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Research Article

A STUDY ON CHANGES IN OVARIAN LUTEAL TISSUE AND UTERINE HAEMODYNAMICS DURING OESTROUS CYCLE IN DAIRY COWS

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ABSTRACT: The objective of present study was to analyze the changes in ovarian luteal tissue and uterine haemodynamics during oestrous cycle in dairy cows. Luteal tissue and uterine perfusion were investigated by performing the trans-rectal Doppler ultrasonography of both the middle uterine arteries in Jersey crossbred cows (N=10) during one oestrous cycle. The diameter and percent vascularity of corpus luteum (CL) during different luteal phases were studied to envisage the luteal tissue changes. Pulsatility and resistivity indices, time averaged mean and maximum velocity, diameter of the artery, volume of blood flow were measured to study the uterine perfusion. CL diameter and vascularity were significantly higher ($p<0.01$) during mid luteal phase as compared to those in early and late luteal phases. A significant difference ($p<0.01$) between the recorded haemodynamic indices *i.e.* pulsatility index (PI), resistivity index (RI), velocity and volume of blood flow along with the diameter of middle uterine artery (MUA), ipsilateral and contralateral to pre-ovulatory follicle were recorded on the day of oestrus and day 10 of oestrous cycle, however, this difference was found to be non-significant ($p>0.05$) with the progression of oestrous cycle into luteal phase. Therefore, the Doppler sonography provided an insight into haemodynamic alterations occurring in luteal tissue and uterus during different phases of oestrous cycle.

Key words: Blood flow, Doppler ultrasonography, Oestrous cycle, Luteal changes, Middle uterine artery.

INTRODUCTION

A basic understanding of oestrous cycle is necessary for effective reproductive management of cattle as the proportion of cows that become pregnant during a breeding season directly contributes to the profitability (Adams *et al.* 2008). The advancement of imaging technology has contributed significantly in the field of bovine reproduction. Doppler sonography also allows to indirectly making the inference about the functionality of CL, follicles and their ability to secrete steroid hormones which are ultimately crucial in sustaining the pregnancy (Rodgers and Irving-Rodgers 2010). The use of real time non-invasive technique to scan tissues and organs offers a tremendous significance in knowing the physiological mechanisms and diagnosis of pathological conditions (Herzog and Bollwein 2007). In recent studies, measurement of uterine blood flow has been made possible with non-invasive colour Doppler ultrasonography. Doppler ultrasonography enables the imaging of anatomical structure (Krueger *et al.* 2009) in order to measure physiological and pathological alterations in uterine blood flow (Steer *et al.* 1990, Scully *et al.* 2015). Blood flow to MUA during oestrus, inter-ovulatory period and pregnancy has been studied using colour Doppler ultrasonography (Arashiro *et al.* 2013). Based on this literature, the present research was carried out to study blood flow to ovarian structures and uterus via application of Doppler ultrasonography during one whole oestrous cycle in dairy cows.

MATERIALS AND METHODS

Jersey crossbred cows (N=10)clinically healthy, normal cyclic, with good genitalia and no history of reproductive abnormality reared in a loose housing system under standard management conditions, fed a total mixed ration (once daily, *ad libitum*) mineral mixture (50 g approx. daily), and unrestricted access to clean potable drinking water were observed for the behavioural signs of oestrus such as mounting, stand still while being mounted by the other cow, bellowing, increased activity,

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vulval oedema and cervico-vaginal discharge hanging from vulva. Following observation of oestrus, the cows having clear cervico vaginal discharge and good uterine tone were selected for the study. These cows were examined ultrasonographically for one whole oestrous cycle to study the diameter and blood flow pattern of CL and uterus at 48 h interval during an entire oestrous cycle using brightness, colour Doppler and spectral mode.

The diameter (mm) of CL was calculated every 48 h after the end of oestrus using two larger perpendicular dimensions until the completion of active oestrous cycle. To measure the blood flow to CL, it was ensured that entire CL falls within the Doppler gate. Vascular perfusion to CL was analysed on the cross-sectional image covering the maximum area of the CL using grids. Vascularity of CL (%) = number of cubes with colour Doppler signal on CL/ number of cubes covering total CL × 100. In present study, luteal phase has been divided into three phases, *i.e.* early, mid and late luteal phase (Fig. 1).

The MUA ipsilateral (i) and contralateral (c) to the pre-ovulatory follicle were scanned by the same operator at 8-10 h after exhibition of oestrus signs and lasted 10 min for each cow. The MUA originates from anterior division of internal iliac artery and moves towards the direction of uterus. Doppler examination was performed using portable ultrasound device (Mindray Z5; VETMODEL 75L50EAV, China) in pulsed-wave mode using a 7.5 MHz linear probe with a power of 50%, filter of 100 Hz and Doppler angle varying between 30º and 60º. The parameters that the device displayed for each waveform by applying the automatic mode were PI, RI, velocity of blood flow (Time averaged mean velocity, TAMEAN; Time averaged maximum velocity, TAMAX) and systole diastole ratio. The diameters (D) of both i and c arteries to the pre-ovulatory follicle were assessed from a Brightness-mode image.

Blood flow volume in mL/min was calculated using the equation (Bollwein *et al.* 2000):

$$\text{BFV-TAMEAN} = \text{TAMEAN} \times \pi \times (D\times0.1/2)^2 \times 60$$

$$\text{BFV-TAMAX} = \text{TAMAX} \times \pi \times (D\times0.1/2)^2 \times 60$$

Numeric data for all the parameters were expressed as mean ± SD. Statistical analysis was performed using a t-test and their level of significance was determined with NCSS 2020, USA (Version 20.0.1). All the experiments have been carried out after the approval of ethical committee of the institute and the principles under Declaration of Helsinki were also taken into consideration.

RESULTS AND DISCUSSION

During recent years, the relationship of CL diameter and vascularity in relation to plasma progesterone secretion has been evaluated in cows using colour Doppler ultrasonography (Pinaffi *et al.* 2015, de Tarso *et al.* 2017). Doppler sonography aids in measurement of blood flow to follicle (Acosta *et al.* 2003, de Tarso *et al.* 2015), CL (Acosta *et al.* 2002), before and after ovulation and in uterus (Scully *et al.* 2015). The growth of the CL and its functions are dependent on blood supply (Acosta and Miyamoto 2004). In present study, the mean CL diameter during early luteal phase was 12.53±0.85 mm, that showed significant increase ($p<0.01$) in diameter *i.e.* 20.07±0.76 mm during mid luteal phase before getting decreased non-significantly ($p>0.05$) to 18.76±0.66 mm during late luteal phase. The significant difference

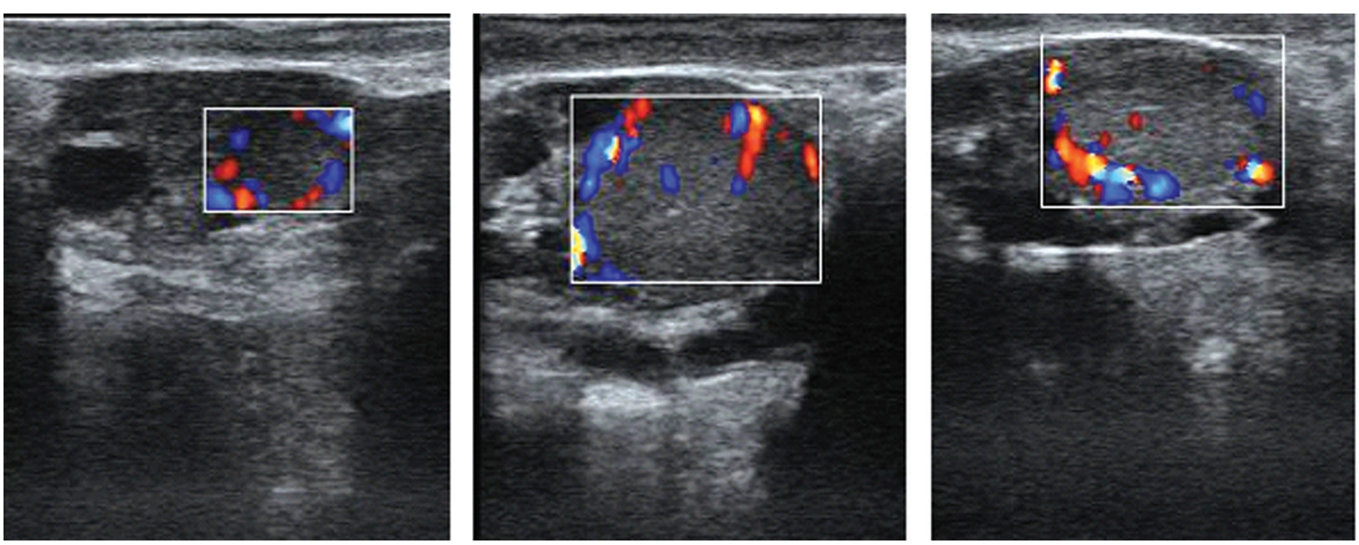

Fig. 1. Corpus Luteum blood flow during early luteal phase (day 2-4), mid luteal phase (day 5-12), and late luteal phase (day 13-21).

Table 1. Changes in Doppler haemodynamic indices of middle uterine arteries in dairy cows (N=10) during an oestrous cycle (Mean ± S.E.).

Day of oestrous cycle	Side of MUA in relation to POF	Resistivity index	Pulsatility index	Time averaged mean velocity (cm/s)	Time averaged maximum velocity (cm/s)	Diameter of MUA (mm)	Blood flow volume-TAMEAN (mL/min)	Blood flow volume-TAMAX (mL/min)	Systolic diastolic ratio
Day 0	psilateral	0.31±0.08^{b}	0.55±0.14^{b}	31.47±2.20^{a}	55.16±1.36^{a}	12.58±0.68^{x}	2353.58±289.81^{a}	4178.13±490.67^{a}	1.58±0.26^{b}
	Contralateral	0.71±0.09^{a}	1.47±0.14^{a}	20.12±2.02^{b}	39.07±2.55^{b}	10.73±0.36^{y}	1072.96±86.18^{b}	2095.55±123.40^{b}	3.39±0.42^{a}
Day 2	Ipsilateral	0.50±0.12	1.00±0.25	25.26±2.33	50.53±3.35	12.27±0.29^{a}	1902.97±214.24^{x}	3580.28±400.36^{x}	2.54±0.41
	Contralateral	0.60±0.04	1.14±0.10	22.18±2.31	41.17±3.61	10.70±0.37^{b}	1260.36±165.16^{y}	2377.54±236.27^{y}	2.60±0.29
Day 4	Ipsilateral	0.66±0.09	1.36±0.14	23.07±5.94	46.88±9.72	11.37±0.38^{a}	1510.29±408.24	2907.99±477.08	3.12±0.41
	Contralateral	0.59±0.14	1.47±0.30	16.52±3.77	31.95±8.24	9.68±0.46^{b}	736.22±267.09	1452.05±541.38	3.20±0.68
Day 6	Ipsilateral	0.45±0.09	0.74±0.17	23.09±3.23	39.98±4.73	11.34±0.76	1546.52±375.54	2500.15±542.88	2.12±0.37
	Contralateral	0.54±0.06	0.97±0.15	23.59±2.04	42.27±3.28	10.46±0.34	1198.72±185.05	2193.21±333.39	2.36±0.32
Day 8	Ipsilateral	0.59±0.06	1.06±0.16	25.41±5.54	49.05±7.48	11.34±0.31	1473.28±351.58	2744.03±559.29	2.39±0.42
	Contralateral	0.50±0.10	0.99±0.42	17.20±2.52	31.73±4.83	10.35±0.59	990.38±220.74	1766.73±397.51	2.62±0.76
Day 10	Ipsilateral	0.69±0.03^{a}	1.39±0.08^{a}	24.65±5.43	38.86±7.43	10.22±0.29	963.31±96.31	1622.78±102.76	3.34±0.26^{a}
	Contralateral	0.48±0.05^{b}	0.94±0.12^{b}	16.09±2.28	28.87±2.72	10.08±0.49	726.19±132.17	1338.74±140.00	1.84±0.16^{b}
Day 12	Ipsilateral	0.69±0.05	1.21±0.12	25.24±4.06	45.83±6.42	10.82±0.52	1467.17±262.27	2695.42±455.68	3.55±0.54
	Contralateral	0.67±0.02	1.14±0.19	24.52±4.19	36.32±3.13	10.34±0.52	1272.96±267.47	1957.97±305.29	2.93±0.18
Day 14	Ipsilateral	0.49±0.12	0.94±0.26	19.33±4.76	35.19±7.27	11.48±0.39	1189.49±352.53	2242.39±610.91	3.35±1.52
	Contralateral	0.69±0.03	1.40±0.16	18.74±1.41	34.02±2.30	10.53±0.43	1007.69±102.66	1782.27±213.53	3.39±0.35
Day 16	Ipsilateral	0.62±0.03	1.29±0.84	21.71±2.62	38.43±3.84	11.08±0.50	1284.05±223.02	2272.78±352.74	2.42±0.26
	Contralateral	0.59±0.05	1.27±0.16	21.01±2.89	35.90±3.39	10.91±0.83	1283.34±306.27	2157.32±406.75	2.91±0.44
Day 18	Ipsilateral	0.63±0.13	1.24±0.39	21.98±1.02^{x}	42.57±6.89	12.50±1.00	1614.5±331.51	3183.48±999.47	2.92±0.59
	Contralateral	0.71±0.01	1.59±0.37	15.39±2.96^{y}	30.63±4.99	10.65±0.65	907.67±207.41	1656.07±448.44	3.44±0.05
Day 20	Ipsilateral	0.74±0.01	1.62±0.02	16.92±1.43	28.63±2.66	11.75±0.15	881.02±212.68	1542.35±311.71	3.81±0.12
	Contralateral	0.76±0.05	1.47±0.14	15.39±2.96	29.15±8.22	10.35±0.95	798.08±49.45	1419.95±95.07	4.60±1.10
Day 22	Ipsilateral	0.76±0.02^{a}	1.19±0.47	-	-	10.30±2.20	-	-	3.93±0.52
	Contralateral	0.42±0.03^{b}	1.01±0.58	-	-	9.15±1.05	-	-	2.58±1.40

^{a,b} Values with different superscripts within the same column for the same parameter and day are significantly different (p<0.01).
^{x,y} Values with different superscripts within the same row for the same parameter and day are significantly different (p<0.05).

(p<0.01) was recorded for size of CL between mid to late and early luteal phase which corresponds to the higher rate of growth during the later phases of oestrous cycle. Similarly, vascularity of CL was 22.80±1.81, that increased significantly (p<0.01) to 38.72±1.67 before decreasing significantly (p<0.01) to 31.98±1.23% during early, mid and late luteal phase, respectively, indicating regression of CL.

Following ovulation, CL size and vascularity increases but no greater increase is seen during early luteal phase as it occurs during mid luteal phase due to angiogenic growth factors such as Fibroblast Growth Factor and Vascular Endothelial Growth Factor (Luttgenau *et al.* 2011). Acknowledging the findings of present study, the blood supply to the CL increases in parallel with its growth, followed by an acute increase in blood flow to the mature CL prior to luteal regression (Matsui and Miyamoto 2009). In further concurrence with our study, Gaur and Purohit (2019) reported a precipitous fall in blood flow to the CL in non-pregnant buffaloes from day

15-21 of the oestrous cycle and thus, represents a quick and reliable diagnostic test for the early pregnancy diagnosis (Siqueira *et al.* 2013). Similarly, the changes in CL diameter and vascularity were also concomitant to plasma progesterone concentration in other studies (de Tarso *et al.* 2017). Herzog *et al.* (2010) also concluded that correlations between progesterone and CL size are not as strong as between corpus luteum blood flow (CLBF) and plasma progesterone, reflecting that Doppler sonography improves the accuracy of diagnosis by providing the physiological information of the organ.

Doppler sonography provides real time blood flow visualization from high velocity flow in large vessels to minimal flow in small vessels (Herzog and Bollwein 2007). The Doppler technology enables the recognition of movement of cells or tissues and is particularly important in the measurement of vascular perfusion. The vascular perfusion to uterus and ovaries is directly under the influence of steroid hormones concentration (Hawkes *et al.* 2016). While studying the uterine haemodynamics, the resistivity, PI and systolic diastolic ratio were found to be significantly lower ($p<0.01$) in ipsilateral artery as compared to contralateral artery on day 0, *i.e.* day of oestrus and day 10 of oestrous cycle, due to decreased impedance to blood flow in the ipsilateral artery. Also, the diameter of MUA and time averaged mean and maximum velocity was recorded to be significantly higher ($p<0.01$) in ipsilateral artery as compared to contralateral artery on the day 0. Similarly, the volume of blood flow to the uterus was found to be significantly higher ($p<0.01$) on day 0 and 2 in ipsilateral as compared to contralateral uterine artery (Table 1).

During oestrus and ovulation, oestradiol (E_2) serum concentration is known to be higher, which bring about increased production of endothelial nitric oxide and results in vasodilation (Acosta *et al.* 2003). In accordance with our findings, Silva (2011) and Hassan (2017) recorded higher value of RI during constriction of distal vascular bed and conversely, a low value of RI, PI and diameter of MUA during decreased impedance to blood flow in the distal vascular bed on the day of oestrus. Also, decrease in RI and PI on the day of emergence of dominant follicles of anovulatory waves during the luteal phase has been mainly due to secretion of 17-β oestradiol by dominant follicle (Bollwein *et al.* 2000). As reported in literature, the diameter of MUA, velocity and volume of blood flow also increase under the influence of potent vasodilator, *i.e.,* oestrogen, therefore, with decrease in concentration of oestrogen, these parameters also start decreasing during the luteal phase (Chen *et al.* 2004).

CONCLUSION

The trans-rectal ultrasonography plays a pivotal role in studying the luteal development during different phases of oestrous cycle. The status of uterine perfusion is well understood via analysis of Doppler haemodynamic indices and also offers a value for the future study by carrying out the investigation during different physiological and pathological conditions of the ovaries and uterus.

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