

## Failure of Reproduction Management in an Inbreeding English Bulldog

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**Abstract :** A two-year-old, female English bulldog was referred for breeding by artificial insemination with frozen semen of male English bulldog, a litter of female bulldog's grandfather. Intrauterine artificial insemination was done two days after the ovulation day. Sperm was evaluated after thawing by computer assisted sperm analyzer, and its motility was 89.8% with normal shape. Pregnancy bearing eight fetuses was diagnosed by ultrasonography and radiography. Cesarean section was performed sixty days after the artificial insemination. Eight pups were delivered with safe, but the entire pup had abnormalities including severe bow-legged malformations, cleft lip, cleft palate, and enlarged cranial part.

**Key words :** abnormalities, artificial insemination, English bulldog, inbreeding.

### Introduction

Health problems in purebred dogs including breed predispositions have recently been highlighted in the media worldwide, and efforts to indicate inheritance of canine disease by breed have been developed (CIDD; Canine Inherited Disorders Database; <http://www.upei.ca/cidd>). Artificial selection focusing on specific morphological characteristics is often major challenges in dog breeding, and many breeds originate from a small number of founders (6). This breeding management has contributed to the unique genetic structure in each breed of dog, making it a precious resource for studying the genetic basis of heritable diseases in both dog and human (2,5). However, breeding program considered only appearance in a small population, where a relatively closed genetic pool has led to reduced diversity and genetic variation within breeds. In addition, accumulation of detrimental genotypes or exaggerated anatomical and mental problems has been resulted from this genetic bottleneck phenomenon in several breeds. For example, cleft lip/palate which is an opening in the lip or the roof of the mouth that occurs due to failure of normal fusion processes during embryonic development may result from hereditary causes. The cleft palate reported with high rates in English bulldogs and low risk in German shepherd dog (8). In the present study, we report several abnormalities in inbreeding English bulldogs puppies after artificial insemination with semen, which was collected from a litter of female bulldog's grandfather.

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### Case

A two-year-old, female English bulldog was referred to Seoul National University Hospital for Animals for breeding by intrauterine artificial insemination with frozen semen of male English bulldog. Semen was collected from a litter of female bulldog's grandfather in England when he was six years old and motility of the semen was 90% with 65% of normal live morphology when collected. It was frozen and stored in liquid nitrogen until thawed. Ovulation was determined by measuring serum progesterone concentration every day or every other day after observation of vaginal bleeding (3,4). Serum was prepared by centrifugation of blood (2 ml) collected from cephalic vein at 1660 g for 10 min and stored at  $-30^{\circ}\text{C}$  until assayed. The serum progesterone level was measured with a DSL-3900 ACTIVE<sup>®</sup> Progesterone Coated-Tube Radioimmunoassay Kit (Diagnostic Systems Laboratories, Inc., Webster, TX). Ovulation was determined when serum progesterone concentration reached 4.0 to 9.9 ng/ml. Intrauterine artificial insemination was done two days after the ovulation day. The dog was anesthetized with 6 mg/kg of propofol (Myungmoon Pharm. Co., Ltd., Seoul, Korea) via intravenous injection, and general anesthesia was maintained with isoflurane (Hana Pharm. Co., Ltd., Seoul, Korea). After abdominal region was prepared aseptically, a midline incision was made, and both uterus horns were exteriorized. Sperm was thawed just before the insemination and evaluated under

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**Fig 1.** Abdomen radiograph of pregnant two-year-old English bulldog with eight fetuses on day 58 after the insemination.



**Fig 2.** Various abnormalities observed in the eight puppies including cleft lip (a), cleft palate (b), severe bow-legged malformations (c) and enlarged cranial part (d).

microscope with computer assisted sperm analyzer. It had 89.8% of motility, and a total of 1 ml was injected into both uterine horns via 22 gauge catheter. Number of corpus lutea palpated from left and right ovaries were four and five, respectively. Pregnancy was diagnosed by ultrasonography and confirmed eight fetuses by radiography on day 58 after the insemination (Fig 1). Parturition was monitored by serum progesterone concentration, rectal temperature and ultrasonography. Fetal heartbeats were decreased to less than 200/min although serum progesterone was 2.53 ng/ml on day 60

after the insemination and Cesarean section was performed. The dog was anesthetized with 6 mg/kg of propofol via intravenous injection, and anesthesia was maintained with isoflurane. All the eight pups were delivered with safe, but the entire pup had at least one abnormality, respectively, including severe bow-legged malformation, cleft lip, cleft palate, enlarged cranial part, and various tail lengths (Fig 2). We consulted the owner that bow-legged malformation could be corrected by orthopedic treatment (11), and cleft lip/palate could be corrected by surgery (1,10). However, the owner did not want to treat any of them, and wanted to euthanize all pups. We recommended to the owner that avoiding inbreeding within the same family for minimizing genetic predispositions or disorder would increase birth rate of healthy normal pups.

## Discussion

Accumulation in knowledge for prevalence/incidence of disease needs for improving success rate of breeding program. In our case, five among eight pups carried cleft lip/palate (Fig 2a, 2b) which occurs less commonly in bulldogs, while brachycephalic syndrome, hip dysplasia, keratoconjunctivitis sicca, pulmonic stenosis, ventricular septal defect occur relatively common in this breed (CIDD, 2013, Feb). Recently, it was suggested that nonsyndromic cleft lip/palate can pass to pups by autosomal recessive inheritance in boxers (7). Therefore, similarly, cleft lip/palate can be occurred in bulldogs after inbreeding between near consanguinity. The second highest incidence in our case was bow-legged malformation which was had in three of eight pups. Two of them showed bilateral abnormal hind legs, and the other carried in only right hind leg. The hind leg malformation in our case was rarely reported and different with swimming puppy syndrome which is also characterized by hind leg abnormality including hyperextension of the stifle and hock joints as well as abduction of the hip joint (9). All the three pups showed adduction of stifle and hock joints (Fig 2c). The most mysterious abnormality was enlarged cranial part, head and neck (Fig 2d), and we cannot find any reference similar to this kind of abnormality. These multiple problems can be occurred by combined effects of loss of heterogeneity, accumulation of detrimental genes, or exaggeration of anatomical features. Therefore, strategies for restricting the rate of inbreeding should be especially important in bull dogs, and veterinarians have to inform and consult to the breeder to reduce health problems and increase breeding goal.

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## 근친교배 잉글리쉬 불독에서 번식 관리의 실패

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**요 약** : 2년령 암컷 잉글리쉬 불독이 근친 유래의 동결정액을 이용한 인공수정을 위해 내원하였다. 배란 후 이틀 뒤 자궁 내 인공수정을 실시하였다. 정액을 해동 후 컴퓨터 기반의 정액 분석기를 사용하여 평가한 결과, 해동된 정자는 정상 모양을 가지고 있었고 운동성이 89.8%였다. 초음파와 방사선 촬영을 통하여 8마리를 임신한 것을 확인하였고, 인공 수정 후 60일 쯤 제왕절개를 실시하였다. 8마리의 산자가 안전하게 분만이 되었지만, 모든 산자들에서 심각한 활모양 다리, 구개열, 구순열, 확장된 두부(頭部) 등을 포함한 기형이 관찰되었다.

**주요어** : 기형, 인공수정, 잉글리쉬 불독, 근친교배