FRI-170

Development of Novel Specific ELISAs for all Inhibins, Activins and Their Binding Proteins FST and FSTL3*

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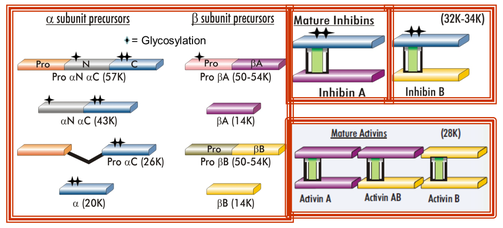
OBJECTIVE

To develop well characterized assays for Inhibin A, Inhibin B, Activin A, Activin B, Activin AB and their binding proteins Follistatin (FST) and Follistatin-like 3 (FSTL3) and to quantify their levels in biological fluids.

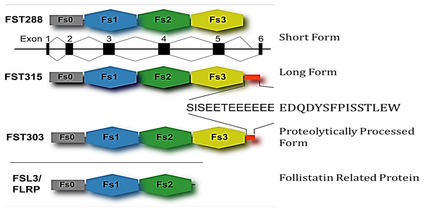
INTRODUCTION

Inhibins and activins were isolated from follicular fluid and have been classically considered as gonadal hormones produced by granulosa cells in the ovary and Sertoli cells in the testis. Inhibins are heterodimers of α-subunit joined together with either the inhibin βA or βB-subunits denoted as inhibin A or inhibin B. The β-subunits themselves can form homo- or heterodimers called Activin A, B or AB. The biological activity of activins is regulated by the monomeric activin-binding proteins FST or FSTL3. Whereas inhibins are mainly considered as reproductive hormones, activins have recently gained attention as mediators of inflammation, regulators of muscle and bone growth and also as important factors regulating cancer cell growth, differentiation and metastatic behavior. Until now, it has been difficult to determine the exact relative proportions of different forms of inhibin and activin dimers and their binding proteins in a given biological sample due to a lack of well characterized assays.

Various Molecular Forms of Inhibins and Activins



Various Molecular Forms of Follistatins



METHOD*

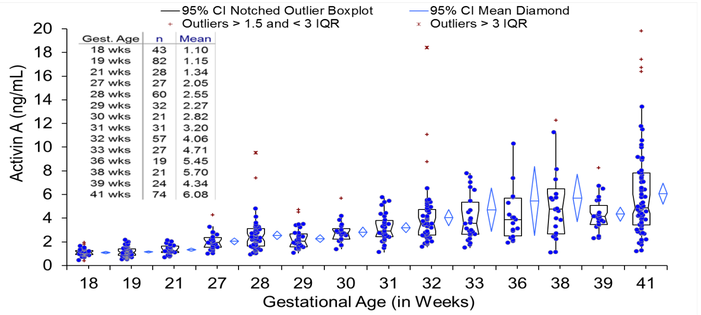
ELISA Reagents	Antibody Binding Region	Dynamic Range (pg/mL)	Analytical Sensitivity (pg/mL)	Imprecision % CV (Concentration)
Inhibin A AL-123	βA-(Capture) & α-(Detection)	10 - 1188	5.4	6.2% (101.3 pg/mL) 5.5% (344.8 pg/mL)
Inhibin B AL-107	βB-(Capture) & α-(Detection)	13 - 1390	1.6	7.4% (68.9 pg/mL) 5.6% (99.4 pg/mL)
Activin-A AL-110	βA-Subunit Mature	100 - 10,000	65.0	5.7% (673 pg/mL) 4.3% (2527 pg/mL)
Total Inhibin AL-134	αC-(Capture) & αN-(Detection)	8 – 525	1.5	4.5% (20.5 pg/mL) 3.9% (69.8 pg/mL)
Activin B AL-150	βB-Subunit Mature	12 – 1400	4.3	4.7% (51.8 pg/mL) 3.1% (225.5 pg/mL)
Activin AB AL-153	βB-(Capture) & βA-(Detection)	1 – 108	1.1	9.2% (87.4 pg/mL) 6.2% (265.2 pg/mL)
Follistatin AL-117	Fs3-(Capture) & Fs1-(Detection)	625 - 20,000	183.0	6.33% (1122 pg/mL) 3.9% (2693 pg/mL)
FSTL3 AL-152	Fs2	1000-12,000	164.0	3.0% (1400 pg/mL) 3.2% (3700 pg/mL)

RESULTS*

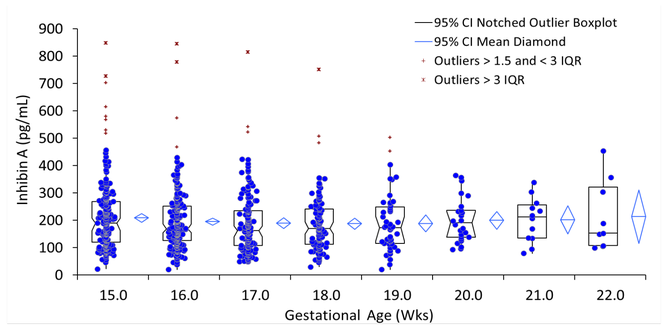
% Cross-Reactivity

ELISA Reagents	Cross-Reactants Spiked at 50 ng/mL in Calibrator Matrix						
	Inhibin A	Inhibin B	Activin A	Activin B	Activin AB	FST 315	FSTL3
Inhibin A	100%	ND	ND	ND	ND	ND	ND
Inhibin B	ND	100%	ND	0.04%	ND	ND	ND
Activin A	ND	ND	100%	ND	ND	ND	ND
Activin B	ND	0.04%	ND	100%	8.9% at 2ng/mL	ND	ND
Activin AB	ND	ND	2.28%	8.9% at 2ng/mL	100%	ND	ND
FST	ND	ND	ND	ND	ND	100%	ND
FSTL3	ND	ND	ND	ND	ND	ND	100%
ND = Non-Detectable							

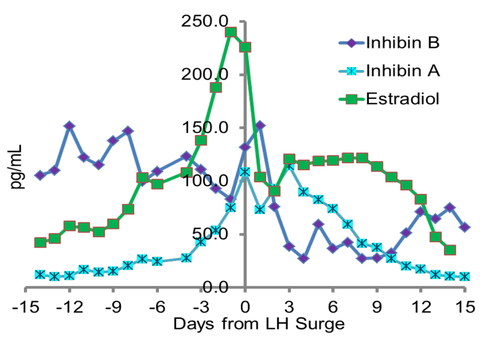
Change in Activin A Concentration With Gestational Age



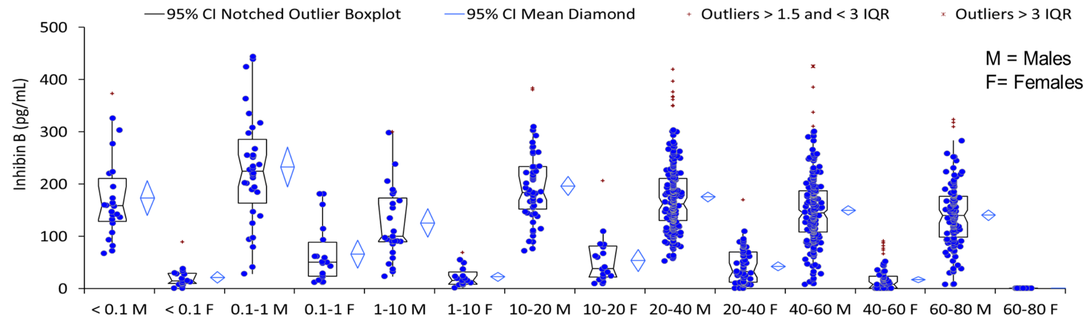
Change in Inhibin A Concentration With Gestational Age



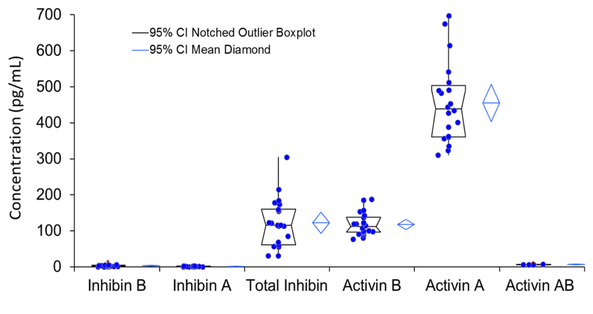
Intra-Cycle Variability of E2, Inhibin B and Inhibin A



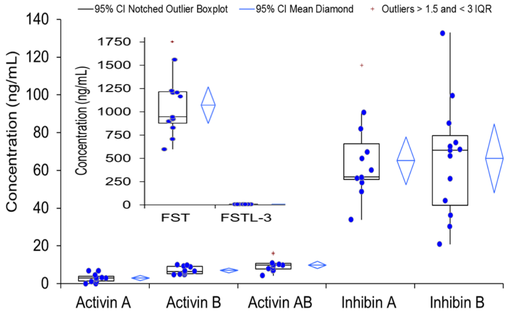
Change in Inhibin B Concentration With Age



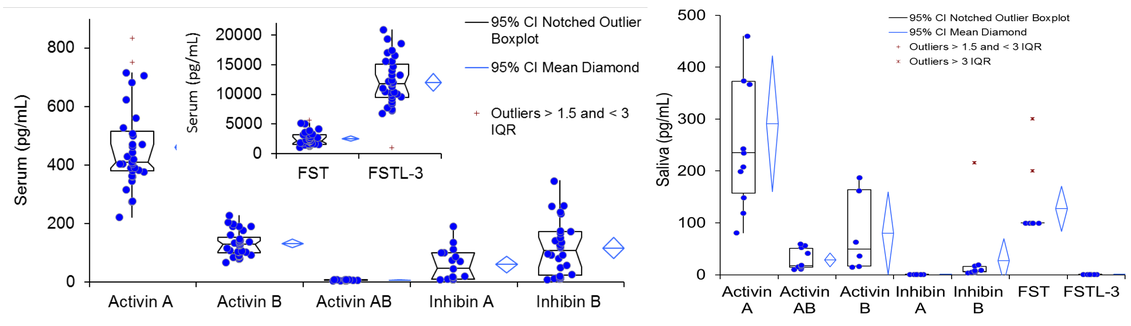
Inhibins and Activins in Azoospermia Subjects



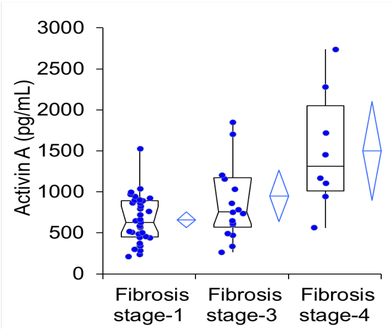
Inh., Act., FST & FSTL3 in Follicular Fluids



Inhibins, Activins, FST & FSTL-3 in Adult Males and Females Serum and Saliva



Serum Activin A in Cystic Fibrosis



CONCLUSIONS

1. Well characterized antibodies and their specificity to Inhibin βA-, Inhibin βB- and Inhibin α- subunits have now enabled accurate determinations of inhibin and activin isoforms in serum, plasma, follicular fluids, urine, saliva, tissue culture extracts using their specific ELISAs.
2. The βB- and α- specific antibodies stain human and mouse ovarian follicles. Alpha-specific antibody can now be used to stain GCT tissues and simultaneously the Total Inhibin ELISA can be used to measuring the circulating total Inhibin levels in the same subjects.
3. Multiple βB- specific antibodies are neutralizing and can be used to study activin B inhibitory activity.
4. Well characterized antibodies to Follistatin, Follistatin-like 3 protein have now enabled accurate determinations of these inhibin and activin binding proteins in serum, plasma, follicular fluids, urine , saliva, tissue extracts using their specific ELISAs. These ELISAs may help estimate the bound vs free inhibins and activins.
5. Well characterized activins, inhibins and their binding proteins FST and FSTL3 ELISAs will serve as important research tools and help quantitate these endocrine and local regulators in physiological and pathophysiological studies .

***Research Use Only**.

* Characteristics updated on Nov 24, 2020

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