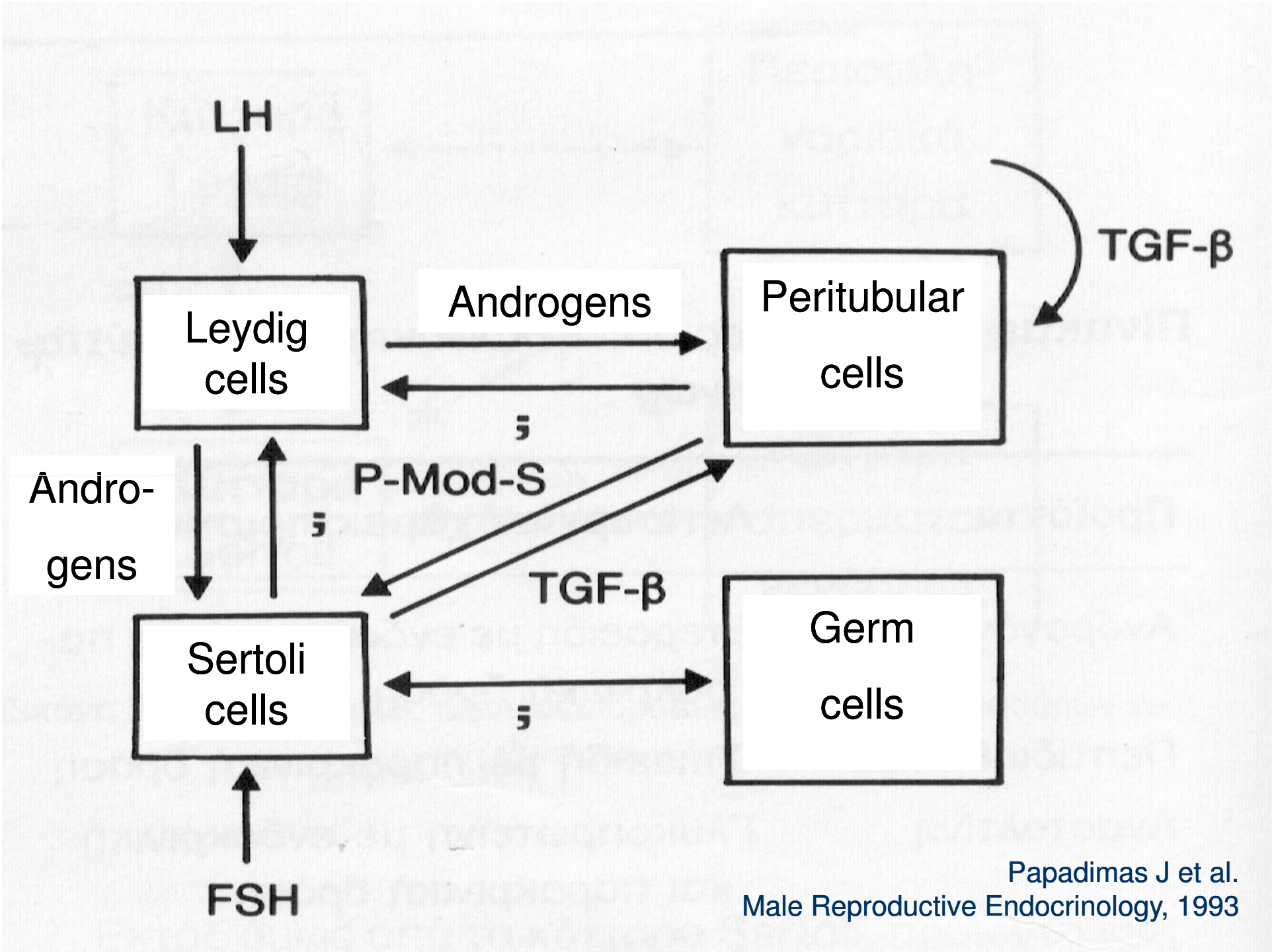
- Serum Inhibin B and AMH levels seems to constitute important diagnostic parameters in male subfertility, as they reflect Sertoli cell status.

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.

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 TESE / FNA in men with azoospermia.



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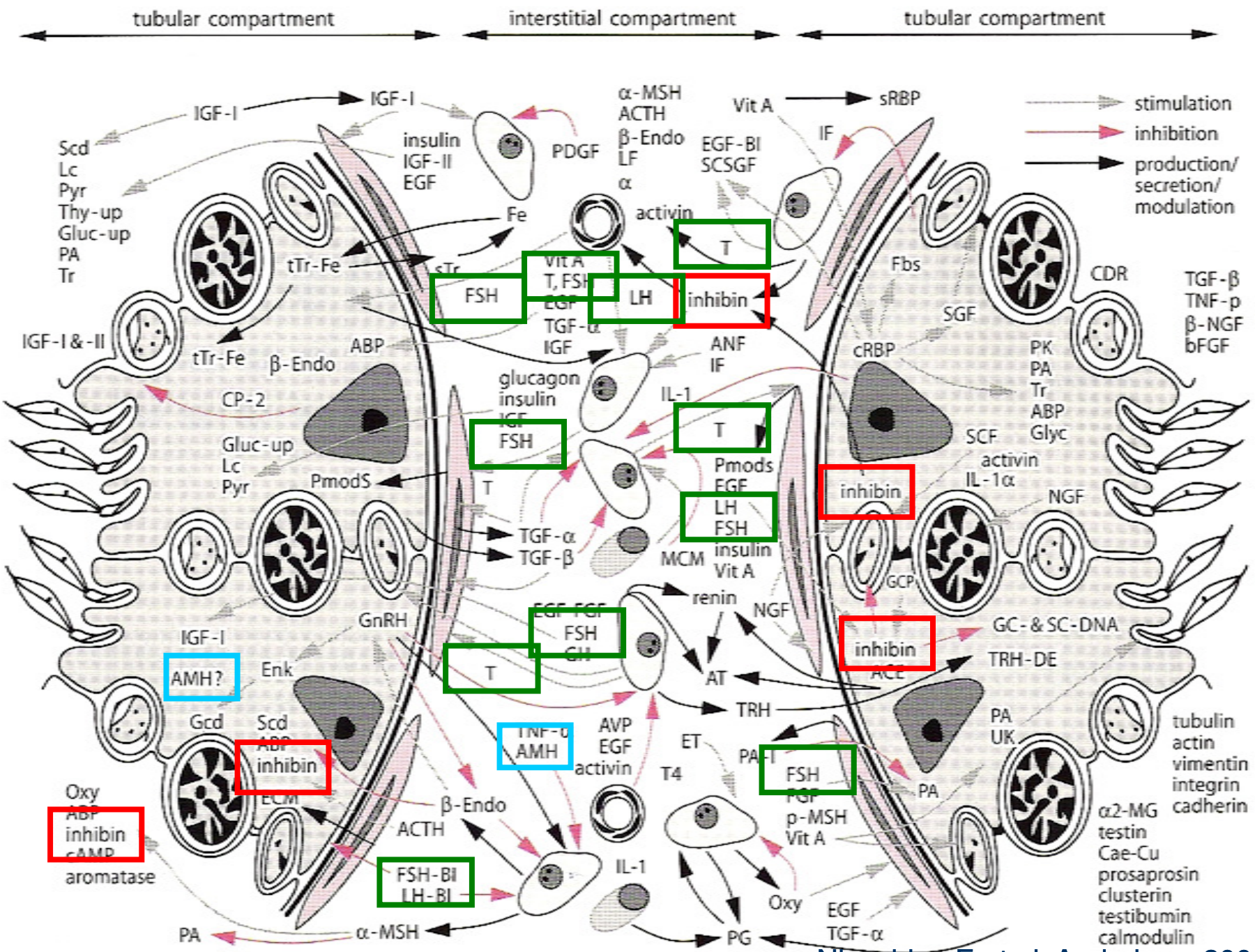

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