

Successful Birth of Pups Produced by GnRH-induced Estrus and Natural Mating in Captive Red Fox (*Vulpes vulpes*)

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ABSTRACT

On January 6, 2010, two months earlier than normal breeding season, a red fox vixen was implanted with synthetic GnRH analogue, Deslorelin. Blood was sampled every 2~3 days from the day of implant to identifying spermatozoa on stains of epithelial cells. Estradiol and progesterone were examined. Even though the vixen was in non-breeding season, she was mated by a male fox. Pregnancy was confirmed by canine pregnancy detection kit that detect relaxin released from placenta. Four healthy pups were born on March 9, 2010. This is the first report showing synthetic GnRH can activate ovarian function and lead to fertile estrus of red fox in non-breeding season.

(Key words : red fox, estrus induction, GnRH implant, natural mating, pups)

INTRODUCTION

Red fox (*Vulpes vulpes*) is listed as "Least Concern (LC)" of conservation status by International Union for Conservation of Nature (IUCN). However, red fox (*Vulpes vulpes peculiosa*) is currently assigned endangered species and protected as class I indigenous animal by ministry of environment in Korea. Compared to advanced research of red fox in European countries, red fox has been little known in Korea, especially reproductive physiology (Bonnin *et al.*, 1978; Smith *et al.*, 1985; Farstad *et al.*, 1992a; Farstad *et al.*, 1992b; Hartley *et al.*, 1994). A lot of research conducted by fur industries in Europe is likely to be fundamental basis for *in-situ* or *ex-situ* institutes of which the purpose is as long as proliferations of endangered, fur animals like red fox. In this study, we tried to induce fertile estrus and produced healthy pups from a red fox vixen using a commercial GnRH implant that has been used mostly for horses.

MATERIALS AND METHODS

1. Animals

A 3-year-old, multiparous, red fox vixen was used for estrus induction in this study. The male red fox was previously proved to have fertile ability and used for natural mating. With free access to water, raw chicken and beef meat have been provided-

once a day, and also rats were fed alive once two weeks. From the beginning of Deslorelin implant, a male fox at same age was with the vixen at the same enclosure.

2. Insertion of GnRH Implant

On January 6, 2010, an implant of synthetic GnRH analogue (2.1 mg of Deslorelin, Ovuplant[®], Peptech Animal Health, Australia) was placed subcutaneously in the lip of vulva. Insertion was performed according to the manufacturer's instruction.

3. Serum Analysis

Blood was collected by saphenous venipuncture from the day of Deslorelin implant to the day of pregnancy diagnosis. The levels of estradiol and progesterone were examined by Neodin Veterinary Laboratory (Seoul, Korea) (Table 1).

4. Vaginal Smear

After finding coital lock accidently, vaginal epithelial cells were smeared on sterile cotton swabs and stained with Diff-Quik stain (Sysmex Co., Kobe, Japan) (Fig 1A).

5. Pregnancy Diagnosis

On February 16, pregnancy was diagnosed by ReproChek kit (Synbiotics corp., San Diego, CA, USA) according to the manufacturer's instruction.

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RESULTS

1. Levels of Estradiol and Progesterone

Estradiol level started increasing until around 5 days after GnRH implant insertion (Table 1). LH peak and ovulation were assumed to occur after day 5 if reproductive physiology related with ovarian cycle in red fox is similar with dogs. When progesterone concentration reaches up to between 4.0 and 7.5 ng/ml, artificial insemination is usually performed (Bouchard *et al.*, 1991; Hase *et al.*, 2000; Park *et al.*, 2009). Ovulation would occur between Day 5 and Day 8 because 0.78 ng/ml of progesterone concentration steeply rose to 5.14 ng/ml during 3 days (Table 1).

2. Existence of Spermatozoa on the Stain of Vaginal Epithelial Cells

On January 14, vaginal epithelial cells were once stained to

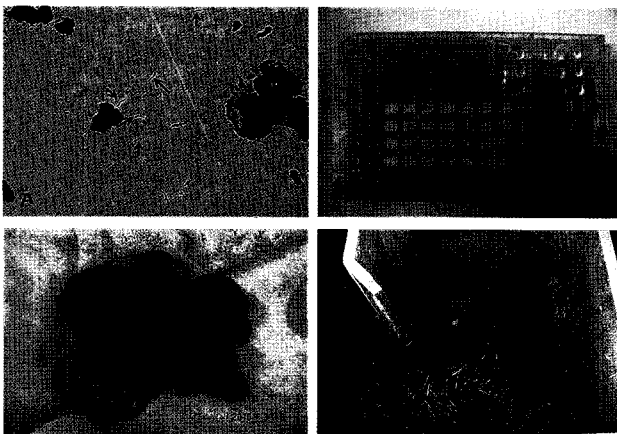


Fig. 1. Pups produced by GnRH-agonist induced estrus and natural mating. (A) Vaginal epithelial cell was stained by Diff-Quik solution after natural mating was monitored. Arrows indicate spermatozoa. (B) Pregnancy diagnosis using ReproCHEK kit performed 41 days after GnRH-agonist insertion. Navy blue color indicates that relaxin hormone released from placenta is detected. (C) Four female pups at birth born on March 9, 2010. (D) 35-day-old pups in a wood box.

identify sperm existence, which performed after finding coital locking. Several spermatozoa were found with red blood cells (Fig. 1A).

3. Pregnancy Diagnosis and Production of Viable Pups

Using a canine pregnancy test kit, ReproCHEK[®], the whole blood sample collected from the vixen that were treated with Ovuplant[®] showed distinct blue color (Fig. 1B). This thick blue color much stronger than neighboring blue colors means the presence of relaxin in the sample. On March 9, 62 days after Ovuplant[®] insertion, four female pups were born, weighed about 150 gram (Fig. 1C and 1D).

DISCUSSION

We showed how to usefulness of GnRH analogue, deslorelin acetate, to induce fertile estrus leading to production of viable pups in red fox. The commercially purchased deslorelin acetate is sold in the type of implant that has been widely used in the field of horse breeding (McKinnon *et al.*, 1993; Samper *et al.*, 2002). In dogs, other GnRH-agonists commercially named Suprelvein[®] (Peptech, Australia) and Gonazon[®] (Intervet, Netherlands) are used for contraception for male dogs or young prepubertal birthes (Concannon *et al.*, 2009). Contrary to this contraceptive action, as treated to anestrus adult dogs, deslorelin acetate can induce a normal proestrus and fertile estrus (Concannon, 1989; Kutzler, 2007; Concannon *et al.*, 2009). Successful production of pus by administration of GnRH analogue was demonstrated in captive wolf and coyote (Asa *et al.*, 2006; Carlson and Gese, 2009). But, in captive red fox, the use of GnRH analogue is little known to induce estrus in breeding or non-breeding season.

In wild animals, pregnancy test is not easy using an ultrasound scanner and X-ray like using in pet dogs and cats. Therefore, simple pregnancy diagnosis kits would be more beneficial in wild carnivores. Most of canine carnivores are spon-

Table 1. Changes of estradiol and progesterone from GnRH-agonist insertion to pregnancy diagnosis

Dates (*)	Jan 6 (Day 0)	Jan 8 (Day 2)	Jan 11 (Day 5)	Jan 14 (Day 8)	Feb 16 (Day 41)
Estradiol ^a	11.1	29.0	74.4	19.0	36.3
Progesterone ^b	2.47	1.77	0.78	5.14	9.67

* Parenthesis means days after GnRH-agonist insertion.

^a The unit of estradiol level is pg/ml.

^b The unit of progesterone level is ng/ml.

taneous ovulator that means ovulation and corpora lutea (CL) formation occur spontaneously (Concannon, 1989). Female red fox in early pregnancy would abort readily if they are manipulated or sedated for the collection of blood samples (Bonnin *et al.*, 1978). In this study, without sedation, blood was collected 5 times by saphenous venipuncture that enables to understand hormonal changes and to assume the time of ovulation. In pseudopregnant red fox vixens, fecal progesterone concentrations are 5 ng/ml after oestrus, and CL have been maintained for 5 months after ovulation (Bonnin *et al.*, 1978).

As a method of differentiating pregnancy and pseudopregnancy, the length and level of progesterone is not optimal (Møller, 1973; Smith and McDonald, 1974; Bonnin *et al.*, 1978). Relaxin test used in this study for pregnancy diagnosis have been applied to discern pregnancy from pseudopregnancy in wild canids and felids (Carlson and Gese, 2007; Braun *et al.*, 2009). Even though the source of relaxin hormone varies according to the species, it comes from CL, placenta, and uterus (Sherwood, 1994). In domestic dogs, relaxin is detected as early as 25 days after ovulation when the commercially available ReproCHEK (Synbiotics Corp., San Diego, CA, USA) (Buff *et al.*, 2001). Relaxin was observed 21 days before parturition in this study. This hormone can be detected after embryonic implantation while remains very low in nonpregnant canids (Carlson and Gese, 2007).

In conclusion, the relaxin detection test kit as a simple pregnancy diagnosis will be very beneficial in monoestrus, seasonal carnivores, and GnRH analogue implant was successfully used for estrus induction in non-breeding season that might have it possible to breed more than once a year.

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