

**ULTRASONOGRAPHIC STUDIES ON POST-PARTUM
UTERINE INVOLUTION AND OVARIAN CYCLICITY IN
MALABARI GOATS**

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(15-MVP-13)**

THESIS

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DECLARATION

I hereby declare that this thesis entitled “**Ultrasonographic studies on post-partum uterine involution and ovarian cyclicity in Malabari goats**” is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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INTRODUCTION

1. INTRODUCTION

According to 2012 census, the goat population in India was 135.2 million, of which Kerala alone holds 12.3 lakh. Thus, a major share of the goat population is in Kerala. Malabari goat, a breed renowned for meat production, high prolificacy (Asha and Naicy, 2012) is adapted to the tropical climate of Kerala. Small group holdings rather than large flocks of Malabari goats are seen throughout the state. This illuminates the importance of conserving this native breed and thus contributing to the state economy.

To achieve optimum reproductive efficiency, a goat should kid thrice in two years. Post-partum period is crucial in reproduction as it involves the involution process of uterus and resumption of ovarian cyclical activity. Uterine involution and resumption of ovarian activity are two important factors determining the future fertility. Normal post-partum uterine involution, early resumption of ovarian activity and oestrus, short inter-kidding interval and absence of puerperal reproductive complications like retention of placenta, metritis, etc. are the key factors to achieve optimum reproductive potential. The assessment of normal uterine involution period is an important criterion that would certainly help to understand and evolve management protocols to reduce the inter-kidding interval in the case of small ruminants. Any prolongation in the normal involution process will severely affect the length of post-partum period and the onset of ovarian activity. In small ruminants, involution is characterised by sudden decrease in uterine size, between the 3rd and the 10th day post-partum. Uterine involution is considered to be complete when there is absence of lochia inside the lumen and there is no significant difference in the cross-sectional diameter of the uterine horn in three consecutive examinations on days 17, 21 and 24 post-partum (Badawi *et al.*, 2014).

Real time B-mode ultrasonography has been used routinely for pregnancy diagnosis in small ruminants since the past decade. But very few works were conducted on imaging the uterine involution and ovarian activity. Many studies on

involution of post-partum uterus in small ruminants like necropsy studies, radiography, laparotomy and laparoscopy, were invasive and difficult to conduct routinely (Ababneh and Degefa, 2005; Badawi *et al.*, 2014). Ultrasonography is a far-reaching technique for the understanding of post-partum involution of the uterus and ovarian activity as it is non-invasive, rapid, accurate, and practically feasible under field conditions (Simoes *et al.*, 2005). Trans-rectal ultrasonography is a convenient method for the direct visualisation of the reproductive tract in case of small ruminants (Zdunczyk *et al.*, 2004). It helps in the measurement of the diameter of uterine horn and caruncles, visualise the presence and character of lochia inside the lumen of the uterus, and assessment of pathological conditions, if any, in the involution process.

The uterine involution occurs in a decreasing logarithmic scale both in size and diameter. The reduction process is faster during the first week post-partum. Following which there is a reduction in frequency, amplitude and duration of uterine contractions leading to a reduction in involution (Noakes, 2009b). The involution process was reported to be completed by day 19 (Ababneh and Degefa, 2005), day 22 (Badawi *et al.*, 2014) and day 28 post-partum (Enginler *et al.*, 2014) in various exotic breeds of goats. Hence, for the proper assessment of involution, imaging needs to be done at frequent intervals, which can only be achieved by routine ultrasonography on a regular basis.

In addition, the functional status of the ovary, the presence and growth of various ovarian structures like follicles and corpus luteum (CL), can also be studied by ultrasonography based on the difference in echogenicity (Vinoles *et al.*, 2004). Estimation of progesterone in serum during the post-partum period also helps to correlate the ultrasonographic study for the presence of CL that in turn reflects the ovarian activity. The progesterone level in serum is considered as one of the most important parameters to determine the reproductive status (Zarkawi and Soukouti, 2001). The follicular development observed during the early post-partum period usually does not reach ovulatory size and gets atrophied. This shows that the resumption of ovarian cyclical activity is not always associated

with behavioural oestrus and ovulation. It is also observed that the first post-partum oestrous cycle is having abnormal cycle length and is often anovulatory. Consequently, the imaging of CL along with serum progesterone estimation is necessary for assessing the resumption of ovarian cyclical activity.

During post-partum, the reproductive system is reconstructed anatomically and physiologically for the establishment of new pregnancy through the process of uterine involution and resumption of ovarian cyclical activity. Hence, a pioneer study was conducted in Malabari goats to understand the pattern of normal uterine involution and resumption of ovarian activity and oestrus. The work was conducted at the Goat farm under the Instructional Livestock Farm Complex (ILFC), Pookode with the following objectives:

1. To study the course of uterine involution during post-partum period by ultrasonography in Malabari goats.
2. To correlate uterine involution with the post-partum ovarian cyclicity using ultrasonography and serum progesterone estimation.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. ULTRASONOGRAPHIC OBSERVATIONS OF THE UTERUS AND OVARIES

2.1.1. Echogenicity of uterine fluid

Hauser and Bostedt (2002) studied uterine involution in ewes by trans-rectal ultrasonography and found hypoechoic lochia within the uterine lumen. They opined that ultrasonography has a greater advantage over post-mortem studies as it allowed assessment of echogenicity of the lochia and gradual reduction in uterine lumen.

Zdunczyk *et al.* (2004) studied uterine involution in Polish Longwool sheep by trans-rectal ultrasonography and stated that the end of uterine involution is characterised by the absence of lochia and small cross sectional diameter of uterine horn. They added that ultrasonography could be used as a reliable research tool for uterine involution studies in sheep.

Ababneh and Degefa (2005) studied post-partum uterine involution in Balady goats by trans-rectal ultrasonography and noticed the presence of lochia and tissue debris as hypoechoic texture in the uterine lumen during the first four days post-partum.

Fasulkov (2014) found that the caruncles and uterine wall were visible as hypoechoic texture and uterine lumen with fluid as anechoic, which was detected up to first week post-partum in local Bulgarian goats by trans-rectal ultrasonography.

2.1.2. Diameter of uterine horns

Van Wyk *et al.* (1972) conducted post-partum involution studies of uterus in ewes and found that on macroscopic examination, the uterine involution appeared to be completed by days 20 to 24 post-partum.

Ali *et al.* (2002) studied post-partum uterine involution in Ossimi ewes by ultrasonography and stated that uterine involution was completed by an average of 16.27 ± 5.1 days (range 12-30 days) post-partum. A sudden decrease in uterine diameter was noticed between first and second weeks (1.49 ± 0.7 cm) than between the second and third weeks (0.49 ± 0.3 cm) and the third and fourth weeks (0.46 ± 0.2 cm).

Rubianes *et al.* (1995) observed macroscopic uterine involution by laparotomy on day 17 and day 30 post-partum in suckling and non-suckling autumn and spring lambed Corriedale ewes. They reported that uterine involution was not affected by weaning.

Hauser and Bostedt (2002) noticed normal physiological regression of cross-sectional diameters the uterus from 4.9 ± 0.87 cm on day one to 1.84 ± 0.14 cm on day 30 post-partum. The lumen diameter of 2.24 ± 0.38 cm on day one reduced rapidly by more than 80 per cent on day 11 post-partum. A uterine lumen diameter of less than two centimeter was detectable after day 11. The end of the involution process was observed by around day 17 post-partum.

Zdunczyk *et al.* (2004) stated that the end of uterine involution was marked by less than two centimeter transverse diameter of uterine horn and lack of contents in the uterus. They observed that 81.8 per cent (18/22) pluriparous ewes completed uterine involution by day 35 post-partum. They opined that breed variation, season of lambing and length of suckling period influenced uterine involution process. They recommended trans-rectal ultrasonography as a useful and reliable method to observe the uterine involution in ewes.

Ababneh and Degefa (2005) stated that ultrasonographic imaging of uterus showed rapid reduction in the diameter of uterine horn during first week post-partum and complete uterine involution occurred by day 19 post-partum and thereafter till day 25 only negligible changes were observed and it was unrelated to parity in Balady goats. They advocated close association between ultrasonographic studies and macroscopic involution process and recommended

ultrasonography as a feasible method to observe uterine involution in field conditions.

Degefa *et al.* (2006), in Balady goats, observed the completion of macroscopic uterine involution by day 19 post-partum with uterus having the size of a non-pregnant uterus.

Hayder and Ali (2008) studied the effect of various factors on post-partum uterine involution by ultrasonography in subtropical ewes and found that the mean time required to complete uterine involution was 31.9 ± 1.2 days. Reduction in size was faster between days seven and 14 but was consistent between days 14 and 32 post-partum. They observed early involution in winter lambing ewes than summer lambing ewes.

Takayama *et al.* (2010) conducted studies on post-partum uterine involution in non-seasonal Shiba goats and stated that suckling had no influence on uterine involution.

Fernandes *et al.* (2013) correlated ultrasonographic findings with the leukocyte count in native Brazilian Pantanal sheep and found that uterine involution in Pantanal ewes occurred between days 14 and 21 post-partum.

Badawi *et al.* (2014), in ultrasonographic studies of uterine involution in Nubian goats, observed a maximum uterine diameter of 32.8 ± 3.6 mm on day three and a minimum of 22.9 ± 3.0 mm on day 31 post-partum. A rapid reduction in uterine diameter was noticed between day three and 14 post-partum ($> 50\%$) while a constant change occurred between days 17 and 27 and decreased subsequently up to day 31. Presence of lochia was detected until first week of post-partum. They concluded the end of involution process by 22 ± 3.3 days. The completion of the involution process was characterised by absence of lochia in the uterine lumen and small uterine diameter with no further change noticed in three consecutive examinations.

Enginler *et al.* (2014) studied the effect of type of gestation (single or twin) and parity upon uterine involution in Saanen does by trans-rectal ultrasonography

and observed that the mean time for the completion of uterine involution in single delivered does were 27.10 ± 0.43 days while in twin delivered does were 28.19 ± 0.54 days, whereas the effect of parity on involution was found to be insignificant. They concluded that uterine involution completed at similar periods in single and twin bearing animals and primiparous and pluriparous Saanen does.

Fasulkov (2014) did ultrasonographic studies in local Bulgarian breed of goats on days 1, 3, 6, 9, 12, 15, 20 and 30 post-partum and measured the mean diameter of uterine lumen on first, third and 30th day as 1.95 ± 0.41 cm, 1.03 ± 0.02 cm and 0.48 ± 0.1 cm, respectively. A significant reduction ($p < 0.001$) in uterine size occurred as early as day three post-partum.

Medan and EL-Daek (2015) conducted studies on uterine involution in Barbary ewes in north Libya found that the diameter of uterine horn decreased rapidly during the first four weeks post-partum and end of involution varied between days 28 and 35 depending on the season of lambing.

Zongo *et al.* (2015), in West African Sahelian does, conducted an ultrasonographic studies to assess the uterine involution and ovarian activity and found the cross-sectional diameter of the uterus with 9.40 ± 0.71 cm on day one post-partum, decreased to 1.72 ± 0.27 cm on day 31 post-partum. They also stated that the end of uterine involution was noticed on 22.44 ± 1.54 days (range 18 - 25 days) post-partum with less than two centimeter transversal diameter of uterine horns and absence of fluid in uterus. Post-partum uterus of single foetus bearing does show an early involution process whereas the does bearing twin foetuses showed a delayed regression.

Ahmed *et al.* (2016) studied the uterine involution in Awassi ewes by trans-rectal ultrasonography and stated that the involution process was completed when there was a small cross-sectional diameter of uterine horns and lack of fluid in the uterine lumen. A sudden drop of more than 50 per cent in the horn diameter was noticed from day three to day 14 post-partum.

2.1.3. Diameter of uterine caruncles

Van Wyk *et al.* (1972) observed that the largest caruncles reached the size of its quiescent stage by day 16 post-partum macroscopically and on microscopic evaluation the complete covering of caruncles with the epithelium occurred by day 28 post-partum.

Rubianes and Ungerfeld (1993) noticed a mean caruncular diameter of 1.5 cm, 0.6 cm and 0.4 cm for ewes ovariohysterectomised at days 1, 17 and 30 post-partum, respectively.

Hauser and Bostedt (2002) stated that after the expulsion of placenta, the caruncles were visible as a concave structure attached to the inner wall of the uterus. They showed a change in shape from concave to convex during the period of involution process. A gradual decrease in size from 2.02 ± 0.16 cm on day one to 1.24 ± 0.17 cm on day eight post-partum was noticed. Due to the similar echogenicity of the caruncles and the endometrium, differentiation of both during day five was difficult, while after day eight caruncles were inappreciable.

Fasulkov (2014) found that the mean outer caruncular diameter on the days 1, 3 and 9 post-partum were 2.86 ± 0.43 cm, 2.27 ± 0.26 cm and 1.33 ± 0.22 cm, respectively and the inner caruncular diameter on the corresponding days were 1.39 ± 0.08 cm, 0.64 ± 0.11 cm and 0.54 ± 0.1 cm, respectively. The visualization of caruncle was possible only up to the day nine post-partum. A statistically significant difference ($p < 0.05$) in caruncular size on day one post-partum as against the caruncular size on day three post-partum could be demonstrated.

Zongo *et al.* (2015) measured the caruncular diameter in West African Sahelian goats and recorded a transverse diameter of 1.78 ± 0.42 cm on day one post-partum, which reduced to 0.12 ± 0.13 cm by day nine post-partum. Caruncles completely disappeared in does having single foetus while it was delayed up to day nine post-partum in those bearing twins.

2.1.4. Resumption of ovarian activity by the presence of corpus luteum

Mascarenhas *et al.* (1995) found that the ovarian activity resumed between days 20 and 50 post-partum in 75 per cent goats during first year.

Orita *et al.* (2000) conducted ultrasonographic studies of ovary during oestrous cycle in Shiba goats and found that the CL could be observed as hypoechoic structure, compared to echogenic ovarian stroma, as round or ellipsoidal structure.

Vinoles *et al.* (2004) mentioned that the follicles were observed as anechoic round structures and CL was visualised as definite, homogeneous hypoechoic structure compared to ovarian stroma and noted a significant relationship between the detection of CL by trans-rectal ultrasonography and post-mortem examination.

Simoes *et al.* (2005) in a study on Serrana goats using trans-rectal ultrasonography opined that the CL could be observed as round or oval structure with a clear contour and a homogeneous granular echogenic luteal tissue. Some CL showed an anechoic central fluid filled cavity by ultrasonography, which was confirmed by slicing technique. They opined that trans-rectal ultrasonography could be used as an ideal, non-invasive technique to detect CL compared to laparoscopy, laparotomy or slicing technique.

According to Hayder and Ali (2008), the first post-partum CL was noticed within 42 days in majority of summer lambed and in few number of winter lambed subtropical ewes.

Kandiel (2008), in a work for the assessment of ovarian activity in does by trans-rectal ultrasonography, observed the CL as a round to oval hypoechoic structure without an obvious margin. Corpus luteum attained a maximum diameter of 8.49 ± 0.47 mm on day 3.0 ± 0.32 post-ovulation. The first ovulation was usually followed by a short luteal phase of mean 5.0 ± 0.32 days.

Takayama *et al.* (2010) studied the post-partum ovarian activity and uterine involution in Shiba goats and opined that suckling controlled the resumption of ovarian activity, which was confirmed by the first ovulation following weaning.

Ovulation occurred early in non-suckling group (9.3 ± 3.2 days post-partum) than in suckling group (12-24 days after weaning). They also opined that the length of luteal phase after ovulation in post-partum nursing goats was much longer than non-nursing ones. Even though short cycles of post-partum oestrus was noticed following ovulation, the proper size of the CL was not attained in such cases.

Kandiel *et al.* (2011) studied the ovarian activity and concentrations of plasma progesterone in Shiba goats from day five to day 70 post-partum and observed a short luteal phase of 5.0 ± 0.3 days following the first post-ovulatory wave, wherein the CL attained a widest diameter of 8.37 ± 0.35 mm.

Zongo *et al.* (2015), in West African Sahelian goats, observed uterine involution by the third and fourth weeks post-partum. However, the ovarian follicular activity could be observed within day seven post-partum.

2.2. SERUM PROGESTERONE PROFILE

Ali *et al.* (2002), in an ultrasonographic study of post-partum rebound of ovarian activity in Ossimi ewes, found that the first increase in serum progesterone level was noticed by around 43.91 ± 5.1 days (range 23-73 days) post-partum. During suckling period, ovarian activity was found to be resumed in 76.9 per cent of goats.

Lewis and Bolt (1983) studied the effect of suckling on post-partum progesterone release in autumn-lambing ewes and found no difference between suckled and non-suckled ewes in the post-partum release of progesterone.

Katongole and Gombe (1985) conducted work on different reproductive hormones in native goats in Uganda and found that during post-partum period, the plasma progesterone levels remained below 3 ng/ml for five or more months in majority of goats and in some cases, it extended for up to eight months.

Mandiki *et al.* (1990) reported that luteal phase could be noted when plasma progesterone concentration increased to more than 0.2 ng/ml for three or more

consecutive days. They also stated that behavioural oestrus was observed after one or more short luteal phases.

Bauernfeind and Holtz (1991) reported that during oestrous cycle, the highest serum progesterone level (7.2 to 7.3 ng/ml) was attained between days 11 and 14 and the level dropped suddenly to 0.12 on day 20 and 0.10 ng/ml on day 22.

Llewelyn *et al.* (1992) studied the post-partum ovarian activity in native goats in Zimbabwe and described that the earliest period, when the plasma progesterone above 2.0 ng/ml after post-partum, could be regarded as the onset of ovarian cyclicity.

Mascarenhas *et al.* (1995) studied the reproductive activity in Serrana goats and opined that the resumption of ovarian activity occurred when two consecutive samples showed serum progesterone level above 0.5 ng/ml. When the progesterone level became less than 0.5 ng/ml in two consecutive samples, it could be concluded as acyclicity.

Rubianes *et al.* (1995) found that in Corriedale ewes early progesterone level up to 0.5 ng/ml could not be related to oestrus behaviour and the length of the corresponding luteal phase was found to be less (9.2 days) than the normal (11-13 days) luteal phase.

Mbayahaga *et al.* (1998) in their study on local Burundian goats considered 0.4 ng/ml or more of plasma progesterone level in two or more consecutive samples as indicative of the presence of CL. Plasma progesterone concentrations above 0.4 ng/ml on days 4 to 10, 11 to 17 and after 17 days of oestrous cycle were defined as short, normal and long luteal phases, respectively.

Mitchell *et al.* (1998) worked on return of oestrus and ovarian function in post-partum ewes and opined that the initial rise in progesterone level above 1 ng/ml in two consecutive samples could be considered as the onset of ovarian activity.

Al-Hozab *et al.* (1999) studied levels of reproductive hormones in post-partum Zomri goats and stated that the serum progesterone concentration remained at low levels throughout the one month period of study indicating the absence of a luteal phase.

Orita *et al.* (2000) conducted ultrasonographic studies of ovary during oestrous cycle in Shiba goats and opined that the level of serum progesterone could be associated with diameter of the CL.

Teleb *et al.* (2003) studied the return of reproductive functioning in post-partum Damascus goats and found that plasma progesterone concentration began to rise much earlier before the onset of behavioural oestrus in Damascus goats.

Kawu *et al.* (2007) studied the serum progesterone profiling in Nigerian Red Sokoto does and observed a mean progesterone concentration of 0.19 ± 0.02 ng/ml between day one and day 30 post-partum. Progesterone peaks were noted on day five and day 17 with maximum concentration on day 17 (0.26 ± 0.07 ng/ml) and minimum on day 25 (< 0.1 ng/ml) post-partum.

Khanum *et al.* (2007) found that the progesterone level remained at basal level between 0.1 and 0.9 ng/ml throughout the post-partum anoestrus period. A concentration above this value was associated with resumption of post-partum ovarian cyclicity. During oestrous cycle, progesterone began to rise from day three and attain a maximum concentration on day 13 (8.5 ± 3.0 ng/ml) of oestrous cycle. Subsequently, it gradually decreased from day 17 and reached the basal level on day 20.

Hayder and Ali (2008) reported a serum progesterone level of above 1 ng/ml in summer lambed ewes by day 39.0 ± 1.2 while winter lambed ewes showed the same level by day 69.3 ± 1.2 .

Kandiel (2008) observed plasma progesterone concentration of 0.78 ± 0.17 ng/ml on day 2.6 ± 0.6 post-ovulation, following first post-partum ovulation. The short rise in serum progesterone level decreased after 3-5 days followed by the onset of a normal ovarian cyclicity, which indicated the relevance of sub luteal

progesterone priming for the resumption of normal ovarian functioning during post-partum.

Mmbengwa *et al.* (2009) stated that the mean progesterone concentration varied with breed and nutritional status of the animal. Elevated serum progesterone level was noticed in animals which received high energy diet irrespective of breed of the animal.

Takayama *et al.* (2010) reported that Shiba goats after parturition exhibited short oestrous cycle without proper development of CL. Following post-partum ovulation, plasma progesterone concentration increased transiently but returned to baseline level suddenly due to lack of proper luteal tissue.

Kandiel *et al.* (2011) found that the plasma progesterone concentration increased from day two post-partum, reached a value above basal level (0.8 ± 0.2 ng/ml) on day five post-partum and this lower concentration might be due to lower proportion of large to small luteal cells when compared to a cyclic CL. They added that sub-luteal progesterone concentration during post-partum period played a major role in resumption of post-partum ovarian cyclicity in ewes.

Mondal *et al.* (2014) worked on hormonal changes during periparturient period in black Bengal goats and observed that the plasma progesterone remained at basal level until day 25 post-partum.

Badawi *et al.* (2014) recorded the maximum and the minimum concentration of progesterone on day 27 post-partum (0.87 ± 0.4 ng/ml) and on day seven post-partum (0.54 ± 0.2 ng/ml), respectively. There was a gradual rise in progesterone level from day seven to day 10 (0.66 ± 0.1 ng/ml) and persisted at this value up to day 17 post-partum and then increased from day 21 (0.77 ± 0.3 ng/ml) and became maximum on day 27 (0.87 ± 0.4 ng/ml) which was dropped suddenly to a lowest value on day 31 post-partum.

2.3. POST-PARTUM ONSET OF BEHAVIOURAL OESTRUS

Mandiki *et al.* (1989) stated that intensity of suckling had no influence on the onset of oestrus in Texel ewes during post-partum.

Mandiki *et al.* (1990) worked on resumption of post-partum reproductive activity in Texel sheep and stated that weaning had a significant effect on post-partum onset of first oestrus and the duration of the post-partum anoestrus in non-suckling ewes were found to be 15 days lesser than in suckling ewes. But the resumption of ovarian activity was noticed much earlier than behavioural oestrus in suckling ewes.

Arora (1992) reported that in Bengal goats, the mean time required for the onset of post-partum oestrus was 89.9 days.

Llewelyn *et al.* (1992) studied post-partum onset of ovarian cyclicity by progesterone estimation in native goats of Zimbabwe and found that the average period for the onset of cyclicity was 97.3 ± 9.5 days and ranged from days 28 to 133. They observed that suckling controls the non-return of ovarian cyclicity, with onset of ovarian cyclicity observed at the time of weaning.

Husain (1993) recorded the mean time for the onset of post-partum oestrus in Black Bengal does as 77.80 ± 1.66 days, 85.20 ± 1.37 days and 68.90 ± 1.54 days in summer, winter and monsoon, respectively.

Rubianes *et al.* (1995) opined that there was no significant difference between suckling and non-suckling ewes in the average duration of first post-partum luteal phase and the first oestrus and in the average number of luteal phases before the first behavioural oestrus was noticed.

Mbayahaga *et al.* (1998) observed post-partum ovarian activity only by 140 ± 52 days in does and 87 ± 25 days in ewes and the number of luteal phases before behavioural oestrus were 0.5 ± 0.8 in does and 2.3 ± 1.6 in ewes. They concluded that the post-partum onset of first behavioural oestrus was one month longer in the does than in ewes.

Teleb *et al.* (2003) recorded the mean period of onset of first post-partum oestrus as 213.3 ± 8.4 days and 206.6 ± 6.7 days in early and late weaned groups of Damascus does, respectively. They observed that though lactation and suckling did not have any effect on resumption of cyclical ovarian activity, season played a major role in the control of ovarian activity.

Freitas *et al.* (2004) found a negative correlation between body condition score during parturition and days of onset of first post-partum oestrus in Saanen goats. The first post-partum oestrus was noticed by day 56 after kidding. They also observed that Saanen goats with one or two parity showed a longer post-partum anoestrus period compared to animals with higher parity.

Fakruzzaman *et al.* (2012) observed that the mean time for the onset of post-partum oestrus during summer, winter and monsoon season in Black Bengal does were 83.33 ± 2.03 days, 94.00 ± 2.31 days and 76.33 ± 6.36 days, respectively.

Rekik *et al.* (2012) stated that the anoestrus period in goats depended up on factors like breed, season, latitude, suckling intensity and presence of buck.

Nascimento *et al.* (2014) opined that a combination of body condition score, body weight, parity, number of lactation and uterine involution had effect on resumption of ovarian activity. They also found a relative negative correlation between the time of onset of first post-partum oestrus and body condition score at kidding.

2.4. SIGNS OF POST-PARTUM OESTRUS

2.4.1. Duration of oestrus

Roberts (1971) noted that the oestrus remained for an average of 40 h with a range of 24-90 h in goats. A similar duration of oestrus in goats, 30–40 h, was reported by Noakes (2009a).

Angora, Mossi and Creole breeds of goats had a short period of oestrus lasting for 22 h (Shelton, 1978), 20 h (Baril *et al.*, 1993) and 27 h (Tamboura *et*

al., 1998), respectively. Evans and Maxwell (1987) opined that in does the duration of oestrus ranged from 20-40 h. Krishnakumar (1992) mentioned that in Malabari goats the duration of oestrus lasted for an average of 30-40 h.

Hafez and Hafez (1993) mentioned that in does the duration of oestrus in normal oestrous cycle would last for 20-35 h. Jainudeen *et al.* (2000) opined that in does the duration of oestrus varied with age, breed, season, and presence of male and was lasted for about 36 h with a range of 24-48 h.

Greyling (2000) reported that in Boer does the oestrus had a mean duration of 37.4 ± 8.63 h. Goel and Agarwal (2002) opined that the Jakharana does had a mean duration of oestrus of 24-36 h. Rajan (2010) reported a mean duration of oestrus 35.81 ± 0.86 h with a range of 18 to 54 h.

Fakruzzaman *et al.* (2012) reported that in Black Bengal does the duration of oestrus varied with season and with a mean duration of oestrus of 36.00 ± 0.70 h in summer, 44.00 ± 1.95 h in winter and 35.54 ± 0.8 h in rainy season.

2.4.2. Intensity of oestrus

Roberts (1971) reported oestrus signs like acceptance of male, restlessness, frequent bleating, intermittent wagging of tail, vulval oedema, hyperaemia, reduced appetite and milk yield.

Mori and Kano (1984) described the behavioural oestrus in Shiba goats as the period when the doe exhibited tail deviation and standing to mount. Llewelyn *et al.* (1992) opined that in does tail wagging from side to side and up and down could be considered as the most reliable signs of oestrus.

Mbayahaga *et al.* (1998) described the post-partum oestrus as when the female stood for mounting.

Senthilkumar (2002) studied the effect of $\text{PGF}_{2\alpha}$ and pregnant mare serum gonadotropin combinations for improving prolificacy in Malabari goats and observed a mean oestrus intensity score of 8.82 ± 1.59 with a range of 2-17 in the untreated group.

Afsal (2003) observed that the main signs of natural oestrus in Malabari does were frequent bleating, wagging of tail, mounting on other animals, standing to be mounted, circling with buck, vaginal hyperemia, vulval oedema, and vaginal discharge.

Teleb *et al.* (2003) noted that there was no significant relationship between lactation and the duration of onset of sexual activity but season played a main role in regulating the ovarian activity.

Khanum *et al.* (2007) studied post-partum reproductive parameters in Dwarf goats of Pakistan and observed that the return of ovarian activity was attained by 15-59 days with an average length of 27.9 ± 14 days. The mean oestrous cycle length was found to be 19.7 ± 1.5 days.

Farshad *et al.* (2008) identified the behavioural oestrus by observing the signs like sniffing the perineum, vocal exchange, courtship, flehmen reaction, standing oestrus and mounting.

Kandiel (2008), using ultrasonography, observed that 83.3 per cent of animals showed at least one ovulation followed by a short luteal phase but none of the animals exhibited behavioural signs of heat prior to the first ovulation.

Noakes (2009a) mentioned that does during oestrus exhibited heat signs like restlessness, anorexia, reduced milk yield, tail wagging, vulval swelling, redness and mounting behaviour.

Rajan (2010) confirmed oestrus in does by observing the behavioural signs like bleating, wagging of tail, frequent urination, standing to be mounted, circling with buck and flehmen reaction and physiological signs like vulval oedema, vaginal moistness and hyperaemia, presence of mucus discharge and relaxation of external cervical os upon speculum examination.

Fakruzzaman *et al.* (2012) reported that in Black Bengal does, the signs of oestrus observed were restlessness, mounting on animals, wagging of tail, bleating, mock fights, licking and sniffing each other. Vaginal mucus discharge

was described as watery and thin during early oestrus, mucinous in mid-oestrus and thick and cheesy in late oestrus.

Rekik *et al.* (2012) reviewed that unlike ewes, progesterone priming was not necessary for exhibiting oestrus behaviour in does and became responsive to oestrogen without progesterone priming.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Twelve post-partum Malabari goats of parity between two to five, with the history of normal kidding were selected for the study. The goats were maintained under semi-intensive system of housing and provided with standard feeding and management conditions. They were fed with a daily ration of 200g concentrate per animal and green grass and water were given *ad libitum*.

3.1. DESIGN OF THE STUDY

The study was conducted at the Goat Farm under Instructional Livestock Farm Complex, College of Veterinary and Animal Sciences, Pookode, during the month of July 2016 to December 2016. Twelve healthy goats without any post-partum complications were selected for the study and experiment started on day 3 after kidding. The kids were allowed to suckle the does. The bucks were housed in separate pens in the same goat farm.

3.2. ULTRASONOGRAPHIC OBSERVATIONS OF THE UTERUS AND OVARIES

Uterine involution and ovarian activity were monitored by ultrasound examination using B-mode ultrasound scanner (My Lab Vet Gold[®], Esaote Pie Medical, Genova, Italy) with a multi frequency 5.0-10.0 MHz trans-rectal probe on days 3, 6, 9, 12, 15, 20, 25, 30, 35 and 40 post-partum. The does were restrained in standing position for trans-rectal ultrasonography. One of the hind legs was folded up at the time of scanning for proper placement of the probe. The rectum was evacuated with the aid of the gloved hand and adequate ultrasound coupling gel was infused into rectum and also applied to the probe to obtain good contact with the scanning surface and rectal wall. The trans-rectal ultrasound probe was fixed to an extension rod for proper maneuvering in the rectum. For scanning of the uterus and ovaries, the probe was moved approximately 60° to each side along its longitudinal axis. Different parameters measured by trans-rectal ultrasonography were:

3.2.1. Echogenicity of uterine fluid

Presence of contents in the uterus and their echogenicity were examined on days 3, 6, 9, 12, 15, 20, 25, 30, 35 and 40 post-partum. Anechoic uterine lumen with a narrow cavity and very little fluid inside on trans-rectal ultrasonography was considered as completion of involution process.

3.2.2. Diameter of uterine horns

Cross-sectional diameter of the uterine horns were recorded and measured. Uterine involution was considered to be complete when there was no further reduction in the uterine diameter for two successive examinations. The transverse diameter of uterine horns of less than two centimeter and lack of contents in the uterine lumen were the parameters recorded for complete involution.

3.2.3. Diameter of uterine caruncles

Cross-sectional diameter of the uterine caruncles were recorded and measured. Caruncles were visualised as an echogenic, circular or C-shaped structures. The mean size of caruncles were recorded by measuring the diameters of three to five caruncles at a time. The interval from parturition to disappearance of caruncles were also recorded.

3.2.4. Resumption of ovarian activity by the presence of corpus luteum

Ovaries were examined by trans-rectal ultrasonography on days 3, 6, 9, 12, 15, 20, 25, 30, 35 and 40 post-partum for detecting the presence of corpus luteum. It could be visualised as round or oval homogeneous hypo-echoic structure compared to echogenic ovarian stroma.

3.3. SERUM PROGESTERONE PROFILE

3.3.1. Blood collection

Blood samples were collected from jugular vein of animals under study in sterile serum vacutainers (Pro-coagulation Tube Clot Activator, Labtech Medico Pvt. Ltd., India) on days 9, 20, 30 and 40 post-partum for serum progesterone estimation by ELISA. The blood samples were stored overnight at 5°C and then centrifuged at 1500 rpm for 20 min for serum separation. The serum samples were stored in plastic vials at -20°C in deep freezer until analysis.

The serum progesterone level in the animals were analysed using the commercial ELISA kits. The ELISA kit used in the present study was Steroid EIA-Progesterone kit (Alkor Bio, Inc, Russia). The Steroid EIA-Progesterone kit is an Enzyme Immunoassay (EIA) designed for the quantitative analysis of total progesterone in serum or plasma. The kit consists of 96 wells coated with anti-Progesterone monoclonal antibodies.

Principle

The competitive ELISA is a quantitative test for the determination of small molecules such as hormones in the blood serum. The test is based on the principle of competitive binding (Van de weil and Koops, 1986; Prakash *et al.*, 1987).

The progesterone in the test serum sample and progesterone-horseradish peroxidase (HRP) conjugate competes for binding with a constant amount of anti-Progesterone monoclonal antibodies. The anti-Progesterone monoclonal antibodies coated wells are incubated with progesterone calibrators, controls, serum samples, and progesterone-HRP conjugate reagent. During incubation a fixed amount of HRP-labeled progesterone competes with the endogenous progesterone in the calibrators and serum sample for fixed number of binding sites of the specific progesterone antibody. Thus, the amount of progesterone-HRP conjugate immunologically bind to the well progressively decreases while the concentration of the progesterone in the serum sample increases. Unbound progesterone- HRP conjugate will remove and the wells are washed. The substrate tetra methyl benzidine (TMB) is then added, resulting in the development of blue colour. The colour development is stopped with the addition of diluted

hydrochloric acid stop solution and the absorbents are measured spectrophotometrically at 450 nm.

The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled progesterone in the serum sample. A standard curve was obtained by plotting the concentration of the calibrators versus the absorbents. The progesterone concentration of the serum samples was calculated from the standard curve.

Procedure

1. The enzyme immunoassay (EIA) kit components, reagents and serum samples were brought to room temperature (20-25°C) prior to the start of the assay procedure.
2. Dispensed 20 µl of all the six calibrators, one control and serum samples into appropriate anti-progesterone monoclonal antibodies coated wells. Wells A1-A2 was kept empty for blank.
3. Added 150 µl of working solution progesterone – HRP conjugate reagent to each well, except well A1-A2.
4. Incubated the strips for 1 h while shaking (500-800 rpm) at 37°C.
5. The well contents were discarded and the empty wells were washed four times with 300 µl of wash solution per well. Finally struck the plate onto a towel to remove any liquid residue.
6. Added 100 µl of substrate solution to all wells including blank and gently mixed for 5 sec and incubated in the dark at room temperature (20-25°C) for 30 min.
7. The reaction was stopped after 100 µl of stop solution was added and gently mixed for 1-2 min, in all wells. The blue colour in the wells changed to yellow colour immediately.

8. The absorbance (optical densities) was read within 20 min using a microplate reader with a 450 nm filter and the mean absorbance value for each set of calibrators, control and serum samples were calculated.

9. A point to point standard curve was constructed by plotting the mean absorbance obtained for each calibrator against its concentration in ng/ml on graph paper, with absorbance values on the Y axis and concentration on X axis.

10. The mean absorbance values for each serum sample were used to determine the corresponding concentration of progesterone in ng/ml from the standard curve.

3.4. POST-PARTUM ONSET OF BEHAVIOURAL OESTRUS

3.4.1. Onset of oestrus

The oestrus was detected based on behavioural and physiological signs of oestrus. Vulval oedema, moist and hyperaemic vestibular mucous membrane, presence of vaginal discharge, frequent bleating and urination, tendency to mount and immobility reflex when left with a teaser buck were recorded.

3.5. SIGNS OF POST-PARTUM OESTRUM

3.5.1. Duration of oestrus

The duration of oestrus was calculated as the period from the time of onset to the end of the behavioural and physiological signs of oestrus. Each animal was exposed to the teaser buck to identify the onset and termination of oestrus. The time halfway between the last toleration and the first rejection by the doe was recorded as the end of oestrus (Holtz *et al.*, 2008).

3.5.2. Intensity of oestrus

Intensity of oestrus was estimated by giving scores to the behavioural and physiological signs associated with oestrus in goats. The score range from 1-5 as recommended by Senthilkumar (2002) were followed in this study.

3.5.2.1. Oestrus signs and its scoring (Senthilkumar, 2002)

No.	Behavioural signs	Score
1	Wagging of tail	2
2	Bleating	1
3	Circling with buck	1
4	Mounting on other animals	1
5	Standing to be mounted	5

No.	Physiological signs	Score
6	Vulval hyperaemia	2
7	Vulval oedema	3
8	Vaginal discharge	5
Total		20

3.6. ANALYSIS OF THE DATA

The data was compiled and subjected to statistical analysis as per the method of Snedecor and Cochran (1994). Variation in uterine diameter in different days post-partum was tested by using Repeated Measures ANOVA followed by post-hoc analysis. Comparison between CL present and CL absent case with uterine diameter in each day post-partum was done by using independent samples t-test. Analysis of data was done using SPSS software Version 21.0.



**Plate 1. Ultrasonography machine (My Lab Vet Gold[®],
Esaote Pie Medical, Genova, Italy)**

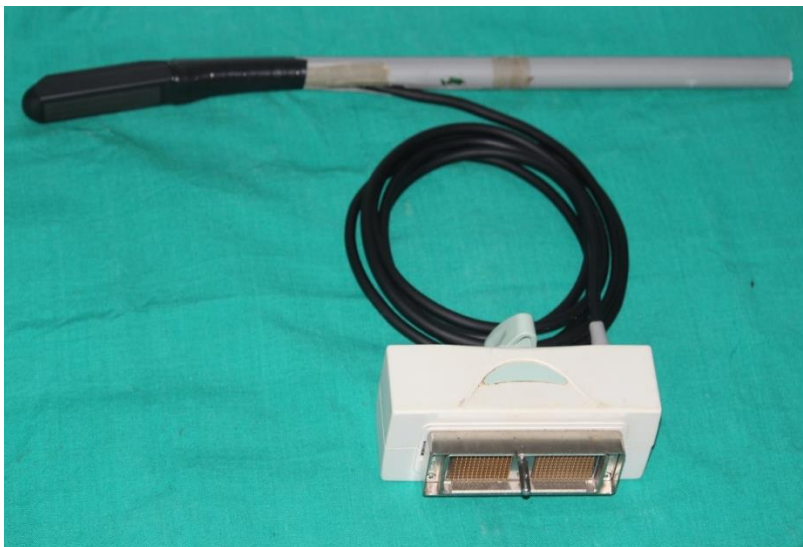


Plate 2 . Trans-rectal probe (Multi frequency 5-10 MHz)

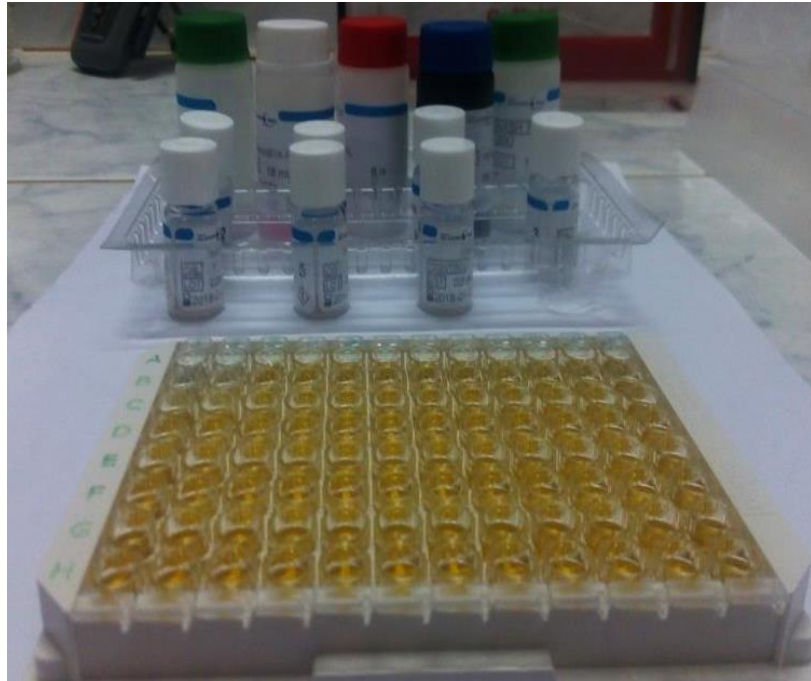


Plate 3. ELISA plate and the reagents

RESULTS

4. RESULTS

Early post-partum uterine involution and resumption of ovarian cyclical activity are two important parameters determining the future fertility of Malabari goats. The present work on “Ultrasonographic studies on post-partum uterine involution and ovarian cyclicity in Malabari goats” was conducted on twelve does with following objectives: (1) To study the course of uterine involution during post-partum period in Malabari goats by ultrasonography. (2) To correlate uterine involution with the post-partum ovarian cyclicity using ultrasonography and serum progesterone estimation. The results of trans-rectal ultrasonography of uterus and ovaries, serum progesterone profile and post-partum onset of behavioural oestrus are discussed here under different headings and sub-headings and are presented in Tables 1 to 10, Plates 4 to 7 and Figures 1 to 6.

4.1. INVOLUTION OF UTERUS

The uterine involution was assessed by considering the changes in parameters like echogenicity of uterine fluid, uterine horn diameter and caruncular diameter. Results of the ultrasonographic assessment of various parameters of the twelve animals are presented in the Tables 1 to 3, Fig. 1 and Plates 4 to 6.

4.1.1. **Assessment of echogenicity of intrauterine fluid by trans-rectal ultrasonography**

The echogenicity of fluid in the uterine lumen assessed by trans-rectal ultrasonography are presented in the Table 1 and Plate 4.

On day one post-partum, by trans-rectal ultrasonographic examination, the uterine fluid was noticed as having a Hypoechoic echotexture due to the presence of tissue debris. By day six post-partum, the uterine fluid became anechoic in eight animals and lacked any tissue debris, but it remained as Hypoechoic echotexture in the rest four animals. By day nine post-partum, the uterine fluid became anechoic in all the animals examined with lack of tissue debris. The fluid

could be appreciated only up to day 12 post-partum. Thereafter the uterine lumen was visible as a narrow anechoic cavity.

4.1.2. Assessment of uterine horn diameter by trans-rectal ultrasonography

The mean uterine horn diameters at different days of post-partum are presented in Table 2 and Plate 5.

The mean uterine horn diameter was found to be less than two centimeter in two consecutive examinations after day 15 post-partum. But statistical analysis of the data revealed that there existed a significant difference ($p \leq 0.01$) in the mean uterine horn diameter up to day 25 post-partum. No significant difference was noticed in the mean diameter of uterine horn after day 25 post-partum.

The mean uterine horn diameter was found to be decreasing on logarithmic scale from day three to day 40 post-partum (Fig. 1). R^2 value of the fitted equation, $y = -0.938 \ln(x) + 4.4069$, is 97 per cent, which showed goodness fit of the regression equation.

4.1.3. Assessment of uterine caruncular diameter by trans-rectal ultrasonography

The mean uterine caruncular diameter at different days post-partum is presented in Table 3 and Plate 6.

The statistical analysis of the data revealed that there was significant difference ($p \leq 0.01$) in the mean uterine caruncular diameter between day three and day six, post-partum. There was no significant difference in the mean uterine caruncular diameter between day six and day nine post-partum. After day nine post-partum, caruncles were inappreciable.

4.2. RESUMPTION OF OVARIAN ACTIVITY

4.2.1. Detection of corpus luteum by trans-rectal ultrasonography

Per cent of animals which resumed ovarian activity during post-partum period was assessed by detecting the presence of CL on trans-rectal B-mode ultrasonography and are presented in the Table 5 and Plate 7. Corpus luteum could be visualised as a roughly circumscribed homogeneous hypoechoic structure compared to the echogenic ovarian stroma.

In all the 12 animals under study, CL was not detected on days 3, 6, 9 and 12 post-partum by the trans-rectal ultrasonography. By day 15 post-partum, CL was detected in 8.33 per cent (n=1) animals. By day 25 post-partum, CL was imaged in 16.67 per cent (n=2) animals. By day 30 post-partum, CL was visualised in 25 per cent (n=3) animals. By day 40 post-partum, CL was detected in 33.33 per cent (n=4) animals.

4.2.2. Detection of corpus luteum by serum progesterone profile

The post-partum ovarian activity as assessed by serum progesterone concentration is presented in the Table 6 and Fig 2 and 3.

By day nine post-partum, 41.67 per cent (n=5) of animals had serum progesterone concentration of more than 1 ng/ml, indicating the resumption of ovarian activity. By day 30 post-partum, 58.33 per cent (n = 7) of animals resumed ovarian cyclicity.

The statistical analysis of the data revealed that there was no significant difference ($p \geq 0.05$) in the mean serum progesterone concentration (ng/ml) of 12 animals between days examined.

4.2.3. Relationship between uterine involution and resumption of ovarian cyclicity using trans-rectal ultrasonography and serum progesterone profiling

Relationship between uterine involution and resumption of ovarian cyclicity using trans-rectal ultrasonography and serum progesterone profiling is presented in the Table 7 and 8.

The statistical analysis of the data revealed that there was no significant relationship ($p \geq 0.05$) between uterine involution and resumption of ovarian cyclicity estimated using trans-rectal ultrasonography and serum progesterone profiling.

4.3. DETECTION OF POST-PARTUM OESTRUS

4.3.1. Onset of post-partum behavioural oestrus

The day of onset of post-partum behavioural oestrus of the 12 animals under study are represented in the Table 9 and Fig 4.

The day of onset of post-partum behavioural oestrus was found to be as early as 47 days and as late as 92 days post-partum with a mean of 62.67 ± 5.249 days.

4.3.2. Signs of post-partum oestrus

4.3.2.1. *Duration of oestrus*

The duration of oestrus noticed during the post-partum behavioural oestrus in the twelve animals is presented in the Table 9 and Fig 5.

The mean duration of oestrus observed in the first post-partum behavioural oestrus was 34.00 ± 2.594 h with a range of 20- 48 h.

4.3.2.2. *Intensity of oestrus*

The intensity of oestrus in the observed animals is scored and classified as high, medium and low and are presented in Table 10 and Fig 6.

The intensity of oestrus was scored based on the behavioural and physiological signs of oestrus observed. The per cent of animals which exhibited high, medium and low intensity of oestrus were 33.33, 41.67 and 25.0, respectively. The mean intensity score recorded was 13.58 ± 0.892 with a score range of 8-17.

Table1. Assessment of echogenicity of uterine fluid on trans-rectal B-mode ultrasonography in post-partum Malabari does (n=12)

Echogenicity of uterine fluid	Days post-partum				
	3	6	9	12	15
Animals with hypoechoic uterine fluid (per cent)	100	33.33	0	0	0
Animals with anechoic uterine fluid (per cent)	0	66.67	100	100	100

Table 2. Assessment of uterine horn diameter (Mean \pm SE), measured by trans-rectal B-mode ultrasonography, in post-partum Malabari does (n=12)

Days post-partum	Uterine horn diameter (Mean \pm SE, cm)	Range (cm)
3	3.495 \pm 0.108 ^a	4.23-2.82
6	2.849 \pm 0.093 ^b	3.45-2.47
9	2.263 \pm 0.103 ^c	2.98-1.67
12	2.004 \pm 0.101 ^c	2.66-1.55
15	1.674 \pm 0.074 ^d	2.29-1.33
20	1.411 \pm 0.045 ^e	1.65-1.17
25	1.308 \pm 0.062 ^{ef}	1.89-1.06
30	1.224 \pm 0.040 ^f	1.43-0.97
35	1.214 \pm 0.022 ^f	1.36-1.09
40	1.163 \pm 0.037 ^f	1.44-0.96
F-value	113.243 ^{**}	
p- value	<0.001	

Means bearing different superscripts in a column differ significantly (p \leq 0.01)

**Significant at 0.01 level

Table 3. Assessment of caruncular diameter (Mean \pm SE), measured by trans-rectal B-mode ultrasonography, in post-partum Malabari does (n=12)

Days post-partum	Caruncular diameter (Mean \pm SE, cm)	Range (cm)
3	1.566 \pm 0.056 ^a	1.72-1.16
6	1.268 \pm 0.048 ^b	1.52-0.89
9	1.120 \pm 0.092 ^b	1.60-0.71
F-value	18.259**	
p-value	<0.001	

Means having different superscripts in a column differ significantly (p \leq 0.01)

**Significant at 0.01 level

Table 4. Post-partum resumption of ovarian activity, by detection of CL on trans-rectal ultrasonography, in Malabari does (n=12)

Days post-partum	Number of animals with CL	Per cent
3	0	0.00
6	0	0.00
9	0	0.00
12	0	0.00
15	1	8.33
20	1	8.33
25	2	16.67
30	3	25.00
35	3	25.00
40	4	33.33

Table 5. Serum progesterone level (Mean \pm SE) in post-partum Malabari does (n=12)

Days post-partum	Serum progesterone level (Mean \pm SE, ng/ml)
9	0.76 \pm 0.152
20	0.53 \pm 0.153
30	0.73 \pm 0.167
40	0.51 \pm 0.140
F-value	0.930 ^{ns}
p-value	0.423

ns- Non-significant; F-value indicates no significant difference ($p \geq 0.05$) in the mean progesterone levels between days examined.

Table 6. Post-partum resumption of ovarian activity, assessed by serum progesterone levels (>1 ng/ml), in Malabari does (n=7)

Days post-partum	Number of animals resumed	Per cent
9	5	41.67
20	5	41.67
30	7	58.33
40	7	58.33

Table 7. Relationship between uterine horn diameter and resumption of ovarian cyclicity, assessed by trans-rectal ultrasonography, in post-partum Malabari does (n=12)

Presence of CL	Uterine horn diameter (cm) at different days post-partum			
	15	25	30	40
Present	1.72 ± 0.000	1.190 ± 0.000	1.120 ± 0.000	1.190 ± 0.000
Absent	1.67 ± 0.081	1.319 ± 0.067	1.234 ± 0.042	1.161 ± 0.040
t- value	0.178 ^{ns}	0.557 ^{ns}	0.779 ^{ns}	0.208 ^{ns}
p-value	0.862	0.590	0.454	0.839

ns- Non-significant

t-value indicates non-significant relationship ($p \geq 0.05$) between the presence of CL and uterine horn diameter

Table 8. Relationship between uterine horn diameter and resumption of ovarian cyclicity, assessed by serum progesterone levels, in post-partum Malabari does (n=12)

Serum progesterone level	Uterine horn diameter (cm) at different days post-partum			
	9	20	30	40
>1 ng/ml	2.380±0.232	1.40±0.080	1.190±0.064	1.190±0.000
<1 ng/ml	2.180±0.0683	1.413±0.053	1.241±0.052	1.161±0.040
t- value	0.827 ^{ns}	0.103 ^{ns}	0.592 ^{ns}	0.208 ^{ns}
p-value	0.448	0.920	0.567	0.839

ns- Non-significant

t-value indicates non-significant relationship ($p \geq 0.05$) between the serum progesterone level and uterine horn diameter

Table 9. Characteristics of post-partum behavioural oestrus in Malabari does (n=12)

Animal no.	Post-partum onset of oestrus (days)	Oestrus duration (h)
1	50	36
2	50	36
3	47	48
4	48	42
5	53	48
6	92	24
7	90	24
8	88	20
9	48	30
10	48	36
11	70	32
12	68	32
Mean ± SE	62.67 ± 5.249	34.00 ± 2.594

Table 10. Number and per cent of Malabari does exhibiting varying intensity of behavioural oestrus post-partum (n=12)

Intensity of oestrus	Number of animal (%)
High (score15-20)	4 (33.33 %)
Medium (score10-15)	5 (41.67%)
Low (score5-10)	3 (25.0%)

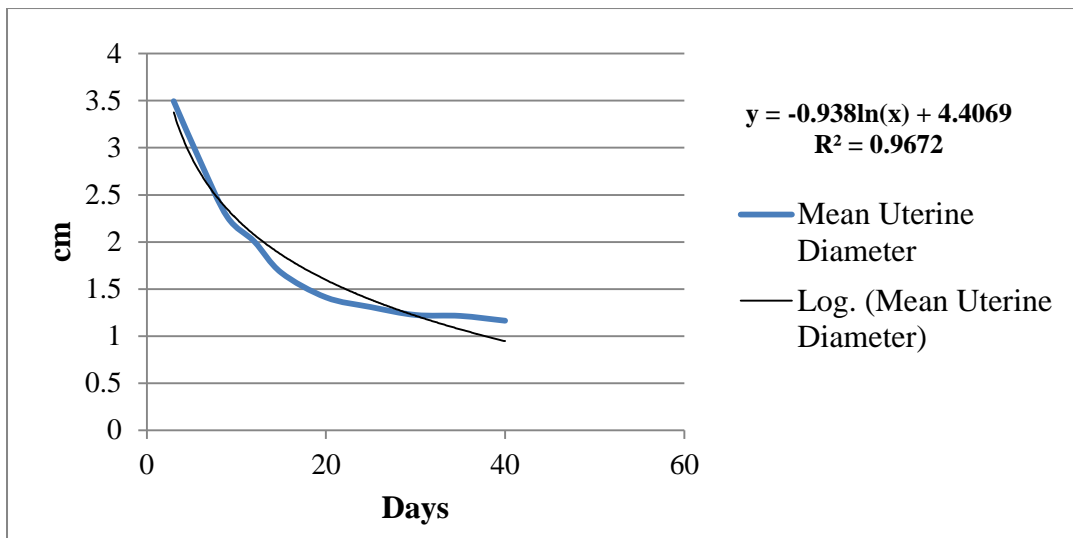


Fig 1. Diameter of the uterine horn in post-partum Malabari does (n=12), decreasing logarithmically between day three and day 40 post-partum

R^2 value of the fitted equation, $y = -0.938\ln(x) + 4.4069$, is 0.9672, showing goodness fit of the regression equation

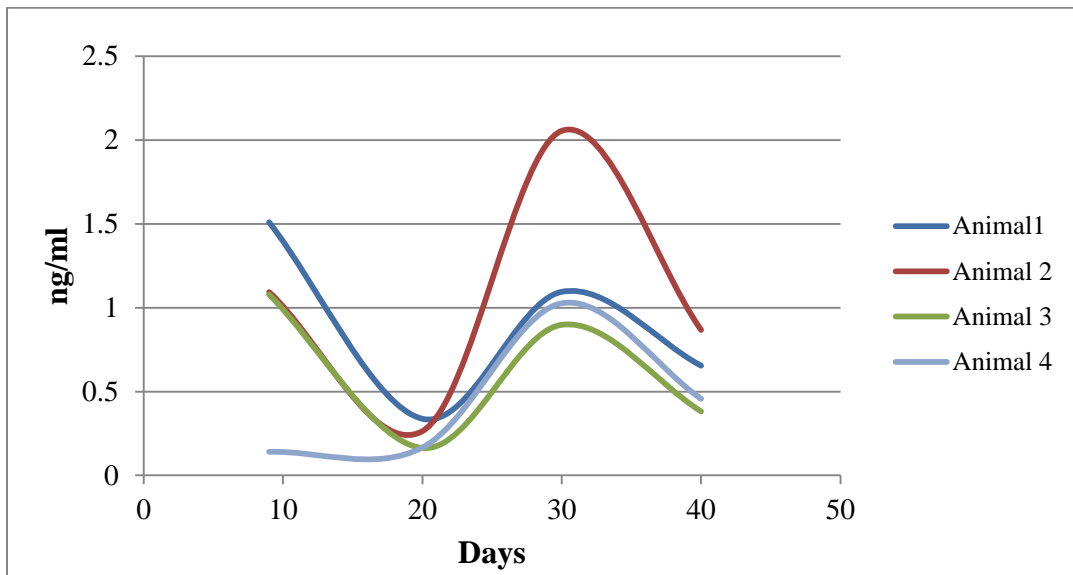


Fig 2. Serum progesterone levels of post-partum Malabari does which resumed ovarian activity (>1 ng/ml)

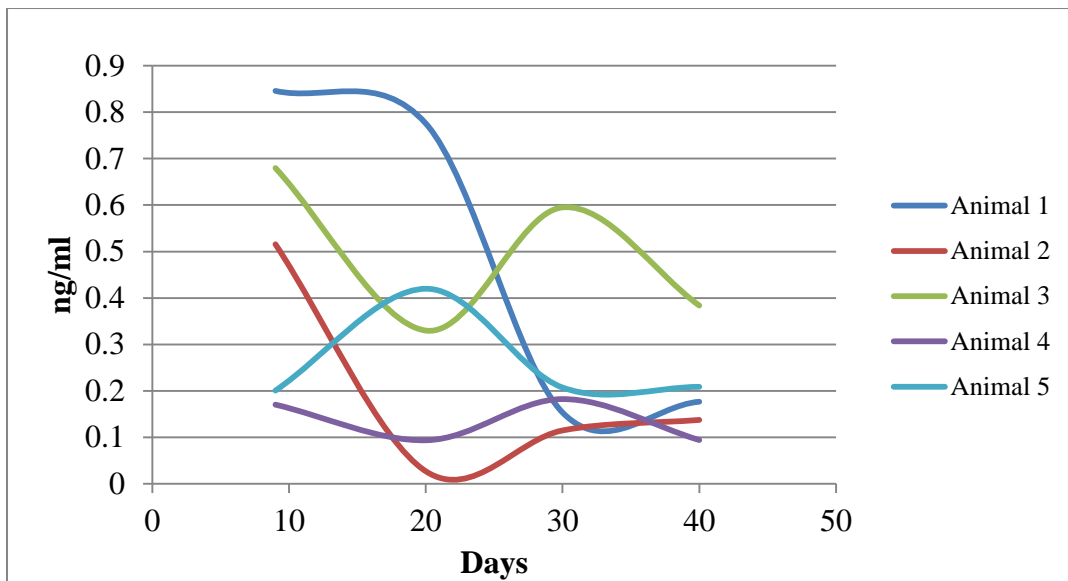


Fig 3. Serum progesterone levels of post-partum Malabari does which did not resumed ovarian activity (<1 ng/ml)

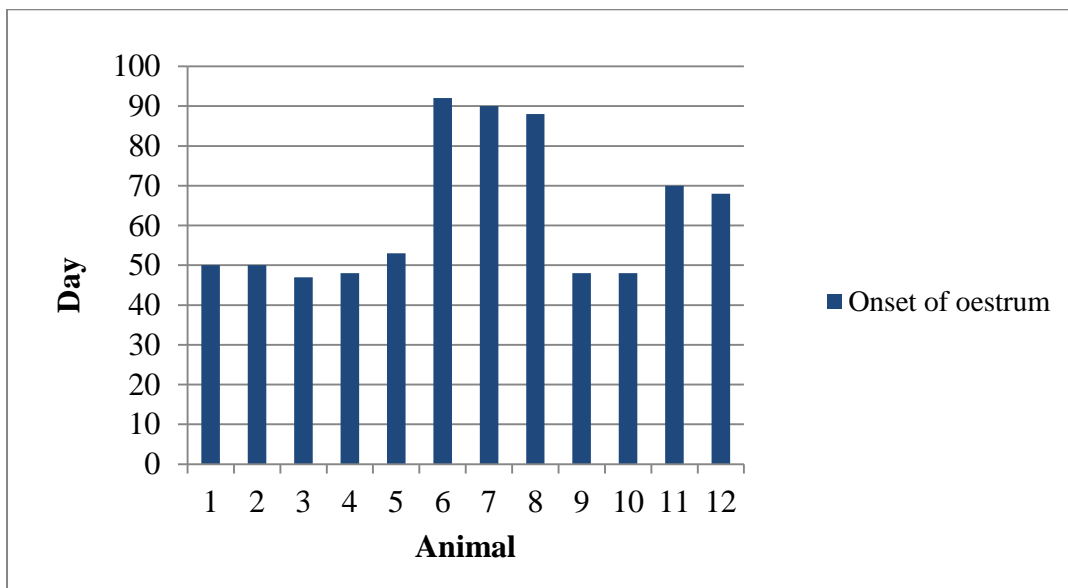


Fig 4. Day of onset of post-partum behavioural oestrus in Malabari does (n=12)

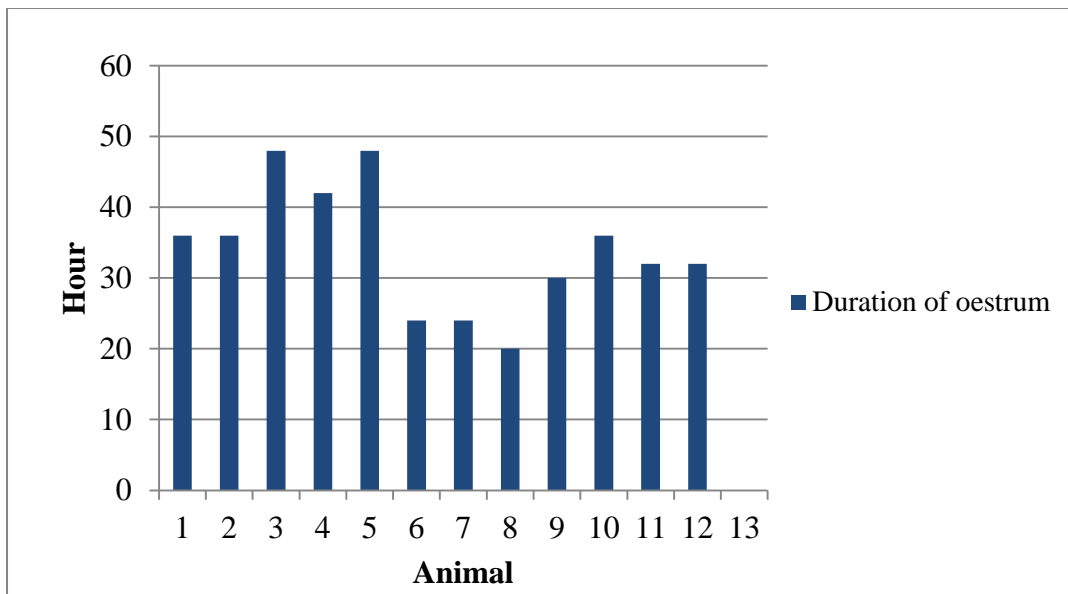


Fig 5. Duration of post-partum behavioural oestrus in Malabari does (n=12)

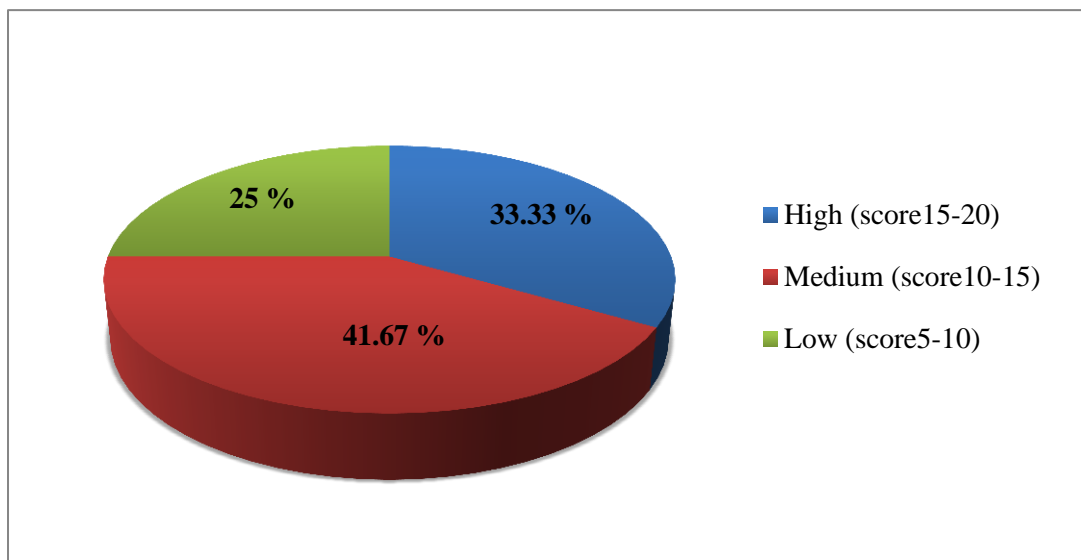


Fig 6. Intensity of first behavioural oestrus (per cent) in post-partum Malabari does (n=12)

Plate 4.

Ultrasonographic images of echogenicity of fluid in the uterine lumen from day 3 to day 12 post-partum



A. Day 3 post-partum



B. Day 6 post-partum



C. Day 9 post-partum



D. Day 12 post-partum

Plate 5.

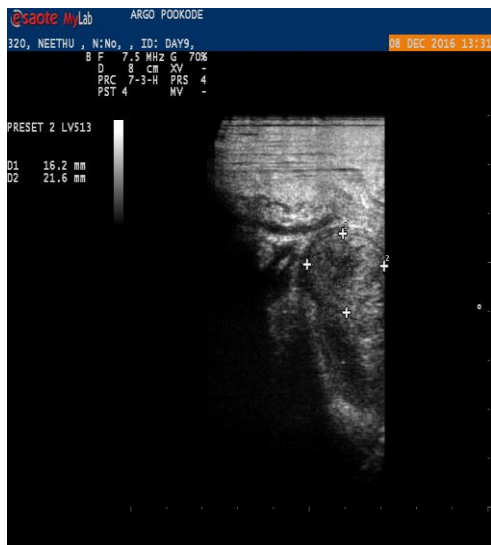
Ultrasonographic measurements of cross-sectional diameter of uterine horn from day 3 to day 40 post-partum



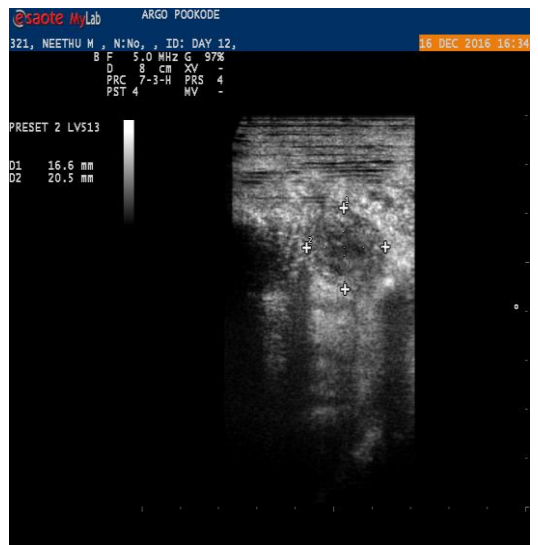
E. Day 3 post-partum



F. Day 6 post-partum



G. Day 9 post-partum



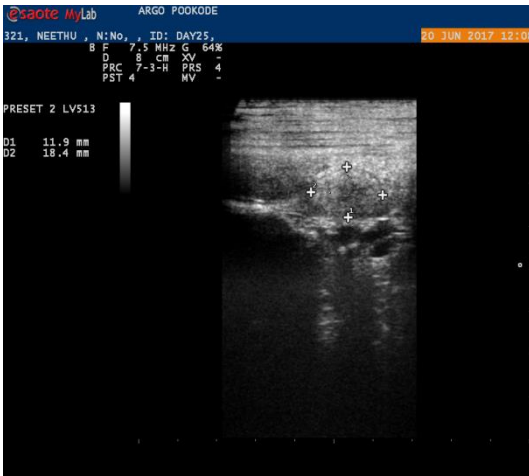
H. Day 12 post-partum



I. Day 15 post-partum



J. Day 20 post-partum



K. Day 25 post-partum



L. Day 30 post-partum



M. Day 35 post-partum



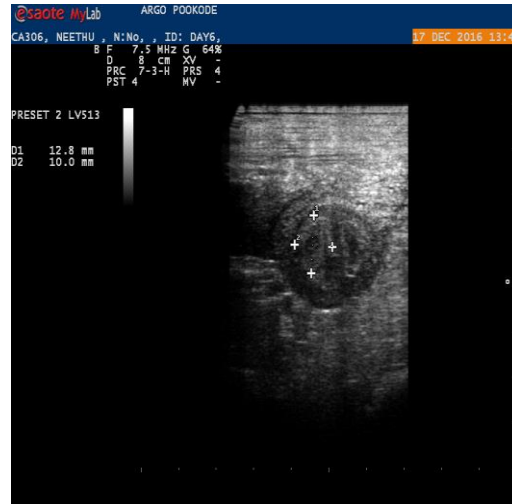
N. Day 40 post-partum

Plate 6.

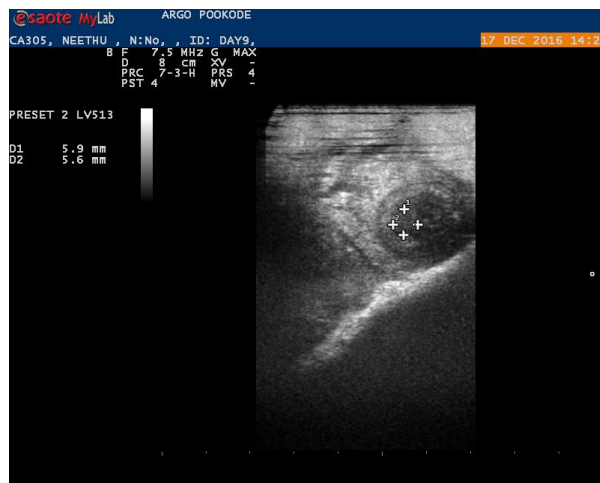
**Ultrasonographic measurements of cross-sectional diameter
of uterine caruncles from day 3 to day 9 post-partum**



O. Day 3 post-partum

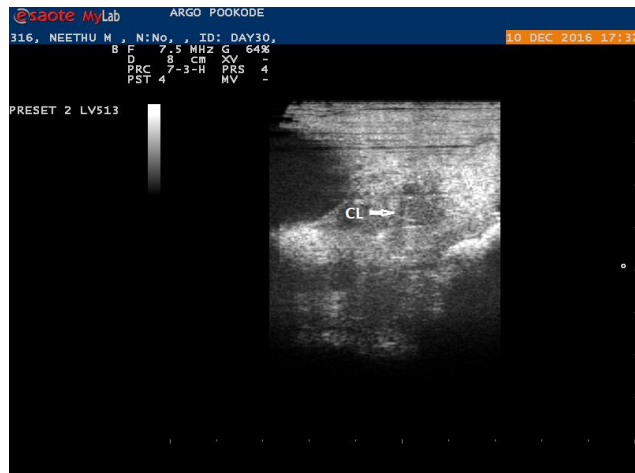


P. Day 6 post-partum



Q. Day 9 post-partum

Plate 7.



**R. Ultrasonographic image of corpus luteum
observed on day 30 post-partum**

DISCUSSION

5. DISCUSSION

The present study was conducted to obtain a baseline data on post-partum uterine involution and resumption of ovarian activity in Malabari goats of Kerala by real time B-mode trans-rectal ultrasonography. Serum progesterone assay was also done to support the findings of the ultrasonography for the assessment of luteal activity in these animals. The examinations were done from day three to day 40 post-partum.

5.1. INVOLUTION OF UTERUS

5.1.1. **Assessment of the echogenicity of intrauterine fluid by trans-rectal ultrasonography**

Uterine fluid and tissue debris were noticed as hypoechoic areas in the uterine lumen on the first day (day three) of ultrasonographic examination. By day six post-partum, uterine lumen was found to be more clear and observed as anechoic echo-texture without tissue debris in 66.67 per cent (8/12) animals but remained hypoechoic in 33.33 per cent (4/12) animals. The lumen became anechoic in all the animals by day nine post-partum and the fluid was appreciated only up to day 12 post-partum. After that, the lumen was visualised as a narrow cavity with a scant fluid inside.

The echogenicity of the lochia on the first day of observation (day three) was in agreement with the reports of Ababneh and Degefa (2005), who noticed the presence of lochia and tissue debris as hypoechoic texture inside the uterine lumen up to day four post-partum and it was completely eliminated between day four to seven post-partum. In the present study, hypoechoic nature of uterine fluid persisted in four animals (4/12) till day six post-partum indicating the presence of tissue debris. Fluid and tissue debris were completely eliminated in all the animals under study by 12-15 days postpartum. But Badawi *et al.* (2014) observed complete evacuation of uterine fluid by 4- 7 days post-partum.

5.1.2. Assessment of uterine horn diameter by trans-rectal ultrasonography

The mean cross-sectional diameter of the uterine horn recorded was 3.50 ± 0.108 cm on day three post-partum and 1.16 ± 0.037 cm on day 40 post-partum. Badawi *et al.* (2014) observed a maximum uterine diameter of 3.28 ± 3.6 cm on day three post-partum in Nubian goats and Zongo *et al.* (2015) observed a mean diameter of 1.72 ± 0.27 cm on day 31 post-partum in West African Sahelian goats. In the present study, no significant difference in the cross-sectional diameter of the uterine horn was noticed after day 25 post-partum in Malabari does. Thus the day of completion of uterine involution in Malabari goats can be considered to be occurring between the days 25 and 30 post-partum, when the mean diameter becomes 1.31 ± 0.062 cm. The day of involution was similar to the findings of Badawi *et al.* (2014), Enginler *et al.* (2014) and Zongo *et al.* (2015) in Nubian, Saanen and West African Sahelian goats, respectively. But Ababneh and Degefa (2005) observed the completion of involution much earlier by day 19 post-partum in Balady goats. The variability in time required for complete uterine involution may be due to difference in breed, reproductive status and body condition.

The completion of uterine involution was characterised by the absence of uterine fluid in the lumen, the absence of uterine caruncle and with no significant difference in the mean diameter of the uterine horn between three consecutive examinations (Badawi *et al.*, 2014).

Zongo *et al.* (2015) stated that the end of involution is marked by less than two centimeter transversal diameter of uterine horn and absence of fluid in the uterus. But, in Malabari goats although the mean uterine diameter was found to be less than two centimeter in two consecutive examinations after day 15 post-partum, there existed a significant difference in the mean uterine horn diameter up to day 25 post-partum. No significant difference was noticed in the mean diameter of uterine horn after day 25 post-partum in Malabari goats.

In the present study, the mean uterine horn diameter decreased logarithmically from day three to day 40 post-partum. A rapid reduction in

uterine horn diameter was observed between day three and 15 post-partum (>50 per cent) while gradual non-significant change was noticed between days 25 and 40 post-partum which was closer to the observations of Badawi *et al.* (2014).

Thus in Malabari does, a post-partum uterine horn diameter of 1.31 ± 0.062 cm might be considered as indicative of uterine involution, rather than two centimeter as reported for other breeds of does.

5.1.3. Assessment of uterine caruncular diameter by trans-rectal ultrasonography

The maximum and minimum mean cross-sectional diameter of the uterine caruncle, observed in the present study, was 1.57 ± 0.056 cm on day three post-partum and 1.12 ± 0.092 cm on day nine post-partum, respectively. Zongo *et al.* (2015) observed caruncles with a transversal diameter of 1.78 ± 0.42 cm on day one post-partum and Hauser and Bostedt (2002) observed a diameter of 1.24 ± 0.17 cm on day eight post-partum.

The statistical analysis of the data revealed that there was significant difference ($p < 0.01$) in the mean uterine caruncular diameter between the day three and six post-partum. A statistically significant difference ($p < 0.05$) on first day post-partum against third day post-partum was demonstrated by Fasulkov (2014). In the present study, no significant difference was noticed in the mean uterine caruncular diameter between day six and day nine post-partum.

After day nine post-partum, in the present study, caruncles were inappreciable. Hauser and Bostedt (2002), Fasulkov (2014) and Zongo *et al.* (2015) also reported similar observations in German improved land breed of sheep, local Bulgarian goats and West African Sahelian goats, respectively.

5.2. RESUMPTION OF OVARIAN ACTIVITY

5.2.1. **Detection of corpus luteum by trans-rectal ultrasonography**

In the present study, CL was not detected in any of the experimental animals up to day 12 post-partum. By day 15 post-partum, CL was detected in 8.33 per cent (n=1) animals. By day 40 post-partum, CL was detected in 33.33 per cent (n=4) animals. Thus in the present study, 33.33 per cent of animals resumed of ovarian cyclical activity within day 40 post-partum which was lower than the observations by Mascarenhas *et al.* (1995) who observed 75 per cent animals with resumption of the ovarian activity between days 20 and 50 post-partum. This lower per cent of detection of CL may be due to the absence of a proper luteal tissue (Takayama *et al.*, 2010) and was in accordance to the observations of Kandiel *et al.* (2011) who noticed post-partum ovarian activity in 83.3 per cent animals with a short luteal phase of 5 ± 0.3 days within 70 days post-partum.

Mandiki *et al.* (1990) noticed the resumption of ovarian activity much earlier than the behavioural oestrus in suckling ewes. In contrast, Hayder and Ali (2008) noticed the first post-partum CL only within 42 days in Farafra adult ewes.

In the present study, the CL was visualised as hypoechoic in echo-texture compared to the echogenic ovarian stroma with a round or oval contour. The echogenicity and shape of the CL were similar to the observations of Orita *et al.* (2000), Simoes *et al.* (2005) and Kandiel (2008).

5.2.2. **Detection of corpus luteum by serum progesterone profile**

By day nine post-partum, 41.67 per cent (n=5) of animals showed serum progesterone concentration of more than 1 ng/ml, indicating the resumption of ovarian activity. By day 40 post-partum, 58.33 per cent (n=7) of animals showed resumption of ovarian cyclicity. But in five animals (41.67 per cent) the concentration remained less than 1 ng/ml, throughout the period of study, without resumption of the ovarian activity.

Katongole and Gombe (1985) considered 3 ng/ml as the baseline progesterone level in plasma and observed that the plasma progesterone levels remained below 3 ng/ml for five to eight months during post-partum. Llewelyn *et al.* (1992) described the plasma progesterone above 2.0 ng/ml after post-partum as the onset of ovarian cyclicity. Khanum *et al.* (2007) in does and Mitchell *et al.* (1998) and Medan and EL-Daek (2015) in ewes considered the resumption of ovarian activity with levels above 1 ng/ml while Mascarenhas *et al.* (1995) and Rubianes *et al.* (1995) considered with level above 0.5 ng/ml as resumption of ovarian cyclical activity. Mbayahaga *et al.* (1998) reported 0.4 ng/ml as the baseline concentration.

The maximum serum progesterone concentration noticed in the present study was 2.05 ng/ml and this lower concentration may be attributed to the absence of a proper luteal tissue (Takayama *et al.*, 2010; Kandiel *et al.*, 2011). A short luteal phase was observed in the case of low serum progesterone concentration (Rubianes *et al.*, 1995; Mbayahaga *et al.*, 1998). In the present study, the serum progesterone concentration began to rise much before the onset of behavioural oestrus which is similar to the findings of Teleb *et al.* (2003).

Al-Hozab *et al.* (1999), Kawu (2007), Mondal *et al.* (2014) and Badawi *et al.* (2014) stated that the serum progesterone level remained low throughout a one month period study.

The statistical analysis of the data revealed that there was no significant difference in the mean serum progesterone concentration (ng/ml) of 12 animals on days 9, 20, 30 and 40 post-partum. The mean serum concentration remained < 1 ng/ml in all the days examined indicating the absence of sufficient luteal tissue.

In the present study, the uterine involution was completed between days 25 and 30 post-partum but the luteal activity was noticed as early as nine days post-partum. The statistical analysis of the data revealed that there is no significant difference in the relationship between uterine involution and resumption of ovarian cyclicity when estimated using trans-rectal ultrasonography and serum

progesterone profiling. The finding is found to have a close association to the observations of Zongo *et al.* (2015).

5.3. DETECTION OF POST-PARTUM OESTRUS

5.3.1. Onset of post-partum behavioural oestrus

The day of onset of post-partum behavioural oestrus was 62.67 ± 5.25 (Mean \pm SE) days with a range of 47-92 days.

A long period for the post-partum behavioural oestrus was observed by Llewelyn *et al.* (1992) (97.3 ± 9.5 days), Mbayahaga *et al.* (1998) (140 ± 52 days) and Teleb *et al.* (2003) (213.3 ± 8.4 and 206.6 ± 6.7 days), while a short period of 15-59 days was noticed by Khanum *et al.* (2007). In Malabari goats, the days of onset of post-partum behavioural oestrus was found to be earlier than other breeds.

A negative correlation between the time of onset of post-partum behavioural oestrus and the body condition score was reported (Llewelyn *et al.*, 1992, Freitas *et al.*, 2004 and Nascimento *et al.*, 2014). Mandiki *et al.* (1990) and Llewelyn *et al.* (1992) stated that suckling had a significant effect on post-partum onset of first oestrus. The post-partum anoestrus will depend upon breed, season, latitude, suckling intensity and presence of buck (Rekik *et al.*, 2012).

Mbayahaga *et al.* (1998) observed that the post-partum onset of behavioural oestrus was one month longer in does than in ewes.

The resumption of ovarian activity depended on breed, nutritional status (Mmbengwa *et al.* (2009), body condition score, energy, body weight, parity, lactation and uterine involution (Nascimento *et al.*, 2014).

5.3.2. Signs of post-partum oestrus

5.3.2.1. *Duration of oestrus*

Mean \pm SE duration of oestrus observed in the first post-partum behavioural oestrus was 34.00 ± 2.594 h with a range of 48 - 20 h.

The mean duration of oestrus observed in the present study was in agreement with the observations by Roberts (1971), Evans and Maxwell (1987) Hafez and Hafez (1993) and Noakes (2009a). But in the present study, the range of oestrus observed was wider. A short period of oestrus lasting for 22 h (Shelton, 1978), 27 h (Tamboura *et al.*, 1998), 20 h (Baril *et al.*, 1993), 37.4 ± 8.63 h (Greyling, 2000), 36 h (Jainudeen *et al.*, 2000) and 24-36 h (Goel and Agarwal, 2002) were also reported in different breeds.

In Malabari goats, a similar observation was reported by Krishnakumar (1992) with 30-40 h and Rajan (2010) with 35.81 ± 0.86 h (range 18 to 54 h) of duration of oestrus.

In does, the duration of oestrus varied with age, breed, season (Fakruzzaman *et al.*, 2012) and presence of male (Jainudeen *et al.*, 2000).

5.3.2.2. *Intensity of oestrus*

The intensity of oestrus was scored based on the behavioural and physiological signs of oestrus observed. In does, oestrus was confirmed by observing behavioural signs like wagging of tail, bleating, frequent urination, circling with buck, mounting on other animals, standing to be mounted and physiological signs like vulval oedema, vestibular moistness and hyperaemia, and presence of vaginal discharge. The per cent of animals that exhibited high, medium and low intensity of oestrus were 33.33 per cent, 41.6 per cent and 25 per cent, respectively. The mean \pm SE intensity score obtained was 13.58 ± 0.892 with a range of 8-17. Oestrus duration and intensity noticed in the post-partum period was apparently similar to a normal cyclic animal.

The signs of oestrus observed was in according to the observations of Noakes (2009a), Llewelyn *et al.* (1992), Afsal (2003), Farshad *et al.* (2008), Rajan (2010) in Malabari goats and Fakruzzaman (2012) in Black Bengal goats. The mean intensity score obtained remained within the range of score measured by Senthilkumar (2002) and Panicker (2011) in Malabari goats.

From the present study, it is concluded that the uterine involution period in Malabari does ranges between day 25 and 30 post-partum with a mean uterine horn diameter of 1.31 ± 0.06 cm. The post-partum resumption of ovarian activity occurs much before the completion of the involution of the uterus. There exist no significant difference in the relationship between uterine involution and resumption of ovarian cyclicity when estimated using trans-rectal ultrasonography and serum progesterone profiling. The resumption of ovarian activity was not found to be associated with the behavioural oestrus.

SUMMARY

6. SUMMARY

The present study was conducted to understand the normal post-partum uterine involution and the resumption of ovarian activity in Malabari goats, the indigenous breed of Kerala. Twelve post-partum Malabari does having parity between two to five and with the history of normal kidding were selected for the study. The study was conducted at the Goat Farm under Instructional Livestock Farm Complex, College of Veterinary and Animal Sciences, Pookode. Healthy animals without any post-partum complications were selected for the study and the experiment started on day three post-partum. Uterine involution and ovarian activity were monitored using B-mode multi-frequency 5-10 MHz trans-rectal probe on days 3, 6, 9, 12, 15, 20, 25, 30, 35 and 40 post-partum. Uterine involution was considered to be complete in the absence of fluid in the uterine lumen, absence of uterine caruncles and no significant reduction in the uterine diameter for two successive examinations. The parameters recorded and measured by trans-rectal ultrasonography were the echogenicity of the uterine fluid and cross-sectional diameter of the uterine horns and uterine caruncles. Visualisation of caruncle was possible only up to the day 9 post-partum. Ovaries were imaged for detecting the presence of corpus luteum, indicating resumption of ovarian cyclicity. The serum progesterone level in the animals was analysed using SteroidEIA-Progesterone kit for correlating the status of CL assessed by ultrasonography. The oestrus was detected based on the behavioural and physiological signs of oestrus observed and recorded. The duration of oestrus was calculated as the period from the time of onset to the end of the behavioural and physiological signs of oestrus. Each animal was exposed to a teaser buck to identify the onset and termination of oestrus. The time halfway between the last toleration and the first rejection by the doe was recorded as the end of oestrus. Intensity of oestrus was estimated by giving scores to the behavioural and physiological signs associated with oestrus in goats.

The lochia was noticed as hypo-echoic due to the presence of tissue debris in day three post-partum on ultrasonographic examination. By day six post-

partum, the uterine fluid became anechoic in eight animals without any tissue debris, but the lumen remained hypoechoic in four animals. By day nine post-partum, the uterine fluid became anechoic without any tissue debris in all the animals examined. The fluid could be appreciable up to day 12 post-partum. Thereafter the uterine lumen was visible as a narrow anechoic cavity.

The mean uterine horn diameter (Mean \pm SE) of 12 animals assessed by trans-rectal ultrasonography on days 3, 6, 9, 12, 15, 20, 25, 30, 35 and 40 post-partum were 3.50 ± 0.108 , 2.85 ± 0.093 , 2.26 ± 0.103 , 2.00 ± 0.101 , 1.67 ± 0.074 , 1.41 ± 0.045 , 1.31 ± 0.062 , 1.22 ± 0.040 , 1.21 ± 0.022 , and 1.16 ± 0.037 cm, respectively. The statistical analysis of the data revealed that there exists a significant difference ($p \leq 0.01$) in the mean uterine horn diameter up to day 25 post-partum. No significant difference was noticed in the mean diameter after day 25 post-partum. The mean uterine horn diameter was found to be decreasing on logarithmic scale from day three to day 40 post-partum.

The mean uterine caruncular diameter (Mean \pm SE) on days 3, 6, and 9 post-partum were 1.57 ± 0.056 , 1.27 ± 0.048 and 1.12 ± 0.092 cm, respectively. The statistical analysis of the data revealed that there exists significant difference ($p \leq 0.01$) in the mean uterine caruncular diameter between day three and six post-partum. There exists no significant difference in the mean uterine caruncular diameter between day six and nine post-partum.

The presence of CL was not appreciated by the trans-rectal ultrasonography up to day 12 post-partum in all the animals examined. By day 15 post-partum, CL was detected in 8.33 per cent (n=1) animals. By day 40 post-partum, CL was detected in 33.33 per cent (n=4) animals, indicating resumption the ovarian activity.

By day nine post-partum, 41.67 per cent (n=5) of animals had serum progesterone concentration more than 1 ng/ml, indicating the resumption of ovarian activity. By day 40 post-partum, 58.33 per cent (n=7) of animals were noticed with resumption the ovarian cyclicity.

Mean (Mean \pm SE) serum progesterone concentration of 12 animals in days 9, 20, 30 and 40 post-partum were 0.76 ± 0.152 , 0.53 ± 0.153 , 0.73 ± 0.167 and 0.51 ± 0.140 ng/ml, respectively. The statistical analysis of the data revealed that there was no significant difference ($p \geq 0.01$) in the mean serum progesterone concentration (ng/ml) of 12 animals in days 9, 20, 30 and 40 post-partum.

On statistical analysis, no significant ($p \geq 0.01$) relationship between uterine involution and resumption of ovarian cyclicity was observed when estimated using both trans-rectal ultrasonography and serum progesterone profiling.

The mean day for the onset of first post-partum behavioural oestrus was found to be 62.67 ± 5.249 days of post-partum with a range of 47-92 days and the mean duration of oestrus observed in the first post-partum behavioural oestrus was 34.00 ± 2.594 h with a range of 20- 48 h. The per cent of animals exhibited with high, medium and low intensity of oestrus were 33.33 per cent, 41.67 per cent and 25 per cent, respectively. A mean intensity score of 13.58 ± 0.892 with a range of 8-17 was obtained in the study.

From the results obtained, the mean uterine involution period in Malabari does was found to be between day 25 and 30 post-partum and the resumption of ovarian activity occurred before the completion of uterine involution in 58.33 per cent animals. No significant relationship was observed between uterine involution and ovarian activity when estimated by trans-rectal ultrasonography and serum progesterone profiling. The resumption of ovarian activity was independent of behavioural oestrus.

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**ULTRASONOGRAPHIC STUDIES ON POST-PARTUM
UTERINE INVOLUTION AND OVARIAN CYCLICITY IN
MALABARI GOATS**

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(15-MVP-13)**

ABSTRACT

Submitted in partial fulfilment of the requirement for the degree of

**MASTER OF VETERINARY SCIENCE
(Animal Reproduction, Gynaecology and Obstetrics)**

2017

**Faculty of Veterinary and Animal Sciences
Kerala Veterinary and Animal Sciences University**



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ABSTRACT

The study was conducted at the Goat Farm under ILFC, College of Veterinary and Animal Sciences, Pookode with the objective to study the uterine involution in post-partum Malabari does by ultrasonography and to correlate it with the ovarian rebound using ultrasonography and serum progesterone estimation. Twelve post-partum Malabari does having parity between two to five, were examined from day three to 40 post-partum. Ultrasonography was done to study the changes in the uterus and ovaries during post-partum and the serum progesterone level was estimated for correlating the status of CL assessed by ultrasonography. The post-partum behavioural oestrus was also studied. The uterine lumen was noticed as hypoechoic due to the presence of fluid and tissue debris in day three post-partum. By day nine post-partum, the uterine fluid appeared as anechoic without any tissue debris. The fluid could be appreciable up to day 12 post-partum. The mean uterine horn diameter was found to be decreasing logarithmically from day three to day 40 post-partum. The involution was considered to have occurred between day 25 and 30 post-partum with a mean uterine horn diameter of 1.31 ± 0.062 cm on day 25 post-partum. No significant difference was observed in the mean uterine caruncular diameter between day six and nine post-partum. The caruncles were inappreciable after day nine post-partum on ultrasonography. Resumption of ovarian activity was noticed during the period of study in 33.33 per cent animals by detecting the presence of CL using trans-rectal ultrasonography and in 58.33 per cent animals by assaying serum progesterone profile, with a concentration > 1 ng/ml, considered as indicative of onset of cyclicity. The relationship between uterine involution and resumption of ovarian activity was found to be non-significant ($p \geq 0.01$), on statistical analysis, when estimated using ultrasonography and serum progesterone profiling. The mean day for the onset of post-partum behavioural oestrus was 62.67 ± 5.249 days. The duration of oestrus observed in the first post-partum behavioural oestrus was with a mean of 34.00 ± 2.594 h with a mean intensity score of 13.58 ± 0.892 .

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KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY
Faculty of Veterinary and Animal Sciences
PROGRAMME OF RESEARCH WORK FOR MASTERS DEGREE
THESIS

(Vide Rule 25(b) of Post Graduate regulations 1988)

- 1. Title of the thesis**
Ultrasonographic studies on postpartum uterine involution and ovarian cyclicity in Malabari goats.
- 2. (a) Title of the departmental/KVASU research project of which this forms a part**
Not applicable
- (b) Code No. if any, and order by which the departmental/KVASU research project is approved**
Not applicable
- 3. (a) Name of student**
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- (b) Admission No.**
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- 4. (a) Name of Major Advisor (Guide)**
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Pookode, Wayanad, 673 576.
- 5. Objectives of the study**
3. To study the course of uterine involution during postpartum period by ultrasonography in Malabari goats.
4. To correlate uterine involution with the postpartum ovarian cyclicity using ultrasonography and serum progesterone estimation.
- 6. Practical / Scientific utility**
- The reproductive performance of small ruminants is of great economic significance, as it determines the annual offspring number. Uterine involution and resumption of ovarian activity are important factors for future fertility. In small ruminants, involution is characterized by rapid reduction of uterine size, most commonly between the 3rd and the 10th day postpartum. On

ultrasonographic examination the absence of intrauterine fluid accumulation after day 4 to 7 postpartum confirms quick regression of uterus.

Estimation of progesterone level has been considered as one of the most important parameter to determine the fertility status.

There are only few reports on ultrasound imaging for assessing uterine involution in goats. The study will help in early re-breeding of does and reduce kidding interval, thus increase the number of kidding per year.

7. Important Publications on which the study is based

Rubianes and Ungerfeld (1993) concluded that in Corriedale ewes, follicle development began immediately after parturition, reaching preovulatory size before day 17 and ovulation (first progesterone increase) occurred after day 17 (Days 18 and 25).

Hauser and Bostedt (2002) observed that the parameters for determination of the endpoint of uterine involution were the transversal diameter of uterine horns of ≤ 2 cm and the lack of contents in the uterine cavity.

Menchaca and Rubianes (2002) recorded a high correlation between the serum progesterone concentration and the development of ovarian follicles in goats.

Zdunczyk *et al.* (2004) reported that complete uterine involution was characterized by a reduced cross-sectional diameter of uterine horns and absence of lochia in the uterus and two successive examinations revealed no further reduction in the uterine diameter.

Ababneh and Degefa (2005) reported that the absence of intrauterine fluid accumulation after day 4–7 postpartum on ultrasound image confirmed quick regression of the uterus in Balady goats.

Degefa *et al.* (2006) reported that involution was completed by 20–25 days postpartum under normal non-pathological conditions and the uterus assumed its pre-gravid size by day 19 in Balady goats.

Kawu *et al.* (2007) studied the ovarian activity in the early postpartum period and observed that it is characterized by fluctuating short-term luteal phases and resumed as early as day 5 postpartum in multiparous Red Sokoto goats.

Kandiel *et al.* (2012) opined transrectal ultrasonography as a useful and reliable method to observe the uterine involution and follicular dynamics in goats.

Machiya *et al.* (2012) observed that the goats after kidding exhibited oestrus at a mean interval of 68.80 ± 6.71 days.

Fasulkov (2014) reported that ultrasound measurement of uterine lumen diameter, outer and inner caruncular diameter showed significant ($P \leq 0.05$) reduction in size as early as day 3 when compared to day 1 postpartum in local Bulgarian goats.

8. Outline of the Technical Programme

The study will be conducted at the Sheep and Goat Farm under Instructional Livestock Farm Complex, College of Veterinary and Animal Sciences, Pookode. Twelve postpartum Malabari goats of parity between two to five and with the history of normal kidding will be selected for the study. The kids will be allowed to suckle the does and the bucks will be housed in separate pens in the same goat farm. Uterine involution and ovarian activity will be monitored by ultrasound examination by B-mode ultrasound machine with a multi frequency 5-10 MHz probe on

day 3, 6, 9, 12, 15, 20, 25, 30, 35 and 40 postpartum.

Uterine involution will be considered to be complete when there is no further reduction in the uterine diameter for two successive examinations, the transverse diameter of uterine horns of ≤ 2 cm and lack of contents in the uterine lumen.

Blood samples will be collected at day 9, 20, 30 and 40 postpartum for serum progesterone estimation by RIA/ELISA.

The data collected will be subjected to statistical analyses as per Snedecor and Cochran (1994).

9. Main items of observations to be made

1. Ultrasonographic observations of the uterus and ovaries.
 - a) Echogenicity of uterine fluid
 - b) Diameter of uterine horns
 - c) Diameter of uterine caruncles
 - d) Resumption of ovarian activity by the presence of corpus luteum.
2. Serum progesterone profile.
3. Postpartum onset of behavioural oestrus
4. Signs of postpartum oestrus.

10. Facilities

(a) Existing

Facilities in the Instructional Livestock Farm Complex, Department of Animal Reproduction, Gynaecology and Obstetrics and Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Pookode will be utilized for the study.

(b) Additional facilities required

Nil

11. Duration of study

Four semesters

12. Financial estimate

Chemicals and biologicals = **Rs. 15,000/-**

Contingencies = **Rs. 10,000/-**

Total = **Rs. 25,000/-**

Signature of Student

Signature of Major Advisor

Place: Pookode

Date: 30-06-2016

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APPENDIX – I

References

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APPENDIX - II

Time frame of work

Semester I

1. Collection of literature.
2. Planning of the program for research work.

Semester II

1. Collection of literature.

2. Selection of animals for research work.

Semester III

1. Screening of the postpartum animals from day 3 postpartum.

2. Assessment of uterine involution and ovarian activity by ultrasonography.

3. Collection of blood at 10 day interval for serum progesterone estimation.

Semester IV

1. Progesterone estimation for assessment of ovarian activity.

2. Interpretation and statistical analysis of results.

3. Preparation and submission of thesis.

CERTIFICATE

Certified that the research project has been formulated observing the stipulations laid down under the Prevention of Cruelty to Animals Act (Amendment, 1998).

Place: Pookode

Date: 30-06-2016

(Major advisor)