Kafkas Univ Vet Fak Derg
26 (1): 65-70, 2020
DOI: 10.9775/kvfd.2019.22274

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Expression of Follicle-stimulating Hormone, Luteotropic Hormone, Estrogen and Progesterone Receptors in Ovary, Oviduct and Endometrium After Estrus Induction in Ewe

Ling YANG [1,†,a] Xiaolei YAO [1,4,†] Xiaoyong CHEN [2,3,†]
Shengqin ZANG [1] Hongxin SUN [3] Yue LIU [3] Weitao DUN [3]

[1] *This work was supported by the Special Fund for Ovine Industry Innovation Team of Modern Agricultural Industry Technology System in Hebei Province (HBCT2018140201, HBCT2018140403)*

† *Ling YANG, Xiaolei YAO and Xiaoyong CHEN contribute equally to this work*

[1] Department of Animal Science, College of Life Sciences and Food Engineering, Hebei University of Engineering, Handan 056021, CHINA

[2] College of Animal Science and Technology, Hebei Agricultural University, Baoding 071000, CHINA

[3] Hebei Institute of Animal Science and Veterinary Medicine, Baoding 071000, CHINA

[4] Huaxing Pet Food Company Limited, Xingtai 054400, CHINA

[a] ORCID: 0000-0003-4385-0024

Article ID: KVFD-2019-22274 Received: 12.05.2019 Accepted: 07.08.2019 Published Online: 07.08.2019

How to Cite This Article

Yang L, Yao X, Chen X, Zang S, Sun H, Liu Y, Dun W: Expression of follicle-stimulating hormone, luteotropic hormone, estrogen and progesterone receptors in ovary, oviduct and endometrium after estrus induction in ewe. *Kafkas Univ Vet Fak Derg,* 26 (1): 65-70, 2020. DOI: 10.9775/kvfd.2019.22274

Abstract

Induction of estrus is an effective management tool for increasing the pregnancy rate in ewes, and there is a long estrus interval after lambing in spring in Small-tail Han ewe. In this study, norgestrel releasing intra-vaginal devices (PRID) and PRID + pregnant mare serum gonadotrophin (PMSG) were used for estrus induction in Small-tail Han ewe, and expression of follicle-stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHR), estrogen receptor (ER) and progesterone receptor (PGR) mRNA in the ovarial stroma, oviduct ampulla and endometrium was analyzed by quantitative real-time PCR. The results showed that the estrus-induction with PRID and PRID + PMSG almost had the same effects on increasing the percentage of animals in estrus and conception rate of the ewes, and the estrus-induction had no significant effect on the expression of LHR, ER and PGR mRNA in the oviduct ampulla, ovarial stroma and endometrium, and FSHR in the ovarial stroma and endometrium. Furthermore, the estrus-induction could significantly decrease the expression of FSHR mRNA in the ovine oviduct ampulla ($P<0.05$). Therefore, it was suggested that down-regulation of FSHR in the oviduct ampulla after estrus induction may be helpful for fertilization and early embryo development.

Keywords: *Ewe, Endometrium, Estrus induction, Ovarial stroma, Oviduct ampulla, Receptor*

Koyunlarda Östrus İndüksiyonu Sonrası Ovaryum, Oviduct ve Endometriyumda Folikül Uyarıcı Hormon, Luteotropik Hormon, Östrojen ve Progesteron Reseptör Ekspresyonu

Öz

Östrusun indüksiyonu, koyunlarda gebelik oranını artırmak için etkili bir yönetim aracıdır ve kısa kuyruklu Han koyununda ilkbaharda kuzulamanın ardından uzun bir östrus aralığı söz konusudur. Bu çalışmada, kısa kuyruklu Han koyunlarında östrus uyarımı için intravaginal salınan norgestrel (PRID) ve PRID + gebe kısrak serum gonadotropini (PMSG) kullanıldı. Ovidukt ampullası, ovaryum stroması ve endometriyumdaki folikül uyarıcı hormon reseptörü (FSHR), luteinleştirici hormon reseptörü (LHR), östrojen reseptörü (ER) ve progesteron reseptörü (PGR) mRNA ekspresyonu düzeyleri kantitatif real-time PCR ile analiz edildi. Sonuçlar, koyunlarda PRID ve PRID+PMSG ile östrus indüksiyonunun, östrustaki hayvan yüzdesi ve konsepsiyon oranını arttırmada neredeyse aynı etkilere sahip olduğunu ve östrus indüksiyonunun ovidukt ampullası, ovaryum stroması ve endometriyumdaki LHR, ER ve PGR mRNA ekspresyonu ile ovaryum stroması ve endometriyumdaki FSHR üzerinde anlamlı bir etkisi olmadığını gösterdi. Bunun yanısıra, östrus indüksiyonunun, koyunlarda ovidukt ampullasında FSHR mRNA'nın ekspresyonunu önemli ölçüde azaltabileceği belirlendi ($P<0.05$). Bu nedenle, östrus indüksiyonundan sonra ovidukt ampullasında FSHR'nin down-regülasyonunun, fertilizasyon ve erken embriyo gelişimi için faydalı olabileceği düşünüldü.

Anahtar sözcükler: *Koyun, Endometriyum, Östrus indüksiyonu, Ovaryum stroması, Ovidukt ampullası, Reseptör*

İletişim (Correspondence)
+86 312 5062050
342317981@qq.com (X. Chen); 1035853426@qq.com (W. Dun)

INTRODUCTION

There are long-day and short-day breeders in seasonally reproductive animals, and ewe is a short-day breeder. Small-tail Han sheep is known as a year-round breeder in Northern China. However, there is a long estrus interval after lambing in spring in Small-tail Han sheep. The induction of estrus is an effective management tool for increasing the pregnancy rate in ewes [1,2], which can control the reproductive process and improve efficiency of extensive production in sheep. Induction of estrus with an intravaginal progesterone-releasing device (IPRD) and GnRH can enhance pregnancy rates in adult ewes, and ewe lambs out of the breeding season [3]. Exogenous gonadotrophins, such as pregnant mare serum gonadotrophin (PMSG), are used to stimulate follicular growth, which increase the estrus response and ovulation rate [4,5], and also lead to a tighter synchrony of ovulation in out-of-season estrus induction and cyclic ewes [6,7].

Pulsatile gonadotropin-releasing hormone (GnRH) is secreted into the hypophyseal portal blood system from hypothalamic neurons. GnRH activates its receptors on the anterior pituitary, which leads to the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [8,9]. FSH and LH exert divergent effects and regulate essential reproductive processes, such as gametogenesis, steroidogenesis, and ovulation [10], which are through binding to the FSH receptor (FSHR) and LH receptor (LHR) on the granulosa cell surface in the gonads, uterus, breasts, and other tissues, respectively [11,12]. Steroidogenesis includes synthesis of the estrogen, progesterone, and androgens. The expression of estrus behavior is related with the peripheral blood estradiol concentrations during estrus in cows, and progesterone also exerts a priming role for the full display of estrus behavior [13]. In general, progesterone blocks the sexual behavior induced by estradiol in female sheep [14]. Estrogen and progesterone regulate the uterine endocrine and paracrine signals through their receptors during the estrus cycle and pregnancy in sheep [15,16]. There was no study on PMSG-estrus induction during estrus interval after lambing in spring in Small-tail Han sheep. Therefore, the present study was aimed at the effects of estrus induction on reproductive efficiency, and expression of FSHR, LHR, estrogen receptor (ER) and progesterone receptor (PGR) in ovarial stroma, oviduct ampulla and endometrium after estrus induction in Small-tail Han sheep.

MATERIAL and METHODS

Animals and Experimental Design

The experiment was carried out under natural conditions during February (a long estrus interval after lambing for Small-tail Han ewes) in Boading, Hebei province, China, and all procedures were approved by the Hebei University of Engineering Animal Care and Use Committee. A total of 70 healthy ewes after weaning, ranging in age from 1.5 to 2 years, were used in this study. During the trial, ewes were group-housed in straw-bedded pens with hay fed ad libitum and supplemented daily according to NRC (2007) based on the nutritional requirements. Norgestrel releasing intravaginal devices (PRID) and PMSG were from Sansheng Biological Technology Co., Ltd., Ningbo, China. PRID was inserted into vagina of the ewe for 14 days. In group 2 (n = 27), 250 IU of PMSG was administered 1 day before sponge withdrawal, but no PMSG was administered in group 1 (n = 27). In the control group (n = 16), ewes were not treated with PRID and PMSG. All of the ewes were monitored daily for estrus using vasectomized rams after the PRID withdrawal, and mated twice with intact rams at a 12-h interval after the detection of sexual receptivity. The pregnancy of ewe was evaluated by transrectal ultrasonography on day 40 post coition. Percentage of animals in estrus is the number of ewes showing estrus/number of total treated ewes in each group x 100, and conception rate is the number of pregnant ewes/number of mated ewes in each group x 100. Ovarial stroma, oviduct ampulla and endometrium were sampled from the ewes of showing estrus and the ewes without estrus (n = 6 for each group) after the PRID withdrawal for 72 h at a local slaughterhouse. The samples were frozen in liquid nitrogen for quantitative real-time PCR (qRT-PCR) assay.

RNA Extraction and qRT-PCR Assay

The samples of ovarial stroma, oviduct ampulla and endometrium were crushed into fine powders in liquid nitrogen, and the powders were digested in TRIzol (Tiangen Biotech Co., Ltd., Beijing, China), and the total RNA was extracted according to the manufacturer's instructions. The cDNA was synthesized with an All-In-One RT MasterMix (with AccuRT Genomic DNA Removal Kit; abm Biotech Co., Ltd., Jiangsu, China), and an EvaGreen qPCR MasterMix-No Dye (abm Biotech) was used for qRT-PCR. The primer sequences of FSHR, LHR, ER, PGR and GAPDH were designed and synthesized by Shanghai Sangon Biotech Co., Ltd. *(Table 1)*. The $2^{-\Delta\Delta Ct}$ analysis method was used to calculate the relative expression values for the qRT-PCR assay, with GAPDH as the endogenous control [17].

Statistical Analysis

The data were subjected to least-squares ANOVA using the general linear models procedures of the Statistical Analysis System Package version 9.1 for Windows (SAS Institute, Cary, NC, USA). Experimental sample groups consisted of six biological replicates for the qRT-PCR assay. Groups were considered significantly different at $P<0.05$.

RESULTS

It is showed in *Table 2* that there was no ewe in estrus in control group, and the majority of ewes came into estrus between 24 and 48 h after PRID removal. It was obvious that estrus induction with PRID or PRID + PMSG

Table 1. *Primers used for qRT-PCR*

Gene	Primer	Sequence	Size (bp)	Accession Numbers
FSHR	Forward	TGTCTTGGAAGCGATAGAGGC	108	NM_001009289.1
	Reverse	GGGAAGGTTCTGGAAGGCAT		
LHR	Forward	CAGCAAGGAGACCAAATAATGAAAC	187	NM_001278566.1
	Reverse	TGAGGGTGTAGACAGAGAGTT		
ER	Forward	GATGGAGTGGCTGGAGTGAG	236	XM_015097472.1
	Reverse	GCCTTTCATTTCTTTTCTTACCTGG		
PGR	Forward	GCTTGAATACATTTATCCAGTCC	114	XM_015100878.1
	Reverse	GAAGAGATTTCACCATCCCT		
GAPDH	Forward	CACCCTCAAGATTGTCAGC	107	NM_001190390.1
	Reverse	CAGTGGTCATAAGTCCCTCC		

Table 2. *Effects of oestrus induction on pregnancy rate of Small-tail Han ewes*

Group	Number of Ewes	Ewes in Estrus (%)				Conception Rate (%)
		0-24 h	24-48 h	48-72 h	0-72 h	
Control	16	0	0	0	0	0
PRID	27	3.70% (1/27)	48.15% (13/27)	33.33% (9/27)	85.19% (23/27)	86.96% (20/23)
PRID +PMSG	27	18.52% (5/27)	44.44% (12/27)	18.52% (5/27)	81.48% (22/27)	90.91% (20/22)

PRID, norgestrel releasing intra-vaginal device; PMSG, pregnant mare serum gonadotrophin

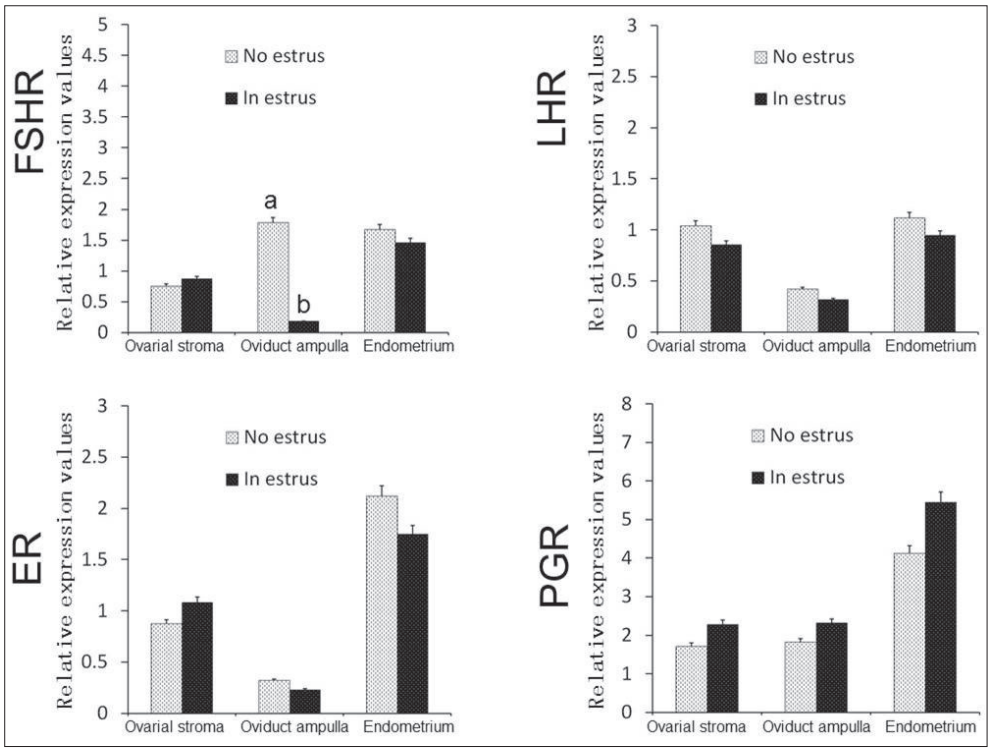

Fig 1. Relative expression values of FSHR, LHR, ER and PGR mRNA in the ovine oviduct ampulla, ovarial stroma and endometrium from the ewes in estrus and ewes without estrus. Note: Significant differences ($P<0.05$) are indicated by different letters within different column

could increase the percentage of animals in estrus and conception rate of the ewes during February. However, there was no significant difference between the group 1 and group 2 in the percentage of animals in estrus (0-72 h after PRID removal) and conception rates.

The qRT-PCR assay revealed *(Fig. 1)* that the relative level of FSHR mRNA was lower in the oviduct ampulla from the ewes in estrus than that from the ewes without estrus ($P<0.05$), but induced estrus had no significant effects on expression of FSHR mRNA in the ovarial stroma and endometrium ($P>0.05$). The relative level of LHR mRNA was low in the oviduct ampulla compared with that in the ovarial stroma and endometrium, and the relative level of ER mRNA was high in the endometrium, and low in the ovarial stroma compared with that in the oviduct ampulla. The relative level of PGR mRNA was high in the endometrium compared with that in the oviduct ampulla and ovarial stroma, but induced estrus had no significant affects on the expression of LHR, ER and PGR mRNA in the ovarial stroma, oviduct ampulla and endometrium ($P>0.05$; *Fig. 1)*.

DISCUSSION

Our results showed that there was a long estrus interval after lambing in spring in Small-tail Han, and estrus induction with PRID was as effective as with PRID + PMSG in increasing the percentage of ewes in estrus and conception rate. It has been reported that intravaginal sponge or controlled internal drug release dispenser (CIDR) can induce majority of ewes come into estrus between 24 and 48 h after sponge or CIDR removal in mature Merino ewes during December in the Great Southern region of Western Australia [18]. The treatment of either CIDR or intravaginal sponge in combination with PMSG can significantly enhance the estrus response, pregnancy and lambing fecundity rates in Awassi ewes during the non-breeding season [19]. There is a uniform effect on the reproductive performance for the different doses of PMSG (300 IU, 400 IU, and 500 IU) following a 12-day treatment with intravaginal sponges in Awassi ewes during the transition period [5]. Therefore, it is suggested that estrus induction with PRID only can increase the percentage of ewes in estrus and conception rate in spring in Small-tail Han ewes.

As a central hormone in mammalian reproduction, FSH is produced and secreted by the pituitary gland, and essential for gonadal development and maturation, and gamete production [20]. Through binding to FSHR, FSH activates the extracellular domain of the FSHR, and induces the maturation of ovarian follicles [21]. The level of FSHR increases until the middle of estrus, and then drops sharply when the LH leads to ovulation [22]. The level of FSHR mRNA is not changed at days 13, 15, and 17 in the same follicles, but decreases in the preovulatory follicles in the pig ovary by day 19 of the estrus cycle [23]. The expression of FSHR protein decreases from early luteal phase to mid and late luteal phases in ovine deep endometrial glands and stroma during the estrus cycle [24]. FSHR expression was decreased in the oviduct induced by insemination or after superovulation [25]. Our results showed that estrus-induction could decrease the expression of FSHR mRNA in the oviduct ampulla, which indicated that down-regulation of FSHR in the oviduct ampulla after estrus induction may be helpful for fertilization and early embryo development.

LHR is essential for LH to regulate follicular maturation and ovulation, and luteal function [26,27]. Estradiol combined with progesterone enhances expression of LHR protein in the porcine oviduct, which is essential for LH-induced relaxation of the porcine oviduct [28]. LHR mRNA is expressed in the immature rat uterus, and declined to an extremely low level after human chorionic gonadotropin (hCG) treatment, which suggests that LH acts on the uterus to block contraction at ovulation [29]. The expression levels of LHR mRNA are relatively lower in uterine tissues and oviduct at the estrus stage in healthy non-pregnant adult Hu sheep [30]. LHR expression is weakly positive or declines in the theca cells of small follicles or large mature follicles on day 0 of the estrus cycle in the porcine ovary [31]. In this study, estrus-induction had no significant effect on expression of LHR mRNA in the oviduct ampulla, ovarial stroma and endometrium, which indicated that LH may have no obvious effect on regulating the physiological status of the oviduct ampulla, ovarial stroma and endometrium of the ewes in estrus.

As a primary female sex hormone, estrogen exerts its actions through ER, and estrogen-ER complex binds to specific DNA sequences, and controls gene expression to activate the transcription of the target genes [32]. ER protein and mRNA are expressed in ovaries of the ewes, and declines from day 2 of the estrus cycle during CL development [33]. The expression of ER mRNA in theca interna tissue and granulosa cells increase continuously during follicle growth in the bovine ovary during estrus cycle [34]. The level of endometrial ER mRNA is highest on day 1, declines between days 1 and 6, and increases between days 11 and 15 of the estrus cycle in ewes [35]. ER mRNA and protein are expressed in the epithelium and smooth muscular layer of the oviduct, but no significant changes are observed during estrus cycle [36]. Our results showed that estrus-induction had no significant effect on expression of ER mRNA in the oviduct ampulla, ovarial stroma and endometrium. Therefore, estrogen may be not obviously implicated in regulating the functions of the oviduct ampulla, ovarial stroma and endometrium of the ewes in estrus.

The biological actions of progesterone are mostly through binding to PGR [37]. PGR is expressed in the ampulla and isthmus of the oviduct, but PGR is not correlated with progesterone concentrations in heifers [38]. There is a reduction of PGR protein expression in most of oviductal and uterine cells after synchronization of estrus with

progestagen in sheep [39]. There is a significant increase of PGR content in the pituitary gland after treatment with GnRH, but PGR content is not altered obviously in the uterus of anestrus ewes [40]. The endometrial and myometrial PGR mRNA levels are highest on day 1 of the estrus cycle, and then decline thereafter in cyclic ewes [35]. The expression of PGR mRNA in follicles and theca interna tissue increases continuously with the development of the follicles in the bovine ovary during estrus cycle [34]. In this study, estrus-induction had no significant effect on expression of PGR mRNA in the oviduct ampulla, ovarial stroma and endometrium, which suggested that progesterone may have no obvious effects on the oviduct ampulla, ovarial stroma and endometrium of the ewes in estrus.

In conclusion, estrus-induction with PRID can increase the percentage of animals in estrus and conception rate of Small-tail Han ewes in spring. The estrus-induction had no significant effects on expression of the mRNA of LHR, ER and PGR in the ovine oviduct ampulla, ovarial stroma and endometrium, FSHR mRNA in the ovarial stroma and endometrium. However, estrus-induction could decrease the expression level of FSHR mRNA in the oviduct ampulla from the ewes in estrus, suggesting that down-regulation of FSHR in the oviduct ampulla after estrus induction may be helpful for fertilization and early embryo development.

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