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Changes of blood biochemical and hormonal profile of female cattle of Mizoram (Zobawng) during oestrous cycle

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Abstract
Zobawng is a rare bovine species and semi-domesticated animal found only in Mizoram. Few studies are available on examining the Zobawng cow's blood biochemicals and the reproductive hormonal status during various stages of the oestrous cycle. Hence, the current study was conducted on ten numbers of healthy female Zobawng cow to determine the changes in serum biochemical and hormonal levels during the oestrous cycle. The oestrus behaviour was initially observed before the blood sampling and the sample was collected on day 0 (the day that the cow exhibited oestrus signs), 4th, 8th, 12th, 16th and 20th. From the blood, serum was separated for biochemical and hormonal profiling as per standard procedures. The results revealed that serum biochemicals, *viz*. glucose, cholesterol, calcium, phosphorus and zinc did not differ significantly during different days of the oestrous cycle. Blood estrogen and progesterone levels were found to differ significantly on different days of the oestrous cycle. This study's findings have provided new perceptions into the blood biochemical and hormonal levels of Zobawng cows during the oestrous cycle and the information may aid in developing a reference range and baseline data for the Zobawng cows for other breeding programs and health monitoring.

Keywords: Blood biochemical, hormonal profile, Zobawng, Mizoram

Introduction
Mizoram, one of India's smallest states and ranked fifth in size, is characterized by its predominantly hilly landscape, with only a few flat regions, encompassing an area of 21,081 square kilometres. Mizoram's climate encompasses a range from moist sub-tropical to moist tropical climates featuring moderately warm summers and mild winters. Winter temperatures typically fluctuate between 11 °C and 24 °C, while summer temperatures range from 18 °C to 29 °C. (Indian state forest report, 2011) [10].

The indigenous cow of Mizoram is known as Zobawng their native name. The meaning of "Zobawng" is the native name where "Zo" means "high land" and "Bawng" means cattle i.e. "cattle of hills" (Rahman and Kalita, 2015) [15]. Zobawng is a rare bovine species and semi-domesticated animal. Zobawng belongs to the phylum Chordata, class- Mammalia, order- Artiodactyla, family – Bovidae, genus Bos, and species- *Bostarus* (hampless). Zobawng are found only in Mizoram. Zobawng is semi-domesticated and ferocious in nature. The size of the body is small. The body colour of the animal is mainly brown, black and grey. Zobawng have a strong and compact body condition and with strong leg (Pundir *et al.*, 2015) [14]. Male animals are much heavier than the female. Cow and bullock are weighed about 169 kg and 200 kg, respectively. The animal is mainly reared for beef purposes and, to some extent for milk and draught purposes. As per the 19th livestock survey, the total cattle population of Mizoram is 34,570. About 67% of the total cattle population accounts for the local cattle Zobawng in Mizoram (Pundir *et al.*, 2015) [14] as we see from the previous census report then we understand that 50% population declined within 28 years. The sharp decline of the local cattle of Mizoram has a negative impact on the socio-economic life of Mizoram people along with an adverse effect on the environment.

A few studies have examined the impact of high altitude stress on Zobawng cattle. For

instance, haematological parameters can be used to assess how well animals have adapted to their environment (Giri *et al*., 2017) [8]. In addition to that, biochemical parameters are responsible for various body functions, and their deficiency results in the impairment of functions and induces structural and physiological abnormalities (Mamun *et al*., 2013) [12]. According to Ahmed *et al.*, (2009) [1], changes in the biochemical and haematological constituents are critical pointers of the animal's physiological or pathological state. Therefore, in high-altitude areas, Zobawng cattle health requires a clinical examination, as well as various biochemical assays and haematology examinations, which aid in the diagnosis or may reveal the type and severity of the disease.

Moreover, hormones regulate homeostasis and enhance essential traits in cattle, including fertility, growth and development, and health (Evans *et al*., 2022) [7]. In this point of view, exploring hepatic metabolism and hypothalamus-pituitary-gonad functioning is crucial for regular physiological and behavioural changes related to oestrous reproduction and is essential for breed improvement (Boro *et al.*, 2022b) [4]. Thus, the present work was conducted to investigate the Zobawng cow's blood biochemical and hormonal profile during the oestrous cycle.

Methods and Materials

The experiment was carried out at the Livestock Farm Complex, College of Veterinary Sciences and A.H., Selesih, Aizawl, Mizoram. The farm is located 965 meters above sea level. A total of 10 Mizoram local cows Zobawng were included in this experiment. The present study was carried out under guidelines approved by the Institute Animal Ethical Committee, College of Veterinary Sciences & A.H., Central Agricultural University, Aizawl, Mizoram, India. The management of cow housing was an intensive system, and according to standards, cows were fed with feed concentrate and dry alfalfa grass, and water was available at all times. Cows were observed twice daily, in the morning and evening, for visible signs of oestrus. Consequently, cow blood samples were taken starting the 0 (zero) day after oestrus symptoms, then on the 4th, 8th, 12th, 16th and 20th day. For estimation of blood biochemical and hormonal levels, ten (10) ml of blood was collected by jugular vein puncture into a vacuum clot activator vial. The vials were kept in a standing position for 20 minutes. After that, it was centrifuged at 3000 rpm for 10 minutes. The obtained serum was kept at -20 ºC until analysis. The serum levels of different biochemical parameters such as glucose, cholesterol, calcium (Ca), phosphorus (P) and zinc (Zn) were analyzed by using commercial diagnostic kit (Mindray, Shenzhen, China) in FUJI dri-chem slide in FUJIFILM (DRI CHEM 4000i) auto analyzer and serum level of estrogen and progesterone were also analyzed with ELISA kit (BT LAB – Bioassay Technology Laboratory, Shanghai, China).

Statistical Analysis

The SPSS software was used to analyze all the data, and values are presented as means and standard errors (SE) with $P<0.05$ as the level of significance.

Results and Discussion

The cow undergoes several changes during the oestrous cycle that are necessary for reproduction, *viz*., the proestrus, oestrus, metestrus, and diestrus stages of the estrus cycle, which lasted 17–21 days, characterized by changes in the uterus, ovary, and hormone levels. The estrus (heat) lasted 6 to 30 hours, and the metestrus lasted 3 to 5 days when ovulation would occur. Diestrus lasted about 13 days, and proestrus lasted 2–3 days, preparing the body for estrus. A few essential minerals, namely Ca, P and Zn exhibit slight variations, which are not significant during the different days of the estrous cycle and they have an important role in reproductive functions in cows (Talukdar *et al.*, 2016) [21]. They represent the value of blood biochemical and hormonal profile at 0, 4, 8, 12, 16 and 20 days of the oestrous cycle of female local cattle of Mizoram (Zobawng) as presented in Table 1.

Glucose

Glucose is the main support system for ovarian functioning (Taludar *et al.*, 2015) [19]. Lack of glucose supply or hypoglycemic condition of the ovum, sperm and embryo suffers from lack of energy Nishimoto *et al.* (2006) [13]. In the present study, it was found that the levels of blood glucose (mg/dl) were recorded as 77.16±10.65, 65.30±3.39, 67.30±6.72, 69.40±6.47 and 83.10±7.97 on day 0, 4, 8, 12, 16 and 20 of the oestrous cycle, respectively. In the present study, it was found that there is no significant difference between the different days of the oestrous cycle but the level was numerically high proestrus and oestrus phase i.e. days 0 and 20. Findings of the Talukdar and Talukdar (2017) [22] showed higher values than the present study.

Cholesterol

The levels of blood cholesterol (mg/dl) were recorded as 172.60±20.97, 142.10±19.35, 130.80±16.46, 150.70±9.46, 196.30± 30.88 and 124.90± 17.37 on day 0, 4, 8, 12, 16 and 20 of the oestrous cycle, respectively. Analysis of variance showed that there was no significant difference in the levels of blood cholesterol at different days of the oestrous cycle. Cholesterol is one of the most important materials for biosynthesis of the steroid hormones (Jeremy, 2010) [11].

Calcium

Ca plays a critical role in the gonadotropic regulation of ovarian steroidogenesis, and a slight deficiency can disrupt the pituitary-ovarian axis and interfere with ovulation (Carnegie and Tsang, 1984) [5]. On days 0, 4, 8, 12, 16 and 20 of the estrous cycle, the level of blood calcium (mg/dl) ranged from 7.83 to 8.53. Analysis of variance showed no significant difference in blood calcium levels at different days of the oestrous cycle. Observation of Talukdar and Talukdar (2017) [22] showed higher value than the present values in the local cattle of Mizoram (Zobawng). The findings of Chandrahar *et al.* (2003) [6] showed similar results to the results of the present study.

Phosphorus

Phosphorus helps in energy transformation at the cellular level and also takes part in phospholipids metabolism. Phosphorus also takes part in CAMP synthesis (Seifi *et al*., 2005) [16]. The present study revealed the level of phosphorus (mg/dl) as 6.64±0.62, 7.22±0.71, 7.18±0.61, 7.78±0.46, 13.62±6.23 and 6.86±0.34 on day 0, 4, 8, 12, 16 and 20 of the oestrous cycle, respectively. Analysis of variance showed that there was no significant difference in phosphorus level at different days of the oestrous cycle. Observation of the Talukdar and Talukdar (2017) [22] showed higher value than the findings in local cattle of Mizoram (Zobawng).

Zinc

Zinc is considered to be a vital element for proper sexual maturity, reproductive capacity, and more specifically, the onset of estrus. Zinc has a vital role in the repair and maintenance of the uterine lining following parturition, speeding the return to normal reproductive function and estrus (Goff, 1999) [9]. The level of zinc (mg/dl) was recorded as 0.514±0.09, 0.750±0.77, 0.638±0.085, 0.56±0.070, 0.50±0.100 and 0.612±0.055 on day 0, 4, 8, 12, 16 and 20, respectively in the present study. Analysis of variance showed that there was no significant difference in zinc level at different days of the oestrous cycle. Results of the Alvi-Shoushtari *et al*. (2012) [2] showed higher values than the present study.

Estrogen

The level of estrogens (pg/ml) was recorded as 8.97±0.46, 6.10±0.52, 3.17±0.44, 3.78±0.50, 5.44±0.52 and 8.74±0.47 on day 0, 4, 8, 12, 16 and 20 of the oestrous cycle, respectively. Analysis of variance showed that there was a significant difference (P<0.01) in the levels of estrogens between different days of the oestrous cycle. The findings of Talukdar (2010) [23] showed similar values to the present findings in local female cattle of Mizoram. The level of serum estradiol 17– β is higher during oestrus and towards the prooestrus (18 days of oestrus) and lower on day 8 of oestrus. A high amount of estrogen, interestingly, has an opposite stimulatory effect on the gonadotropins (Boro *et al*., 2022a) [3]. The LH and FSH start to rise in high mode. When estrogen secretion is high, the LH receptors also more which is made by the theca cells, stirring theca cells to create more androgen that will become estrogen downstream. This type of positive feedback twists LH to spike abruptly, which causes ovulation. Subsequent ovulation, LH stimulates the formation of the corpus luteum. Estrogen has since dropped to negative stimulatory levels after ovulation and therefore serves to maintain the concentration of FSH and LH (Boro *et al*., 2022b) [4].

So, if the level of estradiol is less, it hampers normal physiological function and ultimately it associated with reproductive malfunction and repeat breeding (Talukdar *et al.*, 2024) [18].

Progesterone

In the present study, the level of progesterone (ng/ml) was recorded as 1.03±0.15, 2.75±0.31, 7.95±1.13, 9.35±0.51, 8.38±0.66 and 2.06±0.3 on day 0, 4, 8, 12, 16 and 20 of the oestrous cycle, respectively. Analysis of variance showed that there was a significant difference (p<0.01) in progesterone levels at different days of the oestrous cycle. The findings of Talukdar (2010) [23] showed similar values to the present findings in local female cattle of Mizoram. Progesterone stimulates endometrial gland growth and prepares the uterus to receive and nourish a new embryo. Inadequate luteal progesterone has been considered a cause of abnormal embryonic development and early embryonic loss (Talukdar *et al.*, 2013) [20]. During the oestrous cycle, a low level of progesterone delays the responsiveness for oxytocin at the late luteal phase of another oestrous cycle that increases the $PGF_{2\alpha}$ secretion which interferes with the pregnancy stage (Shaham-Albalancy *et al*., 2001) [17].

Conclusion

In conclusion, the present study was carried out to investigate the Zobawng cow's blood biochemical and hormonal profiles during the oestrous cycle, which indicated that glucose, cholesterol, calcium, phosphorus and zinc were within the normal range. Many reports on oestrous cycle hormones of different cow breeds diverged from Zobawng cows, but the release pattern is almost similar, indicating normal oestrous cycle regulation. This study added a new dimension to our understanding of the Zobawng cow's reproductive physiology, and these findings will be helpful for Veterinary physicians in managing health and reproduction in the extreme climate of high altitude.

Table 1: Level of blood biochemical and hormonal profiles at different days of the oestrous cycle of local cattle of Mizoram (Zobawng)

Parameters	Day 0	Day 4	Day 8	Day 12	Day 16	Day20	F values
Glucose (mg/dl)	77.16±10.65	65.30±3.39	67.30±7.72	69.40±6.47	69.40±6.47	83.10±7.97	0.878^{NS}
Cholesterol (mg/dl)	172.60±20.97	142.10±19.35	130.80±16.46	150.70±9.46	196.30±30.88	124.90±17.37	1.809^{NS}
Calcium (mg/dl)	7.83±0.71	8.30±0.36	8.53±0.73	7.92±1.04	8.0.5±1.23	7.44±1.30	0.158^{NS}
Phosphorus (mg/dl)	6.64±0.62	7.22±0.71	7.18±0.61	7.78±0.46	13.62±6.23	6.86±0.34	1.059^{NS}
Zinc (mg/dl)	0.514±0.09	0.75±0.77	0.638±0.09	0.56±0.09	0.50±0.10	0.612±0.06	1.260^{NS}
Estrogen (pg/ml)	$8.97±0.46^{C}$	$6.10±0.52^{B}$	$3.17±0.44^{A}$	$3.78±0.501^{A}$	$5.44±0.52^{B}$	$8.74±0.47^{C}$	24.544**
Progesterone (ng/ml)	$1.03±0.15^{A}$	$2.75±0.31^{A}$	$7.95±1.13^{B}$	$9.35±0.51^{B}$	$8.38±0.66^{B}$	$2.06±0.39^{A}$	35.947**

** F<0.01

Means bearing different superscripts in a row differed significantly

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