Distribution of Estrogen Receptor Alpha in the Preovulatory Dominant Follicles Before and After a GnRH Injection in Cows

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Introduction

Estrogen receptors (ERs) have been detected in the calf uteri indicate that binding of estradiol by uterus is influenced by the age and hormonal stage of the animal (Sanbom *et al.* 1971). During development. estrogen may have a direct effect on cells that express ERs, but may not affect cells that are devoid of such receptors (Tasende *et al.* 1996). It is generally accepted that there exist two different ER subtypes, namely estrogen receptor-alpha (ERα) and estrogen receptor-beta (ERβ). The presence of ERs in the ovaries of sheep (Tomanek *et al.* 1997), cows (Berisha *et al.* 2002; Van den Broeck *et al.* 2002) and pigs (Slomczynska *et al.* 2001; Slomczynska and Womiak 2001) has already been shown. Presence of ERs in the ovaries plays an important rote in the maintenance of fertility. ERα knockout mice are acyclic, infertile and possess hyperemic ovaries devoid of corpora lutea. Female ERβ knockout mice have small ovaries and show arrested follicular development (Drummond *et al.* 2002). Bovine ERβ mRNA expression in granulosa celts decreases with increase in follicular size (Van den Broeck *et al.* 2002). In contrast, mRNA expression of ERα in theca intema increases continuously during the final growth of bovine follicles, in which it does not increase in granulosa cells (Manikkam *et al.* 2001). A relatively high expression of ERα *is* found in the thecal and stromal cells in comparison with that in granulosa cells (Van den Broeck *et al.* 2002). ERα are present in developing DF on Day 7, early regressing DF on Day 10 and preovulatory DF on Day 18, especially in the TI, and a few were localized in the mGC (Amrozi *et al.* 2004). However, information is limited concerning the specific distribution of ERα in preovulatory dominant follicles (DF). The aim of this study was to determine the distribution *of* ERα in the preovulatory DF after a GnRH administration by means of immunohistochemistry.

Materials and methods

Animals and Experimental Design

Six Japanese black cows (year olds) with BCS about 3.1±0.4 were used in this study. Cows were subjected to receive a progesterone CIDR-B device during mid-luteal phase (CDIR in = Day 0 am) and a $PGF_{2\alpha}$ injection (25 mg, i.m., Pronalgon®F, Pharmacia, Japan) on Day 7 am. CIDR was removed on Day 8 am. They received a GnRH (100 μg, i.m., Supolnen®, Denka, Japan) injection just before ovariectomy on Day 10 pm as Control Group (pregonadotrophin stimulation, n=3). The other cows received same treatments and were ovariectomized on Day 11 am, 12 hrs after GnRH injection as Treatment Group (early preovulatory period, n=3).

Development of the DF and the largest subordinate follicle (SF) were monitored twice daily at 07:00 and 19:00 by means of ultrasonography from the day of CIDR in until the day of ovariec-

tomy by the same operator to maintain a similar evaluating condition. A real time B-mode ultrasound scanner (EUB-405, Hitachi-Medical Co., Tokyo) equipped with a 7.5 MHz transducer (EUP-033J, Hitachi-Medical Co., Tokyo) was used. Appropriate images of follicles were arrested on the screen and maximum diameters of follicles were measured by means of built-in calipers. Ovariectomy was performed by flank laparotomy under epidural anesthesia.

Hormone Determinations

Blood from the jugular vein was collected twice daily from CIDR in until the day of ovariectomy at the same time as ultrasonography. Double antibody RIA was used to determine the concentrations of progesterone by using antisera to progesterone (GDN # 337) (Taya *et al.* 1985).

Processing of Ovaries

After ovariectomy, the ovaries were immediately placed in physiological saline. Follicular walls were immediately put into 10% phosphate- buffered formalin. Follicular tissues were processed for histology, and embedded in paraffin wax.

Immunohistochemistry for ERα

The follicular and luteal tissues were cut to a thickness of 5 μm and mounted on 3-aminopropyltriethoxysilane-coated slides (Dako Cytomation, Kyoto, Japan). Expressions of ERα in the follicular tissues were detected following method which described in our previous study [1]. Stained cells (brown staining) were observed using a light microscope (Eclipse E800, Nikon, Tokyo, Japan) equipped with a digital camera (Digital Camera DXM1200, Nikon, Tokyo, Japan) at x100 magnification.

Data Analysis

Expression of ERα in antral granulosa cells (aGC), mural granulosa cells (mGC), theca interna cells (TI) and theca externa cells (TE) were analyzed according to the method described by Ing and Tornesi (1997). Immunohistological investigation of ERα in each section was located randomly. Intensity of immunohistological staining was graded as 0 (no staining), 1 (weak staining), 2 (moderate staining) or 3 (strong staining). The tissue sample was scored at ten fields over one follicle for each cow by the same observer, who was unknown to which group of the tissue originated from.

Results and discussion

Expression of ERα represented by immunohistological staining intensity in cells was determined using a light microscope equipped with a digital camera. Expression of ERα in antral granulosa cells (aGC), mural granulosa cells (mGC), theca interna cells (TI) and theca externa cells (TE) in Control group were 0.18±0.11, 0.12±0.09, 0.77±0.10 and 0.46±0.06, respectively, and in Treatment group were 0.28±0.18, 0.21±0.15, 1.01±0.14 and 0.48±0.19, respectively. A higher expression of ERα was found in TI than in aGC, mGC and TE within group of cows ($p<0.05$). A moderate expression of ERα was found in TE which significantly higher than in aGC and mGC within group of cows ($P<0.05$). Lower expression was found in aGC and mGC than other follicular cells within group of cows. A tendency of increasing ERα expression after a GnRH injection was found in TI ($P=0.06$, between Control and Treatment group). The results indicate that GnRH

was tend to induce ERα expression in TI and no effects of a GnRH injection on ERα expression in TE, aGC and mGC in bovine preovulatory DF in cows.

ERα was observed in bovine preovulatory DF before and after a GnRH injection with a varying degree of staining intensity. A higher ERα expression was observed in TI in both group cows. It has been known that intermediate to high ERα expression is present in thecal cells bovine ovary (Van den Broeck *et al.* 2002), theca cells rat ovary (Pelletier *et al.* 2000) and in large porcine preovulatory follicle (Slomcynska and Wozniak. 2001). Expression of ERα in theca interna tissue increases continuously and significantly higher during the final follicle growth (Berisha *et al.* 2002). ERα expression has found intensively in the TI preovulatory DF on Day 18 of bovine estrous cycle (Amrozi *et al.* 2004). In the present study, a tendency of increasing ERα expression was found in TI after a GnRH injection. It has been known that FSH and hCG stimulated ERα mRNA in hypophysectomized hamsters ovary (Yang *et al.* 2002).

ERα mRNA is presence in the granulosa cells of porcine preovulatory follicles (Slomczynska *et al.* 2001) and in bovine granulosa cells (Berisha *et al.* 2002). Expression of ERα is localized in the mural granulosa cells in bovine preovulatory DF (Amrozi *et al.* 2004). In the present study, the expression of ERα in GC was not difference before and after 12 hrs of a GnRH injection. Injection of cows with GnRH induced an LH surge within 2 h (Kaneko *et al.* 1991). It has been known that ovulatory hCG unable to change ERα mRNA expression in macaque granulosa cells after 12-36 h administration (Chaffin *et al.* 1999). In contras, a treatment of hCG or GnRH agonist to the culture of human granulosa-luteal cells induces down-regulation of ERα gene expression (Chiang *et al.* 2000). Moreover, ERα mRNA levels are observed during the estrous cycle without an apparent modulation (Byers *et al.* 1997) and ERα transcriptional activity is modulated by ERβ (Hall and McDonnell. 1999; Lindberg *et al.* 2003). Estradiol decrease immunostaining for ERα at 48 h in culture rat granulosa cells (Sharma *et al.* 1999). The results indicate that GnRH was tend to induce ERα expression in TI and no effects of a GnRH injection on ERα expression in TE, aGC and mGC of preovulatory DF in cows.

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