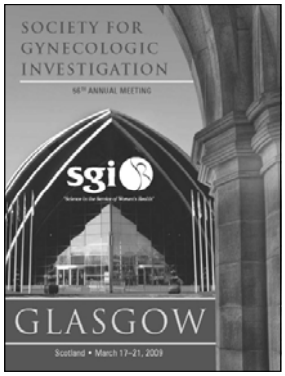


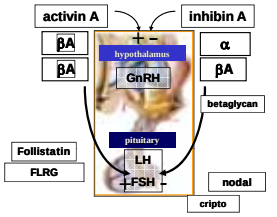
"Endometrial remodelling and regeneration in reproductive disorders satellite symposium 2009"

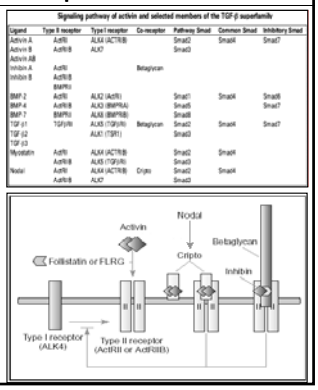
Pasquale FLORIO, *PhD, MD*

The clinical soundness of basic research on activin A in endometriosis and endometrial cancer

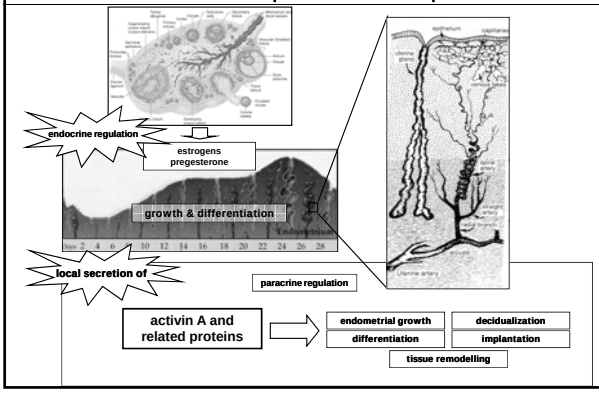
Dpt Pediatrics, Obstetrics & Reprod. Med., Section of Obstetrics and Gynecology University of Siena, Siena, Italy

Activin A and related-proteins

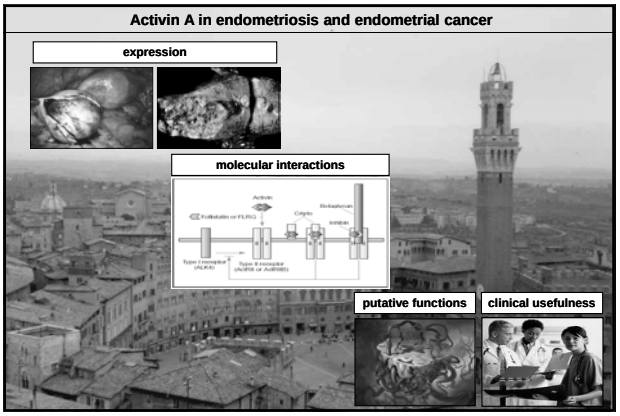




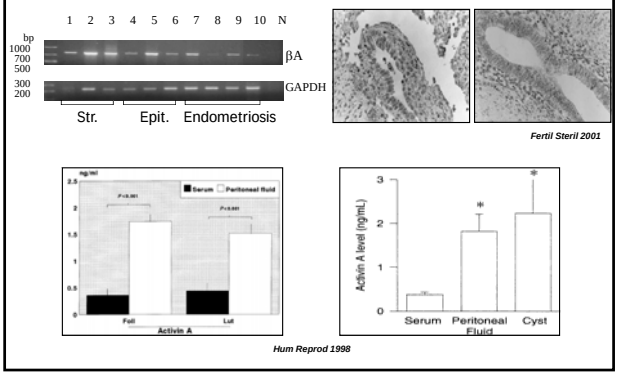
Activin A and related proteins: endometrial products



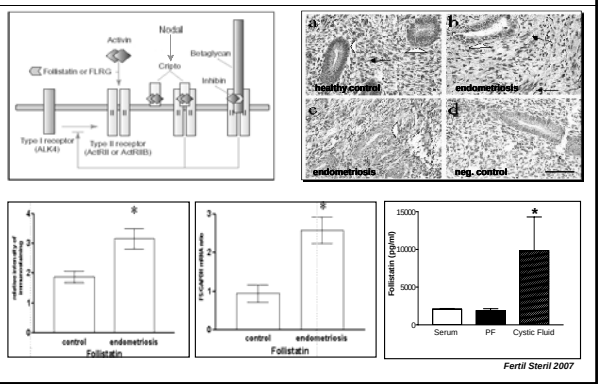
Activin A in endometriosis and endometrial cancer



Activin A and endometriosis



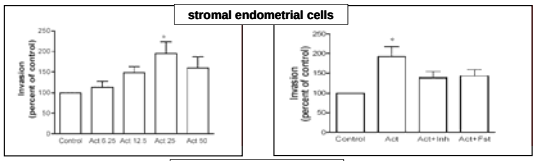
Follistatin and endometriosis



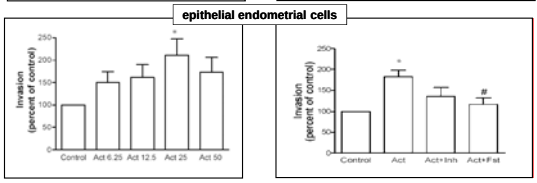
Activin A and follistatin: actions on an in-vitro model of human peritoneum invasion

monolayer of confluent peritoneal mesothelial cells grown in a Matrigel invasion assay

stromal endometrial cells

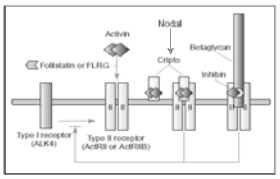


epithelial endometrial cells



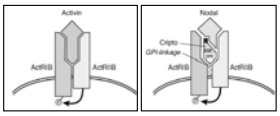
Mol Hum Reprod 2008

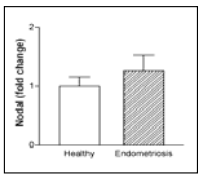
Nodal, cripto and endometriosis

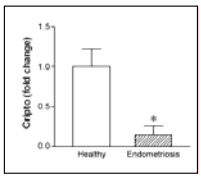


a new role for Cripto as an inhibitor of activin A signaling

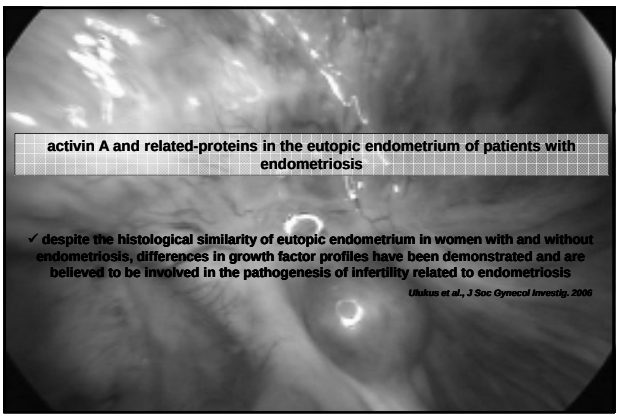
Shen M, J Clin Invest. 2003







Reprod Sci 2009, in press

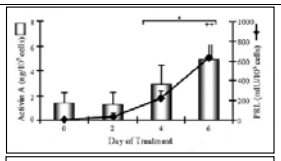


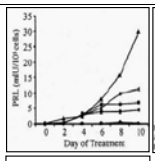
activin A and related-proteins in the eutopic endometrium of patients with endometriosis

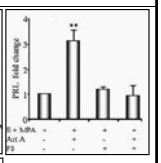
✓ **despite the histological similarity of eutopic endometrium in women with and without endometriosis, differences in growth factor profiles have been demonstrated and are believed to be involved in the pathogenesis of infertility related to endometriosis**

Ulukus et al., J Soc Gynecol Investig. 2006

Activin A and decidualization of the human endometrium

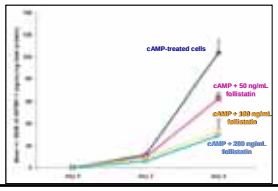






decidualization is associated with the release of PRL and activin A

Jones et al., JCE&M 2002

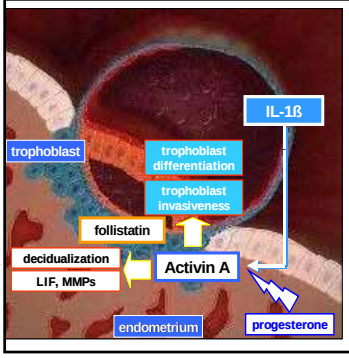


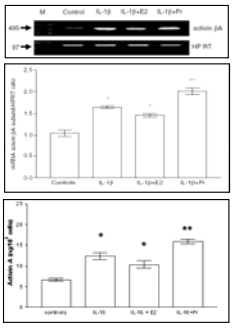
activin A is a key component of pathways related to endometrial stromal cell decidualization

Tierney & Giudice, Fertil Steril 2004

Activin A and the cross-talk between the endometrium and the embryo at implantation

decidual transformation of endometrial stromal cells is a prerequisite for human implantation

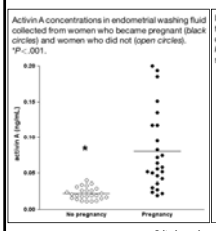


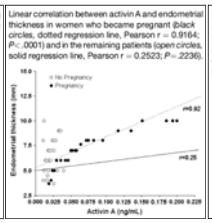


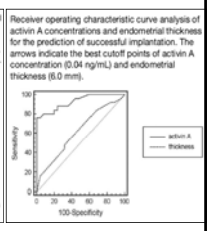
Reproductive Sciences 2007

Activin A as a marker of receptive endometrium

50 women of whom 25 became pregnant after up to three IUI attempts and 25 did not







Criterion values and coordinates of the ROC curve activin A

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>0,04 *	76,00	54,9 - 90,6	100,00	86,2 - 100,0		0,24

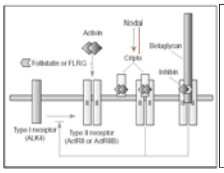
Criterion values and coordinates of the ROC curve endometrial thickness

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>6 *	68,00	46,5 - 85,0	48,00	27,8 - 68,7	1,31	0,67

Fertil & Steril 2009

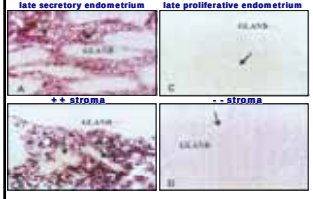
Activin A receptors antagonist: ebaf

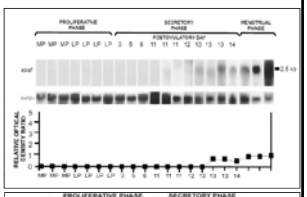
- ✓ protein of 370 amino acids;
- ✓ member of the TGF-ß family;
- ✓ similarity with *lefty-A* (important determinant of vertebrate development such as *left-right asymmetry)*
- ✓ binds the *activin receptor type II B (ACTRIIB)*



	% Similarity
✓ ebaf	100
✓ TGF-ß1	65
✓ activin ßA	65
✓ activin ßB	63

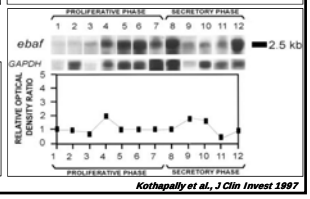
Activin A receptors antagonist: ebaf in the human endometrium





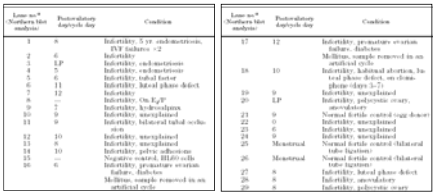
endometrial bleeding

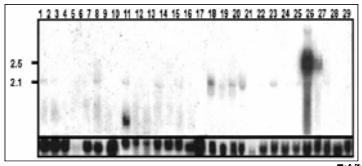
the mRNA of ebaf is strongly expressed in all endometria irrespective of whether the endometrium was in the proliferative or secretory phase of the menstrual cycle



Kothapally et al., J Clin Invest 1997

Dysregulated expression of ebaf, a novel molecular defect in the endometria of patients with infertility - *Northern blot analysis* -



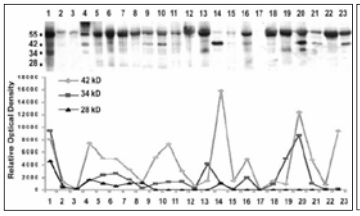


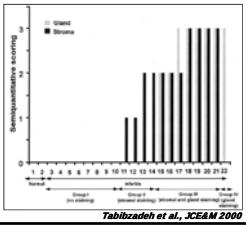
Tabibzadeh et al., JCE&M 2000

Dysregulated expression of ebaf, a novel molecular defect in the endometria of patients with infertility - *Western blot analysis/immunohistochemistry* -

Case no.	Cycle day	Conditions
1	23	Normal
2	25	Normal
3	23	Infertility, male factor
4	23	Infertility, endometriosis
5	23	Infertility, luteal phase defect
6	23	Infertility, luteal phase defect
7	22	Infertility, endometriosis
8	22	Habitual abortion
9	22	Infertility, endometriosis
10	24	Infertility
11	16	Infertility, endometriosis, luteal phase defect

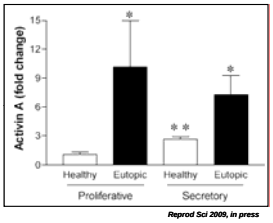
Case no.	Cycle day	Conditions
12	23	Infertility, unexplained
13	22	Infertility, endometriosis
14	23	Infertility, endometriosis
15	23	Infertility, endometriosis
16	23	Habitual abortion
17	23	Infertility, endometriosis, habitual abortion
18	23	Infertility, endometriosis
19	22	Infertility, endometriosis
20	23	Infertility, unexplained, luteal phase defect
21	22	Infertility, endometriosis, luteal phase defect
22	22	Infertility, endometriosis, luteal phase defect



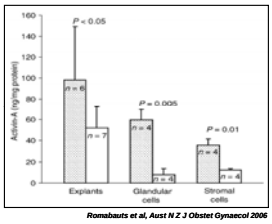


Tabibzadeh et al., JCE&M 2000

Disrupted cyclicity of activin A expression in eutopic endometrium

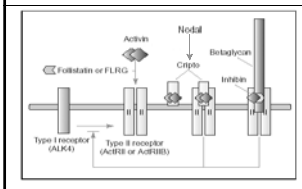


Reprod Sci 2009, in press



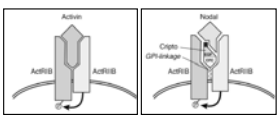
Romabauts et al, Aust N Z J Obstet Gynaecol 2006

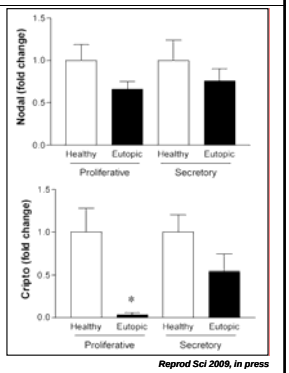
Activin A antagonists: nodal & cripto in eutopic endometrium



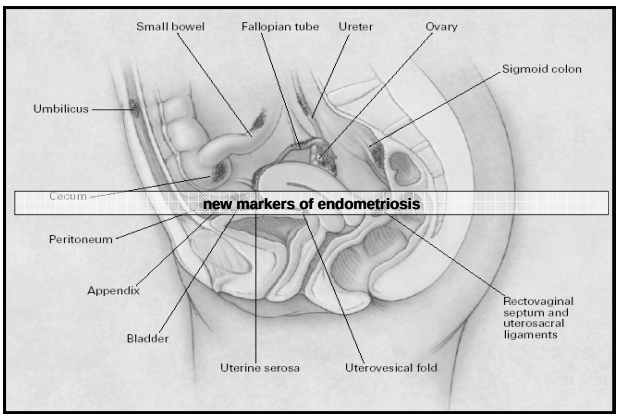
a new role for Cripto as an inhibitor of activin A signaling

Shen M, J Clin Invest. 2003





Reprod Sci 2009, in press



Proposed markers of endometriosis

Tumor markers and polypeptides
(A) CA-125, CA-19-9
(B) SICAM-1 (soluble forms of the intercellular-adhesion molecule-1)
(C) Glycodelin-A (PP 14)

Immunological markers
(A) Cytokines: IL-6, TNF
(B) Autoantibodies
(1) Antiendometrial
(2) Autoantibodies to markers of oxidative stress

Genetic markers
Early growth response (EGR)-1 gene
P450 aromatase
Placental Protein 14 (PP14)

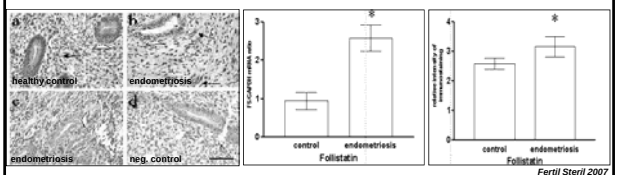
Tissue markers
(A) Aromatase P 450
(B) Cytokeratins
(C) Hormone receptors

Diagnostic accuracy of serum CA-125>/= 20 U/ml in diagnosing patients with endometriosis

	No. of patients	Sensitivity, %	Specificity, %
Collacuri et al. [130]	28	44	90
Fedele et al. [11]	154	85	100
Fisk and Tan [30]	48	43	04
Gurgan et al. [31]	38	0	100
Hornstein et al. [127]	123	16	91
Ismail et al. [133]	30	50	38
Knaitwagen et al. [32]	74	20	82
Lanzone et al. [134]	119	53	86
Medl et al. [122]	368	33	91
Molo et al. [126]	35	100	93
Moloney et al. [128]	60	34	100
Moretuzzo et al. [7]	40	20	90
Muscatello et al. [131]	119	51	86
O'Shaughnessy et al. [129]	100	27	100
Ozaksit et al. [135]	86	80	90
Palton et al. [125]	113	13	93
Pittaway and Fayez [6]	385	17	93

- large range of variability
- not ever specific to endometriosis
- often with low specificity/sensitivity

Follistatin and endometriosis



Fertil Steril 2007

✓ to quantify the concentration of follistatin and CA-125 in the serum of women with ovarian endometrioma and other benign cysts

✓ to investigate the use of follistatin as a marker in the differential diagnosis of benign ovarian cysts

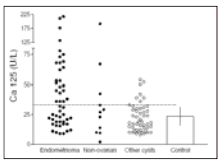
Group A
ovarian endometrioma (n=52)

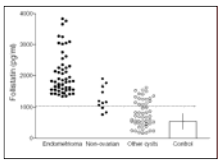
Group B
benign ovarian cysts (n=52)
serous (n=17); mucinous (n=18); demoid cysts (n =7), hemor. corpora lutea cysts (n=10)

Group C
extra-ovarian endometriosis
(stage I or II pelvic endometriosis, n=11)

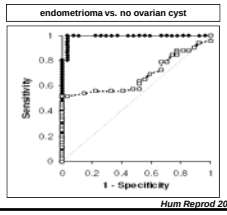
Group D
Healthy controls (n=27)
(laparoscopic tubal sterilization)

High serum follistatin levels in women with endometriosis

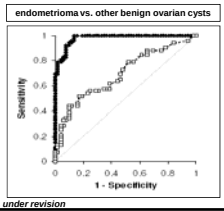




endometrioma vs. no ovarian cyst

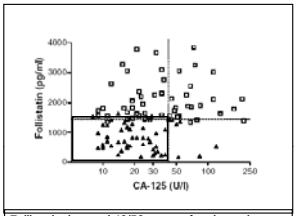


endometrioma vs. other benign ovarian cysts



Hum Reprod 2009, under revision

Serum follistatin measurement in the diagnosis of endometriosis



Follistatin detected 48/52 cases of endometrioma (92% sensitivity) with the cutoff 1433 pg/ml, which corresponds to 96% specificity.

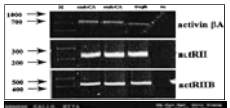
In contrast, CA-125 detected only 44% of the endometriomas with 90% specificity.

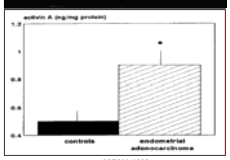
Sensitivity, specificity, likelihood ratios (LR) and area under the receiver operating characteristic (ROC) curve for serum follistatin and CA-125 in the diagnosis of ovarian endometrioma

	Endometrioma vs. no ovarian cyst		Endometrioma vs. other benign ovarian cysts	
	Follistatin	CA-125	Follistatin	CA-125
Best cutoff point	1025 pg/ml	32 U/l	1433 pg/ml	42 U/l
Sensitivity (%)	100 (99-100)	52 (38-65)	92 (85-99)	44 (31-58)
Specificity (%)	96 (89-100)	96 (89-100)	92 (85-99)	90 (82-98)
LR of positive test[a]	25 (4-184)	14 (2-97)	12 (3-31)	5 (2-11)
LR of negative test[b]	0.00 (0.00-0.14)	0.50 (0.37-0.67)	0.08 (0.03-0.21)	0.62 (0.48-0.80)
Area under ROC curve	0.993 (0.978-1.000)	0.681 (0.565-0.797)	0.980 (0.960-1.000)	0.697 (0.596-0.798)

Hum Reprod 2009, under revision

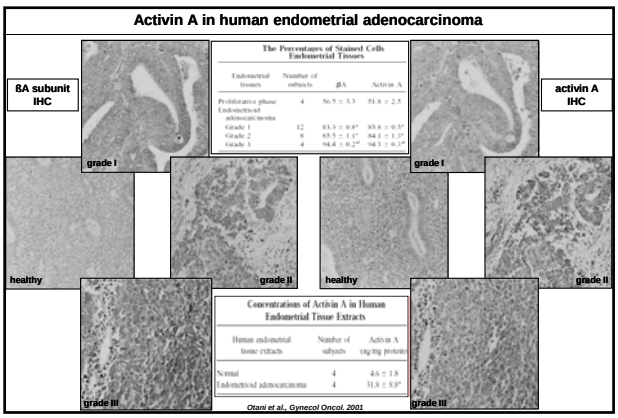
Activin A and endometrial cancer





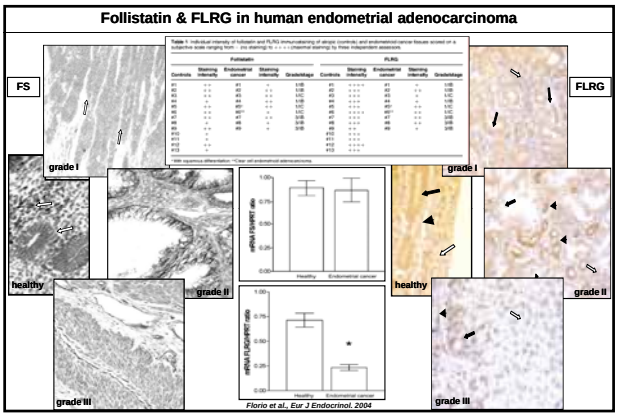
JCE&M, 1998

Activin A in human endometrial adenocarcinoma



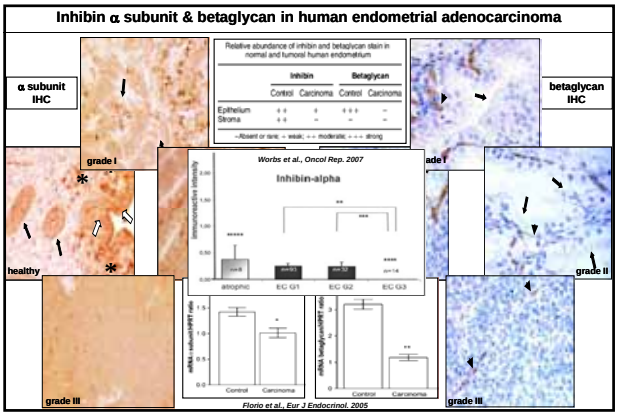
Otani et al., Gynecol Oncol. 2001

Follistatin & FLRG in human endometrial adenocarcinoma



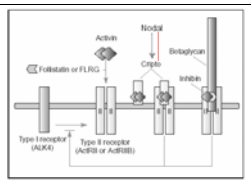
Florio et al., Eur J Endocrinol. 2004

Inhibin α subunit & betaglycan in human endometrial adenocarcinoma



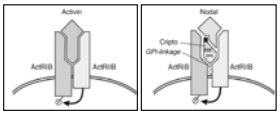
Florio et al., Eur J Endocrinol. 2005

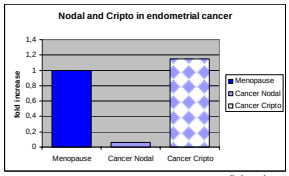
Nodal, cripto and endometrial cancer



a new role for Cripto as an inhibitor of activin A signaling

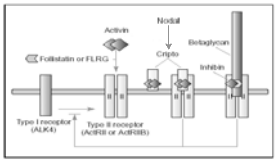
Shen M, J Clin Invest. 2003





preliminary data

Activin and related proteins in and endometrial cancer



↑↑ **activin A**

↓↓ **inhibin A**

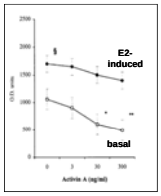
↓↓ **betaglycan**

↓↓ **FLRG**

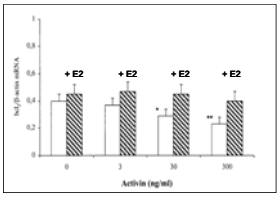
↓↓ **nodal**

Activin A is a regulator of endometrial cancer cell growth

estrogen-responsive Ishikawa (ISH) cells
(well differentiated endometrial adenocarcinoma)



activin caused a dose-dependent inhibition of ISH cell proliferation



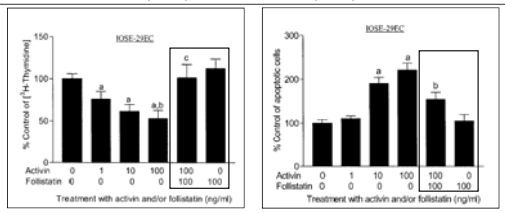
activin caused a dose-dependent decrease of anti-apoptotic Bcl-2 protein level

Choi et al., J Clin Endocrinol Metab 2001

- activin A inhibits endometrial cancer cell line growth & proliferation
- estradiol promotes resistance to the growth-retarding effects of activin through the effect on Bcl-2 levels

Activin A and tumorigenesis: growth inhibition and induction of apoptosis

immortalized ovarian surface epithelium cell line (IOSE-29)
(to study adenocarcinoma tumorigenesis)



activin caused a dose-dependent inhibition of cells proliferation

activin caused increased DNA fragmentation in a dose-dependent manner

cotreatment with follistatin (100 ng/mL) blocked the effects of activin A

Choi et al., J Clin Endocrinol Metab 2001

- activin A inhibits IOSE-29 cell line growth & proliferation
- activin A induces apoptosis increasing DNA fragmentation

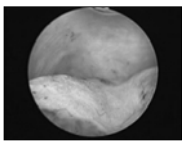
Biochemical screening of endometrial cancer: activin A

Case-control study in patients (n=110) with post-menopausal uterine bleeding

Control group (n=52): endometrial polyps (n=39), atrophic endom (n=16)

Study group (n=52): endometrioid adenocarcinoma (FIGO stage 1A: 24; stage 1B: 18; 1C: 13)

	Study group (n=55)	Healthy controls (n=55)	P
Age (yrs)	63.03 ± 5.28	67.69 ± 8.99	*0.001*
Menarche	12.27 ± 1.09	12.2 ± 1.25	*0.62*
Fertility (yrs)	40.56 ± 5.03	39.9 ± 3.08	*0.01*
Parity	1.98 ± 1.19	1.81 ± 1.11	*0.47*
BMI	26.6 ± 2.16	27.05 ± 4.71	*0.82*
Diabetes	26/55	6/55	*0.000*
Hypertension	11/55	21/55	*0.035*
diab+hypert	3/55	15/55	*0.002*
Tamoxifene	0/55	2/55	*0.51*

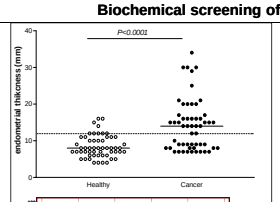


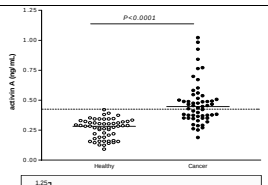
trans-vaginal ultrasounds; operative hysteroscopy with histology; major surgery

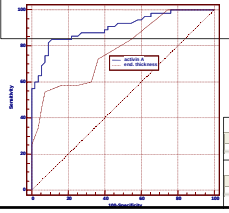
measurement of serum concentrations of activin A by ELISA

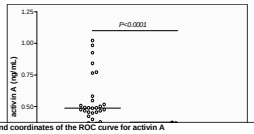
2009, submitted

Biochemical screening of endometrial cancer: activin A









Criterion values and coordinates of the ROC curve for activin A

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
>0.346 ng/mL	81.82	69.1 - 90.9	90.91	80.0 - 96.9	9.00	7.7 - 10.5	0.20	0.07 - 0.5

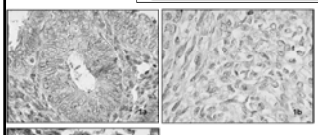
Criterion values and coordinates of the ROC curve for endometrial thickness

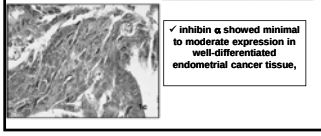
Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
>12 mm	54.55	40.6 - 68.0	92.73	82.4 - 97.9	7.50	5.8 - 9.7	0.49	0.2 - 1.1

2009, submitted

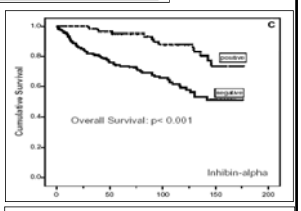
Inhibin-alpha subunit is an independent prognostic parameter in human endometrial carcinomas: Analysis of inhibin/activin-alpha, -betaA and -betaB subunits in 302 cases

Ioannis Mylonas[a,*], Silvia Worbs[a,*], Naim Shabani[a,b], Christine Kuhn[a], Susanne Kunze[a], Sandra Schulze[a], Darius Dian[a], Andrea Gingelmaier[a], Christian Schindlbeck[a], Ansgar Brüning[a], Harald Sommer[a], Udo Jeschke[a], Klaus Friese[a]





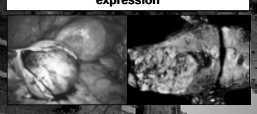
✓ inhibin α showed minimal to moderate expression in well-differentiated endometrial cancer tissue,



✓ associated with histological grading, surgical staging, lymph node status and diabetes
✓ loss of inhibin α expression resulted in a poorer survival

Activin A in endometriosis and endometrial cancer

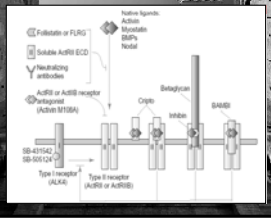
expression



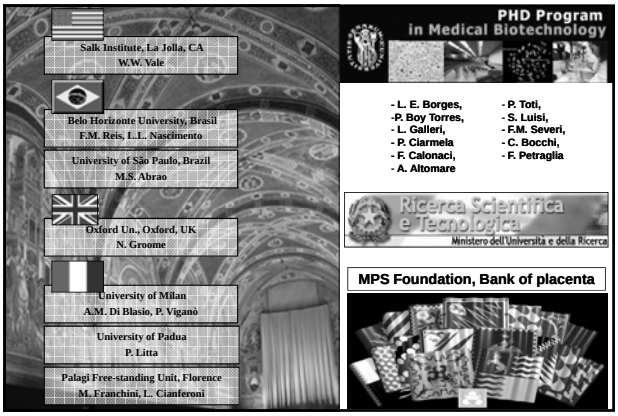
putative functions

clinical usefulness

molecular interactions



Siena, Italy


Salk Institute, La Jolla, CA
W.W. Vale
Belo Horizonte University, Brasil
F.M. Reis, L.L. Nascimento
University of São Paulo, Brazil
M.S. Abrao
Oxford Un., Oxford, UK
N. Groome
University of Milan
A.M. Di Blasio, P. Vigano
University of Padua
P. Litta
Palagi Free-standing Unit, Florence
M. Franchini, L. Cianferoni
PHD Program
in Medical Biotechnology
- L. E. Borges,
-P. Boy Torres,
- L. Galleri,
- P. Ciarmela
- F. Calonaci,
- A. Altomare
- P. Toti,
- S. Luisi,
- F.M. Severi,
- C. Bocchi,
- F. Petraglia
Ricerca Scientifica e Tecnologica
Ministero dell'Università e della Ricerca
MPS Foundation, Bank of placenta