NOTE *Theriogenology*

Serum Progesterone and Estradiol-17β Concentrations, and Lapaloscopic Observations of the Ovary in the Cheetah (*Acinonyx jubatus*) with Pregnant Mare Serum Gonadotropin and Human Chorionic Gonadotropin Treatments

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ABSTRACT. In 3 adult female cheetahs, induced-superovulation treatment was conducted, by means of 200 IU of pregnant mare serum gonadotropin (PMSG) and 100 IU of human chorionic gonadotropin (hCG) 80 hr after PMSG. The administration of PMSG created a sharp increase in the estradiol-17β concentration, resulting in 232 pg/m*l* 8 hr later in one specimen out of three. The hCG administration showed an increase in the progesterone concentration of 2.29 *n*g/m*l* 46 hr later. In addition, after direct observation of the ovary surface by laparoscopy, 5 follicles in the right ovary over 2 mm in diameter, and 7 corpora lutea (5 in the right ovary and 2 in the left) were found. It is assumed that ovulation can be induced with hCG after 80 hr on PMSG during a cheetah's diestrus or proestrus.
KEY WORDS: cheetah, hormone, superovulation.

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The cheetah is one of the most endangered felid species in the world. Cheetahs live on the grassy plains or savannas of eastern and southern Africa, and a small population of cheetah remains in the wild in a narrow region found on the border of Iran and South Russia. Cheetah populations throughout Africa have been steadily declining in the wild because of loss of habitat, poaching and conflict with ranching. It was possible that the cheetah's low genetic variation is characteristic of wild species of felids and that the extreme genetic monomorphism at isozyme and the major histocompatibility complex loci is almost nonexistent in wild species [7]. Such genetic uniformity might have resulted from a population bottleneck followed by inbreeding. This inbreeding "bottleneck", as theorized, led to the present state of cheetah genetics: all cheetahs alive today appear to be as closely related as identical twins. An average of 71% of 18 cheetah spermatozoa in ejaculates was morphologically abnormal, compared to 29% in domestic cats, and this proportion of abnormal spermatozoa has been related to infertility [9]. Reproductive success of this species has been poor, with less than 20% of the mature North American population reproducing [6], and it is important to develop a basic understanding of reproductive function. Interestrous interval, based on behavioral observation and intervals between onset of leukocyte infusions in vaginal smears, were reported to be at least 2 weeks [5] and 10.8 to 12.3 days [1], respectively. Nevertheless, it was demonstrated in our previous study [3] that concentrations of the plasma steroid hormones, especially progesterone, repeated a rhythmic pattern, which ranged from 10 to 12 weeks, although it was debatable as to whether or not spontaneous ovulation always occurred before a rise in the plasma progesterone level. On the other hand, serum LH and estradiol levels were examined in the female cheetah after an exogenous LHRH injection [10]. This study indicated that exogenous LHRH is effective in acutely altering pituitary function: increasing serum LH levels in the female cheetah, but not increasing ovarian estradiol production. Serial injections of FSH followed by hCG administered to female cheetahs resulted in ovarian follicle development, ovulation and/or visible distinct corpora lutea [11]. Moreover, Donoghue *et al.* [4] demonstrated that the mean fertilization rates of oocytes from the cheetah coincubated with cheetah sperm were clearly lower (63.6%) than those of oocytes of the domestic cat (96.6%). It acts as a brake on an artificial reproductive plan in the cheetah, because only a few attempts have so far been made at an effective induced ovulation. Present estimates place their number at 9–12 thousand with about 1/10 of those living in captivity. Over the past 10 years, the cheetah population in Japan was reduced by half resulting in a remaining population of approximately 50 (referred to in "1999th cheetah's domestic register in Japan"). But it is obvious that these subjects under raising play an important role on the preservation of the species and there is a strong desire to establish an effective technique for their artificial reproduction in Japan. The aims of the present study were to investigate hormonal changes and laparoscopic observations of the ovary in the female cheetah after the induced ovulation with pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) treatments. The present study was of the development of induced ovulation techniques for cheetahs to provide knowledge pertaining to the breeding of non-domesticated felids for control or preservation purposes.

Three adult female cheetahs were housed at the Himeji Central Park (Toyotomi, Himeji-shi, Hyogo, Japan; Cheetah 1), Gunma Safari Park (Okamoto, Tomioka-shi, Gunma, Japan; Cheetah 2), and Adventure World, AWS Co. (Shirahama, Muro, Wakayama, Japan; Cheetah 3). The cheetahs raised at these zoos were 5, 3 and 6 years of age, respectively, at a beginning of this experiment.

Female cheetahs were given PMSG and hCG without regard to the stage of the reproductive cycle. In all cheetahs, 200 IU of PMSG (Sigma Co.) and 100 IU of hCG (Sigma Co.) 80 hr after PMSG were administered as a single injection (i.m.). After each animal was placed in a restraint cage, blood samples were collected from the venae cauda at 16 hr before PMSG injection, immediately (0), 8, 32, 56 and 80 hr after PMSG injection, and immediately (0), 24 and 46 hr after hCG injection in Cheetah 1, 16 hr before PMSG injection, immediately (0), 8, 32, 56 and 80 hr after PMSG injection, and immediately (0), 2.5, 24 and 46 hr after hCG injection in Cheetah 2, and 21.5 hr before PMSG injection, immediately (0), 2.5, 26.5, 50.5, 75 and 80 hr after PMSG injection, and immediately (0), 19 and 46.5 hr after hCG injection in Cheetah 3. Blood samples were allowed to clot at room temperature for approximately 20 min, and the serum was collected by centrifugation for 15 min at 3000 rpm. The serum was frozen and kept at –80°C until assay.

Anesthesia for laparoscopy was induced with medetomigin hydrochloride (Meiji Seika Kaisha Ltd., 20 μg/hCG body weight, i.m.), atropin sulphate (Tanabe Seiyaku Co., Ltd., 0.05 mg/kg body weight, i.m.) and ketamine hydrochloride (Sankyo Co., Ltd., 2–3 mg/kg body weight, i.m.) 46 hr after hCG treatment, and when necessary each female was intubated and maintained on isoflurane gas/oxygen in the supine position on a surgical table. A ten millimeter diameter laparoscope (A5294A, Olympus) was inserted into the abdominal cavity through a skin incision made near the umbilicus. The ovaries were evaluated for the numbers of ovarian follicles (2 mm or more in diameter) and corpora lutea. The condition of the oviduct and uterus horn was also observed.

One-tenth to one-fifth milliliter of serum was used for the determination of progesterone and estradiol-17β. For the assay of these serum steroid hormones, serum samples were extracted 3 or 4 times with ethyl ether. The radioimmunoassays of progesterone and estradiol-17β were carried out as described previously [8]. The coefficient of variation of five determinations within a single assay (intraassay precision) for the steroid hormones was within 9%, and that of for five separate assays (interassay precision) for the hormones was within 8%. Rabbit anti-progesterone-3-(*O*-carboxymethyl)-oxime-BSA serum reacts with progesterone, 5α-pregnanedione, pregnenolone, 11-deoxycorticosterone, 17α-hydroxyprogesterone, testosterone, corticosterone, cortisol, cortisone, estrone, estradiol-17β and estriol at 100, 62.2, 6.26, 3.88, 2.25, 1.23, 0.18, 0.07, <0.01, <0.01, <0.01 and <0.01%. Rabbit anti-estradiol-17β-6-(*O*-carboxymethyl)-oxime-BSA serum reacts with estradiol-17β, estrone-3-sulfate, 16-epiestriol, estrone, estriol, estradiol-17α, testosterone, progesterone and 17α-hydroxyprogesterone at 100, 8.00, 5.33, 3.20, 1.77, 0.80, 0.29, <0.08 and <0.08%. Radioactivity was measured with a liquid scintillation spectrometer (Tri-Carb 2500TR, Packard U.S.A.).

Asa, *et al.* [1] have already reported on blood progesterone and estradiol-17β levels in cheetahs after reaching sexual maturity. In their report, blood progesterone and estradiol-17β levels from the time of FSH administration to natural ovulation were studied. During natural ovulation, one case of a plasma progesterone level increase was reported immediately after the plasma estradiol-17β level rose. They further examined the kinds of vaginal epithelial cells and the changes in their number. After examining the relationship between the fluctuations in number and individual hormone levels in the blood, the leukocyte count in the vaginal smear was reported to indicate a cyclic change of 10.8 to 12.3 days on average. Eaton and Craig [5] reported from cheetah's behavioral patterns the estrous cycle to be approximately two weeks, but others, after measuring the sex steroid hormone content in feces, determined the cycle to be 3 to 4 weeks [2], thus failing to present consistent opinions. We [3] have, therefore, obtained and measured blood from pregnant and non-pregnant cheetahs over a long period of time. The blood progesterone level of cheetahs that have never been pregnant fluctuated with a regularity of 0.78–6.20 *n*g/m*l*. The interval of the cycle was 10 to 12 weeks. The blood estradiol-17β level fluctuated at 108–828 *p*g/m*l* (only one specimen had a higher level, and others were 400 *p*g/m*l* or less), and indicated a trend to an increase before the progesterone level had risen. In this experiment, 16 to 21.5 hr prior to the administration of PMSG, the blood progesterone level of three specimens fluctuated at 2.03–3.41 *n*g/m*l*, which was much lower than the above-mentioned blood estradiol-17β level (Fig. 1). The reason for low blood estradiol-17β levels in this experiment has not been clarified, but, with the finding that the Cheetah 1 level increased to 232 *p*g/m*l* eight hr after the PMSG administration, we confirmed a drastic increase in the blood estradiol-17β level due to the administration of exogenous hormones. In fact, the secretion was induced as a result of the PMSG dosage, which had helped ovarian follicles grow. Furthermore, of the female cheetahs in this study, the blood progesterone level of Cheetah 1 showed a tendency to increase 24 hr after hCG was administered following the PMSG administration. Then, by laparoscopy, a total of seven pieces of corpora lutea from both the left and right sides of the ovarian surface were observed (Fig. 2). Those two findings revealed that there was a plural number of ovulation. It was found that with a hormone-administration schedule for a female cheetah by which 100 IU hCG was administered 80 hr after 200 IU PMSG was given on the grounds that the blood progesterone concentration started to increase 3 to 4 days after the peak of the blood estradiol-17β concentration in our previous report [3], the development of ovarian follicles and ovulation could be induced. Nevertheless, in Cheetah 2 and Cheetah 3, no conspicuous fluctuation in the blood steroid hormone level was observed in spite of their hormone-

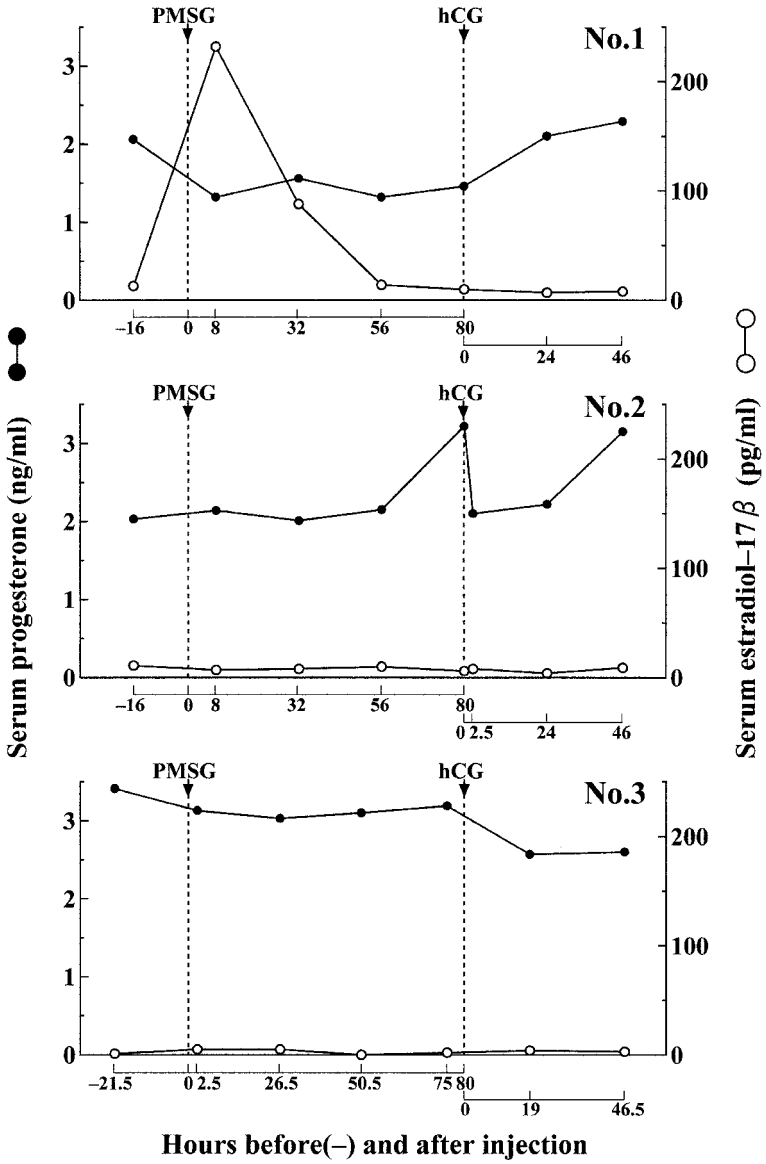

Fig. 1. Serum progesterone and estradiol-17β profiles in three individual female cheetahs treated with 200 IU of PMSG and 100 IU of hCG 80 hr after PMSG.

administration schedules being similar to that of Cheetah 1. Seven to 21 ovarian follicles 2 mm or longer on both the right and left sides could be observed. A translucent retractable fimbria completely covered each ovary or lay free and adjacent to the ovarian surface. A bicornuate uterine horn had a rather light appearance. It is therefore believed that administrating exogenous hormone helped ovarian follicles to develop to a degree not quite enough to induce ovulation. From our results on blood progesterone and estradiol-17β levels of three cheetahs before PMSG was administered, it is presumed that, in all cases, the time PMSG was administered was diestrus or proestrus. Although we were successful in inducing ovulation of one specimen out of three, in two cases out of three, ovarian follicles grew and stopped short of ovulation. For this reason, the optimal administration time for inducing superovulation could not be determined. We observed no active luteal tissue on the ovaries of Cheetah 1 in laparoscopic observation 46 hr after hCG administration and concluded that the cheetah was on induced ovulator. Finally, it is concluded that during the diestrus and proestrus of cheetahs, by administrating hCG 80 hr after giving PMSG, ovulation can be induced. It will also be necessary to further study the intervals between administrations and dosages of PMSG and hCG.

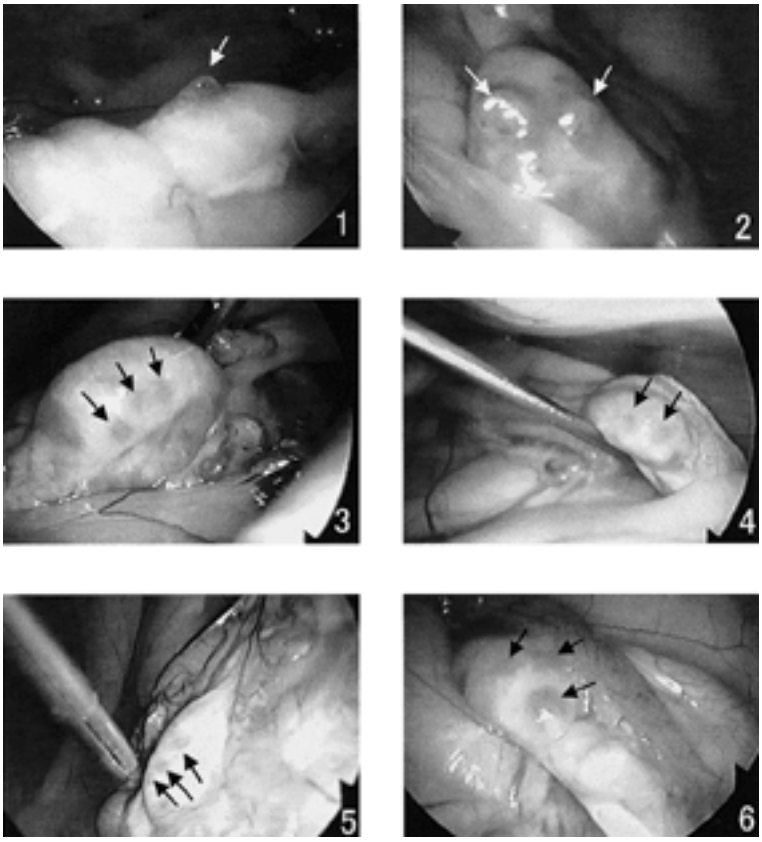

Fig. 2. Left and right ovaries containing corpora lutea (white arrows) and several follicles (black arrows) 46 hr after hCG treatment in three female cheetahs. Five follicles observed 2 days after the last hCG treatment were translucent and spherical (>2 mm in diameter) in the right ovary (1) of Cheetah 1, and generally protruded above the ovarian surface. The hCG administration revealed 5 and 2 corpora lutea in the right and left (2) ovaries, respectively. In Cheetah 2, ten and 21 follicles (<2 mm in diameter) were observed in the right (3) and left (4) ovaries, respectively, but no visible corpora lutea were produced. In the ovaries of Cheetah 3, although no corpora lutea was observed, there were 7 follicles (<2 mm in diameter) in each ovary (right:5, left: 6).

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