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Duration of gestation in pregnant dogs carrying cloned fetuses

Min Jung Kim, Hyun Ju Oh, Jung Eun Park, Geon A. Kim, Eun Jung Park, Young Kwang Jo, Byeong Chun Lee*

Department of Theriogenology and Biotechnology, College of Veterinary Medicine, Seoul National University, Seoul, Korea

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ABSTRACT

The aim of this study was to investigate gestation duration and the physiologic characteristics of pregnant dogs bearing cloned fetuses, especially in the prepartum period. A retrospective study was performed to compare gestation duration in females pregnant with cloned (somatic cell nuclear transfer) fetuses (cloned group) with those bearing noncloned fetuses (control group), and effects of litter size, birth weight, and breed of somatic cell donors on gestation duration in the cloned group were evaluated. Clinical delivery onset signs associated with serum progesterone concentration and rectal temperature were also compared in both groups. The gestation duration calculated from day of ovulation was significantly longer in the cloned (62.8 ± 0.3 days) versus the control group (60.9 ± 0.5 days; $P < 0.001$). There was a negative correlation between litter size and gestation duration including both groups ($r = -0.59$; $P < 0.01$), but there were no differences between birth weights or breed of cell donors and gestation duration in the cloned group. Even though the basal rectal temperature in the prepartum period was not different between control and cloned groups (36.9 ± 0.1 °C and 37.2 ± 0.1 °C, respectively), serum progesterone concentration on delivery day was significantly higher in the cloned group (2.2 ± 0.4 ng/ml) compared with the control group (0.5 ± 0.1 ng/ml; $P < 0.05$). The longer gestation duration of pregnant dogs bearing cloned fetuses might be because of the smaller litter size in this group. Also, the weaker drop in serum progesterone levels in the prepartum period in cloned dog pregnancies indicates that the parturition signaling process might be altered resulting in longer gestation periods.

1. Introduction

Although somatic cell nuclear transfer (SCNT) has been successfully used to clone dogs for the past 7 y [1–7], the efficiency of cloning is still low (<4.2% delivered puppies per transferred embryo). Because cloned offspring are so valuable and hard to produce, safe delivery of full-term puppies is an essential management component in dog cloning, and therefore the mothers must be closely monitored around the time of parturition to check for any signs of maternal distress or difficulty in delivery. Consequently, accurate prediction of gestation duration in pregnant bitches is especially important for the delivery of cloned dogs. Canine pregnancies carrying cloned fetuses have three uncommon features compared with normal, fertilized embryo pregnancies, aside from the embryo production procedures. First, only large breed dogs have been chosen as recipients for transfer of SCNT cloned embryos, because of the easy surgical approach and decreased risk of dystocia. Secondly, it has been frequently reported that only one cloned puppy was delivered from the large breed recipients [1,2,4,6,7]. Lastly, cloned dogs of various breeds from small to large size, including Afghan hound [1,7], Toy poodle [2], Beagle [3], Sapsaree dog [4], Labrador retriever [5], and Pekingese [6], have been produced using a large breed recipient as the recipient mother.

Generally, gestation length is considered as the interval from the day of peak luteinizing hormone (LH) to parturition, with a reference standard of 65.1 ± 0.1 days in Beagles [8]. Factors including litter size and breed have been

* Corresponding author. Tel.: +822 880 1269; fax: +822 873 1269.
E-mail address: bclee@snu.ac.kr (B.C. Lee).

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examined for their influence on gestation duration. Litters fewer than seven [9] or four [10] puppies had a negative correlation with gestation duration, but in other studies, no significant relationship between litter size and gestation duration was reported [11–13]. Because extremely small litters have been frequently reported in dog cloning, we first hypothesized that this could lead to prolonged gestation in pregnancies bearing cloned fetuses. German Shepherd dogs, Golden Retrievers, and hounds were more likely to have a longer average gestation than Labrador Retrievers [10], and the gestation length of Alsatians was reported to be shorter than for Boxers, Bernese mountain dogs, old English sheepdogs, and Bouvier des Flandres dogs [9]. Although the reasons for different gestation durations among different breeds of dogs are not yet identified, breed could be a factor affecting gestation duration. Also, in general, pups delivered from large breed dogs are heavier than those delivered from small breeds. Therefore, we hypothesized that large breed cloned fetuses that lead to increased birth weight make gestation shorter because they can produce appropriate and timely signals for parturition.

To investigate factors affecting accurate prediction of delivery, a retrospective study was performed to compare the duration of gestation in pregnant dogs bearing cloned fetuses with dogs carrying non-cloned fetuses, and to assess effects of litter size, birth weight, and breed of SCNT cell donors on the length of gestation. Clinical signs associated with delivery onset, i.e., serum progesterone (P_4) concentration and rectal temperature, were also compared between the two groups.

2. Materials and methods

2.1. Animal use

Large, mixed breed female dogs aged between 1 and 7 y and weighing 20 to 35 kg were used in this study. All dogs were housed in separate indoor cages. Animal care facilities and procedures followed the standards established by the Committee for Accreditation of Laboratory Animal Care at Seoul National University. The use of animals in this study was according to the Guide for the Care and Use of Laboratory Animals at Seoul National University.

2.2. Determination of ovulation

Ovulation was determined by measuring serum P_4 concentration every day or every other day after observation of vaginal bleeding [7]. Briefly, 3 mL of blood was collected from the cephalic vein and centrifuged at $\times 1660g$ for 10 min to prepare serum. Serum was stored at -30 °C until assayed. The P_4 level was measured with a DSL-3,900 Active Progesterone Coated-Tube Radioimmunoassay Kit (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). The ovulation day was defined as the day when serum P_4 concentration reached 4.0 to 9.9 ng/mL [14,15].

2.3. Determination of delivery

The P_4 concentration, rectal temperature, and sonography of the pregnant bitches were used to monitor the delivery or to decide on a cesarean section. The P_4 level was monitored once a day while it was high ($\geq$4 ng/mL), and then twice a day (8 AM and 4 PM) when it reached $\leq$4 ng/mL or from 3 days before the expected date of delivery. The rectal temperature was monitored 4 times a day while the P_4 level was high ($\geq$4 ng/mL), and every 2 to 3 h after it reached $\leq$4 ng/mL or from 3 days before the expected date of delivery. Fetal heartbeats were monitored by ultrasonography when P_4 and rectal temperatures did not decrease further near the predicted delivery day, and the monitoring interval was shortened when the heart rate decreased to $\leq$220 per min. Pups were delivered either naturally or by cesarean section; the latter was performed when delivery onset did not occur even after P_4 decreased to $<$1 ng/mL, or the fetal heartbeat decreased to $\leq$200 per min. Gestation duration was calculated as the interval from the day of ovulation to the day of parturition.

2.4. Experimental design

A retrospective study was conducted from 2008 to 2012 on 27 pregnant dogs from the Department of Theriogenology and Biotechnology in the College of Veterinary Medicine at Seoul National University. Pregnant dogs were divided into two groups: bitches bearing noncloned fetuses (control group) and those carrying cloned fetuses (cloned group). Bitches were made pregnant by artificial insemination or mating with large, mixed breed male dogs in the control group, and by transferring reconstructed SCNT embryos in the cloned group [7]. Artificial insemination was done with fresh semen via the vagina or uterus. For SCNT, *in vivo* matured oocytes were recovered from oocyte donor dogs, and a donor cell derived from skin of small to large breed dogs was injected into the perivitelline space of enucleated oocytes. The cell and oocyte were fused electrically, and the reconstructed embryos were transferred to a recipient after chemical activation.

2.5. Data analysis

Gestation duration was compared between the cloned group and the control group using an unpaired *t* test. Correlation between litter size or birth weight and gestation duration was examined. Breeds of SCNT cell donors were divided into small to medium breeds (Beagle, Pekingnese, and mixed) and large breeds (Border collie, Laborador retriever, Afghan hound, Pit bull terrier, and mixed) and effect on gestation was compared by an unpaired *t* test. Clinical delivery onset signs of average P_4 concentration the day before and on the day of parturition, and basal rectal temperature near the delivery time, were also compared by an unpaired *t* test. Data were analyzed with GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Gestation duration in the control and cloned groups

A total of 8 and 19 pregnant females was assessed in the control and cloned groups, respectively. The periovulatory P_4 levels were not different between the two groups