B-4476
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SHORT COMMUNICATION Indian Journal of Animal Research

A Study on Some Serum Biological Constituents during Different Days of Normal Oestrous Cycle of Manipuri Pony Mares

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10.18805/IJAR.B-4476

ABSTRACT

Background: The aim of this study was to estimate the serum biochemical (glucose, cholesterol, total protein, calcium, phosphorus and magnesium) and hormonal constituents (estrogen and progesterone) in female Manipuri pony mares during the different days of oestrous cycle.

Methods: A total of 50 numbers of blood samples were collected from 10 numbers of apparently healthy, non-pregnant on the different days of oestrous cycle *i.e.* day 0, 4, 8, 14 and 18 by using commercial diagnostic kits.

Result: The mean serum levels of glucose, cholesterol and phosphorus were found to be non-significant on the different days of oestrous cycle whereas the mean serum level of total protein and calcium was found significantly different (P<0.01). The serum magnesium level differed significantly (P<0.05) amongst the different days of oestrous cycle. Both estrogen and progesterone were also found to be significant (P<0.01) between the different days of oestrous cycle. The results showed significant influence on serum biochemical and hormonal assay during different days of oestrous cycle in female Manipuri pony.

Key words: Biological constituents, Manipuri pony, Oestrus, Oestrous cycle.

Manipuri pony (*Equus ferus caballus*) is one of the rarest breed of pony species and also considered as purest and prestigious equine breeds of India by Indian Council of Agricultural Research (ICAR). Manipuri Pony has been bred over centuries in the erstwhile state of Manipur, India. They are distributed in small number throughout the home tract in different pockets specially, the plain areas of Manipur in the districts of Imphal East, Imphal West, Bishnupur and Thoubal but more dense in Imphal West where the altitude ranges from 700-800 meters above sea level with latitudes of 23°N to 25.68°N and longitudes 93.03°E to 94.78°E.

Normal oestrus and oestrous cycle of domestic animal is a vital physiological phenomenon for effective continuous propagation of its own kind. Similarly in case of widely decreased of the breed comparative evaluation of some of important biological constituents might be effective to know the breeding efficiency of this type of pony during oestrous cycle and under the prevailing condition of Manipur appeared to be scanty. Therefore the present study had been carried out to know about normal serum levels of some biological constituents along with steroidal hormones during different stages of normal oestrous cycle of Manipuri pony mares.

A total of ten clinically healthy, cyclic, non- pregnant female Manipuri pony mares, aged between 4-8 years and weighing between 120-140 kg were taken in the present study. All the animals were fed uniformly and no source of artificial light and no special feed were provided. Mares were provided with water and feed *ad libitum* at all the time. The reproductive status of each mare was determined according to the recorded farm history, in conjunction with the results obtained by clinico-gynaecological examination and physical appearance or visual signs of oestrus status were confirmed by using stallion of high libido. All the mares were kept at paddock whereas the stallions were housed in single pens.

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How to cite this article: Linda, N., Ahmed, F.A., Ali, Chaudhary, J.K., Ali, M.A. and Gali, J.M. (2021). A Study on Some Serum Biological Constituents during Different Days of Normal Oestrous Cycle of Manipuri Pony Mares. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4476.

Submitted: 07-04-2021 **Accepted:** 06-05-2021 **Online:** 14-08-2021

Sampling

Ten (10) ml of blood samples were collected from the jugular vein by using sterile 18 gauge needle fitted with a plastic syringe under the anaerobic conditions. The approved reference number of institutional animal ethics committee: CVSC/CAU/IAEC/19-20/P-1. The samples were collected into a vacuum clot activator vial on different days of oestrous cycles *i.e.* on day 0, 4, 8, 14 and 18th respectively.

The collected blood samples were subjected to remain standing at room temperature for 20 minutes, then the clot

activator vials were centrifuged at 3,000 rpm for 10 minutes and the sera were harvested and stored at -20°C until used for estimation of different biological constituents of this study.

The serum levels of glucose, cholesterol, total protein, calcium, phosphorus and magnesium were analysed by using commercial diagnostic kits. The serum levels of estrogen and progesterone were assayed by using commercial ELISA kits (Cloud- Clone Corp for estradiol having 96 wells) and (Enzo life sciences, Inc, with 96 wells) respectively.

General linear model of one way Analysis of Variance (ANOVA) based on Fisher's least significant difference method was used to determine the significant difference among days (0, 4, 8, 14 and 18 days). The significant values in the ANOVA mean values were compared using Duncan multiple range test. Results are presented as mean ± SE and differences were considered significant when P <0.05. The data obtained were analysed using statistical package SPSS version 25.0.

The present study, the mean value of this investigation shown (Table 1) the mean serum glucose and cholesterol of Manipuri pony at different days of oestrous cycle did not differ significantly between the different days of oestrous cycle which corroborate with the findings of (Ali *et al*. 2004) however lower values were found when compared with the findings of (Abo-ElMatty and El-Shahat 2012). The serum level of glucose tended to be significantly higher (P<0.05) in oestrus stage than those recorded at luteal one in Arabian mares (Abo-ElMatty and El-Shahat 2012) which was found similar that is higher during oestrus phase (day 0) while there was gradual decrease during luteal phase of oestrous cycle (day 14) which might be due to changes of hormones during the oestrus phase (Shoushtari *et al.* 2014).

The analysed of variance test of mean values of cholesterol values were not found to be varied significantly.

Similar findings were also reported by (Nafizi *et al*. 2005) and the present values were found to be lower when compared with the findings of (Abo-ElMatty and El-Shahat 2012). Maintaining higher level of serum cholesterol in present study might be required to maintain for manifestation of reproductive phenomenon of cycles.

In the present study, analysis of variance test revealed that mean values of total protein levels in serum of different days of oestrous cycle were found to be varied significantly (P<0.01). These values were found to be comparable with the findings (Abo-ElMatty and El-Shahat, 2012) but lower with the findings of (Frape, 1986; Ali *et al*. 2004). The higher concentration of total protein during oestrus might be due to higher level of oestrogen which altered the secretary activity of genital epithelium (Modi *et al.* 2017).

The analysis of variance of mean values of serum calcium levels revealed that they varied significantly (P<0.01) at different days of cycle while Duncan multiple range test indicated it was significantly highest o day 0 and on 4 of the cycles and gradually declined and reached lowest level on day 18. Lower production of milk and biological sters (oestrus) with lack of food intake might be cause of retention calcium which became almost stable

However, the present findings have lower values with the findings of (Ali *et al*. 2004; Meliani *et al*. 2011) which might be due to breed differences along with the change of steroidal hormones during oestrus cycles (Shoushtari *et al*. 2014).

Analysis of variance test revealed that the serum phosphorus level did not vary significantly at different days of the oestrous cycle which supports with the findings of Ali *et al.* (2004) but lower when compared with the results of (Ali *et al*. 2009, Meliani *et al*. 2011). However it was appeared that mean values were normal during oestrus cycle of the study.

The present findings revealed that the mean serum magnesium level was differed significantly (P<0.05) among the different days of oestrous cycle with lowest value on day 18. However, these mean level were might be sufficient to maintain body strength including muscle so on while similar findings were also reported by Ali *et al*. (2009) and Meliani *et al*. (2011) which might be due to changes of hormones during oestrus phase (Shoushtari *et al*. 2014).

Significant difference (P<0.01) between the different days of oestrous cycle were observed and corroborate with the findings of AboEl-Maaty (2011) in cyclic mares. Abdenlnaby and AboEl-Maaty (2016) reported that estrogens decreased significantly in a linear fashion from similar level on days 1 and 2 post-ovulation to significantly low concentration on day 7, then a short increase on day 8 and the levels were decreased again to low values on day 10.

Significant difference (P<0.01) between the different days of oestrous cycle was observed in the current study

Table 1: Level of Serum biological constituents (Mean ± SE) at different days of oestrus in cyclic female Manipuri pony.

Biological constituents	Days of oestrous cycle				
	0	4	8	14	18
Glucose (mg/dl) NS	88.13 ± 3.21	89.20± 3.18	87.43 ± 2.96	82.48 ± .66	82.61 ± 2.87
Cholesterol (mg/dl) NS	87.4 ± 4.29	87.82± 4.61	85.94 ± 4.49	81.15 ± 4.63	81.15 ± 4.1
Total Protein (gm/dl)	7.15^{bc} ± 0.14	7.26^{c} ± 0.13	6.97^{abc}± 0.12	6.76^{ab} ± 0.14	6.5^{a} ± 0.16
Calcium (mg/dl)	10.42^{c}± 0.32	10.74^{c}±0.32	10.33^{bc}±0.30	9.46^{ab} ± 0.23	9.37^{a} ± 0.34
Phosphorus (mg/dl)	3.24 ± 0.24	3.34 ± 0.24	3.02 ± 0.22	2.66 ± 0.23	2.56 ± 0.17
Magnesium (mg/dl)	2.41^{bc} ± 0.12	2.46^{c} ± 0.12	2.23^{abc}± 0.11	2.08^{ab} ± 0.10	1.94^{a} ± 0.09

Means bearing different superscript in a row differed significantly. *P < 0.05 (Significant at 5%), **P < 0.01 (Significant at 1%), P > 0.05 (Non Significant).

and similar findings were also recorded by AboEl-Maaty (2011) in cyclic mares. Abdenlnaby and AboEl-Maaty (2016) reported that Progesterone levels were significantly high during static phase than during the luteal growth phase.

It was observed that the mean value of serum magnesium were varied significantly (P<0.01) at different days of oestrous cycle and variation was similar to the findings of AboEl-Maaty (2011) in cyclic mares. Abdenlnaby and AboEl-Maaty (2016) opined that oestrogens decrease significantly in a linear fashion from similar level on days 1 and 2 post-ovulation to significantly low concentration on day 7, then a short increase on day 8 and the levels were decrease again to low values on day 10. It was also observed from the present study the serum mean concentration at different days of the oestrous cycle of the experimental mare varied significantly (P<0.01) lowest value on day 0 and highest on day 14. The trend of variation of progesterone in this study clearly indicated normal to this specific experimental animal.

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