Animal Reproduction Science 143 (2013) 24–29

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Effects in cattle of genetic variation within the IGF1R gene on the superovulation performance and pregnancy rates after embryo transfer

Wu-Cai Yang[a,*], Li-Guo Yang[b], Hasan Riaz[b], Ke-Qiong Tang[b], Long Chen[c], Shu-Jing Li[c,**]

[a] College of Animal Science and Technology, Northwest A&F University, Shaanxi 712100, PR China
[b] Key Laboratory under Education Ministry of China for Agricultural Animal Genetics, Breeding, and Reproduction, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China
[c] Beijing Amber Embryo Technology Co. Ltd., Beijing 100107, PR China

ARTICLE INFO

Article history:
Received 31 May 2013
Received in revised form 8 October 2013
Accepted 17 October 2013
Available online 26 October 2013

Keywords:
Luxi cattle
Chinese Holstein cows
Embryo transfer
Superovulation
IGF1R

ABSTRACT

The insulin-like growth factor 1 receptor (IGF1R) is a membrane glycoprotein mediating most biological actions of IGF1 and IGF2, and has an important effect on ovulation, pre-implantation embryo development and pregnancy rate. The objectives of this study were to detect *IGF1R* gene polymorphisms of cattle and analyze the relationship with superovulation performance and pregnancy rates after embryo transfer (ET), as well as the hormone concentrations at the day of ET. One reported SNP of IGF1R G404T and a novel SNP of IGF1R G399A were analyzed in 170 Chinese Holstein donor cows and 118 Luxi recipients cattle. Statistical analysis revealed that the G404T mutation was associated ($p = 0.019$) with increased ovulation rate and females with this mutation had enhanced performance in producing transferable embryos. For the polymorphic locus G399A, recipients with g.399 GG and g.399 GA genotypes had greater pregnancy rates after ET than that of g.399 AA genotype. Furthermore, the same tendency was observed that the genotype groups with greater pregnancy rates had greater progesterone and lesser estrogen concentrations, but these did not reach statistical significance. Results of the present study showed, for the first time, that the polymorphism in *IGF1R* is associated with superovulation traits, and indicated that the *IGFIR* gene can be used as a potential marker for donor selection.

1. Introduction

Multiple ovulation and embryo transfer (MOET) has become the most powerful tool for animal breeders and scientists to improve genetics in animal herds. The success of a MOET program is determined by many intrinsic and extrinsic factors, including season, breed, age, nutrition, management, stress, quality of gonadotropin products and treatment protocols (González-Bulnes et al., 2004). Most of these factors and sources of variation have been minimized for increasing pregnancy rates with embryo transfer (ET) (Schoolcraft et al., 2001; Spell et al., 2001; Chebel et al., 2008; Jones and Lamb, 2008). The MOET technology is expensive and inefficient with mixed ovarian response to gonadotropin treatments among individuals, and conception rates following ET have improved very little over the years (Hasler, 2003). The candidate gene approach has been widely used in genetic association and biomarker from animals to humans, and many candidate genes of animal

* Corresponding author. Tel.: +86 029 87091148; fax: +86 029 87091148.
** Corresponding author.
E-mail addresses: yangwucai111@163.com (W.-C. Yang), lshujing@hotmail.com (S.-J. Li).

0378-4320/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.anireprosci.2013.10.008

reproduction traits have been detected (Tabor et al., 2002). It would, therefore, be meaningful for donor or recipient selections to use the candidate gene approach to identify potential markers that are correlated with superovulation performance or the pregnancy rates after ET.

Insulin-like growth factor 1 (IGF1) belongs to the IGF superfamily and has a pivotal role in mammalian fertility. Previous studies have shown that IGF1 is involved in physiological processes including ovarian follicular development (Mazerbourg et al., 2003; Beg and Ginther, 2006), ovulation (Echternkamp et al., 2004), pre-implantation embryo development (Velazquez et al., 2005a,b), conception and growth (Adam et al., 2000; Shen et al., 2003; Patton et al., 2007). Genetic studies found that IGF1 knockout mice failed to have ovulations (Lucy, 2000), and when very large doses of gonadotropins were used, the IGF1 knockout mice had oocytes of inferior quality that were not fertilizable (Lucy, 2000). Furthermore, growing evidence indicated that IGF1 has an important role in the success of superovulatory treatments (Herrler et al., 1994; O'Callaghan et al., 2000; Velazquez et al., 2005a,b). Previous reports concluded that oocyte quality and embryo viability are associated with blood concentrations of IGF1 in domestic ruminants undergoing superovulation treatments (O'Callaghan et al., 2000; Velazquez et al., 2005a,b; Velazquez et al., 2009). Owing to the central role of IGF1 action in reproduction, the insulin-like growth factor type 1 receptor (IGF1R) appeared to be a strong candidate gene for mutations associated with reproduction traits. The IGF1R is the main receptor of IGFs, mediating the actions of somatotropic axis on growth, lactation and reproduction (Lucy, 2012). Mouse knockout models have clearly demonstrated the importance of IGF1 and IGF1R for embryonic growth as well as postnatal development. There have been a large number of mutations detected in the *IGF1R* gene, which are associated with postnatal growth in human (Hammer et al., 2004; Kawashima et al., 2005; Inagaki et al., 2007) and on growth and carcass traits in chicken (Lei et al., 2005). However, whether the *IGF1R* polymorphism has any effect on reproduction traits is currently not known.

Therefore, *IGF1R* was chosen as a candidate gene to investigate its effects on superovulation traits and pregnancy rates after ET. PCR-RFLP and sequencing methods were used to detect variants of the *IGF1R* gene in cattle, and the relationships with superovulation traits and the pregnancy rates after ET were evaluated to identify potential markers for the selection of donor and recipient animals.

2. Materials and methods

2.1. Experimental cattle and sampling

All procedures involving animals were approved by the Animal Care and Use Committee of Huazhong Agricultural University. This study was conducted in Beijing Amber Embryo Technology Co. Ltd., Beijing, China. A total of 288 adult female individuals from two breeds of the cattle were analyzed in present study, including Chinese Holstein cows ($n = 170$) which had been successively superovulated one to four times at monthly intervals between 2007 and 2010 using FSH as described in Yang et al. (2011), and Luxi cattle ($n = 118$) which were selected as recipients of Wagyu cattle embryo. Genomic DNA was extracted from blood samples using phenol-chloroform standard protocols (Joseph and David, 2002). Blood samples without anticoagulant were allowed to clot overnight at room temperature and the serum was frozen at −20 °C until it was assayed for progesterone and estrogen concentrations.

2.2. Superovulation procedure and embryos harvest

Superovulation was induced via eight intramuscular injections of FSH (Folltropin-V, Bioniche Animal Health Canada Inc.) for 4 days beginning on Day 4 after the insertion of an intravaginal progesterone-releasing device (PRID1, Ceva Sante Animale, France). The injections were given at 12-h intervals in a dose step-down manner – i.e.; doses of 5.4, 4.4, 3.4, and 2.4 mL on each day-as described in previous study (Tang et al., 2011). Each cow was twice given 4 mL (0.15 mg/mL) of prostaglandin $F_{2\alpha}$ (Qilu Animal Health Products Co., Ltd., China) along with the sixth and seventh FSH treatments. The progesterone-releasing device was removed after the second prostaglandin treatment. Estrous detection by visual observation was conducted twice daily for at least 30 min at approximate 12-h intervals. Any standing to be mounted cow by a herd mate was considered to be in estrus. All cows were inseminated with frozen-thawed semen from one bull of known good fertility 12 and 24 h after standing estrus. Uteri were non-surgically flushed on Day 7 after artificial insemination by an experienced technician using standard techniques. Each uterine horn was flushed with 500 mL of phosphate-buffered saline. Prostaglandin $F_{2\alpha}$ was administered to all cows immediately after the flushing procedure. The recovered lavage was filtered through an embryo filter (Miniflush Embryo Recovery System, mesh size 44 μm, Minitube, Germany). The fluid was examined for oocytes or embryos under a stereomicroscope, and embryos were isolated and graded as A (excellent), B (good), C (fair), or D (poor) according to the criteria of the International Embryo Transfer Society (Robertson and Nelson, 1998). After flushing, recovered ova and embryos were separated into transferable embryos (Grades A, B, and C), degenerate embryos (Grade D), and unfertilized ova (oocytes were defined as unfertilized ova). The number of corpora lutea on the ovaries were counted as an index of the number of oocytes ovulated (Ingman et al., 2006), so unfertilized ova were counted on the basis of corpora lutea. Cows with no oocytes or embryos recovered in two successive superovulation treatments were defined as non-responders or cows without superovulation response. Six cows without superovulation response were found in experimental population as described in Yang et al. (2011). The traits of superovulation included total number of ova, number of transferable embryos, number of unfertilized ova, and number of degenerated embryos.

2.3. Synchronization of estrus in recipients

Luxi cattle ($n = 118$) received a CIDR device (New Zealand DEC Co., Ltd.) on random days of the estrous cycle (Day 0), an injection of 0.6 mg prostaglandin $F_{2\alpha}$