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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Postnatal developmental changes in GnRH- and GnRHR-positive cells in goat submandibular glands

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Abstract

This study investigated the postnatal developmental changes in gonadotropin-releasing hormone (GnRH) and GnRH receptor (GnRHR) in goat submandibular glands, as well as their relationship with postnatal development. The submandibular glands of 0, 30, 60, 90, 120, 150, and 180 day-old female Jining Grey goats were immunostained using the streptavidin–biotin peroxidase complex technique. GnRH-positive cells were distributed in the serous gland and ductal epithelia, and GnRHR-positive cells were only found in the ductal epithelium. The number of these cells remained relatively stable after the growth peak at 90 days of age. We concluded that GnRH may be involved in the postnatal development of submandibular glands.

Keywords: GnRH; GnRHR; immunohistochemistry; submandibular gland; goat

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Introduction

Gonadotropin-releasing hormone (GnRH) is a decapeptide neurohormone secreted by the hypothalamus and a key initiator of the hypothalamic–pituitary–gonadal axis. GnRH regulates reproductive activity by binding to specific receptors of pituitary gonadotrophs (Herrero et al., 2011). Since the first purification of GnRH from the hypothalamus of pigs and sheep in 1971, researchers have found that GnRH and the GnRH receptor (GnRHR) are expressed in the hypothalamus–pituitary–gonadal axis and widely distributed in other organs, such as tumors (Limonta et al., 2012), pancreas (Chu et al., 2010), submandibular glands (Yao et al., 2003), and gastrointestinal tract (Sand et al., 2013a). This finding indicates that these organs synthesize GnRH, and are thus target organs of GnRH. Jin et al. (1998) reported changes with age in the coexistence and relative content of GnRH and its receptor in rat submandibular glands. However, with regard to postnatal developmental changes in GnRH and GnRHR in goat tissues and organs, Liu et al. (2014) only reported changes with age in the coexistence and relative content of GnRH and its receptor in goat pituitary. Postnatal developmental changes in GnRH and GnRHR in goat submandibular glands have not been reported to date.

Female Jining Grey goats are a high-fecundity variety from China and may achieve sexual maturity at the age of 90 days (Dall'Aglio et al., 2011). To determine the postnatal developmental changes in GnRH and GnRHR in goat submandibular glands, as well as their functional relationship with sexual maturity, the distribution and changes with age of GnRH- and GnRHR-positive cells in the submandibular glands of Jining Grey goats were investigated by immunohistochemical staining using the streptavidin–biotin peroxidase complex (SABC) technique.

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Materials and Methods

Sample collection

A total of 40 healthy mature female Jining Grey goats, aged 2 years to 3 years old and weighing 24 kg to 26 kg, were purchased from the goat breeding farm of Shan Dong Kelong Co., Ltd. All these ewes were treated with oestrus synchronization, and then artificial insemination (semen was obtained from male adult Jining Grey goats aged 2 years to 3 years old and weighing 30 kg to 32 kg). The goats were reared under natural conditions with water and feed *ad libitum*. Healthy and strong female goats aged 0 (birth), 30, 60, 90, 120, 150, and 180 days were randomly sacrificed (n = 6 for each age group). Goats were killed each month by severing the carotid artery. Both submandibular glands were immediately removed and stored in Bouin's fluid.

All animals and procedures used in this experiment were approved by the Animal Welfare Protection Committee of Shandong Agriculture University.

Antibodies and chemicals

GnRH (bs-0456R) and GnRHR (bs-1464R) antibodies were purchased from Beijing Biosynthesis Biotechnology Co. Ltd, China. Multiclonal antibodies were used because monoclonal antibodies were unavailable. Biotin-conjugated goat anti-rabbit IgG were obtained from Beijing Zhongshan Goldenbridge Biotechnology Co. Ltd, China (SPN-9001). Enhanced HRP-DAB chromogenic substrate kit was purchased from Tiangen Biotech (Beijing) Co., China. All other reagents were of analytical grade.

Immunohistochemical staining

Specimen preparation

The submandibular gland tissue fixed in Bouin's fluid was conventionally embedded in paraffin and cut continuously into about 5 μm thick slices. Three serial slices were selected at an interval of five and placed on a clean slide treated with polylysine for immune-histochemical and HE staining.

Immunohistochemistry

Immunohistochemistry (SABC method) was performed according to the procedure of Singh et al. (2011). Briefly, the paraffin sections were deparaffinized, rehydrated, and treated with 3% H2O2 for 30 min, and then treated with 10% normal goat serum at 37°C for 30 min. The sections were treated with GnRH and GnRHR antibodies (1:200) in a humid chamber at 4°C for 18 h, and then incubated with biotin-conjugated goat anti-rabbit IgG (1:200) in a constant temperature box (37°C) for 30 min. The sections were incubated with horseradish enzyme-labeled streptavidin avidin (1:200) in the constant temperature box (37°C). DAB was used for colour development of the sections. The sections were stained lightly with hematoxylin, dehydrated, cleared in xylene, and finally mounted.

The sections of the negative control group were incubated with normal goat serum instead of GnRH and GnRHR antibodies in step. Other steps were the same as previously described.

Observations and statistical analysis

Using the SABC technique, the positive cells exhibited a yellow or brown colour, whereas the negative control was colourless in immunostained sections. Manual counting was performed on three randomly selected sections for each experimental animal. Three fields were randomly selected and photographed at 10×40 high magnification for each section using an Olympus BX41 (DP25) microscopic imaging system. Positive cells were counted in each field with a photoshop counting tool. Statistical analysis was done by using SPASS 17.0. All the data analysis was reported as mean±SE.

Results

Distribution of GnRH- and GnRHR-positive cells in the submandibular glands of Jining Grey goats

Yellow or brown GnRH and GnRHR immunoreactive products were localized in the cytoplasm and the nuclei were blue; the negative control was colourless (Figs. 1H and Fig. 2H). On the day of birth, GnRH immunoreactive products are only distributed in the epithelial cells of the intercalated, striated, and interlobular ducts, and some connective tissues (Fig. 1A). At the age of 30 days, the products are widely distributed in the serous acinar cells and ductal epithelial cells at all levels (Figs. 1B-G). On the day of birth, GnRHR immunoreactive products are distributed sporadically in some ductal epithelial cells and connective tissues (Fig. 2A). After 30 days of age, the products are widely distributed in the ductal epithelium at all levels (Figs. 2A-G).

Developmental changes in GnRH- and GnRHR-positive cells in the submandibular glands of Jining grey goats

The number of GnRH-positive cells increased significantly in the submandibular glands of Jining Grey goats from birth to 90 days of age ($P<0.05$), peaked at 90 days of age, and then declined slightly, but not significantly ($P>0.05$) (Table 1). The number of GnRHR-positive cells increased significantly from 0 to 60 days of age ($P<0.05$), continued to increase from 60 to 90 days of age ($P>0.05$), peaked at 90 days of age, and exhibited no significant changes from 120 to 180 days of age ($P>0.05$) (Table 1).

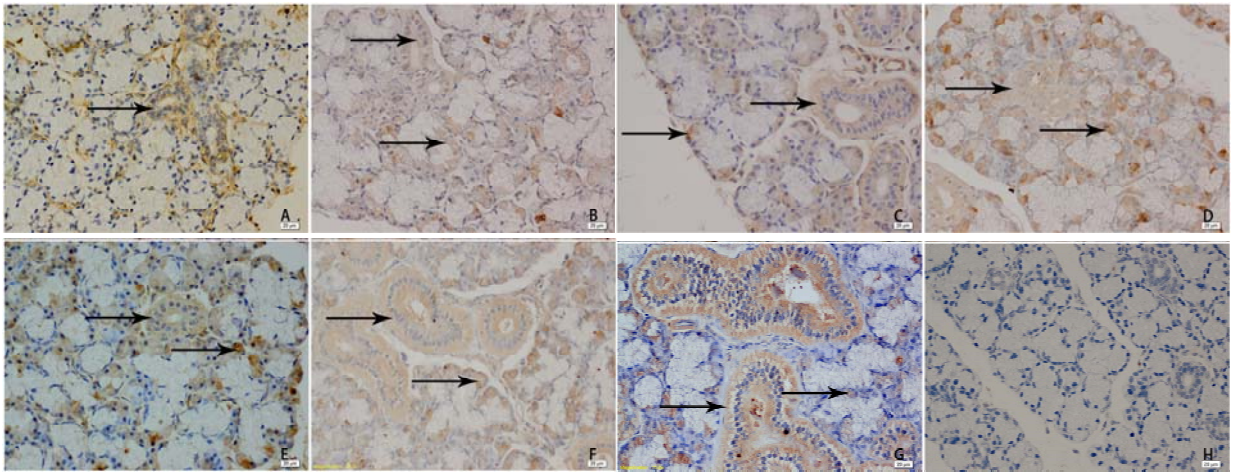

Fig. 1: Submandibular gland stained by immune-histochemistry (400×). A, B, C, D, E, F, G, H were 0, 30, 60, 90, 120, 150, 180 day-old. The arrow showed the GnRH positive cells.

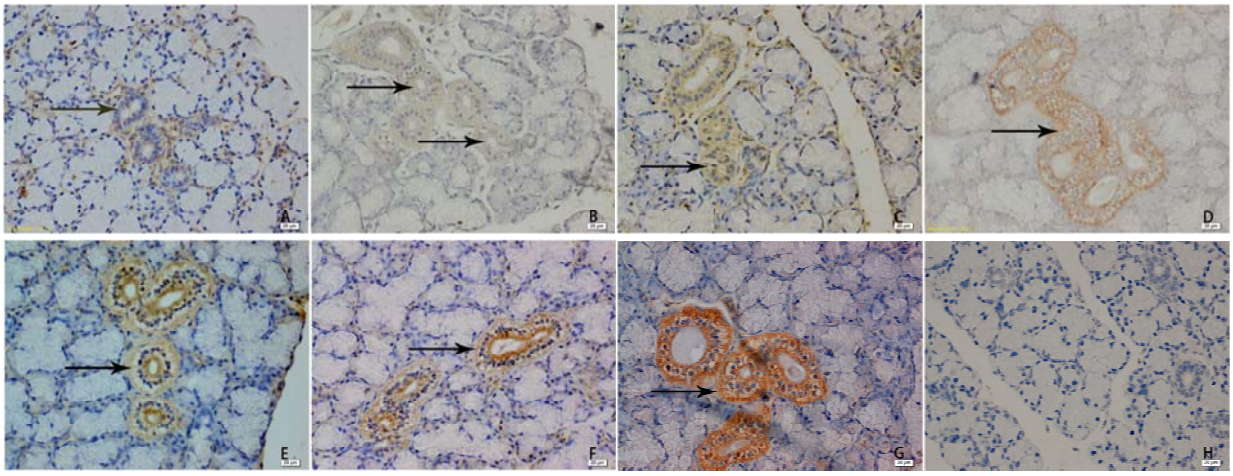

Fig. 2: Submandibular gland stained by immunohistochemistry. A, B, C, D, E, F, G, H was 0, 30, 60, 90, 120, 150, 180 day-old. The arrow showed t GnRHR positive cells.

Table 1: Age-related changes of GnRH & GnRHR positive cells in mandibular salivary gland (number/view)

	0 days	30 days	60 days	90 days	120 days	150 days	180 days
GnRH	35.6±3.8^{a}	80.1±4.9^{b}	95.1±6.5^{b}	129.9±5.7^{c}	116.4±5.0^{c}	113.5±5.9^{c}	113.1±3.8^{c}
GnRHR	34.2±1.6^{a}	58.0±0.8^{b}	72.4±3.0^{c}	77.3±1.7^{c}	73.4±2.5^{c}	70.3±6.3^{c}	73.3±4.5^{c}

Values with different lowercase superscript letters indicate $P<0.05$. Values with the same lowercase superscript letters indicate $P>0.05$.

Discussion

The submandibular gland is a mixed exocrine and endocrine gland that secretes more than 30 types of bioactive peptides (Zhang et al., 2014), such as epidermal growth factor and orexins (Kouidhi et al., 2012). Studies have found that GnRH regulates the functional activities of the pituitary–gonadal axis, as well as different biological functions in different organs, such as regulating the release of chorionic gonadotropin (Herrero et al., 2011), inhibiting the proliferation of cancer cells in tumors (Oktay et al., 2010), regulating the physiological functions of enteric neuronal cells in the digestive tract(Sand et al., 2013b), and regulating the endocrine function of pancreas through GnRHR (Chu et al., 2010).

This study found that GnRH and GnRHR immunoreactive products are localized in the cytoplasm and the nucleus was blue, consistent with the findings of Yao et al. (2003). GnRH-positive cells are distributed in both serous acinar and ductal epithelia in the submandibular glands of Jining Grey goats.

However, GnRH-positive cells are only distributed in the ductal epithelium at birth and found in the serous acinar epithelium at the age of 30 days. This trend is similar to the GnRH-positive cells in the submandibular glands of male SD rat (Jin et al., 1998). The present study suggests that the ability of the submandibular gland to secrete GnRH improves as the postnatal development continues. From birth to 180 days of age, GnRHR-positive cells are only found in the ductal epithelium in the submandibular glands of Jining Grey goats. This finding is different from that in rats, in which GnRHR-positive cells start to appear in serous acinar from approximately 12 days after birth (Jin et al., 1998). Whether or not this finding is related to the difference in animal species or gender, further investigation is necessary.

The structure and function of digestive organs and glands continue to improve with age after the birth of animals; however, time to maturation differs (Oktay et al., 2010). The physiological structure of neuroendocrine cells in acinar epithelium, ducts at all levels, and the ductal system of the submandibular gland gradually improves and matures during differentiation and development. The rates of GnRH and GnRHR-positive cells increase in the pituitary of Jining Grey goats from birth to 180 days of age (Liu et al., 2014). Jining Grey goats become sexually mature at 90 days of age. In this study, the number of GnRH- and GnRHR-positive cells in the submandibular glands of Jining Grey goats gradually increased from birth to 90 days of age (sexually mature), peaked at 90 days of age (sexually mature), and remained relatively stable after sexual maturation (120 to 180 days). Given these results, the present study suggests that the submandibular glands of Jining Grey goats become sexually mature at 90 days of age. This finding coincides with the postnatal developmental pattern of GnRH- and GnRHR-positive cells in the submandibular glands of SD rats (Jin et al., 1998). GnRH synthesized and secreted by the submandibular gland may regulate the development, maturation, and secretory function of the submandibular gland through the autocrine system, and GnRHR mediates this regulation.

Hypothalamus is a major organ that secretes GnRH (Morris et al., 2009). Increased GnRH secretion is a key factor for the initiation of sexual maturation (Zapatero-Caballero et al., 2004). The number of both GnRH- and GnRHR-positive cells in the submandibular glands of Jining Grey goats peaked at 90 days of age (sexually mature); the number of GnRH-positive cells was higher than that of GnRHR-positive cells. Bioactive substances are released into the ductal lumen, pass into the digestive tract along with the saliva, and then absorbed into the bloodstream by the stomach and intestine, having an important factor in regulating the physiological activities of various tissues and cells (Morris et al., 2009). Therefore, the present study suggests that GnRH secreted by the submandibular gland may be essential to the hypothalamus. It also suggested that the submandibular gland promoted and regulated its own development and maturation through autocrine signaling of GnRH and likely participated and contributed to the development and sexual maturation of the pituitary–gonadal axis through paracrine signaling of GnRH. However, this hypothesis needs further investigation.

In the postnatal development of Jining Grey goats, GnRH immunoreactive products were widely distributed in the serous acinar cells and ductal epithelial cells at all levels. GnRHR immunoreactive products were widely distributed in the ductal epithelium at all levels. The number of GnRH- and GnRHR-positive cells increased significantly in the submandibular glands of Jining Grey goats from 0 to 90 days of age, peaked at 90 days of age, and then declined slightly but not significantly. The GnRH in the submandibular gland has an important function in the postnatal development of Jining Grey goats.

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References

Chu C, Xu B and Huang W (2010) A study on expression of FSH and its effects on the secretion of insulin and glucagon in rat pancreas. Tissues Cell, 42: 370-375.

Dall'Aglio C, Zannoni A, Mercati F, Forni M, Bacci ML, Boiti C (2011) Differential gene expression and immune localization of the orexin system in the major salivary glands of pigs. Regul Pept, 172: 51-57.

Jin HS, Huang WQ, Zhang JS, Wen HZ, Zhang CL (1998) Immunohistochemical double-label and aged changes of GnRH and its receptor in submaxillary gland of rats. Acta Anat Sin 29: 94-97.

Kouidhi W, Desmetz C, Nahdi A, Bergès R, Cravedi JP, Auger J, May ME, Canivenc-Lavier, MC (2012) In utero and lactational exposure to low-dose genistein-vinclozolin mixture affects the development and growth factor mRNA expression of the submandibular salivary gland in immature female rats. Toxicol Pathol 40: 593-604.

Oktay K, Türkçüoğlu I, Rodriguez-Wallberg, KA (2010) GnRH agonist trigger for women with breast cancer undergoing fertility preservation by a romatase inhibitor/FSH stimulation. Reprod Biomed Online 20: 783-788.

Herrero L, Pareja S, Losada C, Cobo AC, Pellicer A, Garcia-Velasco JA (2011) Avoiding the use of human chorionic gonadotropin combined with oocyte vitrification and GnRH agonist triggering versus coasting: a new strategy to avoid ovarian hyperstimulation syndrome. Fertil Steril 95: 1137-1140.

Limonta P, Montagnani Marelli M, Mai S, Motta M, Martini L, Moretti RM (2012) GnRH receptors in cancer: from cell biology to novel targeted therapeutic strategies. Endocr Rev 33: 784-811.

Liu X, Wang SY, Huang LB, Hou YM, Shi YZ (2014) Distribution patterns and developmental changes of GnRH and GnRHR-immunopositive cells in the pituitary of Jining gray goats. Pak Vet J 34: 116-119.

Morris KE, Laurent CD, Hoeve RS, Forsythe P, Suresh MR, Mathison RD, Befus AD (2009) Autonomic nervous system regulats secretion of anti-inflammatory prohormone SMR from rat salivary glands Am J Physiol Cell Physiol 296: C514-524.

Sand E Bergvall M, Ekblad E, DAmato M, Ohlsson B (2013a) Expression and distribution of GnRH LH and FSH and their receptors in gastro-intestinal tract of man and rat Regul Peptides, 187: 24-28.

Sand E, Voss U, Harmmar O, Alm R, Fredrikson GN, Ohlsson B, Ekblad E (2013b) Gonadotropin-releasing hormone analog buserelin causes neuronal loss in rat gastrointestinal tract. Cell Tissue Res, 351: 521-534.

Singh P, Krishna A, Stridaran R, Tsutsui K (2011) Immunohistochemical localization of GnRH and RFamide-related peptide-3 in the ovaries of mice during the estrous cycle. J Mol Histol, 42: 371-381.

Yao B, Huang W, Huang Y, Chui Y, Wang Y, Li H, Pu R, Wan L, Zhang R (2003) A study on the localization and distribution of GnRH and its receptor in rat submaxillary glands by immune-histochemical, in situ hybridization and RT-PCR. Life Sci, 25: 2895-2904.

Zapatero-Caballero H, Sanchez-Franco F, Fernandez-Mendez C, García-San Frutos M, Botella-Cubells, LM, Fernandez-Vazquez G (2004) Gonadotropin-releasing hormone receptor gene expression during pubertal development of female rats. Biol Reprod, 70: 348-355.

Zhang Y, Li B, Fu Z, Li S (2014) Calcitonin is expressed in the submaxillary glands of rats. Bosn J Basic Med Sci, 14: 35-39.