

## Hormonal assay in repeat breeding cows and buffaloes

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### ABSTRACT

A study was conducted to assess the hormonal status in 70 repeat breeder and 14 regular breeder cows and buffaloes in Chennai, Tamilnadu. Circulating progesterone level was higher on day 0 and lower on day 7 in repeat breeder than in regular breeder. Estrogen level was lower on day 0 and day 7 in repeat breeder than regular breeder cows and buffaloes. Regular breeder cows had a significant higher level of T3 and T4 on day 0 than the repeat breeder cows and buffaloes. It is concluded from the study that this difference in hormonal concentration of repeat breeders from regular breeders might be responsible for the reproductive disorders in cows and buffaloes.

**Key words:** Estrogen, thyroid hormones, progesterone, repeat breeder

Fertilisation failure or early embryonic mortality contributes to repeat breeding in bovines. Luteal dysfunction leading to inadequate progesterone production may be a cause of early embryonic mortality (Sartori *et al.*, 2002). Early embryonic mortality was high among repeat breeding cows and it has been concluded that embryonic development at 6-7 days after breeding is critical (Albihn *et al.*, 1989). The optimal oviductal environment, including contractile activity for gamete transport, fertilisation and early embryonic development, is mediated by progesterone (P4), estradiol (E2) and prostaglandin (PGF<sub>2</sub>-alpha) (Wijayagunawardane *et al.*, 1999; Doualla-Bell *et al.*, 1998). Hence the present study was undertaken to study the level of serum progesterone, oestrogen and thyroid hormones in repeat breeding cows and buffaloes.

Cows and buffaloes that have had three or more fertile services to bull or artificial insemination but failed to conceive were selected for this study. Such animals exhibited regular estrous cycle with clear mucous discharge without any palpable genital abnormalities on rectal examination. The control animals were the normally cycling cows and buffaloes with normal genitalia exhibiting physiological estrous and regular conception. Approximately 20 ml of blood was collected aseptically for serum separation from seventy repeat breeding syndrome animals (35 cows and 35 buffaloes)

and fourteen regular conceiving bovines (7 cows and 7 buffaloes) on day 0 and day 7 of estrous cycle and stored at -20°C until used. The serum concentration of progesterone, estrogen, triiodothyroxine (T3) and tetraiodothyroxine (T4) were estimated by coat-A-count, a solid phase radio immuno assay procedure using kits supplied by Diagnostic Products Corporation, Los angeles, U.S.A. All samples were assayed in duplicate. The sensitivity of the standard curve ranged from 0.1 to 40 ng/ml for progesterone, 0.1 to 25 pg/ml for estrogen, 0.1 to 10 mg/dl for T4 and 50 to 200 ng/dl for T3. The mean intra- and inter- assay CV calculated from serum pools were 6.4% and 9.7% respectively for progesterone, 11.4% and 13.7% respectively for estrogen, 6.9% and 10.3% respectively for T3 and 6.7% and 11.3% respectively for T4. Statistical analysis of data was carried out as per the standard procedure given by Lewis (1971).

The results of the present investigations are given in the Table 1. The level of progesterone on day 0 (D0) in repeat breeding cows was found to be higher ( $0.558 \pm 0.001$  ng/ml) than in regular breeding cows ( $0.1922 \pm 0.077$  ng/ml). On day 7 (D7) lower level of serum progesterone ( $1.0147 \pm 0.457$  ng/ml) was found in repeat breeding cows than in regular breeding cows ( $3.151 \pm 0.092$  ng/ml). Similar results were noticed in buffaloes. The present observations are in agreement with the findings of Younis *et al.*, (1989). On the contrary, Kurade *et al.* (1993) and Rajasundaram and Rajasekaran (1994) have observed lower concentration of progesterone in repeat breeders than regular breeders on the day of estrous. The regular breeder bovine showed a

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Table 1. Serum concentration of progesterone, estrogen, T3 and T4 in repeat breeding and regular breeding cows and buffaloes

Species	Hormone	Day 0		Day 7	
		Regular Breeder	Repeat Breeder	Regular Breeder	Repeat Breeder
Cow	Progesterone ng/ml	0.1922±0.077 <sup>ad</sup>	0.558±0.001 <sup>P</sup>	3.151±0.092 <sup>Af</sup>	1.0147±0.457 <sup>F</sup>
	Estrogen pg/ml	7.201±1.29 <sup>bo</sup>	6.212±0.216 <sup>BE</sup>	2.99±0.05 <sup>B</sup>	2.104±0.012 <sup>C</sup>
	T3 ng/dl	181.72±14.23 <sup>b</sup>	144.35±15.02 <sup>o</sup>	99.16±0.15 <sup>P</sup>	167.44±15.04 <sup>NP</sup>
	T4 mg/dl	8.86±0.67 <sup>m</sup>	7.3934±0.56	5.92±0.41 <sup>Mq</sup>	7.86±0.70 <sup>R</sup>
Buffalo	Progesterone ng/ml	0.1425±0.012 <sup>s</sup>	1.0405±0.571	4.0525±0.011 <sup>O</sup>	2.6373±0.956
	Estrogen pg/ml	15.009±2.16 <sup>j</sup>	10.76±1.091 <sup>J</sup>	6.123±1.312 <sup>Ik</sup>	3.612±0.125 <sup>K</sup>
	T3 ng/dl	126.253±12.14 <sup>n</sup>	65.31±5.7 <sup>s</sup>	88.23±9.61 <sup>R</sup>	73.86±3.91 <sup>T</sup>
	T4 mg/dl	4.3238±0.29	4.19±0.33	3.5025±0.02	3.602±0.02

Values are Mean ± SE.

Means bearing the small and capital of a letter superscript differ significantly in a row.

Superscript: a,b,g,h,i,k,l,m,o,p,q,r are significant at P < 0.01.

Superscript: c,d,e,f,j,n,s,t are significant at P < 0.05.

high concentration of progesterone than repeat breeder on day 7 (early luteal phase). Belely (1993) has reported a high value of progesterone in regular breeder buffalo than repeat breeder buffalo on day 8. Maurer and Echternkamp (1985) have reported a low serum progesterone concentration in regular breeder cows on day 6, post insemination than pregnant cows. Extended low progesterone concentration during early luteal phase has been reported in 2% of repeat breeding cows by Bulman and Lamming (1978). On the contrary, Linares *et al.* (1982) found no difference in progesterone concentration between regular and repeat breeding cows during the first 7 days of estrous cycle. Table also shows the significance level between repeat breeder and regular breeder cows and buffaloes.

The levels of serum estrogen in regular breeder cows and repeat breeder cows on D0 were 7.201 ± 1.29 and 6.212 ± 0.216 pg/ml respectively. On D7 the values were 2.99 ± 0.05 and 2.104 ± 0.012 in regular breeder and repeat breeder cow, respectively. The serum estrogen levels in regular breeder cows and buffaloes were greater than the value of repeat breeders, which corresponds with the study of Rajasundaram and Rajasekaran (1994). The values on day 7 are lower than day 0 in both regular and repeat breeder cows and buffaloes which correlates the study of Rajasundaram and Rajasekaran (1994). In repeat breeding cows and buffaloes the low estrogen level on Day 0 might be the cause

of delayed ovulation (Dielemann *et al.*, 1986) and also for fertilization failure (Maurer and Echternkamp, 1985).

The table also depicts the thyroid status of regular and repeat breeder cows and buffaloes. The present observation was in agreement with the findings of Dutta *et al.* (1991). The mean serum T3 and T4 level in repeat breeder cows were 144.35 ± 15.02, 7.3934 ± 0.56, respectively on day 0 and 167.44 ± 15.04, 7.86 ± 0.70, respectively which was significantly lower than the regular breeder cows, which was in agreement with the findings of Bondi (1987). In buffaloes the values of T3 were 126.253 ± 12.14 and 65.31 ± 5.7 of regular breeder and repeat breeder, respectively on day 0. In day 7 the values of T3 were 88.23 ± 9.61 and 73.86 ± 3.91 of regular breeder and repeat breeder respectively.

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