ORIGINAL ARTICLE

Veterinary Research Forum. 2024; 15 (2): 83 - 88

doi: 10.30466/vrf.2023.2013227.4033

Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Temporal alteration in ovarian follicle fate with hormonal and hemodynamic perspectives during receptive phase of estrous cycle in endometritic cattle

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Article Info

Article history:

Received: 17 October 2023
Accepted: 06 November 2023
Available online: 15 February 2024

Keywords:

Cattle
Endometritis
Ovulation
Serum progesterone
Uterine hemodynamics

Abstract

The present research was carried out to assess the serum progesterone (P_4) concentration and uterine hemodynamics at estrus till ovulation in cyclic cows (N = 130) with healthy or diseased uterus. At estrus, 85 cows were diagnosed with clinical endometritis (CE; n = 44) and sub-clinical endometritis (SCE; n = 41); whereas, 45 cows being served as control namely no endometritis (NE; n = 45) were included in the study. Serum progesterone estimation at 12 - 14 and 40 hr after the onset of estrus and Doppler sonography of both middle uterine arteries were done to envisage the uterine hemodynamics and ovulation. The serum progesterone concentration was significantly higher at 12 - 14 hr after onset of estrus in CE and SCE cows. At 12 - 14 hr after onset of estrus, a cut-off value of ≥ 0.48 ng mL^{-1} P_4 was obtained, above which 22.72% CE, 26.82% SCE and only 8.88% NE cows failed to ovulate within 36 - 40 hr of estrus onset. Among the Doppler indices, pulsatility and resistance indices were significantly higher; whereas, volume and velocity indices were significantly lower in NE cows. In cows diagnosed with CE and SCE, a higher supra-basal P_4 concentration, and velocity and volume of blood flow to uterus at estrus negatively affected the duration to ovulation.

Introduction

For last few decades, a constant increase in the incidence of uterine diseases in dairy cows has been observed which can be broadly classified into three types of metritis, clinical endometritis (CE) and sub-clinical endometritis (SCE) according to their severity.[1] Uterine infections, in the form of CE and SCE, often manifest fluctuations in the hormonal levels during different stages of estrous cycle; thus, affect the ovarian cyclic activity.[2] Among the hormonal aberrations, supra-basal serum progesterone (P_4) concentration (≥ 0.50 ng mL^{-1}) during follicular phase results in absence or delayed emergence of distinct pre-ovulatory luteinizing hormone (LH) peak;[3,4] thus, perturbance to ovulatory function and reproductive competence of cows ensue.[4]

Eventually, analysis of uterine hemodynamics has been used to assess the nitric oxide, a potent vasodilator, induced vasodilation of uterine arteries, and increased velocity and volume of blood flow.[5] However, no study, to the best of our knowledge, has been effectuated yet to establish the inter-relationship between supra-basal P_4, uterine hemodynamics at estrus and subsequent ovulation in cyclic cows with CE and SCE.

Materials and Methods

At estrus, cyclic cows (N = 130) reared under loose housing system (parity: 2 - 4) with CE (n = 44; turbid cervico-vaginal mucus) and SCE (n = 41; ≥ 1.00% polymorphnuclear cells, endometrial cytotape method)[6] having long duration to ovulation (LDO), and 45 cows being served as control with no endometritis (NE) having clear cervico-vaginal mucus with normal duration to ovulation (NDO) were enrolled for the study. The blood samples were collected through jugular venipuncture to harvest serum for P_4 estimation using progesterone enzyme-linked immunosorbent assay kit (DRG International Inc., Springfield, USA) at 12 - 14 and 40 hr after the onset of estrus. The assay sensitivity was 0.045 ng mL^{-1}.

All the experiments have been carried after the approval of ethical committee of the institute and the

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principles under Declaration of Helsinki were taken also into consideration.

Trans-rectal spectral Doppler ultrasonography (TRUS) of both middle uterine arteries (MUAs) at frequency of 7.50 MHz was carried out to envisage the uterine hemodynamics; whereas, B-mode was used for visualizing the ovarian dominant follicle at estrus (Fig. 1A). Doppler examination was performed in pulsed-wave mode using a 5.00 - 7.50 MHz linear probe with an angle varying between 30° and 60°, 50.00% power and filter of 100 Hz using portable ultra-sound device (Z5 VET; Mindray, Shenzen, China). The MUAs ipsilateral (i) and contralateral (c) to the dominant follicle were scanned by the same operator at 12 - 14 and 40 hr after onset of estrus signs, being lasted 3 - 4 min for each cow. Varying degree of signs of estrus monitored every 6 hr were cervico-vaginal mucus, bellowing and mounting behavior. The parameters displayed for each waveform by applying the automatic mode were pulsatility index (PI), resistance index (RI), time-averaged maximum velocity (TAMAX) or mean velocity (TAMEAN) as mL *per* min, Doppler pulse duration (DPD; msec) and acceleration time (AT; msec).

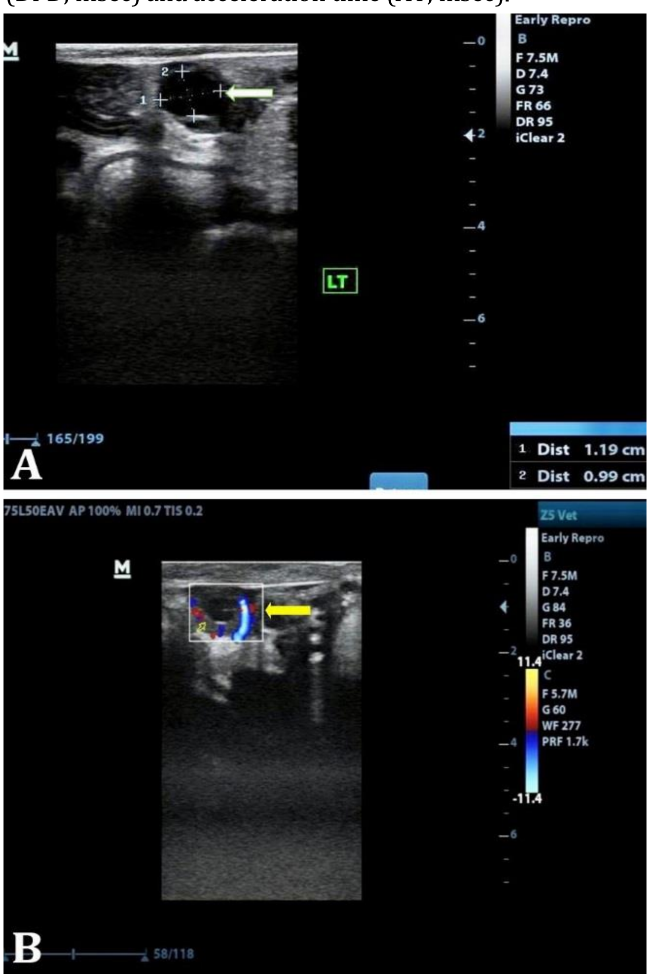

Fig. 1. A) Dominant follicle at estrus (white arrow); **B)** Corpus luteum hemorrhagicum (yellow arrow; Doppler mode).

The diameters (D; cm) of both i and c arteries to the dominant follicle were assessed from a B-mode image and mean of both arteries was taken irrespective of presence of dominant follicle; whereas, the D of dominant follicle (mm) and D of MUA (D-MUA; cm) were measured with automated caliper. Ovulation was detected using B and power Doppler modes (Fig. 1B) through TRUS at 40 hr after the onset of estrus to elucidate the time from estrus onset to ovulation; however, corpus luteum spurium presence on the ovary was further detected during mid-luteal phase (8 - 12 days after onset of estrus) to confirm the ovulatory status in cows. Blood flow volume (BFV; mL *per* min) was calculated using the following equations: [5,7]

$$BFV - TAMEAN = TAMEAN \times \pi \times (D \times 0.10 / 2)^2 \times 60$$

$$BFV - TAMAX = TAMAX \times \pi \times (D \times 0.10 / 2)^2 \times 60$$

Statistical analyses. Numeric data for all parameters are expressed as mean ± SEM and One Way ANOVA for repeated measures, Pearson Correlation Matrix and Receiver Operator Characteristics (ROC) curve, were used to determine the statistical significance of the study using Number Cruncher Statistical System (version 2022; NCSS, Kaysville, USA).

Results

At 12 - 14 hr after onset of estrus, a cut-off value of ≥ 0.48 ng mL^{-1} (sensitivity: 100% and specificity: 89.47%; Fig. 2) serum P_4 was obtained using ROC curve, above which 22.72% CE (10/44), 26.82% SCE (11/41) and only 8.88% NE (4/45) cows failed to ovulate within 40 hr after estrus onset, characterized by the absence of corpus luteum hemorrhagicum. The serum P_4 concentration (ng mL^{-1}) was recorded to be significantly higher ($p < 0.01$), i.e., supra-basal, at 12 - 14 hr after the onset of estrus in CE and SCE cows (LDO group) compared to NE ones (NDO group) with no significant difference ($p > 0.05$) in the D of dominant follicle (Table 1). Eventually, the serum P_4 concentrations did not fall significantly ($p > 0.05$) even at 40 hr after the onset of estrus in CE and SCE cows (LDO group), and no incidence of follicular cysts was recorded.

Among the hemodynamic indices of both MUAs at 12 - 14 and 40 hr after the onset of estrus, PI and RI were significantly higher ($p < 0.01$); whereas, TAMEAN, TAMAX, BFV-TAMEAN, BFV-TAMAX and D-MUA (mm) were significantly lower ($p < 0.01$) in NE cows (NDO group) compared to CE and SCE ones (LDO group; Figs. 3A and 3B). Interestingly, the DPD and AT were significantly higher ($p < 0.05$) in LDO group compared to NDO one at 12 - 14 hr as well as 40 hr after the onset of estrus signs (Table 1). Pearson Correlation Matrix recorded a significant correlation between the D-MUA, and velocity and volume of blood flow (r > 0.75; $p < 0.01$) irrespective of the presence or absence of endometritis and timely or delayed ovulation (Tables 2 and 3).

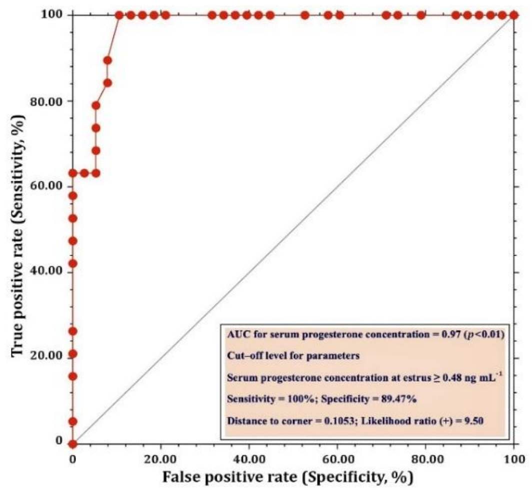

Fig. 2. Receiver operator characteristics (ROC) curve for determining the threshold level of serum progesterone (ng mL^{-1}) at estrus in presence or absence of uterine infection. AUC: Area under the ROC curve.

Discussion

Varying forms of endometritis do not only affect reproduction and production,[8,9] but also pose a great impact on ovarian dynamics, endocrine milieu [2,10,11] and utero-ovarian hemodynamics.[5,12] The average estrus onset to ovulation interval ranges from 27 to 30 hr (range: 24 to 38 hr);[13,14] therefore, the cut-off value of 40 hr was taken as a normal estrus onset to ovulation interval in the present study.

Several authors attributed the presence of supra-basal P_4 concentration (> 0.50 ng mL^{-1}; naturally or exogenously induced) in cows with delayed ovulation, being consistent with our findings and formation of cystic follicles or anovulatory follicles.[3,15,16] The incidence of cystic follicle *per se* was nil in the present study; 20.83% of all the cows enrolled, irrespective of uterine disease presence or absence, showed the incidence of delayed ovulation. Higher P_4 concentration at the time of estrus puts negative feedback on the hypothalamus, resulting in low LH pulse frequency and prevents or delays the LH surge.[3,17] Similarly, the experimental inoculation of endotoxins at estrus leads to delayed ovulation and extended estrus to ovulation interval in nearly 33.00% of experimental cows.[18] However, a significantly higher P_4 and reduced estrogen (E_2) concentrations in follicular fluid of growing follicles in cows affected with endometritis were observed.[19] Therefore, an altered steroidogenic pathway converting P_4 to E_2 in uterine affections can be attributed for the source of higher P_4 concentration at estrus, leading to delayed or no ovulation. Moreover, inflammatory process in the presence of uterine infection also affects the luteolysis by affecting the prostaglandin F2α production;[20,21] therefore, partial or incomplete luteolysis can also be related to supra-basal P_4 concentration at estrus in cows affected with endometritis.

Table 1. Serum progesterone concentration (ng mL^{-1}) and blood flow parameters of middle uterine arteries during clinical endometritis (CE), sub-clinical endometritis (SCE) and no endometritis (NE) in dairy cows at different stages of estrus.

Parameters	12 - 14 hr after the onset of estrus			40 hr after the onset of estrus			
				No ovulation recorded			Ovulation recorded
	CE (n = 44)	SCE (n = 41)	NE (n = 45)	CE (n = 10)	SCE (n = 11)	NE (n = 4)	NE (n = 41)
Diameter of dominant follicle (mm)	12.07 ± 0.17	11.92 ± 0.22	11.89 ± 0.14	12.40 ± 0.38	12.14 ± 0.35	12.24 ± 0.10	-
Serum progesterone concentration (ng mL^{-1})	0.58 ± 0.04^{a}	0.56 ± 0.04^{a}	0.39 ± 0.03^{bB}	0.59 ± 0.03†	0.58 ± 0.04†	0.52 ± 0.06*†	0.74 ± 0.05^{*A}
Pulsatility index	0.96 ± 0.03^{c}	1.10 ± 0.02^{b}	1.18 ± 0.02^{aB}	0.94 ± 0.02^{c}	1.14 ± 0.02^{b}	1.10 ± 0.12^{b}	1.30 ± 0.02^{aA}
Resistance index	0.52 ± 0.01^{c}	0.60 ± 0.01^{b}	0.76 ± 0.01^{aB}	0.50 ± 0.02^{c}	0.63 ± 0.02^{b}	0.72 ± 0.04^{b}	0.80 ± 0.01^{aA}
TAMEAN (cm *per* sec)	12.20 ± 0.13^{a}	11.54 ± 0.12^{b}	9.61 ± 0.14^{cA}	12.11 ± 0.16^{a}	11.40 ± 0.16^{b}	9.46 ± 0.32^{b}	8.04 ± 0.14^{cB}
TAMAX (cm *per* sec)	25.02 ± 0.46^{a}	20.78 ± 0.50^{b}	17.72 ± 0.32^{cA}	25.30 ± 0.62^{a}	20.14 ± 0.76^{b}	17.34 ± 0.70^{b}	15.36 ± 0.27^{cB}
Diameter of MUA (cm)	0.98 ± 0.01^{a}	0.85 ± 0.01^{b}	0.83 ± 0.01^{bA}	0.97 ± 0.01^{a}	0.84 ± 0.01^{b}	0.82 ± 0.05^{b}	0.79 ± 0.01^{cB}
Blood flow volume-TAMEAN (mL *per* min)	558.41 ± 17.76^{a}	402.70 ± 16.50^{b}	331.82 ± 14.52^{cA}	536.53 ± 19.62^{a}	379.80 ± 18.10^{b}	285.35 ± 21.48^{b}	230.34 ± 12.20^{cB}
Blood flow volume-TAMAX (mL *per* min)	1118.77 ± 28.90^{a}	728.15 ± 26.16^{b}	574.96 ± 26.68^{cA}	1104.07 ± 34.62^{a}	683.49 ± 31.22^{b}	549.15 ± 34.26^{b}	451.68 ± 25.08^{cB}
Doppler pulse duration (msec)	818.23± 16.28*	800.66 ± 15.70*†	754.48 ± 14.78†	812.40 ± 20.44*	796.84 ± 18.96*†	752.74 ± 26.68*†	740.32 ± 14.50†
Acceleration time (msec)	106.83± 5.18*	120.12± 5.80*†	128.76 ± 4.72†	104.40 ± 6.48*	124.44 ± 7.96*†	130.90 ± 7.98*†	133.60 ± 3.96†

MUA: Middle uterine artery; TAMAX: Time-averaged maximum velocity; TAMEAN: Time-averaged mean velocity.
^{abc} Values with different superscripts within the same row for the same parameter under their primary category differ significantly ($p < 0.01$).
*,† Values with different superscripts within the same row for the same parameter under their primary category differ significantly ($p < 0.05$).
^{AB} Values with different superscripts under NE category for the same parameter within the same row differ significantly ($p < 0.01$).

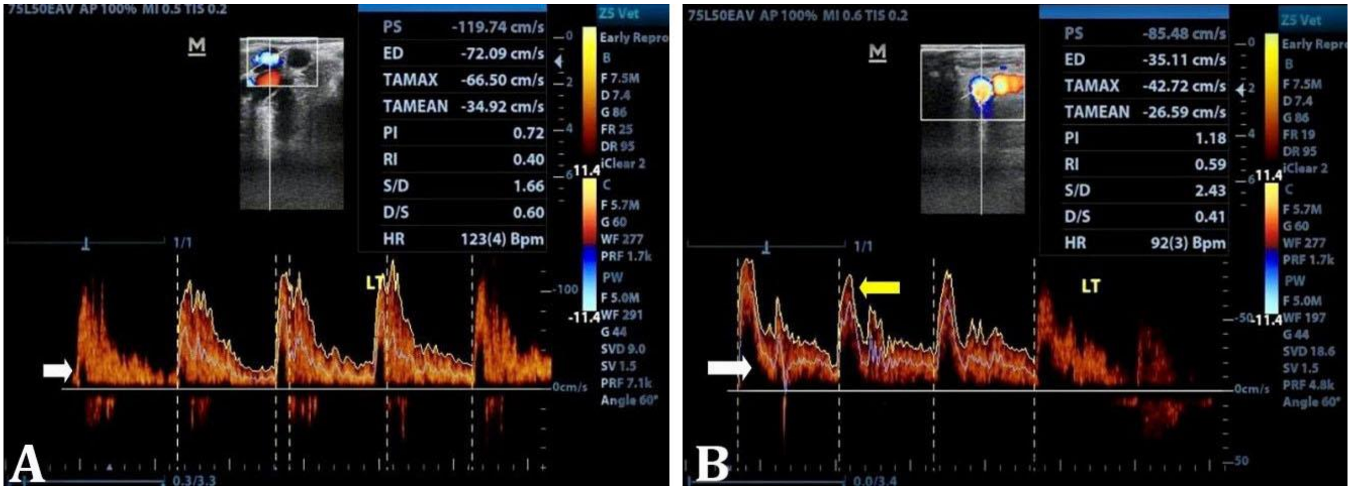

Fig. 3. A) Spectral Doppler waveform of middle uterine artery (MUA) ipsilateral to dominant follicle in a clinical endometritis-positive cow; MUA waveform is characterized by low pulsatility index (PI) and resistance index (RI) along with high time-averaged mean velocity (TAMEAN) and time-averaged maximum velocity (TAMAX), being evidence of MUA vasodilation due to uterine inflammation and spectral window obliteration (white arrow); **B)** Spectral Doppler waveform of MUA ipsilateral to dominant follicle in a non-endometritic cow; MUA waveform is characterized by narrow systolic peak (yellow arrow) and well-demarcated spectral window (white arrow) as well as decreased TAMEAN, TAMAX, being evidence of less MUA vasodilation and no uterine inflammation.

Table 2. Pearson Correlation Matrix for various hemodynamic indices of middle uterine artery in dairy cows (n = 85) with clinical and sub-clinical endometritis at estrus.

Indices	Pulsatility index	Resistance index	TAMEAN	TAMAX	BFV-TAMEAN	BFV-TAMAX	D-MUA
Pulsatility index	1.0000						
Resistance index	0.9088*	1.0000					
TAMEAN	-0.3141	-0.3387	1.0000				
TAMAX	-0.3190	-0.3162	0.9590*	1.0000			
BFV-TAMEAN	-0.3201	-0.3985†	0.7595*	0.7115*	1.0000		
BFV-TAMAX	-0.3601†	-0.3671	0.8488*	0.8428*	0.8068*	1.0000	
D-MUA	-0.2968	-0.3014	0.5614*	0.5860*	0.6904*	0.8303*	1.0000

TAMEAN: Time-averaged mean velocity; TAMAX: Time-averaged maximum velocity; BFV: Blood flow volume; D-MUA: Diameter of middle uterine artery.
*,† Values with different symbols differ significantly at $p < 0.01$ and $p < 0.05$, respectively.

Table 3. Pearson Correlation Matrix for various hemodynamic indices of middle uterine artery in dairy cows (n = 45) without endometritis at estrus.

Indices	Pulsatility index	Resistance index	TAMEAN	TAMAX	D-MUA	BFV-TAMEAN	BFV-TAMAX
Pulsatility index	1.0000						
Resistance index	0.6327†	1.0000					
TAMEAN	-0.2573	-0.2750	1.0000				
TAMAX	-0.2945	-0.2568	0.9398*	1.0000			
D-MUA	-0.2774	-0.1988	0.4342	0.7005*	1.0000		
BFV-TAMEAN	-0.2208	-0.0631	0.5786	0.7094*	0.7894†	1.0000	
BFV-TAMAX	-0.1933	-0.0117	0.3181	0.5694	0.9119*	0.8959*	1.0000

TAMEAN: Time-averaged mean velocity; TAMAX: Time-averaged maximum velocity; BFV: Blood flow volume; D-MUA: Diameter of middle uterine artery.
*, † Values with different symbols differ significantly at $p < 0.01$ and $p < 0.05$, respectively.

Presence of uterine infections also alters the follicular characteristics as cows with genital tract microbial infections had reduced growth rate of the dominant follicles, lower plasma E_2 around the time of maximal follicular diameter and P_4 concentration, and were less likely to ovulate;[10,11] but, did not hold any concurrence with the current study.

Recently, the Doppler ultrasonography, a non-invasive method, has played a pivotal role in diagnosing uterine diseases and understanding hemodynamic changes in uterus during various physiological stages[5] where minimal invasion is required. The role of potent vasodilator, nitric oxide, is pivotal in governing the degree of inflammation during endometritis;[22] however, no literature is available regarding the inverse relationship of hemodynamic changes in MUAs and time of ovulation. In general, higher perfusion to a particular organ is being reflected by a lower RI; whereas, higher PI is indicative of decreased perfusion to distal tissues.[23] Further, it was revealed that hyperemia increased uterine blood flow in women with pelvic inflammatory disease due to the bacterial infection, being associated with low PI and RI indices of the uterine artery blood flow.[24] A similar RI and PI indices pattern was reported in cows during an induced acute uterine affection[25] and spontaneous uterine infection as well.[5,12] Furthermore, an increased PI and RI and decreased uterine blood flow were noted with clearance of uterine infection.[26] In agreement with above studies, the present study also showed significantly higher PI and RI during estrus in cows affected with uterine infection and subsequently, delayed ovulation. The higher volume and velocity of blood flow during endometritis in the present study was consistent with the findings of Debertolis *et al*.;[25] however, delay in ovulation time could be attributed to the changes in follicular fluid being mediated *via* passage of higher levels of lipopolysaccharides and pro-inflammatory cytokines[27] to the ovary containing dominant/pre-ovulatory follicle. Moreover, low DPD in normal cases compared to delayed ovulators was similar to the observations of Satheshkumar.[28]

The present study showed the implications of uterine infections at hormonal level, leading to long term detrimental effects on endocrinological milieu and utero-ovarian enivironment. This work also signified the importance of Doppler sonography and endocrinology in deciphering the molecular implications of disease *per se*. However, more studies are required to investigate the implication of uterine infection on endocrinological milieu and utero-ovarian hemodynamics in dairy cows.

In cows with CE and SCE, supra-basal serum P_4 concentration and higher velocity and volume of blood flow to uterus at estrus negatively affected the timely ovulation after estrus onset, i.e., ovulation within 40 hr after estrus onset.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Conflict of interest

No conflict of interest was reported by the authors.

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