Corpus luteum dimensions and vascularity reflect its functionality in pregnant and non-pregnant buffaloes

M. Gaur[1] and G. N. Purohit[2*]

[1]Department of Veterinary Gynecology and Obstetrics, College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur; [2]Department of Veterinary Gynecology and Obstetrics, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India 334001.

* Corresponding author email:gnpobs@gmail.com

Abstract

With an objective to record the dimensions, vascularity changes in the corpus luteum and progesterone production in pregnant and non-pregnant buffaloes, Surti buffaloes (n=42) were inseminated during natural estrus during the breeding season and scanned by transrectal B mode and color flow mode ultrasonography daily starting from Day 3, till Day 21. The CL dimensions on Day 5, 11 and 13 were significantly higher (P<0.05) in buffaloes that conceived compared to those that did not conceive. On Day 18, the CL diameters were significantly lower in non-pregnant buffaloes. The amount of color pixel in color Doppler evaluation of vascularity of CL showed fluctuating increase in both pregnant and non-pregnant buffaloes up to Day 14 but, thereafter a decrease was recorded. In buffaloes that did not conceive the amount of color pixel was significantly lower (P<0.05) and half of that in pregnant buffaloes. The plasma progesterone concentrations were synchronous to CL diameters and vascularity however, steep decrease in plasma progesterone was observed on Day 20 for non-pregnant buffaloes returning to estrus. It was concluded that the dimensions of the CL and its vascularity reflect its functionality and can be used to predict buffaloes that are likely to be non-pregnant.

Key words: Buffalo; corpus luteum; vascularity; progesterone; Color Doppler.

Introduction

The corpus luteum (CL) is a temporary endocrine gland formed over the ovary following ovulation and secretes progesterone to regulate estrous cycle and support the establishment of pregnancy (Niswender et al., 2000). In the absence of pregnancy in cows, the CL begins to regress by Day 17-18 (Niswender et al., 2000). The functioning of bubaline CL appears to be similar to that in cattle. The progesterone concentration from bubaline CL increased gradually to peak around Day 7 and declined around Day 17-20 coinciding with CL regression (Baithalu et al., 2013). During recent years the relationship of CL diameters and vascularity in relation to plasma progesterone production has been evaluated in cows using color Doppler ultrasonography (de Tarso et al., 2017) as this can be a predictor of fertility following artificial insemination or embryo transfer (Pinaffi et al., 2015). Although one previous report found no difference in the CL vascularity in pregnant and non-pregnant cows during early pregnancy (Beindorff et al., 2010) others found that the CL vascularity provides additional information on the CL function (Luttgenau and Bollwein, 2014) and that the blood flow to the CL on Day 20 represents a quick, reliable, and consistent diagnostic test that enables the early detection of non-pregnant cattle (Siqueira et al., 2013). Moreover, Doppler echo can be used to visualize fetal loss, a situation where the blood flow to the corpus luteum decreases rapidly before the next estrus (Matsui and Miyamoto, 2009). Recently, Pugliesi et al. (2018) found that transferring embryos to cows with a higher blood perfusion and thus better functionality; resulted in higher proportion of pregnancies. A few reports on buffaloes evaluated the vascularity of CL in buffaloes (Caunce et al., 2019; Gaur and Purohit, 2019). The present study evaluated the dimensions and vascularity of CL of Surti buffaloes from Day 3 till Day 21 of insemination during natural estrus using color Doppler ultrasonography and compared them between buffaloes that were subsequently found either pregnant or non-pregnant.

Materials and Methods

Surti buffaloes (n=42) belonging to the elite herd maintained at College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan, India were included in the study during the breeding season (November to March 2015-16). Estrus detection was carried out by parading a teaser buffalo bull early morning and late evening as described previously (Purohit and Rao, 2018). Buffaloes in standing estrus were inseminated twice at 12 h intervals with frozen semen from high fertility buffalo bulls. Buffaloes were restrained in a chute and examined by transrectal B mode and CFM mode ultrasonography daily till pregnancy confirmation at Day 30 using procedures described previously (Zambrano-Varon, 2018). The CL dimensions were recorded, and then the vascularity changes were recorded using CFM mode. The images were stored and the pixel values of color of blood were evaluated as described previously (Gaur and Purohit, 2019).

Blood was collected from buffaloes daily and plasma was separated and stored at -20° C till assay. Progesterone concentration was estimated using ELISA kit (DIA source Immuno assay, Belgium) and the results were read on an Elisa reader as described previously (Gupta and Purohit, 2001). The correlation of plasma progesterone with vascularity of the CL was estimated. The inter- and intra-assay coefficient of variation was less than 10%.

Correlation of CL characteristics and fertility

The relationship between the CL characteristics (CL dimension, pixel values of blood flow and the plasma progesterone concentrations) on different days of estrous cycle were compared between the buffaloes that were subsequently found to be pregnant or non-pregnant to find the relationship between CL characteristics and fertility if any.

Statistical analysis

Statistical computations and data analysis was done as per standard methods mentioned previously and described in standard text (Snedecor and Cochran, 1994).

Results

For the buffaloes studied 20 buffaloes were confirmed pregnant on Day 30, eight buffaloes returned to estrus on Day 21-23 and 14 buffaloes did not return to estrus but were found non-pregnant on Day 30. The results of 20 pregnant and 22 non-pregnant buffaloes have been presented.

CL dimensions

The CL dimensions (Fig. 1) on all days of evaluation in Surti buffaloes that were subsequently found to be pregnant were higher compared to buffaloes that were non-pregnant. Significantly higher (P<0.05) CL dimensions were recorded in pregnant buffaloes on Day 5 (15.84±0.61 mm), Day 11 (20.54±0.48 mm), Day 13 (23.26±0.11 mm) and Day 18 (23.72±0.54 mm) compared to non pregnant buffaloes (11.78±0.41 mm, 16.25±0.37mm, 19.38±0.36 mm and 10.70±0.19 mm on Days 5, 11, 13 and 18 respectively).

Blood flow to CL

The pixel values for blood flow to the CL was significantly higher (P<0.01) on Day 5 (1174.6±112.30) and Day 13 (2563.6±196.54) of the estrous cycle in pregnant buffaloes (Table 1, Fig. 2) compared to non pregnant buffaloes (689.4±54.12 and 1820.25±137.94). In pregnant buffaloes the pixel values increased slightly on Day 15 (3196.2±310.41) and then declined. However, in non-pregnant buffaloes the pixel values were significantly high on Day 15 (3247.8±256.32) followed by a precipitous decline on Day 16 (1432±114.21) that continued till Day 19 (210.21±38.96) of the estrous cycle. On Day 18 the fall in blood supply in non-pregnant buffaloes was significantly higher (P<0.05) compared to pregnant buffaloes.

Plasma progesterone

Plasma progesterone concentrations in pregnant Surti buffaloes were significantly higher (P<0.05) compared to non-pregnant buffaloes on different days of the estrous cycle. Plasma progesterone on Day 5, Day 9 and Day 13 were 0.96±0.04 ng/ mL and 0.65±0.04 ng/mL, 2.64±0.09 ng/mL and 2.27±0.10 ng/mL and 4.79±0.09 ng/mL and 4.09±0.08 ng/mL in pregnant and non-pregnant buffaloes respectively. In pregnant buffaloes the plasma progesterone concentrations were maintained above 1 ng/mL throughout the 21 days of the estrous cycle whereas in non pregnant buffaloes significant decline in plasma progesterone was observed on Day 17 (1.46±0.15 ng/mL) and the decline continued to reach lowest values (0.26±0.08 ng/ml) on Day 20 of the estrous cycle. On Day 20 the progesterone concentrations were significantly higher (P<0.05) in pregnant buffaloes compared to non-pregnant buffaloes.

It was thus noticed that the characteristics of the CL (dimension and blood supply) were significantly related with functionality (progesterone concentration) in Surti buffaloes.

Table 1 Comparison between pregnant and non- pregnant Surti buffaloes for different parameters of corpus luteum

Days of Estrus	CL diameter (mm)		Amount of colour Pixel		Progesterone conc ng/mL	
	Pregnant	Non-Pregnant	Pregnant	Non-Pregnant	Pregnant	Non-Pregnant
3	13.50±0.41	11.21±0.33	689.4±54.12[a]	443±62.51[b]	0.52±0.05	0.45±0.01
4	14.72±0.54	12.95±0.23	712.8±63.24	686.75±74.51	0.65±0.04	0.56±0.03
5	15.84±0.61[a]	11.78±0.41[b]	1174.6±112.30	1013.5±152.36	0.96±0.04[a]	0.65±0.04[b]
6	16.54±0.23	14.38±0.26	783.4±85.32	655±78.45	0.92±0.03	0.83±0.06
7	17.58±0.41	15.41±0.25	1657.4±163.24	1438.75±101.21	1.33±0.11	1.24±0.10
8	18.35±0.35	17.66±0.42	1710±142.31	1666.5±156.45	1.76±0.12	1.60±0.13
9	19.86±0.34	17.18±0.31	1759.4±132.61	1515±164.51	2.64±0.09[a]	2.27±0.10[b]
10	19.84±0.29	18.75±0.46	2162.6±158.23	1820.25±137.94	3.22±0.35	3.20±0.36
11	20.54±0.48[a]	16.25±0.37[b]	2563.6±196.54	2269.75±214.65	3.47±0.34	3.18±0.33
12	20.34±0.36	18.30±0.22	2929.8±263.14	2626.75±197.69	4.73±0.17[a]	4.14±0.12[b]
13	23.26±0.11[a]	19.38±0.36[b]	3196.2±310.41	2669.25±206.54	4.79±0.09[a]	4.19±0.08[b]
14	21.19±0.19	19.68±0.31	3247.8±256.32	3224±254.60	4.78±0.41	4.56±0.51
15	19.94±0.11	17.50±0.24	1397±117.84	1432±114.21	5.10±0.52	5.14±0.47
16	18.18±0.18	17.03±0.29	465.6±89.36	418.25±74.51	4.45±0.45	4.28±0.51
17	15.66±0.26	14.63±0.34	264±52.32	275.75±41.74	3.32±0.48	3.39±0.51
18	16.72±0.54[a]	11.70±0.19[b]	411.6±36.42[a]	210.21±38.96[b]	1.52±0.12	1.46±0.15
19	15.88±0.41	13.75±0.24	192.6±36.21	179.75±23.41	1.74±0.08[a]	0.66±0.11[b]
20	15.28±0.33	13.34±0.51	185.8±30.20	177±31.20	1.65±0.02[a]	0.26±0.08[b]
21	14.10±0.20	13.06±0.48	239±32.21	214±22.19	1.42±0.02	0.23±0.31

Values with different superscripts in a row are significantly different (P<0.05).

Discussion

The CL diameters increased sequentially both in pregnant and non-pregnant buffaloes till Day 13 of estrus, thereafter the diameters decreased slightly. Comparison of CL diameters in pregnant buffaloes on Day 5, 11, 13 and 18 revealed significantly (P<0.05) higher CL dimensions compared to non-pregnant buffaloes. Similar to the present study, Lasheen et al., (2018) recorded increase in CL diameters in pregnant buffaloes yet in non-pregnant buffaloes the CL dimensions started declining from Day 18 of gestation. A previous study on lactating Holstein cows (Ricci et al., 2017) observed that cows that maintained their CL beyond Day 21 are those that underwent embryonic losses. The plasma progesterone concentration increased sequentially both in pregnant and non-pregnant buffaloes up to Day 15. The progesterone concentrations were significantly (P<0.05) higher in pregnant buffaloes compared to non-pregnant buffaloes on Day 5, 9, 12, 13 and 19. Similar increase in plasma progesterone was recorded in pregnant and non-pregnant buffaloes (Batra et al., 1979; Perera et al., 1980; Kamboj and Prakash, 1993; Gupta and Purohit, 2001). A significant (P<0.05) fall in plasma progesterone was observed in non-pregnant buffaloes on Day 19 and 20 of

estrus reflecting luteolysis. Studies on buffaloes have shown that luteolysis in buffaloes occurs between Day 18-20 (Mondal et al., 2004; Mondal et al., 2007).

The blood flow to the CL increased with fluctuations to reach a peak on Day 14, both in pregnant and non-pregnant buffaloes. A previous study observed a precipitous fall in blood flow to the CL in buffaloes during a natural estrous cycle (Gaur and Purohit, 2019). From Day 15 the blood flow to the CL started declining. The blood flow to the CL was significantly higher ($P < 0.05$) in pregnant buffaloes on Day 18 compared to non-pregnant buffaloes. Similar findings have been recorded in previous studies on cattle (Beindorff et al., 2010; Siqueira et al., 2013) and buffalo (Lasheen et al., 2018).

The changes in CL dimensions and vascularity were synchronous to plasma progesterone reflecting that the vascularity of CL changes in relation to its functionality in buffaloes during early pregnancy and corroborates with previous findings in cattle (Beindorff et al., 2010; de Tarso et al., 2017; Lasheen et al., 2018; Gaur and Purohit, 2019). It was concluded that the dimensions of the CL and its vascularity reflect its functionality and can be used to predict buffaloes that are likely to be non-pregnant.

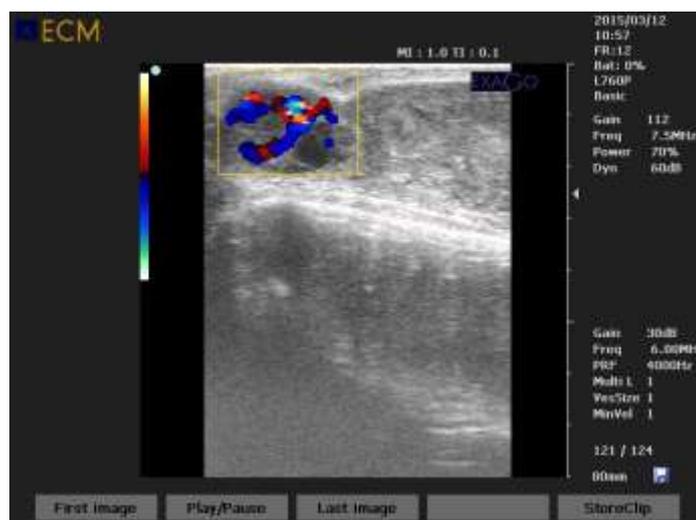Fig 1. The dimension of a CL. A follicle is also visible.

Fig. 2 Colour Doppler sonogram of a Surti buffalo corpus luteum (CL) on Day 13 of the estrous cycle showing high vascularity.

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