Agric. Rev., 4 (2): 119-135, 1983.

HORMONAL STATUS OF FEMALE WATER BUFFALO (*BUBALUS BUBALIS*)—A REVIEW

R.S. Pandey, S.K. Batra and G.S. Pahwa

Hormone Research Laboratory, Dairy Chemistry Division,
National Dairy Research Institute, Karnal-132001 (Haryana), India

ABSTRACT

The discovery of recent analytical techniques like competitive protein binding assay and radioimmunoassay has made it possible to estimate the circulating hormones to the extent of picogram level in biological fluids of the mammalian species including farm animals. An attempt has been made to review briefly the picture of various reproductive harmones as measured by radioimmunoassay, in blood plasma of buffaloes during postnatal period, estrous cycle, pregnancy and post partum phases. Seasonal alteration in the plasma levels of a few hormones in this species has been discussed. Some of the hormones are known to pass from plasma to milk and the concentrations of such hormones in milk have been found to be even higher. The relevance of the hormones in milk has been elaborated and the use of progesterone in pregnancy diagnosis has been presented.

INTRODUCTION

Physiology of reproduction is the most rapidly developing biological science. Although our knowledge is still inadequate many noteworthy advances in understanding the reproductive processes have occurred in the past decade. On one hand, there is pressure to find socially acceptable ways of reducing the reproductive capacity of the world's exploding population, and on the other, there is a great need to maintain reproductive performance of our domestic animals to the maximum so as to provide adequate nutrition for the rapidly expanding human population.

Buffalo is one of the triple purpose farm animals as a major source of milk, meat and tactile energy in the developing countries of Northern tropics and subtropics. India has the largest buffalo population in the world. However, the myriad problems *viz.* late maturity, silent oestrus, delay of post-partum oestrus, repeat breeding and summer anoestrum hamper the productivity of this species. Ample scope exists for increasing the reproductive efficiency of this species if these problems could be controlled and manipulated effectively. Reproductive performance is largely controlled by endocrine glands and the hormones they secrete. Different phases of reproductive cycles are regulated by intricate sequential events and interactions between hormone releasing factors from hypothalamus, pituitary gonadotrophins, sex-steroids and uterine prostaglandin F. Lack of integration in the secretion of these hormones may lead to reproductive failures. The success of hormone therepy lies in accurate identification of the nature of hormonal imbalances and the use of appropriate hormone preparations in a judicious dose schedule.

The complete story of hormonal factors responsible for reproductive failures in buffaloes is far from complete, although information is accumulating with the

NIDRI. Publication No. 83—119.

recent advent of sensitive assay procedures like competitive protein binding and radio-immunoassay. It is therefore imperative to have the knowledge about the levels of various hormones which will lay foundation for elucidating the causes of various reproductive disorders in buffaloes. Though some of the work in this area has already been reviewed (Pandey, 1979; Arora and Pandey, 1982a), the present communication deals with the most upto date literature available as well as the work done in this laboratory on reproductive hormones in blood plasma and milk of water buffaloes.

HORMONES IN BLOOD PLASMA

Post-natal period : Jain and Pandey (1981) reported that progesterone level was below 1 ng/ml during first six months post-birth and above 1 ng/ml during 9–24 month post-birth. However, oestradiol–17β concentration fluctuated between 0.67 ± 0.50 to 12.78 ± 2.24 pg/ml during first 30 months. The higher concentration of progesterone and lower oestradiol level may be responsible for nonovulation and failure to exhibit oestrus in buffalo heifers even at the age of 24 months or more. The higher level of progesterone has the ability to prevent the development of follicles, thereby blocking ovulation (Hansel and Snock, 1970).

Oestrous cycle : There is considerable variation in the length of oestrous cycle in buffaloes, it ranges from 21.77 ± 0.16 to 28.1 days (Rao *et al.*, 1973), Butchaiah *et al.*, 1975) which is longer than that of cows (21 days). Kamonpatana *et al.* (1979b) observed on an average 0.097 ± 0.085 ng/ml of progesterone during pro-estrus which reached to 0.38 ± 0.12 ng/ml at 15.6 ± 3.7 days and declined to basal level 4-7 days preceding next oestrus. But Ahmad *et al.* (1977) while working on cycling Murrah buffaloes reported a higher progesterone concentration at oestrus (0.5 ng/ml), which rose to a peak level of 1.6 ng/ml on day 15. It further increased slightly till day 19 in buffaloes which conceived but declined abruptly in animals which failed to conceive. Goel and Sud (1978) observed still higher level (2.21 ± 0.08 ng/ml) at oestrus which decreased by day 2 to 1.02 ± 0.04 mg/ml which is contrary to the report of other workers and the level remained higher (5.16 ± 0.12 ng/ml) from day 6 to 14 followed by continuous fall till next oestrus.

In our laboratory, Bachlaus *et al.* (1979) reported that the progesterone concentration was 0.16 ± 0.02 ng/ml at oestrus which rose to a peak of 5.21 ± 0.50 ng/ml by day 15 and declined to the basal value of 0.13 ± 0.02 ng/ml at the next oestrus. The similar profile was observed in heifers and lactating animals (Arora and Pandey 1982b) and (Batra *et al.*, 1979b). No significant difference of progesterone concentration between primiparous and multiparous buffaloes could be observed (Pahwa and Pandey, 1983a).

The foregoing discussion suggests that the progesterone concentration increased and decreased with the growth and regression of corpus luteum. Prostaglandin

$F_2\alpha$ ($PGF_2\alpha$) has been demonstrated to play an important role in the regression of corpus luteum in certain species. $PGF_2\alpha$ and its analogues have been effectively used in controlling the oestrous cycle in buffaloes by regressing corpus luteum (Prasad *et al.*, 1979). The exogenous administration of $PGF_2\alpha$ causes the rapid decline of progesterone concentration (Bachlaus *et al.*, 1980), Kamonpatana *et al.* (1979a) and subsequently increases in oestradiol 17β concentration (Pandey *et al.*, 1982). But the peripheral concentration of $PGF_2\alpha$ did not represent the clear picture of its secretion (Batra and Pandey, 1983b) in relation to luteolysis, due to its rapid metabolism (Granstrom, 1972). However, its metabolite 13, 14 dihydro-15 keto $PGF_2\alpha$ (PGFM) reflected the synthesis and release of $PGF_2\alpha$ (Batra and Pandey 1983a), The concentration of PGFM fluctuated around the basal level of 200–250 pg/ml during the luteal phase and started increasing 4 days preceding next oestrus to a peak level of 900 pg/ml 2 days before the next oestrus. The temporal changes around this period showed number of peaks, the major occurring 39–46 h before oestrus, coinciding with the decline of progesterone concentration (Batra *et al.*, 1981).

Bachalus *et al.* (197-) observed a major peak of oestradiol-17β a day before oestrus in most of the animals. The average concentration at the time of oestrus was 19.32±3.73 pg/ml which declined sharply to basal level of 5.73±0.68 pg/ml on day 2 and was almost maintained throughout the luteal phase. It increased gradually from 3 days preceding oestrus to a sharp peak of 35.80±3.48 pg/ml one day before oestrus. However, Kamonpatana *et al.* (1979b) showed a peak concentration of 77±35 pg/ml one day prior to oestrus in Swamp buffaloes which was maintained for about 4 days, followed by a sharp decline to the basal levels of 36±13 pg/ml. These values were much higher than reported by Bachalus *et al.* (1979) in Murrah buffaloes. However, Batra *et al.* (1980a) observed the peak level 23.78 pg/ml on the day of oestrus which declined to the basal level within 2 days and fluctuated around the basal level of 8 pg/ml throughout the luteal phase. In addition, we observed minor elevations on day 4 and 11 after insemination. In animals which did not conceive, the level increased to 21.10±4.10 pg/ml on the subsequent oestrus but the animals which became pregnant (Fig. 2) the basal level was maintained with minor increments on day 4, 11, 22 and 33 of pregnancy. The postoestrus rise in the oestradiol 17β concentration may be associated with triphasic pattern of follicular growth as has been reported for cows. There was no significant difference of oestradiol-17β concentration between primiparous and multiparous buffaloes (Pahwa and Pandey, 1983a). As there was no rise of oestradiol-17β concentration before the increase of $PGF_2\alpha$ and decline of progesterone concentration (Fig. 1), this suggests that basal level of oestradiol-17β is sufficient to elicit $PGF_2\alpha$ secretion. Arora and Pandey (1982b) observed an increase in the levels of androgens 2–5 days before oestrus, suggesting its role in the luteolysis.

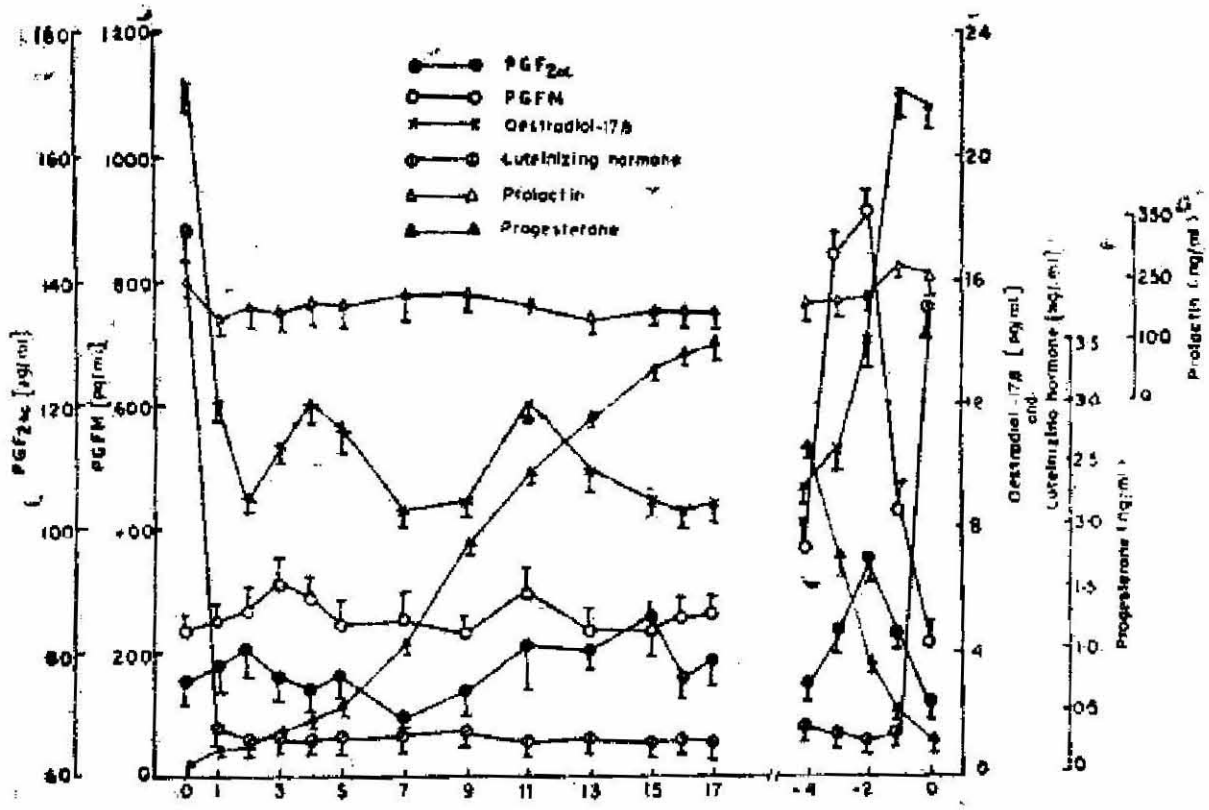

Figure 1. Hormonal changes during oestrous cycle in 18 buffaloes. Vertical lines indicate ± S.E.M.

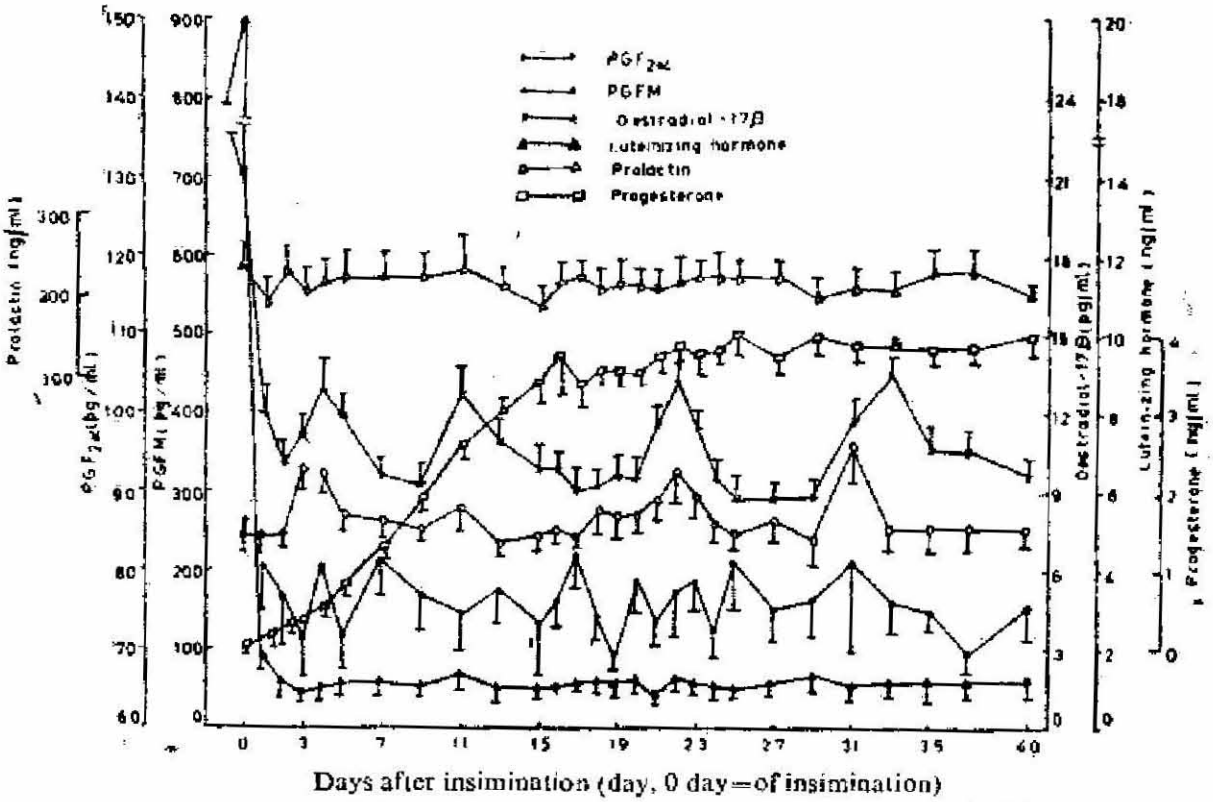

Figure 2. Hormonal changes during early pregnancy in buffaloes. Vertical lines indicate ± S.E.M.

Heranjal *et al.* (1976) observed that the LH concentration at oestrus (14.03 to 38.50 ng/ml) was significantly higher than the level (5.4 to 9.8 ng/ml) during the rest of the oestrous cycle. Albeit Kakar *et al.* (1980) and Arora and Pandey (1982b) observed a similar peak level, but the basal level was 1–2 ng/ml throughout the cycle. The LH peak occurred (0-6 h) after the onset of oestrus and declined to half the level within 6 hr (Batra *et al.*, 1981). The mediation of LH release was the consequence of oestradiol-17β secretion which occurred 8–17 h before the onset of oestrus (Batra and Pandey, 1983c).

Heranjal *et al.* (1979a) observed that the serum prolactin reached maximum concentration (60 ng/ml) on the day of oestrus and remained at the base line value (112–366 ng/ml) during the rest of the cycle. Pahwa and Pandey (1984) also observed higher prolactin concentration than the basal level around oestrus but the difference was not significant. There was significant difference ($P<.001$) of prolactin concentration between primiparous and multiparous animals. The basal concentratin of prolactin in buffalo was much higher than reported in cows by American group of workers (Swanson and Hafs, 1971; Koproski and Tucker, 1973). Though one reason could be the species difference, however the climatic difference between India, being a tropical country and America, a temperate one, seems to be a major factor for higher prolactin concentration in buffaloes.

Kamonpatana *et al.* (1979b) reported basal level of FSH (43.6 ± 8.41 ng/ml) throughout the cycle which peaked (135.7 ± 70.01 ng/ml) at oestrus. A lower basal (20.74 to 29.58 ng/ml) and peak level (52.91 ng/ml) was observed by Heranjal *et al.* (1979a). But both group of workers observed a significantly higher level of FSH at oestrus.

Although there were minor fluctuations in the concentration of cortisol during oestrous cycle, but the differences between the days were not significant (Rao and Pandey, 1981). This implies that cortisol under normal conditions dose not appear to be involved in the regulation of oestrous cycle.

It appears from the aforesaid paragraphs that $PGF_2\alpha$ causes the regression of CL resulting in the rapid decline of progesterone concentration. The pro-oestrus-rise of oestradiol 17β concentration may be responsible for the mediation of LH release which causes ovulation. The occurrence of opposed changes in the plasma progesterone and oestrogen may be responsible for the manifestation of behavioural oestrus.

Arora and Pandey (1982d) reported a variety of temporary disturbances in endocrine profiles with respect to progesterone, oestradiol-17β and LH of 'repeat breeder' buffaloes resulting in reproductive failures.

Pregnancy : Dugwekar *et al.* (1976) reported that progesterone concentration fluctuated between 1.5 to 17.2 ng/ml during pregnancy whereas Ahmad *et al.*

(1979) recorded a range of 1.49 to 2.27 mg/ml between 3 to 9 months of pregnancy. Arora and Pandey (1982c) observed that oestradiol-17β concentration, ranged from 8.29 to 13.00 pg/ml during Ist trimester of pregnancy. Although a plateu of oestradiol-17β concentration was maintained during 2nd trimester of pregnancy, the overall concentration was significantly higher ($P<.01$) during this period as compared to Ist trimester of pregnancy. From 241-243 days of pregnancy, the oestradiol-17β concentration rose gradually till a day before calving when its concentration was highest. Rao *et al.* (1978) measured total oestrogens and progesterone during the last 2 months of pregnancy in 3 buffaloes. They recorded high values of oestrogen on the day of parturition which fell to 1/8th by 2nd day postpartum. An abrupt fall in progesterone level from 1.48 ng/ml maintained till 48 h prior to parturition to a basal level of 0 30 ng/ml was observed within 24 h after parturition by Agarwal *et al.* (1980). LH level did not vary appreciably (1–2 ng/ml) till 256-258 days of pregnancy and declined slowly afterwards to the level of 0.23 ng/ml on the day of parturition (Arora and Pandey, 1982c).

During pregnancy two animals showed gestational oestrus on 112 and 130 days (Batra *et al.*, 1979a). The concentrations of oestradiol-17β and progesterone were high on the day of 'pseudo oestrus'. The heat symptoms disappeared with the decline of oestradiol-17β whereas progesterone level was maintained. The oestradiol-17β secretion during gestational oestrus is due to the follicular development in the ovary which is responsible for oestrus behaviour. The follicular development is either due to an imbalance in the pituitary gonadotrophic complex as a result of neutral stimuli from the foetoplacental unit or some other factors such as stress, leading to endocrine disturbances. However, this amount of oestrogens cannot initiate a massive discharge of LH from the pituitary because this reflex is blocked by progesterone (Short, 1972) and therefore ovulation cannot result from such 'pseudoheats' since progesterone level is also high so resorption of the embryo cannot occur.

Parturition : The recent work from this laboratory (Batra *et al.*, 1982) showed that $PGF_{2}\alpha$ concentration fluctuated before parturition and the peak level was observed 1 day before calving. PGFM, oestradiol-17β and prolactin concentrations, increased gradually over the last 7 days with a significant peak ($P<.001$) a day before calving. The LH concentration remained low without any significant variation with time before calving. The progesterone concentration declined gradually and abrupt fall occurred 1–2 days before calving. The progesterone concentration was negatively correlated with $PGF_{2}\alpha$ ($r= -0.739$) PGFM ($r= -0.8322$) and oestradiol-17β ($r= -0.8896$) before parturition whereas oestradiol-17β concentration was positively correlated with $PGF_{2}\alpha$ ($r=0.986$), PGFM ($r=0.995$) and prolactin ($r=0.908$). Perera *et al.* (1981), observed the decline of progesterone and increased PGFM in conjuction with parturition. The difference may be due to the infrequent

sampling and less number of animals studied by this group of workers. It appears from our study that initial decline of progesterone and simultaneous increase of oestradiol-17β may be stimulating PGF release causing regression of CL, thus ensuing calving.

Post-partum period : The major factor limiting the productivity of the animal is long post-partum heat interval ranging from 76 to 185 days (Bhalla *et al.*, 1967; El-Sheikh and Mohamed, 1976; Singh *et al.*, 1979) which may be due to the non detectable oestrus after parturition. Oestradiol-17β concentration declined significantly after parturition and fluctuated between 8-15 pg/ml until oestrus (Batra and Pandey unpublished data). Progesterone concentration declined gradually (Pahwa and Pandey, 1983b) till the complete regression of the CL of pregnancy which completed between 3 to 29 days post-partum. The delayed regression of CL of pregnancy was also reported by Singh *et al.* (1979). The PGF concentration declined significantly ($P<.05$) on day 1 post-partum (Batra and Pandey unpublished data). This is contrary to the reports in cattle (Edqvist, Kindahl and Stabenfelot, 1978; Thatcher *et al.*, 1980) where almost the same level of PGFM as at parturition was maintained for 4-5 days post-partum. This high level in cow may be responsible for the complete regression of corpus loteum of pregnancy within 2-4 days postpartum in cows. (Labhsetwar *et al.*, 1964; Morrow *et al.*, 1966). The significant decline of prostaglandin F conocntration after parturition in buffaloes may be responsible for the long time taken (3-45 days) for the complete regression of CL of pregnancy (Singh *et al.*, 1979). After the initial decline of $PGF_2\alpha$ no fixed pattern was discernible throughout postpartum period (Batra and Pandey, 1983a). The basal level of PGFM remained higher till 15-20 days postpartum. The higher PGFM concentration after parturition and its slower decrease might be of some significance in uterine involution. $PGF_2\alpha$ may be acting at the hypothalamic level to stimulate the release of prolactin (Thatcher *et al.*, 1980) and growth hormone (Convey, 1974) since both have been shown to be secreted by the exogenous administration of $PGF_2\alpha$.

The progesterone level fluctuated around the basal level during postpartum period and a minor rise was observed before the oestrus (Pahwa and Pandey, 1983b). The measurement of progesterone in blood plasma showed that 50% of buffaloes underwent silent oestrus before the observed oestrus.

In post-partum anoestrus animals, there was higher PGFM level (Batra and Pandey unpublished data) and lower progesterone concentration (Pahwa and Pandey, 1983b), thereby suggesting that re-establishment of normal reoccurring cycles may be dependent upon the removal of uterine inhibition, the agent regulating the inhibition may be $PGF_2\alpha$ acting physiologically at ovarian level via hypothalamus and pituitary. A post partum rise of progesterone that may be critical for initiation of cyclic activity does not occur until the decline of PGFM has been achieved.

LH concentration increased gradually from 5–10 day post-partum and fluctuated between 0.6 to 3.0 ng/ml till oestrus (Batra and Pandey, 1983d). The level increased significantly at oestrus. The basal LH concentration during 2nd and 3rd week post-partum was inversely related to the days to first post-partum ovulation interval. In post-partum anoestrus animals, LH concentration was lower than the normal animals. The similar lower LH level in post-partum anoestrus animals was observed by Heranjal *et al.* (1979b).

There was significant difference of prolactin concentration between primiparous and multiparous (Pahwa and Pandey, 1983b) post-partum buffaloes. Though mean prolactin concentration in post-partum anoestrus animals was higher than the mean of normal animals but in some of the normal animals a level higher than anoestrus animals was observed. Thus anoestrus cannot be attributed to high prolactin concentration. Higher prolactin concentration ranging from 256 to 600 ng/ml was also observed by Heranjal *et al.* (1979b), in anoestrus animals.

SEASONAL ALTERATIONS IN PLASMA HORMONES

Seasonal reproduction is a common device whereby animals respond to climatic extremes which may be detrimental to the survival of the offspring and possibly even to the adults. The important seasonal reproductive problems are weak or silent oestrus, irregular oestrous cycles, low conception rate and early embryonic mortality. Seasonality in buffalo breeding has been reported by many workers which has been reviewed by Razdan and Kakar (1980). Luktuke *et al.* (1973) found that buffalo ovaries were the least active during extreme climatic conditions.

In order to overcome, environmental influences, some work has been done by Roy *et al.* (1964) by protecting the animals against heat and light. They initiated ovarian activity by keeping buffaloes in darkness and cooling the bodies by providing fans. Various managerial practices like providing shelter, wallowing, sprinkling of water and parading the vasectomised bulls have been tried, but these empirical approaches have not met with much success. Therefore, physiological approach could be thought as a possible solution to overcome summer infertility in buffaloes.

Sheth *et al.* (1978) showed that in the peak breeding season, the highest LH concentration (23.9 ± 5.7 ng/ml) was evident at the beginning of oestrus and declined to half the level (11.9 ± 0.9 ng/ml) at the end of oestrus. However, during low breeding season no fluctuations in LH level were noticeable. The overall LH level was low in winter and high in monsoon. Kakar *et al.* (1980) also reported that LH peak (20.80 ± 3.43 ng/ml) was lower in hotter months than cooler months (21.24 ± 0.99 ng/ml) at the onset of oestrus. Razdan *et al.* (1981) from the same laboratory observed a lower LH level in non-cycling (anoestrus) Murrah buffaloes during hotter months. None of the animals exhibited the optimal LH peak unlike in

cycling buffaloes. They correlated it with the ovarian inactivity and anoestrus condition in water buffalo during hot summer months.

While working on 6 non-inseminated buffaloes which were followed for a calander year, Rao and Pandey (1982) observed a lower progesterone level on the day of oestrus and during luteal phase in hotter months than cooler months. The oestradiol-17β concentration was also lower at oestrus in hotter months than cooler months (Rao and Pandey, 1983a), which may be responsible for lower LH peak level on the day of oestrus. Apart from this (Rao and Pandey, 1983b) observed overall higher cortisol level in cooler months as compared to hotter months. These results suggest that progesterone at appropriate level is essential for the expression of oestrus. The expression of oestrus and secretion of LH require an appropriate balance of oestradiol and progesterone. It appears that susceptible animals come under summer stress.

HORMONES IN MILK

Apart from the effect of constituents of milk on the young animal, the varying concentration of milk hormones particularly, in farm animals, are important in studies of maternal endocrinology. The primary impetus behind measuring various steroid hormones in milk residues is their diagnostic value to veterinarians and in aiding the husbandry man to manage reproduction. Most of the work done in milk hormones in buffaloes is from our laboratory.

Progesterone : A comparative study of progesterone in blood plasma and milk of buffaloes has apparently received little attention in the past. A study was, therefore, conducted to estimate the level of progesterone in milk and blood plasma of buffaloes with a view to establish the quantitative relationship of this hormone between the two fluids (Batra *et al.*, 1979b). The pattern of changes of milk progesterone of pregnant and non-pregnant buffaloes was similar to the progesterone in plasma. The average concentration in milk was 4-5 times higher in milk of non-pregnant and pregnant buffaloes (7.67 and 9.19 ng/ml) than that of blood plasma (1.33 and 2.45 ng/ml). In addition, the study showed a very close relationship of progesterone concentration between blood plasma and milk in pregnant ($r=0.98$) and non-pregnant ($r=0.99$) buffaloes. The average progesterone concentration in buffalo milk was higher than cow milk (Batra *et al.*, 1980b) which may be due to the higher fat percentage in buffalo milk (6.6%) as compared to cow milk (4.0%).

A higher progesterone concentration in milk compared to blood plasma was also observed during post-partum period in buffaloes by Pahwa and Pandey (1983b). We revealed that milk progesterone test could be used to monitor post-partum ovarian activity in buffaloes.

There was diurnal rhythm of milk progesterone concentration, being higher in evening milk samples (Suri *et al*, 1981). The higher progesterone in the evening

samples may be due to higher fat percentage in the evening samples. However the similar type of diurnal rhythm was observed in the milk fat also (Suri *et al.*, 1980), which suggests that apart from variation of fat percentage the other factor responsible may be the higher concentration of steroid in the lipid phase in the evening samples. This may be due to the effect of day light, which in sheep has been sown to increase progesterone concentration (McNatty *et al.*, 1973).

The presence of progesterone in milk may be due to its transfer from plasma or synthesis in mammary gland. Heap *et al.* (1975) suggested that progesterone in milk was attributed to its diffusion against concentration gradient from blood. However evidence exists to indicate the synthesis of progesterone in the mammary gland (Slotin *et al.*, 1970).

Oestradiol–17β : The results from this laboratory indicated that oestradiol–17β in blood plasma and milk followed similar pattern with 2–3 times higher concentration in milk (Batra *et al.*, 1980a). In addition, there was a positive correlation of oestradiol–17β in blood and milk of non-pregnant ($r=0.93$) and pregnant ($r=0.89$) buffaloes. The higher level of oestradiol–17β in milk when compared to blood plasma raises the question whether mammary gland of buffaloes are active in the uptake of oestradiol–17β or synthesis of hormone takes place in mammary gland. Pearlman *et al.* (1966) demonstrated the concentration of oestradiol–17β in milk on the day of calving was the higher than plasma (Batra and Pandey unpublished data). But concentration in milk during early post-partum period was equal to that of blood plasma. The shift of oestradiol–17β concentration in milk from higher to lower level may be due to the metabolic conversion of oestradiol–17β. This hypothesis is supported by the relative concentration of oestradiol–17β in milk of cows from day 3 to 25 of lactation which was 14% oestrone, 7% oestradiol–17β and 79% oestradiol–17∝ (Erb *et al.*, 1977). After 15–20 days post-partum, there was gradual increase in oestradiol–17β concentration in milk and the level was higher than plasma. This increase may be due the conversion of oestrone to oestradiol–17β as reported by Challis and Linzell (1973).

Prostaglandin F : The $PGF_{2}\propto$ concentration in milk was higher ($P<.01$) than in blood plasma (Batra and Pandey, 1983b) and was positively correlated in pregnant ($r=0.385$) and non-pregnant buffaloes ($r=0.738$). But the concentration did not vary in accordance with the various stages of the oestrous cycle. However, the concentration of its metabolite, PGFM increased significantly ($P<.001$) in milk with a peak, 2 days preceding oestrus in non-pregnant animals (Batra and Pandey, 1983a). But there was no significant difference of PGFM in blood plasma and milk of buffaloes. The $PGF_{2}\propto$ concentration did not show any trend during post-partum period (Batra *et al.*, 1981) but PGFM declined slowly during early post-partum period. The presence of prostaglandins in milk shows its transfer from plasma as suggested by Manns (1975) or its synthesis in the mammary gland (Maule Walker and Peaker, 1980).

Profein hormones : There was no significant difference of prolactin between blood plasma and milk of buffaloes during post-partum period (Pahwa and Pandey, 1983b) and oestrous cycle (Pahwa and Pandey, 1984). But the concentration was significantly higher in milk of multiparous than in primparous buffaloes. The same level of prolactin in milk as in plasma suggests its transfer from blood. But LH concentration in milk showed erratic fluctuation during oestrous cycle and early pregnancy (Batra and Pandey, 1983c).

PREGNANCY DIAGNOSIS

A striking difference of progesterone content in peripheral plasma (Arora *et al.*, 1979b), milk (Batra *et al.*, 1979b), and milk fat (Suri *et al.*, 1980) from 19 to 27 days after insemination has been used as the basis for a very early pregnancy diagnosis test in buffaloes.

Blood plasma progesterone : The concentration of progesterone in plasma of 32 buffalo heifers 19 to 22 days after insemination was used to diagnose pregnancy (Arora *et al.*, 1979a). A level exceeding 1 ng/ml was taken as indication of pregnancy. The accuracy of laboratory diagnosis for pregnancy based upon the progesterone level on day 22 post insemination was 94.73% and 100% for pregnant and non-pregnant respectively, when compared with the results obtained by rectal palpation carried out 7–8 weeks after insemination. The accuracy of laboratory diagnosis of 'pregnancy' as assessed by the progesterone level on 19–20 days was 75% (Table 1). The low success rate compared to day 22 could be due to the longer oestrous cycle length, therefore, the progesterone level on day 22 post-insemination appears to be a better index for pregnancy diagnosis rather than day 19.

Table 1. Reliability of pregnancy diagnosis based on plasma progesterone level in buffalo heifers

Days post insemination	Laboratory diagnosis	No. of buffaloes	Plasma progesterone (ng/ml, mean±S.E. and range) Day		Accuracy: Judged by non return to oestrus	Accuracy: Judged by rectal palpation 7–8 weeks after insemination
19–20	Non-pregnant	8	0	19–20	7/8 =87.50%	8/8 =100%
			0.12±0.03 (0.03—0.33)	0.18±0.05 (0.11—0.68)		
	Pregnant	24	0.16±0.02 (0.05—0.39)	3.8±0.3 (1.05—12.56)	19/24=79.6%	18/24=75%
22	Non-pregnant	13	0	22	12/12=85.43%	13/13—100%
			0.13±0.02 (0.03—0.33)	0.23±0.05 (0.04—0.60)		
	Pregnant	19	0.17±0.02 (0.05—0.39)	4.39±0.60 (1.82—12.56)	19/19=100%	18/19=94.73%

Perera *et al.* (1980) reported the success rate of 66.7% and 97% for pregnancy and non-pregnancy, respectively, in oestrus synchronised animal. While comparing enzyme-immunoassay (EIA) with radioimmunoassay Kamonpatana *et al.* (1979b) observed a lower level with EIA for the diagnosis of pregnancy.

Milk progesterone : Progesterone is 4 to 5 times higher in milk as compared to blood plasma and the changes in milk tend to reflect the circulating concentrations of blood progesterone (Batra *et al.*, 1979b) therefore collecting milk samples rather than blood would be more practical for dairymen. The study was therefore conducted (Arora *et al* , 1980) to determine the effectiveness of milk progesterone for diagnosing pregnancy or non-pregnancy, in lactating buffaloes. The animals having progesterone concentration more than 10 ng/ml in the samples taken from 19 to 27 days after insemination were classed as 'pregnant' and below this value as 'non-pregnant'. Based upon this criterion, the overall accuracy of diagnosis for pregnancy and non-pregnancy was 79.10% and 76.29% (Table 2). The success rate of pregnancy diagnosis was highest for samples taken on days 23 and 25 after insemination. The comparatively low accuracy of diagnosis on day 27 may be attributed to the high progesterone concentration in the early part of the luteal phase of the second cycle. Similarly the low accuracy observed on day 19 and 21 may be related to the high progesterone concentration due to the variability in the corpus luteum life span with the activity continuing on day 19 and 21 and the regression completed by day 23 or more post-breeding, thereby, resulting in the false pregnancy diagnosis. Thus, day 23 appeared to be the most appropriate for the prediction of pregnancy via milk progesterone. Singh and Puthiyandi (1980) revealed the detection of 100% non-pregnancy on day 20, 24, 28 and 49 days after insemination whereas success rate of pregnancy diagnosis was 66, 68, 81 and 83% respectively.

Table 2. Analysis of buffaloes classified as 'Pregnant' and 'Non-pregnant' by milk progesterone concentration 19 days post insemination

Day	Pregnant			Non-pregnant		
	No. of animals	Diagnosed via milk progesterone	%age accuracy	No. of animals	Diagnosed via milk progesterone	%age accuracy
19	20	26	76.92	24	18	75.00
21	20	26	76.92	24	18	75 00
23	20	23	86.95	24	21	87.50
25	20	24	83.33	24	20	83.33
27	20	28	71.42	24	16	66.66

Milk fat progesterone : The progesterone is highly soluble in milk fat and the concentration of progesterone seems to be influenced by milk fat content (Heap *et al.*, 1975; Hoffmann *et al.*, 1974). In order to overcome completely the mistakes in

milk progesterone test probably due to the variability of fat content, progesterone was estimated directly in milk fat of buffaloes, so as to increase the success rate of pregnancy diagnosis. (Pahwa *et al.*, 1981). A level exceeding 8 ng/100 μl of milk was taken as indication of pregnancy. Using this criterion, the success rate of pregnancy diagnosis increased significantly over milk progesterone test (Table 3). Although success rate of diagnosis increased, some of the factors like short or long cycle, pathologically altered cycle, embryonic mortality, error in oestrus detection which result in incorrect time of insemination and silent oestrus make it unlikely that it can be overcome completely.

Table 3. Pregnancy test from progesterone level in milk fat by single sample on various days after insemination

Days after insemination	Total No. of animal	Pregnant			Non-pregnant		
		Diagnosed via milk fat progesterone	Diagnosed via rectal palpation	% Accuracy	Diagnosed via milk fat progesterone	Diagnosed via rectal palpation	% Accuracy
19	42	25	19	76.00	17	23	73.91
21	42	23	19	82.60	19	23	82.60
23	42	20	19	95.00	22	23	95.65
25	42	21	19	90.47	21	23	91.30
27	42	26	19	73.07	16	23	69.56

CONCLUSION

Prostaglandin $F_2\alpha$ appears significantly in peripheral circulation only when its concentration is tremendously high (around parturition) but its level during other phases of reproduction is low and does not lead to any concrete conclusion. However, the concentration of its stable metabolite PGFM reflects the synthesis and release of $PGF_2\alpha$. The progesterone concentration increases and decreases with the waxing and waning of corpus luteum whose life is longer in buffaloes than cattle. The oestradiol-17 β peak appears before the LH surge around oestrus which suggests that oestradiol-17β is responsible for the mediation of LH release. Albeit, oestradiol-17β concentration shows increasing trends during successive trimesters of pregnancy, but progesterone and LH concentration varies significantly only during 3rd trimester of pregnancy. The higher level of oestradiol at 'pseudo estrus' is responsible for gestational oestrus. The initial decline in the progesterone and increase of oestradiol are the stimuli for the release of PGF which ultimately causes the regression of corpus luteum ensuing calving. The longer time taken for the complete regression of corpus luteum of pregnancy, during post-partum period, higher basal PGFM level, lower LH and progesterone levels are the factors responsible for the long post-partum heat interval in buffalo. The animals not exhibiting oestrus for a longer time after parturition should be treated with GnRH for the

'reawakening' of ovarian activity. The determination of progesterone in blood or milk during post-partum period may be used as a tool to monitor the ovarian activity. The silent oestrus in buffaloes may be due to the poor heat symptoms.

The summer infertility in buffaloes may be due to the imbalance in the secretion of oestradiol and progesterone which are essential for the expression of oestrus behaviour and mediation of LH surge required for ovulation. The low ovarian activity during summer may be associated with the decrease in the level of pituitary activity.

The hormonal changes in milk provides a clue of the circulating level, thereby suggesting that buffalo milk can be used to monitor the endocrine changes. The estimation of progesterone in blood plasma, milk or milk fat on 23 days after insemination can be used as a reliable indicator of pregnancy or non-pregnancy. The concentration of prolactin was higher in multiparous animals than primiparous, however other hormones were not affected by parity.

REFERENCES

Agarwal, S.P.; Agarwal, V.K. and Ahmad, A. (1980) *J. Nuclear Agric. Biol.*, **9** : 1–3.

Ahmad, A.; Agarwal, V.K. and Agarwal, S.P. (1979) *Indian J. Dairy Sci.*, **32** : 386–388.

Ahmad, A.; Agarwal, S.P.; Agarwal, V.K.; Rahman, S.A. and Laumas, K.R. (1977) *Indian J. exp. Biol.*, **15** : 591–593.

Arora, R.C.; Bachlaus, N.K.; Prasad, A. and Pandey, R.S. (1979a) *Indian J. exp. Biol.*, **17** : 124–127.

Arora, R.C.; Bachlaus, N.K.; Prasad, A. and Pandey, R.S. (1979b) *Indian J. exp. Biol* , **17** : 827–828.

Arora, R.C.; Batra, S.K.; Pahwa, G.S.; Jain, G.C. and Pandey, R.S. (1980) *Theriogenology*, **13** : 249–255.

Arora, R.C. and Pandey, R.S. (1982a) *World Rev. Anim. Prod* , **18** : 15–23.

Arora, R.C. and Pandey, R.S. (1982b) *Acta Endocr.*, **100** : 279–284.

Arora, R.C. and Pandey, R.S. (1982c) *Gen & Comp. Endocrinol.* **48** : 403–411.

Arora, R.C. and Pandey, R.S. (1982d) *Anim Prod.*, **34** : 139–144.

Bachlaus, N.K.; Arora, R.C.; Prasad, A. and Pandey, R.S. (1979) *Indian J. exp. Biol.*, **17** : 823–825.

Bachlaus, N.K.; Arora, R.C.; Prasad, A. and Pandey, R.S. (1980) *Theriogenology*, **13** : 297–303.

Batra, S.K.; Arora, R.C.; Bachlaus, N.K. and Pandey, R.S. (1979a) *Zbl. Vet. Med. A.* **26** : 502–505.

Batra, S.K.; Arora, R.C.; Bachlaus, N.K. and Pandey, R.S. (1979b) *J. Dairy Sci.*, **62** : 1390–1393.

Batra, S.K.; Arora, R.C.; Bachlaus, N.K.; Pahwa, G.S. and Pandey, R.S. (1980a) *J. Endocr.*, **84** : 205–209.

Batra, S.K.; Pahwa, G.S.; Suri, A.K. and Pandey, R.S. (1980b) *Anim. Prod.*, **31** : 127–131.

Batra, S.K.; Pahwa, G.S. and Pandey, R.S. (1981) *Indian J. Physiol. Pharmacol.*, **25** (Suppl. 1) 9 (Abst.).

Batra, S.K.; Pahwa, G.S. and Pandey, R.S. (1982) *Biol. Reprod.*, **27** : 1055–1061.

Batra, S.K. and Pandey, R.S. (1983a) *J. Reprod. Fert.*, **67** : 191–195.

Batra, S.K. and Pandey, R.S. (1983b) *Acta Endocr.*, **102** : 314–320.

Batra, S.K. and Pandey, R.S. (1983c) *Anim. Reprod. Sci.*, **5** : 247–257.

Batra, S.K. and Pandey, R.S. (1983d) *Theriogenology*, **19** : 193–200.

Bhalla, R.C.; Senger, S.D. and Soni, B.K. (1967) *Indian J. Dairy Sci.*, **20** : 189–190.

Butchaiah, V.; Tomer, V.K. and Singh, B.P. (1975) *Indian Vet. J.*, **52** : 97–102.

Challis, J.R.G. and Linzell, J.L. (1973) *J. Endocr.*, **57** : 451–457.

Convey, E.M. (1974) *J. Dairy Sci.*, **57** : 905–917.

Dugwekar, Y.G.; Rao, B.R.; Bapat, S.T. and Verman, P.N. (1976) In 'Use of Radiations and Radio isotopes in Studies of Animal Production' I.V.R.I. Izatnagar Dec. 16–18, pp. 276–279.

Edquist, L.E.; Kindahl, H. and Stabenfeldt, G. (1978) *Prostaglandins*, **16** : 111–119.

El-Sheikh, A.S. and Mohamed, A.A. (1976) *Indian J. Anim. Sci.*, **46** : 580–583.

Erb, R.E.; Chew, B.P.; Keller, H.F. and Maluen, P.V. (1977) *J. Dairy Sci.* **60** : 557–565.

Goel, V.D. and Sud, S.C. (1978) *J. Steroid Biochem.*, **9** : 370 (Abst.).

Granstrom, E. (1972) *Eup. J. Biochem.*, **27** : 462–469.

Hansel, W. and Snook, R.B. (1970) *J. Dairy Sci.*, **53** : 945–960.

Heap, R.B.; Henville, A. and Linzell, J.L. (1975) *J. Endocr.*, **66** : 239–247.

Heranjal, D.D.; Sheth, A.R.; Moodbidri, S.B.; Desai, R. and Rao, S.S. (1976) *Indian J. Anim. Sci.*, **46** : 553–555.

Heranjal, D.D.; Sheth, A.R.; Desai, R. and Rao, S.S. (1979a) *Indian J. Dairy Sci.*, **32** : 247–249.

Heranjal, D.D.; Sheth, A.R.; Wadadekar, K.B.; Desai, R. and Rao, S.S. (1979b) *J. Dairy Sci.*, **32** : 383–385.

Hoffmann, B.; Hamburger, R.; Gunjler, O.; Korndorfer, L. and Lohoff, H. (1974) *Theriogenology*, **2** : 21–25.

Jain, G.C. and Pandey, R.S. (1981) *Zbl. Vet. Med. A.*, **28** : 455–458.

Kakar, M.L.; Razdan, M.N. and Galhotra, M.M. (1980) *J. Reprod. Fert.*, **56** : 419–424.

Kamonpatana, M.J.; Kunawangkrit, A.; Bodhipaksha, P. and Luvira, Y. (1979a) *J. Reprod Fert.*, **56** : 445–449.

Kamonpatana, M.; Schams, D. and Wiel, D.F.M. (1979b) In 'Buffalo Reproduction and Artificial Insemination, FAO Animal Production and Health Paper **13** : 226–234. Food and Agriculture Organization of the United Nations, Rome.

Koprowski, J.A. and Tucker, H.A. (1973) *Endocrinology*, **92** : 1480–1487.

Labhsetwar, A.P.; Collins, W.E.; Tyler, W.J. and Casida, L.E. (1964) *J. Reprod. Fert.*, **8** : 85–90.

Luktuke, S.N.; Bhattacharya, A.R.; Singh, S.K. and Khan, B.U. (1973) *Indian Vet. J.*, **50** : 876–881.

Manns, J.G (1975) *Prostaglandins*, **9** : 463–474.

Maule Walker, F.M. and Peaker, M. (1980) *J. Physiol.*, **309** : 65-79.

Mc Natty, K.P.; Revfeim, K.J.A. and Young, A. (1973) *J. Endocrinol.*, **58** : 219-225.

Morrow, D.A.; Roberts, S.J.; Mc Entee, K. and Gray, H.G. (1966) *J. Amer. Vet. Med. Ass.*, **149** : 1596–1609.

Pahwa, G.S.; Batra, S.K.; Arora, R.C. and Pandey, R.S. (1981) *Milchwissenschaft*, **36** : 214–216.

Pahwa, G.S. and Pandey, R.S. (1983a) *Theriogenology*, **19** : 491–505.

Pahwa, G.S. and Pandey, R.S. (1983b) *Anim. Prod.*, **37** : 237–246.

Pahwa, G.S. and Pandey, R.S. (1984) *J. Dairy Sci.* (In press).

Pandey, R.S. (1979) In 8 Buffalo Reproduction and Artificial Insemination. FAO Animal Production and Health Paper **13** : 185–210. Food and Agriculture Organisation of the United Nations, Rome.

Pandey, R.S.; Arora, R.C. and Bachlaus, N.K. (1982) *Zbl. Vet. Med. A.*, **29** : 149-154.

Pearlman, W.S.; De Hertogh, R.; Laumas, K.R.; Brueggemann, J.A. and Pearlmann, M.R.J. (1966) In 8 Steroid Dynamics' pp. 159–177. Eds. G. Pincus. J. Tait and T. Nakao, New York, Academic Press.

Perera, B.M.A.O.; Pathiraja, N.; Abeywardena, S.A.; Motha, M.X.J. and Abeygunawardena, H. (1980) *Vet. Rec.*, **106** : 104–106.

Perera, B.M.A.O.; Abeygunawardena, H.; Thamotharna, A.; Kindahl, H. and Edquist, L.E. (1981) *Theriogenology*, **15** : 463–467.

Prasad, A.; Bachlaus, N.K.; Arora, R.C. and Pandey, R.S. (1979) *Indian J. Dairy Sci.*, **32** : 334–335.

Rao, B.R.; Dugwekar, Y.G.; Bapat, S.T.; Gujrath, K.G. and Verman, P.N. (1978) *Indian J. Dairy Sci.*, **31** : 181–183.

Rao, L.V. and Pandey, R.S. (1981) *Experentia*, **37** : 1118–1119.

Rao, L.V. and Pandey, R.S. (1982) *J. Reprod. Fert.*, **66** : 57–61.

Rao, L.V. and Pandey, R.S. (1983a) *J. Endocr.*, **98** : 251–255.

Rao, L.V. and Pandey, R.S. (1983b) *Anim. Reprod. Sci.*, **6** : 177–184.

Rao, B.R.; Patel, V.G. and Tamhan, S.S. (1973) *Indian Vet. J.*, **50** : 257–264.

Razdan, M.N. and Kakar, M.L. (1980) *Indian Dairyman*, **32** : 459–463.

Razdan, M.N.; Kakar, M.L. and Galhotra, M.M. (1981) *Indian J. Anim. Sci.*, **51** : 286–288.

Roy, A.; Sengupta, B.P. and Misra, M.S. (1964) Proc. Symposium Environmental Physiology and Psychology in Arid Conditions, UNESCO Lucknow.

Sheth, A.R.; Wadadekar, K.B. and Moodbidri, S.B. (1978) *Curr. Sci.*, **47** : 75–77.

Short, R.V. (1972) 'Role of hormones in sex cycles'. In Hormones in Reproduction Eds. C.R. Austin and R.V. Short, Cambridge University Press, London.

Singh, N.; Chauhan, F.S. and Singh, M. (1979) *Indian J. Dairy Sci.*, **32** : 134–139.

Singh, A. and Puthiyandí, R. (1980) *J. Reprod. Fert.*, **59** : 89–93.

Slotin, C.A.; Heap, R.B.; Christensen, J.M. and Linzell, J.L. (1970) *Nature*, **225** : 385–386.

Suri, A.K.; Batra, S.K.; Pahwa, G.S. and Pandey, R.S. (1980) *Zbl. Vet. Med. A.*, **27** : 702–707.

Suri, A.K.; Batra, S.K.; Pahwa, G.S. and Pandey, R.S. (1981) *J. Dairy Res.*, **48** : 503–507.

Swanson, L.V. and Hafs, H.D. (1971) *J. Anim. Sci.*, **33** : 1038–1041.

Thatcher, W.W.; Wilcox, C.J.: Collier, R.J.; Diann, S.E. and Head, H.H. (1980) *J. Dairy Sci.*, **63** : 1530–1540.