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Hormonal profile of repeat breeding buffalo in relation to different phases of oestrus cycle

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Abstract
An experiment was conducted to assess the hormonal profile in normal and repeat breeding buffaloes. Eight clinically healthy normal breeding and eight repeat breeding buffaloes presented to the Veterinary Dispensaries in and around Bidar were selected for the study. Hormonal parameters viz., estrogen, progesterone, tri-iodothyronine (T_3), thyroxin (T_4) were estimated during different phases viz. 0 day (day of oestrus), 11th day (mid luteal phase) and 16th day (early follicular phase) of the oestrus cycle. On the day of oestrus higher progesterone, in mid luteal phase higher estrogen and in early follicular phase lower progesterone was observed in repeat breeders. T_3 and T_4 levels were significantly lower on the day of oestrus in repeat breeders. It may be concluded that the altered hormonal profile may predispose the buffaloes to repeat breeding.

Keywords: Repeat breeder, buffalo, oestrus cycle, oestrogen, progesterone, thyroid hormones

Introduction
The major infertility or sub-fertility causing conditions in buffalo are anoestrus and repeat breeding syndrome (RBS). Sub-fertility is matter of actual concern than infertility and one of the most important and commonly encountered sub-fertility is repeat breeding syndrome. The repeat breeding syndrome is defined as a condition in which dairy animal have regular oestrus cycle and appear normal on superficial clinical examination but fails to become pregnant following three or more breeding (Bartlett *et al*., 1986) [4]. Incidence varies from as low as 0.61 percent (Pandit, 2004) [13] to as high as 55.7 percent (Singh *et al*., 1984) [17]. The different phases of oestrus cycle are regulated by intricate, sequential interplay of different hormones, a suitable hormonal milieu is necessary for ovulation and conception to occur. The concentration of sex steroids like estrogen and progesterone are maintained in balanced way depending on the need of the particular stages of the oestrus cycle and any abnormal variations in the concentration and pattern of secretion can lead to RBS. Metabolic hormones mainly thyroid hormones (T_3 and T_4) concentration in the blood is also another factor that determine the reproductive performance of buffalo. Hence, to improve fertility in repeat breeding buffaloes demands a extensive study on hormonal parameters.

Materials and Methods
Blood samples for hormonal profile estimation were collected from jugular vein on day 0 (day of estrus), 11th day (mid luteal phase) and 16th day (Early follicular phase) of the oestrus cycle from normal breeding as well as repeat breeding buffaloes. Total of 10ml blood was collected and stored in vials coated with clot-activator and serum was separated after one hour and stored at -20 °C until analysis of hormonal profile. The hormonal parameters viz., estrogen, progesterone, tri-iodothyronine (T3) and thyroxin (T4) were estimated by radio immune assay (RIA) technique by using a multi well gamma counter which was calibrated for I125 using RIA kits (Beckman Coulter). The data obtained from the present study was tabulated, and subjected to statistical analysis using one way ANOVA and Student 't' test as per the standard procedure described by (Snedecor and Cochran, 1994) [18].

Result and Discussions
Estrogen
The estrogen concentration (pg/ml) in normal breeding and repeat breeding groups on 0,11th and 16th day of the oestrus cycle were 11.55±0.46, 7.59±0.44, 6.77±0.33 and 10.85±0.49,

9.13±0.51, 7.39±0.34 respectively. Estrogen concentration varied significantly ($p \leq 0.05$) on 0, 11th and 16th day of the oestrus cycle in both groups (Table 1). However, the trend in the estrogen values during entire oestrus cycle was similar in both normal and repeat breeding groups, being highest on the day of estrus, declined in the luteal phase reaching lowest value in follicular phase. When the means of two groups were compared, there was no significant ($p \leq 0.05$) difference in estrogen concentration between normal and repeat breeding buffaloes during oestrus phase and early follicular phase, where as in mid luteal phase, repeat breeding buffaloes had significantly ($p \leq 0.05$) higher concentration of estrogen as compared to normal breeding group. Different workers have reported a wide range of estrogen pattern while comparing normal and repeat breeding animals (Shukla *et al*., 2000; Selvaraju *et al*., 2008 and Barui *et al*., 2015) [16, 14, 05]. The wide variations in the estrogen pattern could be attributed to the fact that the initial steps in reproduction like ovulation, fertilization and implantation of embryo are highly coordinated physiological processes involving interplay of hypothalamic, pituitary and gonadal hormones in relation to time, quantity and pattern of their release. Therefore the estrogen levels are influenced not only by the ovarian follicular activity but also by the feedback mechanisms involved at local, hypothalamic and pituitary level. (Selvaraju *et al*., 2008) [14] have reported that the lower concentrations of estrogen on day 10 of the preceding cycle and during early luteal phase determined conception in repeat breeder cows. (Barui *et al*., 2015) [5] reported that the low level of cholesterol affects the steroidogenesis in the ovaries.

Progesterone

Repeat breeding buffaloes had significantly ($p \leq 0.05$) higher concentration of progesterone on the day of estrus and significantly ($p \leq 0.05$) lower concentration in early follicular phase than the normal breeders, where as no significant variations were observed during mid luteal phase. Progesterone concentration varied significantly ($p \leq 0.05$) within the group on different days of oestrus cycle. Highest progesterone concentration was observed on day 16th and lowest on day 0 of oestrus cycle in both groups (Table 1). Normal early embryonic development and pregnancy were found to be closely associated with progesterone activity initiated from oestrus phase up to early follicular phase and its observed that deficiency or asynchrony in the pattern of secretion of progesterone along with other hormones during this critical phase might cause repeat breeding. Higher progesterone level at the time of oestrus might affect sperm and ovum transport as well as the fertilization process and subsequent embryo passage to the uterus (De Silva *et al*., 1981) [7]. (Anderson and Day, 1994) [4] opined that increased progesterone level at oestrus phase block the LH release and affected oocyte maturation and ovulation. (Duchens *et al*., 1995) [8] reported that suprabasal progesterone level delayed the ovulation and lead to retention of graffian follicle for an extended period in repeat breeders. In agreement to the present findings, (Bage *et al*., 2002) [3] have observed that the repeat breeding heifers have a strong tendency for peri-ovulatory suprabasal progesterone levels. (Campanile *et al*., 2005) [6] reported that embryonic mortality in buffaloes are primarily due to reduced secretion of progesterone by the corpus luteum linked with a reduced capacity of the developing embryo to secrete IFNt interferon at threshold amounts which is necessary to prevent luteolysis. (El-Khadrawy *et al*., 2011) [09] positively correlated the maternal progesterone concentrations with interferon-τ production by the conceptus. As Interferon-τ is responsible for maternal recognition of pregnancy, low level of interferon-τ due to lower progesterone concentration may result in the development of a stronger luteolytic signal thereby causing early embryonic death and reduction in conception rate in repeat breeders. Optimum ratio of estrogen to progesterone could also be a factor for successful fertilization and subsequent conception.

Thyroid Hormones

Tri-iodothyronine and thyroxine levels were significantly ($p \leq 0.05$) lower on the day of oestrus in repeat breeding buffaloes as compared to normal breeder, but no such significant ($p \leq 0.05$) variations were observed between two groups during mid luteal and early follicular phase (Table 2). It is proposed that hypothyroid condition reduces the responsiveness of ovaries to the gonadotropins (Mudgal,1992) [12]. Even for the expression of estrus normal levels of thyroid hormones are essential, so the reduced thyroid hormones can depress the reproductive performance. Therefore the reduced levels of thyroid hormones during the oestrus phase could be one among the key factors responsible for predisposing the buffaloes to repeat breeding condition. In contrast to the present study, (Sharma *et al*., 1999; Agarwal and Sharma, 2002) [15, 01] reported no significant difference in the thyroid hormones between normal and repeat breeding buffaloes. When the data was analyzed within the group, the concentration of T_3 and T_4 were significantly ($p \leq 0.05$) higher on 0 day as compared to 11th and 16th day of oestrus cycle in normal breeder, whereas in repeat breeding buffaloes, only T_3 differed significantly among different phases of oestrus cycle. Similar to the present findings, (Mahendran *et al*.,2001) [11] have also reported that repeat breeding buffaloes had lower T_3 and T_4 values as compared to normal breeder buffaloes on 0 day of oestrus cycle as compared to other phases. In contrast, (Jindal *et al*., 2004) [10] have observed lowered plasma T_3 concentrations in repeat breeders as compared to normal cycling buffalo cows during all the four phases of the oestrus cycle.

Table 1: Serum concentrations (Mean± SE) of estrogen and progesterone at different phases of oestrus cycle in normal and repeat breeding buffaloes

Day of oestrus cycle	Estrogen (pg/ml)		Progesterone (ng/ml)	
	Normal breeder	Repeat breeder	Normal breeder	Repeat breeder
0 day	11.55±0.46^{a}	10.85±0.49^{a}	0.42±0.03^{a}	0.53±0.06^{a*}
11th day	7.59±0.44^{b}	9.13±0.51^{b*}	2.11±0.11^{b}	1.95±0.05^{b}
16th day	6.77±0.33^{b}	7.39±0.34^{c}	3.42±0.13^{c}	2.69±0.11^{c*}

* Significant difference ($p \leq 0.05$) between groups for each parameter on respective day of oestrus cycle.

$^{a, b, c}$ Means with different superscript in each column differ significantly ($p \leq 0.05$) within the group on different days of oestrus cycle

Table 2: Serum concentrations (Mean± SE) of tri-iodothyronine (T_3) and thyroxine (T_4) at different phases of oestrus cycle in normal and repeat breeding buffaloes

Day of oestrus cycle	Tri-iodothyronine (ng/ml)		Thyroxine (ng/ml)	
	Normal breeder	Repeat breeder	Normal breeder	Repeat breeder
0 day	1.71±0.06[a]	1.44±0.02[a]	47.68±1.35[a]	40.93±1.87*
11th day	1.33±0.09[b]	1.16±0.09[b]	42.53±1.25[b]	39.71±1.10
16th day	1.20±0.07[b]	1.14±0.07[b]	41.03±0.87[b]	38.98±0.82

* Significant difference ($p \leq 0.05$) between groups for each parameter on respective day of oestrus cycle. [a, b, c] Means with different superscript in each column differ significantly ($p \leq 0.05$) within the group on different days of oestrus cycle.

Conclusion

It may be consummated that the altered hormonal profile of repeat breeding buffalo as found in this study viz. on the day of oestrus higher progesterone, in mid luteal phase higher estrogen, in early follicular phase lower progesterone and significantly lower T_3 and T_4 levels on the day of oestrus might have prompted the buffalo to repeat breeding.

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