Mineralized deposits in the uterus of a pig without pregnancy loss

Geon A Kim1, Jun-Xue Jin1, Anukul Taweechaipaisankul1, Sanghoon Lee1, Byung Il Yoon2, Jongki Cho3, Byeong Chun Lee1,*

1Department of Theriogenology and Biotechnology, Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul 08826, Korea
2Laboratory of Histology and Molecular Pathogenesis, College of Veterinary Medicine, Kangwon National University, Chuncheon 24341, Korea
3Laboratory of Theriogenology, College of Veterinary Medicine, Chungnam National University, Daejeon 34134, Korea

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Herein, we describe a case of uterine calcification in the uterus of a pig without pregnancy loss. The recipient underwent cloned embryo transfer and Cesarean section for safe delivery of cloned piglets. During the Cesarean section, 4 white, star-like, (2 × 2 × 2) cm, calcified structures were found within the endometrial cavity. Despite dystrophic calcification around the placenta, healthy cloned piglets were produced successfully. To our knowledge, this is the first reported case of dystrophic calcification occurring within the uterus in a pregnant pig.

It is widely accepted that calcium deposition in cellular degenerated areas can be associated with infection and ischemic changes [7,10]. Calcification in the uterine cavity is an uncommon finding in veterinary medicine. In human, results from a few cases have indicated that uterine calcification could be caused by the presence of osseous fragments associated with a previous history of abortion [9]. Alternative etiologies have included osseous metaplasia from multipotent cells, secondary osteogenesis after retention of fetal bone, implantation of embryonic parts, dystrophic calcification of retained necrotic tissue, continuous estrogenic stimulation of the endometrium, and chronic endometrial inflammation. [2,3]. However, there are no reports of uterine calcification in pregnant pig without a history of abortion.

The aim of this study was to present the first case of uterine calcification in a pig with normal piglet delivery through routine Cesarean section. A naturally synchronized female pig was prepared for cloned embryo transfer (ET). Somatic cell nuclear transfer was performed as described in our previous study with slight modification [6]. Cumulus-oocyte complexes were collected and cultured in medium containing TCM-199 with 0.57 mM cysteine, 0.91 mM sodium pyruvate, 5 μL/mL insulin transferrin selenium solution 100X (Invitrogen, USA), 10 ng/mL epidermal growth factor, 10% porcine follicular fluid (vol/vol), 10 IU/mL (IU, international unit) equine chorionic gonadotropin, and 10 IU/mL human chorionic gonadotropin. After 44 h of maturation, cumulus cells of matured oocytes with a first polar body were denuded in 0.1% hyaluronidase. After enucleation in TALP containing 5 μg/mL cytochalasin B and 5 μg/mL Hoechst 33342, a single fibroblast was injected and electrically fused. A total of 260 reconstructed embryos were electrically activated and cultured in vitro. Reconstructed embryos with normal morphology were surgically transferred into both oviducts of a surrogate pig. The female pig used in this study was raised at our private facility. During the ET, progesterone concentration of the naturally synchronized surrogate pig was 7.14 ng/mL. Pregnancy was identified via ultrasonography at 28 days after ET. At 112 days after ET, 5 piglets with no gross structural abnormalities were delivered by Cesarean section. During the Cesarean section surgery, the sow’s measured progesterone concentration was 3.78 ng/mL. The surgical procedure was performed under general anesthesia and efforts were made to minimize any potential suffering of the surrogate. The 5 cloned piglets were produced normally, but abnormal tissue attached to the maternal right uterine horn cavity was observed. All protocols involving animal use were approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-151019-4) in accordance with the Guide for the Care and Use of Laboratory Animals of Seoul National University, Korea.

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*Corresponding author: Tel: +82-2-880-1269; Fax: +82-2-873-1269; E-mail: bclee@snu.ac.kr

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The abnormal uterine tissue was observed to expand outward from the serosal surface of the right uterine horn and attach to the right uterine horn cavity near the bifurcation. A sample of the tissue was examined after staining with hematoxylin and eosin. Histological examination revealed typical porcine epitheliolar placental tissue with an area of calcified tissue (panel A in Fig. 1). Around the area of dystrophic calcification, inflammatory cells, mainly histiocytes, had infiltrated (panel B in Fig. 1).

In guinea pigs, it has been reported that calcification can be induced by hormone administration, including administration of prolactin, human chorionic gonadotropin, estradiol, estrone, and growth hormone [11]. In the present study, pregnancy was maintained with a normal progesterone concentration, and the surgery was performed two days before the due date. Although we do not know why calcification was identified in the uterine cavity, we assume that the phenomenon may be related to metabolic and hormonal changes secondary to the pregnancy.

Although causes of ectopic ossification and mineralization are well described, little has been reported on the pathogenesis of uterine calcification in pregnant animals. In this report, normal cloned piglets were produced and there was no accumulation of mucus within the uterine horns, which is in contrast to observations reported for a case of uterine lithiasis in a dog [5].

In humans, calcification of placenta is considered a part of maturation and aging [12]. Pathogenesis of endometrial calcifications is involved in uterine trauma during instrumentation and/or uterine infection. However, in the present case, we surgically transferred cloned embryos to both oviducts of a sow without a history of uterine trauma or instrumentation. There are few similar reports of uterine calcification; one case of a uterine stone in an 8 years old female child without a history of uterine trauma, and two other cases of uterine stones, one reported in a 28 years old woman with a history of pregnancy loss and the second in a 73-year-old women [1,4]. The female pig in the present study had no history of pregnancy loss in its previous two natural deliveries. Although it has been reported that endometrial calcification in the uterine cavity can induce secondary infertility [8], it did not affect the maintenance and mortality of the cloned littner piglets in the present case.

In summary, herein, we have described a case of uterine calcification, a rarely reported phenomenon in a pregnant farm pig without pregnancy loss.

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**Conflict of Interest**

The authors declare no conflicts of interest.

**References**

1. Alpert LC, Haufrect EI, Schwartz MR. Uterine lithiasis. Am J Surg Pathol 1990, 14, 1071-1075.
2. Cayuela E, Perez-Medina T, Vilanova J, Alejo M, Canadas P. True osseous metaplasia of the endometrium: the bone is not from a fetus. Fertil Steril 2009, 91, 1293.e1-1293.e4.
3. Cicinelli E, Stanziano A, Parisi C, Marinaccco M, Cusio F. Hysteroscopic diagnosis and treatment of endocervical ossification: a case report. J Minim Invasive Gynecol 2005, 12, 159-161.
4. Feyles V, Moyana TN, Pierson RA. Recurrent pregnancy loss associated with endometrial hyperechoic areas (endometrial calcifications): a case report and review of the literature. Clin Exp Obstet Gynecol 2000, 27, 5-8.
5. Iwasaki M, Oliveira CA. Uterine lithiasis in a dog. Aust Vet J 1991, 68, 73-74.
6. Jin JX, Lee S, Khoirinaya C, Oh A, Kim GA, Lee BC. Supplementation with spermine during in vitro maturation of porcine oocytes improves early embryonic development after parthenogenetic activation and somatic cell nuclear transfer. J Anim Sci 2016, 94, 963-970.
7. Misra RP. Calcium and disease: molecular determinants of calcium crystal deposition diseases. Cell Mol Life Sci 2000, 57, 421-428.
8. Onderoglu LS, Yarali H, Gultekin M, Katlan D. Endometrial osseous metaplasia: an evolving cause of secondary infertility. Fertil Steril 2008, 90, 2013.e19-2013.e11.
9. Pereira MC, Vaz MM, Miranda SP, Araújo SR, Menezes DB, das Chagas Medeiros F. Uterine cavity calcifications: a report of 7 cases and a systematic literature review. J Minim Invasive Gynecol 2007, 14, 293.e1-293.e4.
Invasive Gynecol 2014, 21, 346-352.

10. Ryan LM, Cheung HS. The role of crystals in osteoarthritis. Rheum Dis Clin North Am 1999, 25, 257-267.

11. Silva EG, Deavers MT, Parlow AF, Gershenson DM, Malpica A. Calcifications in ovary and endometrium and their neoplasms. Mod Pathol 2003, 16, 219-222.

12. Varma VA, Kim KM. Placental calcification: ultrastructural and X-ray microanalytic studies. Scan Electron Microsc 1985, 1567-1572.