The Effect of PRID on the hemodynamics of preovulatory follicular, luteal and the ovarian arteries in Egyptian buffaloes

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1. Abstract

The aim of the present investigation was to study the comparative efficacy of two estrous synchronization treatments on follicular, luteal changes, and ovarian hemodynamic associated with hormonal changes of progesterone and estrogen in addition to nitric oxide. Six cyclic buffalo cows were scanned daily along three successive estrous cycles transrectal by Doppler ultrasonography to evaluate the normal ovarian hemodynamic during normal spontaneous ovulation and before the start of experiments. Buffaloes in the current study will be categorized according to the experimental design. The buffalo cows in the first experiment were synchronized with progesterone releasing intra-vaginal device (PRID) - PGF2α (7 days inserted PRID and PGF2α injected on the 6th day, n = 6). In experiment 2 the same animals (n = 6)) were taken a rest for two successive estrous cycles. Then, buffaloes were synchronized with PRID- Modified Co- synch (PRID+GnRH) in which, 7 days inserted PRID intra-virginal then animals will receive 10µg of GnRH at day 0, 250µg of PGF2α at day 7, another 10µg of GnRH administered 48h after the PGF2α injection. Blood sampling and ovarian ultrasound examinations (color and power Doppler modes) were conducted on the day of PRID removal, estrous, and luteal phase. Results revealed that buffaloes treated with PRID - PGF2α had a higher total number of follicles and F1 dominant diameter. Color Doppler ultrasonography revealed that, F1 diameter, F1 area/pixel, F1 antrum area/pixel, F1 granulosa area, and F1 colored area/pixels significantly increased in PRID -PGF2α group as compared to normal spontaneous ovulating and PRID+GnRH group. In addition, the CL diameter and their vascularity (CL Area/pixels, CL Color area/pixels, CL Color area %) were markedly increased in PRID - PGF2α group as compared to normal spontaneous ovulating and PRID+GnRH group concurrent with a high level of plasma estrogen. The higher values of resistance index (RI) of ipsilateral and contralateral ovarian arteries were recorded in PRID -PGF2α and PRID+GnRH compared to the normal spontaneous ovulating. A similar tendency was observed in blood flow volume (BFV). However, the ovarian blood flow indices including time average mean velocity (TAMV) and peak systolic velocity PSV) did not change among different groups. A higher level of plasma estrogen was achieved in PRID -PGF2α as compared to other groups. However, the progesterone levels did not vary among groups. The nitric oxide level was significantly higher in PRID+GnRH as compared to others. In conclusion, synchronization with the PRID -PGF2α protocol could be enhanced the follicular activity and hemodynamics of Egyptian buffaloes.

Key words: Buffalo; follicular vascularization; luteal blood flow; spontaneous ovulation; synchronization.

2. Introduction

Buffaloes play a pivotal role in many slow developing countries, as meat and milk producers [1]. In Egypt, buffaloes were supplying more than 70% of milk production as well as 40% of meat production respectively [2]. Many factors were hampered the reproductive efficiency in buffalo cow such as; late puberty, silent heat, long calving intervals, and postpartum anestrous with a low response to super stimulation and embryo transfer [3]. Two important problems of buffalo breeding: silent estrous expression and breeding seasonality, so the estrous synchronization and timed inseminations may be used to overcome it [4]. The use of estrous synchronization techniques may overcome some of the difficulties of estrous detection and increase the efficiency of AI in buffaloes. For estrous control in buffaloes, there are many different synthetic pharmaceutical agents of estrous synchronization (Progestagens, gonadotropin-releasing hormone (GnRH) alone or in combination and prostaglandin F2alpha (PGF2α) regulate follicle growth/regression, ovulation, and corpus luteum (CL) regression with different results [5]. Estrous and ovulation occur within 48-96 hrs after removal of progestagen implant in treated buffaloes [6]. To control the ovulation in buffaloes, the Ovsynch protocol has been used to obtain the optimum conception rate ranged between 33 to 60% without estrous detection [7]. In previous studies, the assessment of ovarian hemodynamic was using the two-dimensional and three-dimensional volumetric analysis by measuring the follicular blood flow 12 h after GnRH treatment using two methods [8]. In Egyptian buffalo, the dominant follicle and the luteal hemodynamics were better after ovsynch-CIDR estrous synchronization protocol compared to CIDR-PGF2α [9]. In spontaneous ovulation of Surti buffaloes, the vascularity of dominant follicle increased gradually to reach the maximum value on the day of estrous. From day 5 till day 13 of estrous, the mean diameter of the corpus luteum (CL), as well as their blood flow, were significantly increased linearly [10]. In cows, the ovarian arteries hemodynamic during the estrous cycle was recorded during their spontaneous ovulation [11], after ovsynch [12], during the development of cystic ovaries [13] and the preovulatory period [14], and in mares after hCG or GnRH administration during diestrous [15]. However, no data are available on the ovarian arteries flow velocities in buffaloes during the estrous cycle whatever synchronized or not. This study aimed to compare the preovulatory follicular, luteal blood flows and the ovarian arteries blood flow velocities, volumes, and indices associated the circulating ovarian hormones and nitric oxide in buffaloes synchronized with PRID with or without GnRH.

3. Materials and Methods

The Institutional Animal Care and Use Committee (IACUC) of Faculty of Science, Cairo University (2018-07V) was ethically approved the present investigation.

3.1. Animals

Six multiparous buffaloes (4-7 years old), weighed 400 ± 50 kg, and the average body condition score was 3.0 ± 0.59. Buffaloes were raised in the farm belonging to the Faculty of Veterinary Medicine, Theriogenology, Cairo University, Egypt (latitude 30'01" N; longitude 31'21" E). Buffaloes were housed under natural temperature and daylight. Each animal received the maintenance requirement composed of commercial concentrate ration (16% crude protein/) and the green clover (*Trifolium alexandrinum*) *ad libitum*. Physical examination was performed to confirm that none of the animals has any evidence of disease. All animals were checked for onset of estrous twice daily and scanned by B-mode and color mode twice a week to confirm the ovarian rebound of animals (i.e. presence of CL) for three successive estrous cycles to follow up the spontaneous ovulations (Spontaneous).

3.2. Estrous synchronization and experimental design:

3.2.1. Experiment 1: Synchronization with progesterone releasing intra-vaginal device -Prostaglandin combination

(PRID -PGF2α): Buffalo cows in first group (PRID - PGF2α, n=6) received PRID containing 1.55gm progesterone (PRID-DELTA, Ceva, France) placed intra-vaginally for 7 days. One day before the PRID withdrawal, a synthetic prostaglandin F2α analog (PGF2α; 250µg cloprostenol sodium/ml, Juramate, juroxpty limited, Australia) was intramuscularly injected. One to three days after the removal of the PRID device, the buffalo cows were checked for the estrous phase. All animals were examined by Doppler ultrasound (3 times/week) to evaluate the degree of ovarian response and determine the difference in ovarian artery blood flow [16]. As well, the ovarian follicular dynamics, and hemodynamic were evaluated along with the induced ovulation.

3.2.2. Experiment 2: Synchronization with progesterone releasing intra-vaginal device - Gonadotropin-releasing hormone (PRID- Modified Co- synch):

The same animals used in the first experiment (n = 6)) were taken a rest for two successive estrous cycles. Buffaloes received 10µg of GnRH (gonadotropin-releasing hormone analog; 5.0 ml Receptal, MSD Animal Health, Intervet International GmBH, Germany) at Day 0 accompanied by insertion of PRID intra-virginal for 7 days, 250µg of PGF2α at Day 7 and another 10µg of GnRH was administered 48h after the PGF2α injection [17]. One to three days after removal of the PRID device, Buffalo cows were checked for estrous.

3.3. Doppler Ultrasonography

All animals were subjected to trans-rectal ultrasonographic Doppler scanning (SonoAceR3, Samsung, Medison, and South Korea) daily to monitor the follicle growth, ovulation, and the development of corpus luteum (CL). The ovarian structures (Figure 1-4) were monitored using Real-Time B-mode 12MHz linear array transducer (SonoAceR3, Samsung, Medison, South Korea). Ovarian follicles were counted and grouped into small (≤5 mm), medium (>5- to <10 mm and large (≥10mm) according to their diameter [11]. The blood flow of follicles and developing CL were recorded. The color flow mode (CFM) and the color signals were activated and used to generate images. So, images and video clips of B-mode, color, and spectral Doppler were saved until further analysis. The dominant follicle (F1) was identified when their diameter reached to at least 10 mm and more than the diameter of others. The disappearance of the F1 and the formation of CL at its site one to two days later was considered the time of ovulation (day 0). The ovarian arteries were first determined by palpating the pulsation then with the color and spectral modes. All the Doppler scans were performed by the same operator as previously reported by [18]. The regressing CL diameter, area, and vascularization were measured [19] during the preovulatory phase extended from day -5 till the day of ovulation (Day 0). The diameter of the ovarian follicle and the CL were recorded by electronic calipers of the ultrasound [18,19]. The blood flow direction and the hemodynamic within the ovarian follicle and the CL were recorded by the color mode. The blood flow of ovarian arteries peak systolic velocity (PSV), end-diastolic velocity (EDV), resistance index (RI) and pulsatility index (PI), systolic/diastolic (S/D) and time average mean velocity (TAMV) for the ipsilateral and the contralateral artery were measured by the spectral Doppler [20]. The blood flow volume was also determined by the scanner depending on the diameter or the area of the blood vessel. The same blood flow volume (BFV) was used and included in the analysis.

3.4 Images analysis and interpretation of the color flow mode (CFM)

The stored Doppler images and video clips in the Doppler scanner were exported and analyzed at each examination. The red and blue areas of Doppler images of color blood flow per pixel were determined by Adobe Photoshop CC (1990-2013, Adobe Systems). The colored area of the antrum and the selected areas were count in pixels as described previously in cows by using a magnetic Lasso tool [21]. The percentage of the color area in the follicle or CL was measured by dividing the color area by their total area [21].

3.5. Blood sampling and progesterone, estradiol and nitric oxide assay

At the time of every ultrasonographic examination, the blood samples were taken from jugular vein punctures in vacutainer tubes with and without anticoagulant (EDTA). After centrifugation of blood samples at 1500 *g* for 20 min, the plasma and sera were separated and stored at -20ºC till further analysis. Progesterone (P_4, EIA-1561), and estradiol 17-β (E_2 EIA-2693) were analyzed using ELISA commercial kits (DRG, Germany). For P4 assay, the sensitivity of the assay was 0.045 ng/ml and test intra- and inter-precisions were 5.4 and 9.96, respectively. The sensitivity of the assay for E2 was 9.7pg/ml and test intra- and inter-precisions were 6.81 and 7.25, respectively. The sensitivity of the nitric oxide (NO) assay was 0.225 mmol/L. The intra- assay and inter-assay coefficients of variation were 5.3% and 6.9%, respectively [22].

3.6. Statistical analysis

Data were presented as Mean± standard deviation. Simple one-way ANOVA was used to study the effect of days within each treatment. While the Two-way ANOVA was used to clarify the influence of days (-5, -4, -3, -2 -1, 0). The Spontaneous ovulation, synchronization treatments (PRID, PRID+GnRH), and their interaction on ovarian follicles, corpus luteum growth with their vascularization, and ovarian arteries blood flow were analyzed using SPSS software [23]. Duncan's Multiple Range Test was used to differentiate between significant means at $P<0.05$.

4. Results

4.1. *Effect of* estrous synchronization treatments *on the follicular dynamic in buffaloes*

In normal spontaneous ovulation, days of estrous cycle were greatly affected the follicular number of small and medium follicles. Similar findings were observed in the diameter of medium follicle, and the dominant F1 diameter, area, antrum area, granulose area, color area, color area%, granulose color area% (Table 1). The spontaneous ovulating buffaloes had the highest number of medium follicles on Day -4 but the lowest number on Day -2. The high number of large follicles is observed on Day -3 but low on Day -4. Maximum diameter of the medium follicle is achieved on Day -4 but minimum on Day -2. The dominant F1 diameter, area, antrum area, color area, color area %, and granulose color area % continued increasing from Day -4 reached the highest values on Day 0.

In PRID -PGF2α group days after PRID removal had pronounced effect on the population of medium and large follicles as well as the follicular diameter of small, medium, and the dominant follicles F1. Diameter, area, antrum area, granulose area, color area, granulose color area% and color area% as shown in table (1). Similar tendency was observed in the PRID+GnRH group. The interaction of treatments and days of cycle significantly influenced the population of large follicles, the diameter of medium follicles, F1 area, F1 antrum area, F1 Granulosa area, and F1 color area %. In PRID -PGF2α group, the number of medium follicles (Figure 1B) declined from Day -5 (1.40±0.13) to -4 (1.33±0.17) and stabilized till Day 0 (1.00±0.00). Meanwhile, the number of large follicles (Figure 1C) significantly increased from Day -5 (0.60±0.13) to -4 (0.67±0.17) and stabilized till Day 0 (1.00±0.00). However, the diameter of small follicles (Figure 1E) reached the maximum diameters on Day -1 (4.37±0.07) and Day 0 (4.45±0.05). Moreover, the diameter of medium follicles (Figure 1F) declined from the maximum diameter on Day -4 (8.50±0.33) to the minimum diameter on Day -2 (6.36±0.23).

Buffaloes treated with PRID- modified Co-synch with two injections of GnRH, the population of small follicles decreased from Day -3 reached the lowest number on Day 0. However, the number of large follicles significantly elevated from day 0 and reached the highest number on Day -1. The medium follicles diameter decreased linearly from Day -4 and achieved the lowest diameter on Day 0. The F1 diameter and antrum area were high on Day 0 compared to Days -1, -4. The F1 area, granulose area, color area, granulose color area % and color area % are high on Day 0 compared to the other days (Figure 1F).

4.2. Effect of estrous synchronization treatments *on blood flow area and the vascularization area of ovarian follicles*

The diameter (Figure 2A) and area (Figure 2B) of the dominant F1 ascended from Day-5

(10.26±0.23mm; 20722±874 pixel) to reach the maximum diameter on Day 0 (12.58±0.20mm; 30953±1140) with a transient decrease on Day -2 (11.42±0.24mm; 25285±854). While F1 antrum area ascended linearly (Figure 2C) from Day -5 (13271±481 pixel) to reach the highest value Day 0 (21055±1117) with nearly the same values on Days -3 and -2, its color area (Figure 2D) and color area % (Figure 2E), granulosa area (Figure 3A), and the granulose color area % (Figure 3B), increased showed a transient decrease on Day -2. Generally, the values of F1 diameter, area, antrum area, color area, color area % of buffaloes treated with PRID were the highest and those treated with PRID+ GnRH were the lowest and the spontaneous ovulations were in between them.

On Day 0, buffaloes synchronized with PRID -PGF2α had the highest population of small, large, total follicles, F1 color area %, and F1 granulosa color area %, but the lowest number of medium follicles compared to PRID+ GnRH and spontaneous ovulating ones. The same trend was observed in diameters of small, medium, and large (F1), area, antrum area, granulose area, colored area, and granulose area are the largest in buffaloes synchronized with PRID -PGF2α (Table 3).

4.3. Effect of the estrous synchronization treatments *on growth and vascularization area of the corpus luteum*

The regressing corpus luteum (CL; Table1) diameter, area color area, and color area % were affected by the days and treatments. The interaction of Days and treatments influenced the regressing corpus luteum diameter, area, and color area. Days within PRID influenced the regressing corpus luteum diameter, area, and color area. The Days within PRID+ GnRH and spontaneous ovulation (Table 1) affected the regressing corpus luteum diameter, area, and color area. The values of the regressing CL diameter (Figure 3C), area (Figure 3D), color area (Figure 3E), and color area % were decreasing from Day -5 till Day 0 and their values of the spontaneous ovulations were the lowest on Day 0 (Table 4). The CL diameter, area, color area, and color area % of buffaloes ovulating spontaneously were the lowest compared to those treated with PRID -PGF2α.

4.4. Effect of the estrous synchronization treatments *on blood flow of the ovarian arteries*

Days pre-ovulation had significantly affected the ipsi Ov. RI, PI, PSV, EDV, TAMV, and BFV and the contra Ov RI, PI, PSV, EDV, TAMV, S/D, and BFV as presented in Table (2). Also, different synchronization treatments significantly affected the ipsi Ov. RI, PI, EDV, TAMV, S/D, and BFV and the contra Ov PI, and BFV (Table 2). Days after removal of PRID within PRID -PGF2α the group influenced the ipsi Ov. RI, PI, PSV, EDV, TAMV, S/D, and BFV and the contra Ov RI, EDV, S/D, and BFV. A similar tendency was recorded in Days within the spontaneous ovulation and PRID+ GnRH (Table 2). The Ipsi Ov RI of buffaloes treated with PRID -PGF2α group was high on Day 0 and -3 compared to the other days but those of the contra Ov RI was higher on Day -1 and -3. The Ipsi Ov PI and the contra Ov PI and EDV were high on Day 0 and low on Day -3. The Ipsi Ov PSV and EDV were low on Day -3 and high on Day -1 but the contra Ov PSV was high on Day -3. The Ipsi Ov TAMV is high on -1 compared to Day -5 whereas the contra Ov TAMV is only high on Day -3. The Ipsi Ov and the contra Ov S/D is high on Day -3. The Ipsi Ov and the contra Ov BFV had the lowest values on Day -3 and the highest on Day 0. In buffaloes treated with PRID+GnRH, the Ipsi Ov RI of were high on Day 0 and low on Day -3 while, the contra Ov RI was low on Day -4. The Ipsi and contra Ov PI were high on Day 0 and low on Day -3. The Ipsi Ov PSV was low on Day -3 and high on Day -1 but the contra Ov PSV. Meanwhile, contra Ov EDV was high on Day 0 and low on Day -4. The Ipsi Ov EDV was low on Day 0 and high on Day -1.

The Ipsi Ov TAMV and S/D were high on -1 compared to Day -4 whereas the contra Ov TAMV was high on Day 0 compared to Days –1, -4. The Ipsi Ov and the contra Ov BFV had the lowest values on Day -3 and the highest on Day 0.

On Day 0 (Table 5), the spontaneous ovulating buffaloes had the lowest ispi Ov. RI, contr Ov. RI, ispi Ov.PI, contr Ov. PI, ispi Ov S/D, ispi Ov BFV, and contr Ov BFV with the highest ispi Ov. EDV. The Ipsi Ov and the contra Ov RI of spontaneous ovulating buffaloes were significantly increased on Day -3 and decreased on Day -4. While the Ipsi Ov PI was elevated on Day -4 and low on Day -5. in contrast, the contra Ov PI was elevated on Day -2 and low on Day -5. On Day -4 the Ipsi Ov

PSV was low and high on Day -0 but the contra Ov PSV and EDV were high on Day 0. The Ipsi Ov EDV was low on Day -4 and high on Day -2. The Ipsi Ov TAMV is high on 0 compared to Days -4, -3 whereas the contra Ov TAMV is high on Day -3 compared to Day -4. The contra Ov S/D is high on Days -3 and -2 compared to Day -4 The Ipsi Ov and the contra Ov BFV had the lowest values on Day -4 and the highest on Day -2.

On Day 0 (Table 5), the spontaneous ovulating buffaloes had the lowest ispi Ov. RI, contr Ov. RI, ispi Ov.PI, contr Ov. PI, ispi Ov S/D, ispi Ov BFV, and contr Ov BFV with the highest ispi Ov. EDV.

4.5. Effect of the estrous synchronization treatments *on progesterone and estrogen and nitric oxide levels*

Progesterone (P4) concentrations (Table 2) tended to be influenced by days but affected by treatment, days following PRID (P=0.040), and those spontaneously ovulated. The nitric oxide (NO) levels are influenced by the interaction of days treatment (P=0.012) and the preovulation days of buffaloes synchronized with PRID+GnRH. On Day 0, NO of buffaloes treated PRID+GnRH are higher than those treated with PRID -PGF2α or spontaneous ovulated (Table 4). In the PRID -PGF2α buffaloes, E2 concentrations are significantly high on Days -3 and 0 whereas the P4 concentrations declined sharply from Day -3 reaching them lowest values on Days -2, 0, and -1. Buffaloes treated PRID+GnRH had the highest NO on Day -1 and the lowest one on Day -4. P4 concentrations of spontaneous ovulating values decreased linearly from Day -5 today 0. (Figure 2F).

5. Discussion

The present study demonstrated more details about the vascular changes in the ovarian follicles, corpus luteum, ovarian arteries associated with hormonal changes of progesterone and estrogen in addition to nitric oxide following normal spontaneous ovulation and different synchronization protocol (PRID+GnRH and PRID -PGF2α) in Egyptian buffaloes for the first time. The present study clarified that, the days of estrous cycle of normal spontaneous ovulating and synchronized buffaloes using PRID+GnRH and PRID -PGF2α were greatly influenced the population and diameter of ovarian follicles. A similar finding was obtained in cows [12]. In buffaloes treated with PRID -PGF2α had significantly highest average diameter of small, large and F1 dominant diameter (3.17±.090, 1.00 ±0.0, 12.58 ±.19mm). Furthermore, PRID -PGF2α group had a significantly higher total number of follicles as compared to the other group. In this respect, the mean diameter of preovulatory follicles was significantly higher in PRID-PGF2α than those obtained in the Ovsynch protocol (14.1±1.5 *vs.* 12.0±2.3 mm, respectively) in Bulgarian Murrah buffalo with inactive ovaries [24]. In previous studies, the diameter of preovulatory ranged from (14.4±0.6) mm to (16.4±0.3) mm in Italian Mediterranean buffaloes using PRID-PMSG-PGF2α treatment [25]. While in Indian Murrah buffaloes, the size of the ovulatory follicles was 15.2±0.2 mm [26]. In Egyptian buffaloes, it was 13.6±0.2 mm [27]. The pronounced effect of PRID -PGF2α on the ovarian rebound at the day's intervals of PRID removal may be attributed to the potent effect of PGF2α. The administration PGF2α one day before the removal of PRID was leading to rapid regression of CL just before PRID removal. So, the inhibitory effect of P4 was rapidly decreased on follicular dynamics [28]. The remarkable increase of follicular diameter recorded herein may have a positive effect on the steroidogenic function of the follicles [29]. Color Doppler ultrasonography in the PRID -PGF2α group revealed that, F1 diameter, F1 area/pixel, F1 antrum area/pixel, F1 Granulosa area and F1 colored area/pixels are significantly increased in the PRID -PGF2α group as compared to normal spontaneous ovulating and PRID+GnRH group. The improvement of vascular changes in the dominant follicles and the follicular area may be accompanied by a high level of E2 recorded in the PRID -PGF2α group (102.4 ±13.20 pg/ml) which lead to rapid infusion of the blood in dominant follicles. In contrast, [9] reported that Ovsynch-CIDR had higher follicle population and 1st-Large follicle diameter at estrous and corpus luteum (CL) volume at the luteal phase accompanied with high level in E2 (at estrous) and P4 (at the luteal phase). They also added that, the blue pixels in the dominant follicle (DF), total blood flow (TBF), and power Doppler pixels

(PDP) were higher in the Ovsynch- CIDR than in the CIDR-PGF2α in Egyptian buffaloes. These differences may be attributed to the use of a different type of synchronization. Also, [24] mentioned that, the start of the therapy (breeding season, low season, or seasonal anoestrous) is a very important factor in Bulgarian Murrah buffalo breeding. In the current finding, a significant increase in the CL diameter and vascularity (CL Area/pixels, CL Color area/pixels, CL Color area %) was observed in the PRID -PGF2α group as compared to normal spontaneous ovulating and PRID+GnRH group concurrent with a high level of plasma progesterone. This is may be attributed to the use of PGF2α on the day before the removal of PRID. In this respect, the blood flow was rapidly increased after injection of PGF2α within 30 min and sustained for 2 hr [30]. At the onset of luteal regression, the endogenous PGF2α released from the endometrium and enhanced the blood flow in CL. In addition, CL with higher blood flow had a higher level of plasma progesterone and more responsive to the effect of administrated prostaglandins [31]. The higher values of RI of ipsilateral and contralateral ovarian arteries were recorded in PRID - PGF2α and PRID+GnRH compared to the normal spontaneous ovulating in the present study. A similar tendency was observed in BFV. This may be attributed to increase of blood flow to the ovary till ovulation and after the day ovulation [32]. buffaloes submitted to PRID protocols induced follicles produce more estrogens (E2) than these after Ovsych stimulation [24]. Similar results in postpartum cows (greater peak of circulating concentrations of E2 in CIDR than Ovsynch treatment) were registered by [33]. Estrogen plays important role in the control of the blood flow of the genital tract due to its vasodilator role [20]. Both E2 levels and follicular blood flow were markedly increased after the administration of GnRH. These will lead to increases in metabolic function in follicular cells [34]. The high level of E2 achieved at the time of estrous will promote the release of endothelial nitric oxide synthase which lead to an abrupt increase the blood flow and vasodilatation [21]

6. Conclusion

Synchronization with the PRID -PGF2α protocol could be enhanced the follicular activity and hemodynamics of Egyptian buffaloes.

7. References

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Table 1. The effect of Days preovulation, treatment and their interaction on follicular and The CL size and vascularization of buffaloes

Follicle	Days	Treatment	Treatment * Days	Day Within treatment		
				PRID - PGF2α	PRID +GnRH	Spontaneous
N small <5mm	.166	.018	.802	.783	.007	.272
N medium >5-<10mm	.020	.034	.137	.001	.331	.046
N large >10mm	.000	.306	.011	.001	.000	.001
N. total follicles	.740	.001	.104	.783	.439	.736
Small diameter	.317	.817	.193	.000	.430	.607
Medium diameter	.000	.197	.000	.003	.000	.000
F1 diameter	.000	.000	.307	.000	.000	.000
F1 area/pixel	.000	.000	.006	.000	.000	.000
F1 antrum area/pixel	.000	.000	.004	.000	.001	.000
F1 granulosa area	.000	.000	.027	.000	.000	.000
F1 colored area/pixels	.000	.000	.186	.000	.000	.000
F1 color area %	.000	.000	.023	.001	.000	.000
F1 granulosa color area %	.000	.101	.670	.000	.000	.000
CL diameter/mm	.000	.000	.000	.007	.000	.000
CL area/pixels	.000	.000	.000	.003	.003	.000
CL color area/pixels	.000	.000	.015	.009	.003	.000
CL color area %	.000	.000	.604	.987	.001	.001

Number (N), dominant follicle (F1)

Table 2. The effect of Days preovulation, treatment and their interaction on the ovarian arteries hemodynamics and the circulating ovarian hormones and NO in buffaloes

Ovarian artery hemodynamics	Days	Treatment	Treatment * Days	Day Within treatment		
				PRID - PGF2α	PRID+GnRH	Spontaneous
Ipsi OvRI	.029	.000	.254	.000	.000	.228
contr Ov RI	.000	.282	.290	.000	.001	.000
Ipsi OvPI	.008	.000	.134	.000	.001	.029
contr OvPI	.000	.002	.087	.090	.000	.001
Ipsi Ov PSV	.000	.672	.000	.037	.000	.000
contr Ov PSV	.015	.404	.010	.137	.021	.036
Ipsi Ov EDV	.004	.001	.000	.000	.001	.000
contr Ov EDV	.005	.597	.010	.001	.008	.013
Ipsi Ov. TAMV	.000	.001	.000	.013	.034	.000
contr Ov. TAMV	.000	.119	.108	.112	.014	.000
Ipsi Ov.S/D	.135	.000	.131	.000	.000	.491
contr Ov.S/D	.000	.714	.010	.000	.649	.000
Ipsi Ov BFV	.000	.000	.014	.006	.000	.000
contr Ov BFV	.010	.000	.016	.001	.000	.003
E2 pg/ml	.865	.937	.638	.453	.439	.513
P4 ng/ml	.066	.002	.450	.048	.148	.023
NO mmol/ml	.128	.824	.012	.182	.032	.714

Table 3. The follicular number, growth and hemodynamic in normal spontaneous and different synchronization treatments (PRID -PGF2α and PRID+GnRH) on the day of ovulation (Day 0). [Mean± SD]

Follicles	PRID -PGF2α	PRID+GnRH	Spontaneous	Total	P-Value
Small number	3.17[b]±.090	2.25 [a]±.131	2.64[a]±.173	2.71±.105	0.007
Medium number	1.00 [a]±.000	1.25 [ab]±.131	1.45 [b]±.116	1.29±.069	.016
Large number	1.00 [b]±.000	.75 [ab]±.131	.73 [a]±.079	.81±.050	.051
Total follicles number	5.17 [b]±.090	4.25 [a]±.250	4.82 [b]±.165	4.81±.108	.014
Small diameter	4.45 [b]±.05	4.05 [a]±.12	4.21 [ab]±.10	4.25±.06	.072
Medium diameter	7.27 [b]±.26	6.55[a]±.33	6.65[a]±.11	6.80±.12	.039
F1 diameter	12.58 [b]±.199	11.63[a]±.17	11.64[a] ±.07	11.90±.09	.000
F1 area/pixel	30953 [b]±1140	25251[a]±706	25137[a]±208	26820±490	000
F1 antrum area/pixel	21055 [b]±1117	16055[a]±515	16446[a]±204	17688±437	000
F1 Granulosa area	9898[c]±64	9196 [b]±223	8691[a]±118	9132±101	000
F1 colored area/pixels	3423 [b]±181	2773[a]±125	2512[a]±32	2822±76	000
F1 color area %	10.95 [b]±.22	10.93 [b]±.20	9.99[a]±.07	10.44±.10	000
F1 Granulosa color area %	34.51 [b]±1.74	30.04[a]±0.82	29.04[a]±0.47	30.79±0.64	.001

Means with different superscripts within the row (a, b, c, d) are significantly different at $P < 0.05$, number (N), dominant follicle (F1)

Table 4. Luteal size and vascularization in normal spontaneous and different synchronization treatments (PRID -PGF2α and PRID+GnRH) on the day of ovulation (Day 0). [Mean± SD]

Variable	PRID -PGF2α	PRID+GnRH	Spontaneous	Total	P-Value
CL Diameter/mm	15.20^{c}±.189	11.15^{b}±.157	9.05^{a}±.285	10.47±.39	.000
CL Area/pixels	45414^{c}±477	23427^{b}±253	16712^{a}±819	22933±1704	.000
CL Color area/pixels	9320^{c}±64	4009^{b}±64	2336^{a}±134	3855±402	.000
CL Color area %	20.53^{b}±.1196	17.11^{ab}±.09	14.92^{a}±.95	16.25±.72	.008
E2 (pg/ml)	102.4±13.20	79.1±13.37	110.2±8.45	102.1±6.42	.195
P4 (ng/ml)	4.59±.43	4.06±.43	4.10±.26	4.23±.19	.526
nitric oxide (mmol/ml)	14.27^{a}±.65	23.3550^{b}±4.45	18.21^{a}±.70	18.06±.99	.007

Means with different superscripts within the row (a, b, c, d) are significantly different at $P < 0.05$.

Table 5. The ovarian arteries blood flow parameters in normal spontaneous and different synchronization treatments (PRID -PGF2α and PRID+GnRH) on the day of ovulation (Day 0). [Mean± SD]

Ovarian arteries	PRID -PGF2α	PRID+GnRH	Spontaneous	Total	P-Value
Ipsi Ov RI	0.85^{b}±0.05	$.83^{b}$±.009	$.77^{a}$±0.09	.80±.007	.000
contr Ov RI	0.77^{b}±0.05	0.7^{b}±.00	$.73^{a}$±.010	.75±.006	.01
Ipsi OvPI	1.84^{b}±0.09	1.8^{b}±.028	1.54^{a}±.026	1.67±.02	.0001
contr OvPI	1.63^{b}±0.012	1.6^{ab}±.01	1.56^{a}±.018	1.59±.01	.012
Ipsi Ov PSV	17.56±.87	15.5±0.7	17.89±0.79	17.36±.5	.225
contr Ov PSV	14.71±0.79	16.0±1.1	15.69±0.57	15.49±.4	.491
Ipsi Ov EDV	2.79±0.11	2.91^{a}±0.2	4.06^{b}±0.21	3.48±.14	.000
contr Ov EDV	3.56±0.17	4.0±.19	3.86±0.22	3.82±.13	.386
Ipsi Ov. TAM	3.80±0.22	3.5±0.2	4.18±0.19	3.95±.13	.141
contr Ov. TAM	3.26±0.25	3.7±0.2	3.43±0.14	3.44±.11	.295
Ipsi Ov.S/D	6.36^{b}±0.28	5.6^{b}±0.3	4.48^{a}±.18	5.24±.18	.000
contr Ov.S/D	3.95±.14	3.9±0.09	4.0±0.13	3.99±.08	.830
Ipsi Ov BFV	45.38^{b}±1.0	47.0^{b}±0.73	40.63^{a}±.86	43.2±.65	.000
contr Ov BFV	41.18^{b}±1.1	42.78^{b}±0.59	37.06^{a}±0.78	39.3±.62	.000

Means with different superscripts within the row (a, b, c, d) are significantly different at $P < 0.05$.

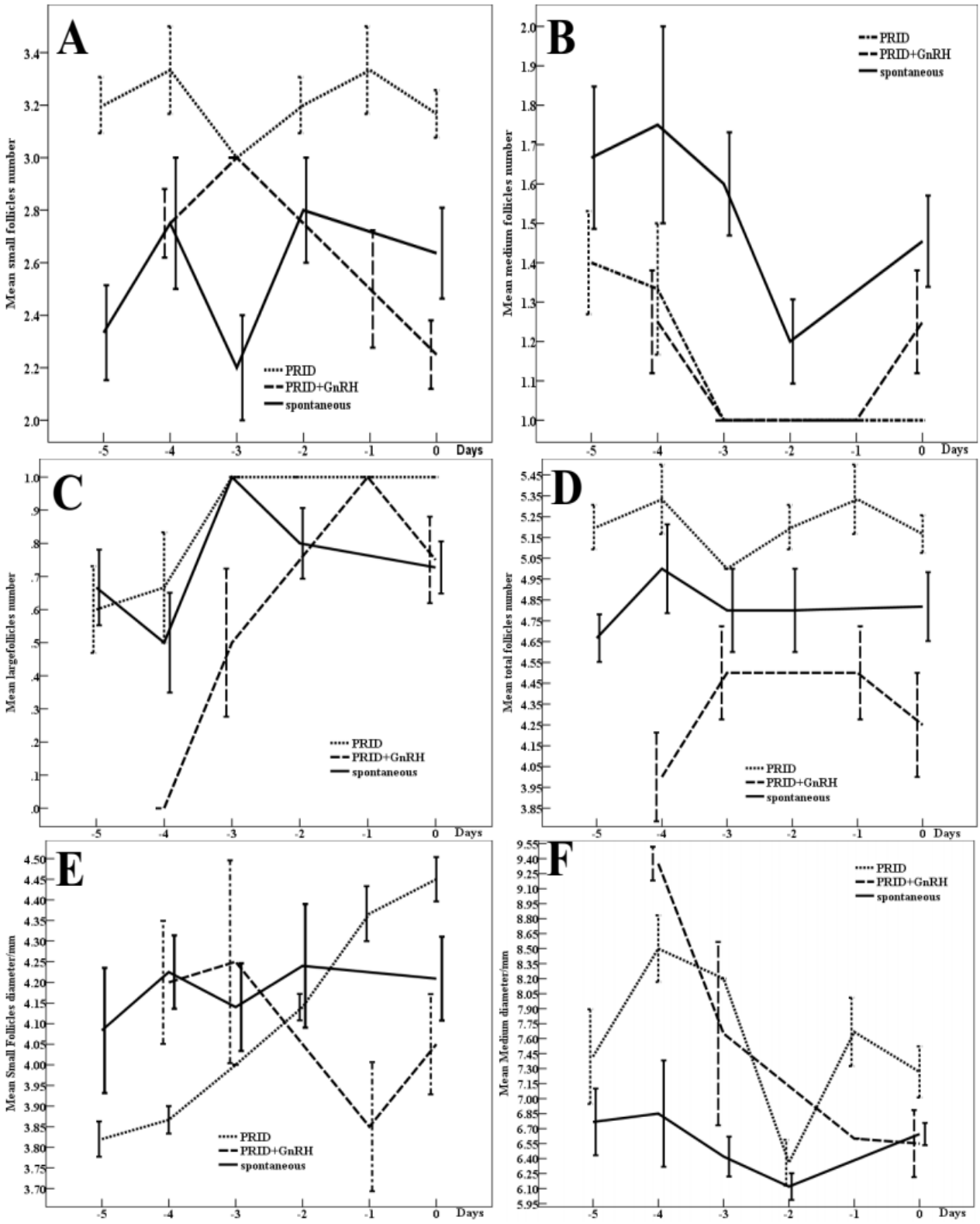

Figure 1. The mean number of small (A), medium(B), Large (C), and total follicles (D), the diameter of small (E) and medium (F) follicles from Day -5 before ovulation till Day 0 (Ovulation) in buffaloes synchronized with PRID -PGF2α, PRID + GnRH and spontaneously ovulated with error bars.

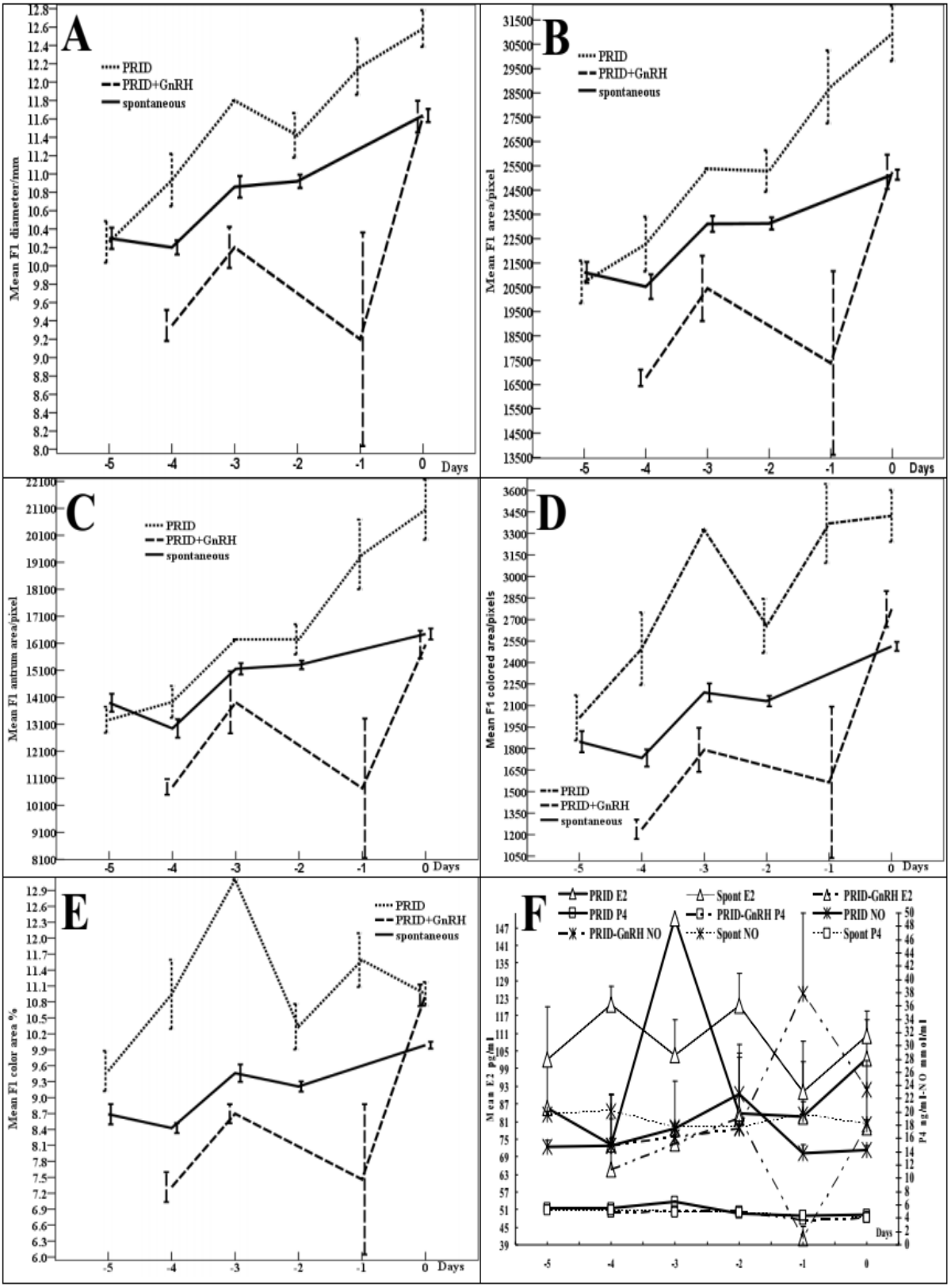

Figure 2. The mean Diameter of the F1 (A), F1 area (B), F1 antrum area (C), F1 color area (D), F1 color area % (E), concentrations of estradiol pg/ml (E2), progesterone ng/ml (P4) and nitric oxide mmol/ml (NO; F) from Day -5 before ovulation till Day 0 (Ovulation) in buffaloes synchronized with PRID -PGF2α, PRID + GnRH and spontaneously ovulated with error bars**.**

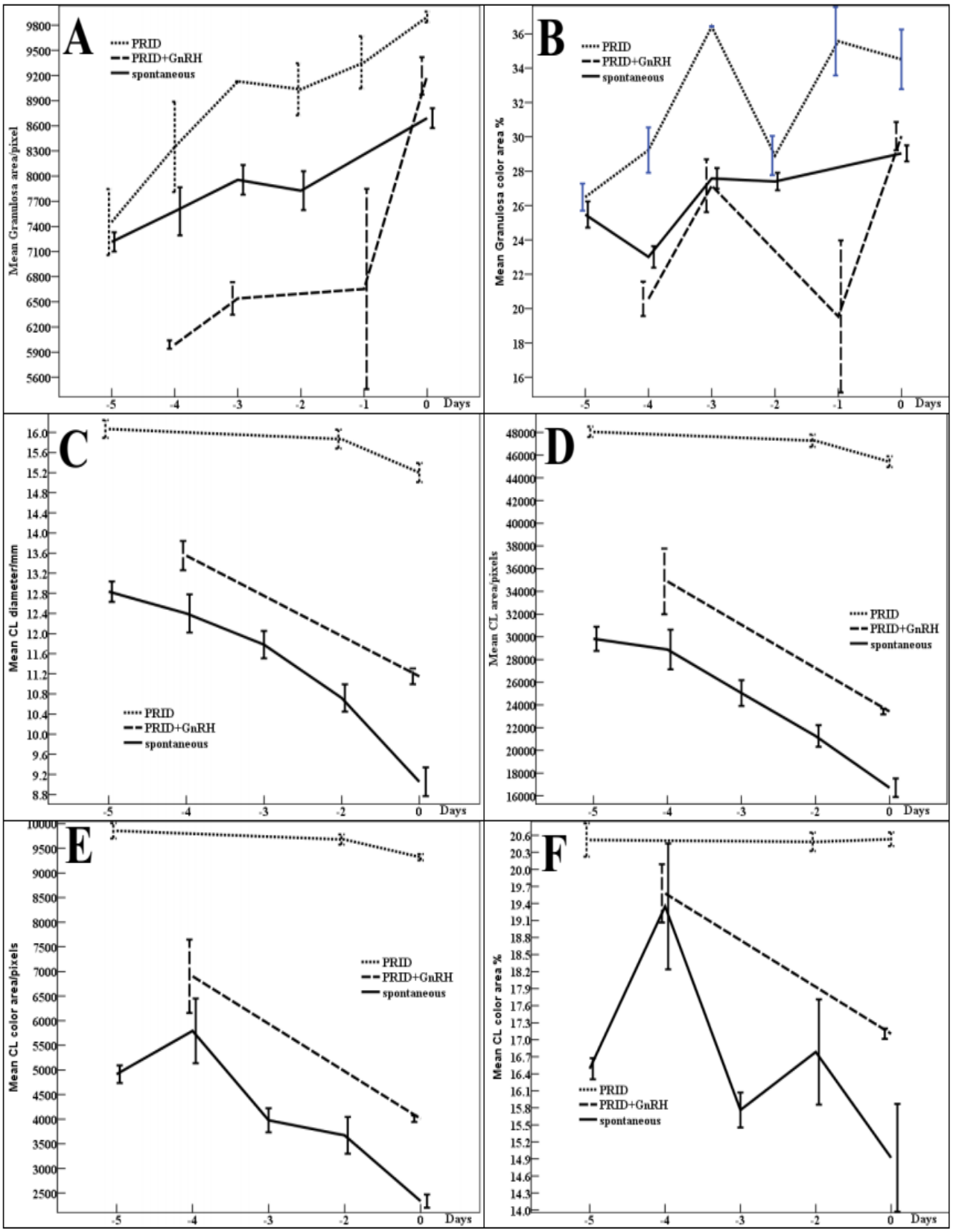

Figure 3. The mean area of F1 granulose (A), color area %of F1 granulose (B), the corpus luteum (CL) diameter (C), CL area (D), CL color area (E), CL color area %(F) from Day -5 before ovulation till Day 0 (Ovulation) in buffaloes synchronized with PRID -PGF2α, PRID + GnRH and spontaneously ovulated with error bars.

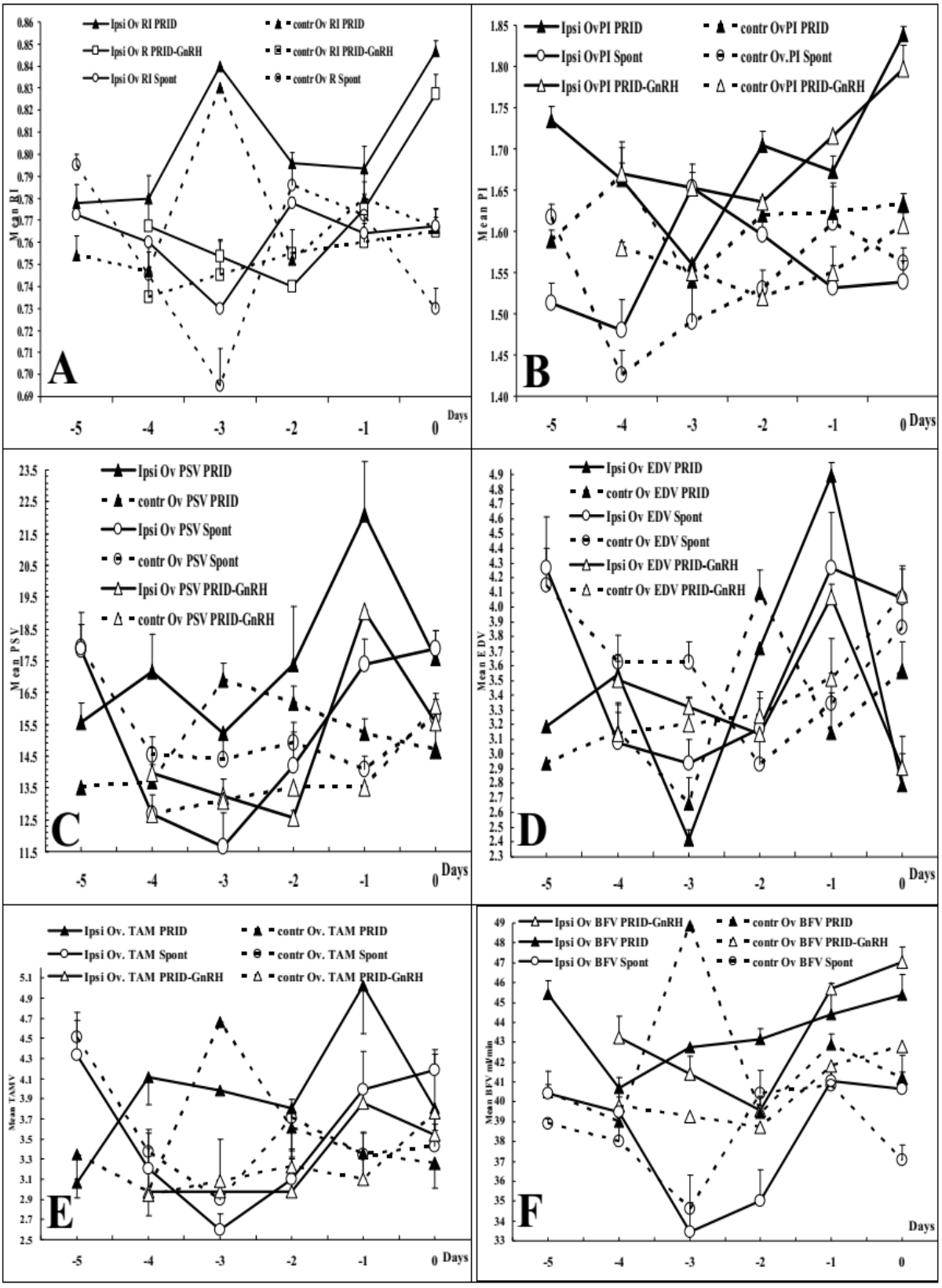

Figure 4. The mean Ovarian arteries RI (A), PI (B), PSVm/s (C), EDV m/s (D), TAMV m/s (E), BFV ml/min (F) from Day -5 before ovulation till Day 0 (Ovulation) in buffaloes synchronized with PRID -PGF2α, PRID + GnRH and spontaneously ovulated with error bars.