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## Postpartum reproductive health monitoring via hormonal and uterine involution investigations in Mehsana buffalo

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### Abstract

This study was undertaken on 26 Mehsana buffaloes maintained at Livestock Research Station, S.D.A.U., Sardarkrushinagar, Gujarat (India). These buffaloes were monitored for their post-partum reproductive performance through per-rectal examination and pregnancy was confirmed at day 60 after natural service. Buffaloes were grouped retrospectively on the basis of fertile estrus in to, Group I (Buffaloes conceiving before 90 days) and Group II (Buffaloes conceiving after 90 days). Blood samples were collected on the day of calving, 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day postpartum. The mean plasma progesterone (ng/ml) ranged between 0.18±0.01 to 0.31±0.02 in Group I and 0.24±0.01 to 0.48±0.02 in Group II with significantly ( $p<0.05$ ) lower P4 in Group I buffaloes. The mean plasma tri-iodothyronine (ng/ml) and thyroxine (nmol/L) ranged between 1.23±0.55 to 2.30±0.68 and 42.67±3.64 to 57.92±4.43 in Group I, 1.02±0.15 to 1.74±0.15 and 40.48±2.40 to 44.07±2.69 in Group II, respectively and were found to be significantly ( $p<0.05$ ) higher at day 20 in group I buffaloes. The gross uterine involution occurred at 28.20±1.10 and 29.80±0.20 days in group-I and group-II, respectively. The average days for the gross uterine involution did not differed significantly between the groups.

**Keywords:** Mehsana buffaloes, progesterone (P4), tri-iodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>), uterine involution

### Introduction

The Buffaloes are used as a source of milk and meat largely in tropical and sub-tropical countries and occupy an important place in the agricultural economy of India. Reproductive performance of dairy cows after the voluntary wait period is highly related to the health status of the uterus after calving (Ferguson *et al.*, 2000) [5]. Postpartum uterine infection has a negative effect on reproductive performance as it increases the calving to first service interval and decreases the conception rate (Fourichon *et al.*, 2000) [6]. The changes in blood constituents can reflect the physiological condition as well as nutritional and health status of cows. Appropriate thyroid gland function and activity of thyroid hormones (TH) are considered crucial to sustain the productive performance in domestic animals and circulating TH can be considered as indicators of the metabolic and nutritional status of the animals (Todini *et al.*, 2007) [18]. Following parturition, Involution of the uterus to its normal non-pregnant size and physiological function is a normal phenomenon in mammals. Several investigators have studied the phenomenon of uterine involution in post-partum buffaloes. Some investigators reported shorter periods (< 35 days) required for completion of involutona and others reported longer periods (> 40 days) in the buffaloes (Nakhshi, 2006; Basem *et al.*, 2017; Vala *et al.*, 2018) [12, 2, 19]. From economical point of view these changes should occur earlier after calving, because of any delay in the completion of uterine involution, re-establishment of ovarian and estrous activities decreases the reproductive efficiency of animal by prolonging subsequent calving interval. Therefore, keeping these facts in view, the study on hormonal profile of Tri-iodothyronine (T<sub>3</sub>), Thyroxine (T<sub>4</sub>), Progesterone (P4) and gross uterine involution during postpartum period in Mehsana buffaloes conducted.

## Materials and Methods

Livestock Research Station, SDAU. Sardarkrushinagar is located in north Gujarat. The average minimum and maximum ambient temperature and relative humidity of the zone ranged from 17.95 °C and 32.25 °C and 48.5% and 73% respectively, during the period of study. All the buffaloes were maintained in well ventilated hygienic sheds and were fed green fodder, hay and compounded concentrate, as per standard feeding schedule followed on the farm. These buffaloes had free access to drinking water. Every Mehsana buffalo was vaccinated according to schedule and had frequent deworming treatments with broad spectrum anthelmintic medications before and after the monsoon. A total of 26 advanced pregnant Mehsana buffaloes irrespective of parity and body weight with a history of normal calving were selected for the study. Blood samples were collected from all the buffaloes on the day of calving, 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day postpartum by puncturing external jugular vein in heparinized vials, centrifuged at 3000 rpm for 10 minutes. The plasma samples were stored at -20 °C till analyzed for hormonal assay. The progesterone in blood plasma was estimated by using commercially available enzyme linked immune-sorbent assay (ELISA) kits manufactured by DBC diagnostics Biochem Canada Inc, Canada. The Tri-iodothyronine (T<sub>3</sub>) and Thyroxine (T<sub>4</sub>) in blood plasma was estimated by using commercially available enzyme linked immune-sorbent assay (ELISA) kits manufactured by Labor Diagnostics Nord GmbH & Co. KG, Germany. The progress of gross uterine involution was felt through per-rectal examination. Uterus and cervix were considered completely involuted when they returned to their normal size, tone and location in pelvic cavity. Measurements were taken by using palm and a centimeter scale following transrectal palpation of the reproductive tract and the findings were recorded as

diameter of cervix (cm), gravid horn (cm) and non-gravid horn (cm). Buffaloes were grouped retrospectively on the basis of first fertile estrous as Buffaloes which conceived before 90 days post-partum (Group-I) Buffaloes which conceived after 90 days post-partum (Group-II). The data obtained from research work were analyzed statistically using 't-test' as per standard statistical procedure. (Snedecor and Cochran., 1994) [16].

## Hormonal Profile

### Progesterone

The mean plasma progesterone (ng/ml) level ranged between 0.18±0.01 to 0.31±0.02 in Group I and 0.24±0.01 to 0.48±0.02 in Group II. The level was found to be significantly ( $p<0.05$ ) lower at 20<sup>th</sup> day postpartum within the groups as compared to that of the other days except with a non-significant difference between 20<sup>th</sup> day and on day of calving in group I buffaloes. It indicated that the plasma P4 concentration remained low (less than 1 ng/ml) up to day 30<sup>th</sup> post-partum in both the group. Similarly a very low or negligible level of progesterone have been reported during postpartum period in buffaloes (Patil *et al.* (2013) [13].

The statistical analysis showed significant ( $p<0.05$ ) lower progesterone levels in Group I as compared to Group II at all the days of postpartum. These findings nearly agreed with the Dhami *et al.* (2012) [3] who reported that value of the control group buffaloes was non-significantly lower than that of the retained fetal membrane groups. Whereas, Dolatkah *et al.* (2013) [4] reported that the P4 concentration had no significant differences between healthy and endometritic postpartum dairy cows. Lower P4 levels observed in the group I may be due to earlier post-partum period (up to 30<sup>th</sup> day) of the study which gets the support of Kalasariya *et al.* (2017) [9] in buffaloes.

**Table 1:** Progesterone (ng/ml, Mean ± SE) at different days in different groups of postpartum Mehsana buffaloes

Days of Postpartum	Groups		Overall mean
	G1 (n=13)	G2 (n=13)	
Day of calving	0.20±0.01 <sup>a</sup> <sub>p</sub>	0.30±0.04 <sup>b</sup> <sub>p</sub>	0.25±0.02
10 <sup>th</sup>	0.31±0.02 <sup>b</sup> <sub>q</sub>	0.45±0.02 <sup>a</sup> <sub>q</sub>	0.37±0.02
20 <sup>th</sup>	0.18±0.01 <sup>a</sup> <sub>p</sub>	0.24±0.01 <sup>b</sup> <sub>r</sub>	0.21±0.01
30 <sup>th</sup>	0.29±0.02 <sup>b</sup> <sub>q</sub>	0.48±0.02 <sup>a</sup> <sub>q</sub>	0.38±0.01

Means with different superscripts within rows & subscripts within columns differ significantly at 5 percent level

### Tri-iodothyronine (T<sub>3</sub>)

The mean plasma tri-iodothyronine (ng/ml) level ranged between 1.23±0.55 to 2.30±0.68 in Group I and 1.02±0.15 to 1.74±0.15 in Group II with an increasing trend from day of calving to 20<sup>th</sup> day postpartum. These findings are in agreement with the reports of Stojic *et al.* (2001) [17] in Murrah buffalo. The T<sub>3</sub> levels were significantly ( $p<0.05$ ) lower at day 20<sup>th</sup> in Group II as compared to Group I buffaloes which is in agreement with the lower concentrations of T<sub>3</sub> in non-conceiving as compared conceiving buffaloes (Ghuman *et al.*, 2011) [7], lower reproductive performance with decrease in T<sub>3</sub> in postpartum in lactating cows (Nahed Saleh *et al.*, 2011) [11]. Variations in the T<sub>3</sub> levels have been attributed to food, environment and especially temperature influence, significant breed differences for thyroid status, Lower T<sub>3</sub> due to negative energy balance and due to high metabolic demands during peak milk production in buffaloes.

### Thyroxine (T<sub>4</sub>)

The mean plasma thyroxine (nmol/L) level ranged between 42.67±3.64 to 57.92±4.43 in Group I and 40.48±2.40 to

44.07±2.69 in Group II with an increasing trend from day of calving to 20<sup>th</sup> day postpartum. Similar to the present study, range of T<sub>4</sub> have been observed in dairy cows at parturition and five days after parturition (Kasagic *et al.*, 2011) [10]. The T<sub>4</sub> levels were significantly ( $p<0.05$ ) lower at day 20 in group II as compared to Group I buffaloes which is in accordance with, lower concentrations of T<sub>4</sub> in non-conceiving as compared conceiving buffaloes (Ghuman *et al.*, 2011) [7], lower reproductive performance with decrease in T<sub>4</sub> in postpartum lactating cows (Nahed Saleh *et al.*, 2011) [11] and non-significant differences between cyclic and acyclic Sahiwal cow at 50 and 60 days postpartum (Sarfraj Ali *et al.*, 2012) [14]. Variations in the T<sub>4</sub> levels have been attributed to food, environment and especially temperature influence, significant breed differences in thyroid status in cows, Lower T<sub>4</sub> due to negative energy balance and due to high metabolic demands during peak milk production in cows.

### Gross Uterine Involution

**Per-rectal Observations:** Per-rectally, the reduction in diameter of uterine horn and cervix, location of genitalia and

gradual increase in tonicity of genitalia was considered to know the gross uterine involution. On 15<sup>th</sup> day postpartum, both the uterine horns and cervix were palpated. Out of 26 buffaloes of both the groups, the genitalia of 18 (69.23%) buffaloes were at pelvic floor within the pelvic cavity while eight (30.76%) buffaloes had uterine horns hanging at pelvic brim. However, these eight buffaloes regained intrapelvic position of genitalia on and before 21<sup>st</sup> day postpartum. The increasing perceptibility in terms of tonicity, elasticity and curling of horns was observed from 15<sup>th</sup> to 21<sup>st</sup> day postpartum and it further continued to be better at 30<sup>th</sup> day postpartum progressing towards that of the normal genitalia in both the groups. The mean gross uterine involution was found to be occurred non-significantly at 28.20±1.10 days in group-I and at 29.80±0.20 days in group-II buffaloes (Table 2). These findings are in close agreement with those of Khasatiya (2003) [20] in Surti buffaloes and Nakhshi (2006) [12] in Mehsana buffaloes. The overall mean gross uterine involution was found to occur at 29.00±1.30 days postpartum in the present study which is in close agreement with the findings in buffaloes (Amer *et al.* 2010) [1]. Variations in days of gross uterine involution observed by different workers could be attributed to the breed of animals, effect of suckling or non-suckling, season of calving, nutritional status and endocrine balance.

**Cervical Diameter**

The cervical diameter (cm) was measured to be 6.31±0.06, 4.61±0.11 and 3.66±0.09 in Group I and 7.07±0.14, 5.64±0.23 and 3.77±0.16 in Group II buffaloes at days 15<sup>th</sup>, 21<sup>st</sup> and 30<sup>th</sup> postpartum, respectively. The statistical analysis revealed a significant (*p*<0.05) reducing trend in the diameter of the cervix from 15<sup>th</sup> to 30<sup>th</sup> day postpartum within the groups which agreed well with the reports in buffaloes (Nakhshi, 2006) [12] and in cows (Jadhav, 2005) [8].

**Uterine Horn Diameter (Gravid and Non Gravid)**

The gravid uterine horn diameter (cm) was measured to be 5.19±0.06, 3.79±0.16 and 2.79±0.13 in Group I and 5.73±0.15, 3.97±0.15 and 2.86±0.11 in group II buffaloes at days 15<sup>th</sup>, 21<sup>st</sup> and 30<sup>th</sup> postpartum, respectively. The non-gravid uterine horn diameter (cm) was measured to be 4.36±0.06, 3.21±0.06 and 2.58±0.11 in Group I and 4.58±0.13, 3.33±0.09 and 2.71±0.10 in Group II buffaloes on 15<sup>th</sup>, 21<sup>st</sup> and 30<sup>th</sup> day postpartum, respectively. A significant (*p*<0.05) reducing trend in the diameter of the gravid and non-gravid uterine horn from 15<sup>th</sup> to 30 day postpartum within the groups corroborates well with the reports in buffaloes (Nakhshi, 2006) [12] and in dairy cows Saut *et al.*, (2011) [15].

**Table 2:** Thyroid hormone level (Mean ± SE) at different days in different groups of postpartum Mehsana buffaloes

Thyroid hormones	Days of Postpartum	Groups		Overall mean
		G1 (n=13)	G2 (n=13)	
Tri-iodothyronine (T <sub>3</sub> ) (ng/ml)	Day of calving	1.23±0.55 <sup>a</sup> <sub>p</sub>	1.02±0.15 <sup>a</sup> <sub>p</sub>	1.12±0.35
	10 <sup>th</sup>	1.80±0.50 <sup>b</sup> <sub>q</sub>	1.45±0.09 <sup>b</sup> <sub>q</sub>	1.62±0.29
	20 <sup>th</sup>	2.30±0.68 <sup>a</sup> <sub>r</sub>	1.74±0.15 <sup>r</sup>	2.02±0.41
	30 <sup>th</sup>	1.64±0.43 <sup>c</sup> <sub>q</sub>	1.47±0.09 <sup>c</sup> <sub>qr</sub>	1.56±0.52
Thyroxine (T <sub>4</sub> ) (nmol/l)	Day of calving	42.67±3.64 <sup>a</sup> <sub>p</sub>	40.48±2.40 <sup>a</sup> <sub>p</sub>	41.57±3.02
	10 <sup>th</sup>	45.05±2.02 <sup>b</sup> <sub>p</sub>	42.87±4.18 <sup>b</sup> <sub>p</sub>	43.96±3.10
	20 <sup>th</sup>	57.92±4.43 <sup>a</sup> <sub>q</sub>	44.07±2.69 <sup>b</sup> <sub>p</sub>	50.99±3.56
	30 <sup>th</sup>	46.22±3.54 <sup>c</sup> <sub>pq</sub>	43.62±4.14 <sup>c</sup> <sub>p</sub>	44.92±3.84

Means with different superscripts within rows & subscripts within columns differ significantly at 5 percent level for each hormones

**Table 3:** Comparative diameter (cm, Mean ± SE) of cervical and uterine horn at different days in different groups of postpartum Mehsana buffaloes

Particulars	Days of postpartum	Groups		Overall mean
		G1 (n=13)	G2 (n=13)	
Cervical diameter (cm)	15 <sup>th</sup>	6.31±0.06 <sup>a</sup> <sub>p</sub>	7.07±0.14 <sup>b</sup> <sub>p</sub>	6.69±0.10
	21 <sup>st</sup>	4.61±0.11 <sup>b</sup> <sub>q</sub>	5.64±0.23 <sup>a</sup> <sub>q</sub>	5.12±0.17
	30 <sup>th</sup>	3.66±0.09 <sup>c</sup> <sub>r</sub>	3.77±0.16 <sup>c</sup> <sub>r</sub>	3.71±0.13
Gravid uterine horn(cm)	15 <sup>th</sup>	5.19±0.06 <sup>b</sup> <sub>p</sub>	5.73±0.15 <sup>a</sup> <sub>p</sub>	5.47±0.10
	21 <sup>st</sup>	3.79±0.16 <sup>c</sup> <sub>q</sub>	3.97±0.15 <sup>c</sup> <sub>q</sub>	3.87±0.15
	30 <sup>th</sup>	2.79±0.13 <sup>d</sup> <sub>r</sub>	2.86±0.11 <sup>d</sup> <sub>r</sub>	2.81±0.12
Non-gravid uterine horn(cm)	15 <sup>th</sup>	4.36±0.06 <sup>a</sup> <sub>p</sub>	4.58±0.13 <sup>a</sup> <sub>p</sub>	4.46±0.09
	21 <sup>st</sup>	3.21±0.06 <sup>b</sup> <sub>q</sub>	3.33±0.09 <sup>b</sup> <sub>q</sub>	3.28±0.07
	30 <sup>th</sup>	2.58±0.11 <sup>c</sup> <sub>r</sub>	2.71±0.10 <sup>c</sup> <sub>r</sub>	2.64±0.10
Gross uterine involution (Days)		28.20±1.10 <sup>a</sup>	29.80±0.20 <sup>a</sup>	29.00±1.30

Means with different superscripts within rows & subscripts within columns differ significantly at 5 percent level for each particulars

**Conclusion**

The study concluded that the statistical analysis showed significant (*p*<0.05) lower progesterone levels in Group I as compared to Group II at all the days of postpartum. Tri-iodothyronine (ng/ml) and Thyroxine (nmol/L) were lower in these buffaloes up to 30 days, with a significantly lower level at the 20<sup>th</sup> day postpartum. It may be due to earlier postpartum period (up to 30<sup>th</sup> day) of the study and the overall mean gross uterine involution was found to occur at

29.00±1.30 days postpartum in both groups, with a significant reducing trend in the diameter of the cervix and uterine horn.

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