

**EVALUATION OF POST-PARTUM UTERINE INVOLUTION
AND RESUMPTION OF OVARIAN CYCLICITY IN VECHUR
COWS**

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(15- MVP- 10)

THESIS

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DECLARATION

I hereby declare that this thesis entitled “**Evaluation of postpartum uterine involution and resumption of ovarian cyclicity in Vechur cows**” 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 of any degree, diploma, fellowship or other similar title of any other University or Society.

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Dedicated to all helping
hands
and
Loving hearts.....

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Introduction

1. INTRODUCTION

India is one of the largest hotspots of world livestock biodiversity possessing 40 recognised indigenous breeds and several other lesser known varieties of cattle. Most of the indigenous breeds are contributing less than one per cent of the total cattle population (Breed Survey, 2013). As far as Kerala is concerned, the only recognised indigenous cattle breed from the State is Vechur cattle. The indiscriminate cross breeding programmes adopted since 1960's paved way for the near extinction of Vechur cows. However, with the conservation efforts of Kerala Agricultural University in 1989, it was saved from extinction. The 19th livestock census points out that Vechur is the least populated indigenous cattle in the country with a total population of 2,479 (Breed Survey, 2013).

Vechur has emerged as a family cow yielding around two to three litres of milk daily sufficient enough for household nutrition. It is also well adapted to the hot humid tropical climate and locally available feeds and fodders (Iype, 2013). This signifies the importance of Vechur cattle as a future sustainable insurance to the livestock sector wherein intensive fodder cultivation and highly sophisticated management systems are prompting farmers to abstain from animal husbandry. The milk of Vechur cows is high in fat and the small fat globule size makes it suitable for infant feeding. The milk has high arginine content and A2 beta-lactalbumin protein. Milk and urine of Vechur cows also find use in Ayurvedic preparations (Bindya and Chinchu, 2016). All these factors contribute to the increasing demand of Vechur cows among farmers of Kerala.

Calving interval, the interval between two successive calvings is an important fertility index of any livestock herd and the ideal calving interval is 365 days giving 'one calf a year'. Vechur cows have an average calving interval of 450 days (Ravi *et al.*, 2006). Calving interval being the sum of calving to conception interval and fixed gestation period, it can be understood that the factor which affects the calving interval is post-partum period (puerperium). In order to achieve ideal calving interval the factor which can be manipulated is calving to conception interval. Knowing the course of normal puerperium of any breed is mandatory before this manipulation.

Puerperium is the period after parturition till complete resumption of normal state of the reproductive tract. According to different studies, puerperium is approximated as 40 days (Sheldon, 2004). It comprised of four important concomitant events following placental expulsion which include involution of uterus, regeneration of endometrium, ovarian rebound and riddance of bacterial contamination. Puerperal period is crucial in assuring the concept of one calf per year in the livestock production sector. Uterine involution, which is the restoration of the reproductive tract after parturition to its previous state, is an important physiological event in puerperium.

After parturition, the ovarian cyclical activity also will be temporarily paused due to the refractoriness of pituitary to the negative feedback effect of the high concentration of steroid hormone (progesterone) established during late pregnancy. Hence resumption of the ovarian activity is essential in re-establishing the ovulation followed by fertilization and next pregnancy. Therefore management practices during this period are very important as it is the critical time for physiological and endocrinological events to accomplish future fertility.

Studies on uterine involution and ovarian rebound have been undertaken years back and are still continuing in many breeds of cattle in different parts of the world using different techniques like slaughter specimen examination, per-rectal palpation and ultrasonographic examination. Some of the breeds studied include Holstein Friesian (Buch *et al.*, 1955; Okano and Tomizuka, 1987; Zain *et.al.*, 1995 and Shwetha, 2016), White Fulani and Sokoto Gudali cows (Eduvie, 1985), Sahiwal cows (Poyam and Tiwari, 2015), Gir cows (Ammu and Dhamsi, 2012) and crossbred cows (Praveen, 2016).

Uterine involution and resumption of ovarian cyclicity of Vechur cows is an unexplored area. Though research in areas related to preservation of semen of Vechur bulls are going on, there is paucity in the knowledge of the gynaecological aspect of Vechur cows. Vechur cow still being a breed of critically endangered status in the domestic animal diversity and is in huge demand, the enhancement of

its reproduction potential is an emergency. Under this context, the present study on the post-partum period of Vechur cows has been undertaken with the following objectives:

1. Gynaecological and ultrasonographic studies to evaluate the post-partum uterine and ovarian changes in Vechur cows
2. To estimate the progesterone profile of Vechur cows to assess the post-partum resumption of ovarian activity

Review of Literature

2. REVIEW OF LITERATURE

2.1. CHARACTERISTICS OF LOCHIAL DISCHARGE

Uterine lochia has been reported to be a normal physiological discharge during uterine involution and composed of portions of sloughed off necrotized caruncles, foetal blood from ruptured umbilicus and foetal fluids. Lochial discharge was seen immediately after calving upto a period of 12 to 18 days post-partum (Dhaliwal *et al.*, 2001 and Sheldon, 2004).

Hajurka *et al.* (2005) observed gradual prolongation of complete uterine involution with increased time of cervical discharge.

Saut *et al.* (2011) collected vaginal secretions using gloved hands in the post-partum crossbred cows and classified the secretions based on its colour, content and odour.

2.1.1. Colour

Roberts (1971) reported that colour of lochial discharge was brown after fourth day post-partum due to an increase in amount of cellular debris. Haemorrhages from exposed surface of caruncles lead to increasingly reddish appearance of lochia from about ten days post-partum. On vaginoscopic examination at 15 to 20 day post-partum, majority of animals exhibited no discharge and a small per cent of animals showed clear mucoid discharge.

Copious lochial discharge had brownish red colour during 8 to 9 days post-partum which became more bloody with white caruncular pieces on the twelfth day and disappeared by 14 to 18 day post-partum (Noakes, 2009 and Sheldon *et al.*, 2011).

2.1.2. Volume

Roberts (1971) observed that the amount of lochia in primipara and pluripara dairy cows were about zero to 50 ml and about 800 to 2000 ml, respectively.

Sane (1994) observed that the amount of lochial discharge in dairy cows were about 1400 to 1600 ml during the first 48 hours after parturition and decreased later on.

2.1.3. Consistency

Saut *et al.* (2011) observed muco-haemorrhagic secretions during third day post-partum which decreased during 21 day and on 28th day post-partum there was complete absence of the discharge. The secretion was classified according to its colour and the content as absent, clear mucus, bloody-mucus, chocolate-coloured mucus, purulent mucus, and purulent.

Shwetha (2016) studied post-partum discharge in ten Holstein cows with normal puerperium and reported that mucoid haemorrhagic discharge was seen in eight post-partum animals on the tenth day post-partum, clear mucoid discharge in nine animals on 20th day and the discharge became scanty or disappeared during 30th day of observation.

2.1.4. Odour

Foetid odour of post-partum vaginal discharge in cattle reflected the increase in bacterial contamination of the uterus (Williams *et al.*, 2005).

Saut *et al.* (2011) evaluated the post-partum vaginal mucus related to odour as odourless, foetid, very foetid, and extremely foetid.

2.2. UTERINE INVOLUTION

Uterine horns and cervix could be palpated ventral to the pelvis perfectly at 4 to 7 days post-partum and observed gross palpable complete uterine involution at 30 days post-partum in dairy cattle (Morrow *et al.*, 1969).

Uterine involution was reported to be a complex biological procedure which involved the expulsion of uterine lochia following myometrial contractions, extracellular matrix turnover and regeneration of the endometrium after caruncular necrosis and sloughing (Sheldon *et al.*, 2011).

2.2.1. Factors affecting the uterine involution

Wagner and Williams (1969) opined that cyclic ovarian activity was not essential for completion of uterine involution.

Eduvie (1985), in his study on Zebu cattle found that factors like suckling, supplementation of concentrate ration, calving season (rainy or dry) and age or parity of dam were not having any effect on period of uterine involution and opined that prolonged calving interval is not an attribute of delay in uterine involution.

Abayawansa *et al.* (1991) related suckling by calves as a cause of faster involution and reported that the earliest time of complete uterine involution was about 3 weeks, but most often it was not attained until 4- 5 weeks post-partum.

Kindahl *et al.* (1999) evaluated uterine involution subjectively by rectal palpation whereas objective evaluation was done using ultrasonography.

Hajurka *et al.* (2005) observed that 97.1 per cent of the pluriparous cows with normal puerperium showed complete morphological uterine involution by Day 40 post-partum. Post-partum complications like retained placenta, post-partum endometritis and high degree of uterine inflammation resulted in prolonged post-partum uterine involution.

Zhang *et al.* (2010) observed that the parity and days for uterine involution were directly proportional to each other.

Poyam and Tiwari (2015) reported that in Sahiwal cows, the body condition score had no significant effect on uterine involution but parity had a significant effect on the cornual diameters and cervical diameter from Day 14 to 44 post-calving.

2.3. CLINICO-GYNAECOLOGICAL EXAMINATION OF THE REPRODUCTIVE TRACT

2.3.1. Per-rectal examination

Per-rectal examination was used as a tool to ascertain uterine involution by many authors (Sane, 1994; Eduvie, 1985; Guilbault *et al.*, 1987; Abayawansa *et al.*, 1991; Kamimura *et al.*, 1993; Kindahl *et al.*, 1999; Hajurka *et al.*, 2005; Saut *et al.*, 2011 and Ammu and Dhami, 2012).

Uterine involution was judged by normal intrapelvic location of the uterus along with its normal consistency, tone and approximately equal cornual size when assessed by per-rectal palpation (Sane, 1994). However, the author also opined that the uterine involution was incomplete after each calving and the disparity between horns could be felt on palpation.

Fonseca *et al.* (1983) reported that the mean time required for involution of uterus and cervix in Holstein cows were 23.8 ± 8.4 and 23.7 ± 6.9 days, respectively. The corresponding values for the involution in Jersey cows were reported to be 21.7 ± 5.3 and 20.9 ± 4.6 days, respectively.

Nigerian indigenous cattle White Fulani and Sokoto Gudali cows were reported to have complete involution of uterus by 24-25 days when assessed by rectal palpation at weekly intervals (Eduvie, 1985).

Guilbault *et al.* (1987) used per-rectal palpation to monitor factors like cervical diameter, the diameter of each horn at external bifurcation and ovarian activity for assessing uterine involution.

Abayawansa *et al.* (1991) performed rectal examination in ten post-partum indigenous cows at an interval of two weeks to ascertain the uterine involution and ovarian activity. They reported a rapid involution of uterus in zebu cattle mean days of 24 ± 4.7 .

Kamimura *et al.* (1993) defined complete uterine involution as resumed positioning of uterus in the pelvic cavity in a contracted form with symmetrical uterine horn diameter when assessed by rectal palpation and observed complete uterine involution by 41.5 days post-partum in dairy cows.

Hajurka *et al.* (2005) observed that pluriparous dairy cows with normal puerperium had complete morphological uterine involution in 27.3 ± 5.5 days.

Saut *et al.* (2011) studied post-partum uterine involution in dairy cows by per-rectal palpation of uterus and observed that uterine involution effectively started from second week after parturition.

Ammu and Dhimi (2012) studied puerperal events and reproductive performance of post-partum Gir cows through per-rectal palpation and observed complete uterine involution at a mean interval of 36.54 ± 0.68 days post-partum.

Scully *et al.* (2013) stated uterine involution as complete when the uterine horn diameter and the uterine fluid volume were minimal and same between two successive observations. They observed complete uterine involution in lactating cows by 43.24 ± 3.94 days post-partum.

Sharawy *et al.* (2015) reported that the mean time taken for complete uterine involution was 20.7 ± 0.49 days in dairy animals.

Theodore (2015) studied gross reduction in uterine size of post-partum cows through per-rectal palpation by assessing the uterine location, increasing the tonicity and reducing the size of uterine horns and cervix. They observed that non-gravid horn involuted faster than the previously gravid. The author reported that by the end of sixth week, both horns and cervix were found to be inside the pelvic cavity. Genitalia were found to be involuted by 24.50 ± 1.14 days when assessed by per-rectal palpation.

Praveen (2016) studied post-partum uterine involution in the crossbred cows under different ecbolic therapy and observed that uterine involution is completed on 31.67 ± 1.67 , 28.33 ± 1.67 , 28.89 ± 1.62 and 32.22 ± 1.21 days in animals treated with oxytocin, cloprostenol sodium, methylethergometrine maleate and control group, respectively when assessed by per rectal examination.

2.3.2. Cervical size and consistency

Morrow *et al.* (1969) observed that the cervix size gradually decreased after parturition and attained its previous size by 39 day post-partum. The diameter of cervix during 10, 20, 30, 40, 50 and 60 days were 6.1, 4.2, 3.5, 3.2, 3.0, 2.9 centimetres respectively.

Fonseca *et al.* (1983) reported that cervix involuted at about 23.7 ± 6.9 days and 20.9 ± 4.6 days in Holstein and Jersey cows, respectively.

Guilbault *et al.* (1987) observed a fall in cervical diameter from 58 mm on sixth day to 32 mm on Day 35 post-partum in Brown- Swiss cows.

Mean external diameter of the cervix was observed to be 15, 11, 6 and 5 cm on post-partum days 2, 10, 30 and 60, respectively (Noakes, 2009).

Wehrend *et al.* (2003) classified involution of cervix into four phases namely phase of fast reduction in size during the first six hours after parturition, protracted reduction in the size without forming folds upto the second day,

protracted reduction in size with formation of folds upto the seventh day and reopening of the cervical canal after the seventh day.

2.3.3. Uterine size and consistency

Morrow *et al.* (1969) opined that uterine horns were smaller than cervix after 25 days post-partum. The mean combined diameters of the uterine horns during 10, 20, 30, 40, 50 and 60 days were 13.6, 7.6, 6.1, 5.7, 5.3 and 5.0 cm, respectively.

Guilbault *et al.* (1987) reported that mean diameters of previously gravid horn and previously non gravid horn were 90 mm and 50 mm on sixth day post-partum, respectively. Decrease in previously gravid cornual diameter was rapid when compared to the previously non-gravid horn and complete uterine involution was attained by Day 30 post-partum.

Theodore (2015) observed that the uterine horns of the post-partum cows showed a progressing trend in curling and tonicity from Day seven to 21.

Shwetha (2016) reported a dorsal corrugation over the previously gravid thick walled uterus and indicated it as a sign of progress in uterine involution which completed at Day 20 post-partum.

2.3.4. Palpable structures on the ovary

Webb *et al.* (1980) carried out weekly palpation of ovaries per-rectally in post-partum dairy cows and reported the presence of developing follicles 15.7 ± 2.0 days after calving.

Abayawansa *et al.* (1991) reported that the size of ovaries in zebu cattle were small when compared to crossbred cows which led to difficulty in palpating and differentiating structures in the ovaries. Further they reported that Zebu cattle achieved resumption of ovarian activity when allowed to be suckled by their calves within 60 days post-calving.

Hanzen *et al.* (2000) palpated follicles as firm, round and smooth structures with diameter ranging from seven to 20 mm during pro-oestrus and oestrus phase of oestrous cycle and which became soft on approaching ovulation. A mature CL had a maximum diameter of 20-30 mm when palpated between six to 18 days after ovulation.

Gitonga (2010) reported that presence of a corpus luteum (CL) or a large follicle in ovary could be considered as an evidence of ovarian cyclicity.

Praveen (2016) reported the presence of a CL within 40 days post-calving in only 33.33 percent of the crossbred dairy cows studied.

2.4. ULTRASONOGRAPHIC EXAMINATION OF THE REPRODUCTIVE TRACT

Okano and Tomizuka (1987) carried out ultrasonographic examination of the reproductive tract in post-partum cows to assess the involutionary changes of uterus and reported complete uterine involution by about 40 days post-partum.

Rajamahendran and Taylor (1990) observed uterine involution at 27 ± 6 days in pluriparous cows studied using 5.0 MHz linear array ultrasonography.

Saito *et al.* (2001) studied correlation between microscopic features and ultrasonographic images of uterine horn in dairy cows and found that longitudinal and circular muscle layers represented slightly echogenic arched layer and slightly echogenic elliptical layer, respectively.

Zhang *et al.* (2010) used ultrasonography to study uterine involution in cows and reported that when both cornual and cervical diameters decreased and were unchanged, the uterine involution was stated to be complete.

Cengic *et al.* (2012) reported that ultrasonography could precisely detect the rate of uterine involution in cows. Uterine involution was observed to be completed by 38- 45 days post-partum.

2.4.1. Cornual diameter

Cengic *et al.* (2012) conducted a study to observe the normal and abnormal uterine involution in dairy cows using ultrasonography and reported that in normal animals the uterine diameter decreased from 45 mm at two weeks post-partum to 20 mm at seven weeks post-partum. The reduction in the cornual diameter was rapid during the first three weeks which later on decreased gradually.

Previously gravid horns of post-partum dairy cows had mean diameter of 12 ± 0.32 , 7.59 ± 0.28 , 4.66 ± 0.16 , 3.67 ± 0.11 , 3.52 ± 0.11 , 3.39 ± 0.11 , 3.16 ± 0.06 , 3.05 ± 0.03 and 3.02 ± 0.02 cm during weeks 1, 2, 3, 4, 5, 6, 7, 8 and 9 post-partum, respectively. Mean diameter of non-gravid horn during the above said weeks were 7.84 ± 0.22 , 4.87 ± 0.15 , 3.7 ± 0.07 , 3.25 ± 0.05 , 3.23 ± 0.05 , 3.15 ± 0.04 , 3.12 ± 0.04 , 3.05 ± 0.03 and 3.02 ± 0.02 cm, respectively (Stephen, 2010).

Abdel-Khalek *et al.* (2015) reported that on the post-partum days of 10, 15, 22, 27 and 32 in Freisian cows, the diameters of pregravid uterine horns were 6.52, 5.16, 4.06, 3.51 and 2.40 cm, respectively and the diameters of non-gravid horns were 3.93, 2.96, 2.42, 2.22 and 2.12 cm, respectively.

Poyam and Tiwari (2015) reported that post-partum Sahiwal cows when examined using trans-rectal ultrasonography, the mean previously gravid uterine horn diameters were 33.9 ± 2.36 , 30.81 ± 2.14 , 28.205 ± 2.03 , 25.49 ± 1.82 , 23.93 ± 1.7 and 21.91 ± 1.51 mm on days 14, 20, 26, 32, 38 and 44 post-partum, respectively.

2.4.2. Presence of fluid in the uterine lumen

Okano and Tomizuka (1987) found presence of lochia in the uterine lumen approximately upto 15th day post-partum on ultrasonographic examination.

Shwetha (2016) during ultrasonographic examination of post-partum uterus on day 10 observed the presence of intrauterine fluid in all the cows studied

which later on reduced to 20 per cent of the animals on Day 20 and no intrauterine fluid in any animal on 30th day post-partum.

Kamimura *et al.* (1993) on ultrasonographic examination of post-partum uterus in Holstein cows observed a large lumen and snowy appearance of lochia till 18 days post-partum.

2.4.3. Changes in the echogenicity of the reproductive tract

2.4.3.1. Cervix and uterus

During initial stages of post-partum, the cross-sectional ultrasound images of the uterus appeared to be darker (anechoic) suggested to be due to its swollen appearance of the uterus and which later became lighter on progression of uterine involution (Okano and Tomizuka, 1987).

Kamimura *et al.* (1993) opined that the echogenicity of the post-partum uterus increased as the uterine involution progressed.

Sutaria *et al.* (2014) concluded that there was an increment in the echotexture of reproductive tract in dairy cows during the progression of post-partum uterine involution. They also reported that the wall of cervical canal appeared as a bright hyperechoic structure, while its lumen was found to be hypoechoic with hyperechoic spots on Day 40th of post-partum cows. The post-partum uterine horns on the same day depicted hyperechoic wall and hypoechoic lumen. The caruncular echogenicity during early post-partum period from 5th to 15th day was seen as bright hyperechoic structures encircled with visible anechoic line.

2.4.3.2. Ovary

Pierson and Ginther (1984), Reeves *et al.* (1984) and Fissore *et al.* (1986) opined that ultrasonography was an efficient and accessible technique for monitoring the luteal and follicular structures in cattle.

Pierson and Ginther (1984) used 5 MHz linear-array ultrasound scanner for the study of luteal and follicular status in heifers and visualised CL on the third day postovulation in most of the animals. Diameter of the largest CL observed was 19 mm. Echogenic pattern of the CL was different from the surrounding tissues with a defined border. They also observed that the echogenic pattern was affected by the presence of a central cavity in some animals which was in correspondence with the maximum size.

2.5. POST-PARTUM SERUM CALCIUM, PHOSPHORUS LEVELS AND Ca: P RATIO

Kamgarpour *et al.* (1999) conducted a study in post-partum dairy cows to ascertain the effect of subclinical hypocalcaemia on uterine involution and ovarian function and reported that the animals with subclinical hypocalcaemia had a total plasma calcium level less than 2.0 mmol/L and these animals had decreased mean size of the first ovulated follicle and an increased time interval for a complete uterine involution.

Mateus and Lopes da Costa (2002) found that plasma Ca concentrations had a significant effect with the occurrence of endometritis but the plasma P values and Ca: P ratios had no significant effects. In animals with normal puerperium, the estimates of plasma calcium, phosphorus and the Ca: P values were 8.0 - 11.3 mg/dl, 4.7- 8.7 mg/dl and 1.04-1.96 mg/dl, respectively.

Dagli *et al.* (2004) observed a gradual increase in mean serum calcium values of post-partum crossbred Gir cows from first to eight week post-partum. They also reported that the serum phosphorus level in the post-partum period was 5.10- 5.76 mg per cent.

The mean serum calcium and phosphorus levels in the cows of Kerala State were reported to be 8.34 and 7.49 mg/ dL respectively. (Cattle Sterility Bulletin, 2013).

Heppelmann *et al.* (2015) suggested 2 mmol/ L as the demarcating value between normocalcaemic and hypocalcaemic cows and found that hypocalcaemia can lead to the prolongation of uterine involution due to reduced myometrial contractility in cows. However, the cornual diameter of the uterus was not affected in this context.

Serum calcium levels during the Day 5, 22 and 42 post-partum in healthy Holstein cows were 1.921 ± 0.123 , 2.122 ± 0.513 and 2.213 ± 0.424 mmol/L, respectively and these values were higher than that observed in cows with endometritis (Brodzki *et al.*, 2016).

2.6. SERUM PROGESTERONE PROFILE

Robertson (1972) reported that the plasma progesterone concentration remained less than 0.5 ng/ml in post-partum dairy cows until resumption of ovarian cyclicity.

After parturition, the serum progesterone level remained less than 0.2 ng/ml until the formation of the CL formation and this later on increased to greater than 1ng/ml (Stevenson and Britt, 1979).

Webb *et al.* (1980) reported that regular ovarian cyclical activity was found to be initiated by 16.6 ± 1.1 days after calving and was assessed by increased plasma progesterone concentrations.

Rajamahendran and Taylor (1990) observed that out of ten cows studied, eight cows presented CL by Day 25 post-partum. Post-partum first ovulation was not affected by the side of pregnancy. The first post-partum oestrous cycle was significantly shorter and had lower progesterone peak levels than the second post-partum cycle. Peak progesterone level observed in the first and second oestrous cycles were 3.0 ± 1.7 and 4.3 ± 1.0 ng/ ml, respectively.

Kamimura *et al.* (1993) observed that presence of central cavity in CL had no effect on plasma progesterone concentration and suggested that

ultrasonographic examination of CL for the assessment of luteal function was a viable alternative for assessment of peripheral progesterone levels.

Tom *et al.* (1998) opined that progesterone concentration reached its minimum value three days prior to ovulation. They also reported that growing phase, static phase and regression of the CL, were from 0 to 6, 6 to 14 and 15 to 20 days post-ovulation, respectively.

Rekwot *et al.* (2000) considered the occurrence of serum progesterone concentration above 1ng/ml in post-partum cows indicated resumption of ovarian cyclicity.

Veronesi *et al.* (2002) observed that the ultrasonographic appearance of the CL was a better indicator of luteal function than diameter of the CL. They classified CL based on the progesterone level as no CL, evolving CL, and mid cycle CL when progesterone levels were below 1ng/ml, 1 to 4ng/ml and above 4 ng/ml, respectively.

Shrestha *et al.* (2005) opined greater than 1ng/ml of skim milk progesterone as an evidence of luteal activity and ovulation was considered to have occurred five days before the luteal activity.

Bicalho *et al.* (2008) considered cows with a serum progesterone level higher than 1 ng/ml had a functional CL.

Siqueira *et al.* (2009) reported that 66 per cent of the ovulations occurred from the right ovary. They also opined that the heterogeneity of luteal tissue assessed by ultrasonography was highly correlated with the circulating progesterone level.

The serum progesterone level higher than 3.2 ng/ml was considered to be indicative of ovulation. The mean serum progesterone concentration in the dairy cows on days 21, 35, 49 and 63 post-partum were 2.47 ± 0.59 , 3.37 ± 0.74 , 6.39 ± 1.21 and 7.12 ± 1.15 , respectively (Stephen, 2010).

Scully *et al.* (2013) reported that dairy cows resumed ovarian cyclicity by Day 21 post-partum through estimation of serum progesterone concentrations of greater than 1ng/ml.

Sharawy *et al.* (2015) observed that the interval from parturition to first ovulation were 20.3 ± 6.1 days when assessed using serum progesterone assay. They assumed that animals with serum progesterone concentrations greater than or equal to 1ng/ml in two consecutive blood samples taken weekly twice had luteal activity.

Praveen (2016) reported that serum progesterone estimation is 100 percent efficient for the detection of luteal activity when compared to per rectal examination (61.90 per cent) and ultrasonographic examination (80.95 percent). The mean serum progesterone values for the post-partum days 10, 20, 30 and 40 were 0.29 ± 0.03 , 0.36 ± 0.06 , 0.82 ± 0.29 and 0.79 ± 0.33 ng/ml respectively.

2.7. OVARIAN REBOUND/ RESUMPTION OF OVARIAN CYCLICITY

Fonseca *et al.* (1983) reported first post-partum ovulation during third week post-partum with a mean interval of 20.8 ± 13.2 and 20.0 ± 14.4 days for Holsteins and Jerseys cows, respectively.

Ghosh *et al.* (1993) observed that supplementing urea-molasses mineral blocks to post-partum Zebu cows enhanced the initiation of post-partum ovarian activity (14- 44 days) and behavioural oestrus (35- 84 days) when compared to control group without supplementation.

Savio *et al.* (1990) observed that mean occurrence of the first dominant follicle after calving was during 11.6 ± 8.9 days with a range of 5- 39 days. They also reported that the interval to first ovulation was 27.4 ± 23.0 days with a range from nine to 85 days.

Taylor and Rajamahendran (1991) defined the day of follicular wave emergence as the day on which follicles of identifiable diameter of two to 5 mm were first observed.

Kamimura *et al.* (1993) observed that the prolonged calving to ovulation interval was due to ovulation failure and not because of absence of follicular development. Hence, they opined that formation of a dominant follicle and CL along with timely luteolysis were necessary for uterine involution and ovarian activity in post-partum cows.

Zain *et al.* (1995) studied about factors in the resumption of ovarian activity and uterine involution of post-partum dairy cows and observed that the interval from calving to resumption of ovarian activity ranged from 13 to 64 days, the average being 25 ± 13 days.

Kindahl *et al.* (1999) opined that involution of uterus was influenced by the ovarian rebound at a high degree. Further, they also reported that compared to normal oestrous cycle, the first oestrus cycle after calving was short.

Viana *et al.* (2000) conducted a study to monitor the follicular dynamics of Gir cattle and found the maximum diameter of dominant follicle as 13.25 ± 0.96 mm.

Montiel and Ahuja (2005) concluded that both inadequate nutrient intake during post-partum period and suckling by calves induced anovulation.

Zhang *et al.* (2010) found that as the parity increased the days from calving to the next ovulation decreased.

Heppelmann *et al.* (2013) monitored the effect of post-partum ovulation on uterine involution and observed that time of first ovulation post-partum was 21.0 ± 6.0 days and the first oestrous cycle length was 19.5 ± 2.0 days in cows without uterine disease.

2.8. OCCURENCE OF POST-PARTUM OESTRUS

Wagner and Williams (1969) reported that the calving to first oestrus mean interval was observed to be 14 days in dairy cows but none of the suckled cows exhibited behavioural signs of oestrus.

The mean length in days from parturition to first observed oestrus was 46.50 ± 39.50 in Sri Lankan Zebu cattle (Abayawansa *et al.*, 1991).

Maina *et al.* (2008) reported that the body condition of the animal had a significant effect over the ovarian activity of the animal.

Ammu and Dhani (2012) observed the first oestrus after calving between 70 and 101 days whereas by progesterone assay it was between 20 to 101 days.

Praveen (2016) observed first post-partum oestrus on 30.67 ± 4.67 days in crossbred dairy cows.

2.9. OESTRUS CHARACTERISTICS

Diskin and Sreenan (2000) opined that standing to be mounted was the only primary sign shown by a cow in oestrus which was accurate and definite. The discharge of long clear elastic strings of mucus was indicative of an imminent heat while thicker cloudier and more viscous mucus was indicative of a recent heat. They further reported that cattle that mount other animals may be in or approaching oestrus. Signs of restlessness such as increased walking, trailing of other cows and bellowing are individual characteristics.

Roelofs *et al.* (2005) studied the relation between various behavioural signs of oestrus and ovulation time and reported that sniffing and chin resting could not be considered as good signs of ovulation as animals in proestrus also display same signs. They also reported that however, standing heat was a useful parameter to assess the oestrus and ovulation, only 58 per cent of oestrus cows displayed a standing heat.

The proportion of crossbred cows that exhibited low, medium and high oestrus intensity for the first post-partum oestrus were 33.33, 44.44 and 22.22 per cent, respectively (Praveen, 2016).

Materials and Methods

3. MATERIALS AND METHODS

3.1. SELECTION OF ANIMALS

The study was conducted at the Vechur Conservation Unit of Kerala Veterinary and Animal Sciences University situated at Pookode and Mannuthy. Eight post-partum healthy Vechur cows (Plate 1) of parity between two and five having normal calving history were selected for this study. The cows were maintained under semi-intensive system of rearing and provided standard feed and management conditions. The calves were allowed to suckle the cows and breeding bulls were maintained in the separate pen of the same shed.

3.2. DESIGN OF THE STUDY

The study was conducted during the period of August 2016 to June 2017 in eight post-partum Vechur cows without any post-partum complications. The period of study in each animal was upto 91 days post-partum. The following observations were made.

3.3. CHARACTERISTICS OF THE LOCHIAL DISCHARGE

Presence of lochial discharge and its physical characteristics like colour, volume, consistency and odour were recorded. Cows were observed from the first day post-partum till the cessation of discharge. Lochia was collected per-vaginally using a gloved hand to assess the colour, approximate volume, consistency and odour (Saut *et al.*, 2011). Colour of lochia was graded according to the physical appearance. Volume of lochial discharge was assessed after collecting in graduated containers. Consistency of the discharge was graded according to the presence of mucus as scanty, thin mucus, moderately thick mucus and thick mucus. Odour of the lochia is graded as neutral or no foetid odour and foetid odour.

3.4. CLINICO-GYNAECOLOGICAL EXAMINATION OF THE REPRODUCTIVE TRACT

Per-rectal examination of the Vechar cows were conducted on post-partum days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91. Reproductive organs were transrectally palpated according to Settergren (1980). Diameter of cervix and uterine horns were approximately assessed using fingers. Complete involution of cervix was ascertained by the absence of significant difference in the measured diameters even after two consecutive examinations at seven days interval. Uterine involution was judged by normal intra-pelvic location of the uterus with no significant difference in diameter between two successive observations along with the normal consistency and tone (Sane *et al.*, 1994). Presence of follicles in the ovaries were assessed by palpating smooth fluid filled fluctuating compressible structures and corpus luteum (CL) as firm non compressible structures on the ovaries (Jackson and Cockcroft, 2002).

3.5. ULTRASONOGRAPHIC EXAMINATION OF THE REPRODUCTIVE TRACT

Transrectal ultrasonography was done using a portable Ultrasound Scanner (My Lab VET Gold[®], Esaote Pie Medical, Genova, Italy) (Plate 2(A)) equipped with a linear array 5.0 to 7.5 MHz frequency transducer (Plate 2(B)). Ultrasound scanning was done on days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91 post-partum to observe the changes in the reproductive tract (Plate 2(C)). Transrectal linear array probe was placed dorsally above the uterine horns and images were freezed and diameters of the uterine horns were taken using inbuilt calipers. If the image of the structure being non spherical, the diameter was estimated by averaging two 90° measurements. The presence of uterine fluid in the lumen of uterus was noted. Changes in the echogenicity of the reproductive tract was observed and graded as hyper-echogenic, hypoechoic and non-echoic (Theodore, 2015). Follicles were defined as non-echogenic spherical structures with a clear demarcation between the follicular wall and antrum. CL were defined as grainy echogenic structures that had a well defined border with less echogenic ovarian stroma (Wolfgang, 1994).

3.6. BLOOD COLLECTION AND SERUM SAMPLE PREPARATION

Ten millilitres of blood was collected into the clot activators (Safelab clot activator vials, India) by jugular venipuncture of the animals using 18G needle on days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91 post-partum. Serum was separated by centrifuging the clotted blood samples at 3000 rpm for 10 minutes. Serum samples were stored below -20° C in multiple storage vials (Shwetha, 2016) until processed for serum Ca, P and progesterone estimations.

3.7. SERUM CALCIUM ESTIMATION

Serum calcium level estimation (mg/dl) was done using Liquick Cor-CALCIUM ARSENAZO (Cormay, USA) kit.

3.7.1. Method principle

Photometric test using arsenazo III. Calcium with arsenazo III at neutral pH yields a blue coloured complex and intensity is proportional to the calcium concentration. Interference by magnesium is eliminated by addition of 8-hydroxyquinoline-5-sulfonic acid.

Mean serum calcium concentration of all Vechur cows on the same post-partum day of observation was calculated and analysed statistically.

3.8. SERUM PHOSPHORUS ESTIMATION

Serum phosphorus level estimation (mg/dl) was done using Liquick Cor-PHOSPHORUS (Cormay, USA) kit.

3.8.1. Method principle

Direct phosphomolybdate reaction without deproteinization. Phosphate ions form with molybdate ions in acid solution proportional amounts of unreduced phosphomolybdate complex. The concentration of the complex formed was determined by measuring its absorbance at $\lambda=340$ nm.

Mean serum phosphorus concentration of Vechur cows on the same day of observation was calculated and analysed statistically.

3.9. SERUM CALCIUM PHOSPHORUS RATIO (Ca: P) ESTIMATION

Serum Ca: P was calculated by dividing the serum calcium concentration with serum Phosphorus concentration. Mean serum Ca: P concentration of Vechur cows on the day of observation was calculated and analysed statistically.

3.10. SERUM PROGESTERONE ESTIMATION

Serum progesterone concentration of the Vechur cows during the post-partum days seven to 91 at weekly intervals were estimated using SteroidEIA- Progesterone kits, Alkor Bio Company, Russia (Plate 3).

3.10.1. Principle of competitive ELISA

SteroidEIA – Progesterone kit is a competitive solid phase enzyme immunoassay. During incubation, progesterone of the tested samples and horseradish peroxidase (HRP) labelled progesterone bind to the antibodies coated on the inner surface of the microplate wells until the balance between them occurs. Separation of free and bound to antibodies progesterone as well as conjugate progesterone- peroxidase occurs while extracting the contents of the wells. The amount of bound conjugate is inversely proportional to the quantity of progesterone in the sample.

Incubation with 3, 3', 5, 5'-Tetramethylbenzidine (TMB) solution the colour is caused by the development of colour and the intensity of the colour is inversely proportional to the progesterone concentration in specimens. Progesterone concentration in the sample is read from a standard curve that is processed in each assay.

3.10.2. Procedure of competitive Enzyme Immuno Assay (EIA)

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. The position of the standards, controls and the serum sample were recorded on the ELISA data recording sheet provided.

3. 20 µl of calibrators, control and serum samples were dispensed into appropriate wells of microplate coated with anti-progesterone monoclonal antibodies after leaving A1- A2 wells for blank.

4. 150 µl of conjugate progesterone – HRP reagent was added to each well except blank.

5. Incubated the microplate for one hour while shaking at 37°C.

7. Washed the microplates using automatic microplate washer set at four cycles and a volume of 300µl of wash solution per cycle.

8. 100 µl of substrate solution was added to all the wells and gently mixed for 5 seconds and incubated in the dark at room temperature (18-25°C) for 15-20 min.

9. The reaction was stopped by adding 100 µl of stop solution and gently mixed for one to two minutes.

10. 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 standards, controls and serum samples were calculated.

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

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

13. Progesterone concentration in ng/ml was calculated by multiplying it with 0.314 (information provided in the kit).

3.11. OVARIAN REBOUND/ RESUMPTION OF OVARIAN CYCLICITY

Resumption of ovarian cyclicity was assessed by an increase in the serum progesterone level above 1 ng/ml in post-partum Vechur cows (Scully *et al.*, 2013).

3.12. OCCURRENCE OF OESTRUS

Animals were closely monitored for the onset of oestrus signs. Mean number of days for the exhibition of first post-partum oestrus was calculated.

3.13. OESTRUS CHARACTERISTICS

The intensity of the oestrus was measured based on the score card described by Rao and Rao (1981) with slight modification in the expression of uterine tonicity. The animals with score from 10 to 15 points, five to 10 points and below five points were graded as intense, intermediate and weak, respectively. The score card is given in Annexure I.

3.14. STATISTICAL ANALYSIS

The data obtained were tabulated and analysed as per standard statistical procedures (Snedecor and Cochran, 1994) using SPSS 21 (Statistical Package for Social Studies) software. Repeated measures analysis was carried out to analyse the data statistically for assessing difference in mean diameter of cervix, uterine horn diameters, serum calcium, serum phosphorus and serum progesterone concentration between the observations. Occurrence of oestrus signs, onset of

post-partum ovarian activity and presence of palpable structures were analysed by descriptive statistics.

Results

4. RESULTS

Results of the present study on evaluation of post-partum uterine involution and resumption of ovarian cyclicity in Vechur cows are presented in the Tables 1 to 7 and Figures 1 to 8.

4.1. CHARACTERISTICS OF THE LOCHIAL DISCHARGE

Characteristics of lochial discharge during first eight post-partum days in Vechur cows are presented in the Table 1. and Fig 1.

Lochial discharge in the post-partum Vechur cows were observed for mean days 5.63 ± 0.38 with a range from four to seven days. Presence of lochial discharge was observed in all the animals for the first four days of post-partum which later on decreased to 87.5, 50 and 25 per cent on 5, 6, and 7 days post-partum, respectively and from eighth day post-partum onwards, the discharge became scanty or nil. Lochial discharge was not observed externally in standing cows and hence, the collection of the same was done per-vaginally.

4.1.1. Colour

Reddish brown lochial discharge was observed in all the cows (8/8) (Plate 4 (A)) on the first day post-partum. On second day post-partum the lochial discharge was having off-white colour with necrosed tissue and blood stains (Plate 4 (B)) in 50 per cent (4/8) of the cows which later on increased to 62.5 per cent (5/8) on third day post-partum. The colour of the lochia in 37.5 per cent (3/8) of the Vechur cows on third day was light brown (Plate 4(C)). Lochial discharge on the fourth day post-partum was off-white, light brown and clear mucoid in 25 (2/8), 50 (4/8) and 25 (2/8) per cent of cows, respectively. On fifth day post-partum cows with light brown, clear and negligible vaginal discharge were 37.5 (3/8), 50 (4/8), and 12.5 (1/8) per cent, respectively. Later on, 50 (4/8) and 25 (2/8) per cent of the total cows examined had clear mucoid discharge (Plate 4(D))

on sixth and seventh day, respectively. Vaginal discharge was absent in all other cows. On eighth day post-partum, none of the animals had a vaginal discharge.

4.1.2. Volume

Mean volume of lochial discharge in post-partum Vechur cows was 93.63 \pm 11.36 ml ranging from 51 ml to 140 ml during the period of observation.

4.1.3. Consistency

Consistency of the lochial discharge varied between first to eighth day post-partum in the Vechur cows studied. On the initial day of lochial discharge, the consistency was moderately thick muco-haemorrhagic which later on became thick with the presence of necrosed tissue and more mucus. Light brown lochial discharge initially had thin mucus content and this became thicker and clearer which later progressed to the final stage of scanty or no mucus.

4.1.4. Odour

All the Vechur cows observed for the odour of lochial discharge were neutral or without any foetid odour.

4.2. CLINICO-GYNAECOLOGICAL EXAMINATION OF THE REPRODUCTIVE TRACT

4.2.1. Cervical size and consistency

The mean cervical diameter and length in Vechur cows during post-partum days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91 assessed by per-rectal examination are presented in the Table 2.

Table 2. Diameter and length of cervix (Mean \pm SE) in post-partum Vechur cows assessed by per-rectal palpation (n=8)

Post-partum days	Diameter of the cervix (cm)	Length of the cervix (cm)
Day 7	4.19 \pm 0.16 ^a	7.25 \pm 0.45 ^a
Day 14	3.44 \pm 0.11 ^b	5.75 \pm 0.28 ^b
Day 21	2.94 \pm 0.10 ^c	4.53 \pm 0.28 ^c
Day 28	2.28 \pm 0.15 ^d	4.00 \pm 0.32 ^d
Day 35	1.94 \pm 0.14 ^e	3.44 \pm 0.15 ^e
Day 42	1.75 \pm 0.08 ^e	3.38 \pm 0.15 ^e
Day 49	1.78 \pm 0.10 ^e	3.50 \pm 0.11 ^e
Day 56	1.78 \pm 0.09 ^e	3.34 \pm 0.14 ^e
Day 63	1.72 \pm 0.07 ^e	3.41 \pm 0.13 ^e
Day 70	1.75 \pm 0.08 ^e	3.38 \pm 0.16 ^e
Day 77	1.78 \pm 0.09 ^e	3.38 \pm 0.15 ^e
Day 84	1.75 \pm 0.11 ^e	3.44 \pm 0.19 ^e
Day 91	1.81 \pm 0.08 ^e	3.41 \pm 0.13 ^e
F- value	144.23**	79.02**
p- value	< 0.01	< 0.01

** - significant at $p < 0.01$

Means with different superscripts in columns varies significantly

On per-rectal palpation of the cervix, the diameter and length of the cervix (mean \pm SE) had a significant difference ($p < 0.01$) between observations upto 35 days. Non-significant difference was observed for diameter and length of cervix from the day 35 post-partum onwards. Hence, the involution of cervix observed between Days 28 to 35 post-partum when assessed by per-rectal examination. The reduction in both diameter and length of the cervix followed a decreasing logarithmic pattern (Fig. 2) with a faster rate of involution in the initial weeks followed by a gradual decline.

Consistency of the cervix was soft and swollen during the seventh day post-partum. Later the cervix attained a firm consistency from Day 21 post-partum.

4.2.2. Uterine size and consistency

The mean diameters of previously gravid uterine horns and non-gravid uterine horns of post-partum Vechur cows during the post-partum days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91 are represented in the Table 3.

Mean diameter of the previously gravid uterine horns showed a significant difference ($p < 0.01$) upto Day 35 post-partum. Non-significant difference was observed in the mean diameter of previously gravid uterine horns from Day 35 post-partum onwards. Therefore, involution of previously gravid uterine horns was observed to be between 28 and 35 days post-partum.

The mean diameters of non-gravid uterine horns was observed to be having a non-significant difference from Day 28 post-partum. Thus involution of non-gravid uterine horns was found to have occurred between Day 21 and 28 post-partum. Complete involution of uterus in Vechur cows was observed between 28 to 35 days post-partum when assessed by per-rectal palpation of the

Table 3. Diameter of previously gravid and non-gravid uterine horns (mean \pm SE) in post-partum Vechur cows assessed by per-rectal examination (n=8)

Post-partum days	Diameter of previously gravid uterine horn (cm)	Diameter of non-gravid uterine horn (cm)
Day 7	3.28 \pm 0.13 ^a	2.56 \pm 0.15 ^a
Day 14	2.75 \pm 0.07 ^b	2.09 \pm 0.13 ^b
Day 21	2.00 \pm 0.13 ^c	1.81 \pm 0.09 ^b
Day 28	1.78 \pm 0.13 ^d	1.56 \pm 0.08 ^c
Day 35	1.69 \pm 0.08 ^e	1.47 \pm 0.06 ^c
Day 42	1.66 \pm 0.05 ^e	1.47 \pm 0.03 ^c
Day 49	1.63 \pm 0.05 ^e	1.41 \pm 0.05 ^c
Day 56	1.60 \pm 0.05 ^e	1.41 \pm 0.05 ^c
Day 63	1.66 \pm 0.07 ^e	1.47 \pm 0.03 ^c
Day 70	1.66 \pm 0.08 ^e	1.44 \pm 0.04 ^c
Day 77	1.63 \pm 0.07 ^e	1.38 \pm 0.05 ^c
Day 84	1.63 \pm 0.05 ^e	1.44 \pm 0.04 ^c
Day 91	1.66 \pm 0.08 ^e	1.41 \pm 0.04 ^c
F- value	46.82**	24.57**
p- value	< 0.01	< 0.01

** - significant at $p < 0.01$

Means with different superscripts in columns varies significantly

reproductive tract. Involution of both uterine horns followed a decreasing logarithmic pattern in its progression (Fig. 3).

Uterus of the post-partum Vechur cows studied showed an increasing trend in curling and tonicity from day seven till complete uterine involution. All animals showed a curled and tonic reproductive tract near the pelvic inlet from Day 21 post-partum on per-rectal palpation.

4.2.3. Palpable structures on the ovary

The mean number of days at which the follicle was palpable through per-rectal palpation method was 30.63 ± 5.11 days. In one of the cows, no developing follicle could be palpated. CL was palpable for the first time on mean 21 ± 8.50 days. Post-partum CL could not be palpated in one of the cows.

4.3. ULTRASONOGRAPHIC EXAMINATION OF THE REPRODUCTIVE TRACT

4.3.1. Cornual diameter

The mean diameters of the previously gravid and non-gravid uterine horns on 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91 day post-partum are represented in the Table 4.

On ultrasonographic examination of the reproductive tract, diameters of the previously gravid horns were observed to have a non-significant difference from Day 42 post-partum observation onwards. Eventhough the mean diameter of non-gravid uterine horn did not differ significantly from day 21 to 35 post-partum, a difference in diameter was observed between Day 35 and 42 post-partum. Thus the involution of previously gravid uterine horns and non-gravid uterine horn were observed to be between Day 35 and 42 post-partum. Involution of uterus was

Table 4. Diameter of previously gravid and non-gravid uterine horns (mean \pm SE) in post-partum Vechur cows assessed by trans-rectal ultrasonography (n=8)

Post-partum days	Diameter of previously gravid uterine horn (cm)	Diameter of non- gravid uterine horn (cm)
Day 7	3.23 \pm 0.08 ^a	2.59 \pm 0.14 ^a
Day 14	2.63 \pm 0.13 ^b	2.16 \pm 0.12 ^b
Day 21	2.07 \pm 0.14 ^c	1.68 \pm 0.10 ^c
Day 28	1.77 \pm 0.11 ^d	1.53 \pm 0.04 ^c
Day 35	1.59 \pm 0.07 ^{de}	1.47 \pm 0.04 ^{cd}
Day 42	1.51 \pm 0.05 ^e	1.43 \pm 0.05 ^d
Day 49	1.51 \pm 0.05 ^e	1.43 \pm 0.04 ^d
Day 56	1.51 \pm 0.05 ^e	1.43 \pm 0.05 ^d
Day 63	1.50 \pm 0.05 ^e	1.42 \pm 0.05 ^d
Day 70	1.49 \pm 0.05 ^e	1.42 \pm 0.05 ^d
Day 77	1.51 \pm 0.05 ^e	1.42 \pm 0.05 ^d
Day 84	1.49 \pm 0.05 ^e	1.42 \pm 0.05 ^d
Day 91	1.50 \pm 0.05 ^e	1.42 \pm 0.05 ^d
F- value	63.86**	34.17**
p- value	< 0.01	< 0.01

** - significant at $p < 0.01$

Means with different superscripts in columns varies significantly

observed to have completed between Days 35 and 42 post-partum in post-partum Vechur cows when assessed through trans-rectal ultrasonography.

Uterine involution in post-partum Vechur cows followed a decreasing logarithmic pattern (Fig 4.) in its progression. The reduction in diameter of the uterine horns were at a faster rate upto Day 21 post-partum followed by a gradual decrease upto Day 42 which became non-significant from Day 42 to 91 post-partum.

Ultrasonographic images of the uterine horns during the post-partum days upto complete uterine involution are presented in Plates 5 to 8.

4.3.2. Presence of fluid in the uterine lumen

Six out of eight animals (75%) studied had fluid in the uterine lumen during Day 7 post-partum when assessed by 7.5 MHz trans-rectal ultrasonography. The fluid in the lumen appeared to be hypoechoic to anechoic (Plate 9). None of the animals had fluid in the uterine lumen from Day 14 post-partum.

4.3.3. Changes in the echogenicity of the reproductive tract

4.3.3.1. Cervix and uterus

Ultrasonographic images showing changes in echogenicity of cervix and uterus are given in Plates 10 and 11, respectively. Echogenicity of the cervix increased from seventh day post-partum. The cervix was hypoechoic with hyperechoic spots in the cervical canal. Cervix became more echogenic from Day 21 post-partum.

Echogenicity of the uterine horns increased from day seven post-partum period. Hypoechoic uterine horns with some caruncular echogenic spots were observed on seventh day post-partum. Echogenicity of the uterus increased as the

diameter of the uterus decreased. Uterus appeared to be more echogenic from 14th day post-partum.

4.3.3.2. Ovary

Echogenicity of the ovary varied according to the structures present in it. Follicles were anechoic with echogenic wall (Plate 12 (A)). The largest diameter of the follicle obtained among all the Vechur cows using ultrasonography was 9.3 mm. CL was observed as a grainy echogenic structure with a well-defined border (Plate 12(B)). The largest CL had a diameter of 19 mm. During the post-partum 28.00 ± 4.58 mean days, the first CL was observed by ultrasonographic examination. Follicles of diameter more than 4 mm were observed on mean 11.38 ± 1.28 days post-partum.

4.4. POST-PARTUM SERUM CALCIUM, PHOSPHORUS LEVELS AND Ca: P RATIO

Serum calcium, phosphorus levels and Ca: P ratio (mean \pm SE) in Vechur cows during post-partum period seven to 91 days on weekly interval are presented in the Table 5. No significant differences were observed in the mean serum calcium, phosphorus level and Ca: P ratio between the days of observation.

4.5. SERUM PROGESTERONE PROFILE

Serum progesterone concentration (mean \pm SE) in Vechur cows on post-partum days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84 and 91 are presented in the Table 6. No significant difference was observed between the mean serum progesterone profiles of different days of observation.

Serum progesterone concentrations were observed to be varying between the animals. Peak serum progesterone value of four cows was between 5 to 8

Table 5. Serum Ca and P levels (mean \pm SE) in the post-partum Vechur cows (n=8)

Post-partum days	Serum calcium (mg/dl)	Serum phosphorus (mg/dl)	Ca : P ratio
Day 7	11.38 \pm 0.50	5.81 \pm 0.52	2.02 \pm 0.11
Day 14	10.00 \pm 0.71	5.28 \pm 0.34	1.89 \pm 0.05
Day 21	11.38 \pm 0.63	5.66 \pm 0.26	2.02 \pm 0.10
Day 28	10.63 \pm 0.71	5.30 \pm 0.34	2.01 \pm 0.08
Day 35	10.75 \pm 0.68	5.57 \pm 0.26	1.93 \pm 0.08
Day 42	10.75 \pm 0.56	5.38 \pm 0.21	1.99 \pm 0.05
Day 49	10.63 \pm 0.57	5.38 \pm 0.35	2.03 \pm 0.17
Day 56	11.00 \pm 0.85	5.57 \pm 0.34	1.98 \pm 0.11
Day 63	10.63 \pm 0.53	5.32 \pm 0.23	2.00 \pm 0.09
Day 70	10.38 \pm 0.57	4.97 \pm 0.17	2.08 \pm 0.08
Day 77	10.13 \pm 0.55	4.98 \pm 0.28	2.09 \pm 0.16
Day 84	10.63 \pm 0.53	5.41 \pm 0.26	1.97 \pm 0.07
Day 91	10.13 \pm 0.40	5.10 \pm 0.19	2.00 \pm 0.09
F- value	0.77 ^{ns}	0.83 ^{ns}	0.31 ^{ns}
p- value	0.54	0.52	0.83

ns- Non-significant

Table 6. Serum progesterone concentration (mean \pm SE) in post-partum Vechur cows assessed by Enzyme immunoassay (n=8)

Post-partum days	Mean progesterone concentration (ng/ ml)
Day 7	0.58 \pm 0.21
Day 14	1.80 \pm 0.53
Day 21	1.92 \pm 0.90
Day 28	1.11 \pm 0.28
Day 35	2.21 \pm 0.60
Day 42	2.93 \pm 0.97
Day 49	1.56 \pm 0.75
Day 56	2.22 \pm 0.75
Day 63	1.98 \pm 0.85
Day 70	1.01 \pm 0.25
Day 77	1.73 \pm 0.40
Day 84	1.12 \pm 0.24
Day 91	0.91 \pm 0.24
F- value	1.73 ^{ns}
p- value	0.194

ns- Non-significant

ng/ml (Fig. 5.) and in three cows, it was between 1 and 3 ng/ml (Fig. 6). Progesterone concentration of cow number four was above 1ng/ml in all the observations with a peak value of 2.45 ng/ml (Fig. 7.). By 14th day post-partum, all the cows had serum progesterone level above 1ng/ml.

4.6. OVARIAN REBOUND/ RESUMPTION OF OVARIAN CYCLICITY

Resumption of ovarian activity was observed to be on 13.13 ± 0.88 days post-partum with serum progesterone level above 1ng/ml. The day of resumption of ovarian activity in individual cows is presented in the Table 7.

4.7. OCCURENCE OF POST-PARTUM OESTRUS

Onset of post-partum first behavioural oestrus signs in Vechur cows were observed on mean days of 49.71 ± 7.76 post-partum with a range of 28 to 84 days. Oestrus signs were not observed even upto 91 day post-partum in one of the cows.

4.8. OESTRUS CHARACTERISTICS

Different characteristics of the first post-partum observed oestrus were recorded and the intensity graded according to the score card as intense, intermediate and weak. This is represented in Fig.8. Per cent of animals exhibited intense, intermediate and weak oestrus were 57.15 (4/7), 29.57 (2/7) and 14.28 (1/7), respectively.

Table 7. Day of resumption of ovarian activity in Vechur cows according to serum progesterone profile (> 1ng/ ml)

Cow No.	Post-partum Days
Cow 1	Day 14
Cow 2	Day 14
Cow 3	Day 14
Cow 4	Day 7
Cow 5	Day 14
Cow 6	Day 14
Cow 7	Day 14
Cow 8	Day 14

Discussion

5. DISCUSSION

5.1. CHARACTERISTICS OF THE LOCHIAL DISCHARGE

Most of the studies on lochial discharge of cows have been done to assess the abnormal puerperium (Williams *et al.*, 2005; López-Helguera *et al.*, 2012 and Shwetha, 2016) and there is paucity of studies on grading of normal lochial discharge of cows. The duration of the presence of normal lochial discharge in cows was found to be highly variable in different studies. Lochial discharge visible immediately after calving and extended upto 12 to 18 days in dairy cows (Sheldon, 2004). However, Shwetha (2016) has observed the presence of lochial discharge upto 20 to 30 days in post-partum Holstein cows.

In the present study, presence of lochial discharge was observed in Vechur cows on mean of 5.63 ± 0.38 days which ranged from four to seven days. Vechur is an indigenous cattle breed with a very low body size and small genitalia when compared to other dairy cattle of Indian or exotic origin. Hence, shorter duration of the lochial discharge could be attributed to the small size of the breed and its genitalia.

5.1.1. Colour

The colour of lochial discharge in Vechur cows changed from the initial reddish brown colour to off white colour with necrosed tissue and blood stains indicating the presence of white caruncular pieces and slight haemorrhage. Later, the discharge became less reddish coloured and transformed into a clear mucoid discharge. Shwetha (2016) also observed that, the disappearance the lochial discharge preceded a clear mucoid discharge. However, on tenth day post-partum the author observed an increase in blood content of the discharge. This was not observed during the present study which might be attributed to the possibility of decreased haemorrhage and shorter duration of presence of lochial discharge in post-partum Vechur cows. The variation in colour of lochial discharge at different days post-partum was also reported by Noakes (2009) and Sheldon *et al.* (2011).

However, Noakes (2009) reported a second cleansing characterised by an increase in fluid and tissue debris content during 7- 10 days post-partum. This was not observed in Vechur cows may be because of the short duration of lochial discharge.

5.1.2. Volume

Mean volume of lochial discharge in post-partum Vechur cows were 93.63 ± 11.36 ml with a range of 51 ml to 140 ml. None of the cows voided lochial discharge while standing. Lochial discharge was observed on per-rectal palpation. The mean volume of lochial discharge in Vechur was found to be much lower than the earlier reports of Roberts (1971) and Sane (1994). According to Roberts (1971), the amount of lochia in primipara dairy cows was about nil to 50 ml and in pluripara, it was about 800 to 2000 ml. Sane (1994) observed that the amount of lochial discharge in dairy cows were about 1400 to 1600 ml during first 48 hours after parturition and decreased later on. The decreased volume of lochia in Vechur cows may be due to its small body size and smaller genitalia when compared to other dairy breeds.

5.1.3. Consistency

Consistency of the lochial discharge was graded according to the content of mucus. A moderately thick mucus discharge was observed on day one post-partum in Vechur cows and this became more mucoïd in the later days. Saut *et al* (2011) observed muco-haemorrhagic lochial discharge on day three post-partum. The blood content in the lochial discharge was very less when compared to other crossbred and exotic dairy cows. This may be due to less haemorrhage from caruncular region. The clear mucoïd discharge prior to disappearance of the lochia was in agreement with the observations of Shwetha (2016).

5.1.4. Odour

The odour of the post-partum vaginal discharge in Vechur cows was neutral or no foetid odour. This finding was in agreement with the observations of Saut *et al.* (2011) and Shwetha (2016). Foetid odour of the post-partum vaginal discharge post-partum has been reported to be an indication of uterine infections (Dolezel *et al.*, 2008). The present study was conducted in cows having normal puerperium, and this might be the reason for the non-foetid or neutral odour of the lochia.

5.2. CLINICO-GYNAECOLOGICAL EXAMINATION OF THE REPRODUCTIVE TRACT

5.2.1. Cervical size and consistency

The mean cervical diameter of the Vechur cows was observed to be highest on day seven post-partum with a diameter of 4.19 ± 0.16 cm. The mean diameter of the cervix reduced to 3.44 ± 0.11 , 2.94 ± 0.10 , 2.28 ± 0.15 and 1.94 ± 0.14 cm during 14, 21, 28 and 35 days post-partum with significant difference ($p < 0.01$). No significant reduction in diameter of the cervix was observed thereafter. The trend in decrease in diameter of the cervix was comparable to that observed by Theodore (2015) with a mean diameter on 7, 14, 21, 28, 35 and 42 days post-partum as 5.13 ± 0.05 , 4.48 ± 0.14 , 3.51 ± 0.16 and 3.15 ± 0.02 , 2.99 ± 0.08 and 2.88 ± 0.09 cm, respectively. Abdel-Khalek *et al.* (2013) observed mean cervical diameters of 4.66 ± 0.23 , 4.10 ± 0.18 , 3.92 ± 0.29 , 3.76 ± 0.22 and 3.22 ± 0.23 cm on post-partum days 7, 14, 21, 28 and 35, respectively. However, both the authors observed no significant difference between the mean diameters on day 28 and day 35 post-partum.

The mean length of cervix of Vechur cows was highest on day seven post-partum (7.25 ± 0.45 cm) and it decreased with significant difference ($p < 0.01$) to 5.75 ± 0.28 , 4.53 ± 0.28 , 4.00 ± 0.32 and 3.44 ± 0.15 cm respectively on days 14, 21, 28 and 35 post-partum. Later on the difference in length of cervix was observed to be non-significant. The trend of decrease in length of cervix over the post-partum days in Vechur was in accordance with the report of Abdel-Khalek *et al.* (2013) except that the differences were no longer significant beyond the 28th day post-partum.

In the present study, the involution of cervix was observed to be between 28 and 35 days post-partum. This finding was in accordance with the observations of Fonseca *et al.* (1983) and Guilbault *et al.* (1987). Fonseca *et al.* (1983) observed involution of cervix on mean 23.7 ± 6.9 days and 20.9 ± 4.6 days in Holstein and Jersey cows, respectively. In Brown-Swiss cows, Guilbault *et al.* (1987) observed cervical involution on 35 days post-partum. However, when compared to the diameter of cervix in other breeds, Vechur cows were found to have a small cervical diameter. The diameter of cervix in Vechur cows was below 2 cm after involution from post-partum day 35 onwards whereas, it was reported to be 2.9 cm in other dairy cows (Morrow *et al.*, 1969) and 3.2 cm in Brown- Swiss cows Guilbault *et al.* (1987).

5.2.2. Uterine size and consistency

The mean diameter of previously gravid uterine horn had a significant difference ($p < 0.01$) from day seven post-partum to Day 35 post-partum with the highest diameter on day seven post-partum. From Day 35 onwards, the diameter of previously gravid uterine horns did not vary significantly. Hence, the involution of previously gravid uterine horn was observed to be between Day 28 and 35 post-partum. This finding was in agreement with Fonesca *et al.* (1983), Guilbault *et al.* (1987), Abayawansa *et al.* (1991), Hajurka *et al.* (2005), Ammu and Dhami (2012) and Praveen (2016) in various dairy cows. Ammu and Dhami (2012) in Gir cows observed involution of uterus on mean 36.54 ± 0.68 days post-partum. Abayawansa *et al.* (1991) in Sri Lankan Zebu cattle observed uterine involution on 24 ± 4.70 days. Praveen (2016) reported uterine involution on mean 32.22 ± 1.21 days in crossbred cows. The mean days required for uterine involution observed by Fonesca *et al.* (1983) in Holstein and Jersey cows were 23.80 ± 8.40 and 21.70 ± 5.30 days, respectively. Guilbault *et al.* (1987) in Brown Swiss cows and Hajurka *et al.* (2005) in pluriparous dairy cows observed uterine involution on 35 days and 27.30 ± 5.50 days, respectively.

In present study, involution of uterine horns occurred slowly than that observed by Eduvie (1985) in Nigerian indigenous cattle White Fulani and Sokoto Gudali cows (24-25 days), Sharawy *et al.* (2015) in dairy animals (20.7 ± 0.49 days) and Theodore (2015) in Crossbred cows 24.50 ± 1.14 days.

In the present study, decrease in diameter of the uterine horns was in a faster rate upto Day 21 post-partum followed by a gradual reduction in the diameter. Uterine involution in Vechur cows found to progress in a decreasing logarithmic pattern and this was in agreement with the reports of Noakes (2009) and Theodore (2015).

Genitalia of all the animals were observed to have tonicity and curling on Day 21 post-partum and during this time, whole uterus was palpable inside the pelvic-cavity. The observation of the present study was in agreement with the findings of Theodore (2015). Theodore (2015) also reported that the tonicity of the uterine horns increased from day seven post-partum and observed a highest tonicity on Day 21 to 28 day post-partum.

5.2.3. Palpable structures in the ovary

Palpation and differentiation of the structures in the ovary was found to be very difficult in Vechur cows. This could have been due to the small ovary size of the breed, conforming to the findings of Abayawansa *et al.* (1991) in Zebu cattle. The mean number of days at which follicles were palpable was higher than that observed by Webb *et al.* (1980) in post-partum dairy cows. CL in Vechur cows was palpable for the first time on mean 21 ± 8.50 days with a range of 14 to 49 days. Praveen (2016) reported that the presence of CL was observed in only 33.33 per cent of the crossbred dairy cows studied within 40 days post-calving.

5.3. ULTRASONOGRAPHIC EXAMINATION OF THE REPRODUCTIVE TRACT

5.3.1. Cornual diameter

Diameter of both previously gravid and non-gravid uterine horns had highest measurement on seventh day post-partum. The mean diameter of the previously gravid uterine horns on day seven post-partum was 3.23 ± 0.08 cm which differed significantly ($p < 0.01$) with the diameters on days 14, 21, 28, 35 and 42 as 2.63 ± 0.13 , 2.07 ± 0.14 , 1.77 ± 0.11 , 1.59 ± 0.07 , 1.51 ± 0.05 cm, respectively. From the Day 42 onwards, there was no significant difference in the mean diameter of previously gravid uterine horn.

The decrease in mean diameter of non-gravid uterine horn also followed the same trend. The highest diameter was noted on the seventh day of observation *i.e.*, 2.59 ± 0.14 cm which decreased significantly ($p < 0.01$) to 2.16 ± 0.12 , 1.68 ± 0.10 , 1.53 ± 0.04 , 1.47 ± 0.04 and 1.43 ± 0.05 cm on days 14, 21, 28, 35 and 42, respectively. From the Day 42 post-partum, no significant difference was observed between the mean diameters. Hence, the day of uterine involution was identified to fall between Day 35 and 42 post-partum.

The findings were in agreement with the reports of Okano and Tomizuka (1987), Rajamahendran and Taylor (1990), Kamimura *et al.* (1993), Cengic *et al.* (2012) and Theodore (2015) as they observed uterine involution on 40 days, 27 ± 6 days, 41.5 days, 38- 45 days and 31.97 ± 1.82 days, respectively. However, Stephen (2010) observed no significant difference in the diameter of uterine horn from 35 day post-partum. Poyam and Tiwari (2015) observed significant reduction in both the uterine horns of Sahiwal cows from 14 to 44 days post-partum. Scully *et al.* (2013) reported a slightly high range of time periods for complete uterine involution in Holstein cows (43.24 ± 3.94 days).

Uterine involution was rapid during first 21 days post-partum which later on decreased gradually following a logarithmic pattern. Cengic *et al.* (2012) also observed a same pattern of reduction in diameter of the post-partum uterine horns.

After 35 days post-partum, the diameter of previously gravid uterine horns of Vechur cow was around 1.5 cm. Diameter of the uterine horns of Vechur cows were observed to be smaller than that of other breeds. However, (Stephen, 2010)

reported a diameter more than 3 cm after 35 days post-partum in dairy cows. Uterine diameter of more than 2 cm was reported in dairy cows and Sahiwal cows by Cengic *et al.* (2012) and Poyam and Tiwari (2015), respectively. The variation in the diameter of the uterine horn in Vechur cows may be attributed to breed difference.

5.3.2. Presence of fluid in the uterine lumen

Fluid was observed in the uterine lumen in 75 per cent of the Vechur cows during day seven post-partum which disappeared later on by 14 day post-partum. Presence of fluid in uterine lumen has been reported for a longer time by Okano and Tomizuka (1987), Kamimura *et al.* (1993) and Shwetha (2016). The variation in the present study may be due to the decreased volume of lochial discharge and a shorter duration of the presence of lochial discharge.

In Vechur cattle, the lochia appeared slightly hypoechoic under ultrasonography. However, Kamimura *et al.* (1993) observed snowy appearance of the lochia in the uterine lumen till 18 days post-partum. This variation may be due to apparently lesser content of necrosed tissue and cellular debris in the uterine fluid of post-partum Vechur cows.

5.3.3. Changes in the echogenicity of the reproductive tract

5.3.3.1. Cervix and uterus

Echogenicity of the reproductive tract in Vechur cows were found to increase from the day seven post-partum. The echogenicity of the cervix was more from the Day 21 post-partum and uterine horns showed more echogenicity from Day 14 itself. The hypoechoic appearance of the reproductive tract on day seven post-partum was due to its swollen oedematous condition. The present findings were in agreement with the reports of Okano and Tomizuka (1987), Kamimura *et al.* (1993) and Sutaria *et al.* (2014) in various dairy cows.

5.3.3.2. Ovary

In the present study, the echogenicity of the ovaries varied with the structures presented in it. Follicles were anechoic with hyperechoic wall. CL was observed as a grainy echogenic structure with well-defined border. The observation was in agreement with the reports of Pierson and Ginther (1984) and Wolfgang (1994). The highest diameter of the follicle obtained in the present study among all the Vechur cows using ultrasonography was 9.3 mm and this measurement was found to be lower than that in Gir, another Indian cattle breed, reported by Viana *et al.* (2000). The largest CL observed in the present study had a diameter of 19 mm which was in agreement with the observation of Pierson and Ginther (1984).

5.4. POST- PARTUM SERUM CALCIUM, PHOSPHORUS LEVELS AND Ca: P RATIO

Mean weekly serum calcium levels in the Vechur cows during the post-partum days seven to 91 had no significant difference between them. Kamgarpour *et al.* (1999), Heppelmann *et al.* (2015) and Brodzki *et al.* (2016) observed serum calcium level below 8 mg/dl in animals having endometritis. Kamgarpour *et al.* (1999) opined that the cows with serum calcium level below 8 mg/dl can be considered as hypocalcaemic. The estimates of serum calcium levels in the present study was higher than 8 mg/dl indicated that Vechur cows under study were neither hypocalcemic nor suffering from endometritis and they had a normal uterine involution in line with the reports of Kamgarpour *et al.* (1999), Heppelmann *et al.* (2015) and Brodzki *et al.* (2016).

Mateus and Lopes da Costa (2002) and Theodore (2015) also observed comparable results in the animals having normal puerperium with a plasma calcium level of 8-11.3 mg/ dl and 8.18 ± 0.23 mg/ dl, respectively.

Serum calcium profile in the post-partum Vechur cows did not vary ($p > 0.05$) significantly in contrary to the findings of Dagli *et al.* (2004) where he observed a gradual increase in the serum calcium levels from first to eight week

post-partum. The different trend in Vechur cows is attributable to the lesser milk production of these cows when compared to other crossbred and exotic dairy cattle

Serum phosphorus level in the post-partum Vechur cows did not vary significantly ($p > 0.05$) during the post-partum period. The results were in agreement with the findings of Mateus and Lopes da Costa (2002), Ammu (2011) and Theodore (2015).

Serum Ca: P level in the post-partum Vechur cows also did not vary significantly ($p > 0.05$) during the post-partum period. The results were in agreement with the findings of Mateus and Lopes da Costa (2002), Ammu (2011) and Theodore (2015).

5.5. SERUM PROGESTERONE PROFILE

Mean serum progesterone profile did not show any significant difference ($p > 0.05$) between the days of observation. However, all animals except one, had serum progesterone concentration below 1 ng/ml on the seventh day post-partum. All the animals had serum progesterone concentration above 1 ng/ml on Day 14 post-partum. Webb et al. (1980) reported an increase in progesterone level above 1ng/ml on mean 16.6 ± 1.1 days after calving in dairy cows. However, Scully *et al.* (2013) reported serum progesterone concentration greater than 1ng/ml by Day 21 post-partum in dairy cows. Theodore (2015) observed plasma progesterone concentration below 1 ng/ml upto Day 24 post-partum and Praveen (2016) reported serum progesterone concentration below 1ng/ml upto 40 days in post-partum crossbred cows. Serum progesterone concentration was varying between the animals. This observation was in accordance with Robertson (1972)

5.6. OVARIAN REBOUND/ RESUMPTION OF OVARIAN CYCLICITY

Serum progesterone concentration above 1ng/ml was considered to be an indication of resumption of ovarian cyclicity in Vechur cows in the present study.

Similar assumption has been made by Rekwot *et al.* (2000), Shrestha *et al.* (2005), Bicalho *et al.* (2008), Scully *et al.* (2013) and Sharawy *et al.* (2015).

In the present study, the resumption of ovarian activity was observed to be on 13.13 ± 0.88 days post-partum when the serum progesterone level increased to 1 ng/ml. The time period for resumption of ovarian cyclicity estimated in Vechur cows under the present study was found to be much lower than the earlier reports in crossbred cattle (Sharawy *et al.*, 2015; Webb *et al.*, 1980 and Scully *et al.*, 2013). Sharawy *et al.* (2015) observed resumption of luteal activity on mean 20.3 ± 6.1 days. However, Webb *et al.* (1980) observed ovarian rebound on mean 16.6 ± 1.1 days after calving in dairy cows whereas, Scully *et al.* (2013) observed resumption of ovarian activity on 21 days post-partum. The variations in the day of resumption of ovarian activity may be due to the difference in the breed and management practices followed. The present finding is advantageous in that the Vechur cows regain their normal ovarian activity earlier than crossbred cattle.

5.7. OCCURRENCE OF POST-PARTUM OESTRUS

The day of first observed oestrus post-partum in the present study varied widely between the animals. In Vechur cows the first observed behavioural oestrus was on mean 49.71 ± 7.76 days ranging from 28 to 84 days. One of the animals was not observed to be in behavioural oestrus during the period of observation upto Day 91 post-partum. The days for onset of post-partum oestrus in Vechur cows estimated under the study is in close accordance with the values reported for Sri Lankan Zebu cattle (Abayawansa *et al.*, 1991). Much higher and lower estimates have also been reported by many authors *viz.*: in Gir (Ammu and Dhani, 2012), crossbred cows (Praveen, 2016) and exotic cattle (Wagner and Williams, 1969).

Ammu and Dhani, (2012) also observed a wide range of 70 to 101 days for the observation of first oestrus after calving in Gir cows. The mean length in days from parturition to first observed oestrus was 46.50 ± 39.50 in Sri Lankan Zebu cattle (Abayawansa *et al.*, 1991). However, in crossbred cattle, Praveen (2016)

observed first oestrus in shorter interval of days of 30.67 ± 4.67 . Whereas, in exotic cattle Wagner and Williams, (1969) observed oestrus on 14 days post-partum. The time lag for the onset of first post-partum behavioural oestrus in Vechur cows may be attributed to the more frequent occurrence of silent oestrus in indigenous cattle during the initial post-partum days, justified with serum progesterone assay.

5.8. OESTRUS CHARACTERISTICS

According to the score card, the per cent of animals that exhibited intense, intermediate and weak oestrus were 57, 29 and 14, respectively. The proportions under the present study varied from earlier reports (Praveen, 2016) in which the author observed low, medium and high oestrus intensity in 33.33, 44.44 and 22.22 per cent of crossbred cows, respectively.

Summary

6. SUMMARY

Enhancement of reproduction in Vechur cattle is an emerging necessity as it is a critically maintained breed with huge demand. Puerperium is the period involving critical physiological and endocrinological events which determines the future fertility of an animal. The present study was conducted in eight Vechur cows of parity between two to five, with a normal calving history and normal puerperium. Objectives of this study were gynaecological and ultrasonographic studies to evaluate the post-partum uterine and ovarian changes and to estimate progesterone profile to assess the post-partum resumption of ovarian activity.

Cows were examined for the presence of lochial discharge from day one post-partum till the cessation of discharge. The characteristics of lochial discharges such as its colour, volume, consistency and odour were assessed. Per-rectal examinations of the cows were done on weekly interval from day seven to day 91 post-partum in order to evaluate the diameter of cervix, diameter of both uterine horns and palpable structures on the ovary. Ultrasonographic examination of the uterus was also done on the same days to assess the diameter of both uterine horns, presence of fluid in the uterine lumen and echogenicity of the reproductive tract. Blood collection was carried out to estimate the serum progesterone, calcium and phosphorus concentration of Vechur cows during the days of observation.

The lochial discharge was observed in the Vechur cows for a mean 5.63 ± 0.38 days ranging from four to seven days with a mean volume of 93.63 ± 11.36 ml ranging from 51 to 140 ml. According to the colour and consistency of the lochial discharge, it was graded as reddish brown moderately thick mucus discharge, off- white thick mucus discharge, light brown thin mucus discharge and clear transparent thick mucus discharge. Odour of the lochial discharge was neutral or non-foetid.

On per-rectal examination, the cervix was observed to be involuted between day 35 and 42 post-partum. Mean diameter and length of cervix when assessed per-rectally decreased significantly ($p < 0.01$) between days seven to 42 post-

partum. Diameter (mean \pm SE) of cervix were 4.19 ± 0.16 , 3.44 ± 0.11 , 2.94 ± 0.10 , 2.28 ± 0.15 , 1.94 ± 0.14 , 1.75 ± 0.08 cm on weekly intervals from day seven to 42 post-partum. Cervical length (mean \pm SE) were 7.25 ± 0.45 , 5.75 ± 0.28 , 4.53 ± 0.28 , 4.00 ± 0.32 , 3.44 ± 0.15 and 3.38 ± 0.15 cm on weekly interval from day seven to 42 post-partum. Thereafter no significant difference in cervical diameter and length were observed.

Per-rectally, the uterine horns were palpable in the pelvic cavity with tonicity and curling on day 21 post-partum in all the cows. Non-gravid uterine horns of the cows were observed to be involuted faster than the previously gravid uterine horn when assessed by per-rectal examination. Significant difference ($p < 0.01$) was observed upto day 28 post-partum in non-gravid uterine horns suggesting the involution of non-gravid horn to be occurred between day 21 and day 28 post-partum. The mean diameter of previously gravid uterine horn was observed to have significant difference ($p < 0.01$) upto day 35 post-partum. Therefore, the involution of previously gravid uterine horn was occurred between day 28 and day 35 post-partum. Per-rectal examination of the ovaries revealed the presence of palpable follicles and corpus-luteum on mean 30.63 ± 5.11 days and 21 ± 8.50 days post-partum, respectively.

Ultrasonographic examination of uterine horns revealed complete involution of uterus between day 35 and 42 post-partum. Both uterine horns had a significant difference in diameter between the weekly observations upto day 42 post-partum which was absent later on. Fluid in the uterine lumen was observed to be hypoechoic to anechoic in 75 per cent on day 7 post-partum and was absent on day 14 observation. Echogenicity of the cervix and uterus was observed to be increased during post-partum because of the reduction in swelling. Ovaries had different echogenicity during post-partum period according to the structures presented in it. The largest diameter of the follicle obtained among all the cows using ultrasonography was 9.3 mm. The largest corpus luteum had a diameter of 19 mm.

Serum progesterone concentration was below 1ng/ml in all the cows except one. But in all the animals it was above 1ng/ml on day 14 post-partum. Thus the resumption of ovarian activity was observed to be on mean 13.13 ± 0.88 days in post-partum cows.

Serum calcium, phosphorus levels and Ca: P ratio had no significant difference between the days of observation. All the animals were normocalcaemic throughout the period of the study.

Onset of first post-partum behavioural oestrus signs post-partum was observed on mean days of 49.71 ± 7.76 . The intensity of oestrus was graded as intense, intermediate and weak oestrus in 57.15, 29.57, and 14.28 per cent of animals, respectively.

Since there is no post-partum studies reported in Vechur cows, the present study can be considered as a base work for future post-partum studies. It can be concluded that lochial discharge in post-partum Vechur cows were observed for mean 5.63 ± 0.38 days which is a shorter interval when compared to studies in other cattle breed. Ultrasonographic studies revealed complete uterine involution between 35 to 42 days post-partum. Resumption of ovarian activity was observed to be on mean 13.13 ± 0.88 days post-partum. Though the animals were found to resume their ovarian cyclicity at an earlier post-partum period, the observed oestrus was appreciated later which may be due to the occurrence of silent oestrus in post-partum period of Vechur cows. Since the ovarian cyclicity in Vechur cows occurs earlier, interventions for reproductive enhancement can be carried out during early post-partum to ensure an optimum calving interval.

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Abstract

ABSTRACT

The study was conducted in eight post-partum Vechur cows having normal calving history and puerperium with the objectives of gynaecological and ultrasonographic studies to evaluate the post-partum uterine and ovarian changes in and to estimate progesterone profile to assess the post-partum resumption of ovarian activity. The characteristics of lochial discharge such as colour, volume, consistency and odour were assessed. Per-rectal examination, ultrasonographic examination and blood collection of the cows were performed on weekly interval starting from the day seven to day 91 post-partum to assess the cervical and uterine involution and ovarian activity. On per-rectal examination, cervix was observed to be involuted between 35 to 42 day post-partum. Previously gravid and non-gravid uterine horns were observed to be involuted between 28 to 35 days and 21 to 28 days post-partum, respectively. On ultrasonographic examination, the echogenicity of the cervix and uterus was observed to be increased as the involution proceeded and both uterine horns involuted between 35 to 42 days post-partum. Per-rectal examination of the ovaries revealed the presence of palpable corpus-luteum and follicles on mean 21 ± 8.50 and 30.63 ± 5.11 days post-partum, respectively. Resumption of ovarian activity was found to be achieved on mean 13.13 ± 0.88 days post-partum as evidenced by serum progesterone level above 1 ng/ml. The estimated serum Ca, P levels and Ca: P ratios were not having any significant difference between the days of observation. Onset of post-partum behavioural oestrus signs were observed by mean 49.71 ± 7.76 days with a graded oestrus intensity of intense, intermediate and weak in 57.15, 29.57, and 14.28 per cent of cows, respectively.

Appendices

ANNEXURE-1

Score Card for assessing intensity of oestrus

SI No.	OESTROUS CHARACTERS	POINTS		
		Split- up	Maximum	Total
1.	Behavioural changes		5	5
	1. Restlessness and alertness	1		
	2. Mounting	1		
	3. Standing to be mounted	1		
	4. Posture	1		
	5. Chin resting and rubbing	1		
2.	Physiological changes			5
	1. Vulval oedema			
	• Highly oedematous	2	2	
	• Oedematous	1		
	• Not oedematous	0		
	2. Urination intermittent, incomplete	1	1	
	3. Genital discharge			
	• Large volume, clear, stringy	2		
	• Moderate volume, stringy to viscous	1		
• Sparse volume, viscous, flecks of pus	0			
3.	Gynaecological observation			5
	1. Fern pattern			
	• Typical fern pattern	2		
	• Atypical fern pattern	1		
	• No fern pattern	0		
	2. Cervical relaxation	1		
	3. Uterine tonicity			
	• Highly tonic	2		
	• Tonic	1		
• Flaccid and atonic	0			
	TOTAL			15
	GRADING	Intense- 10 to 15 points Intermediate- 5 to 10 points Weak- 0 to 5 points		

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

Evaluation of postpartum uterine involution and resumption of ovarian cyclicity in Vechur cows

Department of Animal Reproduction, Gynaecology and Obstetrics,
College of Veterinary and Animal Sciences, Pookode, Wayanad, 673 576.

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

Meenuja M.S.

(b) Admission No.

15-MVP-10

4. (a) Name of Major Advisor (Guide)

Dr. Abdul Azeez C.P.

(b) Designation

Assistant Professor

5. Objectives of the study

3. Gynaecological and ultrasonographic studies to evaluate the postpartum uterine and ovarian changes in Vechur cows.
4. To estimate progesterone profile to assess the postpartum resumption of ovarian activity.

6. Practical / Scientific utility

The study on normal uterine involution and resumption of ovarian activity in a population will help to plan the postpartum breeding programmes. This will

also help to differentiate between physiological and pathological uterine changes after parturition.

Postpartum reproductive changes has been studied in detail in different breeds of cattle, but there is a paucity of information with respect to Vechar cattle. The breedable population of Vechar cows all over the state is alarmingly low. Hence the present study is envisaged to acquire scientific knowledge regarding postpartum changes in Vechar cattle to suggest some valuable strategies for the multiplication of the available stock.

7. Important Publications on which the study is based

Kamimura *et al.* (1993) observed that uterine involution in Holstein cows occurred by 41.5 days in normal postpartum. They also characterised uterine involution as resumption of uterine horns with symmetrical diameters in a contracted form within the pelvic cavity.

Zain *et al.* (1995) reported early uterine involution in dairy cows

without puerperal complications (at 22 days) than animals with puerperal complications (>30 days).

Opsomer *et al.* (1996) opined that to achieve an economically optimal calving interval of 12 months, the postpartum cow should be inseminated in 50 to 60 days postpartum during an observed oestrus following resumption of ovarian activity.

Kindahl *et al.* (1999) noted that breed, management and environmental factors influence postpartum reproductive changes in dairy cows.

Sheldon *et al.* (2000) used ultrasonography to study the effect of uterine involution and ovarian activity in reproductive performance of dairy cows.

Dagli *et al.* (2004) observed a gradual increase in mean serum calcium values of postpartum cows from first week to eight week postpartum.

Hajurka *et al.* (2005) observed that uterine involution was completed by 40 days in primiparous (100%) and pluriparous (97.1 %) cows with normal puerperium.

Ammu *et al.* (2012) observed uterine involution at 35.66 ± 1.31 days in postpartum Gir cows.

Cengic *et al.* (2012) reported that ultrasonography could precisely detect the rate of uterine involution in cows.

Sutaria *et al.* (2014) studied transrectal ultrasonography in postpartum Kankrej cows and reported variation in echogenicity of postpartum reproductive tract with an increment in echogenicity as days advanced.

8. Outline of the Technical Programme

The study will be conducted at the Vechur Conservation Unit, Instructional Livestock Farm Complex of College of Veterinary and Animal Sciences, Pookode. Eight postpartum Vechur cows (parity between two to five) having

normal calving history will be selected. Clinico-gynaecological and trans-rectal ultrasonographic examination (5.0 to 7.5 MHz) of the reproductive tract to monitor postpartum changes in the uterus and ovaries, estimation of serum progesterone profile for the assessment of resumption of ovarian activity and estimation of serum calcium and phosphorus levels will be done at weekly interval starting from day 7 to day 91 postpartum. The animals will be closely observed for the occurrence of oestral signs upto day 91 postpartum.

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

9. Main items of observations

1. Characteristics of lochial discharge.
2. Clinico-gynaecological examination of the reproductive tract.
 - Uterine size and consistency.
 - Cervical size and consistency.

- Palpable structures on the ovary.
3. Ultrasonographic examination of reproductive tract.
 - Cornual diameter.
 - Presence of fluid in the uterine lumen.
 - Changes in the echogenicity of the reproductive tract.
 4. Serum progesterone profile.
 5. Serum calcium and phosphorus level.
 6. Occurrence of oestrous signs.
 7. Oestral characteristics.

10. Facilities

(a) Existing

Facilities in the Department of Animal Reproduction, Gynaecology and Obstetrics, Veterinary Biochemistry and Central Instrumentation Laboratory, College of Veterinary and Animal Sciences, Pookode and Mannuthy will be utilized for the study.

(b) Additional facilities required

Facilities available in the Instructional Livestock Farm Complex, Livestock farms under KVASU and Artificial Insemination Centre under KVASU will be utilized for the study.

11. Duration of study

Four semesters

12. Financial estimate

Chemicals and biologicals:

Rs.15,000.00

Contingencies :

Rs.10,000.00

Total :

Rs.25,000.00

Signature of Student

Signature of Major Advisor

Place: Pookode

Date:

Name, Designation and Signature of members of the Advisory Committee

Major Advisor

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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. Preparation of synopsis.
3. Selection of animals for research work.

Semester III

1. Clinico-gynaecological examination of the cows.
2. Ultrasonographic evaluation for assessing uterine involution and blood sampling at weekly interval.

Semester IV

1. Progesterone profiling for assessment of ovarian activity.
2. Compiling 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:

Dr. Abdul Azeez C. P.

(Major advisor)

CURRICULUM VITAE

1. **Name of the candidate** : Meenuja M.S.
2. **Date of Birth** : 15th September 1990
3. **Place of Birth** : Thiruvananthapuram
4. **Marital Status** : Married
5. **Permanent Address** : Meleveedu
Kariavattom P.O
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6. **Email id** : meenujadilip@gmail.com
7. **Major Field of Specialization** : Animal Reproduction,
Gynaecology and Obstetrics
8. **Educational Status** : B.V.Sc & AH
9. **Publications Made**
 - a) **Full paper published** : 1
 - b) **Conference abstract** : 4
10. **Membership of Professional Societies** :
 - i) Indian Veterinary Association
 - ii) Indian Society for Advancement of Canine Practice
 - iii) Indian Society for Study of Animal Reproduction

