A new model to study the effects of gonadotropins on an "in vitro" prepubertal artificial porcine mini-testis

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At present, there is no reliable experimental model "in vitro" to analyze the complex interactions between gonadotropins on the pre-pubertal Sertoli cells (SC) and Leydig cells (LD). Considering that, in the pre-pubertal period, only the anti-mullerian hormone (AMH) is upregulated by FSH and down-regulated by androgens [1-2], AMH could be considered a potential marker of pre-pubertal testis function. The aim of our work was to study the effects of FSH, LH and HCG on an *in-vitro* model of "mini-testis". SC and LD, obtained from 15-20 days old neonatal pigs, were isolated and evaluated in terms of purity by AMH (unique pre-pubertal SCs marker), INSL3 (LD marker), ASMI (peritubular cells marker) and PGP9.5 (gonocytes and spermatogonial cells marker). Finally, purified SC and LD were co-cultured to obtain the "mini-testis" and were stimulated with gonadotropins. We have then evaluated: a) AMH, inhibin B and testosterone levels released in the culture medium (by ELISA), both in basal conditions and after stimulations; b) analysis of the follicle-stimulating hormone receptor (FSHR), MAPkinasi (Erk1/2, AKT) by Real Time PCR. We show an increase in inhibin B levels after FSH and FSH/LH stimulation and a selectively increase in testosterone production after LH treatment. AMH secretion was downregulated by FSH treatment. These data seem to preliminarily suggest that ERK1/ ERK2 expression was up-regulated by FSH and FSH/LH stimulation while FSHreceptor expression was down-regulated by FSH and increased by FSH/LH treatment; AKT was up-regulated in all conditions. The proposed model, by creating an artificial mini- testis, could help better understanding the complex and still partially unknown interactions between human gonadotropins, SC and LD possibly creating a novel background to shed light inside a future therapy of male infertility.

References

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Keywords

Gonadotropins; Sertoli cells; Leydig cells.