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ESTRADIOL-17B CONCENTRATION IN DIFFERENT BIOLOGICAL FLUIDS DURING THE OESTROUS CYCLE IN BUFFALO

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The present study was designed to determine the matched estradiol-17B concentration in serum, fore-milk and saliva during different phases of buffalo oestrous cycle by radioimmunoassay technique. Estradiol- 17B concentration in all experimental biological fluids was higher during pro-oestrus followed by oestrus, di-oestrus and met-oestrus. There was a close positive correlation between the salivary estradiol-17B concentration (r=:=0.92)fore-milk (r=0.87) and serum samples (r=0.88). It was concluded that saliva can be used to determine estradiol-L'ZBconcentration as an alternate of milk and serum.

Key words: buffaloes, estradiol-17B, fore-milk, oestrous cycle, saliva, serum

INTRODUCTION

The progesterone and estradiol-17B assay of blood and mil)i has emerged as a useful tool to determine an' appropriate time of insemination, monitoring of cyclicity and pregnancy diagnosis in cattle (Pope et al., 1976; Gunzler et al., 1979) and buffaloes (Batra et al., 1980; Batra and Pandey, 1983). Salivary hormonal concentrations an; highly correlated with plasma (Walker et al., 1981;' Sorgo et al., 1982; Campbell, 1985) and it' has been shown that the saliva contents of some steroids may be used as an index of their unbound plasma. This information is being used to monitor ovarian functions in women (Galvin and Short, 1987) and cattle (Gao et al., 1988). The authors of the present paper have already assayed progesterone hormone levels in serum, fore-milk and saliva during different phases of oestrous cycle in buffaloes (Qureshi et al., 1999). The aim of the present project was to determine estradiol-17B levels in serum, fore-milk and saliva to investigate whether salivary estradiol-17B concentration reflected the same trend as that by serum and fore-milk. Saliva was further investigated to find out if it could serve as an alternate of milk and serum for estradiol-17B determination.

MATERIALS AND METHODS

Forty healthy non-pregnant lactating and cycling buffaloes with the history of normal calving and normal reproductive tract were selected from the animals brought to the clinic of the Department of Animal Reproduction for artificial insemination. The first oestrus of selected buffaloes was missed for the collection of samples during different phases of oestrous cycle from the same animals.

Restraint, venipuncture and blood collection were accomplished as rapidly as possible and samples were rejected, from the animals which displayed excessive excitement in order to minimize disproportionate changes of plasma hormones. In all 480 serum, fore-milk and saliva samples were collected during oestrus (d1), met-oestrus(d2-5), di-o~strus (d6-18) and pro-oestrus (d19-20) phases of oestrous cycle. Blood was collected by venipuncture from the jugular veinand the serum was separated. Fore-milk samples were collected by hand stripping in clean sodium azide quoted vials. Saliva was collected in clean sterilized glass vials by giving gentle pressure on the middle of the dorsum of tongue. All samples wer'e" stored at -20°C until assayed.

Estradiol-17B concentration' in all biological fluids was measured by radioimmunoassay (RIA) using immuchem TM, estradiol kit provided by ICN, Biomedicals, Inc. Diagnostic Division 3300 Hyland Avenue, Costa Mosa, CA 92626. All the laboratory work was done at the Nuclear Institute of Agriculture and Biology, Faisalabad. The serum and fore-milk samples were processed according to the method described by Batra et al. (1980). The saliva samples were processed as described by Kanchev et al, (1988),

Mean ± SD values for estradiol-17B concentrations in serum, fore-milk and saliva during different phases of oestrous cycle were calculated. Correlation of estradiol concentration with all experimental biological fluids were also computed (Steel and Torrie, 1980).

RESULTS

The mean ± SD values recorded for estradiol-17B in serum, fore-milk and saliva during different phases of oestrous cycle are presented in Table 1. The seral estradiol concentration was higher in pro-oestrus (30.73±0.68) follow~dby oestrous (15.72 ± 0.58), di-oestrus (11.51 ± 0.50) and met-oestrus (7.32 ± 0.29) phases. The corresponding values for fore-milk were -13.25± 0.54, 32.50 ± 0.32, 19.23 ± 0.33 and 10.09 ± 0.19, respectively. The salivary estradiol-17B concentrations in the same order were 35.04 ± 0.60, 21.64 ± 0.77" 19.68 ± 0.56 and 9.95 ± 0.22. The hormonal trerid during different phases of oestrous cycle in all experimental biological fluids was similar but comparatively the values were higher in fore-milk, followed by saliva and serum (Fig. 1). In addition, there was a close correlation (P<O.OI) between levels of estradiol-17B in serum (r=0.88), fore-milk (r=0.87) and saliva (r=0.92).

Table 1. Estradiol-17B concentrations (pg/ml) in serum, fore-milk and saliva during different phases of oestrous cycle (n=40)

	Pro-oestrus	Oestrus	Met-oestrus	Di-oestrus
Serum	30.73	15.72	7.32	11.51
Fore-milk	43.25	32.50	10.09	19.23
Saliva	35.04	21.64	9.95	19.68

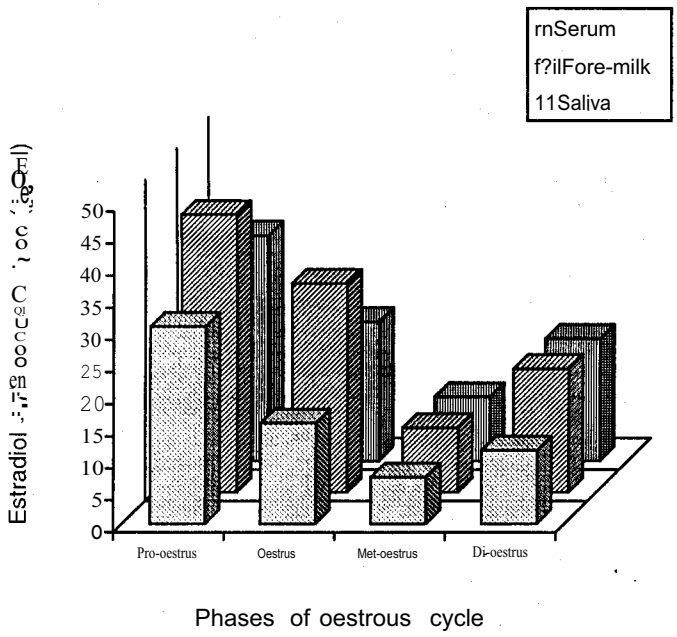

Fig. 1 Bubaline estradiol-17B concentrations (pg/ml) in serum, fore-milk and saliva during different phases of oestrous cycle (n=40)

DISCUSSION

In the present study the concentration of estradiol-17B in fore-milk and serum showed that the changes in its level were similar in both fluids during oestrous cycle in buffaloes. However, the concentration of hormone was comparatively higher in milk. This agrees with the results of Kaminpatha et al. (1976), Bachalus et al. (1979) and Batra et al. (1980). The minor peak of estradiol-17B on day 11 (di-oestrus) corresponded closely to the peak reported by Wettemann et al. (1972). The increasing estradiol-17B concentration on day 11 reflects the mid-cycle follicular growth (Rajakoski, 1960; Hobson and Hansel, 1972). Although the results of present study clearly showed three peaks of estradiol-17B during oestrous cycle (mid-luteal, oestrus and pro-oestrus) but the highest concentration was obtained in the pro-oestrus phase of oestrous cycle. This is also supported by the finding' of Henrick et al. (1971); Shemesh et al. (1972); Dobson and Dean (1974).

The rise of estradiol-17B around oestrus may be involved in the mediation of LH release via positive feed back, which triggers the LH surge responsible for ovulation. Although there is no evidence of this positive feed back in buffaloes, but it has been demonstrated. in ewes (Hauger et al., 1977) and cattle (Hebson and Hansel, 1972). The higher level of estradiol-17B in milk compared to blood, raises the question of whether the mammary glands of buffaloes are active in the uptake of estradiol-17B or the synthesis of this takes place in the mammary tissue. Evidence exists to indicate that mammary tissue of rat and man is able to concentrate oestrogens (Pearlman et al., 1966). There is a possibility of conversion of steroids (Slotin et al., 1970). A close correlation of estradiol-17B concentrations between blood and milk samples in the present study is corroborated by that of Batra et al. (1980). The present authors have not been able to trace any published work on salivary estradiol-17B concentration and its correlation with serum and milk. However, some reports on salivary progesterone concentration and its correlation with serum and milk are available (Kanchev et al., 1988; Gao et al., 1988). Desaulniers (1989) reported measurement of steroids in different body secretionts)/ excretions i.e. saliva, urine and faeces. Similarly, Schramm et al. (1990) reported a progesterone determination in situ urine. In this study too, a significant (P<0.10) positive correlation of salivary estradiol-17B concentration was found

with that of serum and milk (1'=0.92).The results of the present study clearly show that the salivary estradiol-17B trend is similar to that of serum and fore-milk during different phases of oestrous cycle in buffaloes which indicates that salivary estradiol-17B concentration can be used as an index for the assessment of ovarian functions in buffaloes. However, a large scale study in buffalo is suggested to further elucidate information on estradiol-17B concentration in certain other body fluids i.e. tears and body excretions such as urine and faeces to establish the normal levels during different phases of oestrous cycle and early pregnancy.

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