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Maternal dietary supplementation of arginine increases the ratio of total cloned piglets born to total transferred cloned embryos by improving the pregnancy rate of recipient sows

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ABSTRACT

The extremely low full-term developmental efficiency of cloned pig embryos limits the practical application of pig cloning techniques. Maternal dietary supplementation of the nutritionally important amino acid, arginine, can enhance prenatal developmental rate of *in vivo* fertilization-derived pig embryos. It was hypothesized that maternal dietary addition of arginine can also improve the developmental capacity of cloned pig embryos. To test this hypothesis, there was a comparison of the reproductive performance between recipient sows fed an L-arginine-supplemented diet (L-Arg group) and those fed the control diet (control group). There was a subsequent comparison of the developmental indexes of cloned piglets farrowed in the L-Arg and control groups of surrogate sows. Dietary supplementation of L-arginine during gestation days 14–75 increased the plasma concentrations of arginine and arginine metabolites, including nitric oxide, spermidine, and putrescine in recipient sows of transferred cloned pig embryos. Although maternal arginine addition did not affect the birth weight and placental development indexes of newborn cloned piglets, it significantly increased the ratio of total cloned piglets born to total transferred cloned pig embryos by increasing the pregnancy rate of recipient sows. The results of this study suggest that nutritional management of recipient sows is an effective approach to improve the developmental rate of cloned pig embryos.

1. Introduction

The pig somatic cell nuclear transfer (SCNT) technique is a valuable application opportunity in the swine industry and in the fields

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of bioscience and biomedicine. This technique currently, however, has a low success rate of approximately 1% (number of born cloned piglets/number of transferred cloned embryos; Liu et al., 2015). The extremely low efficiency of the pig SCNT approach is mainly due to the greater death rate of transferred cloned embryos than what occurs normally in pig production, which is supposedly caused by SCNT-induced erroneous reprogramming, in the reproductive tract of recipient sows (Zhao et al., 2010; Huili et al., 2014; Niemann, 2016).

In several recent studies, the dietary supplementation of arginine, a multifunctional amino acid, to pregnant sows or gilts increased the number of viable fetuses per litter by 1.7–3.7 (Bérard and Bee, 2010; Li, 2011; Li et al., 2014) or the number of live-born piglets per litter by 1–2 (Ramaekers et al., 2006; Mateo et al., 2007; Gao et al., 2012). Arginine improves embryonic/fetal survival in pigs by several mechanisms, including the direct enhancement of the anti-oxidative defense, activation of the mammalian target of rapamycin signaling pathway to stimulate protein synthesis in the placenta, uterus, and fetus, and the indirect increase in placental development and uteroplacental blood flow *via* the arginine metabolites ornithine, proline, spermidine, putrescine, and nitric oxide (Wu et al., 2013).

Because maternal dietary supplementation of arginine enhances the prenatal survival of fertilization-derived pig embryos or fetuses, it was hypothesized that addition of arginine to the diet of recipient sows also can improve the developmental capacity of cloned pig embryos or fetuses. To test this hypothesis, in this study, comparisons of the reproductive performance were made between recipient sows fed the control diet (control group) and those fed an L-arginine-supplemented diet (L-Arg group). Subsequently, there were comparisons of the developmental indexes of cloned piglets farrowed by two different groups of surrogate sows.

2. Materials and methods

2.1. Ethics statement

This study was conducted in strict accordance with the “Instructive Notions with Respect to Caring for Laboratory Animals,” issued by the Ministry of Science and Technology of China. The animal experimental protocol was approved by the Institutional Animal Care and Use Committee of South China Agricultural University. All efforts were made to minimize animal suffering.

2.2. Scnt

The SCNT procedure was performed as described previously (Li et al., 2013; Shi et al., 2015). The SCNT experiment was conducted in Guangdong province of China during about 4 months from April to August, which is during the summer season in Guangdong province. During this period the local temperature is 25 to 36 °C.

During the SCNT experiment, 70 replicates were conducted. In each of 59 replicates, about 450 cloned embryos were produced and transferred to two recipient sows; one of them was randomly allocated to the L-Arg group while the other one was classified into the control group. In each of 11 replicates with more oocytes obtained from the slaughter house, about 700 cloned embryos were produced and transferred to three recipient sows. In these cases, one recipient sow was randomly allocated to the L-Arg group and the other two recipient sows were assigned to the control group. After 70 replicates were conducted, 70 and 81 recipient sows were randomly allocated to the L-Arg and control groups, respectively.

Cloned embryos were produced using the same protocol with the same source-derived donor cells and oocytes. Cloned embryos were transferred to the oviducts of Yorkshire recipient sows at 24 h after activation (at 1–2 cell stage). Each recipient sow had transfers of 220–240 cloned embryos. The sows used as recipients were similar in genetic makeup, and were developed and maintained in the same conditions. Only the sows that were in the second to fifth parity were used as recipients, and the 2 or 3 recipient sows used in each replicate were in the same parity.

2.3. Animal management

Recipient sows were housed individually in gestation crates (2.1 m × 0.6 m) in the same pig farm. From gestation days 14–75, control recipient sows were fed a control diet (see Table 1 for the ingredients and nutrient amounts of the diet), whereas the L-Arg group of recipient sows were fed a diet supplemented with 1% L-arginine − HCL (Hebei Huaheng Biological Technology Co. Ltd., Hebei, China), equivalent to 0.83% L-arginine. The diet was designed to meet the nutrient requirements for gestating sows set by the United States National Research Council. During gestation days 1–30, 31–60, 61–90, and 91–110, sows were provided with 2.0, 2.2, 2.6, and 3.2 kg of diet per day, respectively, and had free access to drinking water. On gestation day 30, all sows were examined using an ultrasonic machine to determine pregnancy status. On gestation day 110, sows were transferred to individual farrowing crates (2.2 m × 1.5 m). Recipient sows were allowed to farrow cloned piglets *via* spontaneous labor. If spontaneous farrowing did not occur until gestation day 116, then recipient sows were injected with a prostaglandin analog (cloprostenol, 200 μg/recipient) to induce parturition. The number of total piglets born and piglets born alive was recorded. The birth weights of new born piglets were measured within 12 h after birth.

2.4. Blood collection and analysis

To reduce the risk of abortion in recipient sows that was caused by stress resulting from the blood collection process, which