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Location of oocyte-specific linker histone in pig ovaries at different developmental stages postpartum

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ABSTRACT

This study was conducted to determine the location of oocyte-specific linker histone (H1foo) in pig ovaries at different developmental stages postpartum using histologic, immunohistochemical, and immunofluorescent protocols. The pig ovaries were divided into three periods: proliferation of oogonia (P1, 3 days postpartum), slow growth of follicles (P2, from 40 days to 60 days postpartum), and rapid growth of follicles (P3, from 72 days to 165 days postpartum). With the development of the pig ovary, the boundary between the cortex and medulla gradually became obvious, and the cortex became thinner while the medulla thickened. The rete ovarii could only be observed in P1. The number of oogonia gradually declined after birth, whereas primordial follicles and early growing follicles all underwent an increasing trend followed by a decreasing trend. Developing antral follicles and antral follicles were first observed in 72 and 95 days postpartum, respectively. Both the immunohistochemistry and immunofluorescence detection showed that H1foo was mainly located in the cytoplasm of oogonia and apoptotic oogonia, as well as in the ooplasm of follicles and apoptotic follicles. Moreover, with the development of the pig ovary, the range of the positive signals became larger.

1. Introduction

The mammalian ovary is a heterogeneous organ, producing eggs inside of the follicles which succeed growth, recruitment, dominance selection, ovulation, CL formation, or fall to atresia. Moreover, the ovary can secrete many kinds of endocrine substances, such as estrogen, progesterone, growth factor, inhibin, activin, androgen, oxytocin and so on. As an important economic animal and as an animal model frequently used in medicine and biology research, the study of the morphophysiology of pig ovary development has been a concern of researchers.

Black and Erickson [1] found that in the prenatal pig, primordial germ cells appear in the region of the germinal ridge at 18 days postcoitum (dpc). Mitotic activity of the germ cells begins as early as 13 dpc and ends at 7 days postpartum (dpp). Meiosis began at 40 dpc and ended at 35 dpp. The number of germ cells was approximately 500,000 at birth [1] and declined to approximately 420,000 at puberty [2]. Wei Ding et al. studied the histological structures of 70 dpc, 90 dpc, 1 dpp, and 120 dpp pig ovaries [3,4]. Oxender et al. [5] studied the morphologic changes in pig ovaries from 49 dpc to 90 dpp. Pelliniemi studied the ultrastructure of the gonadal ridge and cord in the early fetal pigs [6–8]. Bielańska-Osuchowska [9] studied oogenesis and oocyte degeneration in the embryos of domestic pigs by electron microscopy and stereological analysis, as well as described the ultrastructure of eggs and follicles.

Apoptosis of germ cells is very important for mammals. According to Bielańska-Osuchowska [9], the majority of oogonia and follicles degraded during the development of the pig ovary. Kim et al. showed that programmed cell death involved in follicular development of pigs was dependent on different pathways. In normal pigs, programmed cell death depended on the expression of autophagy-associated genes, whereas in miniature pigs, it was regulated by apoptosis-associated genes [10].

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Chromosomes in eukaryotic cells are composed of nuclear DNA, histones, and nonhistones. Histones include core histones (H2A, H2B, H3, and H4) and linker histones (H1 family and H5). Linker histones are both species- and tissue-specific. Eleven subtypes of H1 linker histones have been reported in mammals. Six of these subtypes are somatic subtypes (H1.0, H1.1, H1.2, H1.3, H1.4, and H1.5), four are germ cell–specific subtypes (H1 testis, H1 oocyte, H1T2, and H1LS1), and one has been newly identified (H1x) [11–13].

The oocyte-specific linker histone H1 (H1foo) was initially found in the mouse [14]. In the mouse, H1foo was first noted in the germinal vesicle stage oocyte, persisted in the metaphase II stage oocyte, the first polar body, and the two-cell stage embryo, and then disappeared at the four- or eight-cell stage [14]. During oogenesis and early embryogenesis, H1foo reportedly serves an important function in the regulation of gene expression by changing the chromatin structure [14]. Tanaka et al. [15] reported that H1foo was expressed in oocytes of primary, secondary, and preovulatory follicles, and with the development of follicles, the expression of H1foo gradually increased, indicating that H1foo is significant in the growth and maturation of oocytes. According to Mizusawa et al. [16], H1foo was found in follicles of different stages in human ovaries. Mcgraw [17] studied the bovine embryo development process *in vitro* and found that the highest levels of H1foo expression were in germinal vesicle oocytes; such level gradually declined to the eight-cell embryo stage. After intracytoplasmic sperm injection, H1foo was also detected in sperm chromosomes [16]. In both nuclear and somatic cell nuclear transfers, the somatic H1 linker histone is rapidly replaced by H1foo, which is necessary for the accomplishment of nuclear reprogramming [18,19]. To explore the molecular mechanism of this process, Becker et al. [20] studied the binding dynamics of somatic H1 and H1foo, thereby finding that H1foo bound to the chromatin correctly and tightly.

However, few reports have been published on the location and distribution of H1foo in pig ovaries at different ages after birth. In this research, we chose 3, 40, 50, 60, 72, 86, 95, and 165 dpp Changbai pig ovaries as experimental materials and studied the histologic features to provide more proof for the reproductive physiology of pig ovaries. We also analyzed the location of H1foo in the pig ovary at different developmental stages after birth to provide more theoretical evidence for further research into the functions of H1foo.

2. Materials and methods

2.1. Sample selection

All animals were clinically healthy food animals slaughtered routinely in an abattoir in Yangling, Shaanxi Province, China. Immediately after veterinary hygienic inspection, 3, 40, 50, 60, 72, 86, 95, and 165 dpp pig ovaries were collected for histologic examination.

2.2. Histology

The ovaries were fixed in cold Bouin's solution. Each ovary was transversally dissected at two and three levels, sectioned at 5 μm after being embedded in paraffin, and stained by hematoxylin and eosin with a standard procedure. Sections were observed and photographed with a Motic digital microscope (Motic, Xiamen, China) [21].

2.3. Immunohistochemistry

Immunohistochemistry of H1foo was performed on 5-μm tissue sections. Briefly, paraffin sections were dewaxed with a xylene substitute (Haohua, Luoyang, China) and rehydrated in a graded series of ethanol (Haohua). Antigen retrieval was performed by heating at 95 °C in citrate buffer (MXB, Fuzhou, China) for 15 minutes. Sections were pretreated with 50 μL of endogenous peroxidase blocker (MXB) for 25 minutes at room temperature to quench endogenous peroxidase activity before the application of 1% goat serum (MXB) for 25 minutes at room temperature. Sections were labeled with antimouse H1foo (1:250; Abcam, USA) for 1 hour in a humid chamber at room temperature before the use of biotinylated secondary antibody (MXB) at 37 °C for 25 minutes. Sections were then incubated with streptavidin–biotin peroxidase solution (MXB) at 37 °C for 25 minutes and subsequently counterstained with 3,3′-diaminobenzidine tetrahydrochloride detection kit (MXB) for 5 minutes before being mounted with Faramount Aqueous Mounting Medium (MXB).

Negative controls were made by replacing the primary antibody with PBS (Youcon, Beijing, China) or preincubating it with specific blocking peptides to H1foo (ab194535; Abcam). Results were detected by a Motic digital microscope (Motic) [21].

2.4. Immunostaining intensity measurements

According to developmental stage, six experimental groups were established: oogonia, primordial follicles, primary follicles, secondary follicles, developing antral follicles, and antral follicles. Immunohistochemical staining of H1foo was qualitatively examined in 20 serial sections from each experimental group. The captured digital images were evaluated by ipwin32 (Media Cybernetics, USA). The intensity of immunohistochemical reaction was expressed as relative optical density and was calculated with previously reported methods [22]. The results of all groups are expressed as overall mean ± standard error of the mean.

2.5. Immunofluorescence

For immunofluorescence, microwave antigen retrieval was performed in antigen retrieval buffer (Dingguo Changsheng, Beijing, China) at 95 °C for 15 minutes. Subsequently, blocking was performed in 1% donkey serum (MXB). Sections were incubated in rabbit antimouse H1foo (1:250; Abcam, USA) overnight at 4 °C in a humid chamber. Subsequently, the Alexa Fluor 594 AffiniPure Donkey Anti-Rabbit Immunoglobulin G (H+L) (1:200; Jackson, MS, USA) was applied and incubated for 2 hours while being protected from light. Then, 4′,6-diamidino-2-phenylindole was applied for 5 minutes while protected from light. Sections were mounted with Fluorescent Mounting Medium (KPL, USA).