Validity of Retrograde Corpora Lutea of Pregnancy As an Index of Past Gestation in Sika Deer, *Cervus nippon*

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ABSTRACT. Corpora lutea of the sika deer (*Cervus nippon*, Cervidae) were histologically examined in order to elucidate their retrograding processes after parturition. They showed a remarkable regression within one month as luteal cells decreased in number and, by nine months after parturition, turned into masses of blood vessels containing many arterioles with a diameter of 30 μm or less. In retrograde corpora lutea (RCL), older than 1.5 years after parturition, arterioles of these dimensions decreased markedly, while those measuring 50 μm or more became dominant. These findings indicate that an examination of RCL can distinguish females less than nine months after parturition from those more than 1.5 years after parturition. Based on the findings of RCL from the ovaries of a 10.5-year-old female, it is suggested that RCL remain in the ovaries for at least 8.5 years after parturition. It was, however, difficult to distinguish RCL of pregnancy from retrograde accessory corpora lutea (RACL). In order to arrive at a complete count of RCL, the ovaries should be examined at thicknesses of 0.5 mm. To check the presence/absence of RCL of pregnancy within nine months or less after parturition, sectioning at thicknesses of 2 mm is sufficient. — **KEY WORDS:** *Cervus nippon*, deer (sika), past gestation, reproductive history, retrograde corpus luteum.

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To determine the past ovulation or parturition of wild animals, ovaries have sometimes been examined for RCL (e. g., Japanese serow [5], cetacea [4]). In deer species of the subfamily Odocoileinae including the white-tailed deer, *Odocoileus virginianus* [1], black-tailed deer, *Odocoileus hemionus* [3, 15], and caribou, *Rangifer tarandus* [2], for which the morphology and lifetime of RCL have been elucidated, similar morphological examinations have been conducted to estimate reproductive performance. However, for species belonging to the subfamily Cervinae, such as the red deer, *Cervus elaphus*, and sika deer, a comparable method has not yet been established. The authors have previously clarified the histological characteristics of RCL of estrus and pregnancy, and estimated their lifetimes to be up to three months and more than seven years, respectively [6]. In some monotocous animals such as the cow [10] and the Japanese serow [12], the number of RCL of pregnancy in the ovaries is consistent with the number of past parturitions. However, since a pregnant sika deer often has two corpora lutea (the smaller one is referred to as an accessory corpus luteum, ACL) in spite of her monotocous nature, the total number of RCL in both ovaries may be greater than that of her past parturitions. This makes it difficult to estimate the actual number of past parturitions in the sika deer. If we can obtain pairs of ovaries from females with known reproductive histories and find histological differences in the retrograded bodies, they can be used as criteria to show the time of formation of an individual corpus luteum, or to show the year of pregnancy.

The sika deer of Japan usually mates in October and delivers a single fawn in the following June or July. Evidence of pregnancy in fawns (0.5 years old) is not

reported. About one half of the females become pregnant at one year old and more than 90% at two years old [14]. In the present study, we collected the samples from a population inhabiting Wadayama, Hyogo Prefecture, western Japan. The pregnancy rate of females older than 1.5 years was high 73.3–89.9% [7], and ACL were found in 80.7% of the pregnant females [13]. Therefore, for this population, we can estimate the approximate number of past parturitions and that of RCL in the ovaries from the age of individuals. The objective of this study was to determine the time of past pregnancies of the female sika deer by using the ovaries from females with a presumable reproductive history and by examining RCL histologically.

MATERIALS AND METHODS

Eight pairs of ovaries from adult females of sika deer shot at Wadayama, Hyogo Prefecture, in March 1992 were used as the subjects of the presumptive reproductive histories. To observe the regression process of corpora lutea during the period from late pregnancy to five months after parturition, we used twenty pairs of ovaries from adult females (8, 2, 4, 2 and 4 in June, July, September, October and November, respectively) shot at Mt. Goyo, Iwate Prefecture, from November 1990 to October 1991 and one pair of ovaries from an adult female shot at Shodoshima Island, Kagawa Prefecture, in June 1993. The ovaries were fixed in 10% formalin and then embedded in paraffin. The samples from Hyogo Prefecture were serially sectioned at a thickness of 100 μm , and those from Iwate and Kagawa Prefectures were sectioned at 2 mm. The sections were then stained with hematoxylin-eosin and Weigert's

resorcinfuchsin. The age of the deer was estimated by counting annual cement rings of the first incisors.

RESULTS

Retrograding process of corpora lutea after parturition: Corpora lutea at the late stage of pregnancy were filled with normal luteal cells, which suddenly decreased in number after parturition with degenerated ones scattering in the parenchyma (Fig. 1). At 1–3 months after parturition, the parenchyma was occupied by arterioles measuring 50 μm or less in diameter and by connective tissues containing a small number of degenerated luteal cells (Fig. 2). At 5–9 months after parturition, the parenchyma was largely occupied by arterioles 50 μm or less in diameter, most of which measured 30 μm or less; debris of degenerated luteal cells were found in very small amounts; and on capsules, a small number of elastic fibers began to be seen (Figs. 3 and 4). At 1.5 years after parturition or later, arterioles measuring 30 μm or less in diameter decreased markedly, with the parenchyma occupied mainly by those measuring 50 μm or more, and elastic fibers appeared on capsules (Fig. 5). As regression of corpora lutea proceeded, the number of arterioles decreased and elastic fibers proliferated on the arterial walls, making less clear the border between retrograde bodies and surrounding tissues (Fig. 6).

In retrograde bodies 1.5 years or older after parturition, arterioles 30 μm or less in diameter abruptly decreased in density (Table 1). RCL of pregnancy and RACL could be distinguished by their different dimensions by nine months after parturition, while thereafter no morphological differences were found between the two.

Various types of RCL and their occurrence: RCL were classified into four types according to phases of the histological characteristics:

Type I: Many luteal cells and arterioles 30 μm or less in diameter in the parenchyma; the border with surrounding tissues is clear with no elastic fibers on capsules (Fig. 1).

Type II: A few luteal cells and a number of arterioles 30 μm or less in diameter; the border with surrounding tissues is clear with elastic fibers appearing on arterial walls and capsules (Figs. 2–4).

Type III: The parenchyma has turned into a mass of arterioles without showing any luteal cells; arterioles 30 μm or less in diameter have decreased in number while those 50 μm or more are dominant; the border with surrounding tissues has become less clear, with elastic fibers appearing on arterial walls and capsules (Fig. 5).

Type IV: The parenchyma is composed of connective tissues and a few arterioles; on the blood vessel walls we observed a large number of elastic fibers in the process of disintegration; the border with surrounding tissues is not clear (Fig. 6).

Type I, Type II and Type III–IV corresponded to post-parturition intervals of one week, 1–9 months and 1.5 years or more, respectively. Table 2 shows the frequency of each type detected in pairs of ovaries from seven females by a detailed microscopy of their serial sections. Type I RCL

were not found in any female, whereas one or two RCLs of Type II were found in all of the females. Type III was found in all females 4.5 years or older and increased with age. RCLs of Type IV were observed only in a single female of 10.5 years (Table 2).

Thickness of sectioning for calculating the number of RCL: Length, width and thickness of 50 RCLs found in pairs of ovaries from eight females were measured by the reconstruction of RCL obtained from serial sections (Table 3). The lowest renunciation limit of the cube root of the product of the three diameters was 0.45 mm. The same figure for eight RCLs of pregnancy about nine months after parturition was 1.85 mm.

DISCUSSION

At which gestation were the individual RCLs formed? This estimation has been attempted in several animal species. Kita *et al.* [5] have classified RCL of pregnancy of Japanese serows into five types based on the number of remaining luteal cells and the extent of degeneration of the small arteries in the parenchyma. They have reported that the presence of RCL of the freshest type is indicative of pregnancy during the previous year. Mansell [9] stated that, for the white-tailed deer, RCL within one year after parturition were distinguishable from older ones through their sizes and histological characteristics. For the caribou [2] and red deer [8], it has been reported that RCL within five months of parturition can be distinguished from those aged one year or more. For the sika deer, however, estimation of RCL's age remains difficult because, unlike for the white-tailed deer, the indices or criteria such as shape, dimension, and amount of "pigmented lutein cells" [9] have not been available. In the present study, however, we could distinguish RCL within nine months from those of 1.5 years or older by using the number of arterioles of 30 μm or less in diameter found in the parenchyma. That is, the presence of RCL within nine months after parturition can be regarded as positive evidence of pregnancy having occurred the year before.

What is the maximum thickness of slices for a complete count of RCLs in the ovaries? From the measurements of 50 RCLs, the lowest renunciation limit proved to be 0.45 mm. Since a 10–20% contraction of tissues due to paraffin embedding is given [11], 0.5 mm thick sectioning of formalin-fixed ovaries would be appropriate to observe all the RCL without omission. To examine the presence/absence of RCL within 9 months after parturition, 2 mm slices would suffice.

Of eight sika deer from Hyogo Prefecture, 7 females of known age were examined for appearance of RCL types. Based on the reproductive condition of sika deer in Japan [7, 14], we have presumed that there were no twins, and no pregnancies at 0.5 years among the seven. For Type I ("within one week after parturition"), no RCL were detected. This is because all the ovaries examined were obtained in March. For Type II, one or two RCLs were observed in each pair of ovaries, a finding that can be attributed to the

Fig. 1. Corpus luteum within a week of parturition (Type I). Degenerating luteal cells are scattering. $\times 75$, Hematoxylin-eosin staining (HE).

Fig. 2. Corpus luteum approximately one month after parturition (Type II). Parenchyma is occupied by arterioles and connective tissue which contains degenerated luteal cells (arrows). $\times 150$, HE, Bar = $50\ \mu\text{m}$

Fig. 3. RCL approximately nine months after parturition (Type II). Parenchyma was mostly occupied by blood vessels. $\times 30$, Weigert's resorcinofuchsin staining (W).

Fig. 4. Higher magnification of Type II RCL. Many arterioles of $<30\ \mu\text{m}\phi$ are seen. $\times 150$, HE, Bar = $50\ \mu\text{m}$.

Fig. 5. RCL of 1.5 year after parturition (Type III). Few arterioles of $<30\ \mu\text{m}\phi$ are seen. Parenchyma is occupied mainly by arterioles of $>50\ \mu\text{m}\phi$. $\times 150$, HE, Bar = $50\ \mu\text{m}$.

Fig. 6. RCL presumed to be more than 6.5 years after parturition (Type IV). $\times 75$, W.

Table 1. Density of arterioles (<30 $\mu\text{m}\phi$) within RCL of pregnancy

Time after parturition	Number of RCL examined	Density of arterioles (Mean $\pm$ SD, /mm ²)
<1 week	4	66.0 $\pm$ 22.6
1~3 months	4	72.3 $\pm$ 5.1
ca. 9 months	8	98.8 $\pm$ 6.1
>1.5 year	8	34.0 $\pm$ 7.0

Table 2. Number of RCL in females of various ages

Animal	Age (Year)	Number of RCL				
		Type I	Type II	Type III	Type IV	Total
A97	2.5	0	2	0	0	2
A108	3.5	0	2	0	0	2
A101	4.5	0	1	1	0	2
A102	5.5	0	2	5	0	7
A109	6.5	0	2	7	0	9
A 90	7.5	0	2	6	0	8
A 98	10.5	0	2	9	6	17

Table 3. Size of RCL (Mean $\pm$ SD, mm)

	Number	X (length)	Y (Width)	Z (Thickness)	$\sqrt[3]{X \cdot Y \cdot Z}$
RCL of pregnancy and RACL	50	2.0 $\pm$ 0.7	1.1 $\pm$ 0.5	1.6 $\pm$ 0.8	1.5 $\pm$ 0.5
RCL of pregnancy within 9 months after parturition	8	2.6 $\pm$ 0.4	1.6 $\pm$ 0.3	2.8 $\pm$ 0.4	2.3 $\pm$ 0.2

high pregnancy rate and high frequency of ACL among adults in the study population. Type III and Type IV are problematic. Figure 7 compares the number of RCL expected from the age with the number actually observed. For the animal A 97, it was expected from her age (2.5 years) that she had delivered, if ever, only once 0.5 years before and that she would have one or two RCLs of Type II (two were in fact observed). The animal A 108 (3.5 years) could have delivered once or twice, and therefore four RCLs could be expected. However, only two (Type II) were actually observed, suggesting that she had delivered only once (0.5 years before). Similarly, the animal A 98 (10.5 years) could have delivered nine times and would have had 18 RCLs at most, whereas 17 were detected, two of Type II, nine of Type III and six of Type IV. This female was considered to have had successive parturitions starting 8.5 years before and to have delivered nine times. From these results, it seems plausible that RCLs of Type III are 1.5–5.5 years after parturition, and those of Type IV are 6.5 years or more. In addition, four of the seven females (A 97, A 98, A 102 and A 109) had probably delivered every year, while two females (A 101 and A 108) had failed to deliver once and the other (A 90) had failed to deliver once or twice. The fact that the oldest retrograde body was 8.5 years after parturition suggests that the lifetime of RCL is 8.5 years or more. Further observations using older females will be necessary.

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Fig. 7. Relationship between each type of RCL and years after parturition. ○: RCL expected from the age, ●: RCL actually observed.

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