 Printed in U.S.A.

ABSTRACTS

SCIENTIFIC PAPERS TO BE PRESENTED AT THE CONJOINT MEETING OF THE AMERICAN FERTILITY SOCIETY AND THE CANADIAN FERTILITY AND ANDROLOGY SOCIETY

OCTOBER 11–14, 1993

MONTREAL, QUEBEC, CANADA

The abstracts of this booklet are provided to the members of The American Fertility Society for their use in planning for the Annual Meeting.

The abstracts are those that will be presented in both oral and poster sessions and are published in the order of their presentation. Abstracts of special lectures and clinical seminars are not included.

The abstracts are printed exactly as submitted.

Published by:

The American Fertility Society
1209 Montgomery Highway
Birmingham, Alabama 35216

THE AMERICAN FERTILITY SOCIETY

THE SOCIETY FOR REPRODUCTIVE MEDICINE AND BIOLOGY

1209 Montgomery Highway, Birmingham, Alabama 35216-2809

Tel.: 205-978-5000 — Fax: 205-978-5005

LA SOCIÉTÉ CANADIENNE DE FERTILITÉ ET D'ANDROLOGIE

THE CANADIAN FERTILITY AND ANDROLOGY SOCIETY

2065, Alexandre de Sève, #409, Montréal, Québec, Canada — H2L 2W5

Tél.: 514-524-9009 — Fax: 514-524-2163

elicited IGF-like effects including growth stimulation. Des^{1-3} IGF-I and Long R_3 IGF-I, two analogues with reduced binding affinity for IGFBPs but unaltered binding to the Type I receptor, increased IGFBP-4 levels in CM and increased stromal cell growth. When CM from IGF-I and IGF-II treated cells were incubated with ^{125}I-IGFBP-4 for 6 hours at 37°C, ^{125}I-IGFBP-4 levels decreased 90% compared to incubation with control CM.

Conclusions: (1) IGF peptides and analogues with reduced affinity for IGFBPs have mitogenic effects on endometrial stromal cells probably mediated via the Type I IGF receptor. (2) Stromal production of IGFBP-4 is increased by peptides that bind to the Type I receptor. (3) Regulation of IGFBP-4 levels in endometrial stromal cells are regulated by IGF peptides through receptor and non-receptor mediated mechanisms. The latter may involve (a) their binding to IGFBP-4 and subsequent activation of a BP-4-specific protease or (b) their binding to a protease that shares sequence homology with IGFBP-4 with subsequent activation and proteolysis of IGFBP-4. It is postulated that IGF peptide is more available upon partial proteolysis of IGFBP-4 for interaction with the Type I IGF receptor. Supported by NIH HD25220 (LCG).

O-151

Quantitative Relationship Between Pineal Melatonin (M) Secretion, 6-Hydroxymelatonin Sulfate (6-MS) Excretion, and Lowering of Plasma Tryptophan (TRP) Levels. R. C. Zimmermann[1,2], C. J. McDougle[2], J. Olcese[3], M. Schumacher[3], G. R. Heninger[2], L. H. Price[2]. [1]Section of Reproductive Endocrinology, Mayo Clinic, Rochester, MN; [2]Department of Psychiatry, Yale University, New Haven, CT; and [3]Institute for Hormone and Fertility Research, University of Hamburg, Hamburg, Germany.

Objectives: Melatonin secretion can be abnormal in a variety of reproductive endocrine disorders. For example M secretion is reported to be increased in hypothalamic amenorrhea, and decreased in some forms of depression, which is one of the cardinal symptoms encountered in premenstrual syndrome. The 2 most pertinent mechanisms influencing pineal M production are light, and alterations in the beta-adrenergic nervous system. Melatonin is produced from TRP, an essential amino acid. The relationship between plasma TRP concentration and M production and secretion has not been well characterized. The purpose of this study was to see whether lowering of plasma TRPlevels influences pineal M secretion.

Design: The influence of active or sham TRP depletion on M secretion and 6-MS excretion was studied by using a double-blind, placebo-controlled design.

Material and Methods: Eleven subjects, 5 men and 6 women, received an amino acid mixture (AAM) with or without TRP (2.3 g) at 15:00 h, which induces synthesis of labile protein stores. Blood samples were collected hourly throughout the night, and urine was collected in 8-hour intervals starting at 07:00 h of the study day. Melatonin and 6-MS concentrations were measured with radioimmunoassay kits, whereas total and free TRP were assayed by high-performance liquid chromatography. Paired t-tests were used to compare the active with the sham group. Linear regression was used to correlate the time point of maximal TRP depletion (+5 h) with M secretion expressed as area under the curve (AUC; 16:00 h–08:00 h) and 6-MS excretion.

Results: Plasma M concentration (AUC: 455 ± 86 pgxh/ml) and nocturnal 6-MS excretion (23:00 h–07:00 h: 5.38 ± 1.19 μg/8 h) were consistently decreased when compared to a sham drink containing the same AAM plus 2.3 g of TRP (M: 738 ± 152 pgxh/ml, $p < 0.025$; 6-MS: 8.38 ± 1.81 μg/8 h, $p < 0.025$). In addition, the amount of M secreted during the depletion experiment expressed as AUC and nocturnal 6-MS excretion correlated significantly with the lowest value of free but not total TRP levels, which was encountered 5 h after the intake of the drink (correlation M AUC with free TRP level: $r = 0.77$, $p = 0.05$; correlation of nocturnal 6-MS with free TRP concentration: $r = 0.83$, $p < 0.02$). No significant correlation was encountered between change in TRP levels during the control experiment and M secretion or nocturnal 6-MS excretion.

Conclusion: These findings demonstrate that M secretion and 6-MS excretion are dependent on plasma TRP concentration. Therefore, we propose that in addition to light and alteration of the noradrenergic nervous system, plasma TRP levels can influence pineal M secretion profoundly. Consequently, some of the abnormalities seen in M secretion in reproductive endocrine and depressive disorders might be caused by alterations in plasma TRP levels. Studies are in progress to test this hypothesis.

O-152

Ovarian Intrabursal Administration of Recombinant TGFβ1 Inhibits Follicle Rupture in Gonadotropin-Primed Mouse. S. Juneja, R. S. Williams, N. Chegini and [1]G. Ksander, Department of Obstetrics and Gynecology, College of Medicine, University of Florida, Gainesville, FL and [1]Celtrix Pharmaceuticals Inc, Santa Clara, CA.

Objective: The expression of TGFβs mRNA and production of TGFβs proteins have been demonstrated in the ovarian tissue of several species including human. TGFβs have been shown to influence ovum maturation and theca and granulosa cell proliferation and steroidogenesis. The aim of this study was to find out the effect of intrabursal treatment of TGFβ1 on PMSG/hCG-primed ovulation in mouse.

Design: Ovulation in PMSG/hCG-primed mice treated with rTGFβ1 was compared to control mice treated with vehicle.

Materials and Methods: Female mice ($B_6D_2F_1$; age, 4–6

wks) were primed with PMSG followed 48 hr later by hCG. Recombinant transforming growth factor β1 (rTGFβ1, 100 ng) was injected into the bursa of each ovary 1 hr before hCG injection. Ovaries from control mice received 1 μl of vehicle containing 1 mg BSA/ml saline. Cumulus-enclosed ova were retrieved from the mice at 14 hr post-hCG. Ovulation rate was determined by the number of ova recovered from mice. Ovaries from each mice were processed for histological studies.

Results: The number of ova recovered from rTGFβ1-treated mice was significantly lower than that from vehicle-treated mice (7.30 ± 1.14 vs. 28.81 ± 2.75, $p < 0.0001$). The data was analysed by Student's t-test. Morphological observations on H & E staining of ovaries indicated significantly higher number of unruptured preovulatory follicles in rTGFβ1-treated mice, whereas, control mice showed higher number of corpora lutea.

Conclusion: This study shows that intrabursal treatment of mice with rTGFβ1 inhibits ovulation. This suggests that TGFβ1 may play a role in the regulation of ovulation.

O-153

Hormonal Regulation of Insulin-like Growth Factor Binding Protein-4 Gene Expression in Cultured Rat Granulosa Cells. D. S. Choi, L. T. Putowski, R. M. Rohan, and E. Y. Adashi. Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Maryland School of Medicine, Baltimore, MD.

Objective: Insulin-like growth factor binding protein-4 (IGFBP-4) is expressed in rat granulosa cells where it may be involved in the processes of follicular atresia and IGF action. Levels of ovarian IGFBP-4 transcripts are regulated in vivo by FSH and estrogen treatment. To further characterize the hormonal regulation of this IGFBP in a more defined system, IGFBP-4 expression was studied in cultures of rat granulosa cells.

Design: The amount of IGFBP-4 mRNA was measured by Northern blot hybridization after extraction of total RNA from rat granulosa cell cultures before and after various hormonal treatments.

Materials and Methods: 2×10^6 viable granulosa cells from diethylstilbestrol (DES)-primed (s.c. silastic implant at 21 d.o., ovaries collected at 25 d.o.) intact rats were obtained by follicular puncture and cultured in 12 × 75 mm polypropylene tubes with 2 ml McCoy's 5A serum free media with glutamine. Cultures were treated with FSH, IGF-I, Epidermal Growth Factor (EGF) or Activin-A for up to 72 h. RNA was extracted from individual tubes by the acid phenol method (RNAZOL-B), and electrophoresed in 1% agarose gels after glyoxal denaturation. An aliquot of each sample was run on a parallel gel and stained with ethidium bromide to evaluate RNA integrity and quantity. The amount of IGFBP-4 mRNA was measured by northern blot hybridization to a ^{32}P-labelled portion of the rat IGFBP-4 cDNA. Hybridization signal was quantified in a BETASCOPE 603. The relative amount of IGFBP-4 transcripts was normalized to the constitutively-expressed probe CHOB (encoding ribosomal protein S2).

Results: In primary cultures of granulosa cells from DES-primed rats, IGFBP-4 transcript levels declined dramatically by 6 h but then increased by 48–72 h to 1.3-fold of initial values. FSH (100 ng/ml) had a tendency to increase IGFBP-4 transcripts after 48 h in cultures but decreased IGFBP-4 transcripts to 70% of untreated controls ($P < 0.05$) by 72 h. The effect of FSH after 72 h was dose-dependent (3–100 ng/ml). IGF-I (100 ng/ml) did not alter the amount of IGFBP-4 transcripts after 6 h, 24 h, or 48 h but did decrease them to 74% of control ($p < 0.05$) after 72 h. Addition of EGF (10 ng/ml) to either FSH or IGF-I treated cultures resulted in a further decrease of IGFBP-4 transcripts after 72 h. Treatment with Activin-A (50 ng/ml) alone lowered the amount of IGFBP-4 transcripts after 24 h, 48 h, and 72 h of culture.

Conclusion: Rat granulosa cells in culture maintain a high basal level of IGFBP-4 transcript which can be altered, albeit modestly, by FSH, IGF-I, EGF, or Activin-A. These factors may enhance granulosa cell health by decreasing the level of the antigonadotropic IGFBP-4.

O-154

Effects of Atrial Natriuretic Peptide on Rat Ovarian Granulosa Cell Steroidogenesis In Vitro. [1]K. M. Johnson, [2]F. M. Hughes, Jr., [1]R. S. Mather, [1]H. O. Williamson, [2]W. C. Gorospe, [1]Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, South Carolina; [2]Division of Molecular and Cellular Endocrinology, Department of Anatomy and Cell Biology, Medical University of South Carolina, Charleston, SC.

Objectives: Evidence now suggests not only the existence of an intrinsic ovarian renin-angiotensin system (RAS), but that this system may play an important modulatory role in reproductive processes. Atrial natriuretic peptide (ANP) is an important counter-regulatory hormone to the RAS in several organ systems. Yet, studies to date have not explored the potential role of ANP in preovulatory follicular steroidogenesis. The objective of this study was to determine the effects of ANP on estrogen, progesterone, and 20 α-hydroxypregn-4-en-3-one (20 α-OH-P) production by both undifferentiated and differentiated rat ovarian granulosa cells in vitro.

Design: Undifferentiated and differentiated rat ovarian granulosa cells treated with increasing doses of rat ANP compared to two concurrent control groups.

Materials and Methods: Granulosa cells were obtained from ovaries by a non-enzymatic needle puncture technique. Isolated cells were washed three times by centrifugation (250 × g, 10 minutes) in sterile McCoy's 5a me-