Zoo Biology 10:35–41 (1991)

Estrous Synchronization in the Gaur (*Bos gaurus*): Behavior and Fertility to Artificial Insemination After Prostaglandin Treatment

R. W. Godfrey, D. D. Lunstra, J. A. French, J. Schwartz, D. L. Armstrong, and L. G. Simmons

USDA-ARS, R.L. Hruska U.S. Meat Animal Research Center, Reproduction Research Unit, Clay Center (R.W.G., D.D.L.), Department of Biology, University of Nebraska-Omaha, (J.A.F., J.S.), and Omaha's Henry Doorly Zoo, Omaha (D.L.A., L.G.S.), Nebraska

The gaur (*Bos guarus*) is an endangered species that is a wild ancestor of domestic cattle. This study was conducted to evaluate 1) the efficacy of prostaglandin $F_{2\alpha}$ (PG) to synchronize estrus in gaur, 2) behavior of male and female gaur around estrus, and 3) fertility after artificial insemination (AI) at the PG-induced estrus. Six female gaur were utilized, along with a vasectomized gaur bull used to aid in detecting estrus. All females were given two i.m. injections of PG (25 mg/injection) 11 days apart, and monitored for estrus for 120 hr (48 hr via chinball marks, followed by 72 hr continuous observation) after the second PG injection. Three of the six females were in estrus during the 120-hr evaluation period. One female was in estrus prior to 48 hr after the second PG injection, and two females were observed in estrus during the 72-hr continuous observation period. When a female was observed in estrus (standing to be mounted by the vasectomized bull), she was bred by AI at 12 and 24 hr after the onset of estrus. The four females not observed in estrus, including the one marked during the first 48 hr, were bred by AI at 80 and 92 hr after the second PG injection. Of the two gaur females observed in estrus, one female was first mated by the vasectomized bull at 77 hr and the second female was mated at 98 hr after PG. Both females exhibited very short durations of receptivity (less than 4 hr). The second female observed in estrus became pregnant after AI and gave birth to a healthy gaur calf after a 299-day gestation. It appears that female gaur can be synchronized with PG techniques developed for domestic cattle. These data should provide useful information for programs studying and maintaining this endangered species and may have rele-

Received for pubication December 11, 1989; revision accepted April 20, 1990.

Address reprint requests to Dr. R. W. Godfrey, Center for Reproduction of Endangered Wildlife, Cincinnati Zoo, 3400 Vine Street, Cincinnati, OH 45220.

vance for the cattle industry, since the gaur could provide a source of diverse ancestral genetic material.

Key words: zoo, wild cattle, reproduction

INTRODUCTION

The ability to manipulate the estrous cycle of domestic cattle with prostaglandin $F_{2\alpha}$ (PG) and use of artificial insemination (AI) has enabled producers to control bovine reproduction and to utilize labor and resources more efficiently. Use of estrous synchronization in exotic species along with AI can aid in increasing the number of animals within a species as well as increasing the genetic diversity of a species [Durrant et al., 1986]. Gaur (*Bos gaurus*) cattle are an endangered species of wild cattle native to the jungles of southeast Asia. The gaur is a browsing species that feeds on leaves, twigs, and brush; and the gaur is exceptionally heat-tolerant and resistant to tropical diseases and insects [Popenoe, 1983]. Chromosome studies have suggested that the gaur is the wild ancestor of domestic cattle [Winter et al., 1984], and the gaur could be a source of diverse ancestral genetic material that may have considerable value in cross-breeding programs with *Bos indicus* and *Bos taurus* cattle [Popenoe, 1983; Dhillon, 1985]. Gaur females have been shown to be responsive to superovulation procedures and embryo transfer techniques developed for domestic cattle [Stover and Evans, 1984; Pope et al., 1988], and a gestation length of approximately 9 mo has been reported for the gaur [Asdell, 1964]. Wild cattle exhibit some types of sexual behavior similar to those seen in domestic cattle [Reinhardt, 1983], but little is known about the sexual behavior of the gaur. By combining estrous synchronization techniques and behavioral observations, it may be possible to utilize AI in *Bos quarus*. The objectives of this study were to determine the efficacy of PG to synchronize estrus in gaur (*Bos gaurus*), observe behavior of male and female gaur during estrus, and evaluate fertility after AI at the PG-induced estrus.

MATERIALS AND METHODS

Synchronization, observations, and AI were conducted during September 1988. Six female gaur ranging in age from 2.3 to 7.5 years of age and one vasectomized gaur bull (9 years old) were used. Two of the females had had at least one calf previously, although none of the females were nursing a calf at the time of the study. The mature gaur bull, representative of the existing zoo-herd gene pool, was vasectomized 5 weeks before and electroejaculated 1 and 2 weeks before the study to ensure sterility. This bull was used for detection of estrus after no sperm were found in an ejaculate. All females were given two 25 mg injections i.m. of PG (Lutalyse, Upjohn) 11 days apart. Ovaries were palpated rectally 1 to 2 days prior to each PG injection to detect corpora lutea (CL). The vasectomized bull was fitted with a chinball marking harness, placed with the females immediately after the second PG injection, and marks recorded twice daily to identify any females mounted prior to the beginning of continuous observation.

Beginning at 48 hr after the second PG injection, continuous observation of behavior was conducted for 72 hr (i.e., from 48 through 120 hr after PG injection). Night vision telescopes (courtesy of U.S. Army Communication-Electronics Command, Ft. Monmouth, NJ) were utilized to observe the animals during hours of

darkness (21:00 through 07:00). Behavioral observations were conducted from platforms atop two towers (5 m high) which were placed approximately 25 m from the animals to minimize disturbance of the animals. Events of sexual behavior were recorded as event codes (range 1–10) of increasing duration or intensity, with code 0 = no activity; 1–2 = sniffs; 3–4 = disoriented mounts; 5–8 = oriented mounts that did not culminate in intromission; 9–10 = services (weak or strong lunges with ejaculation). Only those females that stood to be mounted and serviced by the vasectomized bull (i.e., onset of estrus) were considered to be in estrus. Females observed in estrus were artificially inseminated at 12 and 24 hr after the onset of estrus [Hardin et al., 1980b]. Gaur females not observed in estrus by 80 hr after the second PG injection were inseminated at 80 and 92 hr after the second PG injection. Frozen gaur semen in .5 ml French straws (60×10^6 sperm/ml) was used for all inseminations. Semen had been collected from gaur bulls by electroejaculation and frozen using a procedure similar to that of Hopkins et al. [1988]. Manual clitoral massage (3-sec duration) was administered to each female after each insemination [Randel et al., 1975; Lunstra et al., 1983]. Pregnancy was determined by rectal palpation at 45–60 days after breeding, and gestation length was determined from actual calving date [Lunstra et al., 1983].

RESULTS AND DISCUSSION

At the time of the first PG injection, three of the six females had a palpable CL, and five of the six females had a CL at the time of the second PG injection. Three of the six female gaur exhibited estrus during the 120-hr period after the second PG injection. One female gaur was in estrus before the beginning of the continuous observation period (prior to 48 hr after PG), as evidenced by chinball marks and by the level of interest that the vasectomized bull continued to express toward her after the continuous observation began. Two out of the six female gaur were observed in estrus during the 72-hr continuous observation period (Fig. 1). Each of these two females was mated twice by the vasectomized bull. The first female observed to be in standing estrus during continuous observation was mated by the vasectomized bull at 77 and 80 hr after PG injection. This female was a 2.6-year-old heifer. The second female observed in estrus stood to be mounted and mated 98 and 99 hr after PG injection. This female was a 2.4-year-old heifer. The time to estrus after PG in these two gaur females was similar to that of domestic cattle [Hardin et al., 1980a; Hansen et al., 1987; Godfrey et al., 1989].

Average estrous cycle length within groups of *Bos taurus* and *Bos indicus* females is similar (approximately 20 days), but *Bos indicus* females show more variation and a larger proportion have long cycles, particularly during winter months [Plasse et al., 1970]. Estrous cycle length in gaur females is unknown, and gaur may exhibit variability in cycle length. The length of the follicular phase in gaur may be such that the 11-day interval between PG injections is not adequate to synchronize estrus. If the follicular phase is altered in gaur, the period of sensitivity of the CL to PG may also be altered. Although CL were palpable in several females (5 of 6) at the time of the second PG injection, it was not determined if these CL were functional or at a stage in their development when they are sensitive to PG. These factors may explain why only three gaur females exhibited estrus within the 120 hour period after the second PG injection. Another possible explanation is that some of the females

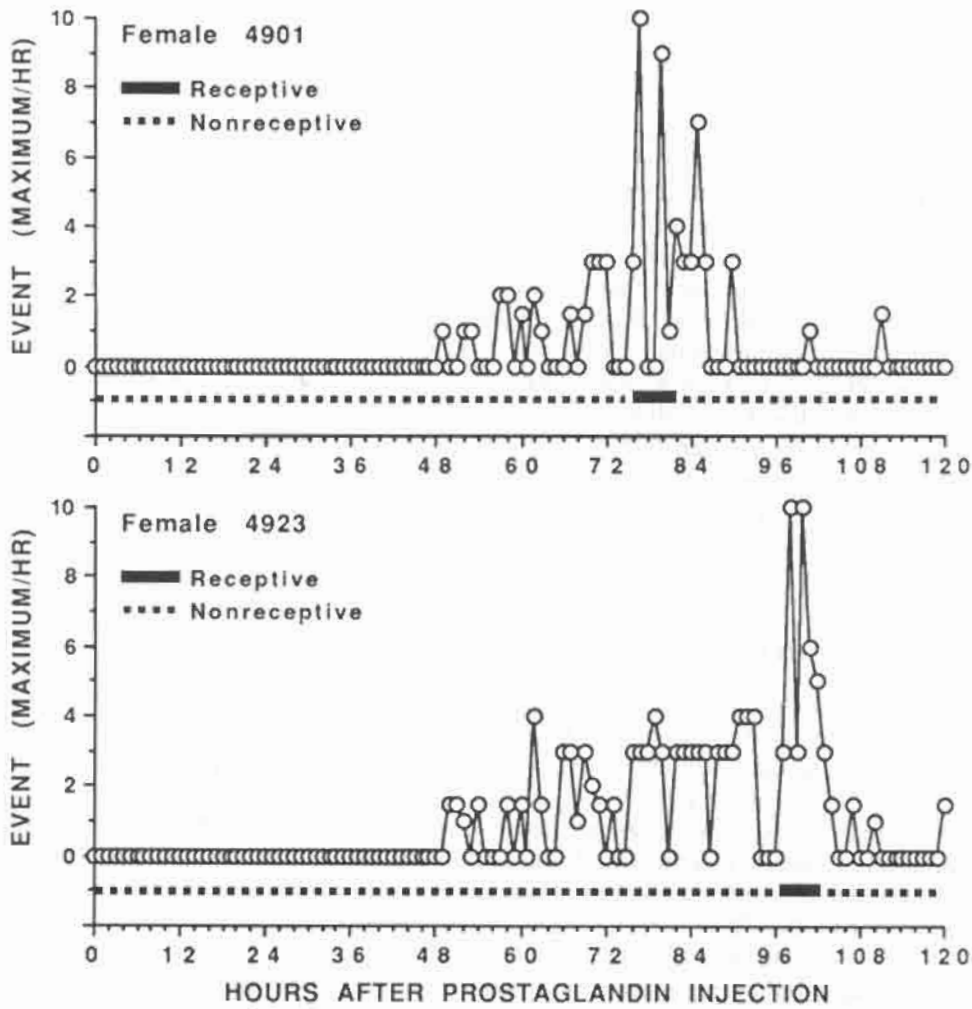

Fig. 1. Events of sexual behavior around estrus recorded during 120-hr period after last prostaglandin injection in two *Bos gaurus* females that exhibited standing estrus (receptivity). Observation of sexual behavior began at 48 hr after the prostaglandin injection and continued for 72 hr. Events of sexual behavior were recorded as event codes (range 0–10) of increasing duration or intensity, with codes 0 = no activity; 1–2 = sniffs, 3–4 = disoriented mounts; 5–8 = oriented mounts that did not culminate in ejaculation; 9–10 = services (weak or strong lunges with ejaculation).

may have been in seasonal anestrus [Reinhardt, 1983] at the time of PG treatment (i.e., September). In southeast Asia, the gaur breeds primarily in November through December, since most births occur in August through September [Asdell, 1964], although seasonal periods of complete anestrous have not been confirmed. All of the commercial PG products available for estrous synchronization are only effective in animals that have ongoing ovarian cycles and that have a functional corpus luteum. Prostaglandin treatments to synchronize estrus are relatively ineffective during periods of postpartum and seasonal anestrus. However, the presence of a palpable CL in three of the six gaur females before the first PG injection and in five of the six gaur

females before the second PG injection indicated that the majority of these females were not anestrus and that the gaur ovary exhibited a level of response to PG similar to that induced by PG in domestic cattle.

Only the second female gaur that exhibited standing estrus during the continuous observation period became pregnant after AI, based on palpation at 45 to 60 days post-estrus. This female gave birth to a healthy gaur calf 299 days later. This gestation length agreed with limited gaur data available [Asdell, 1964] but was longer than the 275–290-day gestation length typical of domestic cattle [Ford and D'Occhio, 1989]. Pregnancy rate per female observed in estrus (1 of 2 inseminated after standing estrus) was comparable to the success rate achieved in *Bos taurus* cattle. However, pregnancy rate per female inseminated (1 pregnant of 6 inseminated) was lower than that obtained in domestic cattle. The timing of AI after estrus was similar to that used in Brahman cattle with a high degree of success [Hardin et al., 1980a,b]. However, Hardin et al. [1980b] reported a decrease in fertility of Brahman (*Bos indicus*) cattle using timed AI after PG without regard to estrus compared to females bred only after observation of estrus. In the present study, the dose and treatment schedule for PG was as recommended by the manufacturer for use in *Bos taurus* cattle. Several studies have shown that the dose of PG analogs recommended for use in *Bos taurus* cattle is too high and will suppress fertility when used in *Bos indicus* cattle [Hansen et al., 1987; Godfrey et al., 1989]. Because gaur are a tropical cattle species, they may have physiological responses to PG treatment (i.e., fertility and estrous response) that are similar to those seen in the Brahman (another tropical species of cattle). It may be necessary to reduce the dose of PG, develop a different regimen of PG injection, and modify the timing of insemination after estrus in the gaur to utilize estrous synchronization technology most effectively.

During the continuous observation period, the gaur bull and females exhibited sexual behavior that was similar to that seen in domestic cattle. There were marked flehmen reactions by the vasectomized bull after investigation of the vulva or urine of females. Olfactory signals play a key role in detection of estrus in domestic cattle [Jacobs et al., 1980; Hradecky et al., 1983; French et al., 1989; Lunstra et al., 1989], and this also appeared to be true in gaur. However, there was no incidence of female to female mounting among the gaur, in contrast to that seen in female domestic cattle [Coe and Allrich, 1989]. This may be due to the presence of the bull, since the vasectomized bull remained very close to and protective of the female as estrus approached. Approximately 6–8 hr before onset of standing estrus, the vasectomized bull began attempted mountings of the female although she was nonreceptive and would not stand (Fig. 1). Once the female became receptive, the vasectomized bull mounted several times and then mounted and exhibited several rapid pelvic thrusts followed by a strong lunge, which was indicative of ejaculation. The vasectomized bull serviced each estrual female twice within a short period of time (3 and 1 hr), and then the female rapidly became nonreceptive (Fig. 1). This resulted in a very short duration of receptivity (less than 4 hr), compared to *Bos indicus* [approximately 7 hr; Plasse et al., 1970] and *Bos taurus* females [approximately 15 hr; Coe and Allrich, 1989].

Both females observed in estrus were first serviced by the vasectomized bull in the daytime (10:00 and 07:00 hr). Because very little or no sexual activity had been observed by the keepers prior to this study, zoo personnel had thought that the gaur were breeding at night. Other zoos also have reported very few observations of

mating activity during daytime hours in gaur [Stover and Evans, 1984]. *Bos indicus* females are more nocturnal in breeding behavior [Plasse et al., 1970], but there is very little sexual activity at night in *Bos taurus* females [Boyd et al., 1989] and a similar pattern appeared to exist in the gaur. After our study was completed, the keepers reported a higher level of daytime sexual activity among the gaur. This is likely due to increased awareness of gaur sexual behavior, including the rapidity of the mating act and the short period of female receptivity, rather than an actual increase in the amount of activity of the animals.

These results show that prostaglandins can be used to synchronize estrus in gaur. Estrous response to PG treatment and fertility after AI was lower than expected, but this may be due to variable cycle length, dose of PG utilized, or timing of the insemination after estrus. More research needs to be done to improve fertility to AI after estrous synchronization with PG in gaur females. Further studies are also needed to obtain a detailed characterization of the length and endocrinology of the estrous cycle in the gaur, if reproductive technology from domestic cattle is to be adapted successfully for use in the gaur.

ACKNOWLEDGMENTS

The authors thank Alan J. Kruger and Donna M. Griess of the U.S. Meat Animal Research Center for assistance in data collection and typing of the manuscript, respectively. We thank the U.S. Army Communication-Electronics Command, Fort Monmouth, NJ, for temporary loan of night vision telescopes. Appreciation is also extended to the staff and volunteers from Omaha's Henry Doorly Zoo and the students from Dr. Jeff French's Animal Behavior class for assisting with the behavior observations.

REFERENCES

Asdell, S.A., ed. PATTERNS OF MAMMALIAN REPRODUCTION. Second Edition, Cornell University Press, Ithaca, NY, 1964.

Boyd, G.W.; Lunstra, D.D.; Corah, L.R. Serving capacity of crossbred yearling beef bulls. I. Single-sire mating behavior and fertility during average and heavy mating loads at pasture. JOURNAL OF ANIMAL SCIENCE 67:60–71, 1989.

Coe, B.L.; Allrich, R.D. Relationship between endogenous estradiol-17b and estrous behavior in heifers. JOURNAL OF ANIMAL SCIENCE 67: 1546–1551, 1989.

Dhillon, R.K. The Malaysian Selembu, a new animal through Gaur/cow hybridisation. WALLACEANA 42:9–10, 1985.

Durrant, B.S.; Oosterhuis, J.E.; Hoge, M.L. The application of artificial reproduction techniques to the propagation of selected endangered species. THERIOGENOLOGY 25:25–32, 1986.

Ford, J.J.; D'Occhio, M.J. Differentiation of sexual behavior in cattle, sheep and swine. JOURNAL OF ANIMAL SCIENCE 67:1816–1823, 1989.

French, J.M.; Moore, G.F.; Perry, G.C.; Long, S.E. Behavioural predictors of oestrus in domestic cattle, *Bos taurus*. ANIMAL BEHAVIOUR 38:913–919, 1989.

Godfrey, R.W.; Guthrie, M.J.; Neuendorff, D.A.; Randel, R.D. Evaluation of luteolysis and estrous synchronization by a prostaglandin analog (Luprostiol) in Brahman cows and heifers. JOURNAL OF ANIMAL SCIENCE 67:2067–2074, 1989.

Hansen, T.R.; Randel, R.D.; Peterson, L.A. Bovine corpus luteum regression and estrous response following treatment with Alfaprostol. JOURNAL OF ANIMAL SCIENCE 64:1280–1284, 1987.

Hardin, D.R.; Warnick, A.C.; Fields, M.J. Artificial insemination of subtropical commercial beef cattle following synchronization with cloprostenol (ICI 80996): II. Estrous response. THERIOGENOLOGY 14:259–268, 1980a.

Hardin, D.R.; Warnick, A.C.; Wise, T.H.; Schultz, R.H.; Fields, M.J. Artificial insemination of subtropical commercial beef cattle following synchronization with cloprostenol (ICI 80996): I. Fertility. THERIOGENOLOGY 14: 249–258, 1980b.

Hopkins, S.M.; Armstrong, D.L.; Hummel,

S.K.C.; Junior, S. Successful cryopreservation of Gaur (*Bos gaurus*) epididymal spermatozoa. JOURNAL OF ZOO ANIMAL MEDICINE 19: 195–201, 1988.

Hradecky, P.; Sis, R.F.; Klemm, W.R. Distribution of flehmen reactions of the bull throughout the bovine estrous cycle. THERIOGENOLOGY 20:197–204, 1983.

Jacobs, V.L.; Sis, R.F.; Chenoweth, P.J.; Klemm, W.R.; Sherry, C.J.; Coppock, C.E. Tongue manipulation of the palate assists estrous detection in the bovine. THERIOGENOLOGY 13:353–356, 1980.

Lunstra, D.D.; Hays, W.G.; Bellows, R.A.; Laster, D.B. Clitoral stimulation and the effect of age, breed, technician, and postpartum interval on pregnancy rate to artificial insemination in beef cattle. THERIOGENOLOGY 19:555–563, 1983.

Lunstra, D.D.; Boyd, G.W.; Corah, L.R. Effects of natural mating stimuli on serum luteinizing hormone (LH), testosterone (T) and estradiol-17b (E2) in yearling beef bulls. JOURNAL OF ANIMAL SCIENCE 67:3277–3288, 1989.

Plasse, D.; Warnick, A.C.; Koger, M. Reproductive behavior of *Bos indicus* females in a subtropical environment. IV. Length of estrous cycle, duration of estrus, time of ovulation, fertilization and embryo survival in grade Brahman heifers. JOURNAL OF ANIMAL SCIENCE 30:63–72, 1970.

Pope, C.E.; Dresser, B.L.; Kuehn, G.; Kramer, L.; Gillespie, D. Live birth of a Gaur (*Bos gaurus*) calf following nonsurgical embryo transfer to a Holstein (*Bos taurus*) recipient. THERIOGENOLOGY 29:289, 1988.

Popenoe, H., ed. LITTLE-KNOWN ASIAN ANIMALS WITH A PROMISING ECONOMIC FUTURE: REPORT OF AN AD-HOC PANEL. United States National Academy of Sciences, National Academy Press, Washington, D.C., 1983.

Randel, R.D.; Short, R.E.; Christensen, D.S.; Bellows, R.A. Effect of clitoral massage after artificial insemination on conception in the bovine. JOURNAL OF ANIMAL SCIENCE 40: 1119–1123, 1975.

Reinhardt, V. Flehmen, mounting and copulation among members of a semi-wild cattle herd. ANIMAL BEHAVIOUR 31:641–650, 1983.

Stover, J.; Evans, J. Interspecies embryo transfer from Gaur (*Bos gaurus*) to domestic Holstein cattle (*Bos taurus*) at the New York Zoological Park, 10th INTERNATIONAL CONGRESS ON ANIMAL REPRODUCTION AND ARTIFICIAL INSEMINATION 2:243.1–243.3, 1984.

Winter, H.; Mayr, B.; Schleger, W.; Dworak, E.; Krutzler, J.; Burger, B. Karyotyping, red blood cells and haemoglobin typing of the mithun (*Bos frontalis*), its wild ancestor and its hybrids. RESEARCH IN VETERINARY SCIENCE 36: 276–283, 1984.