Saliva progesterone in sea lion

Research note

Measuring Saliva Progesterone in the California Sea Lion Zalophus californianus

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カリフォルニアアシカ（Zalophus californianus）の唾液による早期妊娠診断とモニタリングの試み

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ABSTRACT. Two captive mature female California sea lions (Zalophus californianus) were trained to provide saliva samples for the purpose of diagnosing of pregnancy at an early stage and monitoring reproductive cycles. Saliva was collected from 1 to 4 times per week from autumn of 1996 to spring of 2000, and the progesterone concentration in the saliva was measured by enzyme immunoassay (EIA). In spite of differences between the two sea lions used in the study, the data thus obtained allowed us to plot their annual reproductive cycles and to diagnose pregnancy a few months earlier than possible using an ultrasonoscope. A comparison of progesterone concentrations in plasma and in saliva failed to show correlation in one of the two animals included in this study, however it was suggested that refining the sampling method will improve this correlation.

Key words: California sea lion, saliva, progesterone.


Diagnosis of pregnancy at an early stage is highly important for the proper management of captive wild animals. In land mammals, pregnancy is widely diagnosed and monitored by examining blood levels of progesterone [1], while for marine mammals, blood samples can be drawn from small cetaceans without restraint by utilizing operant-conditioned methods, and reproductive status is thus easily monitored throughout the year [2]. For pinnipeds, on the other hand, physical restraint is often necessary to draw blood samples, making it difficult to do so frequently. Furthermore, delayed implantation has been reported in a number of pinnipeds species [3] and plasma progesterone remains at a relatively high value for several months after conception, so it is difficult to distinguish pregnancy during this period. As a result, by the time it becomes possible to accurately diagnose pregnancy by blood sample, the sampling process is likely to be stressful for the mother. In the present study, we measured progesterone from saliva samples that were collected non-invasively and with no
stress to the animal in order to assess the validity of saliva progesterone for diagnosing pregnancy and for monitoring the reproductive status of California sea lions *Zalophus californianus*.

Two adult female California sea lions in captivity were used in this study. Both were sexually mature at the time of the experiment: one (#1) was born in 1990 and the other (#2) was born in 1992. They were kept in captivity for show performances and were given basic training for saliva sampling. Saliva collection was made while the animals were out of the water or after wiping the mouth with a cotton swab to prevent water contamination. The samples were collected during routine training sessions by experienced trainers. A cotton swab was placed in the animal’s mouth while the trainer showed the sea lion a bucket of fish to stimulate salivation. If the cotton swab seemed wet enough, it was removed from the sea lion’s mouth and saliva was pressed out of the swab into a plastic tube using a syringe. Saliva samples were collected 1 to 4 times per week depending on training conditions. To prevent metabolism of progesterone [4], saliva samples were frozen at -20°C immediately after sampling, though it took 5 to 10 minutes to finish all sampling processes. Blood samples were also collected for comparison from veins of the hind flippers while the sea lions were physically restrained. During gestation, blood samples were taken infrequently out of consideration for the safety of the mothers. The sampling periods for saliva were from September of 1996 to April of 2000 for #1 and from October of 1996 to July of 1999 for #2; sampling periods for plasma were from November of 1995 to November of 1999 for #1 and from July of 1997 to October of 1999 for #2.

Plasma and saliva progesterone were measured using double antibody enzyme immunoassay (EIA) as previously described [5]. The plasma and saliva were extracted by diethyl ether. Interassay variation was 13.2% and 9.8% for saliva.

**Fig 1.** Progesterone concentrations in the saliva of two California sea lions (Upper: #1, Lower: #2). Arrows indicate the gestation periods of each animal. BS: Breeding seasons, estimated by behavior and by the appearance of the vulva. ★: The day when pregnancy was confirmed by ultrasonic examination.
progesterone and plasma progesterone, respectively, and intraassay variation was 10.8% and 11.2%.

For comparison, ultrasonic examination (SSD610 with 3.5MMz liner probe, Aloka Co., Ltd., Tokyo, Japan) was performed in January, February and March of each test year on each animal that had shown mating behavior during the preceding breeding season. The estrus of each animal was determined by the appearance of the vulva, and behavior observations were recorded during the breeding season for each animal. The ultrasonic diagnosis of pregnancy was done by visual confirmation of vertebrae and a heart of fetus.

The progesterone concentrations in the saliva and the plasma of the two sea lions are shown in Figs. 1 and 2, respectively. The progesterone concentrations in the saliva of the two sea lions were quite different in their range at undetectable to 2.77ng/ml for #1 and undetectable to 1.03 ng/ml for #2 over the entire sampling period of each animal. The progesterone concentration in saliva followed a relatively similar pattern to that in plasma, although the correlation between saliva and plasma levels was 0.671 for #1 and 0.013 for #2 (P<0.05).

For #1, the saliva progesterone concentration remained low from September of 1996 to May of 1998, while the plasma concentration showed a similar pattern. Observations of the behavior of #1 indicated that her ovaries were nonfunctional during this period, since neither changes in the appearance of the vulva nor mating behaviors were observed. Due to improvements in husbandry, #1 later underwent estrus and mating behavior was observed during the breeding season of 1998. Subsequently, both behavioral observation and saliva and plasma progesterone levels indicated that the breeding cycle of #1 resumed its normal cyclic pattern. Mating behavior was observed during the breeding seasons of both 1998 and 1999, and parturition was successful in June of 2000. The saliva progesterone levels of #1 remained relatively high from December of 1999 to April of 2000. The fetus was confirmed by ultrasonic examination in March of 2000.

The reproductive cycles of #2 were deemed to be normal based on the plasma concentrations of progesterone and behavioral observations. Her saliva progesterone concentrations were variable but tended to rise prior to breeding season. Mating behavior was observed during the 1997 and 1998 breeding seasons, and parturition was successful in July of 1999. Sea lion #2's saliva progesterone levels remained relatively high from January of 1999 to July of 1999. The diagnosis of pregnancy was confirmed by ultrasonic examination in March of 1999.

Analysis of saliva is widely conducted in the fields of human clinical and biological research [6]. Steroid measurement is one of the most reliable procedures for screening assays in humans since plasma and saliva concentrations are well correlated due to passive diffusion through the cell membrane of the salivary epithelium [7]. Saliva progesterone concentrations have been measured in several different species [8-10], and the ranges of values and their correlation coefficients differ according to the

![Fig 2. Progesterone concentrations in the plasma of two California sea lions.](image-url)
species. In the present study, the concentration of progesterone in both saliva and plasma was comparatively low although saliva levels were found to be relatively high from December to June of the gestation period of each animal. Individual differences were noted between the two individuals included in this study, and, despite the high correlation coefficient between saliva and plasma progesterone levels reported for the Hawaiian monk seal Monachus schauinslandi [11], such correlation was found in only one of the sea lions. Meanwhile, a use of salivary progesterone concentration for pregnancy detection in a California sea lion was reported [12], so it was suggested that our result might have been due to differences in training since the sea lions, especially #2, were sometimes indolent and contamination by water was suspected. Modification of the sampling method is expected to improve the accuracy of future data. Furthermore, progesterone is known to be metabolized by saliva [4], delayed freezing or partial thawing during transportation to laboratory might affect the measurement especially when the levels of progesterone were low. Additionally, the low frequency of blood sampling during the gestation periods when the concentration of progesterone was high might also contribute to ambiguity of the result.

Nevertheless, the use of saliva progesterone levels to diagnose pregnancy was successful and diagnosis was possible a few months earlier than by ultrasonic analysis; it was also possible to accurately monitor the reproductive status of the animals. Taking saliva samples is a safe and easy method of examining the reproductive status of California sea lions; it is also less stressful for the animals. Given that there might be a certain amount of variation in the progesterone concentrations found in saliva, repeated samples must be taken to confirm a diagnosis of pregnancy.

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REFERENCES


要約

飼育下のカリフォルニアアシカ雌２頭の唾液を非侵襲的に採取し、唾液中プロゲステロン（P）濃度を測定することにより、早期妊娠診断と性周期のモニタリングに用いることが可能であるかを検討した。その結果、個体差はあるものの、唾液中P濃度の年間変動をモニタリングすることができ、妊娠診断も超音波エコーを用いるより2か月以上早期に行うことが可能であることが判明した。

キーワード：カリフォルニアアシカ、唾液、プロゲステロン

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