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Cell-specific immuno-localization of progesterone receptor alpha in the rabbit ovary during pregnancy and after parturition

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ABSTRACT

Progesterone receptor alpha (PRA) has a central coordinator role in the ovarian functions in mammals. The aim of this study was to investigate the immunolocalization of PRA in the rabbit ovary during pregnancy and after parturition. The rabbit ovary during pregnancy and after parturition had moderate cytoplasmic and moderate to intense nuclear PRA immunostaining in the ovarian surface epithelial cells, stromal cells and interstitial gland cells. The PRA was also present in granulosa cells and theca interna cells of the growing, small antral and mature Graafian follicles. Theca interna cells of the atretic antral follicle in addition to endothelial and fibroblast cells had PRA immunoreactivity. The PRA were also observed in the theca externa smooth muscle-like cells of the growing and antral follicles and in the telocytes. In the present study, the corpora haemorrhagica and early developing corpora lutea had, slight cytoplasmic and nuclear PRA immunostaining in the large lutein and small lutein cells. The endothelial cells of the corpora haemorrhagica and corpora lutea had an intense nuclear PRA immune signal. The corpora lutea at an advanced stage of development had moderate cytoplasmic and nuclear PRA immunostaining in the large lutein cells and intense nuclear PRA immunostaining in the small lutein cells. The regressed corpora lutea did not have PRA immunostaining in the apoptotic large lutein cells and moderate cytoplasmic and intense nuclear PRA immunostaining in the small lutein cells.

1. Introduction

Progesterone is a steroid sex hormone essential for all aspects of female reproductive function in vertebrates. These include ovulation, growth and differentiation of ovarian structures, uterine and mammary gland development, pregnancy establishment and maintenance and the neuro-behavioral expression associated with sexual responsiveness (Clarke and Sutherland, 1990; Lydon et al., 1995; Pinter et al., 1996; Conneely et al., 2003; Gava et al., 2004; Gadkar-Sable et al., 2005). The physiological effects of progesterone are mediated by an interaction of the hormone with specific intracellular progesterone receptors (PR). The PR are produced as a result of expression of a single gene as two protein isoforms; progesterone receptor alpha (PR-A) and progesterone receptor beta (PR-B) and these receptors are members of the nuclear receptor superfamily (NRS) of transcription factors (Conneely et al., 2003; Kubota et al., 2016). The NRS regulate gene transcription by discriminative binding to DNA regulatory sequences as well as by specific interactions with co-activator and/or co-repressor proteins to regulate the activity of the RNA polymerase complex (McKenna and O'Malley, 2001). The ovarian progesterone receptor gene is regulated by pituitary gonadotropins (Pinter et al., 1996). Several *in vivo* and *in vitro* studies have demonstrated that the PR-A and PR-B proteins have different transcription activation

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properties when progesterone binds to the receptors (Conneely et al., 2003, 2001, 1989; Lydon et al., 1995; Mulac-Jericevic et al., 2003, 2000; Mulac-Mulac-Jericevic and Conneely, 2004; Seagroves et al., 2000). These studies elucidated that the ablation of PR-A does not affect response of the mammary gland or thymus to progesterone but results in severe abnormalities in ovarian and uterine function leading to female infertility. The ablation of PR-B does not affect ovarian, uterine or thymic responses to progesterone but reduces mammary ductal morphogenesis and alveologenesis during pregnancy.

Increased information of progesterone on conceptus-endometrial interactions is necessary to understand and elucidate the causes of early embryonic losses and for development of new strategies to improve pregnancy outcomes and female fertility (Spencer et al., 2016). Thus, investigation of PR-A in both the uterus and ovary is necessary to elicit the progesterone-dependent reproductive responses necessary for female fertility. The immunopresence of PR in the ovary has been investigated in many species such as humans (Iwai et al., 1990; Maybin and Duncan, 2004), primates (Hild-Petito et al., 1988), cattle (Van den Broeck et al., 2002; D'Haeseleer et al., 2007), pigs (Słomczyńska et al., 2000; Durlej et al., 2010), rabbits (Korte and Isola, 1988; Iwai et al., 1991; Lan et al., 2014; Parillo et al., 2013), dogs (Vermeirsch et al., 2001) and rodents (Lydon et al., 1995; Park-Sarge et al., 1995; Hułas-Stasiak and Gawron, 2010). There is limited previous research focusing on whether the localization of PRA varies during pregnancy and after parturition especially in rabbits. The aim of the present study, therefore, was to describe the normal cellular distribution of PRA in the rabbit ovary at different stages of pregnancy and after parturition.

2. Materials and methods

2.1. Animals

Sixteen sexually mature New Zealand white rabbit does (4–5 months old) with a mean weight of 2.97 ± 0.2 kg were reared in the animal house, faculty of medicine, Assiut University. The rabbits were housed in separate cages under controlled and consistent conditions of light (12 h light), temperature (22–25 °C), ventilation and humidity. The does were mated with fertile New Zealand white bucks. The day of mating is designated Day 0. Pregnancy can be detected 7–10 days after mating by abdominal palpation.

2.2. Samples

Ovaries (right and left) were obtained at 12 h, 3, 7, 14 days post-mating, the time of parturition and 10 days post-partum. Ovaries were dissected within ¼ hr after slaughtering and were immediately fixed with Bouin's fluid. Two to three animals were used for each experimental period.

2.3. Immunohistochemistry of PRA

Immunohistochemical detection of PRA in paraffin sections was performed using PR (Clone SP2) rabbit monoclonal antibody (Cat.#RM-9102S0) and an Ultravision Detection System (Anti-Polyvalent, HRP/DAB; Thermo Fisher Scientific, USA).

The fixed ovaries were dehydrated in ascending grades of ethanol, cleared in methyl benzoate and then embedded in paraffin wax. Sections (3–5 μm) of paraffin-embedded tissue were dewaxed by immersion of slides in xylene three times for 5 min each time. Subsequently, rehydration of tissue on the slides occurred by placement in 100%, 100%, 95%, and 80% ethanol for 3 min at each immersion, and slides were rinsed in PBS with a pH of 7.4 (three times for 5 min each time). Endogenous peroxidase actions were inhibited by adding 3% hydrogen peroxide for 10 min at room temperature followed by washing in PBS at a pH of 7.4 (four times for 5 min each time). For antigen detections, the slides were placed in 10 mM sodium citrate buffer (pH 6.0) and samples were heated to near boiling (95–98 °C) in a water bath for 20 min followed by cooling for 20 min at room temperature. Sections were then rinsed in PBS at a pH of 7.4 (three times for 1 min each time). Sections were covered with Ultra V block for 5 min at room temperature to inhibit non-specific background staining. Sections were then incubated with the primary antibodies for 30 min at room temperature. The slides were washed with PBS at a pH of 7.4 (four times for 5 min each time), followed by incubation with a biotinylated secondary antibody [TP-015-BN, Anti-Mouse IgG (H + L), Anti-Rabbit IgG (H + L), Thermo Fisher Scientific, USA] by adding drops on the section for 10 min at room temperature. The slides were subsequently rinsed in PBS (pH 7.4, three times for 5 min each time) followed by incubation with drops of streptavidin- peroxidase complex (Thermo Fisher Scientific,USA) for 10 min at room temperature. The slides were subsequently rinsed in PBS pH 7.4, (four times for 5 min each time). Visualization of the bound antibodies was conducted by adding one drop of DAB (diaminobenzidine) Plus chromogen to 2 ml of DAB Plus substrate by mixing and swirling and subsequently applying a drop on the tissue. Incubation occurred for 5–15 min at room temperature. The sections were counterstained in Harris hematoxylin for 30 s. The sections were dehydrated in a graded series of alcohols (ethanol 95%, and ethanol 100% two times), cleared of xylene, and mounted with DPX. Negative controls did not have the primary antibodies added during slide processing. Immunohistochemical staining was examined using an OLYMPUS BX51 microscope and the photos were taken using an OLYMPUS DP72 camera adapted to the microscope. With assessing the intensity of the immunostaining, the staining of the nucleus and/or cytoplasm was characterized by the following amount and color of immunostaining: intense (dark brown to black), moderate (brown), slight (light brown) and negative immunostaining (no immunoreactivity).

2.4. Negative image study: by using CMEIAS color segmentation

An improved computing technology was used to process color images by segmenting foreground object pixels from the background (Gross et al., 2010) with this technique being especially useful because of the complex color micrographs that existed for the present study. This done