Seasonal changes in plasma progesterone concentrations in buffalo cows (*Bubalus bubalis*)*

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Summary. Blood samples were taken from 6 non-inseminated cyclic buffaloes for 1 calendar year. Plasma progesterone concentrations on the day of oestrus and also in the luteal phase of the cycle were significantly higher ($P < 0.01$) in the cooler (warm and cold) seasons than in the hot and dry and hot and humid seasons.

Introduction

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. India is a tropical country and in the north extreme climatic conditions prevail. In summer temperatures reach 42°C during the day and 30°C during the night. Daylength goes up to 13–14 h. Karnal is situated at 245 m above mean sea level on 29°43'N latitude and 76°58'E longitude. Seasonality in buffalo breeding has been reported by many workers (Rife, 1959; Bhattacharya, Luktuke & Roy, 1970; Roy, Bhattacharya & Luktuke, 1972). Luktuke, Bhattacharya, Singh & Khan (1973) found that buffalo ovaries were least active during extreme climatic conditions. The important seasonal reproductive problems are weak or silent oestrus, irregular oestrous cycle lengths, low conception rates and early embryonic mortality.

The mechanisms whereby biometeorological cues are transduced into the endocrine messages that govern gonadal functions are not clearly understood. There are controversial reports regarding the variations of progesterone secretion in dairy cows in natural climatic conditions (Roussel, Beatty & Lee, 1977; Rosenberg, Herz, Davidson & Folman, 1977). However, none of these workers followed the same animals throughout the year, and the sampling frequency was low. The present study was undertaken to investigate circannual changes of progesterone concentrations in buffalo.

Materials and Methods

Animals and blood collection

Six normally cyclic Murrah buffaloes (2nd lactation) were selected from the Institute herd and they were not inseminated for 1 complete calendar year. They were fed daily Berseem, maize, lucerne and concentrate mixture to provide 365 g digestible crude protein, 4.54 kg total digestible nutrient, 7.52 g crude protein and 8.60 kg dry matter/500 kg body weight. The animals were kept in a loose housing system and the occurrence of oestrus was checked 3 times a day by means of a vasectomized bull and behavioural symptoms. The day of oestrus was

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designated Day 0. Blood samples were collected on alternate days via jugular venepuncture into tubes packed in ice. The blood was centrifuged at 4°C within 1 h of collection and plasma was stored at -20°C until analysis.

Experimental conditions

The months were grouped into four seasons (hot and dry, hot and humid, warm, and cold) in relation to the basic differences in the average maximum temperature and relative humidity. Climatic data are shown in Table 1. The animals were periodically checked for any clinical abnormalities.

Duncan's multiple range test was used for statistical analysis of the data.

Table 1. Average monthly environmental variables throughout the calendar year

	Hot dry			Hot humid			Warm			Cold		
	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March
Average temperature mean (°C)	28	32.6	31.5	29.2	29.5	28	25	24.5	14.7	13.6	16	19
Average minimum temperature (°C)	18.7	24.4	25.9	25.7	25.5	22.9	18.1	12.1	8.0	8.1	8.9	11.7
Average maximum temperature (°C)	37.3	40.9	37.1	32.6	33.5	33.2	31.9	27.0	21.5	19.2	23.1	26.4
Relative humidity (%)	55	41	72	90	89	89	88	89	91	94	86	85
Rainfall (mm)	2.8	1.0	125.8	487.8	67.8	118.4	12.0	7.8	53.8	41.2	2.4	19.8
Wind velocity (km/h)	6.1	7.5	10.5	7.5	4.4	3.7	3.6	3.2	3.9	4.9	6.3	5.3
Sunshine (h)	11.7	10	10.4	6.3	8.4	10.1	9.4	8.4	7.1	7.2	8.2	8.4

Radioimmunoassay

Plasma progesterone concentrations were determined by the radioimmunoassay described by Arora, Bachlaus, Prasad & Pandey (1979). The antiserum was raised in a rabbit to carboxymethyl progesterone-7 α -BSA. The accuracy was assessed by 8 duplicate estimations of 0.25, 0.5, 1.0, 2.0 ng progesterone added to hormone-free plasma (0.2 ml), and the recoveries were 0.27 ± 0.01 , 0.56 ± 0.03 , 1.08 ± 0.03 and 2.04 ± 0.04 ng respectively. The sensitivity of the assay was 12.59 pg/tube and was calculated from the smallest amount of standard that was significantly different from zero dose at the 95% confidence limit. Intra- and inter-assay coefficients of variation were 11.2% ($n = 16$) and 14.8% ($n = 8$) respectively. The antiserum cross-reacted 7% with 3 β -hydroxy-5-pregnane-20-one, 6% with 11 α -hydroxy-4-pregnane-3,20-dione, 0.3% with 17-hydroxyprogesterone and <0.2% with corticosterone, cortisone, cortisol, testosterone and oestradiol-17 β .

Results

The progesterone values throughout the oestrous cycle in the four seasons are shown in Table 2.

In each season the progesterone changes throughout the cycle followed the normal pattern reaching maximum values on Day 18. However, progesterone concentrations were higher on the day of oestrus, and during the luteal phase of the cycle in the warm and cold seasons (Table 3).

Table 2. Circannual changes of plasma progesterone concentrations (ng/ml) in buffalo cows during the oestrous cycle

Day of cycle	Hot and dry		Hot and humid		Warm		Cold	
	No. of observations	Mean $\pm$ s.e.m.	No. of observations	Mean $\pm$ s.e.m.	No. of observations	Mean $\pm$ s.e.m.	No. of observations	Mean $\pm$ s.e.m.
0	9	0.14 $\pm$ 0.05	19	0.16 $\pm$ 0.04	14	0.44 $\pm$ 0.09	14	0.49 $\pm$ 0.06
2	15	0.27 $\pm$ 0.05	19	0.77 $\pm$ 0.11	16	0.74 $\pm$ 0.08	11	0.96 $\pm$ 0.19
4	14	0.95 $\pm$ 0.03	20	0.83 $\pm$ 0.09	16	0.96 $\pm$ 0.09	12	1.28 $\pm$ 0.15
6	16	0.96 $\pm$ 0.12	17	0.91 $\pm$ 0.09	16	1.47 $\pm$ 0.23	11	1.74 $\pm$ 0.20
8	16	1.07 $\pm$ 0.09	18	0.98 $\pm$ 0.16	13	1.73 $\pm$ 0.41	12	1.97 $\pm$ 0.22
10	14	1.23 $\pm$ 0.13	18	1.16 $\pm$ 0.10	14	1.99 $\pm$ 0.37	10	2.06 $\pm$ 0.69
12	11	1.24 $\pm$ 0.31	17	1.24 $\pm$ 0.18	12	2.19 $\pm$ 0.30	12	2.22 $\pm$ 0.19
14	13	1.44 $\pm$ 0.21	17	1.29 $\pm$ 0.18	12	2.33 $\pm$ 0.38	11	2.54 $\pm$ 0.28
16	13	1.76 $\pm$ 0.17	20	1.57 $\pm$ 0.14	11	2.45 $\pm$ 0.33	10	2.80 $\pm$ 0.57
18	12	2.05 $\pm$ 0.16	19	1.72 $\pm$ 0.16	11	2.67 $\pm$ 0.49	14	3.11 $\pm$ 0.20
-3	15	0.56 $\pm$ 0.10	15	0.54 $\pm$ 0.06	12	1.22 $\pm$ 0.28	11	1.24 $\pm$ 0.31
-1	17	0.36 $\pm$ 0.05	18	0.27 $\pm$ 0.07	13	0.73 $\pm$ 0.09	12	0.94 $\pm$ 0.15

Table 3. Analysis of progesterone concentrations in different seasons

Day of cycle	Between seasons		Within seasons		
	d.f.	MS	d.f.	MS	F
0	3	0.532	52	0.054	9.69**
2	3	1.19	57	0.17	6.71**
4	3	0.516	58	0.14	3.67*
6	3	2.22	56	0.40	5.48**
8	3	3.39	55	0.77	4.40**
10	3	3.16	52	1.42	2.22
12	3	4.01	48	0.75	5.34**
14	3	5.15	49	0.88	5.82**
16	3	4.34	50	1.06	4.08*
18	3	5.93	52	0.88	6.73**
-3	3	2.34	49	0.48	4.83**
-1	3	1.41	56	0.11	1.2

* $P < 0.01$.** $P < 0.05$.

Discussion

Progesterone at appropriate levels is essential for the expression of oestrus, to prepare the animals for pregnancy and to maintain the pregnancy once initiated. In the present study we found significantly different progesterone concentrations in the four seasons (Table 3). Preliminary measurements of oestradiol concentrations also showed that values in the hotter months were lower than in cooler months. These observations support the view that expression of oestrus and secretion of LH require an appropriate balance between oestradiol and progesterone (Karsch, Legan, Ryan & Foster, 1980). Higher concentrations of progesterone from Day 0 to Day 13 have been demonstrated in animals which conceive than those which do not conceive (cows; Batra, Pahwa, Suri & Pandey, 1980; buffalo: Batra, Arora, Bachlaus & Pandey, 1979; Suri, Batra, Pahwa & Pandey, 1981). The low progesterone level in hotter

months in comparison to cooler months is probably responsible for the inadequate expression of oestrus and low conception rates in buffaloes (Table 4).

Table 4. Conception rates of contemporary herd mate buffaloes during the different seasons

	No. of inseminations	Conception %
Hot and dry	74	22.98*
Hot and humid	94	25.11*
Warm	201	42.41
Cold	196	28.43

* Significantly different from conception rate in cooler months, $P < 0.05$ (χ^2 tests).

Mills, Thatcher, Dunlap & Vincent (1972) and Abilay, Johnson & Madan (1975) observed increased progesterone secretion during chronic exposure to heat in climatic chamber studies. These studies have been valuable in establishing the basic parameters of the stress but such an application to field conditions is often difficult because of the diurnal variation in ambient temperature, relative humidity and difficulty in controlling other aspects of the animals' environment.

Our results agree with those of Rosenberg *et al.* (1977) for cows exposed to natural climatic conditions in summer: reduced fertility and lower plasma progesterone concentrations throughout the luteal phase of oestrous cycle were reported. However, Roussel *et al.* (1977) found a significantly higher level of progesterone (monthly samples) in cows in the hot season and suggested that this was related to the mobilization of body fat, indicated by substantial weight loss. In the present study, we could not find any appreciable decrease in the body weight of the experimental animals.

The effects of each climatic condition separately cannot be distinguished but lower concentrations in the hot and humid season indicate that humidity reinforces the effect of high temperature to suppress progesterone secretion and ultimately lead to reproductive failures in buffaloes.

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