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Predicting conception in red foxes (*Vulpes vulpes*) by monitoring vaginal electrical resistance with coital-lock time

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Abstract

This study aimed to find a practical tool for predicting conception in red fox vixens following natural mating. Basal vaginal electrical resistance (VER) during post-breeding seasons in Japan consistently ranged from 90 to 220 units. The VER displayed a single peak after the vulva became well-swollen during breeding seasons, and median VER magnitude was 350 units among all vixens that became pregnant, 230 units among vixens that did not become pregnant, and 190 units among vixens that did not mate. Conception rates in vixens with VER above 350 units were dramatically higher when coital-lock time was more than 40 min. These findings suggest that a peak magnitude of VER with coital-lock time may be an expedient predictor of conception in red foxes.

Key Words: coital-lock time, red fox vixen, vaginal electrical resistance

The red fox (*Vulpes vulpes*: *V. v.*) inhabits a wide geographic area in the northern hemisphere including Eurasia, North Africa, and Central America. Two subspecies, *V. v. schrencki* and *V. v. japonica*, live in Japan. Red fox vixens are seasonally monoestrous, exhibiting estrus from January to March[3,7)], with a duration recognized to be 2–3 days[3)].

In captivity, some red fox vixens do not become pregnant following vaginal (natural) mating. If non-pregnant vixens can be identified immediately after mating, they may be excluded from the gestation group, reducing labor and other costs of caring for pregnant animals. Frequent pregnancy diagnosis by palpation, ultrasound or X-ray techniques at the early stage is accompanied by a high risk of abortion because conscious vixens are extremely nervous. Although typical dynamic patterns of blood or fecal estrogen and progesterone have been confirmed during breeding seasons, late conception, and parturition in foxes[8,11)], these do not allow for quick determinations in the clinical field.

The present study was carried out to find a

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practical tool for predicting conception in red fox vixens following mating. The level of vaginal electrical resistance (VER) in the cervical mucus of conscious vixens has become a routine technique in modern fox theriogenology[1,5]. VER has been reported to increase during pro-estrus, to reach a maximum in the curve around ovulation, and to decline thereafter[4,8]. The VER is used for detecting the optimum conception time of farmed vixens in foreign countries. Meanwhile, based on our practical observations, the insertion time of the penis in the vagina of vixens (coital-lock time) seemed related to pregnancy occurrence. Thus, we focused on the VER magnitude immediately prior to mating and coital-lock time during mating as non-invasive indices for predicting conception. First, VER was monitored throughout the year to clarify the estrus profile (*e.g.* monoestrous) in Japan. Next, a single peak magnitude of VER prior to mating was classified into three groups based on the outcome of mating attempts. Finally, we examined whether there was a relationship of coital-lock time with conception rates using vixens with VER above 350 units. These data collected in 2008–2017 were retrospectively analyzed.

Red foxes (*V. v. schrencki*) maintained in the privately owned Zao Fox Village (latitude: 38°02'14"; longitude: 140°32'02"; altitude 700 m, Miyagi, Japan) were used in the study. Since its opening in 1985, approximately 200 foxes have been kept in the petting zoo. All foxes are identified individually with microchips. Foxes were fed 300–500 g of mixed feed consisting of dog food and equal parts fish, horse, and beef meal (total energy: approximately 150 kcal/100 g wet feed), in the morning once daily according to body nutrient conditions, with tap water provided *ad libitum*. Vixens for propagation were kept in individual outdoor breeding wire cages (90 cm × 120 cm × 60 cm). Wooden boxes used as nursery cases (90 cm × 50 cm × 40 cm) were divided into three enclosed, dark sections and combined with breeding wire cages via an entrance tunnel[2,9,10] during pre-parturition and nursing stages for 7 weeks. All procedures were carried out in accordance with the Guidelines for Animal Experimentation[6] with the animal welfare rules of the Zao Fox Village.

The VER of vixens was measured twice at 2-min intervals with an ovulation detector (Draminski Dog Ovulation Detector; Draminski, Olsztyn, Poland) attached to a vaginal probe. The probe rod (7 mm in diameter) was inserted approximately 7–10 cm up to the cervix for 30 sec until the VER value stabilized. The lower value at 2-min intervals was adopted. VER units were expressed according to the methods specified by the manufacturer. Based on the result of the preliminary study, one unit in the VER measurement used in this investigation corresponded to one ohm (Ω) in the VER determined by VER equipment provided from another manufacturer (SI-LI 3 D type, A/S Lima, Co., Sandnes, Norway).

In the first study conducted in 2008, four healthy mature vixens (animal Nos. 1 to 4), aged 23–24 months and weighing 8–10 kg, were utilized. VER was monitored at a fixed time in the early morning during post-breeding (April to December) and breeding seasons (January to March). Foxes were regarded as "healthy" based on appetites, the condition of feces and urine, the appearance of the fur and clinical observations.

In the second study performed in February to March of 2009 and 2010, 32 healthy vixens (Nos. 5 to 36), aged 23–24 months and weighing 8–10 kg, were chosen to examine the effect of a single peak magnitude of VER on conception rates. After visually confirming well-swollen vulva, the VER was measured for 2 consecutive days prior to mating. Nineteen male foxes that had successfully bred in the past, aged 36–48 months, were used as stud foxes. Most of the males utilized were imported as original studs from Norway and their first filial generations were also used. Using semen obtained in advance by electroejaculation, only individuals showing high viability of spermatozoa were selected as studs. The mean number and its range of active

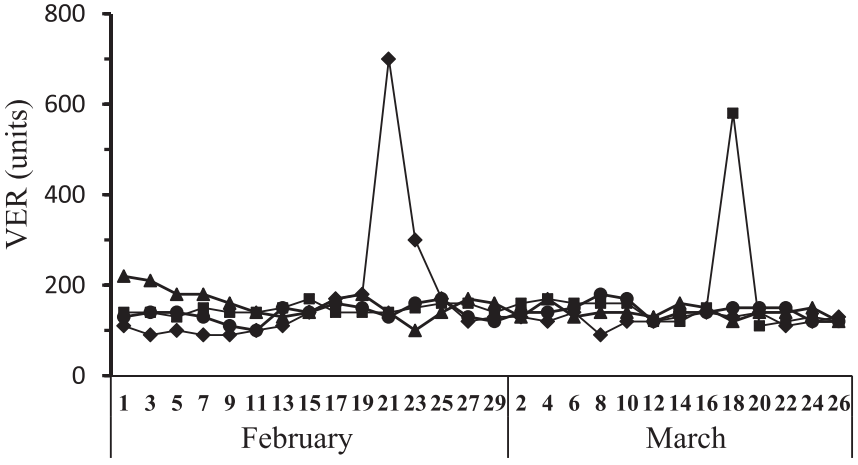

Fig. 1. Continuous changes in vaginal electrical resistance (VER) of vixens in February to March in the first study. Two (closed diamonds and squares) of four vixens elicited high peak VER values, but two other vixens (closed circles and triangles) consistently had values close to the basal mean.

spermatozoa in studs used were 516×10^6/m*l* and $272–692 \times 10^6$/m*l*, respectively, indicating that their counts were good enough to make vixens pregnant. One stud was introduced to each vixen with estrus in the breeding wire cage for 1 hr in the morning and their interactions were observed via a digital video cassette (DCR-TRV950, SONY, Tokyo, Japan) to determine whether coupling had succeeded. In cases where successful copulation was not observed, another male fox was introduced to the vixen. Abortions during gestation were assessed by bleeding on the cage floor under the video monitor.

In the third study carried out in 2009–2017, 44 healthy vixens with VER above 350 units were selected to examine the relationship of the coital-lock time with conception rates after the first mating challenge. Coupling of a vixen showing aggressive or avoidance behavior against the male in the first mating challenge was attempted a maximum of three times with changing males at intervals of 24 hr. The data of the first mating was selected in this trial because of the initial breeding event in vixens used. Thirty-five males, which included original stud foxes imported from Norway and their first and second filial generations, were used in this study. The high viability of spermatozoa in each stud prior to mating was confirmed microscopically. Age and bodyweight of females and males at the initiation of the trial were 24–60 months and 8–11 kg, and 24–60 months and 9–11 kg, respectively. Based on each coital-lock time obtained, vixens were divided into 4 groups; 20 min or less, 21–30 min, 31–40 min, and more than 40 min, and their conception rates (number of pregnancies / number of vixens divided) were represented as percentages.

Numerical data are expressed as the mean $\pm$ the standard error of the mean (SEM). Statistical evaluation was analyzed by Welch's t-test between two groups. A probability level of $P < 0.05$ indicated statistically significant.

In the first study, estrus signs were observed only in February and March under the conditions of this study. A single peak VER after the vulva became well-swollen was observed in two (720 units in No. 1, and 590 units in No. 2) of the four vixens (Fig. 1). The findings were well consistent with the results of previous studies in red fox vixens[1,3,7]. The baseline anestrous VER values before and after peak levels ranged consistently from 90 to 220 units with a mean value of 140 ± 2 units [calculated from 108 points taken from the same vixens (N = 4) on different days, from 1 February to 26 March, excluding 2 days representing VER above 350 units]. The reason the remaining two vixens (Nos. 3 and 4) showed

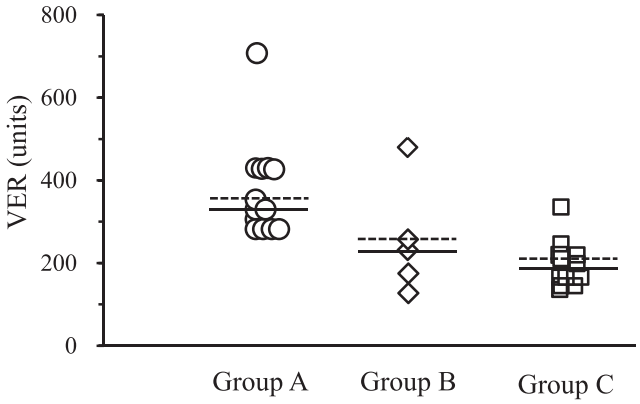

Fig. 2. Scatterplots showing changes in vaginal electrical resistance (VER) of vixens in the second study. Group A: 13 vixens that became pregnant after mating; Group B: 5 vixens that did not become pregnant after mating; Group C: 14 vixens that did not mate. Dotted and solid lines represent the mean and median values, respectively, of each group.

no changes in VER despite the breeding seasons may be explained by immature sexual susceptibility in the vixens.

In the second study, 32 vixens were divided into three groups, namely, vixens that became pregnant after mating (Group A), those that did not become pregnant after mating (Group B), and those that did not mate (Group C) (Fig. 2). The mean, non-estrous, basal anestrous VER of 32 vixens measured on a day randomly selected during February and March was 144 ± 4 units, broadly corresponding with the data (140 units) obtained in the first study. The median VER value prior to mating was 350 units (range: 280 to 700 units; mean ± SEM: 381 ± 30 units) in the 13 vixens of Group A, 230 units (range: 160 to 480 units; 264 ± 51 units) in the 5 vixens of Group B, and 190 units (range140 to 340 units; 202 ± 13 units) in the 14 vixens of Group C (Fig. 2). One vixen in Group B and one vixen in Group C had high VER values (480 and 340 units, respectively), but did not become pregnant. Moreover, animal No. 10 did not become pregnant following repeated mating challenges thereafter. Briefly, although the mating was conducted once a year for 3 consecutive years, this vixen did not become pregnant. The cause remains still unclear. Animal No. 36, which showed estrus signs without mating, was highly neophobic, and engaged in aggressive or avoidance behaviors when introduced sequentially to different males. The cause of such behaviors, which have been occasionally observed, remains unresolved. According to our observations, all studs showed in common enlargement (3 to 5 folds) of the testes during the breeding seasons compared to during post-breeding seasons. No individual difference in mating behaviors of studs selected was noted under the conditions of this investigation. The visual signs of estrus in Group C were considerably subtle relative to those in Group A. No abortion was visually observed in any of the groups throughout gestation. These data suggest that VER values between 270 and 280 units may become a marginal zone for predicting conception.

Although it was not measured exactly in the second study, coital-lock time in Group A was likely to have been longer than that in either Group B or C. Thus, we focused on the coital-lock time as another index.

In the third study, conception after the first mating challenge occurred in 23 of the 44 vixens with VER above 350 units (conception rate: 52%). The median coital-lock time (40 min) in pregnant vixens (N = 23) was significantly longer than that (25 min) in non-pregnant vixens (N = 21) (Fig. 3A). Conception rates at the coital-lock times 20 min or less, 21–30 min, 31–40 min, and more than 40 min were 0% (0/4 vixens), 27.8%

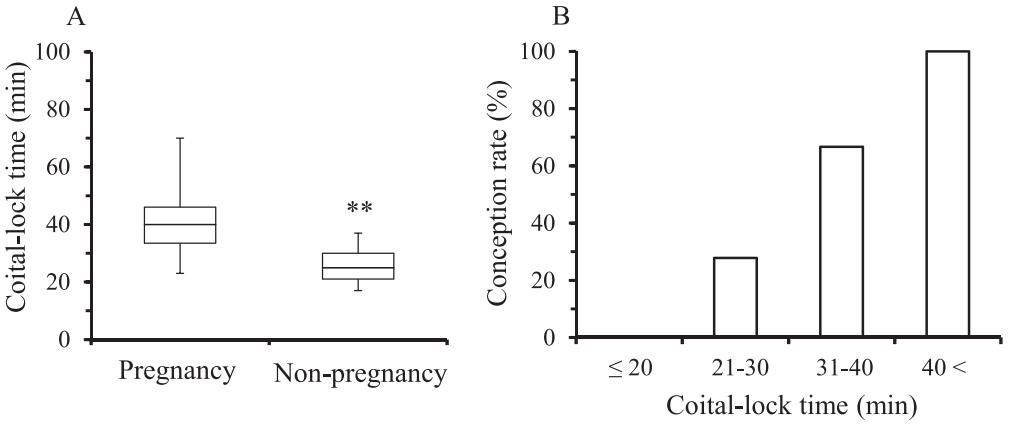

Fig. 3. Coital-lock time in pregnant and non-pregnant vixens (A), and conception rates at each coital-lock time (B) classified in the third study. A: Coital-lock time in pregnant (N = 23) and non-pregnant (N = 21) vixens are depicted as box and whisker plots. Median and quartiles are shown in the box. Upper and lower bars represent maximum and minimum values, respectively. $^{**}P < 0.01$ vs. pregnant vixens.B: Based on each coital-lock time, vixens are divided into 4 groups; 20 min or less (N = 4), 21–30 min (N = 18), 31–40 min (N = 12), and more than 40 min (N = 10), and their conception rates are calculated.

(5/18), 66.7% (8/12), and 100% (10/10), respectively (Fig. 3B). These values were coital-lock time-dependent with increasing conception rates. There was no relationship between coital-lock time and litter size (3–4 cubs in all groups). According to the report in blue foxes from Møller *et al*[8], the maximum estradiol-17β in plasma took place 2 days before a peak magnitude of VER and coincided regularly with the luteinizing hormone (LH) surge followed by ovulation. The levels and duration of the major LH surge was considered to be 1–3 days[8]. Taken together, one possibility is raised that the sexual receptivity with longer coital-lock time or conception rate may depend largely on the estradiol-17β level that corresponded with the VER magnitude. Alternatively, further studies are necessary to collect cumulative background data for VER and coital-lock time with estimation of blood or fecal estrogen, LH and progesterone levels during pro-estrus and estrus periods in red foxes.

In conclusion, monitoring of a single peak magnitude of VER prior to mating together with coital-lock time during mating as a non-invasive tool may provide additional, valuable information about the likelihood of conception after mating in red fox vixens.

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