FSH increases the different LH- and hCG-dependent intracellular signalling and the downstream life/death signals in vitro

Livio Casarini 1,2, Laura Riccetti1, Francesco De Pascale1, Alessia Nicoli3, Simonetta Tagliavini4, Tommaso Trenti5, Giovanni Battista La Sala6,7,5, Manuela Simon2,6,2

1. Unit of Endocrinology, Dept. Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy. 2. Center for the Genomic Research, University of Modena and Reggio Emilia, Modena, Italy. 3. Unit of Obstetrics and Gynecology, IRCCS-Arcispedale Santa Maria Nuova, Reggio Emilia, Italy. 4. Dept. of Clinical Pathology, Azienda USL Modena, Italy. 5. Dept. of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, Reggio Emilia, Italy. 6. Dept. of Medicine, Endocrinology, Metabolism and Geriatrics, Azienda USL Modena, Italy

Introduction
Luteinizing hormone (LH) and chorionadotropin (hCG) are glycoprotein hormones regulating ovarian function and pregnancy. They were equivalently used in assisted reproduction techniques (ART) due to their binding to a common receptor (LH|CR). However, differences between LH and hCG were demonstrated at molecular and physiological level [1]. Our previous study revealed that LHCR mediates hCG-dependent steroidogenesis-related signalling and LH-dependent proliferative and anti-apoptotic events in human granulosa cells [2].

Aim
The aim of this study is to evaluate how follicle-stimulating hormone (FSH) co-treatment, in the ART therapeutic doserange, affects the different LH- and hCG-specific responses in vitro.

Study design
We evaluated phospho-CREB, ERK1/2 and AKT activation by Western blotting, gene expression by real-time PCR, cAMP, progesterone and estradiol production by ELISA, and cell viability by MTT assay in human granulosa-lutein cells (hNCGL). LH and hCG dose-response experiments (0.1 pM-1.0 nM range) were performed, in the presence of 10 nM FSH.

Results
In the presence of FSH, hCG biopotency is higher than that of LH, and is about 5-fold increased, in terms of cAMP activation (fig.1), compared to previous data obtained in the absence of FSH [2]. Moreover, different LH and hCG dose-response curves were observed, in terms of 50% effective doses (EC50s), hill-slopes and maximal levels (Mann-Whitney's U-test; p<0.05; n=6), suggesting hormone-specific receptor cooperativity and biopotency. In the presence of FSH, the range of effective hCG doses increased, in terms of CREB phosphorylation (fig.2). FSH increased the LH-dependent ERK1/2 and AKT phosphorylation, the expression of the X-linked inhibitor of apoptosis (XIAP) gene (fig.3), and the cell viability (Mann-Whitney's U-test; p<0.05; n=4), resulting in anti-apoptotic effects (fig.4). Consistently with the effect on cAMP and CREB activation, steroid production increased under hCG and FSH co-treatment (figs.5, 6).

Discussion
FSH potentiates the LH-dependent anti-apoptotic and the hCG steroidogenic (and pro-apoptotic) potential in vitro. The different modulatory activity of FSH on LH and hCG action in vitro reflects their different physiological functions, consisting in proliferative effects exerted by LH during the follicular phase and before trophoblast development, and the high steroidogenic potential of hCG requested to sustain pregnancy. These findings were recently published [3].

References