Refined Porcine Follicle Stimulating Hormone Promotes the Responsiveness of Rabbits to Multiple-Ovulation Treatment

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Abstract: We investigated whether refined follicle stimulating hormone (FSH) with only a little contaminating LH can promote the responsiveness of rabbits to multiple-ovulation treatment. One group of female rabbits was stimulated with refined porcine FSH (pFSH), an FSH source with low LH activity, and another group was treated with pFSH. The mean number of eggs recovered from donors stimulated with refined pFSH (27 ± 3) was significantly greater (P<0.05) than that with pFSH (20 ± 2). Furthermore, the mean number of remaining follicles of donors stimulated with refined pFSH (19 ± 4) was significantly greater (P<0.05) than that with pFSH (12 ± 1). To decrease the number of remaining follicles in donors treated with refined pFSH, the dose of human chorionic gonadotropin (hCG) was increased from 75 to 150. However, there were no differences in the numbers of eggs and remaining follicles. The results of the present study suggest that refined pFSH with little contaminating LH promotes the responsiveness of rabbits to multiple-ovulation treatment compared with pFSH.

Key words: follicle-stimulating hormone, multiple-ovulation

The extension of transgenic technology to rabbits has led to new insights into lipoprotein metabolism [6, 11] and the impact of transgene expression on atherosclerosis [11]. In mice [2] or rats [4], the substantial loss of viability of manipulated embryos during pregnancy, following their transfer to the oviducts of recipients, leads to low production efficiency in the object animals. Thus, a large quantity of eggs is required for DNA microinjection. A multiple ovulation of rabbits with multiple injections of FSH is commonly used to increase the number of embryos. However, this multiple injection method is time and labor consuming, and also gives strong stress to donor rabbits. Recently, it has been reported that a single injection of FSH dissolved in polyvinylpyrrolidone (PVP) solution given to rabbits induced multiple ovulation and was a practical alternative to 6 times injection of FSH dissolved in saline [10]. However, the yield and quality of embryos raised after multiple ovulation are variable and unpredictable owing to variations in ovarian response.

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Variability in the FSH:LH ratio of gonadotropin preparation is considered to be a factor causing variability of multiple ovulation responses [5, 13]. To decrease LH contamination in FSH preparations isolated from the pituitary gland, refined pig FSH with only a little containing LH, or recombinant bovine FSH without LH has been used for multiple ovulation of cattle. However, multiple ovulation of cattle with these FSH preparations gave varied responses ranging from a complete lack of ovulation [9] to a normal number of embryos [5, 18]. On the other hand, recombinant human FSH without LH has been shown to be more effective for stimulating multiple follicular development than urinary-derived FSH [8, 12, 14] and is used widely in treatments of human infertility. In rabbits, there is no information on the effects of FSH with only a little LH, or recombinant FSH, on the responsiveness of rabbits to multiple-ovulation treatments.

To know the effects of refined porcine FSH (pFSH) on the responsiveness of rabbits to multiple ovulation treatment, we examined the numbers of recovered eggs and remaining follicles at 18 h after human chronic gonadotropin (hCG) administration and mating.

Multiple ovulation: Refined pFSH (Antrin R, Denka Pharmaceuticals Inc., Kanagawa, Japan) and pFSH (Antrin 20, Denka) were used for multiple-ovulation treatment. Refined FSH has decreased LH contamination and contains 8.8 AU FSH and 0.0013 IU LH per 1 mg protein (Hirako et al. personal communication). Experiments were performed in accordance with the guidelines of Kitayama Labes Co., Ltd. for the care and use of laboratory animals. Specific-pathogen-free/virus antibody-free Kbs:JW rabbits (3.0 to 4.0 kg) were housed in an environmentally controlled room with a 12-h dark: 12-h light cycle, at a temperature of 23 ± 3°C, and humidity of 60 ± 15%. They were given free access to pelleted rabbit chow (Oriental Yeast Co., Shizuoka, Japan) and filtered water. The multiple ovulation of female rabbits was induced by a single subcutaneous injection of 3-AU of either refined or crude pFSH dissolved in 10% PVP (168-03115, K-90, Wako Chemical Industries, Japan). The females were then intravenously given 75 or 150 IU hCG (Teikoku Zouki, Japan) at 72 h after the injection of FSH and were mated with male Kbs:JW rabbits. Gonadotropin-releasing hormone (GnRH, 1 µg Fertilelin acetate: Denka) was administered 1–1.5 h before the hCG injection [17].

Egg recovery: The oviduct ampullae were flushed with TCM 199 supplemented with 10% fetal bovine serum (FBS, Equitech Bio, TX, USA) 16 to 18 h after hCG injection and the recovered eggs were kept in TCM 199 supplemented with 10% FBS at 38.5°C in 5% CO2 in air. The number of recovered eggs was counted under a microscope. The number of remaining follicles was assessed at 18 h after hCG administration.

Statistical analysis: The data are shown as mean percent ± SEM. The numbers of recovered eggs and remaining follicles were compared using the unpaired t test. A P value less than 0.05 was considered to be significant.

The mean number of eggs recovered from donors stimulated with refined pFSH (27.1 ± 2.5, n: 30) was significantly greater (P<0.05) than that with pFSH (20.1 ± 1.8, n: 50, Fig. 1). Furthermore, the mean number of remaining follicles of donors stimulated with refined pFSH (19.2 ± 4.1, n: 18) was significantly greater (P<0.05) than that with pFSH (12.4 ± 1.4, n: 31, Fig. 1). To promote the ovulation, the dose of hCG was increased, however, there were no differences in the numbers of recovered eggs (35.8 ± 9.8 (75 IU, n: 4) vs 21.3 ± 5.9 (150 IU, n: 4), Fig. 2) and the numbers of remaining follicles (14.5 ± 4.0 (75 IU, n: 4) vs. 23.8 ± 11.7 (150 IU, n: 4)).

The variability in the FSH:LH ratio of gonadotropin preparation is considered to be a factor causing vari-
ability of multiple ovulation responses [5, 13]. As usually available FSH preparations are isolated from the pituitary gland and are crude preparations, prevention of such variations in the FSH:LH ratio is difficult. To circumvent this problem, we used refined pig FSH with only a little contaminating LH for multiple ovulation of rabbits. It has been reported that refined pFSH contains less than 1/15 of the LH contamination of crude preparations [1]. In this study, refined FSH promoted the responsiveness of rabbits to multiple ovulation treatment compared with pFSH. It has been shown that the high levels of LH could result in luteinization of follicles [3, 13], desensitization of LH receptors [15], or premature ovulation [3, 7, 15]. Thus, refined FSH with only a little contaminating LH may promote the responsiveness of rabbits to multiple ovulation treatment.

In cattle, donors treated with FSH without contaminating LH lacked LH secretions during final follicle maturation, and final follicle maturation was impaired in these donors [16]. The increment of remaining follicles that were observed in this study might be caused by immaturity of follicles due to reduced contamination of LH as suggested by cattle [16].

The results obtained from this study indicate that refined FSH with only a little contaminating LH can promote the responsiveness of rabbits to multiple ovulation treatment.

References