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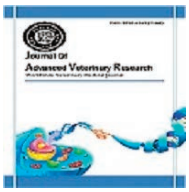

Ovsynch Produced Larger Follicles and Corpora Lutea of Lower Blood Flow associated Lower Ovarian and Uterine Blood Flows, Estradiol and Nitric Oxide in Cows

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ABSTRACT

The use of ovsynch and its modifications for synchronizing estrous cycle and ovulation in ruminants is widely used with lowered conceptions rates. This study aimed to investigate the follicular (F1) and the corpus luteum (CL) hemodynamics, ovarian (OvA) and uterine arteries (UtA) blood flow volumes (BFV) and dynamics associated with the circulating estradiol (E2), progesterone (P4), glucose and nitric oxide (NO) in cows treated with the ovsynch protocol. Eight Friesian cows underwent trans-rectal Doppler scanning and blood sampling each other day throughout two successive non-treated (Spontaneous) and two treated successive estrous cycles (ovsynch). The results revealed that the existed dominant follicles (F1) area, antrum area, color area, and the CL area and the color area on the ovaries declined following the first dose of the gonadotropin-releasing hormone (GnRH, Day-11) till Day -5 then another dominant follicle started growth and reached ovulation (Day 0). Ovsynch pre-ovulatory phase (Day-5 to 0) had more ($P<0.01$) small, medium, and total follicles. The ovsynch F1 ($P=0.001$) and the CL ($P=0.006$) had higher areas but lower color areas percent. The ovsynch increased ipsi-lateral OvA pulsatility index (PI) that associated decreased diameter and time average mean velocity (TAMV) and blood flow volume except Day 6 (BFV, $P<0.05$). The ovsynch improved ($P<0.01$) the ipsilateral UtA PI but lowered its diameter, peak systolic velocity (PSV), TAMV, and BFV except Day 10. Ovsynch ovulation was characterized by low E2, NO, and high glucose but the late luteal phase had high ($P<0.001$) P4 and glucose with low E2. In conclusion, the decreased follicle and luteal vascularization with lowered uterine blood flow and estradiol may adversely affect the quality of the oocyte and the decreased progesterone from Day 7 till Day 10 and the ipsilateral uterine artery BFV may not support the implantation and disturb the maintenance of the embryo after timed insemination.

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Introduction

Several hormonal treatments were used to synchronize oestrus and ovulation to perform timed insemination for maximizing conception rate and managing delivery and lactation (Nowicki *et al.*, 2017). Doppler ultrasound was used to study the uterine blood flow with no hormonal treatment throughout the estrous cycle in lactating Sahiwal cows (Hassan *et al.*, 2017), to study the follicle growth and hemodynamic, corpus luteum development and ovarian and uterine arteries blood flow following spontaneous ovulation in Friesian cows (Abdelnaby *et al.*, 2018), and after estradiol benzoate administration in lactating Holstein-Friesian dairy cows (Rawy *et al.*, 2018). Moreover, Doppler was used for assessing the endometrial blood perfusion in Angus cows (Owen *et al.*, 2018), and follicle vascularity with both corpus luteum blood flow and progesterone in Aberdeen Angus beef cows during the synchronized estrous cycle using Co-Synch plus CIDR protocol (de Tarso *et al.*, 2017). To predict fertility, both preovulatory follicle and luteal vascularization were studied during spontaneous and induced ovulation using gonadotropin-releasing hormone (GnRH) and ovsynch in lactating Holstein cows (Acosta *et al.*, 2003; Bolwein *et al.*, 2010; Varughese *et al.*, 2017). The luteal blood flow was studied after treatment with human chrioin gonadotropins (hCG, Beindorff *et al.*, 2009), after the induction of luteolysis using prostaglandin F2α (PGF2α, Kaya *et al.*, 2017), and the use of estradiol benzoate (Araujo *et al.*, 2009). Preovulatory follicular dynamics, uterine artery blood flow, and ovarian hormones were studied after a combined 5-day progesterone releasing device with two doses of GnRH and PGF2α in Bos indicus beef cows (Moonmanee *et al.*, 2018).

Protocols based on gonadotropic releasing hormone (GnRH) have been used extensively for timed artificial insemination of dairy (Pursley *et al.*, 1995) and beef cattle (Geary *et al.*, 2001). Ovsynch protocols as one of the intensively used GnRH protocols resulted in pregnancy rates similar to those obtained after artificial insemination with estrous detection in lactating dairy cattle (de La Sota *et al.*, 1998). Ovsynch, as one of the most popular hormonal protocol was used both for routine cycle synchronization and for treatment of cystic ovarian disease, silent heat, or heat stress (Nowicki *et al.*, 2017). It was

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more effective for cows rather than heifers with several modifications to improve insemination and pregnancy rates (Nowicki *et al*., 2017).

Estrogen is a vasodilator and hypotensive agent and can induce vascular relaxation by stimulating the release of endothelium-derived vasodilator nitric oxide (NO) or by acting directly on the vascular smooth muscle (White, 2002). NO production increased during the preovulatory phase when estrogen levels were highest (Kharitonov *et al*., 1994).

Information on the ovarian and uterine arteries blood flow concerning the growth and the vascularization of the dominant follicle, the corpus luteum, and uterus during the ovsynch program is not reported. Therefore, the present study aimed to evaluate the dynamics of the dominant follicle and the developing corpus luteum vascularization, the ovarian and uterine arterial blood flows, progesterone, estradiol, glucose, and nitric oxide of Friesian cows treated with Ovsynch synchronization program in comparison to the spontaneous ovulation.

Materials and methods

Animals, treatments and Ultrasound scanning

This study was performed in accordance with the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Science, Cairo University Egypt. The current study used eight mature, healthy cycling Holstein Friesian cows of 3-5 years old, 3.5±0.5, of body condition score (BCS) and 420.0±20.0 kg body weight. Animals belonged to the research farm of Faculty of Veterinary Medicine, Cairo University (30.0276°N, 31.2101°E). Cows were maintained under uniform conditions of feeding and management and were kept individually in an indoor paddock under natural light and temperature with artificial lighting at night. Cow's nutritional maintenance requirements composed of commercial concentrated ration and hay with clean water. Ovulation was affirmed by each other day ultrasound examination for two successive ovulations.

Non-treated cows were examined for two successive (spontaneous ovulations) inter-ovulatory intervals (April-May, 2017) followed by two consecutive treatments of the Ovsynch (ovsynch, GPG) protocol (May-June, 2017). Cows were administrated the first intramuscular injection of GnRH 20mg/head (Receptal® 0.004 mg/ml, Msd Animal Health India) after Day 15 (Fig. 1) of the second spontaneous ovulation (Day-11 ovsynch). Seven days later (Day-4), animals received a dose PGF2α per intramuscular injection (25mg; Lutalyse®, Upjohn). Two days after the PGF2α injection (Day -2), a second GnRH dose was injected. Animals were examined each other day till Day 15 after the induced ovulation. The second ovsynch treatment started from Day 15 after the ovulation of the first ovsynch treatment (Fig. 1).

A trans-rectal pulsed-wave Doppler ultrasound scanner equipped with 7.5MHz linear-array trans-rectal transducer (EXAGO, Echo Control Medical, France) was used for the examination of the ovaries, uterus, ovarian arteries, and the uterine arteries by both color and spectral modes and for counting and measuring the diameters of different classes of the ovarian follicles included small follicles of diameter ≤0.5cm diameter), medium follicles of >0.5 to ≤1.0cm diameter), and large follicles of diameter >1.0 cm (Siregar *et al*., 2016). The same operator performed all scans using the same Doppler ultrasound settings early in the morning. Ovulation was determined by the disappearance of a large dominant preovulatory follicle (>10mm) and the development of a corpus luteum at its location. The last day the dominant follicles were monitored was considered the day of ovulation (day 0) and days after ovulation included days from day 1 to day 15 (Abdelnaby and Abo El-Maaty, 2017a; Abo El-Maaty and Abdelnaby, 2017). The onset of luteal regression was defined as the first day when the CL diameter began to decline and continued to decrease thereafter. The CL diameter was measured throughout the growth (Days 1 to 5), static (Days 6 to 10), and regression phases (Days 11-15, Abdelnaby and Abo El-Maaty, 2017b). During both spontaneous and induced ovulation, the preovulatory phase included the ovulatory wave growth from Day -5 till ovulation (Day 0), the early luteal growth phase included days from Day 1 to 5, the mid-luteal static phase included days from Day 6 to 10 and the late luteal regression phase included days from Day 11 to 15 (Fig. 1). In the induced ovulation, the extended luteal phase (Post-GnRH) included days from the first GnRH (Day-11) to Day-6. The electronic calipers of the ultrasound determined the largest diameter of each follicle or the corpus luteum (CL) per ovary, ovarian and uterine arteries (Abdelnaby and Abo El-Maaty, 2017a,b). The color mode determined the direction of blood flow and the vascularization area within each follicle, CL and uterine horns. The spectral Doppler blood flow was set to measure the ovarian and uterine arteries peak systolic velocity (PSV), end-diastolic velocity (EDV), resistance index (RI) and pulsatility index (PI), and time average mean velocity (TAMV; Hassan *et al*., 2017; Satheshkumar *et al*., 2017).

Using the diameter of either the ovarian or the uterine artery and their corresponding mean blood flow velocity, the blood flow volume (BFV) was calculated from both velocity and cross-sectional surface area of the same blood vessel (Herzog and Bollwein, 2007) as follows: BFV ml/min=TAMV (m/s)x π x (D in cm/2) x(D in cm/2) x 60; where BFV= blood flow volume (ml/min), D/2= (the diameters/2=radius/cm of the ovarian or the uterine arteries in cm), TAMV = Time average mean blood flow velocity (cm/s). The vessel diameter was calculated from the mean of three measurements of the diameter made from frozen two-dimensional color mode just

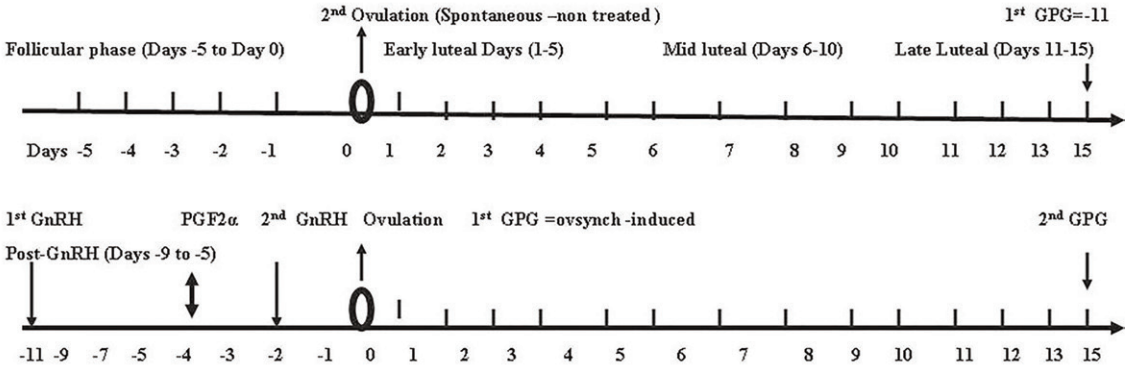

Fig. 1. The experimental design and the days of starting the first and the second ovsynch (GPG) protocols and the phases of the estrous cycle

before spectral Doppler measurements (Rawy *et al*., 2018).

Image analysis

Real-time B mode/color Doppler images were stored in the hard drive of the Doppler scanner then images and video clips were exported at the end of the experiment using a removable hard disk to a computer for blood flow area analyses of follicles and corpora lutea with the ovarian and uterine arterial blood flow spectral Doppler data collection. The follicular and luteal color blood flow red and blue areas of Doppler images were counted per pixel using Adobe PhotoShop CC software (1990-2013, Adobe Systems). A magnetic Lasso tool was used to outline the area, the antrum area and colored area and then each measurement was used to count the selected areas in pixels as described in cows (Acosta *et al*., 2003). The percent of the color area within each follicle or corpus luteum (CL) was counted by dividing the color area by its total area.

Blood sampling and hormone assaying

Blood samples were collected via jugular vein punctures in plain vacuum tubes of all cows following each ultrasound Doppler examination. Serum was harvested and stored at -20°C until hormone assaying. Progesterone (P4, EIA-1561), and estradiol (E2 EIA-2693) were analyzed using ELISA commercial kits (DRG, Germany). The sensitivity of the assay was 0.045 ng/ml and test intra- and inter-precisions were 5.4 and 9.96 for P4. The sensitivity of the assay was 9.7pg/ml and test intra- and inter-precisions were 6.81 and 7.25 for E2. For measuring nitric oxide metabolites (NO), serum samples were mixed with an equal volume of freshly prepared Griess reagent and incubated for 10 minutes at room temperature and absorbance was measured at 540nm using a microtiter plate reader. Nitrite (NO_2) standards (0–50 mM) were used to determine NO concentration in each well as previously measured in our laboratory in mares (Abdelnaby *et al*., 2016). The sensitivity of the assay was 0.225 mmol/L. The intra- assay and inter-assay coefficients of variation were 5.3% and 6.9%, respectively. Spectrum Diagnostics liquizyme glucose reagent was used to measure glucose in the serum. The sensitivity of the test was 5 mg/dL. Intra- and inter-assay precision were 1.09% and 1.17%.

Statistical analysis

Descriptive statistics are presented as Mean± standard error of the mean (SEM). Simple one way ANOVA was used to study the effect of the days and phases of the ovsynch and the spontaneous ovulations on ovarian follicles growth and vascularization, corpus luteum growth and vascularization, and ovarian and uterine arteries hemodynamics using SPSS software (2016). Duncan's Multiple Range Test was used to differentiate between significant means at $P<0.05$. Independent sample Student t-test was used to compare the differences of each parameter between spontaneous and induced ovulation within each phase of the estrous cycle.

Results

Days of ovsynch and spontaneous ovulations influenced ($P<0.0001$) the numbers (Fig. 2A), and the diameters (Fig.2B) of the medium, the large, and the total follicles; F1 diameter, area, vascularization, and color area %; CL diameter, area, and the luteal color area; blood flow volumes and indices of both uterine and ovarian arteries blood flows ($P<0.0001$), including ipsilateral ($P=0.045$), and contra-lateral uterine arteries (UtA) RI ($P=0.032$), the ipsilateral and contralateral uterine horns color area ($P=0.0001$).

The first dose of gonadotropin increased the medium follicles number and diameter, induced luteolysis (Table 1), and improved the ovarian (Table 2) and uterine (Table 3) blood flows before the administration of the prostaglandin.

During the follicular phase, the ovsynch treatment obtained higher number of small ($P=0.0001$), medium ($P=0.0001$), total follicles ($P=0.002$), small follicles' diameter ($P=0.0001$), F1 area ($P=0.067$), F1 antrum ($P=0.066$), but lower F1 color % ($P=0.065$; Table1), smaller ipsi-lateral ovarian (OvA) diameter ($P=0.009$), higher PI ($P<0.001$), RI ($P=0.004$), and S/D ($P<0.005$) with lower EDV ($P=0.047$), TAMV ($P=0.021$), and BFV ($P=0.005$). The contra-lateral OvA had higher PI ($P=0.001$), but lower TAMV ($P=0.009$), and BFV ($P=0.058$; Table 2). The ipsilateral uterine horn (Table 3) had a higher color area ($P<0.0001$), lower ipsilateral UtA diameter ($P=0.003$), PSV ($P=0.001$), EDV ($P=0.003$), and BFV ($P<0.044$) but higher S/D ($P<0.012$). A lower ($P=0.005$) contra-lateral uterine horn color area accompanied a slightly low UtA diameter ($P=0.076$), PSV ($P=0.009$), EDV ($P<0.033$). The ovsynch follicular phase was characterized by a higher glucose ($P=0.0001$) but lower NO levels ($P=0.0001$) than those of the spontaneous ovulation (Table 4). On the Day of ovulation, the ovsynch ovulating follicle obtained the same diameter (12.55±0.07 vs 12.54±0.07mm,Fig.1B), higher area (3605±31 vs 2880±113 pixel; $P=0.0001$;Fig. 1C), higher antrum (3144±30 vs 2412±111 pixel; $P=0.0001$;Fig. 1C), color area (1076±11.05 vs 1056±16.22;Fig. 1C), lower color area % (29.89±0.35 vs 38.72±1.42%; $P=0.0001$;Fig. 1C), compared to the spontaneous one.

During the early luteal phase after ovulation, the CL ovsynch area increased ($P<0.001$; Table 1) whereas the ipsilateral OvA obtained a higher PI ($P=0.026$), but lower TAMV ($P=0.026$), and BFV ($P<0.073$; Table 2). The contra-lateral OvA had a higher diameter ($P<0.001$), PI ($P<0.012$), but lower TAMV ($P=0.012$). The insignificant decrease of the ipsilateral UtA blood flow was marked by decreased RI ($P<0.003$), TAMV ($P=0.049$), S/D ($P=0.006$) and BFV ($P<0.067$; Table 3), with higher P4 ($P=0.078$), and glucose ($P<0.0001$; Table 4) concentrations.

During mid-luteal phase, the ovsynch CL presented higher area ($P=0.005$), but lower color area % ($P=0.001$; Table 1). The ipsi OvA showed higher PSV ($P=0.042$), whereas contra-lateral OvA had a lower TAMV ($P=0.021$) but tended to have higher PI ($P=0.082$) with lower BFV ($P<0.083$; Table 2). Only the contralateral uterine horn (UH) had a higher color area ($P=0.035$), associated with increased contralateral UtA PSV ($P=0.048$; Table 3) with lower E2 ($P=0.068$) and P4 ($P=0.007$; Table 4) concentrations compared to the spontaneous ovulation.

In the late luteal phase, the ovsynch cycle had higher ($P<0.001$) number of medium follicles with lower diameters and the mean diameter of the F1 did not reach 10mm with a lower color area ($P=0.025$), the CL had higher area ($P=0.01$), but lower color area % ($P=0.006$; Table 1). The ovsynch ipsilateral OvA had higher PI ($P=0.026$), RI ($P=0.011$), and S/D ($P=0.009$), but tended to have low EDV ($P=0.085$; Table 2). The increased ipsilateral UtA PI ($P=0.003$), accompanied lower PSV ($P=0.001$), EDV ($P=0.023$), TAMV ($P=0.001$), and BFV ($P=0.023$; Table 4). The decreased contra UH color area ($P=0.041$) was associated with increased values of PI ($P=0.029$), and RI ($P=0.0001$) with decreased values of PSV ($P=0.006$), EDV ($P=0.0001$), and S/D ($P=0.0001$; Table 3). The decrease of E2 ($P=0.019$) concentrations was accompanied by an increase of P4 ($P=0.0001$) and glucose ($P=0.0097$; Table 4). The ovsynch ipsilateral uterine horn vascularization was low ($P<0.05$) during the follicular phase whereas that of the contralateral was low ($P<0.05$) during the follicular and late-luteal phases but high during the mid-luteal one (Table 3).

In contrast to the spontaneous ovulation, number of small (P<0.05) and total (P<0.05) follicles) were high since the first GnRH treatment throughout all days of the estrous cycle (Fig. 2A). The number of medium (P<0.05) follicles of ovsynch (Fig. 2A) was high after the first GnRH treatment and during Days 4-13 (P<0.0001) compared to the spontaneous ovulation (Fig. 2A. The small follicle's diameters (Fig. 2B) descended (P<0.0001) following the first GnRH (P<0.05) but was nearly similar the spontaneous ovulation except Day -5 and -11. The diameters of the medium follicles of the ovsynch ovulation (Fig. 2B) was lower (P<0.05) than those of the spontaneous ovulation from Day 5 to Day 15 but tended to be high (P>0.05) on Days 3 and 5.

.On the day of ovulation (Day 0), the ovsynch and spontaneous ovulating follicles achieved nearly the same maximum diameter (1.25±0.01vs 1.26 ±0.01), whereas the ovsynch F1 (Fig. 2C) obtained higher (P=0.005) area (3618±45 vs 3149±149), antrum area (P=0.005; 3156±44 vs 2682±129), lower (P=0.0001) color area (1081±44 vs 1111±23), and lower the color area % (29.92±0.54 vs 36.56±1.71). After the administration of the prostaglandin, the F1 color area % started decreasing reaching minimum value on Day 0 (Fig. 2D) but those

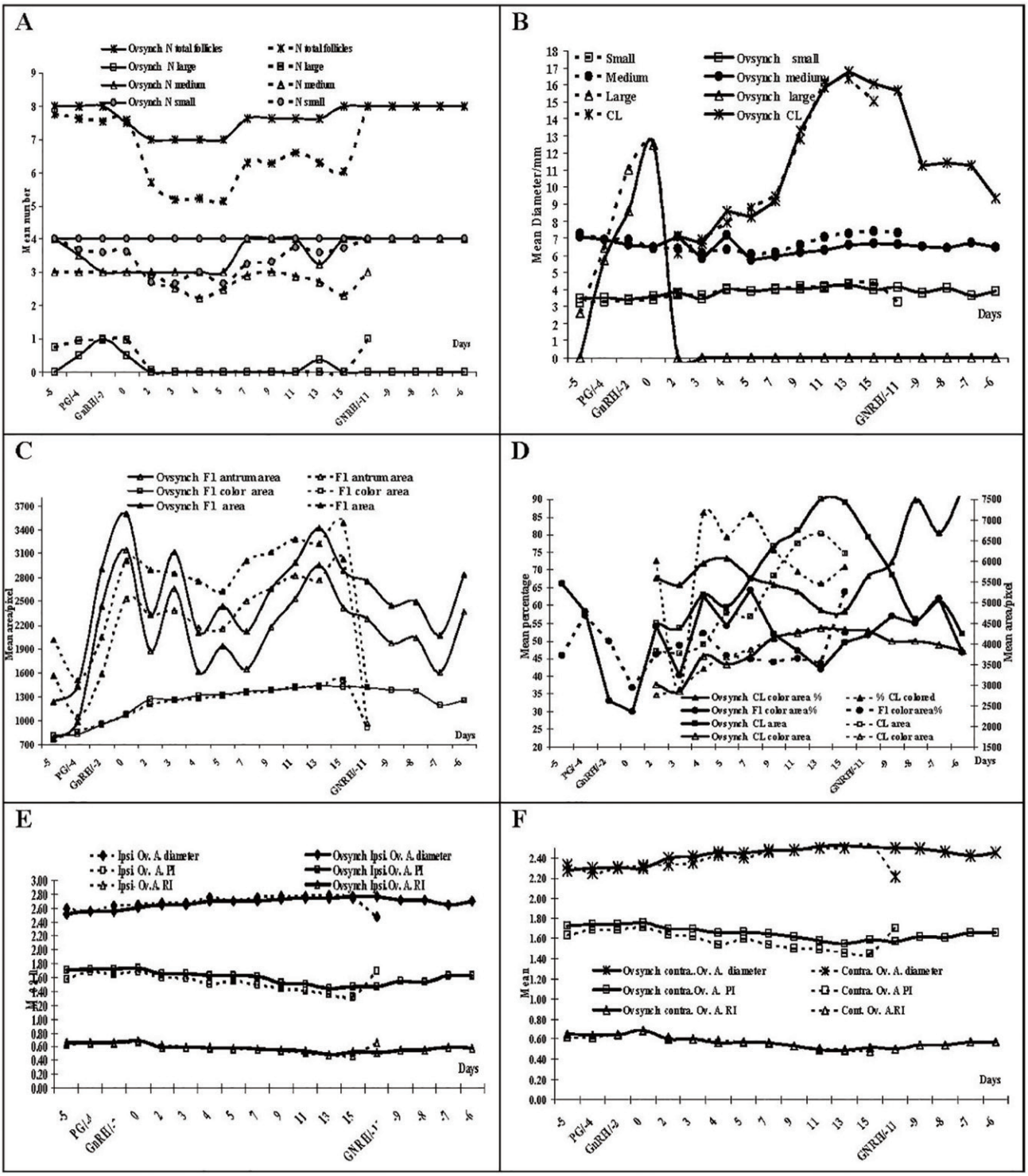

Fig. 2. Mean numbers (A) and diameters (B) small, medium, large and total follicles; area, antrum area and colored area of the ovulating preovulatory follicle and the non-ovulating follicle (C); area, color area/pixel and colored area % of the corpus luteum (D); ipsilateral (E) and contralateral (F) ovarian artery cross section diameter (CS/mm), RI (Resistance index), and PI (Pulsatility index) in induced (Ovsynch) and normal cycling cows from the start of ovsynch till day 13 after ovulation (Day 0)

developed after ovulation obtained lower color area % compared to the spontaneous.

Contrary to the spontaneous ovulation with two waves (preovulatory follicular and a non-ovulatory mid-luteal), the large next ovulating follicles >10mm (10.1± 0.04) started growth on Day -5 before the administration of the PG (Fig. 2B) and the non ovulating F1 started growth from Day 7for ovsynche and Day 5 for the spontaneous (Fig.2C)

The ovsynch CL showed similar mean diameter (Fig. 2B) to that the spontaneous CL during all phases except Days 2, 4, 15 obtained higher diameter and Days 5, 7 obtained lower diameters (Fig. 2B). The ovsynch CL area increased (P<0.05) from Day 2 till Day 15 (Fig. 2D). The Ovsynch CL color area (P<0.016; 3861±4.4 vs 3905±16) descended from Days 5 to 7 and was higher on Days 2 and 4 (Fig. 2D). The Ovsynch CL color area % was low (P<0.05; 73.89±5.39 vs 78.89±4.65) descended from Day 2 till Day 15 except Dat 3).

The diameter of the ovsynch ipsilateral OvA (Fig. 2E) was slightly lower than the spontaneous (P<0.05) except Day 5 but the contralateral one was slightly higher than the spontaneous (Fig.2F). The PI of the ovsynch ipsilateral (Fig. 2E) and contralateral (Fig.2F) OvAs were higher than the spontaneous (P<0.05) throughout the cycle except Day 15. The RI of the ovsynch ipsilateral ovarian arteries were higher (P<0.05) than the spontaneous one except Day 15 (Fig. 2E). The EDV (Fig. 3A) of the ovsynch ipsilateral ovarian artery was lower (P<0.05) than the spontaneous from Day-5 till Day -2, Day 11 and Day 15 (Fig.3A). The TAMV of the ovsynch two ovarian arteries (Fig. 3A, B) were lower (P<0.05) compared to the spontaneous ones during all days except Day 0. The PSV of the ovsynch of the two ovarian artery were lower (P<0.05) than the spontaneous one from Day -5 till Day -2 and throughout the luteal phases except Day 4, 15 (Fig.3A, B). The blood flow volume (BFV) of the ovsynch ipsilateral ovarian arteries waere lower (P<0.05; Fig. 4) than the spontaneous one but the ipsilateral obtained higher value on Day 6 and the contralateral had higher value on Day 13. (Figure 3).

In general, the diameter (Fig. 3C) of the ovsynch ipsilateral UtA was lower (P<0.05) than the spontaneous one. On the day of ovulation, the ipsilateral PI of the ovsynch uterine arteries (Fig. 3C) decreased (P<0.05) but the contralateral ovsynch PI increased (Fig. 3D) in comparison to the spontaneous ones. The RI of the ovsynch two uterine arteries (Fig. 3C) were higher (P<0.05) than the spontaneous one on Day 0 (P<0.05; Fig. 3D). The PSV (Fig. 3E, F) of the ovsynch two uterine arteries (Fig. 3E, F) were lower (P<0.05) than the spontaneous ones from Day-5 till Day -2 and increased from Day 0 till Day 11. The decrease of the EDV (Fig. 3E) of the ovsynch uterine arteries compared to the spontaneous one was demarcated (P<0.05) from Day -5 to Day 0 with showing no differences till Day 9. The TAMV of the ovsynch ipsilateral ovarian artery were lower (P<0.05) than the spontaneous one from Day 3 to Day 7, Day 11 and Day 15 (Fig.3E). Generally, the ovsynch ipsilateral uterine artery showed lower (P<0.05) blood flow volume (Fig. 4) compared to the spontaneous one from Day -5 till Day -2, from Day 3 to Day 13 except Day 9.

Table 1. Differences in follicles numbers and diameters during different phases of both spontaneous and induced ovulations of Friesian cows

Parameters	Ovulation type	Phases of the estrous cycle					P-value
		Follicular	Early luteal-	Mid-luteal-	Late-luteal	Post GnRH	
N. Small follicles	induced	4.00±0.00*	4.00±0.00*	4.00±0.00*	4.00±0.00*	4.00±0.00	
	spontaneous	3.67±0.05c	2.71±.05a	3.31±0.08b	3.75±.05c		0.001
N. medium follicles	induced	3.30±0.06b*	3.00±0.00a*	4.00±0.00d*	3.57±0.14c*	4.00±0.00d	0.001
	spontaneous	3.00±0.00b	2.57±0.06a	2.94±0.03b	2.60±0.06a		0.001
N. large follicles	induced	0.72±0.00b	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.001
	spontaneous	1.00±0.00c	0.00±0.00a	0.61±0.00b	0.00±0.00a		0.001
N. Total follicles	induced	7.86±0.05cd*	7.00±0.00a*	7.53±0.12b*	7.79±0.11c*	8.00±0.00d	0.001
	spontaneous	7.61±0.06c	5.30±0.08a	6.31±0.09b	6.34±0.08b		0.001
Diameter of small/cm	induced	0.35±0.003a*	0.38±0.0.005b	0.41±0.004cd	0.42±0.005d	0.39±0.003c	0.001
	spontaneous	0.33±0.002a	0.37±0.004b	0.41±0.003c	0.42±0.005d		0.001
Diameter of medium/cm	induced	0.67±0.01b	0.64±0.01b#	0.59±0.01a*	0.66±0.02b	0.65±0.01b	0.001
	spontaneous	0.68±0.005c	0.62±0.004a	0.64±0.007b	0.72±0.004d		0.001
Diameter of large/cm	induced	0.79±0.03b	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00	0.001
	spontaneous	1.08±0.03c	0.00±0.00a	0.65±0.07b	0.00±0.00a		0.001
F1 area /pixel	induced	2463±134a#	2497±68a	2482±77a*	3175±97b#	2633±55a	0.008
	spontaneous	2190±91a	2673±65b	3132±58c	3404±45d	±	0.001
F1 antrum area/pixel	induced	2000±133a#	2020±71a	2012±79a*	2707±96b#	2171±55a	0.008
	spontaneous	1727±91a	2174±72b	2654±63c	2940±46d		0.001
F1 color area/pixel	induced	926±18a	1290±6b#	1381±9c	1420±8c*	1385±7c	0.001
	spontaneous	957±14a	1265±7b	1367±6c	1468±8d		0.001
F1 color area%	induced	43.72±2.19a#	52.85±1.44b	56.55±1.63b*	45.34±1.62a	53.58±1.07b	0.001
	spontaneous	47.93±1.27b	49.54±1.24b	44.64±0.89a	43.63±0.55a		0.001
CL diameter	induced		0.77±0.02a	1.19±0.07b	1.59±0.05c	1.16±0.04b	0.001
	spontaneous		0.74±0.01a	1.16±0.04b	1.56±0.02c		0.001
CL area/pixel	induced		4743±107a#	6099±194b*	7115±209c*	5600±197ab	0.001
	spontaneous		4117±110a	5296±145b	6283±128c		0.001
CL color area/pixel	induced		3268±67a	3979±66b	4299±38c	4118±33c	0.001
	spontaneous		3163±50a	4002±29b	4307±13c		0.001
CL color area %	induced		69.66±1.75b	66.24±2.03b	61.04±1.65a*	77.25±2.47c	0.001
	spontaneous		94.51±12.75c	78.89±2.03b	70.71±1.39a		0.081

Data were expressed as Mean ± SEM (standard error of the mean)

Mean with different superscripts (a,b,c,d) within row are significant at P<0.05; *: significant at P<0.05; #: P>.05, Post GnRH phase after the first GnRH; N: number

E2 (Table 4) concentrations of the ovsynch ovulation were lower (P<0.05) than the spontaneous one during the late luteal phase, Days -5, -4, 2, 6, and Days 9 to 13. The progesterone (P4) concentrations of the ovsynch ovulation were lower (P<0.05) than the spontaneous one during the mid-luteal phase but higher (P<0.05) during the late luteal phase, Days 4 and Days 6 to 9. After the ovsynch ovulation, P4 increased linearly reached a maximum and stable value from day 10 to day 13, but after the spontaneous ovulation P4 increased from basal values reached high values from day 6 to day 10 then decreased linearly reaching low values on day 13. The ovsynch NO levels were lower (P<0.05) than the spontaneous one during the follicular phase (Table 4) and peaked after the first GnRH, on Day -4 and Days 4 to 5 whereas that of the spontaneous ovulation reached a high peak on Day -2 and another one on Days 4 to 5. Glucose levels of the ovsynch ovulation were higher (P<0.05) than the spontaneous one during all phases except the mid-luteal one. Though glucose levels of the ovsynch and spontaneous ovulations reached a maximum value on day 7, but the ovsynch had higher glucose levels except on Days 9 and 9 (Fig. 5).

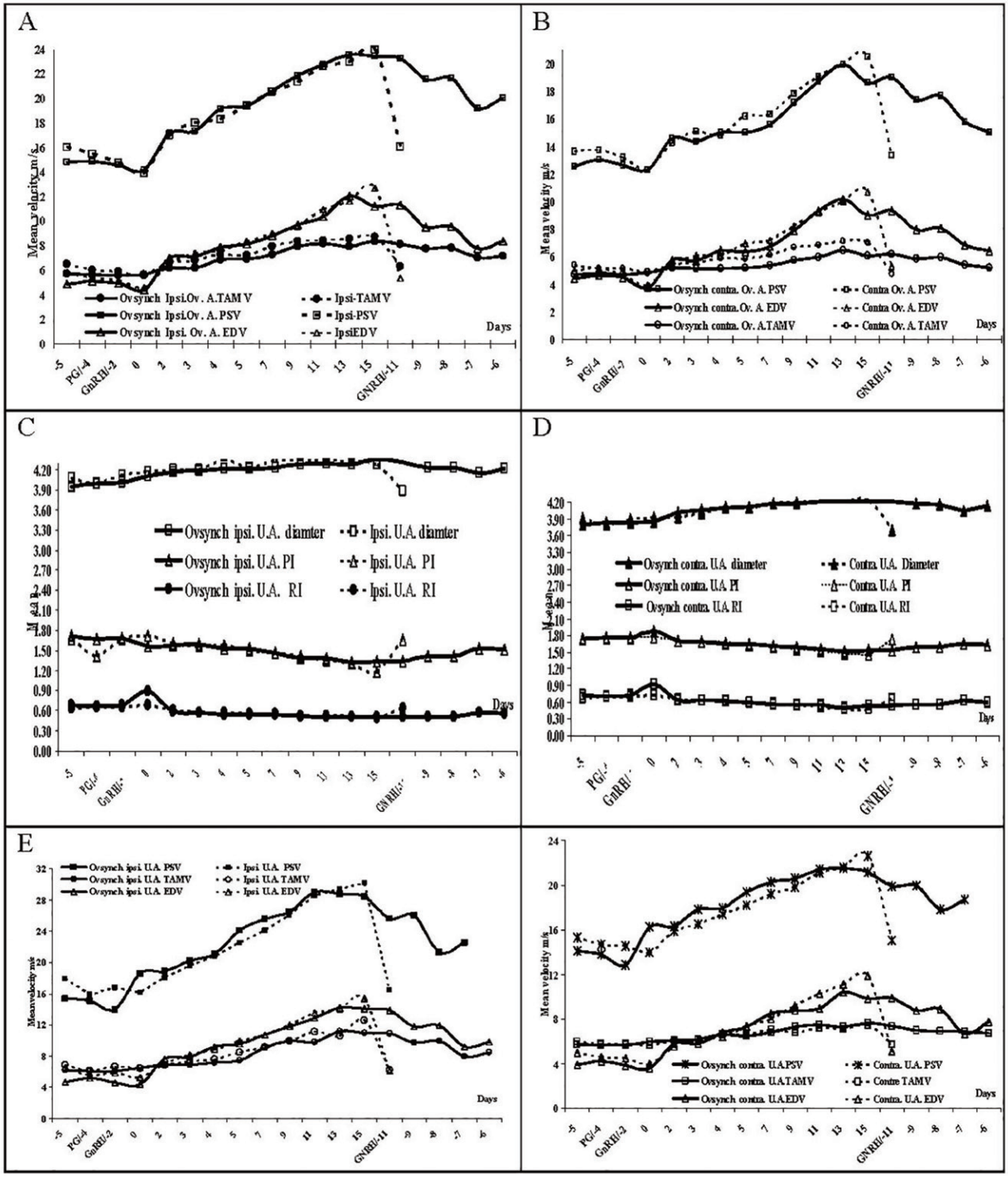

Fig.3. Mean ipsilateral (A) and contralateral (B) ovarian artery PSV (Peak systolic velocity), EDV(End diastolic velocity) and time average man velocity (TAMV); ipsilateral (C) and contralateral (D) uterine artery cross section diameter (CS/mm), RI (Resistance index), and PI (Pulsatility index); ipsilateral (E) and contralateral (F) uterine artery PSV (Peak systolic velocity), EDV(End diastolic velocity) and time average man velocity (TAMV) from the start of ovsynch till day 13 after ovulation (Day 0)

Discussion

In dairy cows, Doppler ultrasound was used with (De Souza *et al*., 2013; Varughese *et al*., 2017) and without (Abdelnaby *et al*., 2018) reproductive synchronization protocols. In response to the first GnRH treatment of this study, both follicles and corpora lutae on the ipsilateral ovary showed decreasing areas and color areas. In agreement with our observation, the decrease in blood flow to the regressing CL was associated with a decrease in blood flow to adjacent follicles (Ginther *et al*., 2016). This study found a significant effect of the days and phases of the estrous cycle after the induction of synchronization using ovsynch protocol on the number and diameter of ovarian follicles of cows. Similar to the increase in the number and diameters of the medium follicles >0.5 cm in response to the first dose of GnRH on Day 15 (1st GnRH, Day -11) of the ovsynch ovulation, Atkins *et al*. (2008) started their first GnRH dose in the earlier part of the estrus cycle (≤Day 10) and reported an increase in the proportion of dominant follicle that was large enough to respond to GnRH second dose with an increase of the ovulatory response. Though the ovsynch preovulatory follicle obtained higher area, compared to the previous spontaneous ovulations of the same cows, this increased area resulted from the increase of their antral areas which in turn declined both color area and % of the color area that might threaten the fertility. On the other hand, the increase of the size of the preovulatory follicle at the final GnRH indicated high fertility and was a significant predictor of pregnancy 35 days later (Busch *et al*., 2008; Udin *et al*., 2017). Both spontaneous and ovsynch ovulation had nearly similar medium follicles' diameters falling in the range from 6-8 mm at the second dose of GnRH as reported by Atkins *et al*. (2008). The mean largest diameter of the medium follicle observed during the preovulatory (follicular phase) in either spontaneous (0.68±0.01) and ovsynch (0.67±0.01) ovulation in the current study was nearly similar but the current study disagrees with the ovulation that defined as the disappearance of large follicle when the medium one was 8 mm (Filho *et al*., 2010). Depending only on preovulatory follicle diameter, cows got diameters between 13.5 and 17.5 mm (Keskin *et al*., 2016), and heifer between 8 and 16mm (Patterson *et al*., 2011) became pregnant. Both spontaneous and ovsynch ovulating cows of this study had preovulatory follicles of high color blood flow area exceeding 900 pixels. Similarly, Holstein Friesian cows ovsynch to ovulate using ovsynch protocol with follicles attaining a high color area of >550 pixels underwent a normal pregnancy when inseminated (Varughese *et al*., 2017). In agreement with our results, the color blood flow areas of the ovulating (F1) follicles achieved an area > 900 pixels on the day of ovulation, pregnant cows previously synchro-

Table 2. Differences in ipsilateral (Ipsi.OV. A.) and contralateral ovarian arteries (Contra.OV. A) diameters/mm blood flow velocities and volume flow (mL/min) during different phases of both spontaneous and induced ovulations of Friesian cows

Parameter	Ovulation type	Phases of the estrous cycle					P-value
		Follicular	Early luteal-	Mid-luteal-	Late-luteal	Post GnRH	
Ipsi.OV. A. diameter	induced	2.57±0.01^{a*}	2.67±0.01^{b}	2.72±0.01^{c}	2.74±0.02^{c}	2.74±0.01^{c}	0.001
	spontaneous	2.61±0.01^{a}	2.70±0.02^{a}	2.76±0.02^{b}	2.79±0.02^{b}		0.001
Ipsi.OV. A.PI	induced	1.72±0.003^{e*}	1.64±0.003^{d*}	1.56±0.011^{c}	1.47±0.014^{a*}	1.53±0.009^{b}	0.001
	spontaneous	1.66±0.02^{d}	1.56±0.02^{c}	1.47±0.03^{b}	1.34±0.02^{a}		0.001
Ipsi.OV. A.RI	induced	0.67±0.003^{e*}	0.58±0.002^{d}	0.56±0.003^{c}	0.51±0.009^{a*}	0.54±0.004^{b}	0.001
	spontaneous	0.65±0.003^{a}	0.59±0.004^{b}	0.55±0.003^{c}	0.49±0.003^{d}		0.001
Ipsi.Ov. A.PSV	induced	14.59±0.15^{a}	18.29±0.21^{b}	21.47±0.19^{c*}	23.19±0.22^{e}	22.13±0.18^{d}	0.001
	spontaneous	14.73±0.12^{a}	18.12±0.11^{b}	21.02±0.12^{c}	23.31±0.13^{d}		0.001
Ipsi.Ov. A.EDV	induced	4.83±0.09^{a*}	7.54±0.10^{b}	9.41±0.14^{c}	11.30±0.29$^{e\#}$	10.10±0.18^{d}	0.001
	spontaneous	5.07±0.08^{a}	7.46±0.09^{b}	9.33±0.06^{c}	11.89±0.12^{d}		0.001
Ipsi.Ov. A.TAMV	induced	5.67±0.05^{a*}	6.56±0.07^{b*}	7.71±0.09^{c}	8.08±0.03^{d}	7.88±0.06^{c}	0.001
	spontaneous	5.87±0.07^{a}	6.94±0.10^{b}	8.17±0.16^{c}	8.74±0.18^{d}		0.001
Ipsi.Ov. A.S/D	induced	3.05±0.04^{d*}	2.45±0.01^{c}	2.28±0.02^{b}	2.06±0.04^{a*}	2.20±0.02^{b}	0.001
	spontaneous	2.93±0.03^{d}	2.46±0.02^{c}	2.26±0.02^{b}	1.97±0.01^{a}		0.001
Ipsi.Ov. A. BFV	induced	17.59±0.19^{a}	22.11±0.29$^{b\#}$	26.97±0.43^{c}	28.64±0.39^{cd*}	27.90±0.33^{d}	0.001
	spontaneous	18.97±0.39^{a}	24.36±0.79^{b}	30.18±1.29^{c}	32.89±1.36^{c}		0.001
Contra.Ov. A. diameter	induced	2.29±0.01^{a}	2.43±0.01^{b*}	2.48±0.01^{c}	2.52±0.01^{d}	2.49±0.00^{c}	0.001
	spontaneous	2.29±0.01^{a}	2.37±0.01^{b}	2.47±0.01^{c}	2.52±0.01^{d}		0.001
Contra.Ov. A.PI	induced	1.74±0.004^{e*}	1.68±0.004^{d*}	1.62±0.005$^{c\#}$	1.56±0.008^{a}	1.60±0.04^{b}	0.001
	spontaneous	1.69±0.01^{d}	1.61±0.02^{c}	1.53±0.03^{b}	1.45±0.03^{a}		0.001
Contra.Ov. A.RI	induced	0.66±0.005^{d}	0.59±0.003^{c}	0.54±0.006^{b}	0.49±0.005^{a}	0.53±0.005^{b}	0.001
	spontaneous	0.65±0.005^{d}	0.59±0.003^{c}	0.54±0.003^{b}	0.49±0.004 ^{a}		0.001
Contra.Ov. A.PSV	induced	12.66±0.12^{a}	14.76±0.11^{b}	16.68±0.30^{c}	19.44±0.26^{e}	17.74±0.24^{d}	0.001
	spontaneous	12.96±0.17^{a}	15.05±0.11^{b}	17.16±0.15^{c}	19.81±0.13^{d}		0.001
Contra.Ov. A. EDV	induced	4.33±0.10^{a}	6.07±0.09^{b}	7.60±0.23^{c}	9.78±0.17^{e}	8.31±0.18^{d}	0.001
	spontaneous	4.51±0.13^{a}	6.13±0.08^{b}	7.76±0.10^{c}	10.02±0.12^{d}		0.001
Contra.Ov. A. TAMV	induced	4.78±0.03^{a*}	5.18±0.03^{b*}	5.59±0.08^{c*}	6.29±0.12^{e}	5.89±0.06^{d}	0.001
	spontaneous	5.03±0.08^{a}	5.64±0.11^{b}	6.37±0.17^{c}	7.18±0.25^{d}		0.001
Contra.Ov. A. S/D	induced	2.98±0.05^{d*}	2.43±0.02^{c}	2.21±0.03^{b}	2.00±0.02^{a}	2.15±0.02^{b}	0.001
	spontaneous	2.96±0.04^{d}	2.46±0.02^{c}	2.22±0.01^{b}	1.99±0.02^{a}		0.001
Contra.Ov. A.BFV	induced	11.91±0.12^{a*}	14.40±0.09^{b}	16.17±0.18$^{c\#}$	18.86±0.35^{e}	17.16±0.18^{d}	0.001
	spontaneous	12.61±0.29^{a}	15.12±0.42^{b}	18.43±0.64^{c}	21.62±0.88^{d}		0.001

Data were expressed as Mean ± SEM (standard error of the mean)
Mean with different superscripts (a,b,c,d,e) within row are significant at P<0.05; *: significant at P<0.05; #: P>.05, Post GnRH phase after the first GnRH;

nized by ovsynch protocol had follicular blood flow of 841.33±155.22 pixel on the day of AI (Day-1) including cows with high follicular vascularization (Varughese *et al*., 2017). Moreover, when cows were synchronized with the co-synch (CIDR+Ovsynch), the average diameter (12.3±0.01mm vs 11.7±0.01mm) was nearly similar but the percentage of colored area (55.5±1.7 vs 43.72±2.19) of the preovulatory follicle and the CL (80.2±1.3 vs 69.66±1.75) were higher (de Tarso *et al*., 2017) compared to those belong to the follicle synchronized with ovsynch alone during the current study. As well as, Simmental cows showed no significant (P>0.05) effect of ovsynch protocol on follicle size and clinical sign of estrus (Udin

Table 3. Differences of ipsilateral (Ipsi.Ut. A.) and contralateral uterine arteries (Contra.Ut. A.) diameters blood flow velocities and volume flow during different phases of both spontaneous and induced ovulations of Friesian cows

Parameter	Ovulation type	Phases of the estrous cycle					P-value
		Follicular	Early luteal-	Mid-luteal-	Late-luteal	Post GnRH	
Ipsi.UT. A. diameter	induced	4.02±0.01^{a*}	4.19±0.01^{b}	4.27±0.01^{c}	4.29±0.03^{c}	4.28±0.01^{c}	0.001
	spontaneous	4.10±0.02^{a}	4.25±.03^{b}	4.34±0.04^{c}	43.36±.03^{c}		0.001
Ipsi.UT. A.PI	induced	1.70±0.01^{d}	1.56±0.01^{c}	1.42±0.01^{b}	1.35±0.01^{a*}	1.40±0.01^{b}	0.001
	spontaneous	1.68±0.01^{d}	1.55±0.01^{c}	1.43±0.00^{b}	1.26±0.01^{a}		0.001
Ipsi.UT. A.RI	induced	0.74±0.06^{b}	0.56±0.004^{a*}	0.53±0.005^{a}	0.51±0.005^{a}	0.52±0.004^{a}	0.001
	spontaneous	0.66±0.01^{d}	0.58±0.00^{c}	0.53±0.00^{b}	0.51±0.00^{a}		0.001
Ipsi.UT. A.PSV	induced	14.91±0.25^{a*}	19.70±0.22^{b}	24.89±0.37^{c}	27.99±0.52^{e*}	26.33±0.32^{d}	0.001
	spontaneous	16.37±0.33^{a}	20.20±0.28^{b}	25.21±0.21^{c}	29.34±0.13^{d}		0.001
Ipsi.UT. A.EDV	induced	4.75±0.14^{a*}	8.62±0.17^{b}	11.50±0.18^{c}	13.62±0.25^{e*}	12.37±0.21^{d}	0.001
	spontaneous	5.58±0.21^{a}	8.46±0.13^{b}	11.45±0.10^{c}	14.40±0.13^{d}		0.001
Ipsi.UT. A.TAMV	induced	6.21±0.15^{a}	7.13±0.08^{b*}	9.47±0.23^{c}	10.66±0.29^{d*}	10.00±.16^{c}	0.001
	spontaneous	6.44±0.14^{a}	7.56±0.13	9.64±0.13^{c}	11.91±0.14^{d}		0.001
Ipsi.UT. A.S/D	induced	3.23±0.06^{c*}	2.29±0.02^{b*}	2.17±0.02^{ab}	2.05±0.02^{a}	2.13±0.02^{ab}	0.001
	spontaneous	3.04±0.05^{d}	2.39±0.02^{c}	2.20±0.02^{b}	2.04±0.001^{a}		0.001
Ipsi.UT. A.BFV	induced	47.37±1.21^{a}	59.14±0.64$^{b\#}$	81.39±2.08^{c}	92.22±1.86^{e}	86.27±1.52^{d}	0.001
	spontaneous	51.55±1.49^{a}	65.32±2.09^{b}	86.67±2.59^{c}	107.48±2.68^{d}		0.001
Ipsi.UT. H. color area	induced	1080±13^{a*}	1340±10^{b}	1443±19^{c}	1580±13^{d}	1490±15^{c}	0.001
	spontaneous	1159±15^{a}	1337±9^{b}	1464±8^{c}	1593±6^{d}		0.001
Contra.UT. A. diameter	induced	3.84±0.01$^{a\#}$	4.08±0.01^{b}	4.19±0.01^{c}	4.21±0.01^{c}	4.19±0.01^{c}	0.001
	spontaneous	3.88±02^{a}	4.03±0.02^{b}	4.19±0.01^{c}	4.24±0.01^{d}		0.001
Contra.UT. A.PI	induced	1.79±0.03^{c}	1.68±0.01^{b}	1.59±0.00^{a}	1.53±0.01^{a*}	1.57±0.03^{a}	0.001
	spontaneous	1.75±0.01^{d}	1.67±0.00^{c}	1.58±0.01^{b}	1.47±0.01^{a}		0.001
Contra.UT. A.RI	induced	0.77±0.06^{b}	0.62±0.00^{a}	0.57±0.01^{a}	0.53±0.01^{a*}	0.55±0.01^{a}	0.001
	spontaneous	0.70±0.01^{d}	0.62±0.00^{c}	0.56±0.01^{b}	0.49±0.00^{a}		0.001
Contra.UT. A.PSV	induced	13.67±0.24^{a*}	17.08±0.16^{b}	19.95±0.19^{c*}	21.04±0.14^{d*}	20.31±0.15^{c}	0.001
	spontaneous	14.39±0.16^{a}	16.93±0.11^{b}	19.60±0.08^{c}	21.94±0.12^{d*}		0.001
Contra.UT. A. EDV	induced	3.89±0.12^{a*}	6.42±0.13^{b}	8.62±0.16^{c}	9.72±0.30^{d*}	9.08±0.14^{c}	0.001
	spontaneous	4.32±0.14^{a}	6.36±0.09^{b}	8.65±0.14^{c}	11.39±0.12^{d}		0.001
Contra.UT. A. TAMV	induced	5.76±0.13^{a}	6.35±0.05^{b}	7.14±0.10^{c}	7.37±0.13^{c}	7.16±0.08^{c}	0.001
	spontaneous	5.75±0.39^{a}	6.32±0.05^{b}	6.95±0.08^{c}	7.37±0.08^{d}		0.001
Contra.UT. A. S/D	induced	3.60±0.07^{c}	2.67±0.03^{b}	2.32±0.03^{a}	2.18±0.06^{a*}	2.25±0.03^{a}	0.001
	spontaneous	3.46±0.05^{d}	2.68±0.02^{c}	2.29±0.03^{b}	1.98±0.02^{a}		0.001
Contra.UT. A.BFV	induced	39.93±0.90^{a}	49.90±0.63^{b}	58.99±1.02^{c*}	61.57±1.25^{c}	59.25±0.82^{c}	0.001
	spontaneous	40.97±0.59^{a}	48.74±0.82^{b}	57.59±0.77^{c}	62.63±0.93^{d}		0.001
Contra.UT. H color area	induced	1326±223	1216±13	1426±16	1510±16*	1458±11	0.801
	spontaneous	1056±12^{ab}	1347±130^{bc}	1397±6	1556±8^{c}		0.001

Data were expressed as Mean ± SEM (standard error of the mean)
Mean with different superscripts (a,b,c,d,e) within row are significant at P<0.05; *: significant at P<0.05; #: P>.05, Post GnRH phase after the first GnRH; Blood flow volume :BFV

Table 4. Ovarian hormones, glucose and estradiol during different phases of spontaneous and induced ovulations (Ovsynch)

	Ovulation type	Phases of the estrous cycle					P-value
		Follicular	Early luteal-	Mid-luteal-	Late-luteal	Post GnRH	
E2 (pg/ml)	induced	248±11.85^{b}	237±9.63^{b}	216±13.32$^{b\#}$	138±1.41^{a*}	153±2.57^{a}	0.001
	spontaneous	253±9.4^{b}	244±10.97^{b}	244±7.82^{b}	159±3.9		0.001
P4 (ng/ml)	induced	1.19±0.10^{a}	6.82±0.33$^{b\#}$	12.62±0.36^{d*}	14.57±0.43^{e*}	10.36±0.52^{c}	0.001
	spontaneous	1.02±0.14^{a}	6.08±0.29^{b}	14.44±0.13^{c}	9.39±0.22^{d}		0.001
NO (µmol/L)	induced	47.96±2.55^{b*}	50.07±3.26^{b}	49.14±1.20^{b}	37.42±1.59^{a}	45.64±1.21^{ab}	0.11
	spontaneous	62.49±1.39^{c}	49.47±1.88^{b}	47.89±1.02^{b}	35.72±0.65^{a}		0.001
Glucose (mg/dL)	induced	59.54±0.89^{b*}	68.62±0.36^{c*}	68.16±0.81^{c}	60.64±1.23^{b*}	51.96±1.34^{a}	0.001
	spontaneous	50.32±0.49^{a}	62.79±0.62^{c}	67.85±0.72^{d}	55.40±0.75^{b}		0.001

Data were expressed as Mean ± SEM (standard error of the mean)
Mean with different superscripts (a,b,c,d) within row are significant at P<0.05; ; *: significant at P<0.05; #: P>.05, Post GnRH phase after the first GnRH;

et al., 2017). From our results, the diameter of the preovulatory follicle (F1) alone is not a predictive of the future fertility where the diameter did not vary in cows but the increase of the antral area with the decrease of color area and % of the color area could be used as a predictive for the future fertility. In agreement with our findings, synchronized beef cows and the pregnant cows obtained larger follicles of greater blood flows (de Tarso *et al.*, 2016). In agreement with the current study, after induction of luteolysis with PGF2α analogue, cows ovulated spontaneously or treated with a single injection of GnRH had ovulating follicle wall of the same color area but cows spontaneously ovulated showed a gradual increase of the flow area in parallel with plasma estradiol concentration and remained high until ovulation and cows of ovsynch ovulation with GnRH showed increased TAMV synchronously with the flow area and remained relatively unchanged until ovulation (Acosta *et al.*, 2003).

Cows of this study subjected to the ovsynch had higher CL diameter and area but lower color area percent compared with the spontaneous ovulation. This coordinated decrease of the preovulatory follicle and CL percent of the colored area agreed with the coordination between the follicle blood flow and luteal vascular perfusion (de Tarso *et al.*, 2017). The studies evaluated the luteal blood flow in cows after induction of luteolysis with PGF2α a and treated with a single injection of GnRH or received no treatment obtained the highest value of TAMV and the increase of their early luteal the blood flow area and volume two- to three-folds, indicating active angiogenesis and normal corpus luteum development (Acosta *et al.*, 2003). In agreement with the modified ovsynch protocol (Bollwein *et al.* (2010), a similar decrease of the follicular and luteal areas and blood flow of cows treated with a second GnRH 40 h after PGF2α but not with those received a second GnRH 60 h or not. Though, Lüttgenau and Bollwein (2014) recommended the use of the luteal blood flow to evaluate luteal regression instead of the use of luteal size for the better association of luteal blood flow color area with progesterone than with its size. From the findings of this study, we suggest the use of CL color area % as a marker for the initiation of luteolysis. This suggestion was proved here after the first GnRH caused a sharp linear decrease in luteal blood flow area. P4 and luteal diameter were associated with an increase in the CL color area % and the day when CL color area % started to decrease was Day-7 and also after ovulation on Day 11 indicated and confirmed the start of luteolysis and this decrease of CL color area % was associated with an increase in the diameter, area, color area, and a stable P4 concentrations. In agreement with Aslan *et al.* (2011), who ovsynch ovulation with either GnRH or hCG and found no differences in the luteal blood flow and size on Days 9 and 12, this study categorized Day 9 within the mid-luteal phase and Day 12 within the late-luteal phase showed no changes between ovsynch and spontaneous CL diameter and color area.

The ipsilateral and contralateral ovarian arteries of the ovsynch cows showed similar patterns of blood flow dynamics to the spontaneous ovulation where the ipsilateral ovarian artery had larger diameter and blood flow volumes compared to the contralateral one (Abdelnaby *et al.*, 2018).

As well as, the increased ipsilateral uterine horn vascularization and uterine artery blood flow expressed by higher diameter, PSV, EDV, BFV with lower PI, RI along the estrous cycle was also observed during the normal estrous cycle of Angus cows where the endometrial perfusion was greater ipsilateral to the mature CL compared with the contralateral and the similar increase observed during the luteal phases compared to the follicular phase in both spontaneous and ovsynch cycles indicated higher blood perfusion in the luteal phase compared to follicular one which agreed with the dependence of the uterine blood flow on the ovulating ovary (Owen *et al.*, 2018). Conflicting blood flow reported when cows were treated with estradiol benzoate where the ipsilateral uterine artery had lower blood flow as expressed by higher PI and lower blood flow volume and TAMV two days before and 7 days after the injection (Rawy *et al.*, 2018). Even though the increased uterine artery diameter indicated increased blood flow in our study, it was associated with decreased PI and blood flow volume in cows treated with estradiol benzoate (Rawy *et al.*, 2018). In contrast to Friesian cows, where the ipsilateral uterine artery

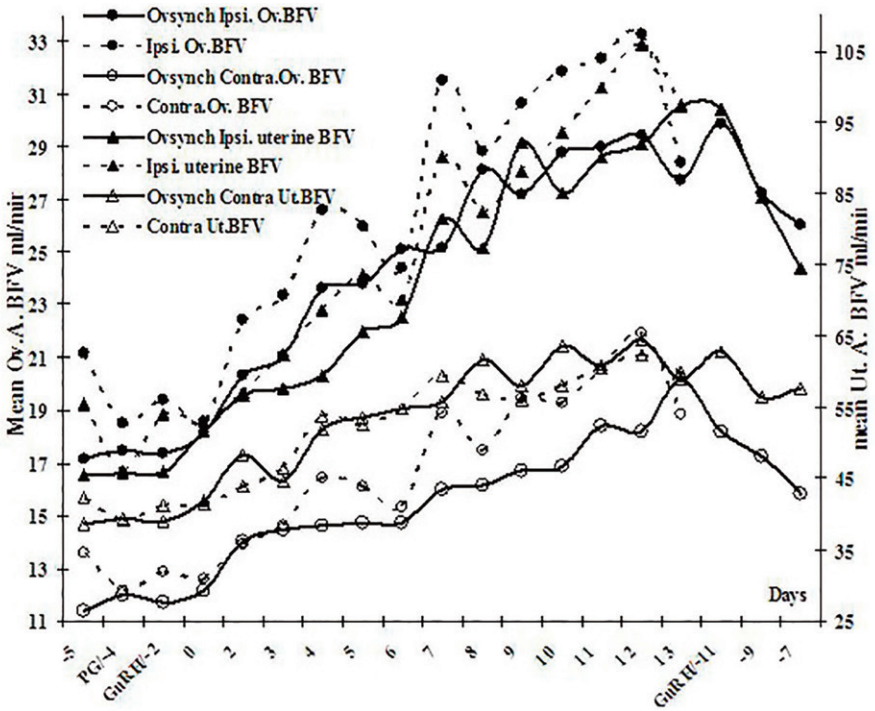

Fig. 4. Mean blood flow volume (ml/min.) of the ovarian and uterine arteries ipsi-lateral and contra- lateral to the ovulating follicle and the developing corpus luteum from the start of ovsynch treatment till day 13 after ovulation (Day 0)

RI of the ovsynch and spontaneous ovulations reached their highest values on the day of ovulation, Sahiwal cows had higher values of their uterine artery RI on day -1 (estrus) than on day of ovulation and also two days after ovulation and this variability of the RI between cows were attributed to the variation of their milk production (Hassan *et al*., 2017). Contrary to the normal estrous cycle of Sahiwal cows where uterine artery PI was higher on Day 10 rather than on Day 0 and Day 2 (Hassan *et al*., 2017), both the ovsynch and spontaneously ovulated cows of this study showed an increase of the uterine artery PI from day -4 and -2 till day 0. Both PI and RI of ovsynch and spontaneous ovulations were low during all luteal phases compared to the follicular ovulatory phase, the RI of uterine arteries was considerably low, while the PI was substantially elevated during diestrus compared to estrus and ovulation (Hassan *et al*., 2017). Contrary to the current results, cows synchronized with modified ovsynch protocol showed no difference in uterine blood flow velocity (TAMV) and uterine blood flow resistance (PI) between the right and left sides of the uterus on Days –1 and the location of the preovulatory follicle and corpus luteum did not affect, therefore, the mean blood flow values for both sides of the uterus were used for analysis (Bollwein *et al*., 2010).

Similar to cows ovsynch or spontaneously ovulated of this study, mares' spontaneously ovulated showed increased production of follicular E2 and NO concentrations from day -5 till ovulation in association with uterine blood flow vascularization (Abdelnaby *et al*., 2016). E2 is likely not the only factor responsible for vasodilation (Mattioli *et al*., 2001). The association of increased ovarian blood flow in our study indicated by decreased uterine PI with the increased E2 during follicle growth when ovaries were stimulated in cows (Honnens *et al*., 2008), and women (Weiner *et al*., 1993) referring this increase of uterine blood flow to the vasodilatory effects of circulating E2 that was postulated to be mediated by NO (White, 2002). E2 stimulated the release of NO from vascular cells by mechanisms that dependent (Binko and Majewski, 1998) or independent (Caulin-Glaser *et al*., 1997) on the expression of the gene. Similarly, the peak of increased NO during follicle development followed by increased estradiol and ovarian blood flow and the other peak of NO during the early corpus luteum development phase (Days 4,5) associated with the increased uterine blood flow were also noted during the estrous cycle in mares (Abdelnaby *et al*., 2016). As well as, the treatment with an organic NO donor as a regulator of the blood flow in the genital tract (Musicki *et al*., 2009) improved the uterine blood flow ipsilateral to the corpus luteum for the first 11 days of the estrous cycle (Zoller *et al*., 2016). The decreased NO during the follicular phase accompanied by decreased follicle color area percent correlated a decrease in the quality of the oocyte and decreased conception that motivated veterinarian to use co-synch or modified ovsynch protocols to improve conception rate (Whittier *et al*., 2013). Also, the follicular estradiol enhanced NO production and may cause a rapid dilation of blood vessels by activating angiogenic factors such as endothelial nitric oxide synthetase (eNOS) (El-Sherry *et al*., 2013). The increased NO during the luteal development in coordination with increased luteal color blood flow and % of colored pixels on day 5 was attributed to its secretion by the luteal endothelial cells which also regulate P4 secretion. Therefore, the blood vessels and endothelial cells of the CL played an important role in the functionality of the CL (Miyamoto and Shirasuna, 2009). In Bovine, NO was identified as the main mediator of increased luteal blood flow (Shirasuna *et al*., 2008). The significant increase of P4 on Day 9 after the spontaneous ovulation and Day 13 after the ovsynch ovulation disagree with the increase of P4 on Day 12 after the spontaneous ovulation (Hassan *et al*., 2017).

The significant increase of glucose levels of the ovsynch ovulation compared to spontaneous one during all phases with nearly similar values during the mid-luteal one was associated with decreased E2 and increased P4 in the ovsynch compared to the spontaneous ovulation. In contrast, when glucose metabolism was assessed in ewes from Days 7 to 11 in association with follicle and corpus luteum growth dynamics and functionality from Days 6 and 11, the glucogenic treatment increased both plasma levels of P4, E2, and the number of 2–3-mm follicles with improved oocyte quality (Berlinguer

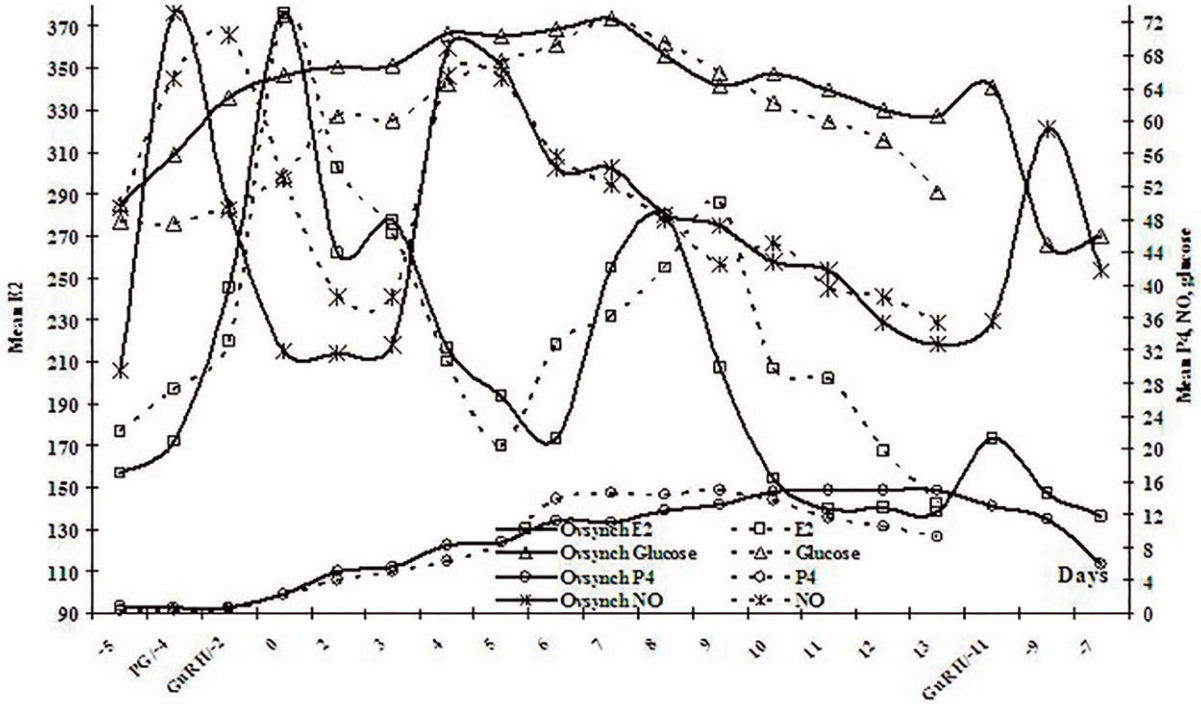

Fig. 5. Mean peripheral estradiol pg/ml (E2 pg/ml), progesterone ng/ml (P4 ng/ml), nitric oxide mmol/ml (NO µmol/L) and glucose concentrations from the start of ovsynch till day 13 after ovulation (Day 0)

et al., 2012). The increased concentrations of glucose during all phases of the ovsynch ovulation, but the mid-luteal one to compensate for the reduction of the blood flow pre-ovulatory and the next ovulatory follicles and their adverse effects on the follicle quality. In mammals, elevated P4 production is consistent with the homeostatic role of P4 in glucose regulation. It regulated follicle growth and/or atresia within the ovary. Ovaries can regulate glucose homeostasis in addition to their primary role in reproductive activity (Wilsterman *et al*., 2017).

Conclusion

Phases and days of the estrous cycle had influenced all the follicular, luteal, ovarian and uterine arteries blood flow parameters of the spontaneous and the ovsynch ovulation. The estrous cycle of ovsynch ovsynch one follicular ovulating wave but no mid-luteal non-ovulating waves. On the day of ovulation and along with the follicular phase, the ovulating follicle of both ovsynch and spontaneous ovulations had the same diameter but when ovulation was ovsynch the increased follicle area was associated with increased antrum area that resulted in lowered blood flow vascularization and percent of color area. After ovulation induction, the developed corpus luteum had similar diameter but larger area and lower percent of color area. Along the ovsynch estrous cycle, the ipsilateral ovarian and uterine arteries and uterine horns vascularization had higher blood flows compared to their contra-laterals and similar differences between the two ovarian and uterine arteries were observed in the spontaneous ovulation. Compared to spontaneous ovulation, NO decreased only during the follicular, but the progesterone decreased during the mid-luteal then increased during the late luteal and was associated with decreased estradiol whereas glucose levels increased during all phases except the mid-luteal one.

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Conflict of interest

The authors declare that they don't have any conflict of interest.

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