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Serum glucose & cholesterol levels in anestrous and estrous Kenguri ewes

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Abstract
The study was conducted to evaluate the levels of serum glucose and cholesterol at different time interval i.e, -15D, -7D, 0D, 7D, 12D and on the day of estrus in synchronization protocols in postpartum anestrous Kenguri ewes in non-breeding season. Thirty healthy ewes of 60 days postpartum were selected and divided in to 5 groups, each containing 6 animals (n=6). All the five groups were fed with maize grains (250 gm/ewe/day) for 15 days prior to synchronization protocol as flushing protocol. G-I served as control group with only maize feeding without treatments. After 15 days of Maize feeding, 4 treatment groups received CIDR intravaginal device (0.3 gm of progesterone) left intravaginal for 12 days. After 12 days, during the removal of CIDR, G-II and G-IV animals received I/M inj.PGF2α @125µg /ewe whereas G-III and G-V received I/M inj. PMSG @ 500 IU/ewe. Upon exhibition of estrus and natural mating, G-IV and G-V animals received I/M inj. hCG @500 IU/ewe. In conclusion the blood serum glucose levels in the present study were found to be decreased on the day of estrus whereas the blood serum cholesterol levels were found to be increased on the day of estrus in experimental animals.

Keywords: Kenguri, flushing, estrus synchronization, blood serum glucose and cholesterol

1. Introduction
Blood is an important and reliable medium for assessing the health status of individual animals. Variations in blood parameters of animals are due to several factors such as altitude, feeding level, age, sex, breed, diurnal and seasonal variation, temperature, and physiological status of animals. Serum biochemical tests are widely used for the diagnosis of serious animal diseases which can lead to economic losses in animals like reduced fur, wool, and milk production (Kiran *et al*., 2012) [1]. The patterns of reproductive activity in the adult ewes are dominated by two distinct rhythms. The first of them is a 16 to 17 day long estrous cycle. The other is an annual rhythm of ovarian cyclicity characterized by a season-dependent cessation (anoestrus) and restoration (breeding season) of ovulatory ovarian cycles (Goodman, 1994; Gordon, 1996) [2, 3]. In the longer days of spring, there is a break in the reproductive period, whereas the shorter days of autumn are associated with the onset of estrus (Dogan and Nur, 2006) [4]. Thus, reproductive seasonality is an important factor that limits the productivity of small ruminants (Zarazaga *et al*., 2003) [5].
Along with the breeding receptivity the energy levels and Glucose is one of the most important metabolic substrates required for proper function of the reproductive processes in livestock (Naqvi *et al*. 2011) [6]. Glucose is the primary metabolic fuel used by the central nervous system, and inadequate availability of utilizable glucose reduces hypothalamic release of GnRH. The concentration of glucose in the blood of animals may influence the rate of steroidogenesis and gonadotropin synthesis and secretion in goats (Rufai *et al*., 2013) [7]. Glucose is the primary metabolic fuel used by the central nervous system, and inadequate availability of utilizable glucose reduces hypothalamic release of GnRH (Hess *et al*. 2005) [8].
In sheep and other ruminants, cholesterol is naturally produced in the liver and intestinal walls. It is also a source of energy and is a precursor of steroid hormones, bile acids and it is also required for normal cell function (Khan *et al*., 2013) [10]. It plays a vital role in the formation of cell membranes, the production of hormones, bile, and metabolism of fat-soluble vitamins, acting as an antioxidant (Okonkwo *et al*., 2010) [11]. Cholesterol is essential for the proper functioning of the body, but it can be harmful when it is consumed or produced in the body in excessive amounts.

In veiw of their importance in the cyclic functionality of the animals the study was undertaken to ascertain the levles of the serum glucose and cholesetrol in anoestrus, after induction and at estrous in Kenguri ewes.

2 Materials and Methods

2.1 Location of the study: The present study was carried out on Kenguri ewes maintained at Department of Instructional Livestock Farm Complex, Veterinary College, Nandinagar, Bidar and field flocks (private farmers) in and around Bidar District. The study was conducted during the period of March 2018 to May 2018.

2.2 Selection of Animals

Thirty healthy ewes, aged about 2-5 years which have not shown estrus up to 60 days postpartum were selected for this study. Animals were already vaccinated against PPR. Deworming was carried out with Fenbendazole @ 7.5 mg/kg BW. Animals were allowed to graze during day hours from 10:00 a.m. to 5:00 p.m. and provided clean water ad libitum. These animals were randomly divided into five different groups. Number of animals in each group was six.

2.3 Synchronization Protocols

Animals were divided into 6 groups. Group I (n=6), the group was treated as control. Each animal was fed with 250 g of maize daily (morning) for 15days and later observed for estrus signs without receiving any of the treatment. Group II(n=6) recieved Flushing (maize feeding for 15 D) with CIDR device (for 12 D intra vaginally) followed by Inj PGF2α at CIDR removal. Group III (n=6) recieved similar maize feeding for 15 Days followed by CIDR device kept intravaginally for 12 D followed by Inj PMSG at CIDR removal. Group IV (n=6) Recieved Flushing (maize feeding for 15 D) followed by CIDR device kept intravaginally for 12 Days followed by Inj PGF2α at CIDR removal also Inj hCG upon Natural service .Group V(n=6) Flushing with maize feeding for 15 Days followed by CIDR device kept intravaginally for 12 Days followed by Inj PMSG at CIDR removal along with Inj hCG upon Natural service. The animals were tested for pregnancy and different serum hormonal levels were analyzed to find out best protocol with high pregnancy rate.

2.4 Serum Profiles

Blood samples were collected by jugular vein puncture from all the groups on day -15, -7, day 0, 7, 12 and at detected estrus. Serum samples were harvested by subjecting through centrifugation at 3000 rpm for 10 minutes and using micro pipette serum sample was made into aliquot in duplicate and stored at -20 ˚C until analysis. The serum samples were subjected for serum progesterone analysis performed by ELISA (CALBIOTECH Progesterone ELISA kit). Serum glucose and cholesterol analysis was performed as per the assay procedures mentioned in the kits (Swemed Diagnostics, Bengaluru) using auto chemistry blood analyzer (Artos Elita, Swemed Biomedicals Pvt Ltd, Bengaluru).

2.5 Statistical Analysis

The data obtained was analysed by SAS 9.3 software using one – way ANOVA (Kaps and Lamberson, 2017) [12].

3. Results

There was a significant (p<0.05) increase in the serum glucose levels across the groups after flushing (Day 0) with respect to the values observed prior to initiation of flushing (Day -15) as observed in table 1. This increase in glucose in the blood serum might be due to the production of more propionic acid in concentrate supplemented groups (McDonald *et al.* 1996) [13]. There was non-significant decrease in glucose value on the day of estrus when compared prior to flushing among the groups which might be due to stimulation of insulin secretion from isolated islets by prostaglandin (Goodman and Gilman, 1980) [14] or by progesterone treatment (Lenzen, 1978) [15].

There was significant (p<0.05) increase in the serum cholesterol on the day of estrus when compared to other intervals of time (Table 2).

This increased level of cholesterol on the day of estrus may be due to estrogen hormone which effect on the carbohydrate metabolism inturn cause increased production of cholesterol in endocrine gland tissue from acetate (Purohit and Kohli, 1977) [16].

Table 1: Mean ± S.E values of serum glucose (mg/dL) in different groups at different intervals (-15D, -7D, 0D, 7D, 12D and on the day of estrus)

Grp \ Days	-15	-7	0	7	12	ESTRUS
G I	$47.39^{ac} \pm 1.60$	$44.20^{a} \pm 1.17$	$55.39^{b} \pm 0.69$	$48.38^{acAC} \pm 0.67$	$47.89^{acA} \pm 0.55$	$42.11^{cA} \pm 0.13$
GII	$46.01^{a} \pm 0.52$	$49.68^{bde} \pm 0.88$	$52.93^{c} \pm 0.62$	$50.22^{deA} \pm 0.42$	$48.96^{e\ A} \pm 0.43$	$44.75^{aAB} \pm 0.85$
GIII	$44.79^{a} \pm 3.87$	$50.33^{ab} \pm 1.92$	$56.47^{b} \pm 0.79$	$55.52^{bB} \pm 0.94$	$51.85^{abB} \pm 0.57$	$45.90^{aB} \pm 0.76$
GIV	$47.52^{a} \pm 2.44$	$51.19^{a} \pm 3.79$	$54.03^{a} \pm 4.29$	$51.24^{a\ AD} \pm 1.02$	$50.00^{a\ AB} \pm 0.80$	$45.02^{aAB} \pm 0.52$
G V	$44.20^{\ a} \pm 1.17$	$45.70^{a} \pm 1.41$	$50.57^{b} \pm 0.98$	$46.48^{abC} \pm 1.01$	$44.32^{aC} \pm 0.86$	$44.00^{aAB} \pm 0.44$

Note: 15th day: first day of flushing, -7th day: 7 days after flushing, 0th day: End of the flushing and first day of CIDR insertion, 7th day: 7 days post CIDR insertion, 12th day: day of CIDR removal, Estrus day: Day when animal exhibit estrus.

G I: Control (only Flushing), G II: Flushing+ CIDR+ PGF2α, G III: Flushing+ CIDR+ PMSG, G IV: Flushing+ CIDR+ PGF2α + hCG, G V: Flushing + CIDR + PMSG + hCG

Means with different superscripts differs significantly at p<0.05

^{abcde} superscripts indicate the difference between serum glucose values at different time interval within group.

^{ABCD} superscripts indicate the difference between serum glucose values at different groups within the time intervals

Table 2: Mean ± S.E values of serum cholesterol (mg/dL) in different groups at different intervals (-15D, -7D, 0D, 7D, 12D and estrous)

Days / Grp	-15	-7	0	7	12	ESTRUS
G I	$55.34^{a} \pm 0.27$	$56.62^{a} \pm 0.28$	$56.48^{a} \pm 0.46$	$56.53^{a} \pm 0.65$	$56.95^{a} \pm 3.39$	$70.28^{b} \pm 0.95$
GII	$56.12^{a} \pm 0.64$	$57.40^{a} \pm 0.62$	$57.04^{a} \pm 0.73$	$56.85^{a} \pm 0.47$	$57.58^{a} \pm 0.40$	$72.86^{b} \pm 0.52$
GIII	$57.14^{a} \pm 0.99$	$56.47^{a} \pm 1.05$	$57.96^{a} \pm 0.88$	$56.75^{a} \pm 0.62$	$58.03^{a} \pm 0.69$	$70.35^{b} \pm 0.96$
GIV	$53.62^{a} \pm 1.66$	$54.00^{a} \pm 1.16$	$55.02^{a} \pm 1.25$	$54.86^{a} \pm 0.94$	$54.46^{a} \pm 0.91$	$71.24^{b} \pm 0.36$
G V	$55.12^{a} \pm 1.33$	$56.75^{a} \pm 1.16$	$55.84^{a} \pm 3.27$	$53.27^{a} \pm 2.10$	$54.45^{a} \pm 1.19$	$70.37^{b} \pm 0.98$

Note: 15th day: first day of flushing, -7 th day: 7 days after flushing, 0th day: End of the flushing and first day of CIDR insertion, 7 th day: 7 days post CIDR insertion, 12th day: day of CIDR removal, Estrus day: Day when animal exhibit estrus.
G I: Control (only Flushing), G II: Flushing+ CIDR+ PGF2α, G III: Flushing+ CIDR+ PMSG, G IV: Flushing+ CIDR+ PGF2α + hCG, G V: Flushing+ CIDR+ PMSG+ hCG
Means with different superscripts differs significantly at $p<0.05$
^{abcde} superscripts indicate the difference between serum glucose values at different time interval within group.
^{ABCD} superscripts indicate the difference between serum glucose values at different groups within the time interval.

4. Discussion

From the results it is clear that there was a decrease in the serum concentration of glucose at estrous and there was an increase in the value of cholesterol level at estrous. Decreased level of glucose on the day of estrus may be due to temporary decrease in feed intake or from changes in the metabolic status of the animal while under the influence of high level of estrogen (Singh and Dutt, 1974) [17] or estrogen hormone had an effect on the carbohydrate metabolism (Purohit and Kohli, 1977) [16].

The estrogens influence lipid metabolism through liponeogenesis which in turn caused increased production of cholesterol in endocrine gland tissue from acetate. The increased level of cholesterol at estrus may be due to withdrawal of stored tissue cholesterol in the blood for estradiol 17-β synthesis (Honnappagol and Patil, 1991) [18].

5. Conclusion

Based on the present research findings, the following conclusions were drawn: Supplementation of 250 gm maize to experimental animals in non breeding season has shown the increased blood serum glucose levels after 15 days. Whereas blood serum glucose levels in the present study were found to be decreased on the day of estrus in experimental animals when compared to pre-estrous levels.

Blood serum cholesterol levels in the present study were found to be increased on the day of estrus in experimental animals significantly.

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