Theriogenology 103 (2017) 143–148

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Association of gene polymorphisms of estrogen receptor, follicle-stimulating hormone β and leptin with follicular cysts in Large White sows

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ARTICLE INFO

Article history:
Received 14 June 2016
Received in revised form
13 March 2017
Accepted 14 March 2017
Available online 7 April 2017

Keywords:
ESR
FSHβ
Leptin
Mutation
Follicular cyst
Sow

ABSTRACT

Ovarian follicular cysts are one of leading causes of infertility and financial loss in pig breeding program. This study was carried out to investigate the association between polymorphisms of estrogen receptor (ESR), follicle-stimulating hormone (FSH) β, and leptin genes and follicular cysts in sows. A total of 47 and 120 sows with follicular cysts and normal follicles, respectively, were selected to evaluate whether these candidate loci affect the formation of follicular cysts in sows. The polymorphisms of ESR, FSHβ, FSHβ/HaeIII and leptin genes were tested by PCR and PCR-RFLP methods. Cyst–normal case data analysis showed that ESR/PvuII polymorphisms are highly associated with follicular cysts and that sows ESR/PvuII genotype have lower rate of suffering from cysts ($P = 0.021$). Unfortunately, FSHβ, FSHβ/HaeIII, and leptin C3469T polymorphisms were found no significant difference in follicular cysts sows and normal sows. These results suggest that FSHβ, FSHβ/HaeIII, and leptin C3469T genotypes are not able to effect the presence of follicular cysts ($P > 0.05$). In addition, the haplotype EBCM and EBTM within four loci of genes had significant dominance effect on follicular cysts ($P < 0.05$). The detection of ESR/PvuII polymorphisms and haplotype EBCM and EBTM can positively improve the development of biological biomarkers, which is thereby beneficial in breeding and ovary-protective therapy of reproductive disease in pigs.

1. Introduction

Follicular cysts are considered to be one of the most common reproductive disease in sows that cause the inhibition of ovulation [1] and infertility that affecting approximately 2.4–4.0% sows [2,3]. It is characterized by the diameter of follicles that were greater than 21 mm and contain liquid-filled structure with thin walls where corpus luteum was inexistent [4]. Follicular cysts can be caused by endocrine disturbance [5]. Abnormal secretion of LH was detected in cystic follicles [6]. The reason may be LH can impact the action of the granulosa and theca interna layers [7] then adjusted steroidogenesis to cause the development of follicular cysts [8]. And the hypothalamus examination revealed that the concentrations of GnRH is fluctuant in the swine with experimentally-induced cystic ovaries [9]. As glycoprotein hormone, FSH is produced by the anterior pituitary and consists of α [10] and β subunits [11]. It is widely acknowledged that FSH plays an important part in follicular maturation and mediating follicular development and ovulation rate [12]. Prolonged stimulation of FSH was needed for the cause of ovarian cysts by human chorionic gonadotropin in the hypophysectomized mice [13]. An overexpression of inhibinα in mice suppress serum concentrations of FSH and estradiol can lead to the development of follicular cysts [14,15]. And serum levels of FSH were also found fluctuant between normal pigs and follicle cystic pigs [16]. These studies led us to speculate that FSH levels are able to affect the development of follicular cysts.

Estrogens play important role in regulation of ovarian maturation by binding to estrogen receptors. The levels of estrogens in small cysts are apparently higher than in the normocyclic follicles prior to ovulation [17]. However, only a small quantity of estrogen were detected in large cysts [18]. Our previous finding

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http://dx.doi.org/10.1016/j.theriogenology.2017.03.030
0093-691X/

has also indicated that the concentrations of oestradiol in serum was lower in follicle cystic sows than that in the normal sows [16]. In addition, treatment with adrenocorticotrophic hormone (ACTH) has been proposed as contributing to end ovulation and cause cystic ovaries by reducing the oestrogen secretion in pigs [19]. Estrogen receptor (ESRs) are well known as nuclear receptors identified to be potential candidates in ovarian responsiveness because of the effect of estrogens on follicle growth and maturation [20]. When combined with follicle-stimulating hormone (FSH), ESRs can also stimulate the occurrence of ovarian follicle [21]. Indeed, knockout of ESR in transgenic female mice suffered from haemorrhagic cystic ovaries and arrest the follicle formation by the effect of half of normal ESR level [22]. All these data suggesting an association of follicular cysts with abnormal oestradiol signaling.

Leptin was initial studied to improve the body fat content and energy balance [23]. Nevertheless, subsequent finding verified that the leptin gene performs a predominant role in granulosa cell function and follicle development in pigs [24]. Leptin mRNA expression was detected in porcine oocytes at different phases of oocyte formation and follicular maturation [25]. Furthermore, finding indicated that leptin in supraphysiological concentration could cause the development of follicular cysts by promoting progesterone and testosterone secretion in pigs [26]. It is possible that leptin concentration disturbance can play a key role in the formation of follicular cysts.

On the other hand, there are numerous studies demonstrate that ESR polymorphisms and mutations that are associated with disorders such as breast cancer, spontaneous abortions, coronary and endometriosis [27–30] and FSHβ polymorphisms has been confirmed to affect on the polycystic ovary syndrome and hypogonadism [31,32]. Leptin polymorphisms have also been shown that involved in the hypertension and depressive disorder [33,34]. In addition, our earlier study indicated that inhibin-α polymorphisms are highly related with follicular cysts and that sows with c.-42GG and c.3222GG genotypes have less chance of suffering from follicular cysts [35]. Collectively, we propose the hypothesis that gene mutations of ESR, FSHβ, leptin may have roles in the formation of follicular cysts because of their potential influence on follicular development. Thus, this paper aims to examine the association of gene polymorphisms of ESR, FSHβ, FSHβ/HaeIII, leptin with ovarian follicular cysts in Large White pigs.

2. Materials and methods

2.1. Tissue samples

Ovaries with follicular cysts from 47 Large White sows and normal ovaries from 120 sows in the age range of 5–6 months old were collected from local slaughterhouse and transported to the laboratory within 30 min in phosphate buffer at 37 °C. Ovaries with cysts were separated via measuring diameters (21–45 mm) and observing the existence of fluid-filled structures with smooth morphology and inexistence of corpus luteum [4]. In subsequent experiments, a small portion of follicle tissue was obtained from each ovary and placed into a homogenizer (TIANGEN, Beijing, China) to grind on tissue homogenate. All experiments were performed thrice, and each follicular tissue sample was removed from only one follicle.

2.2. Forced restriction fragment length polymorphism (RFLP)

DNA was extracted from 167 follicle tissue homogenates by using the Mammalian Genomic DNA Miniprep Kits (Sigma, Japan). PCR amplification was performed in a total reaction volume of 25 μL, which contained 50 ng genomic DNA, 1 U Taq DNA Polymerase (Promega, USA), 1X PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2), 0.2 mM of each dNTP (Promega, Germany), and 30.0 pmol of primers (Sigma, Japan). The PCR-amplified conditions and primers of ESR [36], FSHβ [11], FSHβ/HaeIII [37], and leptin [38] are listed in Table 1. Genotyping of FSHβ can be obtained directly via PCR method [39]. Afterward, the PCR-amplified products of ESR, FSHβ/HaeIII, and leptin were digested for an overnight genotyping at 37 °C; restriction enzymes PvuII, HaeIII, and HinfI were also used according to the manufacturer's instruction (Takara Bio) to ensure complete digestion. Agarose gels (3%) stained with ethidium bromide can separate the digestion products.

2.3. Statistical analysis

Statistical analysis was performed using the SPSS software (IBM SPSS Statistics 22). The haplotype analysis and pairwise linkage disequilibrium in ESR, FSHβ, FSHβ/HaeIII, and leptin were operated by http://analysis.bio-x.cn/SHEsisMain.htm. Hardy–Weinberg equilibrium was also tested via x^2-test to analyze the genotypic distribution of the groups. Odd ratios (ORs) and 95% confidence intervals (CIs) related to various factors with more frequency between normal and cyst group were analyzed via logistic regression model. The statistical significance of allele and genotype distribution in normal and follicular cyst groups was assessed by the Pearson chi-square test. The significant difference was confirmed when the P-value <0.05.

3. Results

3.1. Forced RFLP analysis

RFLP analysis was performed using PvuII [36], HaeIII [37], and HinfI [38] restriction enzymes, as described previously. The PCR-amplified products of ESR, FSHβ-HaeIII, and leptin gene in 167 (47 with cyst; 120 without cyst) gene samples were digested and genotyped based on fragment length(bp): ESR: EE (120, 65, 55), EF (65, 55; Fig. 1); FSHβ: AA (500), AB(500,200), BB(200; Fig. 2); FSHβ-HaeIII: TT (332, 208, 84), TC (332, 208, 173,4), and CC (208, 173, 84; Fig. 3); leptin: MM (465, 4), MN (465, 347, 118, 4), and NN (347, 118; Fig. 4).

The ESR/PvuII (P = 0.021, Table 2) polymorphisms showed statistical difference between the cyst and normal groups. Moreover, the EE genotype frequency in the cyst group (51.1%) was clearly lower than that in the normal group (70%, P = 0.021). No association was found in the FSHβ, FSHβ-HaeIII, and leptin C3469t polymorphisms (p > 0.005) of the cyst and normal groups.

3.2. Association analysis

The ORs and 95% CIs for all genotype and haplotype were analyzed to confirm the confidence of data obtained. The analysis results demonstrated that the ESR (OR, 0.447; CI, 0.224–0.894; P < 0.05) and haplotype EBCM (OR, 0.000; CI, 0.000–0.008) and EBTM (OR, 3.674; CI, 1.395–9.672; Table 3) are involved in the occurrence of follicular cysts. Furthermore, sows with ESR/PvuII polymorphisms and haplotype EBCM and EBTM have lower risk of forming follicular cysts.

3.3. Polymorphism analysis

The distributions of FSHβ/HaeIII genotype frequencies within cyst and normal population were within the Hardy Weinberg equilibrium, whereas the FSHβ polymorphisms deviated from it.