Animal Reproduction Science xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Postnatal ovarian development and its relationship with steroid hormone receptors in JiNing Grey goats

YunZhi Shi [a,b], ShuYing Wang [a,*], Shu Bai [a], LiBo Huang [a], YanMeng Hou [a]

[a] College of Animal Science and Technology, Shandong Agriculture University, Taian, Shandong 271018, China
[b] School of Basic Medical Sciences, Taishan Medical University, Taian, Shandong 271016, China

ARTICLE INFO

Article history:
Received 15 October 2014
Received in revised form
23 December 2014
Accepted 4 January 2015
Available online xxx

Keywords:
Goat
Postnatal development
Ovary
ERα
ERβ
PR

ABSTRACT

In this work, we examined the ovarian development and its relationship with steroid hormone receptors levels and the precocious puberty in JiNing Gray goats by using optical microscopy, immunohistochemistry, quantitative real-time RT-PCR (qPCR) and Western blotting. We found that in the ovaries of neonatal kids, high level of receptors for estrogen (ERα and ERβ) and progesterone (PR) and their mRNA were observed along with growing follicles. From 0 to 30 days of age, the weight and volume of ovaries increased significantly and the boundary between the inner and outer cortex disappeared, while the expression of ERα, ERβ and PR and their mRNA decreased sharply. When 60 days old, the animals began to ovulate; the expression of ERα, ERβ and PR and their mRNA significantly increased, and the animals reached puberty. On day 90, the animals manifested sexual maturity with biggest mature follicles 6.18 mm in diameter, the expression of ERβ and PR protein and their mRNA was maintained at a high level, with decreased expression of ERα and its mRNA. Before puberty, the expression of ovarian ERα (prepubertal dominant receptor) and it's mRNA was significantly higher than that of ERβ (dominant receptor after sexual maturity). The results showed that JiNing Grey goats' ovaries had fast development and early maturation, and ERα, ERβ and PR protein and mRNA expression in the ovary had distinct specificity for time and space, which may be closely related to the strain's progenitive characteristics.

1. Introduction

The JiNing Gray goat, a first-class strain cultivated in the southwest of Shandong province, China, has early sexual maturity and high progenitive rate. According to China Goat Strains, compared with other breeds, the JiNing Gray goat has the strongest fertility (from an average of 2.94 lambs up to 6–7 per breed) (Tu, 1989). The JiNing Grey goats reached puberty (time to first ovulation) when 2 months old, which was 4 months earlier than that of Hu sheep (6 months of age) (Chen et al., 1989). Further, the time of sexual maturity was significantly earlier in JiNing Grey goats (90 days) than in the Boer goat (157.2–191.1 days) (Greyling, 2000), Angora goats (180–240 days) and Inner Mongolia Cashmere goats (150–180 days) (Tu, 1989). Our lab reported ovarian histogenesis, the number of cumulus oocyte complexes (COC) ovulated and lambing percentage from 1990 s (Han et al., 1993; Shuying et al., 1993; Liu et al., 2014); besides, other researchers tried to screen the genetic relationship between gene mutation of KISS-1 and GPR54 and the JiNing Gray Goat's precocious puberty and prolificity by the method of PCR–SSCP (Feng et al., 2009; Chu et al., 2012). However, ovarian development and its relationship with steroid hormone receptors levels and the animal's precocious puberty remains poorly understood.

* Corresponding author. Tel.: +86 18263827699.
E-mail address: sywang@sdau.edu.cn (S. Wang).

http://dx.doi.org/10.1016/j.anireprosci.2015.01.001
0378-4320/

The ovary is not only the major organ to produce estrogen and progesterone but an important target organ of these hormones (Pencharz, 1940). Estrogen and progesterone play an important role in regulating the normal development and function of the ovary by binding to their specific receptors, estrogen receptors (ERα and ERβ) (Green et al., 1986; Kuiper et al., 1996) and progesterone receptors (PR) (Misrahi et al., 1987), respectively. Experiments using gene knockout animals showed that ERα knockout female mice reportedly developed multiple hemorrhagic ovarian cysts and had no corpus luteum or did not ovulate (Lydon et al., 1995; Couse and Korach, 2001), while PR knockout female mice developed multiple genital malformations and did not ovulate (Lydon et al., 1995); ERβ knockout female mice were able to conceive but had significantly less embryos (Emmen and Korach, 2003). In recent years, a few studies focused on postnatal development of the ovary, but the results were quite inconsistent (Revelli et al., 1996; Byers et al., 1997; Yang et al., 2004; Ni et al., 2007). To reveal the postnatal ovarian development pattern and its relationship with ERs and PRs in the JiNing Grey goat, we examined the ovarian histological structure and the expression of mRNA and protein for the estrogen receptors and progesterone receptor in ovaries of the animal by during maturation of the animalby using optical microscopy, immunohistochemistry, quantitative real-time RT-PCR (qPCR) and Western blotting, which would help to provide a theoretical basis for revealing the possible biological mechanism of the hormone receptors in the ovarian development and sexual maturity.

2. Materials and methods

2.1. Collection of tissue samples

All experiments were approved and supervised by Animal Welfare Protection Committee of Shandong Agriculture University. Forty healthy, mature female goats aged 2–3 years old (purchased from ShanDong KeLong husbandry Co. Ltd., ShanDong province, China), weighing 24–26 kg and six healthy, mature male goats, weighing 30–32 kg, had been conventionally raised in Livestock Technology Experiment Station of ShanDong Agricultural University. Forty-two healthy lamps sired by natural conception after estrus synchronization of all animals mentioned above were randomly divided according to their age into seven groups: group of goats aged 0 day on born day (D0), 30 days (D30), 60 days (D60), 90 days (D90), 120 days (D120), 150 days (D150) and 180 days (D180), six in each group that having similar height, body length and weight.

Based on the experimental group, each goat was slaughtered and both ovaries were immediately removed. After being observed, measured and weighed, one of the two ovaries was stored at −80 °C, the other kept in Bouin's fluid. Ovary tissue fixed in Bouin's fluid was conventionally embedded in paraffin and serial slices (5 μm) were cut on a Leica microtome (RM2235, German). All these slices were stuck on the clean slide coated with 10% poly-L-lysine, air dried, and stored at −20 °C until use.

2.2. Optical microscopy

The slices were deparaffinated and hydrated before stained with hematoxylin for 10 min, flushed with water till no color could be washed off; nextly they were differentiated with hydrochloric acid alcohol, turned blue in tap water, incubated in eosin for 1 min, and then dehydrated with alcohol, cleared with xylene, and sealed with neutral gum. At least 10 stained slices were randomly selected from each group. Microscope camera system (Olympus BX41, Japan) was adopted to observe the slices and take photos for them. The number of corpora lutea or corpora albicantes was recorded under a 10× objective lens. The diameter of follicles (in the cross-section containing the oocyte nucleolus), the thickness of the cortex and medulla were measured using an ocular micrometer.

2.3. Immunohistochemistry

The slices were processed through standard protocols of immunohistochemistry with minor modifications (Singh et al., 2011). Briefly, following 3% H_2O_2 quenching endogenous peroxidase, the slices were repaired in microwave to further expose nuclear antigens. Rabbit anti-ERα (1:200, Santa Cruz, USA), rabbit anti-ERβ (1:200, BIOSS, China), rabbit anti-PR (1:200, BIOSS, China) were used to coincubate with the slices for 2 h at 37 °C. IgG marked by biotin (1:200) and SABC hypersensitivity reagent kit were bought from Beijing Zhongshan Golden Bridge Reagent Company. Negative contrast slices were treated with the same measures as above except that the first antibodies was replaced with normal rabbit serum IgG. Binding specificity and purity of all antibodies had been tested by Western blotting.

The immune positive products were tan in the immunohistochemically stained slices while the negative contrast slices could not be stained. Stained slices randomly choosen from each specimen were photographed in random visual fields at high magnification (400×). The images were then analysed with Image Pro-Plus 4.5, the average optical density (AOD) of positive area was obtained to evaluate the stained level of cells and reflect the quantity of target antigen. At least 10 specimens from each of six animals were examined for all investigations.

2.4. Quantitative real-time RT-PCR (qPCR)

qPCR was performed to determine the level of expression of mRNA for ER subtype and PR in the ovaries of each group. The relative level of expression of each mRNA was standardized against GAPDH (internal control) as previously described (Qiu et al., 2013). Ovaries (collected from three goats at each group and randomly cut two parts from each ovary) were handled as individual samples for this analysis. Total RNA from each sample was extracted with RNAiso Plus (Takara, DaLian, China) following the manufacturer's directions. Quality and quantity of isolated total RNA were evaluated using RS323C ultraviolet spectrophotometer (Eppendorf, Germany)

Please cite this article in press as: Shi, Y., et al., Postnatal ovarian development and its relationship with steroid hormone receptors in JiNing Grey goats. Anim. Reprod. Sci. (2015), http://dx.doi.org/10.1016/j.anireprosci.2015.01.001