



SERUM AUTOANTIBODIES DIRECTED TO THE GONADOTROPIN RELEASING HORMONE RECEPTOR ECL2 ARE DIAGNOSTIC OF PCOS

PRESENTED BY

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I have no financial or other conflict of interest to report

PCOS BACKGROUND

8-10% of women of reproductive age

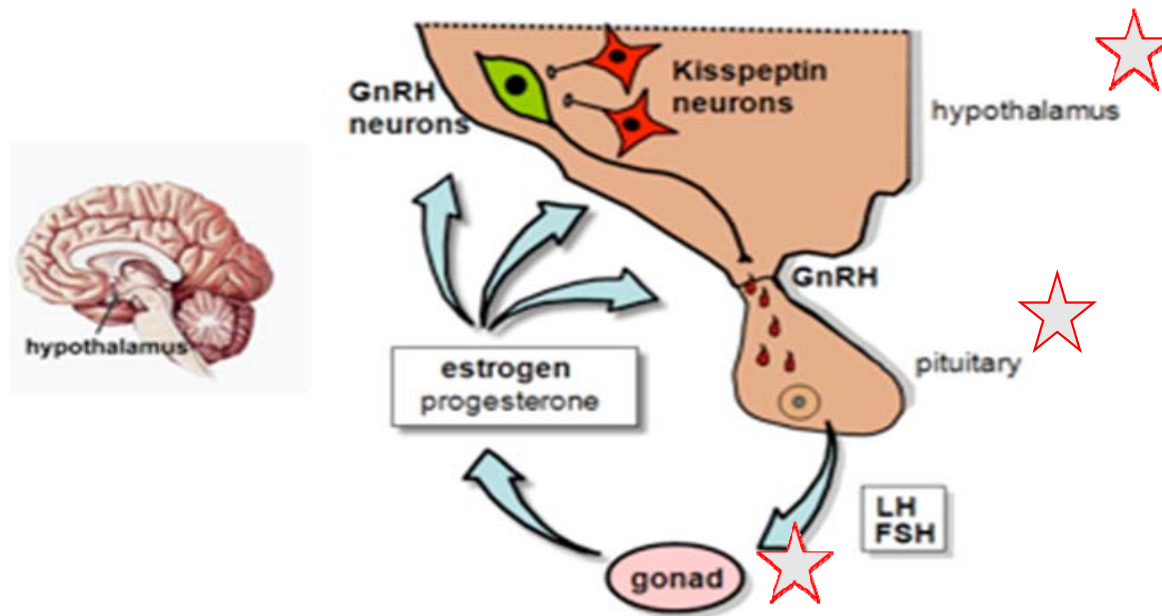
Two out of three are required to make the diagnosis:

- **Oligo and/or anovulation**
- **Clinical and/or biochemical signs of hyperandrogenism**
- **Polycystic ovaries (by ultrasound)**

Diagnosis of exclusion: No viable laboratory-based assay has been available

PCOS BACKGROUND

- In ovulatory women, GnRH is released in a pulsatile manner resulting in differential secretion of LH and FSH



- In PCOS there is a variable elevation of LH, presumed to be caused by accelerated GnRH-LH pulsatile activity

From The Physiological Society of New Zealand <http://www.physoc.org.nz>

HYPOTHESIS

- **Our lab has previously demonstrated the presence of autoantibodies against the second extracellular loop of different G proteins including alpha 1AR, Beta 1/2AR, M2/3R, AT1R etc. in selected diseases of previously unknown etiology**
- **We hypothesized that autoimmune antibodies to the second extracellular loop of the GnRH receptor would be present in PCOS and be of pathogenic and diagnostic value**

METHODS: SAMPLES

- **Obtained from the Reproductive Endocrinology and Infertility clinic and were blinded to the lab:**
 - 32 PCOS patients (based on the Rotterdam criteria)
 - 38 age and BMI matched, ovulatory infertile women
 - 7 patients with Tubal Factor (TF)
 - 12 patient with Male Factor (MFI)
 - 19 Unexplained infertile women

METHODS: ELISA

- **Day 1:**

Coated the plates with the antigen at a concentration of 10mcg/ml

- **Day 2:**

Sera diluted at 1:50 and added to the plates

- **Day 3:**

Secondary antibody added (goat antihuman IgG 1:2000)

Substrate added (para-nitrophenyl-phosphate 104)

- **The optical density (OD) values are read at 405 nm at 10 min intervals**

FIG.1

ELISA DATA

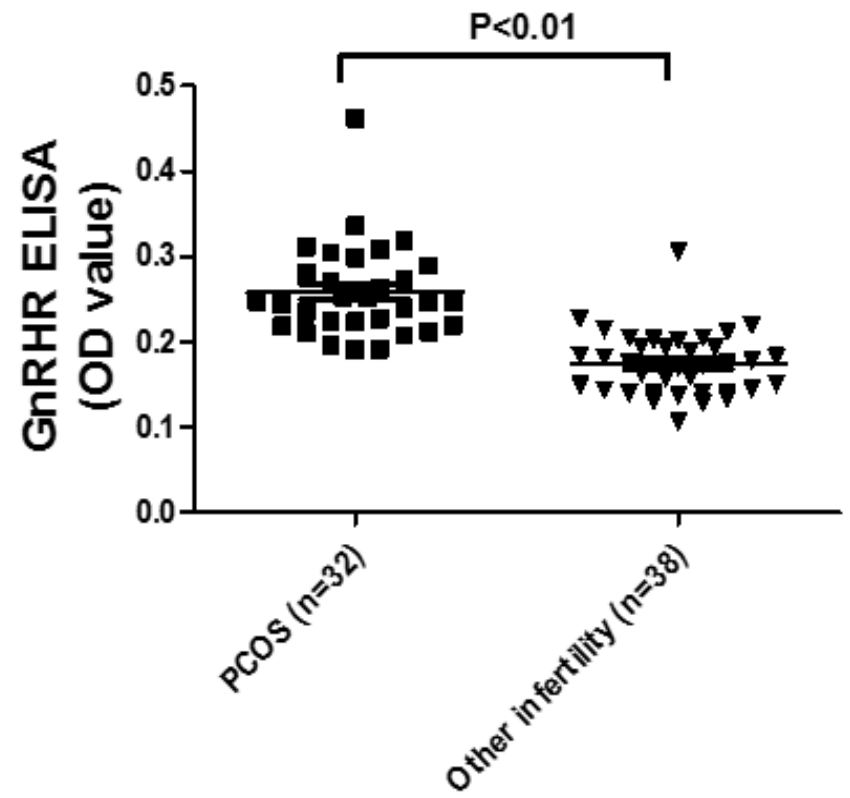
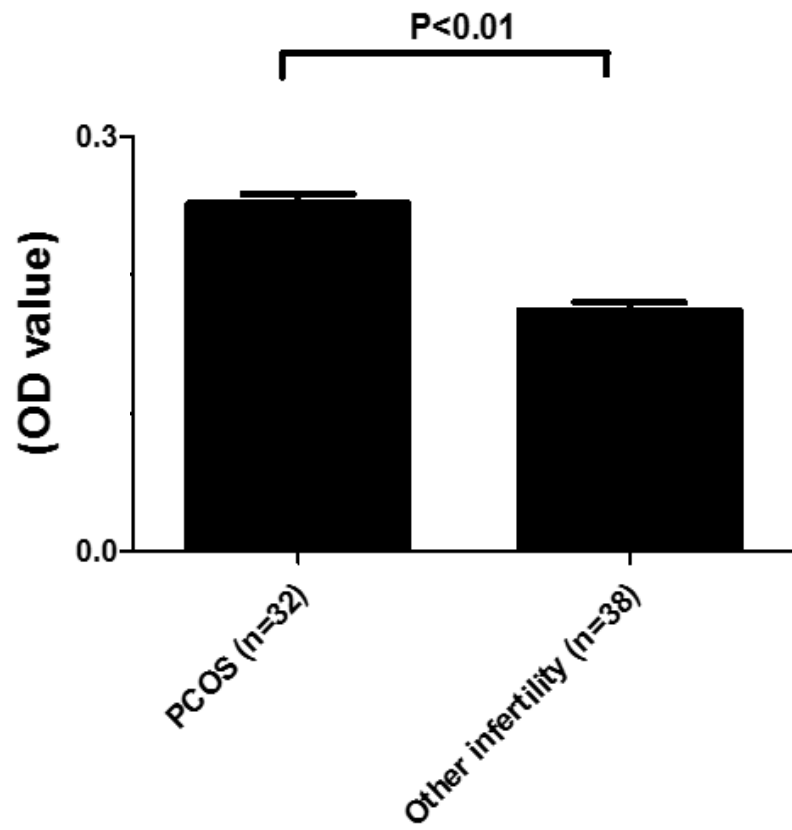


FIG. 2

PCOS vs Infertile subgroups

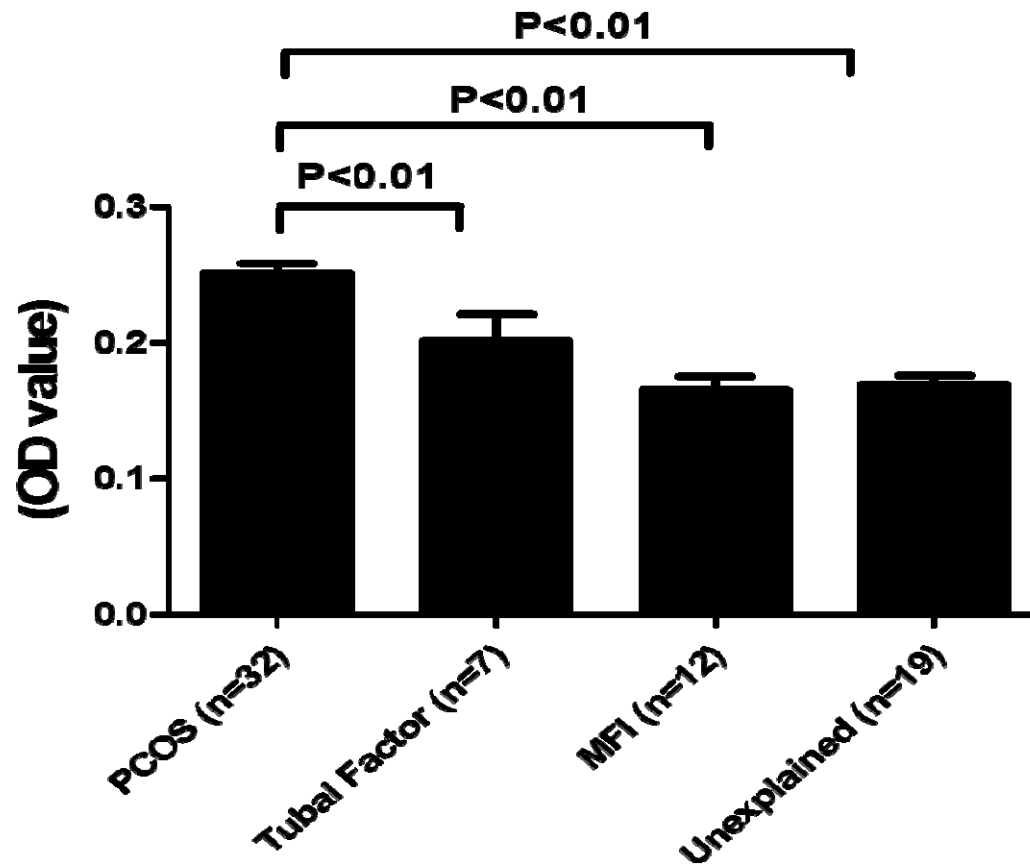
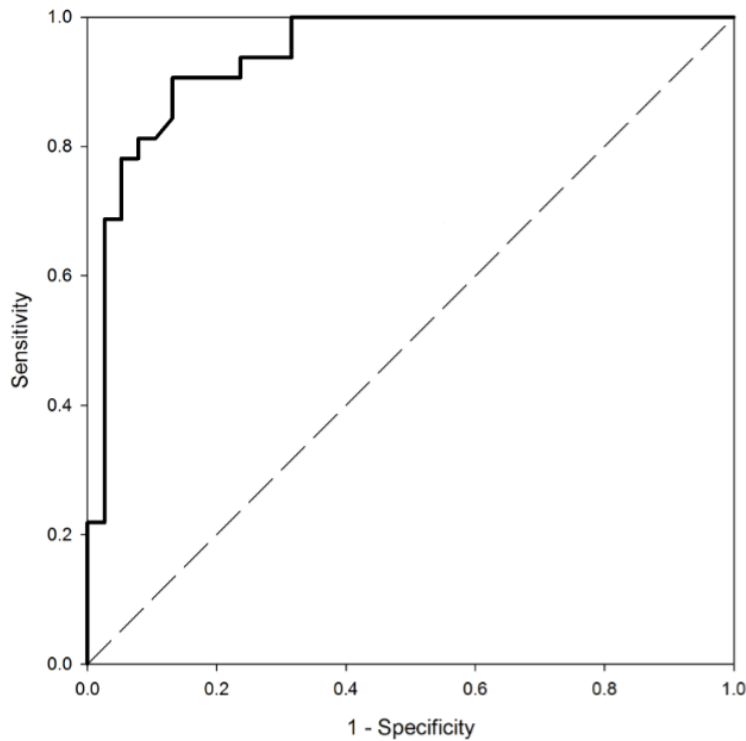


FIG.3

ROC CURVE



- **AUC=0.94 ± 0.03 p<0.0001**
 - **GnRHR Ab > 0.2062 indicates presence of PCOS**
- Sensitivity=91%**
Specificity=87%

FIG.4 Effect of PCOS IgG on GnRHR mediated Ca²⁺I in CHO cells

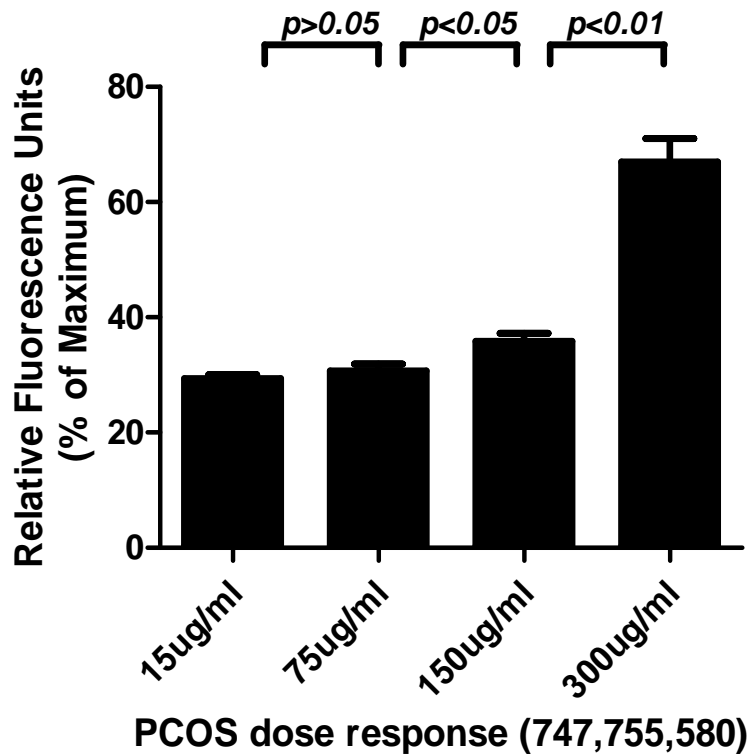
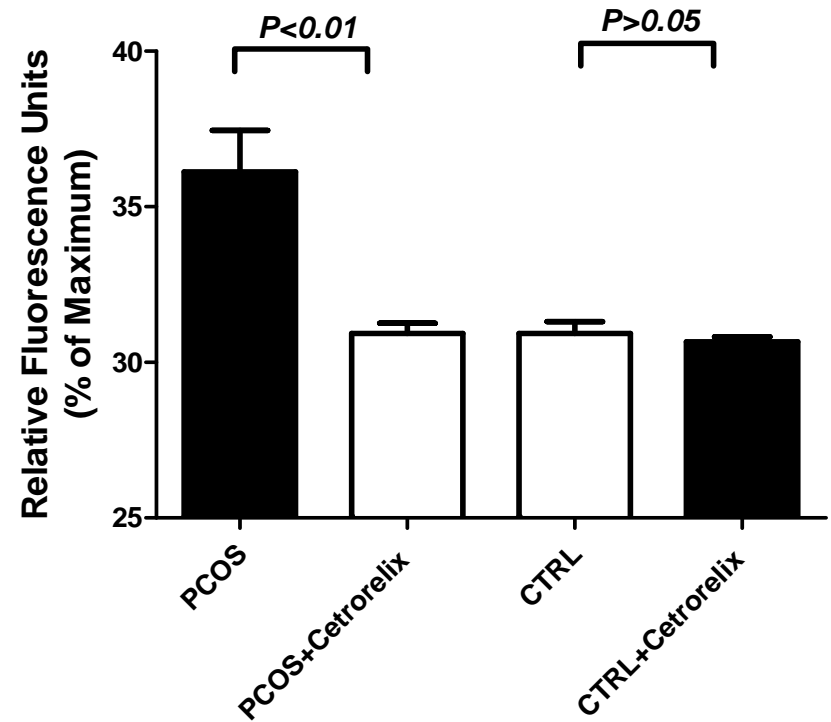


FIG.5 Cetorelix (antagonist) blocks IgG induced GnRHR activity in PCOS



CONCLUSIONS

- PCOS subjects harbor *activating* autoantibodies directed toward the GnRHR ECL2
 - ELISA positive
 - IgG specific
 - Dosage responsive activation of specific GnRHR transfected cells
 - Activity is blocked by a GnRH-specific antagonist
- It is likely these activating autoantibodies disrupt hypothalamic and pituitary LH/FSH signaling and lead to abnormal gonadal signaling in afflicted individuals
- The ELISA assay appears to fulfill the need for an inexpensive, sensitive *and* specific test to identify subjects with PCOS-AAb

FUTURE PLANS

- **Animal studies**
 - **Passive transfer of IgG to mice**
- **Decoy peptide**
 - **Block the antibodies**
- **The variable presence and activity of these autoantibodies in females and in males raises the possibility that they are active in other conditions:**
 - **Hyperandrogenism including acne**
 - **Metabolic Syndrome**
 - **Hypogonadotropic hypogonadism**
 - **Premature pubarche and puberty**
 - **Cancers including ovarian, endometrial, breast, prostate**

Thank you to the following associates

OUR LAB:

- My mentor David C. Kem, MD, George Lynn Cross Prof of Research
- Xichun Yu MD
- Hongliang Li MD, PHD
- Zachary Nuss
- Jonathan Liles

REI DEPARTMENT:

- Latasha Craig MD, Chief of the Section of Reproductive Endocrinology & Infertility
- Anna C. Reynolds MS4

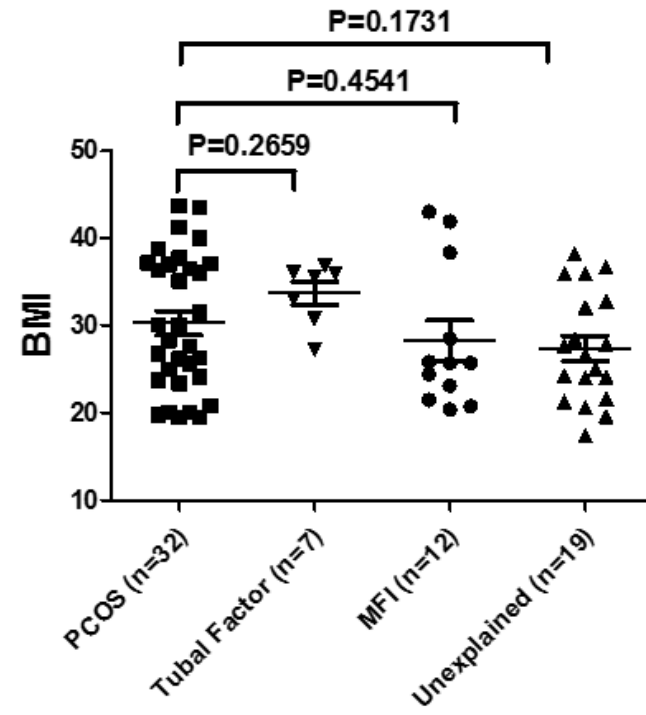
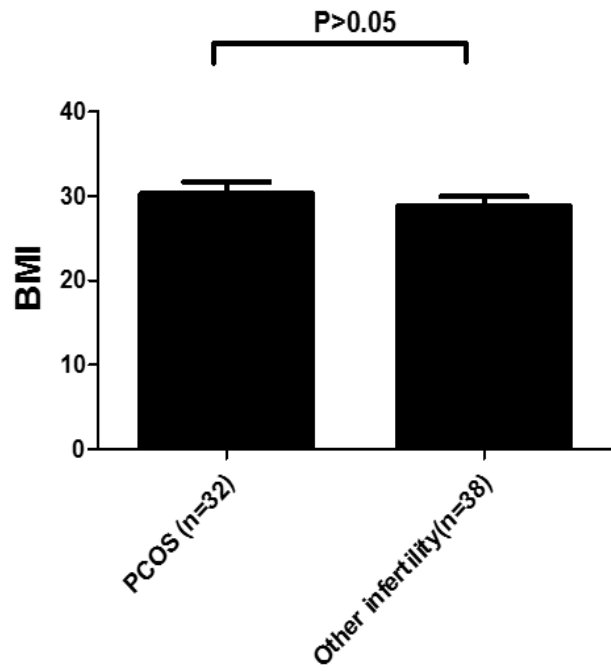
PEDIATRICS DEPARTMENT (BIOSTATISTICS)

- Christopher Aston PHD

Тяк си ти!

FIG.6

BMI



WITEBSKY'S POSTULATES FOR AUTOIMMUNE DISEASE

- **Direct evidence from transfer of pathogenic Ab or pathogenic T cells**
- **Indirect evidence based on reproduction of the autoimmune disease in experimental animals**
- **Circumstantial evidence from clinical clues**