
Individual murine follicles were cultured in the presence of human recombinant Follicle Stimulating Hormone (rFSH), human FSH (FSH) or in the absence of gonadotrophins, to determine the relative contribution of FSH and Luteinizing Hormone (LH) to follicular development. The culture system permits the in vitro growth of primary mouse follicles to the Graafian stage in the normal physiological time course. These follicles can be ovulated in response to the presence of LH and fertilized. Follicles were microdissected and cultured in 25 µl droplets of a Minimal Essential Medium under mineral oil. Medium was supplemented with human transferrin and 5% serum obtained from either Hypogonadal (hpg) or hypophysectomized mice. Follicles grown in medium supplemented with 1 IU/ml FSH (NIH: biological potency = 570 IU/ml) were compared to those grown in the absence of FSH and to those grown in medium supplemented with 1, 3 or 5 IU/ml FSH (Serono: biological potency = 7596 IU/ml). Follicles were transferred to fresh medium daily, and samples from spent medium were analysed for Estradiol (E) using a Serono assay kit. Follicles grown in the absence of FSH developed little beyond the early antral stage while those grown in FSH generally developed to Graafian stage over 5 days of culture. rFSH was less successful than FSH at supporting follicular development, particularly at the lower doses, with fewer follicles reaching the Graafian stage, although most developed at least to the early antral cavities. Medium obtained from follicles grown in the absence of gonadotrophins contained negligible or undetectable (<4 pg/day) levels of E on Day 5. Day 5 rFSH follicles produced measurable E levels, if significantly lower than those of the control, FSH group (290, cf 891 pg/day E <0.05).

Results suggest that both FSH and LH are necessary for full antral development, and that pure, recombinant FSH can stimulate reduced levels of E from ovarian follicles. The latter result, while different to that found in vivo, may reflect the sensitivity with which the in vitro system is able to detect aspects of development that may be masked in the whole animal.

LONG TERM EFFECTS OF A GnRH AGONIST WITH OR WITHOUT pFSH ON FOLLICULAR GROWTH REGULATION IN THE BOVINE.
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Macroscopic and microscopic populations of ovarian follicles were determined following daily injections of a potent GnRH agonist (480 µg/day) for 12.8 days, with or without a six day stimulation, LH contamination lower than 0.01%) initiated on Day 12. On Day 2 of an estrous cycle (estrus=D0), heifers were assigned to one of the following three groups: 1) GnRH from D2 to D12 (n=4); 2) GnRH from D2 to D18 (n=9) and 3) GnRH from D2 to D18 plus pFSH (from D12 to D18 (n=6). As determined by daily ultrasonography, the number of follicles S10 and >10mm and the diameter of the largest follicles (Fl) remained relatively low in heifers treated with the GnRH agonist for 12 or 18 days. However, pFSH injections from Days 12 to 18 along with GnRH, increased the number of follicles between 5-10mm from 1.0 to 6.0 (P<0.01) while in heifers with GnRH alone, its decreased from 1.0 to 0.75 follicles (P=0.10). A dominant follicle (>10mm) was observed at Day 17 in pFSH treated heifers only. The total number of microscopic follicles = 1.8mm was similar in the different groups. Atresia was influenced by the duration of GnRH treatment; the proportion of atretic follicles among the total number of follicles increased from 83.6 to 95.3% (P<0.05). Among the atretic follicles, the proportion of both large and small atretic follicles was greater in heifers treated with GnRH alone than in those treated with pFSH (41.2 vs 20.8; P<0.09). In the total follicular population, the number of Class 1 follicles (1.58-3.78mm) was lower in pFSH treated heifers than in those treated with GnRH alone or with GnRH plus pFSH (18.13 vs 32.8; P<0.03). In contrast, the numbers of Class 2 (3.68-5.85mm) and 3 (>5.56mm) follicles were greater in heifers treated with pFSH than in those treated with only GnRH for 18 days. Atresia increased with the duration of GnRH treatment for Class 1 follicles (77.1 vs 91.9; P=0.07). In heifers treated with pFSH, most of Class 2 and 3 follicles were atretic (10.2/10.9 and 14/15.3 respectively) but these follicles were in early atresia, as compared to follicles resulting from GnRH treatment for 12 or 18 days (P=0.05). These results indicate that a GnRH agonist imposed during an estrous cycle inhibits follicular development by accumulating small follicles at a late stage of atresia and that pure FSH prevents atresia of small follicles and allows further growth to ovulatory size but needs LH support to assure healthy condition. Supported by F.C.A.R. Quebec.

40 EFFECTS OF FSH, SST AND PROGESTERONE ON FOLLICULAR DYNAMICS IN POSTPUBERTAL SUCKLED BEEF COWS. Yacin Yavas*, Sylvie Roberge1, Mary M. Buhr, Walter Johnson2, Robert M. Liptrap2 and John S. Walton1. Dept. of Animal Sciences, Dept. of Population Medicine1, Dept. of Biomedical Sciences2, Univ. of Guelph, Guelph, Ontario, Canada.

In beef cows, numerous follicular waves resume soon after parturition; however, their follicles fail to ovulate. The objective of this study was to determine whether follicular stimulated (FSH), bovine somatostatin (SST) and progesterone releasing intravaginal device (PRID) treatments on follicular dynamics of postpartum suckled beef cows (cows). Two groups of postpartum beef cows were investigated. On day 21 (parturition = day 0), cows (n=6/treatment) received an sc injection of either SALINE (10 ml), FSH (200 µg) or SST (40 µg), or a PRID for 10 days. Follicular dynamics were monitored by ultrasonography from day 18 through day 50. Daily blood samples were collected during the same period for progesterone measurement to confirm ultrasonographic observations. FSH injection was followed, in all cows, by development of a dominant follicle which ovulated in 2 of 6 cows. PRID insertion was followed, in all cows, by selection of a medium follicle which became dominant and was sustained until PRID removal. In 50% of cows (3/6) receiving PRID treatment, PRID removal was followed by ovulation within 5 days and a luteal phase of normal length. In the remaining cows, the dominant follicle was sustained for another week before it started to regress. The BST treatment did not affect follicular development. Days to first postpartum ovulation were not different (P>0.05) among PRID (37.5±17.3), FSH (41.0±17.3), SST (50.3±17.3) and SALINE (44.0±17.3) groups. However, PRID treatment reduced the incidence of short luteal phase to 0% (0/3) in the cows that ovulated within 5 days of PRID removal. Although persistence of the first dominant follicle did not differ (P>0.05) among treatment groups, maximum diameter of that dominant follicle was greater in control (53±10 mm) and FSH (9.3 mm) groups than in SALINE (7.3 mm) and SST (6.8 mm) groups. These data indicate that there are follicular waves in postpartum beef cows and these follicles are responsive to FSH but not to BST. PRID treatment sustains these follicles in a pattern which is similar to that in normal cycling cows. Short cycles induced by PRID treatment may be a useful tool to study the establishment of ovulatory follicles and corpora lutea following ovulation in postpartum cows.