

Bioactivity of the HP urinary FSH (Fostimon®) versus the recombinant FSH (Gonal-FÆ) evaluated by the follicle bioassay

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Objective: FSH molecules show biochemical heterogeneity, reflected in the occurrence of isoforms exhibiting differences in physico-chemical and pharmacological properties. Dependent on their source, gonadotrophin preparations used in assisted reproductive technologies can differ regarding their clinical efficacy. Gonadotrophins target the ovary supporting and regulating follicle development, and determine steroid hormone production and oocyte maturation. In this study the bioactivity of a HP urinary FSH was assessed in comparison with a recombinant FSH preparation. Design: The mouse follicle bioassay, a highly standardized in vitro model mimicking ovarian function, was used to investigate the impact of the gonadotrophins on folliculogenesis, steroidogenesis and oogenesis in a dose range covering sub- to supra-physiological conditions. Materials and Methods: Preantral ovarian follicles from pre-pubertal mice were individually cultured in 96-well plates during 12 days up to the pre-ovulatory stage. Meiosis resumed upon hCG stimulation, resulting in mature metaphase II oocytes on day 13. Follicles were exposed continuously to 1, 5, 10 and 100 IU/L of HP urinary FSH (Fostimon® = F) or recombinant FSH (Gonal F® = G). Per dose 60 follicles were cultured in 6 test replicates in 3 independent repeats. Follicle development was scored morphologically (follicle survival, differentiation stage, mucification of the cumulus-oocyte complex) and the steroid hormone profile was monitored in the medium throughout culture. Oocyte yield and nuclear maturation were analysed upon in vitro ovulation. Endpoint parameters are presented as mean values \pm SD ($P < 0.05$ was considered as significantly different). Results: Follicle development, oocyte yield and maturation were similar for Fostimon® and Gonal F®. Folliculogenesis and oogenesis were not supported by the 1 IU/L dose, as follicles did not reach the antral stage and few oocytes extruded a polar body. When exposed to 5 IU/L FSH, $98 \pm 4\%$ (F) and $92 \pm 10\%$ (G) of the follicles survived, $83 \pm 16\%$ (F) and $67 \pm 14\%$ (G) developed an antral cavity and $95 \pm 8\%$ (F) and $91 \pm 10\%$ (G) responded to the HCG stimulus, whereas oocyte maturation rates were $94 \pm 6\%$ (F) and $84 \pm 25\%$ (G). Optimal follicle development was observed at 10 and 100 IU/L dose levels: $> 90\%$ of the follicles survived and formed an antrum; $> 90\%$ of the cumulus-oocyte complexes mucified in response to ovulatory stimulation; and $\geq 90\%$ of the oocytes extruded a polar body. The hormone secretion profile was not significantly different for both preparations. Testosterone was measurable in all the replicates at the 100 IU/L dose level. Estradiol production increased dose-dependently, while progesterone secretion remained at basal levels throughout the follicular phase for 5 and 10 IU/L dose levels but increased in function of time at the 100 IU/L dose level. Conclusion: We demonstrated that equal doses of HP urinary Fostimon® and recombinant Gonal F® induced similar responses in the follicle bioassay. Folliculogenesis, oogenesis and steroidogenesis characteristics were comparable. The quantitative, multi-parametric test approach seemed highly effective to investigate gonadotrophin bioactivity at the target organ in a broad dose range, as the system allows the separate evaluation of gonadotrophin effects on different physiological processes in the ovary.