

72. STEREOLOGICAL ASSESSMENT OF GONADOTROPIN EFFECTS ON OVARIAN FOLLICLE DEVELOPMENT IN MOUSE OVARY

Yuan Wang, Jenny A. Spaliviero, Charles M. Allan, David J. Handelsman and Peter J. Illingworth

Department of Reproductive Medicine, Westmead Hospital, University of Sydney, Westmead, NSW 2145, Australia; ANZAC Research Institute, Sydney, NSW 2139, Australia

Although ovulation requires concerted action of gonadotrophins, the specific effects of FSH on ovarian follicle populations remains difficult to isolate from LH effects in vivo. We therefore aimed to study the effects of FSH and LH in the gonadotrophin-deficient *hpg* mouse using unbiased stereology to study gonadotropin effect on ovary. Female *hpg* mice were treated for 20 days (days 21-41 days of age) with daily i.p. injections of hFSH (10 IU/day) or hCG (1 IU/day) alone or combined. Ovaries were fixed in paraformaldehyde and embedded in glycol methacrylate; thick sections (25 μ m), stained with PAS, were subjected to stereological counting of follicle number (primordial, primary, secondary, antral, preovulatory) using the oocyte nucleolus as index particle. Sections and frames for counting were selected by random uniform sampling and the particle count obtained through the middle 18 μ m of the section (CAST-grid system, Olympus). Follicle number per ovary was obtained by combining particle density by optical disector with ovarian volume by Cavalieri's principle. The number of primordial follicles was higher in untreated *hpg* compared with phenotypically normal littermates (2771 ± 415 v. 1800 ± 216) and was reduced by FSH treatment alone (1793 ± 186) or with hCG (1147 ± 273) but not by hCG alone (2715 ± 600). By contrast, primary follicle numbers were increased by hCG treatment alone (994 ± 62), or with FSH (889.5 ± 104.0) compared with untreated (325 ± 52), FSH-treated *hpg* mice (426 ± 79) and normals (403 ± 27.09). Antral follicles were absent in the untreated *hpg* and hCG-alone-treated animals, but appeared following treatment with FSH alone (101 ± 20) or with hCG (187 ± 34) and in normals (116 ± 21.42). Preovulatory follicles were only seen in the wild type (5 ± 3.58) and combined treatment groups (10 ± 6.86). We conclude that, using unbiased quantitative methods, gonadotrophins have distinct effects on early follicle recruitment with prolonged FSH reducing primordial follicle number, whereas hCG had no effect, while the reverse effects were evident in primary follicle numbers. Nevertheless, antral, pre-ovulatory and ovulated require joint action of both LH and FSH. Further studies are required to investigate the mechanisms involved. Supported by NHMRC.