遗 传 学 报 *Acta Genetica Sinica*, September 2006, 33 (9): 800–807 ISSN 0379-4172

The Correlation of Reproduction-Related Gene Expression with Germ Cell Number in DM and PLL Gilts

HU Yan-Biao[1], PAN Zeng-Xiang[1], XU Dan[1], XU Yin-Xue[1,①], LIU Hong-Lin[1,①], HUANG Rui-Hua[1], HU Zhi-Gang[2]

1. *College of Animal Science and Technology, Nanjing Agricultural University, Nanjing* 210095, *China*;
2. *Fumin Agricultural Sideline Production Base in the Garrison Command of Shanghai, Chongming County, Shanghai* 202181, *China*

Abstract: In this study, the ovarian germ cell number was counted in 3-week-old Duroc × Meishan (DM, *n*=30) and *PIC* × (Landrace × Large White) (PLL, *n*=53) gilts, and the mRNA expression levels of four reproduction-related genes were investigated by quantitative RT-PCR. Correlation of germ cell number with the expression level of these genes was analyzed. Results showed that the germ cell number of DM was significantly higher than that of PLL gilts ($P<0.01$), although there was no significant difference between the ovarian weight of DM and PLL gilts ($P=0.269$). No significant correlation existed between germ cell number and ovarian weight in the two gilt groups ($R=0.335$, $P=0.07$; $R=0.119$, $P=0.398$, respectively). A significant correlation was found between the germ cell number and expression level of *ESR* and *IGF1R* mRNA in DM gilts ($R=0.648$, $P<0.05$; $R=0.757$, $P<0.01$, respectively), but the correlation between the germ cell number and expression level of *FSHR* and *INHBA* mRNA did not reach statistical significance. Significant correlation was found between the germ cell number and the expression level of *ESR*, *FSHR*, and *IGF1R* mRNA in PLL gilts ($R=0.435$, $P<0.01$; $R=0.438$, $P<0.01$; $R=0.292$, $P<0.05$, respectively), but not with *INHBA* mRNA in PLL gilts.

Key words: pigs; ovary; germ cell number; gene expression

Prolificacy in pigs is generally defined as the number of piglets born alive, which is an important limiting factor affecting sow productivity. The number of piglets born alive per litter is also an important economic quantitative trait. There is a significant difference between Chinese pig breeds and breeds from other parts of the world in this respect. Some Chinese breeds exhibit exceptional prolificacy, making them important genetic resources for breeding. For example, Chinese Meishan pigs (MS, one of the local strains of Taihu pig) farrow on average 3–5 more piglets per litter and possess more teats and reach sexual maturity 60–90 days earlier than their European counterparts [1,2]. These substantial differences in breed offer a unique model to study the regulatory mechanisms of reproductive efficiency.

From the perspective of reproduction, higher farrowing rate is based on higher ovulation rate, and the latter is closely related to germ cells [3, 4]. McCoard *et al.* [2] compared the dynamics of germ-cell population in two swine breeds that differ in prolificacy, White Composite (WC) and Meishan (MS) sows. Wise *et al.* [5] investigated the relationship between the weight of ovary and the number of oocytes and the ovulation rate in fetal and neonatal life of MS and WC. However, they only focused on the developmental morphological characteristics of germ cells.

The development and maturation of germ cells

Received: 2005-09-13; Accepted: 2006-03-07

This work was supported by Chinese National Program for High Technology Research and Development(“863” Project) (No. 2002AA242031-3) and the Shanghai Key Project of Science and Technology Service for Agriculture (No. 2004-56).

① Corresponding author. E-mail: xuyinxue@yahoo.com; E-mail: liuhonglin@263.net; Tel: +86-25-4395278; Fax: +86-25-4395314

are affected by many factors, among which, genetic and neuroendocrine factors are the two most important intrinsic factors. Mammalian germ cells achieve their highest number during the fetal period, and then gradually reduce their numbers decrease [6]. Similarly, sow germ-cell growth also fits this developmental model, but there are differences between breeds. For example, at postnatal 7–25 days, the total number of germ cells of MS gilts is more than that of WC; at postnatal 25 days, there is a certain amount of Graafian follicles in the MS ovary, whereas there was none in the WC ovary. Together with a number of other observations, researchers proposed that ovarian maturity is quicker and sexual maturity is earlier in MS than in WC [2]. On the other hand, germ-cell development is regulated by a variety of hormones, which act on ovaries through their corresponding receptors, stimulating or inhibiting the growth of follicles [7, 8]. Therefore, the gene expression of the relevant hormones and their receptors is likely to directly influence the development of germ cells. Whether this phenomenon exists in MS hybrid pigs and other synthetic breeds has not been reported up to date. This study compares the differences in the number of germ cells of 3-week-old DM (Duroc × Meishan) and PLL (*PIC* × (Landrace × Large White)) gilts by collecting their ovaries and examines the relationships between germ-cell development-related gene expression and the number of germ cells to provide the basis for further exploring the mechanisms of germ-cell growth and improving reproduction performance.

1 Materials and Methods

1. 1 Materials

Experimental herd and sample collection: Thirty 3-week-old DM hybrid gilts were gathered from JiangPu Pig Farm; 53 3-week-old PLL hybrid gilts were from Fumin Agricultural Sideline Production Bases in the Garrison Command of Shanghai. Bilateral ovaries were collected. One was kept in liquid nitrogen, and the other was preserved in PBS solution under freezing.

Reagents used: Bovine serum albumin (BSA), Collagenase Ⅰ(Nanjing Dazhi Biological Engineering Company, Limited); M-MLV reverse transcriptase, DNA polymerase (Promega)； SYBR Green Ⅰ (Eastwin Innovative Biotechnology Limited Company).

1. 2 Methods

1. 2. 1 Germ cell counting

Frozen-preserved ovaries were trimmed to remove excess oviduct tissue, which was weighed and then subjected to collagenase digestion to dissociate the somatic and germ cells for the estimation of total germ cell number per ovary. In detail, intact ovaries were placed in 5 mL of collagenase digestion buffer [2] (0.03% collagenase type Ⅰ, 200 U/mg; 0.5% bovine serum albumin in Hank's balanced salt solution, pH 7.6) and incubated overnight at 37℃ in a shaking water bath. The next morning, complete disaggregation of the cellular suspensions was carried out by pipetting through successively smaller tips. Germ cells were counted by a hemacytometer. Oocytes were easily distinguished because of their large size (approx. 30 μm or larger) and spherical shape (Fig.1). The whole process was performed by one operator. The germ cells were counted in two counting chambers, with a total of eight squares. Finally, the total germ cell number was estimated using the following formula, total germ cells = (the total number in eight squares/8) × 50 000, and the procedure was repeated for ten times.

1. 2. 2 RNA extraction and cDNA synthesis

Total RNA was extracted from the ovary by the guanidine isothiocyanate-phenol-chloroform method. Five micrograms of RNA was then used for agarose gel (containing 1.5% formaldehyde) electrophoresis to examine the quality of RNA (Fig.2). The 28S and 18S bands were clear, indicating that RNA extraction was effective.

First strand of cDNA was synthesized according to the manufacturer's instruction, with 2 μg of RNA and oligo-d$(T)_{15}$, using the M-MLV reverse transcriptase kit. The quality of the cDNA was evaluated by PCR amplification of the β-actin gene, followed by SDS-PAGE and silver staining (Fig. 3). The PCR