Original Article
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Reproductive ability of a cloned male detector dog and behavioral traits of its offspring

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In 2007, seven detector dogs were produced by somatic cell nuclear transfer using one nuclear donor dog, then trained and certified as excellent detector dogs, similar to their donor. In 2011, we crossed a cloned male and normal female by natural breeding and produced ten offspring. In this study, we investigated the puppies' temperaments, which we later compared with those of the cloned parent male. The results show that the cloned male had normal reproductive abilities and produced healthy offspring. All puppies completed narcotic detector dog training with a success rate for selection of 60%. Although the litter of cloned males was small in this study, a cloned male dog bred by natural mating produced puppies that later successfully completed the training course for drug detection. In conclusion, cloning an elite dog with superior genetic factors and breeding of the cloned dog was found to be a useful method to efficiently procure detector dogs.

Keywords: behavioral traits, cloned dog, drug detection dog, reproductive normality

Introduction

It is possible to propagate animals with specific genetics or elite genetics through somatic cell nuclear transfer (SCNT). A number of breeds of dogs have been produced by SCNT [9-11, 13,16,17], and it has been shown that both male and female cloned animals have normal reproductive characteristics and can reproduce normally [12,15,21]. For example, one report demonstrated that cloned females have normal reproductive hormone levels and ovarian follicle development [8]. Another study reported that healthy offspring were produced when cloned females were artificially inseminated using semen of cloned males, both cloned males and females are derived from adult somatic cells [18]. However, no studies have investigated the behavioral characteristics of the offspring of cloned dogs to date.

Behavioral traits and aptitude for various roles in dogs are also likely to be influenced by genetic factors [14]. Indeed, it has been shown that genetic factors contribute significantly to several behavioral traits such as fearfulness, aggressiveness, reactivity and general activity [2,20]. In particular, it was reported that behavioral traits such as courage, nervous stability, hardness and affability have positive genetic correlations [19]. Accordingly, dogs exhibiting superior detection ability are recommended for breeding to increase the production of such animals, thereby reducing the burdens associated with training and selection of detection dogs. Because of its high efficiency, SCNT using donor cells from dogs with superior talent represents an excellent approach for producing drug detection dogs [4].

In this study, we investigated the reproductive ability of a cloned male dog by natural breeding. We also investigated the behavioral traits of these puppies and their performance in the selection test to evaluate their normality.

Materials and Methods

Natural breeding and pregnancy monitoring

Seven male cloned Labrador Retriever dogs were born in 2007. All dogs were trained in the narcotic detection dog training course at the Detector Dog Training Center (DDTC) and passed all final assessments successfully, as previously

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reported [4]. A female dog born in 2006 had a good personality suitable for breeding. In previous breeding, she produced normal puppies. To predict her fertile period, serum progesterone was measured once a day (in the morning) from the time of observation of estrus bleeding. Moreover, serum progesterone was measured twice a day from the 55th day of first mating to predict the delivery time. Progesterone concentrations were measured with a DSL-3900 ACTIVE Progesterone Coated-Tube Radioimmunoassay Kit (Diagnostic Systems Laboratories, USA). Following mating with a cloned male dog, the bitch lived in a kennel with other dogs for 30 days, after which she was moved to the delivery room located in the DDTC. A specialized veterinarian and technician monitored the pregnant bitch from mating to delivery, throughout which time progesterone concentrations and body temperature were measured. To assess the mature weights, the body weights of the ten puppies were checked at 2 years of age.

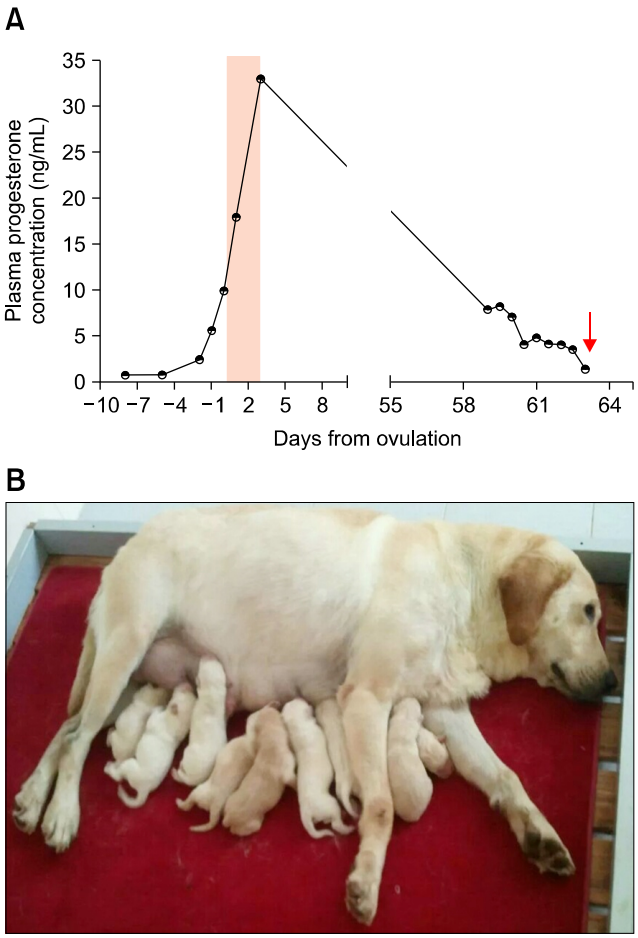

Fig. 1. Birth of puppies. (A) Hormonal change in a female dog from estrus to delivery. The square indicates the fertile period of a female dog. While in these periods, she stayed with a male cloned dog in a room. The arrow indicates the delivery time. (B) Appearance of puppies at 1 day after delivery.

Training and evaluation in the DDTC

All ten puppies were assessed at 7 weeks of age by the Volhard puppy aptitude test (Volhard test) and Toman litter test. In the final evaluation, instructors assessed the results of the puppies' training to determine their potential as detector dogs. During the course of this study, puppies learned the sequence of searches under various situations. Based on their boldness, concentration, sequence of search, reaction to targets and eagerness, each test factor was divided into five categories [4]. Success was defined as a score of 60 or higher, while a score of less than 60 indicated failure. All of the cloned dog's offspring were trained according to the aforementioned training and evaluation course.

Analysis of Volhard tests in puppies

We used the modified Campbell (Volhard test) and evaluation [1]. The Volhard test consisted of nine subtests and was conducted by the test leader, who was an experienced instructor but a complete stranger to the puppies. The test location was the one of the training buildings in the DDTC, and it was the puppies first time visiting there. The duration of each subtest was 30 sec. After testing, puppies were classified into 6 types according to the Volhard test evaluation method as previously described [4].

Statistical analysis

Statistical analysis was conducted using GraphPad Prism

Table 1. Characteristics including sex, coat color, mature weight and final score in parent dogs (a cloned male and a wild female) and the first offspring (F1; P1–P10) produced by natural mating

ID of dog	Sex	Coat color	Mature weight (kg)	Final score*
Cloned male	Male	Yellow	28	90 (success)
Wild type female	Female	Yellow	29	
P1	Male	Yellow	26.5	81 (success)
P2	Male	Yellow	27	74 (success)
P3	Male	Yellow	29	37 (failure)
P4	Male	Yellow	27	86 (success)
P5	Male	Yellow	28	74 (success)
P6	Female	Yellow	25.8	65 (success)
P7	Female	Yellow	20	68 (success)
P8	Female	Yellow	19	34 (failure)
P9	Female	Yellow	20	48 (failure)
P10	Female	Yellow	28	53 (failure)

Cloned male data is already suggested in previous study [4]. *Final score was that obtained based on the regular drug detector dog training test according to the Detector Dog Training Center detector dog training manual.

4.02 (Graphpad Software, USA). The correlation data between mature weight and final score were analyzed by Pearson's *r*. A p value < 0.05 was considered to indicate significance.

Results

Breeding and birth of puppies after breeding a cloned male dog and a female dog

Natural breeding was performed to evaluate the fertility of the cloned male dog. At 61 days after mating, ten offspring (male: 5, female: 5) were born by natural delivery (Table 1; Fig. 1). The puppies were healthy, had a yellow coat color, and no abnormalities in morphology (Table 1; Fig. 2).

Final score of offspring for selection of detector dogs

Among the ten puppies, six passed the detector dog training course with scores of 60 or above. However, the score that the offspring received in the final test was lower than the score of their father (cloned male) in the training program. The final selection success rate for offspring evaluated for drug detection was 60% (Table 1).

Correlation between final training score and mature weight

We examined whether final training score and mature weight were significantly correlated within the ten offspring (Table 1). The mature weight of puppies at 2 years of age was not significantly correlated with the final selected outcome score.

Behavioral traits in Volhard tests in puppies

Among the ten puppies, six obtained a total score of Type 2 or Type 3 as indicated in Table 2. Four puppies achieved scores of Type 4, Type 5 and Type 6.

Discussion

We examined the reproductive ability of a male cloned dog

Fig. 2. Mature appearance of F1 (P1–P10) produced by breeding a male cloned dog and a female dog. F1 developed without health problems until the end of training course. The image was taken at 2 years of age.

Table 2. Volhard puppy aptitude test results for a cloned male dog and F1 (P1–P10) at 7 weeks of age

ID of dog	Social attraction	Following	Restraint	Social dominance	Elevated dominance	Retrieving	Touch sensitivity	Auditory sensitivity	Sight sensitivity	Total type of Volhard puppy aptitude tests
Cloned male	1	1	2	2	3	–	–	–	–	2
P1	3	2	5	3	3	6	1	6	1	3
P2	1	2	2	2	3	3	4	4	4	2
P3	3	2	5	4	3	6	1	6	3	4
P4	2	2	3	2	3	3	5	4	3	3
P5	1	2	3	3	3	4	4	4	3	3
P6	5	6	6	6	3	6	1	4	5	6
P7	4	4	4	4	3	5	5	4	3	4
P8	1	2	4	1	3	3	5	4	3	3
P9	1	2	4	1	3	4	5	4	3	3
P10	6	5	5	4	3	5	6	5	4	5

and the behavioral traits of his offspring. Several previous studies reported that cloned animals had normal reproductive ability [3,5,18]. In this study, ten healthy puppies were produced by natural mating between a cloned male dog and a normal female dog in the DDTC. It was previously reported that although the first cloned male dog exhibited normal libido, natural mating could not be performed because of his inexperience in the clasping method [18]. However, we found that the cloned male dog had a normal libido along with other normal mating behavior and reproductive ability.

His puppies showed normal growth and matured well without congenital defects or infectious disease until final selection. In our report, seven of the ten puppies showed mature weights above 25 kg, which is similar to that of their parents. All of these offspring had similar mature weights that were within the normal reference range [6,7]. Among the four puppies that failed the final selection test, two showed lower weights than the other dogs. However, we could not find any correlation between final test score and mature weight in puppies because the population was too small to lead to a solid conclusion. Behavioral traits of the puppies were monitored to evaluate their trainability and compare similarities between the cloned parent dog and his puppies because it was assumed that behavioral traits of the puppies were inherited from the cloned male dog. To determine the behavioral traits of puppies, Volhard tests were performed. As in our previous report, a cloned male dog scored subtype 2 in a modified Campbell test [4]. However, only one of the puppies, P3, scored subtype 2, which was identical to his father's score. Interestingly, six puppies showed the 2 or 3 subtype in the Volhard test, indicating suitability for training. Notably, none of the puppies receiving a 2 or 3 subtype score in the Volhard test were among the final selected dogs.

Furthermore, the cloned dog's offspring achieved a 60% success rate in the final selection tests in the present study. When compared with the approximately 30% success rate of the Japan detector dog training program [14], as well as the 0–40% success rate of the US customs service (unpublished data), our 60% training outcome success rate was remarkable.

The cloned detector dog received an excellent grade in training; therefore, it was concluded that cloning elite dogs using the SCNT technique was a better approach for producing qualified detector dogs [4]. We showed that cloned dogs could breed normally, which led us to hypothesize that this method will allow generation of better candidate dogs with higher potential to pass the training course. Furthermore, while the behavioral traits of puppies correlate with the genetic factors of a cloned male dog, and although only one litter was investigated for this study, it is generally accepted that genetic factors are also important in training dogs.

In conclusion, a cloned male dog had a normal libido and could mate with females naturally. The pairs could produce puppies that later successfully completed the training course for drug detection with a greater than 60% success selection rate. It is expected that breeding cloned dogs with excellent abilities will be an optimal method to produce more candidate dogs for unique purposes.

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Conflict of Interest

There is no conflict of interest.

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