Immunohistochemical Studies of Insulin-Like Growth Factor-I in Corpora Lutea of Cycling and Pregnant Mice, Rats, Hamsters, Guinea Pigs and Cattle

Hiromichi TAMADA, Mika OHNO, Tsutomu SAWADA and Junichi MORI

Department of Animal Reproduction, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka 593, Japan

Abstract. Immunohistochemical staining of insulin-like growth factor-I (IGF-I) in the corpora lutea (CL) during estrous cycle and pregnancy was compared among mice, rats, hamsters, guinea pigs and cattle. During the estrous cycle, while clear staining was not detected in the hamster CL, the CL of the mouse, rat and guinea pig showed modest staining except for the mouse CL 1 day after ovulation which showed very weak staining. In contrast, immunoreactive IGF-I in the bovine CL showed distinct changes. While very weak staining was noted during the follicular and early luteal phases, the intensity increased markedly thereafter, peaking at the late luteal phase. During pregnancy, the mouse and rat CL showed positive staining, and the intensity increased at late pregnancy, especially in the rat CL intense focal staining was noted. In the hamster CL, clear positive staining was detected only at late pregnancy. In contrast, in the guinea-pig and bovine CL, although intense staining was detected during mid-pregnancy, the staining decreased at late pregnancy. The results revealed species differences in the accumulation of immunoreactive IGF-I in CL.

Key words: IGF-I, Corpus luteum, Immunohistochemistry.

Recent evidence suggests that polypeptide growth factors influence proliferation and functional differentiation of ovarian follicle cells [1]. Among the growth factors, insulin-like growth factor-I (IGF-I) has been the subject of intensive investigation [2]. However, these studies focused mainly on follicle cells and studies on the corpora lutea (CL) have been limited. In the CL of the cattle [3-5], rabbit [6] and rat [7, 8], stimulatory effects of IGF-I on progesterone production in vitro have been reported, and the expression of IGF-I in the CL of rats [7, 9] and cattle [4] has been detected.

The present comparative study documents changes in the intensity of immunostaining of IGF-I in CL of five different mammalian species during estrous cycle and pregnancy.

Materials and Methods

The ovaries from 33 ddY mice (30-40 g), 31 Sprague-Dawley rats (165-330 g), 31 golden hamsters (100-180 g), 20 Hartley guinea pigs (490-980 g), 14 cycling Japanese Black and 10 pregnant Holstein cattle were used. The bovine ovaries were collected from a local slaughter house. All other species of animals were maintained at 24 ± 1 °C under a lighting schedule of 14 h light (0500-1900 h), and had access to commercial chow and water freely. The mice, rats and hamsters were killed under deep ether anesthesia, and the guinea pigs...
were anesthetized by intraperitoneal injection of sodium pentobarbital. In the mouse, rat and hamster, the stages of the estrous cycle were confirmed by examination of the vaginal smears. Rats and hamsters showed regular 4-day cycles. In the guinea pig, the stages of the estrous cycle were verified by examination of vaginal opening and smears. The day when vaginal epithelial cells showed maximum cornification was defined as day 0 of the estrous cycle. Mean (± s.d.) length of the estrous cycle was 16.5 ± 0.5 days (n = 6). The stages of the estrous cycle in the cattle were estimated by the gross appearance of ovaries according to Ireland et al. [10]. In the mouse, rat, hamster and guinea pig, the morning of finding a vaginal plug or spermatozoa in the vaginal smear was defined as day 1 of pregnancy. In the pregnant cattle, the crown-rump length of the fetus was measured to evaluate the stage of pregnancy. The stages when ovaries were collected were as follows. Mice: proestrus, estrus, diestrus, and days 1, 3, 8, 13, 16 and 20 of pregnancy; rats: proestrus, estrus, metestrus, diestrus, and days 1, 3, 8, 15, 19 and 21 of pregnancy; hamsters: proestrus, estrus, metestrus, diestrus, and days 1, 3, 6, 10 and 14 of pregnancy; guinea pigs: days 1, 5, 10 and 14 of the estrous cycle, and days 5, 15, 25, 35, 50 and 65 of pregnancy; cattle: days 1-4, 5-10, 11-17 and 18-20 of the estrous cycle, and months 1, 3-4, 5-6, 7, 8 and 9 of pregnancy. The numbers of animals used in each stage were 3-4 in mice, rats and hamsters, 2 in guinea pigs, 3-4 in cycling cows and 1-2 in pregnant cows.

Immunolocalization was based on the procedure described previously [11-13]. In brief, ovaries were excised, cleaned of fat, and fixed in Bouin’s solution for 24 h. The bovine ovaries were cut into small pieces before fixation. Paraffin-embedded tissue blocks were sectioned at 7 μm. Sections were deparaffinized, hydrated in phosphate buffered saline (PBS) for 20 min and then incubated in blocking solution (10% normal goat serum) for 10 min prior to incubation in primary antibodies for 42-45 h at 4 C. A rabbit antiserum (UBK-487) raised against human IGF-I, a gift of Drs L. Underwood and J. J. Van Wyk, and distributed by the Hormone Distribution Program of the NIDDK was used as the primary antibody at a dilution of 1:1000 in PBS. This antiserum has 0.5% crossreactivity with IGF-II, and crossreacts minimally with insulin at 10−6 molar. Immunostaining was performed using a Zymed Histostain-SP kit for rabbit primary antibody (Zymed laboratories, San Francisco, CA). This kit utilizes biotinylated secondary antibody (goat anti-rabbit IgG), a horseradish peroxidase-streptavidin conjugate and a substrate chromogen mixture [14]. Blocking of endogenous peroxidase activity was achieved by a 45 second incubation in 0.23% periodic acid in PBS following secondary antibody incubation [15]. Some sections were counterstained lightly with hematoxylin. Red deposits indicated the sites of immunostaining. For comparison of immunoreactivity, sections from specific stages or days of the estrous cycle or pregnancy were mounted onto the same slide, and the staining was repeated at least 3 times to confirm differences in intensities. Control experiments included incubation of sections with normal rabbit serum or primary antibodies neutralized with excess of recombinant human IGF-I (Bachem Inc., Torrance, CA).

**Results**

Ovarian sections after incubation with normal rabbit serum or antibody preincubated with excess antigenic peptides showed no staining in all the species examined. Representative photographs of sections of rat CL are shown in Fig. 1. The staining

---

**Fig. 1.** Photomicrographs showing immunohistochemical staining of IGF-I in the rat CL on day 19 of pregnancy. (A) Anti-IGF-I antibody showed intense intracellular focal staining in the luteal cells. (B) No staining was observed in sections incubated in the antibody pre-neutralized with excess antigen. ×198. Bar=30 μm.
IGF-I IN CORPORA LUTEA

pattern of immunoreactive IGF-I was intracellular, and no-nuclear staining was noted in any sections studied.

The representative photomicrographs of sections of the CL of each of the species examined are presented in Figs. 1A and 2A-H. During the estrous cycle, modest staining of IGF-I in CL was noted in the mouse, rat and guinea pig (data not shown). Exceptionally, the mouse ovary at estrus contained two types of CL; the newly formed CL (Fig. 2A) showed very weak staining, while the older CL showed modest staining. In the hamster, staining

Fig. 2. Photomicrographs showing immunohistochemical staining of IGF-I in ovarian sections. (A) Mouse CL and interstitial glands (IG) on day 1 of pregnancy. IG showed modest staining, while only very weak staining was detected in CL 1 day after ovulation. (B) Mouse CL on day 20 of pregnancy. Intense staining was detected. (C) Rat CL on day 1 of pregnancy. Modest staining was detected. (D) Hamster CL on day 14 of pregnancy. Modest staining was detected. (E) Guinea-pig CL on day 35 of pregnancy. Intense staining was observed in some cells. (F) Bovine CL on days 1-4 of the estrous cycle. Very weak staining was detected. (G) Bovine CL on days 5–10 of the estrous cycle. Some cells showed modest staining. (H) Bovine CL on days 11–17 of the estrous cycle. Many cells showed intense staining. (I) Rat follicle and IG on day 1 of pregnancy. Modest staining was detected in IG. × 198. Bar = 30 μm.
was not observed during the estrous cycle (data not shown). In contrast, immunoreactivity in the bovine CL showed distinct changes (Figs. 2F-H). During pregnancy, the intensity of the staining changed in all the species examined (Figs. 1A and 2A-E). While as a whole the staining was observed evenly in the cytoplasm of both large and small luteal cells, in the rat CL on days 19 and 21 of pregnancy intense focal staining was detected (Fig. 1A). The relative intensities of immunostaining in CL of the estrous cycle and pregnancy are summarized in Figs. 3 and 4, respectively.

In the hamster and guinea pig, no clear positive staining was observed in ovarian cells other than CL, although some intercellular spaces in follicles showed weak staining (data not shown). In the mouse and rat, interstitial glands showed clear positive staining only at estrus and on day 1 of pregnancy, and weak staining was detected in the intercellular spaces in follicles (Figs. 2A and 2I). In the cattle, granulosa and theca cells showed weak staining (data not shown).

Discussion

We used an antiserum raised against human IGF-I for immunostaining in 5 different mammalian species, because evolutionarily conserved protein and RNA sequences for IGF-I have been reported [16-22]. By using this antibody, positive immunostaining has been noted in ovarian sections of all the species examined.

Hansson et al. [9] examined immunostaining of IGF-I in various tissues of the cycling rat. According to their report, intense staining was observed in granulosa cells of the primordial and primary follicles, and theca cells, interstitial glands and CL also showed positive staining. In this study, although granulosa cells did not show intense staining, the other types of cells showed positive staining, and we further observed distinct changes in intensity of the staining in CL during pregnancy in five species and during the estrous cycle in the cattle. In the bovine CL, IGF-I gene expression has been examined [4], and intensity of staining was consistent with the extent of the gene expression. On the other hand, in the rat CL, IGF-I mRNA levels are high during early pregnancy [7] as compared with the intense focal staining at the late stage of pregnancy. Since IGF-I is internalized via type I IGF receptor in target cells [23, 24], this inconsistency may partly be explained by immunodetection of the antigen not only synthesized in CL, but also internalized after binding to the receptor.

Since IGF-I stimulates progesterone production by CL in vitro in the cattle [3-5], rat [7, 8] and rabbit [6], the regulatory role of IGF-I in CL function has been suggested. As shown in Figs. 3 and 4, this study shows that in the cycling cattle, immunoreactivity of IGF-I in CL increased during the active phase of CL function, while in the mouse and rat that was high at the late stage of preg-

![Fig. 3. Relative intensities of immunostaining of IGF-I in CL of the mouse, rat, hamster, guinea pig and cattle during estrous cycle. Intensities of immunostaining were subjectively graded; ±, no or very weak staining; +, intense staining. *CL 1 day after ovulation showed very weak staining.](image)

![Fig. 4. Relative intensities of immunostaining of IGF-I in CL of the mouse, rat, hamster, guinea pig and cattle during pregnancy. Intensities of immunostaining were subjectively graded; ±, no or very weak staining; +, intense staining. *CL 1 day after ovulation (immunoreactivity of the other CL was +).](image)
nancy when CL function declines [25, 26]. Furthermore, while the hamster CL showed positive staining only at the late stage of pregnancy, the immunoreactivity in guinea-pig CL decreased at this stage when CL function is still maintained in both the species [27, 28]. From these findings it may be concluded that the intensity of immunostaining of IGF-I in CL does not always parallel the function of CL.

In this study, the interstitial glands of the rat and mouse ovary showed clear staining only at estrus and on day 1 of pregnancy. The reason for the absence of the staining at other stages of the estrous cycle is not clear. It may be possible, however, that follicle stimulating hormone and luteinizing hormone surges on the previous day are concerned in the immunoreactivity for IGF-I in the interstitial glands because equine chorionic gonadotropin distinctly increased it in the mouse and rat (our unpublished data).

Recently IGF-binding proteins [29, 30] and IGF-I receptor [5, 7, 8], which are involved in the expression of IGF-I action, were detected in CL. Further studies on the expression of IGF-I, its receptor and binding proteins are needed for elucidation of physiological roles of IGF-I in CL function.

Acknowledgements

The authors wish to thank Professor S. K. Dey, Department of Obstetrics and Gynecology and Physiology, University of Kansas Medical Center for revising the manuscript and for valuable and stimulative advice, and Professor F. Sasaki, Department of Anatomy, University of Osaka Prefecture for valuable advice. This work was supported in part by the grant-in-aid 04304023 for Cooperative Research from the Ministry of Education, Science and Culture, Japan.

References


14. Hsu S-M, Raine L. The use of avidin-biotin-per-


27. Rowlands IW, Short RV. The progesterone content of the guinea-pig corpus luteum during the reproductive cycle and after hysterectomy. J Endocrinol 1959; 19: 81–86.

