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 (Sanborn *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; Siomczynska and Womiak 2001) has already been shown. Presence of ERs in the ovaries plays an important rote in the maintenance of fertility. ERa 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 at.* 2002). Bovine ER[3 mRNA expression in granulosa celts decreases with increase in follicular size (Van den Broeck *et al.* 2002). In contrast, mRNA expression of ERa in theca interna 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 immunohis-tochemistry.

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 ovariectomy 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 a!***. 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.

Immunohistochemistryfor ERa

The follicular and luteal tissues were cut to a thickness of 5 urn and mounted on 3-aminopropyl-triethoxysi lane-coated slides (Dako Cytomation, Kyoto, Japan). Expressions of ERa 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 x 100 magnification.

Data Analysis

Expression of ERa in antral granulosa cells (aGC), mural granulosa cells (mGC), theca interna cells (Tl) and theca externa cells (TE) were analyzed according to the method described by Ing and Tomesi (1997). Immunohistological investigation of ERa 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 ERa represented by immunohistological staining intensity in cells was determined using a light microscope equipped with a digital camera. Expression of ERa in antral granulosa cells (aGC), mural granulosa cells (mGC), theca interna cells (TI) and theca externa cells (TE) in Control group were 0.I8±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 ERa was found in TI than in aGC, mGC and TE within group of cows ($p<0.05$). A moderate expression of ERa 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 ERa 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 ERa expression in Tl and no effects of a GnRH injection on ERa expression in TE, aGC and mGC in bovine preovulatory DF in cows. ERa was observed in bovine preovulatory DF before and after a GnRH injection with a varying degree of staining intensity.

ERa was observed in bovine preovulatory DF before and after a GnRH injection with a varying degree of staining intensity. A higher ERa expression was observed in TI in both group cows. It has been known that intermediate to high ERa 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 (Stomcynska and Wozniak. 2001). Expression of ERa in theca interna tissue increases continuously and significantly higher during the final follicle growth (Berisha *et al.* 2002). ERa expression has found intensively in the TI preovulatory DF on Day 18 of bovine es-**trou5** cycle (Amrozi *et al.* 2004). In the present study, a tendency of increasing ERa expression was found in TI after a GnRH injection. It has been known that FSH and hCG stimulated ERa mRNA in hypophysectomized hamsters ovary (Yang *et at.* 2002).

ERa mRNA is presence in the granulosa cells of porcine preovulatory follicles (Slomczynska *et at.* 2001) and in bovine granulosa cells (Berisha *et al.* 2002). Expression of ERa is localized in the mural granulosa cells in bovine preovulatory DF (Amrozi *et al.* 2004). In the present study, the expression of ERa 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 ERa 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 ERa gene expression (Chiang *et al.* 2000). Moreover, ERa mRNA levels are observed during the estrous cycle without an apparent modulation (Byers *et al.* 1997) and ERa transcriptional activity is modulated by ERP (Hall and McDonnell. 1999; Lindberg *et al.* 2003). Estradiol decrease immunostaining for ERa at 48 h in culture rat granulosa cells (Sharma *et al.* 1999). The results indicate thai GnRH was tend to induce ERa expression in TI and no effects of a GnRH injection on ERa expression in TE, aGC and mGC of preovulatory DF in cows.

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