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Exploring the Role of Iodine and Thyroid Hormone in Abnormal Metestrus Bleeding Associated Repeat Breeding and Silent Estrus in Cows of the North Eastern State of Assam in India

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ABSTRACT

Background: The present study documents the association of repeat breeding and silent estrus in cows with iodine deficiency, hypothyroidism and abnormal metestrus bleeding in high-producing Jersey and Holstein Friesian crossbred cows of Assam in India.

Methods: The field based study was conducted involving 470 Holstein Friesian and Jersey crossbred cows in Assam of North East India during the period August, 2021 to July, 2022. Out of 470 crossbred cows investigated, 62 (13.00%) with repeat breeding and abnormal metestrus bleeding (Group-A), 18 (3.83%) were diagnosed as silent estrus (group-B) and 6 with normal breeding history (Group-C) without the signs of metestrus bleeding were randomly selected for the study. The cows belonging to all the 3 groups were subjected to serum T_3, T_4 and iodine estimation.

Result: 42 (67.74%) crossbred cows in group A (n=62) and 13 (72.22%) in group B (n=18) were diagnosed to have hypothyroidism including Iodine deficiency. The overall incidence of hypothyroidism out of 470 cows was 11.70%. Group C showed significantly higher T_3 and T_4 levels than groups A and B. Thus, it concluded that iodine deficiency -induced hypothyroid condition might be attributed to the causes of abnormal metestrus bleeding associated with repeat breeding and silent estrus in high-producing crossbred cows of Assam in India.

Key words: Abnormal metestrus bleeding, Cows, Hypothyroid, Infertility, Iodine deficiency.

INTRODUCTION

Metestrus bleeding or metrorrhagia is observed in about 48% of cows and 75% of heifers 24 to 48 hours after the end of estrus and is said to be of physiological origin (Roberts, 1986). During estrus, the caruncular capillaries become hyperaemic and get congested under the influence of high estrogen levels and at an early part of the metestrus, on withdrawal of estrogen the capillaries break and metrorrhagia occurs (Larsen, 1986). Although the condition is regarded as normal, there are reports which suggest an association of metestrus bleeding with repeat breeding conditions in cows (Kishore *et al*., 2008). Human gynaecology has long been using the term Abnormal Uterine Bleeding to denote any type of bleeding that does not fall within the normal range of amount, frequency, duration and cyclicity (Byna *et al*., 2015), without any clinically detectable organic pelvic pathology *viz*: tumour, inflammation or pregnancy. According to Byna *et al*. (2015) hypothyroidism is responsible for causing metrorrhagia in men and animals. Moreover, hypothyroidism has been attributed as one of the etiologies for silent estrus in cattle (Spielman *et al*., 1945). Hypothyroidism in women and animals is associated with various reproductive disorders, such as the delayed onset of puberty (Silva *et al*., 2012), anovulation, ovarian cysts and menstrual/estrual irregularity (Peter *et al*., 1989). In animals, hypothyroidism occurs mostly due to iodine deficiency rather than thyroid-gland diseases (Singh and Beigh, 2013). Generally, the soil of high rainfall and hilly areas is deficient in many micro minerals including Iodine. So, naturally under the influence of the soil-plant-animal chain the cattle also suffer from Iodine deficiency. The North Eastern region of India is identified as an iodine-deficient area (Bora and Baruah, 2017). Hence, it can be hypothesized that the cows from this zone of the country might have also been suffering from iodine deficiency leading to hypothyroidism, abnormal metestrual bleeding, repeat breeding, silent estrus, *etc*. Recently, we observed that the crossbred cows of Assam with abnormal metestrus bleeding have an association with repeat breeding syndrome. Here the volume of blood discharged was typically more (20-100 ml) and release lasted for a longer duration (48 to 196 hours)

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following estrus. This was further related to conception failure. As there were no preliminary data and studies related to abnormal metestrus bleeding and its association with the iodine status in the said area, the present study was intended to evaluate the association of thyroid dysfunction with repeat breeding syndrome and abnormal metestrus bleeding and silent estrus in crossbred cows in Assam. The aim was to bring to record the updated line of treatment for bovines suffering from repeat breeding/silent heat associated with abnormal metestrus bleeding in the region.

Therefore, the present study aimed to evaluate the association of thyroid dysfunction with repeat breeding and abnormal metestrus bleeding and silent estrus in crossbred cows in Assam.

MATERIALS AND METHODS

Experimental animals

The present study was a field-based study conducted involving 470 Holstein Friesian and Jersey crossbred cows belonging to 52 households of 5 districts in Assam of North East India during the period August, 2021 to July, 2022. A total of 62 crossbred cows having symptoms of abnormal metestrus bleeding (Group-A), 18 crossbred cows with silent estrus (Group-B) and 6 crossbred cows with normal breeding history (Group-C) without the signs of metestrus bleeding were randomly selected for the study. The cows were within 4-8 years of age and produced 10-20 liters of milk per day. All the cows in groups A, B and C were subjected to serum T_3, T_4 and iodine estimation.

The blood discharged by the cows from 48 hours to 3-5 days post estrus relatively in a higher volume of 50-100 ml or even more was considered Abnormal Metestrus Bleeding and the cows those were with active corpus luteum without the visible signs of estrus were considered as silent estrus under this study. The blood samples were collected from each of the cows irrespective of the stages of the estrous cycle. Serum was separated and stored at -20°C.

The thyroxine and triiodothyronine in serum were estimated in duplicate with the help of Radio Immuno Assay using commercial RIA kit ^{125}I labeled-T_4tracerRIA (REF 1447-IM 3286) and 1251-T_3 RIA (REF 1699, IM3287) kits provided by the IMMUNOTECH s.r.o.-Radiova 1-102 27 Prague 10-Czech Republic.

The serum iodine was estimated as per the method described(Bird and Jackson, 1962). For this 0.2 ml of serum or standard iodine solution or distilled water was taken in test tube followed by addition of 2 ml of chloric acid (125 g potassium chlorate, 225 ml distilled water and 9 ml 70% perchloric acid) and 0.1 ml of 0.4% sodium chromate solution. The test tubes were placed in sand bath at 140-150°C for 1 hour. Thereafter 2 drops of chloric acid were added to each tube during 20, 30, 40, 50 and 55 minutes while on the sand bath. After one hour the tubes were allowed to cool to room temperature and observed for red coloured crystals. Then 10 ml of solution containing 1% NaCl in 1 N H_2SO_4 and 2 ml of arsenic acid solution (0.9% Arsenic (III) oxide and 0.7% NaOH, pH 7.0) was added to the test tube. The absorbance of the solution was recorded at 415 nm immediately after addition of 2% ceric sulphate solution in $1NH_2SO_4$.

Statistical analysis

For statistical analysis of data, one-way ANOVA was performed with SPSS statistics for Windows Version 28.0, 2021 software and differences between the means were compared by Post Hoc Duncan LSD test and the $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

A total of 62 (13.00%) cows were detected with abnormal metestrus bleeding (Fig 1) associated with the problem of repeat breeding and 18 (3.83%) with silent estrus out of a total of 470 crossbred cows investigated. Out of 62 cows with abnormal metestrus bleeding 42 (67.74%) and out of 18 silent estrus cows 13 (72.22%) were diagnosed as having hypothyroidism including Iodine deficiency, respectively. Based on the total number of cows surveyed (n=470) the overall incidence of hypothyroidism in cows was 11.70% (55 numbers). The mean±SE values of serum T_3, T_4 and Iodine levels are presented in Table 1 and Fig 2. The group C showed significantly higher T_3 and T_4 levels than groups A and B. There was no significant difference between group A and B in the case of serum T_3 levels however, the group B had a significantly higher T_4 level than group A. Depicting hypothyroidism the serum T_4 (nmol/L) in Group A and B differed significantly ($p<0.05$) from the value of normal breeding cows in Group-C (97.013±7.929).

In the recent years, there is substantial increase in bovine infertility especially the repeat breeding and silent estrous with abnormal amount of metestrous bleeding in high producing cows of Northeastern India. Our study hypothesized that the Iodine deficiency origin hypothyroidism in affected cattle resulting in abnormal metestrus bleeding with repeat breeding and silent estrus. The critical difference test revealed a significant ($p<0.05$) difference between T_4 values in Group A and B. Under the present study the serum T_3 and T_4 levels in both abnormal metestrus bleeding and silent estrus groups of cows were significantly lower than that in normal breeding group, but the T_4 level was only found to be substantially lower than the physiological range (54.0-110.7 nmol/L) in cattle, whereas T_3 value was within the physiological range (0.63-2.61 nmol/L, Capen and Martin, 2003). The estimated T_3 and T_4 values were in accordance with the finding of Tadayonfar and Noaman, (2013). Moreover, the values of T_4 (90.361 nmol/L) and T_3 (2.28 nmol/L) of healthy cows were in conformity with the present finding in respective group of cows. The cows detected with significantly ($p<0.001$) lower concentration of serum T_4 and iodine in the present study were considered hypothyroid despite having normal T_3 values because it was stated that in moderate to severe serum iodine deficiency, the T_4 level always remains lower but T_3 level is either normal or even higher as a protective

mechanism (Silva *et al*., 1978). Goff, (2015) also mentioned the similar pattern of T_4 and T_3 levels in serum of cows suffering from Iodine deficiency. According to our apprehension significantly ($p<0.001$) lower serum iodine (µg/L) was found in the cows of Group A (30.944±1.998) and Group B (38.844±3.675) as compared to the value in Group C (86.068±4.407) and the critical level of serum Iodine in cattle is 50 µg/L (Radostits *et al*., 2007). However, a nonsignificant difference existed between the serum iodine level of Group A and B. In consonant with the present finding, Tadayonfar and Noaman, (2013) also recorded 38.80±1.52 µg/l of serum iodine in hypothyroid cows. It was stated that the animals and man living in more distance from costal region are most likely to suffer from iodine deficiency because the soil (0.8-150 µg/g) plant and water within the area of 50 km from the sea contain high level of Iodine as



Fig 1: Abnormal metestrus bleeding/discharge noticed 24-48 h after estrus in crossbred cows suffering from repeat breeding syndrome.

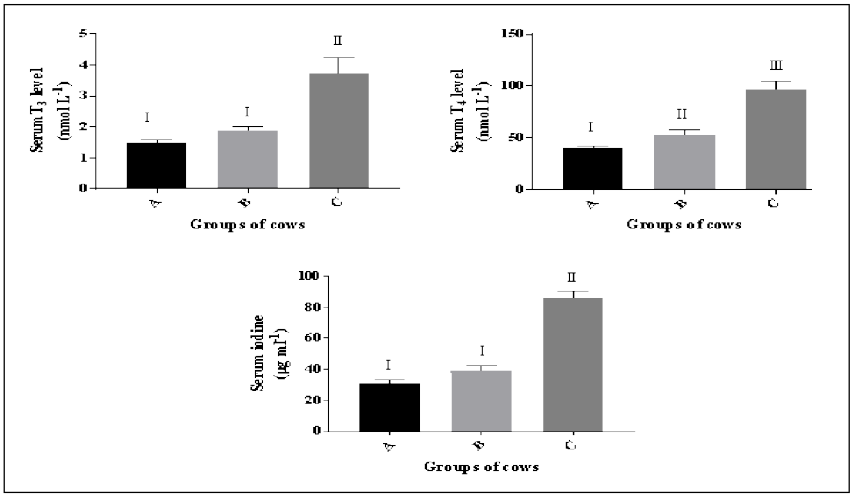

Fig 2: Serum T_3, T_4 and iodine level (bars depicting different Roman letters indicate statistically significant difference ($p<0.05$).

Table 1: Serum T_3 (nmol/L), T_4 (nmol/L) and Iodine (µg/L) levels of crossbred cows.

Animal group	No of Hypo-thyroid cow	No of Iodine deficient cow	T_3 (nmol/L)	T_4 (nmol/L)	Iodine (µg/L)
A (n=62)	42 (67.74%)	42 (67.74%)	1.489±0.101^a	40.719±1.234^a	30.944±1.998^a
B (n=18)	13 (72.22%)	13 (72.22%)	1.893±0.129^a	53.243±4.425^b	38.844±3.675^a
C (n=6)	0 (0.00%)	0 (0.00%)	3.733±0.531^b	97.013±7.929^c	86.068±4.407^b

The values bearing different superscripts bear statistically significant difference ($p<0.05$).

compared to the soil at a distances more than 50 km (0.4-14 µg/g). Hence, Bora and Baruah, (2017) might have got significantly lower Iodine content in soil and crop of Assam. The majority of grass and forages used as fodder usually contains very less amount of iodine (20-50 g/kg dry matter) whereas the recommended level of iodine supplements is 120-250 g/kg dry matter for cattle (Schone and Rajendram, 2009). This may be the reason behind these iodine deficiency and hypothyroidism in crossbred cows of Assam. In high lactating cows these problems may become more severe as considerable amount of thyroid hormone is secreted through milk (Iveta *et al*., 2011). The term Abnormal Metestrus Bleeding used in this report, perhaps, for the first time in veterinary science to denote a pathological condition of cows that conforms with the term "abnormal uterine bleeding" applied in human gynaecology, where the abnormal uterine bleeding is mostly associated with hypothyroidism (Verma *et al*., 2017). Byna *et al*. (2015) stated that hypothyroidism caused E_2: P_4 ratio disturbances which are basically responsible for causing abnormal uterine bleeding in women and animals. Although, (Roberts, 1986) failed to get any relationship between metestrus bleeding with conception but later on he suggested that the thyroid hormones might be involved with certain types of female infertility. Further, he mentioned that during early metestrus the epithelium over the uterine caruncles becomes very hyperemic and as a consequence of estrogen withdrawal some capillary hemorrhage occurs and is called metestrus bleeding. Hence, more amount of bleeding for prolonged duration might be associated with relatively higher estrogen over progesterone concentration. Hypothyroidism stimulates the release of TRH from hypothalamus to work upon the pituitary for TSH secretion. Interestingly, both the thyrotrops and lactotrops contain receptors for TSH (Reimers, 2003). So, the TRH induces hyperprolactinemia in response to increased TSH secretion from the pituitary. This higher prolactin alters the GnRH pulsatile release by preventing kisspeptin secretion (Kokay *et al*., 2011) that leads to defective or delayed LH response causing luteal phase defect and anovulation (Anasti *et al*., 1995), ovarian cysts, menstrual/estrus irregularity in animals and women (Peter *et al*., 1989). This delayed ovulation or anovulation may result in relative estrogen excess for prolong. On the other hand, thyroid hormones affect progesterone production by directly influencing LH receptors in ovaries. Hypothyroidism also alters the peripheral metabolism of estrogens by decreasing Steroid Hormone Binding Globulin (SHBG) production leading to abnormal feedback at pituitary level. Additionally, metabolism of estrogen is altered and peripheral conversion of androgens to estrogens is increased. So, on withdrawal of these relatively excess amounts of estrogens both uterine and vaginal bleeding or menorrhagia or metestrual bleeding occur in hypothyroid conditions (Swarupa *et al*., 2016). Besides the hypothyroidism impair conception by decreasing the level of PGE_2 as well as decreasing the expression of the receptivity factors homeobox A10 and osteopontin (Kowalczyk-Zieba *et al*., 2021). Moreover, the hypothyroidism was also detected in the silent estrus group of examined cows. The etiology of silent estrus in these cows may be attributed to the hypothyroidism as stated by (Spielman *et al*., 1945). Now the reason behind these different manifestations under the same nature of patho-physiology might be under the degree of deficiencies in Iodine and thyroid hormones in these groups of cows or their interactions with other hormones and growth factors influencing the release of GnRH (Silva *et al*., 2012).

CONCLUSION

In the light of the foregoing results, it can be concluded that iodine deficiency and hypothyroid condition might be attributed to the causes of abnormal metestrus bleeding associated with repeat breeding and silent estrus in high-producing crossbred cows of northeastern state of Assam in India. However, elaborate studies on these issues covering more numbers of animals and parameters are required to be conducted to come to a concrete solution to these problems.

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Conflict of interest: None.

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