ELSEVIER

EFFECT OF CRESTAR™ ON ESTRUS SYNCHRONIZATION AND THE RELATIONSHIP BETWEEN FECAL AND PLASMA CONCENTRATIONS OF PROGESTAGENS IN BUFFALO COWS

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ABSTRACT

In buffaloes estrus synchronization provides an opportunity for enhanced use of AI; however, changes in hormone secretion during synchronization are poorly understood. The aim of this investigation was to determine if the concentration of progesterone metabolites in feces would correlate with the concentration of progesterone in blood and thus, could be used for noninvasive monitoring of the reproductive status in buffalo cows. Additionally, the influence of a norgestomet-estradiol treatment (CRESTAR™-ear implant) was investigated. According to the clinical examination and the progesterone profile in blood samples during the three wks before the treatment, the 17 animals were allotted to 3 groups: 1) CL = presence of corpus luteum throughout the period of 3 wks before the treatment (n = 8); 2) CY = cyclic, corpus luteum present for less than 3 wks (n = 6); and 3) AE = anestrous, with inactive ovaries (n = 3).

In the first group, 4 animals started an estrous cycle after implant withdrawal and conceived after natural mating. In the second group one of the cyclic cows showed estrus two d after implant withdrawal, the other 3 had a delayed estrus (12 to 16 d). The two cows which had had inactive ovaries at the beginning but were cyclic before the treatment started, remained cyclic after implant withdrawal but did not become pregnant. The 3 anestrous cows of the third group remained anestrous after the treatment.

The progesterone concentration in blood clearly correlated with the concentration of the metabolites in feces. Therefore, this noninvasive method is a valuable tool for determining the luteal status, and such information may be useful for developing estrus synchronization regimens in buffalo cows.

Key words: buffalo cows, progesterone, blood, feces, norgestomet

INTRODUCTION

In tropical regions, buffaloes have a greater potential as an animal protein source (meat and milk) than cattle (32, 33). For successful application of reproductive biotechnology,

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further studies of the reproductive physiology of buffaloes are needed to establish procedures such as AI and embryo transfer. Under subtropical conditions, estrus synchronization provides an opportunity for the enhanced use of AI.

Numerous researchers have evaluated the potential of using a norgestomet-estradiol treatment as a tool to synchronize estrus in cyclic and anestrous cattle (4, 5, 6, 16, 27, 28, 29) and buffaloes (15, 26, 31). Sanchez et al. (18) and Kojima et al. (8) demonstrated that synthetic progestins have less activity than progesterone for physiological functions other than suppression of estrus. The variable rate of follicular development after synchronous decline in plasma progesterone levels is the most limiting factor in achieving synchrony of estrus and ovulation (9).

In order to be able to monitor the corpus luteum function of buffaloes without restraining the animals, it would be helpful to measure the concentration of progesterone metabolites in feces. Fecal samples can be easily collected without interfering with the animals. Therefore, the method of measuring steroid metabolites in the feces has been applied for monitoring the reproductive function in a variety of species (for review see 21). However, there are great differences even among species of domestic livestock as regards percentage and delay of excretion (12). Although steroid metabolites are excreted into the bile in the conjugated form (30), in ruminants the fecal metabolites are present mainly in an unconjugated form (12, 21). Using group-specific antibodies, several fecal progesterone metabolites (20-oxopregnanes) could be quantified by EIA during the estrous cycle of cattle (22).

Therefore, the first aim of this study was to determine whether the concentration of progesterone metabolites in fecal samples of water buffaloes would reflect the concentration of progesterone in blood. A secondary objective of this study was to assess the reproductive status of the animals before and after the treatment with CRESTAR™ by monitoring progesterone profiles in blood and feces.

MATERIALS AND METHODS

Animals

The study was carried out at the buffalo farm related to Sakha Animal Production Research Station, Kafr El- Sheikh, Egypt, between September and December 1996. Seventeen buffalo cows *(Bubalus bubalis)* aged 4 to 9 yrs were selected for this study. The animals were living in a semi-sheltered open yard with bulls of known high fertility, all under the same feeding and management conditions. All cows had a history of normal calving and had no clinically detectable abnormalities in their genital tracts. All cows were rectally examined ten d before and at the start of the experiment. Some enlargement of the uterine cavity (determined by rectal palpation) in cows Numbers 1 to 4 indicated early pregnancy.

According to the results of the rectal palpation and the blood progesterone profile during the 3 wks before treatment, the animals were allotted to 3 different groups: 1) CL = presence of corpus luteum throughout the period of 3 wks before the treatment; 2) CY = cyclic, corpus luteum present for less than 3 wks; and 3) AE = anestrous, with inactive ovaries.

The first group (n = 8) comprised those animals with a rectally palpable corpus luteum which was present throughout the 3 wks before implantation (CL). The second group (n = 6) had varying functional structures on the ovaries and varying concentrations of progesterone in blood during the 3 wks of observation and were therefore regarded as cyclic (CY). The third group (n = 3) had no functional structures on the ovaries and did not display clinical signs of estrus. The progesterone concentration in blood was well below 1 ng/mL throughout the 3 wks before treatment. These animals were classified as anestrous (AE).

Treatment

CRESTAR™ (Intervet, Boxmeer, The Netherlands) consists of 2 components: an injection of estradiol valerate (5 mg) with norgestomet (3 mg) and a silicone ear implant containing norgestomet (17α-acetoxyl-11ß-methyl-19-nor-pregn-4-ene-3,20-dione). The injection was administered im and at the same time the implant was inserted sc at the outer edge of the ear in all animals. After 9 to 10 d the norgestomet implants were removed. Five buffalo cows (Numbers 6, 9, 10, 11, and 16) were injected im upon implant withdrawal with 500 IU of eCG (Chronogest, Intervet, Boxmeer, The Netherlands), as it was reported to enhance the ovarian stimulation (15). After norgestomet implant withdrawal, clinical signs of estrus manifestation were checked by daily observations for a period of 3 wks. Pregnancy was tested by palpation per rectum 60 d after implant withdrawal.

Blood Progesterone Assay

Before the treatment, 4 blood samples were collected from the jugular vein of each animal at weekly intervals (Day -21, -14, -7, 0). After the treatment, up to 17 blood samples were collected from each animal at Days 1, 7, 8, 9, 10, 11, 13, 14, 19, 20, 21, 28, 29, 30, 31, 32 and 37. Blood samples were chilled on ice, transported to the laboratory and centrifuged at 3,000 x g for 15 minutes. Serum was kept at -20° C until assayed. Progesterone was measured using a commercial radioimmunoassay ("Coat-A-Count" progesterone kit; Diagnostic Products Corporation, Los Angeles, CA, USA). Intra- and inter-assay coefficients of variation were 2.7 and 9.7 %, respectively. The Coat-A-Count progesterone antiserum is highly specific for progesterone.

Assay of Progesterone Metabolites in Feces

About 10 grams of feces were collected rectally from each animal in small plastic bags. The fecal samples were collected at the same time as the blood samples. They were kept frozen at -20° C until shipment via air-freight to Vienna. After thawing, progesterone metabolites in 0.5 g of fecal samples (wet weight) were extracted by the addition of 1 mL of distilled water and 4 mL methanol as described by Palme et al. (13). Previous experiments showed that the moisture content of ruminant feces was within narrow limits and did not influence the steroid concentration (21, 22). After shaking for 30 min, the suspension was centrifuged at 2,500 x g for 15 min. An aliquot of the supernatant was diluted with assay buffer and the EIA for 20-oxopregnanes was performed as described by Schwarzenberger et al. (20, 23).

Statistical Analysis

The Pearson Product Moment Correlation (Sigma Stat™, SPSS Inc., Erkrath, Germany) was used to calculate the coefficient of correlation and the regression line values of progesterone in blood and metabolites in feces of the corresponding same-day samples.

RESULTS

Animals were allotted to 3 groups retrospectively according to the results of the clinical examination and the blood progesterone profile of the 3 wks before the treatment: 1) Group CL: Cows 1 to 4 were pregnant as determined by palpation per rectum, which revealed a mature corpus luteum and an enlargement of the uterine cavity. Additionally, we found a high concentration of progesterone in blood and 20-oxopregnanes in feces. Cows 5 to 8 were either having a persistent corpus luteum or they were in the early stages of pregnancy. Cows 1 to 4 maintained the pregnancy throughout and after the treatment; cows 5 to 8 became cyclic after the treatment. 2) Group CY: Cows 9 to 14 had a variation in progesterone profile indicating cyclicity before the treatment and they continued to be cyclic after the treatment. 3) Group AE: Cows 15 to 17 remained inactive throughout treatment. Table 1 lists the experimental design and the clinical data of the 17 buffalo cows.

Table 1. Experimental design and clinical data of the 17 buffalo cows.

Animal number	Status on the day of implant insertion	Group	eCG treatment	Days from implant removal to estrus	Status 60 days after implant removal
1	Pregnant	CL	No	-	Pregnant
2	Pregnant	CL	No	-	Pregnant
3	Pregnant	CL	No	-	Pregnant
4	Pregnant	CL	No	-	Pregnant
5	Cyclic or pregnant	CL	No	10 to 12	Cyclic
6	Cyclic or pregnant	CL	Yes	10 to 12	Cyclic
7	Cyclic or pregnant	CL	No	10 to 12	Cyclic
8	Cyclic or pregnant	CL	No	10 to 12	Cyclic
9	Cyclic	CY	Yes	2	Cyclic
10	Cyclic	CY	Yes	16	Cyclic
11	Cyclic	CY	Yes	12	Cyclic
12	Cyclic	CY	No	12	Pregnant
13	Cyclic	CY	No	-	Anestrus
14	Cyclic	CY	No	-	Anestrus
15	Anestrus	AE	No	-	Anestrus
16	Anestrus	AE	Yes	-	Anestrus
17	Anestrus	AE	No	-	Anestrus

The concentration of progesterone metabolites in feces (as measured by the determination of 20-oxopregnanes) followed that of progesterone in blood. According to the calculation of the regression line (fecal 20-oxopregnanes = 157 (plasma progesterone) + 144) and the coefficient of correlation ($r = 0.77$; $P < 0.001$), there was a high correlation between the gestagen values in feces and blood.

In 13 out of 279 cases the gestagen concentration in feces did not match with the range of expected values according to the progesterone levels in blood. These 13 samples were omitted from any further statistical analysis. However, in this longitudinal study it was not so important to have a high correlation between the concentrations in blood and feces, as for the determination of the luteal status of the animals we could use the matching pair of samples of either the preceding or the following day. If the 13 aberrant values had been included and the predictive value of the gestagen concentration in feces been evaluated, in 4 cases (= 1.4%) false positive and in 9 cases (= 3.2%) false negative results would have been obtained.

At Day 60 after implant withdrawal, palpation per rectum of the cows 1 to 4 proved that they were approximately 3 to 4 months pregnant. The blood progesterone concentration of the 4 pregnant group CL animals ranged from 4.80 to 8.50 ng/mL (median = 5.50) before, 2.32 to 7.20 ng/mL (4.16) during, and 1.59 to 6.6 ng/mL (3.53) after the treatment. The corresponding values of gestagen metabolites in feces ranged from 521 to 1430 ng/g (836), 396 to 1551 ng/g (891) and 374 to 2827 ng/g (831), respectively.

In the other 4 animals of this group (Numbers 5 to 8) the treatment with CRESTAR ™ interrupted luteal function, as it was shown by an estrus 10 to 12 d after withdrawal of the implant. The median of the gestagen concentration in these 4 animals was maintained at a high level (5.30 ng/mL blood and 935 ng/g feces) during the 21 d before treatment. Clinical data revealed that these animals had a mature corpus luteum and no pathological changes in their uteri. During the period of norgestomet implantation the median of the gestagen concentration was 4.75 ng/mL in blood and 952 ng/g in feces. A sharp decrease in the gestagen concentration was observed on Day 4 (Number 8) and on Day 6 to 10 after implant withdrawal (Numbers 5 - 7). Animals in this group exhibited signs of estrus 10 to 12 d after implant withdrawal, which was in agreement with very low levels of progesterone in blood and of its metabolites in feces. All animals conceived after natural mating at the synchronized estrus, as could be confirmed by pregnancy diagnosis performed rectally on Day 60 after implant withdrawal.

Only one of 6 animals of Group CY displayed estrus as expected on Day 2 (Number 9). Another 3 showed a delayed estrus on Day 12 (Numbers 11 and 12) or Day 16 (Number 10) after withdrawal of the implant. The low levels of progesterone in blood (0.25 ng/mL) and of fecal 20-oxopregnanes (47 ng/g) in the first two samples collected from cows Numbers 13 and 14 as well as the results of the clinical examination pointed towards inactivity of the ovaries at that time. However, the increase in the levels of progesterone in blood and metabolites in feces on Day -7 revealed that the ovarian activity of these animals occurred without any hormonal administration.

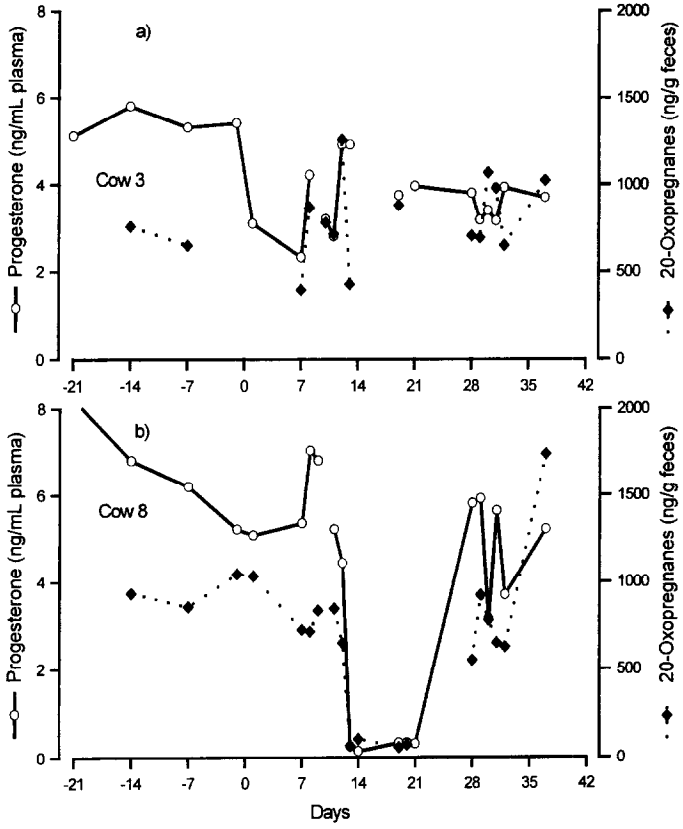

Figure 1. Concentration of progesterone in blood (ng/mL) and of 20-oxopregnanes in feces (ng/g) of buffalo cows, which had consistently high gestagen concentrations before the treatment and which a) retained a functional corpus luteum or b) resumed cyclic ovarian activity after the treatment. Treatment with CRESTAR™ started on Day 0 and the implant was withdrawn 9 to 10 d later.

Clinical data and the progesterone profile indicated that one buffalo cow (Number 12) had conceived at estrus after treatment. Although the females were living with fertile bulls and the progesterone profile indicated they were cyclic before the treatment, the others in this group failed to conceive.

The three buffalo cows in group AE, which upon clinical examination revealed smooth inactive ovarian structure before the experiment and had low gestagen levels in blood and feces, did not respond to the treatment with CRESTAR™ and remained anestrous. The

median of the progesterone concentration maintained low levels of 0.19 ng/mL before and 0.15 ng/mL during the period of treatment. The corresponding levels of progesterone metabolites in feces were 66 ng/g and 51 ng/g of feces, respectively. After implant withdrawal, the progesterone concentrations remained very low in all samples collected from two cows (Numbers 15 and 17) except for two small peaks of 1.15 and 1.45 ng/mL in blood on Days 10 and 11 after implant withdrawal, respectively. However, cow 16, which received an injection of eCG, showed an increase in the progesterone concentration from 1.59 ng/mL on Day 11 after implant withdrawal to 7.30 ng/mL on Day 19, which remained high (> 3 ng/mL) for another 4 d and declined afterwards. This increase and decline was reflected in the concentration of progesterone metabolites in the feces as well.

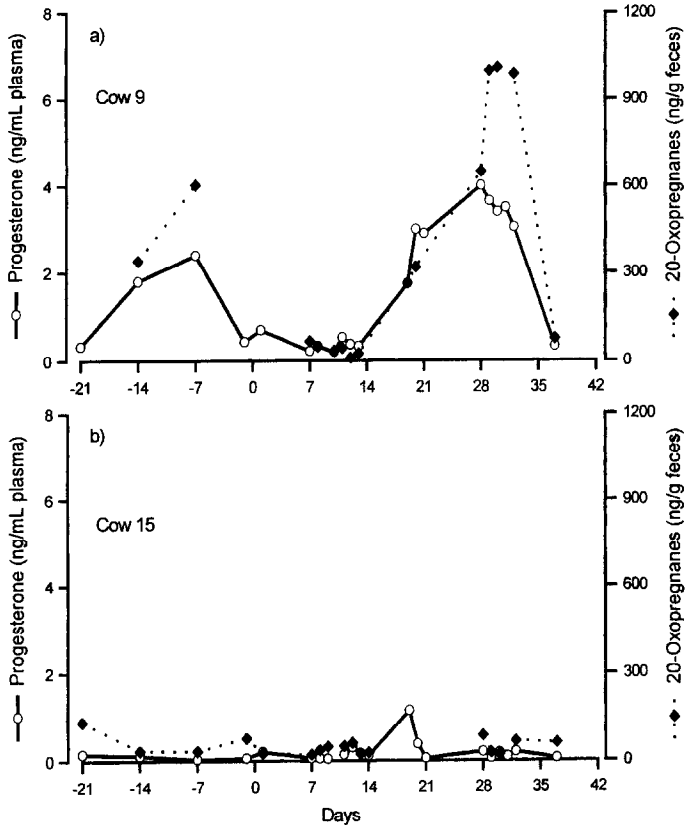

Figure 2. Concentration of progesterone in blood (ng/mL) and of 20-oxopregnanes in feces (ng/g) of buffalo cows, which before the treatment were a) cyclic or b) anestrous. Treatment with CRESTAR™ started on Day 0 and the implant was withdrawn 9 to 10 d later.

The application of eCG to 5 buffalo cows in this investigation had no effect on their reproductive performance. Cow Number 6 returned to estrus within 10 to 12 d after implant removal. Cows 9 to 11 started estrus 2, 16 or 12 d after implant withdrawal, respectively, and cow 16 remained anestrous as did the other two in that group.

DISCUSSION

As the clinical examination 60 d after implant removal verified, 4 of 8 buffalo cows which had continuous high progesterone concentrations throughout the previous 21 d period maintained the pregnancy after norgestomet-estradiol treatment. The treatment was tolerated probably due to the minimal effect of this hormonal therapy on the endogenous hormones. In the other 4 animals of this group the CRESTAR™ treatment interrupted the maintenance of the functional corpus luteum, which could have been an indication of pregnancy.

Sheldon (24) described several factors which influence early embryo survival in cattle. Favero et al. (4) reported that the administration of estradiol valerate and norgestomet implants on Day 21 post insemination caused a severe decrease in the calving rate in beef heifers. Only 1 out of 21 (5 %) of the heifers calved according to the initial AI.

It appeared that, on the basis of the variation in blood progesterone levels, the cows in group CY were cyclic before the treatment. The values of progesterone before treatment were similar to those obtained by other authors in cyclic buffaloes. Peak concentrations (0.86 to 4.97 ng/mL) were reported between Days 13 to 16 (1, 3, 11, 19, 25).

The delay of displayed estrus after implant withdrawal may be attributed to an inhibitory effect of norgestomet on follicular growth and development by suppression of LH or FSH secretion. Robinson et al. (17) hypothesized that PRID treatment could inhibit endogenous progesterone production by reducing the luteotropic effects of circulating LH. The long interval to the onset of estrus after implant withdrawal may reflect the time that is necessary for body clearance of norgestomet in the buffaloes. The precision of synchrony of estrus depends on the synchrony in follicle development (14), the stage of estrous cycle at which the treatment is initiated, and the effects of exogenous progesterone on ovarian structures and endogenous hormone production (10). An norgestomet ear implant on the day of embryo transfer slightly increased the pregnancy rates in cows and heifers, but did not alter the plasma progesterone concentration of pregnant and nonpregnant animals on Days 6 and 7 (28).

In anestrous buffaloes, we found only low progesterone levels and these results agree with those obtained by previous authors (2, 7). These reports and our results show that irregular progesterone profiles indicate disturbances in the normal development of the corpus luteum. In summer-anestrous buffaloes kept at 2 levels of management (village vs farm), those treated with norgestomet had higher rates of ovulation and conception than PRID treated animals. None of the control buffaloes exhibited estrus during the period of the study (26).

The concentration of progesterone in blood was reflected by the concentration of progesterone metabolites in feces, thereby allowing for the monitoring of the functional stage of the corpus luteum with this noninvasive method. A single progesterone analysis does not provide sufficient information to evaluate the ovarian status accurately. However, progesterone profiles obtained from a series of samples taken over a period of time give good evidence of the reproductive status of the animals. Therefore, in our study weekly samples were taken for a period of 3 wks before the treatment. It is not easy to obtain blood samples from buffalo cows under farm conditions or in the field, and the ovary of buffalo cows is rather small and difficult to palpate rectally. Therefore, the use of fecal samples for monitoring luteal activity would be advantageous. Another method of noninvasive monitoring of ovarian activity or pregnancy is the determination of progesterone in milk samples or in urine. However, milk samples are not available from nonlactating animals and it is simpler to collect feces than urine. Nevertheless, the extraction of gestagen metabolites from fecal samples and the quantification by a relevant EIA using group specific antibodies (13, 21, 22) requires a specialized laboratory, limiting the applicability of this method to research projects and well organized logistics of the transfer of samples and results.

In conclusion, the utilized CRESTAR™ treatment was not effective in synchronizing estrus in buffalo cows, since only 1 of 6 cyclic animals responded with an estrus 2 d after implant withdrawal and the treatment had no effect in any of the 3 anestrous buffalo cows. According to our results, the determination of progesterone metabolites in fecal samples of water buffalo cows enables the monitoring of the ovarian activity (corpus luteum function). Such information may be useful for developing estrus synchronization regimens in buffalo cows.

REFERENCES

1. Batra SK, Arora RC, Bachlaus NK, Pandey RS. Blood and milk progesterone in pregnant and non-pregnant buffalo. J Dairy Sci 1979; 62: 1390-1393.
2. El-Belely MS. Hormonal state in buffalo cows affected with ovarian inactivity and possible trials for oestrus induction. Ph.D. Thesis Vet. Med. Cairo University, 1984
3. El-Belely MS. Progesterone, oestrogens and selected biochemical constituents in plasma and uterine flushings of normal and repeat-breeder buffalo cows. J Agric Sci, Cambridge 1993; 120: 241-250.
4. Favero RJ, Faulkner DB, Kesler DJ. Norgestomet implants synchronize estrus and enhance fertility in beef heifers subsequent to a timed artificial insemination. J Anim Sci 1993; 71: 2594-2600.
5. Ghallab AM, Ott RS, Cmarik GF, Kesler DJ, Faulkner DB, Hixon JE. Effects of repetitive norgestomet (a) treatments on pregnancy rates in cyclic and anestrous beef heifers. Theriogenology 1984; 22: 67-74.
6. Hixon DL, Kesler DJ, Troxel TR, Vincent DL, Wiseman BS. Reproductive hormone secretions and first service conception rate subsequent to ovulation control with Synchro-Mate B. Theriogenology 1981; 16, 219-230.

7. Khattab RM, Barkawi AH, Aboul-Ela MB. Patterns of ovarian and oestrous activity in problem buffaloes as monitored by plasma progesterone concentration. Buffalo J 1988; 4: 173-181.
8. Kojima N, Stumpf TT, Cupp AS, Werth LA, Roberson MS, Wolfe MW, Kittok RJ, Kinder JE. Exogenous progesterone and progestins as used in estrous synchrony regimens do not mimic the corpus luteum in regulation of luteinizing hormone and 17ß-estradiol in circulation of cows. Biol Reprod 1992; 47: 1009-1017.
9. Macmillan KL, Peterson AJ. A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anoestrus. Anim Reprod Sci 1993; 33: 1-25.
10. Macmillan KL, Taufa VK, Barnes DR, Day AM. Plasma progesterone concentrations in heifers and cows treated with a new intravaginal device. Anim Reprod Sci 1991; 26: 25-40.
11. Niansheng Y, Peichien W. Peripheral plasma levels of progesterone and 17 beta-oestradiol during normal and low breeding seasons and induction of estrus during low breeding season in Chinese Swamp buffaloes. Buffalo Journal 1988; 4: 217-223.
12. Palme R, Fischer P, Schildorfer H, Ismail MN. Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock. Anim Reprod Sci 1996; 43: 43-63.
13. Palme R, Möstl E, Brem G, Schellander K, Bamberg E. Faecal metabolites of infused ^{14}C-progesterone in domestic livestock. Reprod Dom Anim 1997; 32: 199-206.
14. Pierson RH, Ginther OJ. Follicular populations during the estrous cycle in heifers. 1. Influence of day. Anim Reprod Sci 1987; 14: 165-176.
15. Rao AR, Rao SV. Synchronisation of oestrus in buffaloes with norgestomet. Veterinary Record 1979; 105: 256.
16. Rentfrow LR, Randel RD, Neuendorff DA. Effect of estrus synchronization with Syncro-Mate B® on serum luteinizing hormone, progesterone and conception rate in Brahman heifers. Theriogenology 1997; 28: 355-362.
17. Robinson NA, Leslie KE, Walton JS. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in holstein cows. J Dairy Sci 1989; 72: 202-207.
18. Sanchez T, Wehrman ME, Kojima FN, Cupp AS, Bergfeld EG, Peters KE, Mariscal V, Kittok RJ, Kinder JE. Dosage of the synthetic progestin, Norgestomet, influences luteinizing hormone pulse frequency and endogenous secretion of 17ß-estradiol in heifers. Biol Reprod 1995; 52: 464-469.
19. Sanwal PC, Varshney VP, Pande JK, Sharma NC, Rahman M. Peripheral plasma levels of progesterone in buffalo during oestrous cycle and early pregnancy. Indian J Anim Sci 1980; 50: 480-482.
20. Schwarzenberger F, Francke R, Göltenboth R. Concentrations of faecal immunoreactive progestagen metabolites during the oestrous cycle and pregnancy in the black rhinoceros (Diceros bicornis michaeli). J Reprod Fert 1993; 98: 285-291.
21. Schwarzenberger F, Möstl E, Palme R, Bamberg E. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Anim Reprod Sci 1996; 42: 515-526.

22. Schwarzenberger F, Son CH, Pretting R, Arbeiter K. Use of group-specific antibodies to detect fecal progesterone metabolites during the estrous cycle of cows. Theriogenology 1996; 46: 23-32.
23. Schwarzenberger F, Tomasova K, Holeckova D, Matern B, Möstl E. Measurement of fecal steroids in the black rhinoceros (Diceros bicornis) using group-specific enzyme immunoassay for 20-oxo-pregnanes. Zoo Biol 1996; 15: 159-171.
24. Sheldon M. Bovine fertility - practical implications of the maternal recognition of pregnancy. In Practice 1997; 19: 546-566.
25. Singh G, Sharma SS, Singh GB, Sharma RD. Progesterone assays and rectal palpation in cycling and non-cycling buffalo heifers. Indian J Anim Reprod 1982; 2: 1-5.
26. Singh G, Singh GB, Sharma RD, Nanda AS. Experimental treatment of summer anoestrous buffaloes with norgestomet and PRID. Theriogenology 1983; 19: 323-329.
27. Singh U, Khurana NK, Inderjeet. Plasma progesterone profiles and fertility status of anestrus Zebu cattle treated with norgestomet-estradiol-eCG regimen. Theriogenology 1998; 50: 1191-1199
28. Smith AK, Broadbent PJ, Dolman DF, Grimmer SP, Davies DAR, Dobson H. Norgestomet implants, plasma progesterone concentrations and embryo transfer pregnancy rates in cattle. Vet Rec 1996; 139: 187-191.
29. Spitzer JC, Burrell WC, LeFever DG, Whitman RW, Wiltbank JN. Synchronization of estrus in beef cattle. I. Utilization of a norgestomet implant and injection of estradiol valerate. Theriogenology 1978; 10: 181-200.
30. Taylor W. The excretion of steroid hormone metabolites in bile and faeces. Vitam Horm 1971; 29: 201-285.
31. Uoc NT, Long DD, van Ty L, Chupin D, Renard JP, Nguyen BX. Effects of estradiol-17ß and hCG supplementation on superovulatory responses and embryo quality in swamp buffalo (Bubalus bubalis) implanted with norgestomet. Anim Reprod Sci 1997; 47: 181-187.
32. Villares JB. Social aspects of buffaloes breeding for social economics conditions progress of the mankind. Proc IV World Buffalo Cong 1994; 1: 190-195.
33. Zicarelli L. Management in different environmental condition. Buffalo J 1994; (Suppl. 2): 17-38.