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ER-α EXPRESSION IN THE HYPOTHALAMUS-PITUITARY-GONAD AXIS OF THE BACTRIAN CAMEL (*Camelus bactrianus*)

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ABSTRACT

The aim of this study was to examine the expression of estrogen receptor (ER-α) in the Bactrian camel's hypothalamus-pituitary-ovary (HPO) axis and its significance. Immunohistochemistry (SABC) and Image-Pro Plus 6.0 were used to study the expression of Estrogen Receptor (ER-α). Immunohistochemical analysis revealed that the expression of the ER-α was found in all of three organs in the hypothalamus-pituitary-ovary (HPO) axis. The ER-α immunopositive neurons were found in the main hypothalamus nuclei, which were stained in various degrees. A lot of ER-α immunopositive cells were observed in the pars intermedia of the adenohypophysis. Meanwhile, a small amount of ER-α immunopositive cells were found in the pars distalis near the pars intermedia. In contrast, no ER-α expression was observed in the neurohypophysis. The ER-α immunopositive production was detected in the follicular granules, interstitial gland, corpus luteum and mesenchyme of the ovary. These results suggested that estrogen of the camel acted not only on the sexual gland, but on the various areas of the central nervous system. Thus, we speculated that ER-α took part in the regulation of reproduction, endocrine and cognition in the brain.

Key words: Bactrian camel, estrogen receptor (ER), hypothalamus-pituitary-ovary (HPO) axis

Animals mainly regulate reproduction by Hypothalamus-pituitary-gonadal axis (HPG axis) (McGowan *et al,* 2008). As one of important hormones of regulating animal reproduction on the HPG axis, estrogen acts on target organs by means of estrogen receptor to regulate functions of target organs. It is proved that ER positive product existed in the wide ranges of some nucleus in the hypothalamus, hypophysis and some peripheric organs, such as ovary, spermary, vascular endothelium, smooth muscles, digestive tract, bone tissue, prostate, uterine, oviduct and so on (Gao *et al,* 2008; Takashi *et al,* 2007; Pedram *et al,* 2010; Elvira *et al,* 2009; Qian *et al,* 2011). Estrogen receptor has two subtypes: ER-α and ER-β, distributing different organs and playing different roles. ER-α mainly takes part in reproductive regulation, highly expressed in hypothalamus and brains regions of regulating reproduction, such as bed nucleus of the stria terminalis (BNST). (Corina *et al,* 2007; Deepak *et al,* 2010; Heather, 2007; Liu *et al,* 2008). So far, the study of the ER-α expression on the HPG axis in camels has not been reported. In this paper, we shall review the data obtained in our laboratory regarding the localisation of ER-α on the HPG axis, using the highly-sensitive streptavidin-biotin-peroxidase complex method (SABC). By mean of these results, the connection of different exsit of ER-α on the hypothalamus, pituitary and ovaries is detected. The study offers the morphological basis for the further study of the mechanism of ER-α action, partial rationale for the research of livestock reproductive physiology, animal reproduction, and thermatology.

Materials and Methods

Six aged female Bactrian camels, similar weight and health in clinic, were sampled from Allashan Right Banner of Inner Mongolia in China.

Sampling and preparing histotomy

After arteria carotis communis being chopped up, the heads of the camels were cut open to remove brains. Then the brains were cut sagittally, hypothalamuses were disported. The pituitary glands were taken out and were fixed in the 4% PFA. Ovaries were also removed and fixed in the 4% PFA. After having been fixed for 12h, tissues were fixed in the new 4% PFA 48h. Then tissues were dehydrated using graded ethanol, vitrificated by dimethylbenzene,

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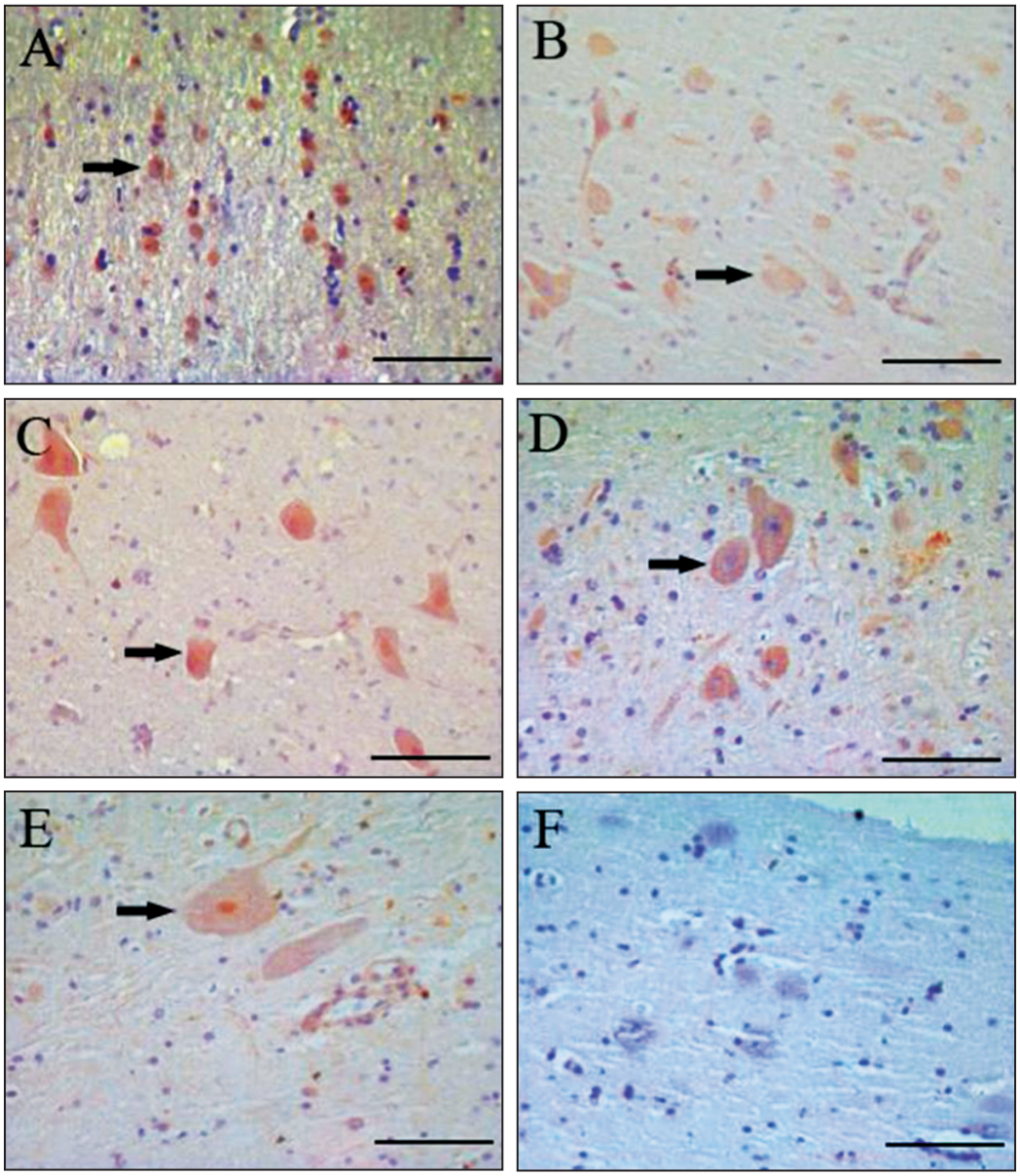

Fig 1. Immunohistochemical localisation of ER-α on the principal Nucleus in the hypothalamus of the camel (400×). **A.** ER-α immunoactive neurons in suprachiasmatic nucleus. **B.** ER-α immunoactive neurons in arcuate nucleus. **C.** ER-α immunoactive neurons in preoptic nucleus. **D.** ER-α immunoactive neurons in ventromedial nucleus. **E.** ER-α immunoactive neurons in posterior nucleus. **F.** None ER-α immunoreactivity neurons in control group. Scale bars, 100μm.

embedded in paraffin, cut into successive slices which were 5 μm thick.

Three suits of slices were attained from these three tissues of every camel for different experiments, including immunohistochemistry, Nissle staining (the hypothalamus nuclei positioning) and HE staining (the histological structure observation to pituitary and ovary), negative control. The hypothalamus nuclei positioning was based on relative atlas in the Systematic Anatomy (Blechman *et al,* 2007; Shimogori *et al,* 2010).

Immunohistochemistry procedures

(1) Sections were deparaffinised in xylene and dehydrated in graded ethanol. (2) Antigen retrieval: after being washed by PBS, the sections were boiled in citrate buffer (10 mM, pH 6.0) for 15 min. Then the buffer was boiled again, followed by a period of cooling. (3) Endogenous peroxidase was blocked by incubation in 0.3% hydrogen peroxide. (4) Following washes in PBS three times for 5 min, the sections were blocked for 1h with 1% bovine serum albumin. (5) The sections were incubated with

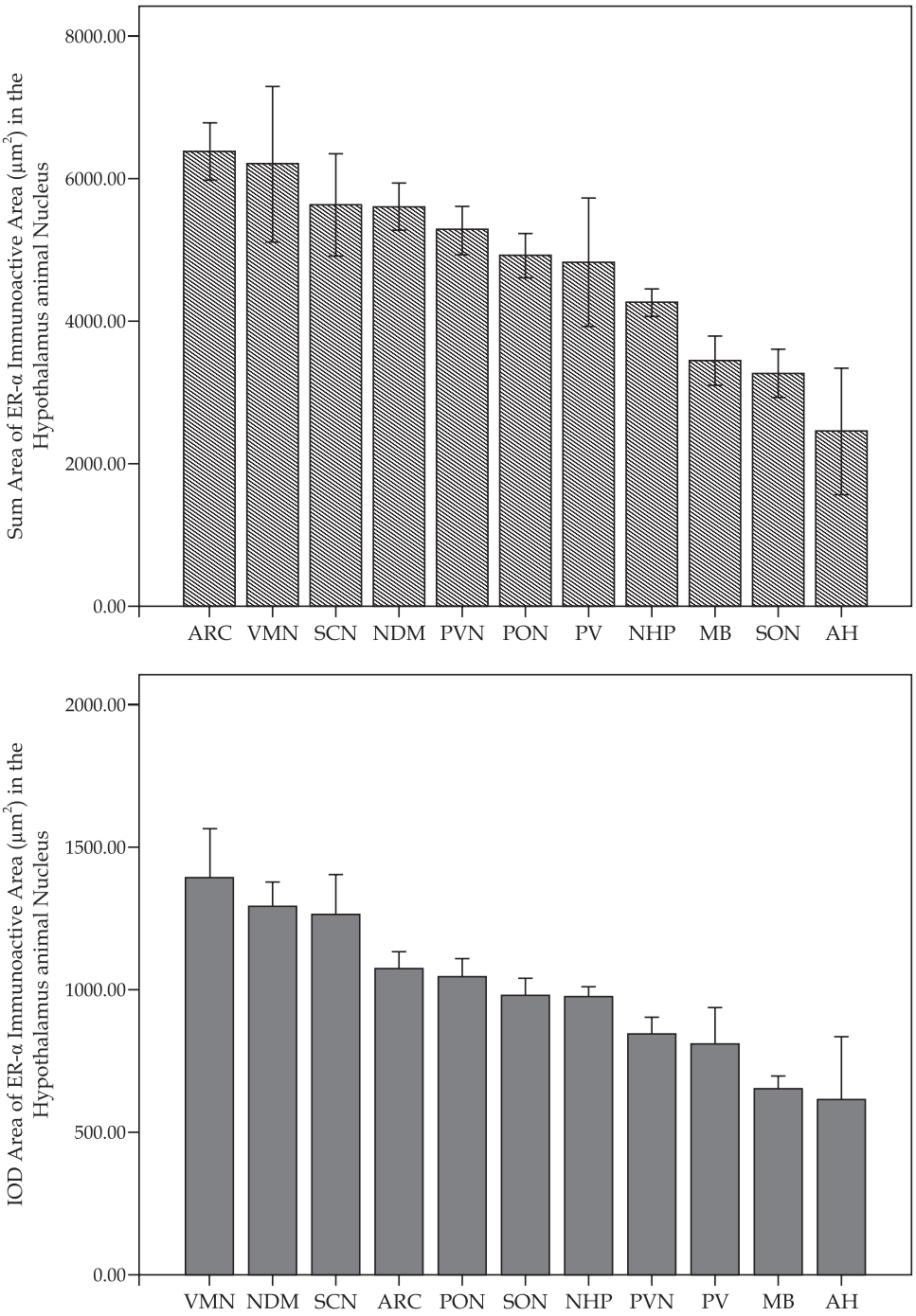

Fig 2. Sum area and IOD of ER-α Immunoactive area (μm^2) in the hypothalamus nucleus.

the primary antibody (ER-α monoclonal antibody, 1:200). (6) Following washes in PBS three times for 5 min, sections were incubated in the biotinylated goat anti-rabbit secondary antibody (1:200) for 2h at room temperature. (7) Following washes in PBS three times for 5 min, sections were incubated in the streptomycin-biotin-HRP complex for 2h at room temperature. (8) Following washes in PBS three times for 5 min, the 3, 3- diaminobenzidine (DAB) were used as chromogen. (9) Sections were then counterstained, dehydrated and coverslipped. For the negative control, sections were incubated in PBS (pH=7.4) instead of the primary antibody (Xu *et al,* 2010).

Observation and Statistical Analysis

The sections of hypothalamus, pituitary and ovary were viewed using the Olympus microscope. 5

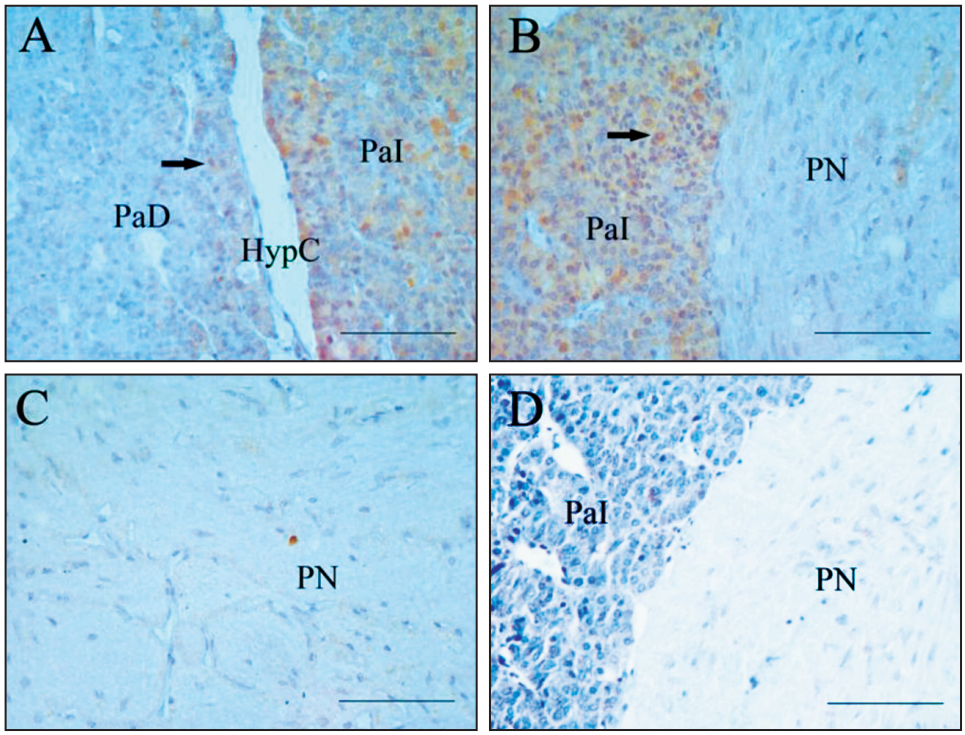

Fig 3. Immunohistochemical localisation of ER-α on the pituitary of the camel. **A.** ER-α immune active in pars distails and pars intermedia; **B.** ER-α immune active in neurohypophysis and pituitary pars intermedia; **C.** ER-α immune active in neurohypophysis; **D.** Negative control. PaD: Pars distails, PN: Neurohypophysis; PaI: Pituitary pars intermedia. HypC : hypophysial cleft, Scale bars=100µm.

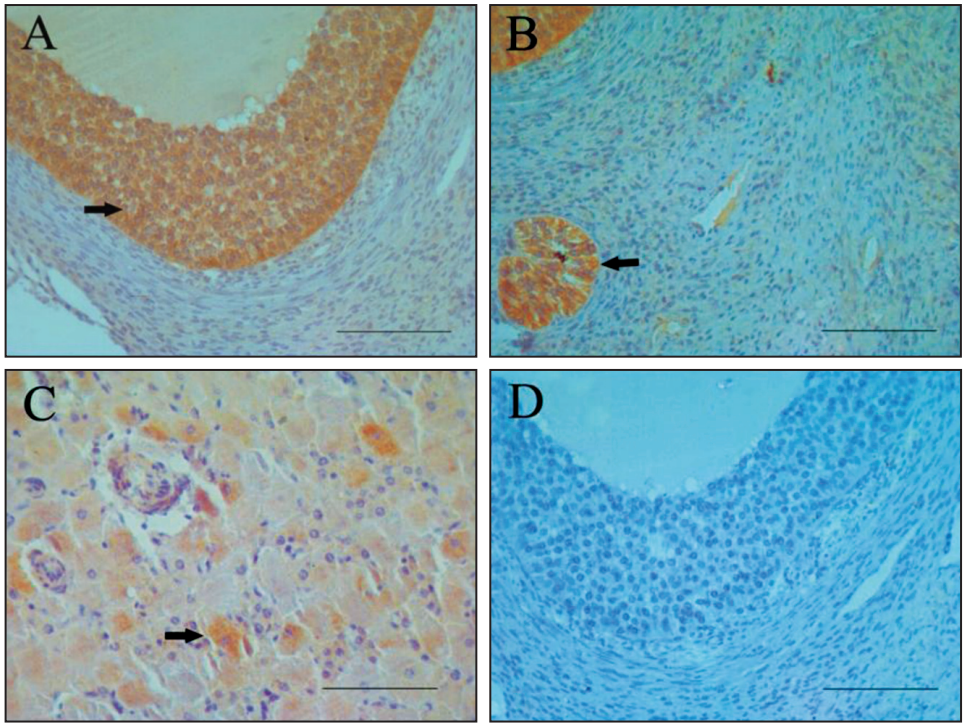

Fig 4. Immunohistochemical localisation of ER-α on the ovary of the camel. **A.** ER-α immune active production in ovarian follicle; **B.** ER-α immune active production in interstitial gland; **C.** ER-α immune active production in corpus luteum verum; **D.** None ER-α immune active production in ovarian follicle. Scale bars=100µm.

fields of vision from two different sections in different areas of each sample using Motic electric microscope. The optical density of ER positive product in the 60 pictures from different areas of 6 camels were analysed by Image-Pro Plus 6.0. All data were assessed for statistically significant differences via a one-way ANOVA and t test. All statistical analyses were carried out using SPSS16. 0.

Results

Results showed that ER-α expression was observed in hypothalamus, pituitary and ovary, but varying in different areas. Especially, the expressing areas and integral optical density (IOD) of ER positive product in the hypothalamus nuclei had more obvious difference.

The distribution of ER-α positive neurons and nerve fibres in the hypothalamus

Examination of Immunohistochemistrical sections (Sagittal plane) revealed that the neurons were unequal positive for ER-α expression in the nucleus, cytoplast and protuberance, various from brown to brownish yellow. In addition, the background was colourless or light brown. On the basis of different nucleius, the styles of neurons were different, including multipolar neurons and two polar neurons. These neurons existed together or dispersedly, having the irregularly round, oval, spindle, triangle somas, etc. ER-α positive nerve fibres were shallow brown filaments or beads in shape between neurons. In accordance with staining intensity of reaction particles in the cytoplasm, the ER-α expression was divided into three grades-strong, moderate and weak expressions. The cytoplasm of strong positive cells was stained in nigger-brown, because of brownish yellow particles fusing together. As for the weakly positive cells, they were slightly stained, approaching to the background. The profiles of these cells were unclear. Of course, the features of moderately positive cells were somewhere in-between. According to the result that the negative controls were stained in blue and had no positive products, the method used in the study was reliable.

In the hypothalamus, ER-α positive neurons existed in the eleven nuclei, such as anterior hypothalamic nucleus (AH), mamillary nucleus (MB), ventromedial nucleus (VMN), arcuate nucleus (ARC), periventricular nucleus (PVN), dorsomedial nucleus (NDM), posterior nucleus (NHP), suprachiasmatic nucleus (SCN), supraoptic nucleus (SON), paraventricular nucleus (PV) and preoptic nucleus (PON). Remarkably, the level of expressions in the different nuclei was variable (Fig 1). Optical density analysis and multiple comparison data analysis revealed that the expressed areas and integral optical density (IOD) of ER positive product of ARC, NDM, SCN and VMN were larger than other nuclei ($P< 0.001$) (Fig 2).

ER-α expression in pituitary

In the pars intermedia of the adenohypophysis, the most gland cells were stained in nigger-brown, being strong positive. In the positive cells, most of which were round, the reaction particles mainly were observed in the cytoplasm (Fig 3A and B). It was analysed that the positive area was 13,072 μm^2 and IOD was 2,798. A small amount of ER-α immunopositive cells were found in the pars distalis of the adenohypophysis. One part of these positive cells existed round the sinusoid capillary and another part unevenly scattered. In contrast, no ER-α expression was observed in the neurohypophysis (Fig 3B and C).

ER-α expression in ovary

ER-α immunopositive production was detected in the follicular granules, interstitial gland, corpus luteum and mesenchyme of the ovary. In the granular cell layers, a mass of ER-α positive cells were found, which mainly were round and round to oval. Majority of these positive cells were strong positive and the reaction particles mainly were observed in the cytoplasm (Fig 4A). According to the statistic data, the positive area had amounted to 19,576μm^2. Meanwhile, the IOD had also reached to 6,514. A lot of positive cells were also examined in the interstitial gland (Fig 4B), whose positive area was 4858μm2 and IOD was 1,679. In the case of corpus luteum, ER-α strongly positive cells were observed in the corpus luteum peripheral. However, weakly positive cells were found uniform distribution in the whole corpus luteum (Fig 4C). Besides, some positive cells were detected in the mesenchyme of the ovary (Fig 4).

Discussion

The (ER-α) expression was found in all the three tissues in the hypothalamus-pituitary-ovary (HPO) axis.

According to this study, ER-α positive neurons existed in the eleven nuclei. ER positive cells of ARC, NDM, SCN, SON, PON and VMN were more than other nuclei, by means of optical density analysis (the expressed areas and integral optical density) and multiple comparison data analysis. Hence, it is

construed that ER-α neurons of ARC, NDM and SCN may play a dominant role in regulating reproduction.

The ER-α expression in PVN and SON suggested that ER-α partly mediated the secretion of oxytocin neurons and pitressin neurons activities in PVN and SON, which was in accordance with the result from Zhao and Qing (2005). The fact that ER-α positive cells existed widely in the female hypothalamus indicated some relative conclusions. The hypothalamus was one of the important target organs of estrogen. ER-α participated in or regulated neuroendocrine activities of the hypothalamus. Estrogen played a role in neuron growing development and differentiation, hormone secretion, neurotransmitter synthesis and release and sexual behaviour and so on (Sergei *et al,* 2007). ER-α positive product existed both in cell nucleus and cytoplasm, which was in accordance with the results from Blaustein *et al* (1992) and Mei and Zhang (2009).

A lot of ER-α immunopositive cells were observed in the pars intermedia of the adenohypophysis. Meanwhile, a small amount of ER-α immunopositive cells were found in the pars distalis near the pars intermedia. In contrast, no ER-α expression was observed in the neurohypophysis. Eosinophil was main cells around the pars intermedia. Those Eosinophil cells just right were cellgen of prolactin cells and ER-α immunopositive cells existed around prolactin cells. It was the same with the result from Zhang and Cai (2010) that ER-α play a main role in regulating the lactation central in the hypophysis. In addition, Pelltier and Liao (1988) revealed that ER was relative to the estrogen feedback inhibition of gonadotropin secretion and affected the development of anterior pituitary by mediating estrogen.

ER-α was also found in the ovaries, especially in the granular cells and interstitial gland. Besides, the expression in the corpus luteum was similar with interstitial cells. Above results agreed with the studies from Greene *et al* (1984) in the mammal. Estrogen was mainly produced by the granular cells (Zhan *et al,* 2005) from which the conjecture could be got that ER-α which was highly expressed in the granular cells was involved in the estrogen production. Similarly, on the basis of the facts that the interstitial gland had strongly positive reaction products and could secrete estrogen (Salvetti *et al,* 2009; John *et al,* 2006; Xiong *et al,* 2012), it could be guessed that ER-α from the interstitial gland also took a part in the estrogen production. A small amount of positive cells were observed in the mesenchyme. John *et al* (2006) found that estrogen inhibited the development and functions of the mesenchymale cells via ER-α.

The corpus luteum can synthetise corpus luteum hormone, oxytocin and norepinephrine and so on. In turn, those hormones affect the function of corpus luteum by with the feedback mechanism. Especially, oxytocin directly acts on luteal cells by means of oxytocin receptors on the luteal cellular membranes (Okuda *et al,* 1992). Mature corpus luteum may regulate the synthesis of thyroxine and luteal hormone through autocrine and paracrine (Mutinati *et al,* 2010). Hence, it was envisaged that in the corpus luteum estrogen interacted with ER-α and cooperated with oxytocin, norepinephrine and luteal hormone to regulate hormonal balance and maintain internal environment homeostasis.

Earlier studies indicated that female ER-α gene knockout mice were sterile. The reason was that the development of follicles would stop in preovulation stage and then what happened was not ovulation, but atresia or haemorrhagic cyst (Judith *et al,* 2005; John *et al,* 2004). ER-α expression in the hypothalamus and hypophysis, possibly resulted in ER-α gene deletion and disappearance of negative feedback E2 on the hypothalamus – hypophysis axis. Hence, LH was promoted to release, which led to the ascent of LH in the serum. The increasing secretion of LH would further trigger the ovulation obstacles (John *et al,* 2004; Zhan *et al,* 2018). Thus, it can be guessed that ER-α regulated ovulation by means of negative feedback loop.

The ER-α expression was observed in all the three tissues in the hypothalamus-pituitary-ovary (HPO) axis. The result suggested that estrogen of the camel not only acted on the sexual gland, but also on the various areas of the central nervous system. Thus, it was construed that ER-α took part in the regulation of reproduction, endocrine and cognition in the brain.

The female camel is a kind of induced ovulation animal, and the ovulation inducing factor (OIF) in the sperm of the male camel (Chen and Yun, 1980). The preliminary research of the mechanism of the OIF in the female camel was done by Pan Guangwu using the radiation mark. The result suggested that OIF of the camel acted on the pituitary (Pan and Chen, 2002; Pan and Xie, 2003), but the particular pathway of the OIF regulating the pituitary to control the ovulation remain unclear. According to this study, ER-α immune active production existing on the Hypothalamus-Pituitary-Gonad Axis of Camel was similar with other non-induced ovulation

animals such as goats, so clarifying the reproductive mechanism of the female camel depends on a deep research (Zhu *et al,* 2019; Mijidodorj *et al,* 2012; Zhou *et al,* 2019).

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References

Blaustein JD, Lehman MN, Turcotte JC and Greene G (1992). Estrogen receptors in dendrites and axon terminals in the guinea pig hypothalamus. Endocrinology 131(1):281-290.

Blechman J, Borodovsky N, Eisenberg M, Nabel-Rosen H, Grimm J and Levkowitz G (2007). Specification of hypothalamic neurons by dual regulation of the homeodomain protein Orthopedia. Development 134:4417-4426.

Chen BH and Yun ZX (1980). Reproductive patreroductive pattern of the Bacttrian camel—Section 2. Sexual activities of the camel. Chinese Journal of Animal and Veterinary Sciences 2:65-76.

Corina E Andreescu, Bogdan A Milojkovic, Elize D Haasdijk, Piet Kramer, Frank H De Jong, Andrée Krust, Chris I De Zeeuw, Marcel TG De Jeu (2007). Estradiol improves cerebellar memoryformation by activating estrogen receptor β. The Journal of Neuroscience 27(40):10832-10839.

Deepak P Srivastava, Kevin M Woolfrey and Feng Liu (2010). Estrogen receptor βactivity modulates synaptic signaling and structure. The Journal of Neuroscience 30(40):13454-13460.

Elvira Haas, Indranil Bhattacharya, Eugen Brailoiu, Marlen Damjanović, G Cristina Brailoiu, Xin Gao, Laurence Mueller-Guerre, Nicole A Marjon, André Gut, Roberta Minotti, Matthias R Meyer, Kerstin Amann, Emerita Ammann, Ana Perez-Dominguez, Michele Genoni, Deborah J Clegg, Nae J Dun, Thomas C Resta, Eric R Prossnitz and Matthias Barton (2009). Regulatory role of G protein-coupled estrogen receptor for vascular function and obesity. Circulation Research 104:288-291.

Gao H, Fält S, Sandelin A, Gustafsson JA and Dahlman-Wright K (2008). Genome-wide identification of estrogen receptorα-binding sites in mouse liver. Molecular Endocrinology 22(1):10-22.

Greene GL, Sobel NB, King WJ and Jensen EV(1984). Immunochemical studies of estrogen receptors. Journal of Steroid Biochemistry 20(1):51-56.

Heather A Harris (2007). Estrogen receptor-β: recent lessons from *in vivo* studies. Molecular Endocrinology 21(1): 1-13.

John F Couse, Mariana M Yates, Karina F Rodriguez, Jo Anne Johnson, Donald Poirier, Kenneth S Korach (2006). The intraovarian actions of estrogen receptor-alpha are necessary to repress the formation of morphological and functional Leydig-like cells in the female gonad. Endocrinology 147(8):3666-3678.

John F Couse, Mariana M Yates, Ryan Sanford, Abraham Nyska, John H Nilson and Kenneth S Korach (2004). Formation of cystic ovarian follicles associated with elevated luteinising hormone requires estrogen receptor-β. Endocrinology 145(10):4693-4702.

Judith MA, Emmen, John F Couse, Susan A Elmore, Mariana M Yates, Grace E Kissling and Kenneth S Korach (2005). In vitro growth and ovulation of follicles from ovaries of estrogen receptor (ER) α and ERβ null mice indicate a role for Erβ in follicular maturation. Endocrinology 146(6):2817-2826.

Liu Feng, Day Mark, Muñiz Luis C, Bitran Daniel, Arias Robert, Revilla-Sanchez Raquel, Grauer Steve, Zhang Guoming, Kelley Cody, Pulito Virginia, Sung Amy, Mervis Ronald F, Navarra Rachel, Hirst Warren D, Reinhart Peter H, Marquis Karen L, Moss Stephen J, Pangalos Menelas N and Brandon Nicholas J (2008). Activation of estrogen receptor-β regulates hippocampal synaptic plasticity and improves memory. Nature Neuroscience 11:334-343.

McGowan BM, Stanley SA, Donovan J, Thompson EL, Patterson M, Semjonous NM, Gardiner JV, Murphy KG, Ghatei MA and Bloom SR. (2008). Relaxin-3 stimulates the hypothalamic-pituitary-gonadal axis. AJP - Endo August 295(2):278-286.

Mei CX and Zhang JQ (2009). Advance in study of estrogen receptor. Chemistry of life, 2010 30(4):590-594.

Mijiddorj Tselmeg, Kanasaki Haruhiko, Purwana Indri N, Oride Aki, Sukhbaatar Unurjargar and Miyazaki Kohji (2012). Role of neurokinin B and dynorphin A in pituitary gonadotrophand somatolactotroph cell lines. Endocrine Journal 59(7):631-640.

Mutinati M, Desantis S, Rizzo A, Zizza S, Ventriglia G, Pantaleo M and Sciorsci RL (2010). Localisation of thyrotropin receptor and thyroglobulin in the bovine corpus luteum. Animal Reproduction Science 118(1):1-6.

Okuda K, Miyamoto A, Sauerwein H, Schweigert FJ and Schams D (1992). Evidence for oxytocin receptors in cultured bovine luteal cells. Biology of Reproduction 46(6):1001-1006.

Pan GW and Chen YF (2002). Iodination in the radioisotope of the ovulation-inducing bioactive protein in Bactrian camel. China Herbivores (04):22-24.

Pan GW and Xie QG (2003). Observation and analysis of hypophysis section in Bactrian camel. China Herbivores, (05):7-8.

Pedram A, Razandi M, O'Mahony F, Lubahn D and Levin ER (2010). Estrogen receptor-β prevents cardiac fibrosis. Molecular Endocrinology 24(11):2152-2165.

Pelletier G and Liao N (1988). Distribution of estrogen receptors in the rat tiruitary as studied by in situ hybridisation. Molecular and Cellular Endocrinology 56(1-2):29-33.

Qian Wu, Ken Chambliss, Michihisa Umetani, Chieko Mineo, and Philip W. Shaul (2011). Non-nuclear estrogen

receptor signaling in the endothelium. The Journal of Biological Chemistry 286:14737-14743.

Salvetti NR, Baravalle C, Mira GA, Gimeno EJ, Dallard BE, Rey F and Ortega HH (2009). Heat shock protein 70 and sex steroid receptors in the follicular structures of induced ovarian cysts. Reproduction in Domestic Animals 44(5):805-814.

Sergei Musatov, Walter Chen, Donald W Pfaff, Charles V Mobbs, Yang XJ, Deborah J Clegg, Michael G Kaplitt, Sonoko Ogawa (2007). Silencing of estrogen receptor α in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. PNAS 104(7):2501-2506.

Shimogori T, Lee DA, Miranda-Angulo A, Yang Y, Wang H, Jiang L, Yoshida AC, Kataoka A, Mashiko H, Avetisyan M, Qi L, Qian J and Blackshaw S (2010). A genomic atlas of mouse hypothalamic development. Nature Neuroscience 13:767-775.

Takashi Nakamura, Yuuki Imai, Takahiro Matsumoto, Shingo Sato, Kazusane Takeuchi, Katsuhide Igarashi, Yoshifumi Harada, Yoshiaki Azuma, Andree Krust, Yoko Yamamoto, Hiroshi Nishina, Shu Takeda, Hiroshi Takayanagi, Daniel Metzger, Jun Kanno, Kunio Takaoka, T John Martin, Pierre Chambon and Shigeaki Kato (2007). Estrogen prevents bone loss via estrogen receptor[alpha] and induction of fas ligand in osteoclasts. Cell 130(5):811-823.

Xiong DS, Lu YZ and Dan YY (2012). Study on the coexistence of estrong receptor with PRL,FSH and LH in adenohypophysis of pregnancy goats. Progress in Veterinary Medicine 33(10):33-36.

Xu YQ, Wang JL, Song GQ and Shao BP (2010). Expression of GnRH in the hypothalamic-pituitary-ovarian axis of the pregnant cattle. Acta Veterinaria et Zootechnica Sinica 41(12):1649-1654.

Zhan M, Lian XL and Liu JN (2018). Overview of experimental study on regulation effect of different exterior factors on hypotha-lamic-pituitary-ovarian axis. Gansu University Of Chinese Medicine 35(03):92-96.

Zhan XQ, Wang XZ and Zhang JY (2005). Progress on estrogen receptor and female reproduction. Progress In Veterinary Medicine 26(12):35-39.

Zhang JQ and Cai WQ (2010). Research progress of brain estrogen receptor. Journal of Regional Anatomy and Operative Surgery 18(1):1-2.

Zhao HY and Qing SZ (2005). ER immunoreactivity in the hypothalamus-pituitary-gonad axis of young goats. Chinese Journal of Veterinary Science 25(1):46-48.

Zhou KR, He YQ and Ge WB (2019). Study on PRLR gene expression and tissue distribution in pituitary and ovary of Ganjia Tibetan sheep (Ovis aries) during estrous cycle. Journal of Agricultural Biotechnology 27(10):1894-1900.

Zhu YX, Liu J and Ai Y (2019). Comparative transciptome analysis of lactation pituitary gland in yak. China Animal Husbandry and Veterinary Medicine 46(3):824-831.