Changes of Estradiol, Progesterone and Vaginal Epithelial Cells of a Pseudopregnant Captive Coyote (*Canis latrans*) during Breeding Season

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ABSTRACT

Most captive canids and felids at Zoos in advanced countries have been examined enough to apply artificial reproductive techniques to them. We investigated reproductive hormones and vaginal epithelial cells of a 6-year-old, female coyote, hoping these data could eventually be extended to artificial insemination with frozen-thawed conspecific semen at Seoul Zoo. As a relative of pet dogs, coyote exhibited a similar appearance with only minor differences. In vaginal smear, an increase in the number of superficial cells suggests that the bitch has reached a state close to estrus. A sudden decrease of estradiol and increase of progesterone is considered as a preovulatory event. Vaginal epithelial cells and hormones might be useful for determining the optimal time of artificial insemination in coyotes' breeding.

(Key words : coyote, pseudopregnant, estradiol, progesterone, vaginal epithelium)

INTRODUCTION

Coyote (*Canis latrans*) is a species of canids which is so widely distributed in North America that the reproduction patterns of these animals would resemble that of conspecific coyotes that are raised at Seoul Zoo, located in a similar range of latitude. They are generally known as being socially monogamous (Bekoff and Wells, 1986; Gese, 2001), seasonally monoestrous (Stellflug *et al.*, 1981; Bekoff and Wells, 1986), and territorial (Bekoff and Wells, 1986; Gese, 2001).

Coyote is known to be so strongly pair-bonded that artificial captive breeding is also needed at Seoul Zoo. However, applying artificial reproductive technology must be preceded by understanding their reproductive biology at the present habitat (Goodrowe *et al.*, 2001; Zindl *et al.*, 2006; Shin *et al.*, 2008; Thomassen *et al.*, 2009).

During a single breeding season, from March to June, here at Seoul Zoo we examined the serum levels of estradiol and progesterone of a pseudopregnant coyote separated from males in concurrence with microscopical observations of exfoliated vaginal epithelium. In this study, we show endocrine patterns and vaginal cytology of sequestered female coyote.

MATERIALS AND METHODS

1. Animals

The subject of our study was a female coyote which was born in captivity, over 6 years of age, primiparous, that has been living a long time without sexual intervention of males. The animal was hospitalized in an indoor enclosure. Unlimited access to water was permitted and fresh chicken with bones was provided twice a day. Fifty ug of gonadorelin acetate (Gonadon®, Dongbang, Korea) was injected i.m. on April 3 because this bitch bled over much of the enclosure and was considered to have finished the proestrus period.

2. Hormone Analysis

Blood samples were collected March 30 to May 28, weekly from cephalic or saphenous veins late afternoon without sedation or anesthesia. The blood collected in evacuated tubes was centrifuged at 4,000 rpm for 15 min and stored at −20℃ until the serum estradiol and progesterone were analyzed by ECLIA (electrochemiluminescence immunoassay) and RIA (radioimmunoassay), respectively at Neodin Vet. Lab. (Seoul, Korea).

3. Vaginal Smear

Vaginal epithelial cells were collected at the same day of blood collection using a cotton swab. The cells, which were rotated against the lumen of the vagina, were gently removed and rolled along a slide glass in 2 rows (Feldman and Nelson, 2004). After air drying at room temperature, the cells were fixed and stained with Diff-Quik® (International Reagents Corp., Kobe, Japan) staining solution.

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RESULTS

1. Hormone Analysis

After injection of gonadorelin to stop prolonged proestrus, the bloody vaginal discharge decreased prominently and estradiol level started decreasing from 26.4 pg/ml on April 3 to 19.8 pg/ml on April 9 while progesterone increasing from 2.31 ng/ml on April 3 to 9.07 ng/ml on April 9 (Fig. 3).

2. Microscopical Observations of Vaginal Epithelial Cells

Typically, parabasal cells with red blood cells, mucus and neutrophils were shown in proestrus (Fig. 1, A), anuclear or nuclear superficial cells in estrus (Fig. 1, B and C), and advent of parabasal cells means the bitch enters into diestrus (Fig. 1, D).

At the beginning of this study, the bitch already showed serosanguineous vaginal discharge (Fig. 2, A-E). The presence of red blood cells was also observed in the vaginal exudate. Anuclear or nuclear superficial cells were seen with a clear background in estrus (Fig. 2, F-H). A sudden advent of parabasal cells were characterized in diestrus (Fig. 2, I and J).

DISCUSSION

Back-calculation from May 2, the parturition date of a dominant pair at the exhibition enclosure of coyotes suggested the breeding season of coyotes here at Seoul Zoo starts in early March. Unfortunately, we couldn't examine the hormones of mated females and compare it to nonpregnant groups. The other two coyotes at the same excluded place exhibited anuclear superficial cells when we started investigating hormone

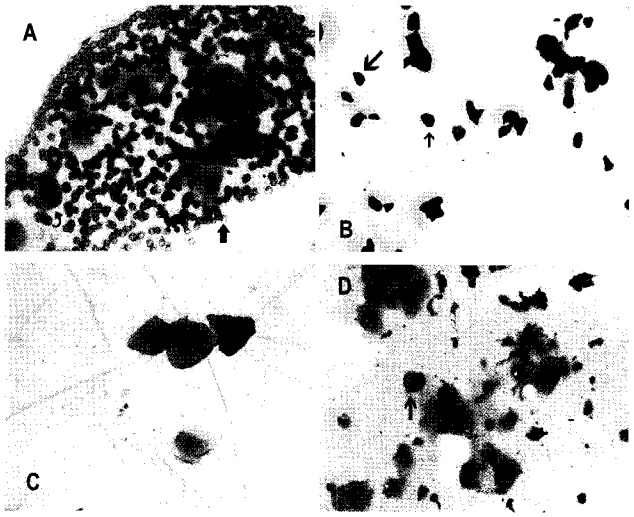

Fig. 1. Cell types of a coyote's exfoliated vaginal epithelium during mating season. (A): Parabasal cells (hair-pin arrow), red blood cells (open arrow) and a neutrophil (straight arrow) are seen in proestrus. (B, C): Anuclear (thin straight arrow) and nuclear (thick straight arrow) cells are observed in estrus. (D): parabasal cells (white arrow) were shown on the vaginal smear.

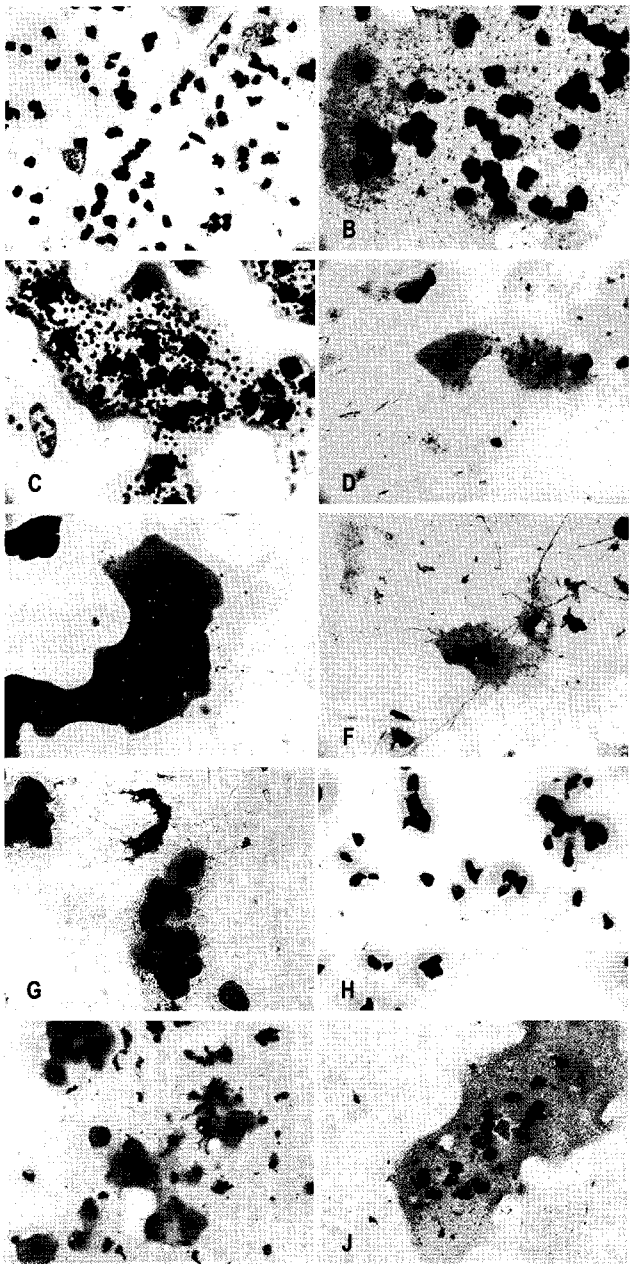

Fig. 2. Vaginal epithelial cells of a coyote before and after GnRH treatment. (A) Mar 30, 100X, (B) Mar 31, 200X, (C) Apr 3, 200X, (D) Apr 17, 200X, (E) Apr 29, 200X, (F) May 8, 200X, (G) May 15, 400X, (H) May 22, 400X, (I) June 1, 400X, (J) June 5, 200X.

levels and vaginal epithelial cells (not shown). Soon they were moved to exhibition enclosure of coyotes but failed to become pregnant.

Red blood cells, mucus, and cell debris were typically observed on vaginal smear even though vaginal discharge was not present on gross examination of the female coyote (Fig. 1, A; Fig. 2, A-E). The vaginal discharge of the bitch in proestrus stopped after injection of gonadorelin acetate. In the meanwhile, estradiol levels also started going down with an increase of progesterone. Therefore, we conclude the use of GnRH analogues like gonadorelin acetate could be applied to coyotes showing protracted proestrus period as similar protocols has positively affected artificial insemination of companion dogs (Feldman and Nelson, 2004; Thomassen and Farstad, 2009). With progression toward latter stages, admixtures of parabasal, various size of intermediate and superficial epithelial cells presented on the vaginal smear (Fig. 1, B and C; Fig. 2, F-H). Superficial epithelial cells that are keratinized or retained pyknotic nuclei are the predominant cell type from approximately day-4 through ovulation to day 7 (Carlson and Gese, 2008). The appearance of superficial cells against a clear background represented the characteristic of estrus in the coyotes, particularly after ovulation (Fig. 2, H) (Hodges, 1990; Green *et al.*, 2002; Carlson and Gese, 2008). At the end of estrus, vaginal smears showed advent of parabasal cells as the coyote entered diestrus (Fig. 2, I and J). In this study, gonadorelin acetate could dramatically decrease serosanguineous vaginal discharge, but its effect on the length of the proestrus period was not clear.

Male dogs are promiscuously accepted by a bitch in a wide range of proestrus and estrus while alpa female coyotes that have a strong pair-bond with their counterparts suppress the other fertile females within their group. Sexual acceptance period of coyotes is 4~5 days, between 2 days before and after ovulation (Carlson and Gese, 2008). Ovulation induction and luteinization can be predicted by a sudden increase of progesterone and a rapid decrease of estradiol after GnRH treatment (Fig. 3). Serum progesterone concentration and vaginal cytology is considered critical standards to estimate the ovulation time in dogs and the progesterone concentration reached or exceeded to 4.0 ng/ml was so properly regarded as the ovulation time that recovering oocytes is good during its time in the oviduct

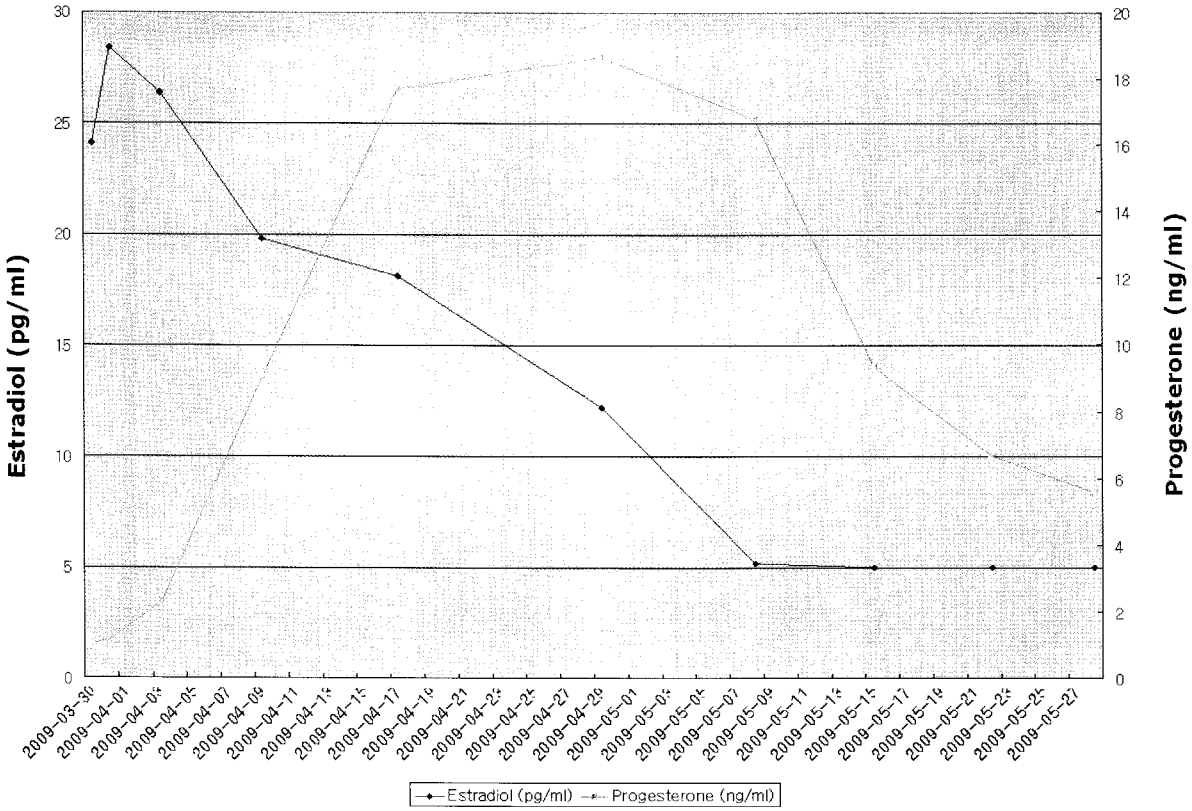

Fig. 3. Concentrations of estradiol and progesterone in the coyote.

(Hase *et al.*, 2000). As in dogs, vaginal cytology and serum progesterone fluctuation could be used for proper timing of artificial breeding of coyotes. We do not suggest the exact time of ovulation, but we could propose that the sudden rise of progesterone after GnRH treatment might be an indicator of right time for inseminating frozen-thawed semen. According to the results of other studies, we could assume that the highest serum progesterone level at the preovulation was 9.0 ng/ml (Stellflug *et al.*, 1981; Carlson and Gese, 2008). Subordinate female coyotes were so sexually suppressed by an alpha female that the relations between vaginal cytology and hormone levels are sometimes unclear (Carlson and Gese, 2008). Like gray wolves (*Canis lupus*), coyotes are seasonally monestrus and only the alpha female in each group breeds and rears young, and behaviorally induced reproductive inhibition is common among subordinate canid females (Mech, 1970; Knowlton *et al.*, 1999). The coyote in this study, as one of a subordinate group, protracted its proestrus that was terminated by injection of gonadorelin. Due to the presence of alpha female, subordinate group members may ovulate and generally fail to breed with prolonged proestrus or estrus (Hodges, 1990). Interestingly, the coyote in our concern, having been separated from the alpha female for a long time, showed prolonged proestrus. Meanwhile, another two female coyotes showed cytological and hormonal changes at a similar time as the alpha female (not shown). In addition, the female coyote investigated in this study showed a raised level of estradiol concentration again after June 16 (not shown) and nesting behavior using its own shedded fur. Extended or splitted estrus is rare in the reproduction cycle of coyotes in the wild (Kennelly and Johns, 1976; Kennelly *et al.*, 1977; Hodges, 1990). In addition, the chance of females producing pups dramatically reduces with a drop in mean litter size especially with mothers between the age of 8 and 12 years (Green *et al.*, 2002). When blood sampling can be routinely performed in the captive breeding of wild canids, serum hormone levels can provide evidence of ovulation, and distinguish pregnancy from pseudopregnancy (Carlson and Gese, 2007). Also, the relative prevalence of exfoliated epithelial cells, red blood cells, leukocytes, and mucus on a vaginal smear can be helpful in predicting reproductive potential of an animal (Zindl *et al.*, 2006; Shin *et al.*, 2008; Thomassen and Farstad, 2009).

To our knowledge, this is the first report showing changes of vaginal epithelial cells and ovarian hormones during estrus of a captive coyote in Korea. Even though coyotes are not indigenous to Korea, the species is scientifically valuable for understanding how to succeed in breeding for species conservation and application of artificial breeding techniques to other canids in captivity.

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