Contraceptive Effect of Proligestone on Spotted Seals and Crossbreeds of Spotted Seals and Harbor Seals

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ABSTRACT. Spotted seals (*Phoca largha*) and related crossbreeds maintained at Kamogawa Sea World breed seasonally from the end of January to middle of March. For contraception in these animals, the effect of a single administration of a contraceptive synthetic luteal hormone for dogs, proligestone (PRG), was investigated. The animals tested were 10 seals aged 4–24 years old, and a total of 35 trials were performed over five years. PRG was administered in 23 trials during January, which was one month before the estimated estrus, and in 12 trials during December of the previous year, which was two months before the estimated estrus. The dose of PRG was 5 mg/kg in 32 trials and 10 mg/kg in 3 trials. The effect of the contraception was judged by the presence or absence of delivery. Among 23 animals treated in January, 2 animals treated with 5 mg/kg PRG became pregnant, but the contraception was successful in the other 21 animals. Contraception was successful in all 12 trials treated with 5 mg/kg PRG in December. Overall, contraception was successful in 94.3% (33/35). Therefore, a single administration of 5 mg/kg PRG in December may be an effective method of contraception for seals.

KEY WORDS: estrus prevention, proligestone, seal.

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Although aquariums try to breed Pinnipeds, the capacity of breeding facilities is limited and a method of scheduled reproduction that maintains genetic variety is important. Moreover, the conception rate among seals increases when different species are kept together [1, 5, 9, 13]. Thus, contraception is required to prevent crossbreeding. However, studies using hormones for contraception in seals have not progressed [12].

Spotted seals (*Phoca largha*) and harbor seals (*Phoca vitulina*) are monoestrous animals with spontaneous ovulation, and are seasonal breeders with delayed implantation [2, 3]. Furthermore, seals become reproductive at 4 years old [2, 5]. Spotted seals and kuril harbor seals inhabiting Hokkaido enter estrus in April and June, respectively, subsequently becoming pregnant and delivering their young [5, 7]. In Kamogawa Sea World (Kamogawa, Chiba, Japan), non-pregnant seals enter estrus from the end of January to the middle of March, although this varies annually (unpublished data). Males are also seasonal breeders, as shown by histological findings of the testis and the peripheral blood testosterone level [2, 4]. The age of sexual maturity, initiation of the breeding season, and the duration of delayed implantation are considered to be affected by light, temperature, and nutritional conditions. The delivery period in spotted seals and crossbreeds of spotted seals and harbor seals is February–March in our aquarium (Table 1), and estrus onset occurs soon after delivery.

There are 18 species of seals known to date, but little has been clarified regarding the endocrinology related to reproductive physiology. There is one report on the time courses

of progesterone (P₄) and estradiol-17 β (E₂) levels during gestation and non-gestation periods in harbor seals [8]. According to this report, the P₄ level is high even during the period of delayed implantation, and the blood P₄ level is high during the late gestation period due to P₄ secretion from the placenta. The blood P₄ level in non-pregnant animals shows a course similar to that during the early gestation period, but the level decreases within about six months. These findings suggest that pregnancy may be diagnosed by measuring the blood P₄ level six months after estrus and thereafter [3].

A common contraceptive method using a drug is the administration of synthetic luteal hormone. Synthetic luteal hormone includes chlormadinone acetate [11], an implant developed for dogs, and proligestone (PRG) for injection [14]. However, the implant was developed for long-term contraception over 1–2 years and requires anesthesia for subcutaneous implantation and excision. PRG is injected subcutaneously at 3–5 month intervals in dogs. Thus, in non-pregnant seals in our aquarium, a single injection may inhibit estrus during the breeding season between January and March. The dose of PRG for subcutaneous administration is 20 mg/kg in dogs, and the dosing volume for a large dog is less than 6 ml (600 mg). Thus, we estimated a dose of 5 or 10 mg/kg for seals weighing about 100 kg.

In this study, we administered a single dose of PRG to spotted seals and related crossbreeds, then investigated the contraceptive effect.

MATERIALS AND METHODS

Animals: The animals used were 10 female spotted seals and crossbreeds of spotted seals and harbor seals kept at Kamogawa Sea World. The age, body weight, and past

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Table 1. Animals tested

No.	♂ × ♀ (Breed)	Birthday (age) ^{a)}	Body weight (kg) ^{b)}	Parity ^{c)}
1. Spotted Seals	(Wild)	1971 (25)	116	9
2. Spotted Seals	–	1979.3.5 (15)	104	2
3. Crossbreed	Spotted Seals × Harbor Seals	1979.3.7 (15)	120	2
4. Crossbreed	Spotted Seals × Harbor Seals	1982.2.26 (12)	117	1
5. Crossbreed	Crossbreed × Crossbreed	1984.2.14 (10)	90	1
6. Crossbreed	Crossbreed × Harbor Seals	1984.3.5 (10)	101	1
7. Spotted Seals	–	1987.3.18 (7)	86	0
8. Crossbreed	Crossbreed × Crossbreed	1991.2.24 (4)	75	0
9. Crossbreed	Crossbreed × Crossbreed	1991.3.7 (4)	80	0
10. Crossbreed	Crossbreed × Spotted Seals	1995.2.19 (3)	64	0

a), b), c): Age, body weight, and past delivery at the time the experiment was initiated.

Table 2. Temperature and water temperature of outdoor environment in the animal facility for seals (°C, 1994–1999)

		January	February	March	April	May	June
Temperature	range	7.0–9.2	7.6–8.9	9.5–12.5	13.5–16.9	17.9–20.2	20.8–21.7
	Mean	8.2	8.3	10.8	15.3	18.9	21.1
	± SE	0.4	0.2	0.4	0.5	0.4	0.2
Water temperature	range	12.8–15.0	12.8–4.7	13.4–16.8	15.7–18.2	18.9–20.9	21.5–24.3
	Mean	13.6	13.3	14.4	17.0	19.8	22.4
	± SE	0.3	0.3	0.6	0.5	0.4	0.5
		July	August	September	October	November	December
Temperature	range	23.9–26.7	24.9–29.3	22.4–27.2	18.1–23.1	14.5–16.9	10.6–11.2
	Mean	24.7	27.3	24.4	20.7	15.8	11.0
	± SE	0.5	0.7	0.9	0.7	0.4	0.1
Water temperature	range	22.3–27.6	24.5–28.9	23.7–28.1	20.3–24.6	17.9–21.0	14.5–16.6
	Mean	24.7	26.7	25.6	22.6	19.1	15.6
	± SE	0.9	0.8	0.9	0.8	0.6	0.5

deliveries of the animals are shown in Table 1. No. 1 was wild-born and the other 9 animals were born and raised in our aquarium. Males and females were kept separated before initiation of this experiment.

The mean temperature and mean water temperature for each year and month in the outdoor environment of this study (1994–1999) are shown in Table 2. The animal facility measured 61.6 m² with a 1.5 m water depth. Overall, 11–15 animals were kept in this facility. The water was chlorinated natural sea water (0.4 ppm chlorine). The animals were fed 3–6 kg of defrosted mackerel, Atka mackerel, and smelt, in the morning and evening daily.

To obtain reference values for the experiment, blood was collected from five animals (non-treatment group) for measurement of the blood P₄ level during the gestation and non-gestation periods. The time-course of the blood P₄ level was observed in three seals used in this experiment (Nos. 1, 2, and 3) and in No. 11 and No. 12 during the gestation period eight times in three pregnant animals and the non-gestation period seven times in four animals between 1982 and 1994. Blood was collected 1–8 times from each animal.

Dose and timing of proligestone administration and observation of adverse effects: PRG used in the experiment was a commercial estrus prevention agent for dogs (Kobinan®, Sankyo Co., Ltd., Tokyo, Japan). The doses were 5 and 10 mg/kg PRG. A total of 35 trials in 10 animals were made over five years. Of these 35 trials, 32 used 5 mg/kg PRG and the other 3 used 10 mg/kg PRG.

PRG was administered to 6, 8, and 9 animals in January, which was one month before the estimated starting time of the breeding season, in 1994, 1995, and 1996, respectively. For estrus in 1997 and 1999, PRG was administered to 7 and 5 animals in December of the previous year, 1996 and 1998, respectively, which was two months before the estimated starting time of the breeding season. All animals, excluding No. 10, received repeated contraceptive treatment for 2–5 consecutive years. Males and females were kept separate before the experiment. PRG was subcutaneously injected to the root of the tail. The blood P₄ level was confirmed to be 1.0 ng/ml or lower before administration of PRG to ascertain that seals were not pregnant [10].

Animals treated with PRG were kept with males that had

experience in copulation and demonstrated ability to fertilize and produce offspring. Non-treated animals were kept separately from males.

Adverse effects after PRG administration were determined by observing the condition of the administration site, appetite, behavior, and changes in body weight.

Judgment of contraceptive effect: The contraceptive effect was judged by the presence or absence of delivery in the animals tested. The presence or absence of ovulation was determined by measuring the blood P_4 level between April and September. When the P_4 level was 1.0 ng/ml or lower, it was judged that ovulation was inhibited. Blood samples were sporadically collected after PRG administration for measurement of the P_4 level.

As a control for evaluation of the P_4 level in animals that received contraceptive treatment, the time course of the blood P_4 level was observed in spotted seals and crossbreeds of spotted seals and harbor seals. The control animals were raised in our aquarium between 1982 and 1994. Blood samples were collected before April and March of the following year in 8 pregnant years from 3 animals and in 7 non-pregnant years from 4 animals. Blood samples were collected 1–8 times in each year.

Measurement of peripheral blood progesterone level: To collect blood samples for measurement of the P_4 level, the animal was restrained in a squeeze cage, and blood was collected from the plantar vascular network at the root of the rear leg. The blood samples were centrifuged immediately and the plasma was stored at -30°C until the P_4 measurement. P_4 was measured by EIA [6].

RESULTS

Blood progesterone levels in pregnant and non-pregnant animals: The time-course blood P_4 levels in the untreated pregnant and non-pregnant animals are shown in Fig. 1. The peripheral blood P_4 level was 1.5–3.5 ng/ml in April and the

mean level was 7.6 ± 0.6 (SE) ng/ml in pregnant animals. The P_4 level increased thereafter and the mean level was 8.9 ± 1.1 ng/ml in June. The P_4 level varied, 7.1–18.2 ng/ml, between October and immediately before delivery, but the level was high, about 10 ng/ml, in many cases.

The time-course of the P_4 level in non-pregnant animals was similar to that in pregnant animals between April and July, but the level after October was 0.1–2.6 ng/ml with a mean level of 0.8 ± 0.5 ng/ml.

Contraceptive effect of proligestone administration: The contraceptive effects of 5 and 10 mg/kg PRG administered one (January) or two months (December of the previous year) before the breeding season are shown in Table 3.

Among 23 trials treated with 5 or 10 mg/kg PRG in January between 1994 and 1996, 2 animals treated with 5 mg/kg PRG delivered, showing that contraception was unsuccessful, but contraception was successful in the other 21 trials (91.3%). Contraception was successful in all 12 trials (100%) treated with 5 mg/kg PRG in December. The overall success rate of contraception was 94.3% (33/35).

Of 2 animals, in which contraception failed, No. 3 delivered a normal neonate, but No. 8 aborted at six months of gestation.

Among cases of successful contraception, the peripheral blood P_4 levels were measured 29 times in 9 animals, as shown in Table 4.

In 19 cases of successful contraception, the P_4 level was measured in April–September and the values were at the baseline level in 17 cases (89.5%) excluding No. 7 (measured on April 7 in 1994) and No. 6 (measured on November 11 and December 11 in 1999).

There was no adverse effect caused by PRG administration.

DISCUSSION

There is no concrete data on the conception rate for seals

Fig. 1. Changes in the peripheral blood progesterone level in pregnant and non-pregnant spotted seals and crossbreeds of spotted seals and harbor seals.

Table 3. Contraceptive effect of the administration of proligestone

Days of administration	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8	No.9	No.10	Conception rate
1994.1.30	—	○	○ ^{a)}	○ ^{b)}	○ ^{c)}	○	○	—	—	—	6/6 (100%)
1995.1.23	—	○	○	○	○	○	○	○	○	—	8/8 (100%)
1996.1.20	○	○	×	○	○	○	○	×	○	—	7/9 (77.8%)
1996.12.27	○	○	—	—	○	○	○	○	○	—	7/7 (100%)
1998.12.27	—	—	○	—	○	○	—	○	—	○	5/5 (100%)

* : 10 mg/kg PRG ○ Successful contraception ×: Unsuccessful contraception (pregnant) —: Not used in the experiment.

Table 4. Blood progesterone levels (ng/ml) in seals in which the contraception succeeded

Date of blood sampling	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9
1994 2/28		0.08		0.17	0.28	0.40	0.33		
4/7		1.00		0.59	0.15	1.00	5.66		
8/11						0.21			
11/4							0.32		
11/26		0.27	0.64	0.34	0.12	0.69	0.02		
1995 6/18							0.67		
7/31		0.10							
8/20		0.59					0.43		
9/9							0.22		
10/13		0.03							
11/27		0.63	0.39	0.69	0.36	0.69	0.10	0.17	0.21
1996 7/1							0.32		
7/10		0.10							
7/16	0.69	0.10							
8/21			Gestation				0.43	Gestation	
9/20				0.10				(abortion)	
9/27				0.10					
12/2	0.98	0.59			0.79		0.85		0.10
1997 4/22	1.01				0.37	1.00	0.02	0.67	0.42
1998 11/19									
11/26									
1999 11/9					0.80				
11/11			0.60			5.30			
12/11						3.00			

under animal maintenance. The conception rate in nature reported by Hayama *et al.* [5] was almost 100%. Therefore, the results of this study represent adequate contraceptive effects. The contraceptive effect was observed in seals treated with 5 and 10 mg/kg PRG in this experiment. Two of 9 animals treated with 5 mg/kg PRG on January 20, 1996 became pregnant, showing that contraception was unsuccessful. This failure may not have been due to an inadequate PRG dosage, but may have been due to the timing of administration because contraception was successful in all animals treated with 5 mg/kg PRG in December of the previous year. Since PRG administration is initiated three months before estimated estrus in dogs [14], a few months may be required to obtain a contraceptive effect after PRG administration.

According to Reijnders [10], the blood P₄ level during

the luteal phase was maintained at 5–10 ng/ml in kuril harbor seals. The blood P₄ level was low (1.01 ng/ml or lower) between April and September in animals in which contraception was successful in this study, suggesting that ovulation was inhibited and no corpus luteum was formed. Of two animals in which the P₄ level increased, the level was 5.66 ng/ml in No.7 in April but conception did not occur. In No.6, the P₄ level increased in November and December, suggesting that spontaneous abortion may have occurred. Since signs of estrus are unclear in seals maintained at our aquarium, they show no pudendal swelling (unpublished), it was difficult to observe the inhibition of estrus. However a single administration of 5 mg/kg PRG in December of the previous year induced a contraceptive effect on the seals maintained in our aquarium successfully inhibiting ovula-

tion. This may be the first report of contraception for seals using a synthetic luteal hormone.

Of the seals used in the experiment, 90% were born in our aquarium and the onset of labor occurred between February 14 and March 18, which was several months earlier than that in wild seals inhabiting Hokkaido. As shown in Table 2, the higher temperature and water temperature of the outdoor environment of this aquarium compared to those in their natural habitat as well as stable feeding may affect the onset of labor.

This experiment did not determine the minimal effective dose of PRG for seal contraception and thus, it remains necessary to investigate the optimal dose for clinical application. It will also be necessary to confirm the restoration of conception ability after contraceptive treatment with PRG in case breeding subsequently becomes desirable.

The P_4 level was high during the late gestation period in the control pregnant animals in this experiment. This finding is consistent with the study reported by Reijnders *et al.* [10], in which the P_4 level was maintained at a high level until the late gestation period due to P_4 secretion from the placenta in harbor seals. Since we did not collect blood samples immediately before delivery, the P_4 level one month before delivery was unknown. The course of blood P_4 level in non-pregnant seals in this study was almost the same as that reported by Reijnders *et al.* [10].

In this study, we measured the blood P_4 level before PRG administration and confirmed that the level was low. This finding supports the study reported by Gardiner *et al.* [3] showing the usefulness of measuring the P_4 level in the determination of pregnancy in harbor seals.

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