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# The Fertility Outcomes of Egyptian Buffalo Cows after Ovsynch and Presynch-Ovsynch Protocols

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## ABSTRACT

The present study aimed to compare the reproductive outcomes after the blind application of the standard Ovsynch and Presynch Ovsynch protocols on Egyptian Buffaloes (*Bubalus bubalis*) during the breeding season. Fifty multiparous Egyptian buffalo cows of an unknown stage of the estrous cycle were randomly divided into two groups: 1) the standard Ovsynch protocol (first GnRH (G1) at d0, $PGF_{2\alpha}$ at d7 , and second GnRH; G2 56 h later and 2) the pre-synch Ovsynch (G6G-Ovsynch) protocol ($PGF_{2\alpha}$ and GnRH 2 days apart 6 days before starting G1 of the standard Ovsynch). Cows were subjected to timed artificial insemination (TAI) 16 and 40 h after the G2 injection in both groups. Blood sampling and ovarian transrectal ultrasonography were performed at three time points, PGF2α, G2, and 2 days after G2. Serum progesterone ($P_4$) (ng/mL) concentrations were significantly higher in the G6G-Ovsynch group than in the Ovsynch group at the time of $PGF_{2\alpha}$ and two days after the G2 injection, but they were lower ($P < 0.05$) at the G2 injection. The Vascularity index of the corpus luteum (CL) and dominant follicle (DF) wall area (%) were higher ($P < 0.05$) in the G6G-Ovsynch group compared to the Ovsynch group. The ovulatory response indicated by the presence of CL at d7 was significantly higher in G6G-Ovsynch than in Ovsynch (73 vs. 51%, respectively). Moreover, a higher ($P < 0.05$) pregnancy rate was observed in G6G-Ovsynch than in the Ovsynch group. In conclusion, the blind application of the G6G-Ovsynch improved the ovulatory response in the early stages of the synchronization protocol and raised the pregnancy rates in Egyptian buffaloes.
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## INTRODUCTION

Domestic water buffaloes (*Bubalus bubalis*) contribute a major sector to the agricultural economies of many developing countries (**Perera, 2011**). Buffaloes are renowned for being able to produce high-quality milk and meat from low-quality roughage. They are more efficient at digesting feed than cattle (**Khedkar *et al.*, 2003**). Buffaloes are also crucial livestock for rural areas because of their resistance to illnesses and tropical environments (**Singh and Balhara, 2016**). In addition, buffaloes supply emerging nations with milk, meat, and draught power (**Perera, 2011**). Over the past 30 years, there has been a considerable rise in the global buffalo population. In 2018, there were more than 200 million buffalo. Egypt had ~ 3.4 million buffalo heads (69.1% cows and 30.9% bulls), producing 43.1% and 42.8% of total meat and milk production, respectively (**FAOStat, 2019**).

The main element affecting productivity is reproduction efficiency, which is hampered by issues with management, nutrition, illnesses, and the climate (**Perera, 2008**). Delays in puberty and longer postpartum anestrus length are caused by poor nutrition, which is typically associated with seasonal variations in feed availability and quality (**Perera, 2008**). The success of controlling the estrous cycle and fixed timed inseminations in dairy and beef cattle (**Bridges and Lake, 2011**) has resulted in increased adoption of these techniques in the buffalo, a species with problems of poor estrus expression, poor heat detection, and seasonality of reproduction. The ovarian activity and reproductive efficiency of buffaloes in Egypt are influenced by the season. Winter and spring are the best times to breed since

they have greater pregnancy rates, pre-ovulatory follicle and mature CL diameters, and large follicle counts than summer and fall (**Ali, 2015**).

The natural mating of estrus buffalo cows remains the main breeding form in the world, while AI remains the main technique utilized for genetic improvement and fertility management in modern beef and dairy farms (**Bó *et al.*, 2016**). Due to poor estrus detection, the variable duration (4–64 h) of oestrus (**Baruselli, 2001**), and the difficulty of predicting the time of ovulation, AI in buffaloes was limited for decades. However, only in the recent years, AI has progressively risen due to better understanding of buffalo reproductive physiology, particularly the knowledge of ovarian dynamics through ultrasonography (**Perera, 2011**), usage of hormones for controlling the estrous cycle, and advances in semen cryopreservation (**Neglia *et al.*, 2020**).

Similar to cattle, methods for estrus synchronization in buffalo have used prostaglandins, progestins, and GnRH either separately or in combination (**De Rensis and Lopez-Gatius, 2007**). These hormonal drugs control follicular growth and regression, ovulation, and corpus luteum (CL) regression. The mechanism of synchronization protocols involves either the growth of DF to ovulation after luteolysis (prostaglandins), or the functional removal of existing DF, followed by the emergence of a new follicular wave that progresses to ovulation (progestins and GnRH-based protocols).

It was claimed that using Ovsynch protocols decreases the period between the first insemination, days open, and culling of infertile cows (**Tenhagen *et al.*, 2004**). In addition to scheduled pregnancy diagnosis and re-synchronization of non-pregnant buffalo cows, accurate estrus detection approaches can be used to re-inseminate returned to estrus cows (**Galvão *et al.*, 2013**). In buffaloes, the application of synchronization protocols, followed by fixed-timed insemination without estrus detection, can effectively manage less pronounced estrus signs (**Roy and Prakash, 2009**). On most buffalo farms, the basic plan of reproductive management still heavily relies on the artificial insemination of detected estrus (**Caraviello *et al.*, 2006**). However, submitting buffalo cows for AI-based exclusively on estrus observation results in poor reproductive performance (**López-Gatius *et al.*, 2004; Lopez *et al.*, 2004**) mainly due to two limitations: the human factor (i.e., the subjective and time-consuming visual observation of estrus signs) and cow-related factors (i.e., the lack of estrus expression due to high milk production or ovulation failure conditions).

The use of $PGF_{2\alpha}$ and its analogues due to their luteolytic properties on the CL is one of the oldest approaches for the synchronization of estrus in buffaloes (**De Rensis and Lopez-Gatius, 2007**). In both cattle, (**Schallenberger *et al.*, 1984**) and buffalo (**Shah *et al.*, 2014**), $PGF_{2\alpha}$ -induced luteolysis takes place from days 5 to 18 of a typical estrous cycle. After determining whether CL is present, a single $PGF_{2\alpha}$ injection will often cause estrus 60 hours later. Two injections, 11–14 days apart, are required for synchronization with $PGF_{2\alpha}$ without evaluation of CL, this increases the number of animals exhibiting estrus after the second injection (**Neglia *et al.*, 2020**). Due to limited projection on the ovarian surface, palpation of a CL in buffaloes seems challenging (**Sharifuddin and Jainudeen, 1983**). Large variability in estrus behaviour, estrus duration, and ovulation, has also been recorded according to the follicular population (**Neglia *et al.*, 2020**). The onset of estrus can be delayed for up to 5 days if a DF is absent, but it is shortened to 2-3 days when a DF is present (**De Rensis and Lopez-Gatius, 2007**).

Due to the challenge of heat detection in buffaloes (**Neglia *et al.*, 2020**), the management of follicular growth and ovulation is the fundamental basis of estrus synchronization protocols. This enables fixed-timed AI, avoids the need for heat observation, and is more efficient for reproductive management of the herd. To regulate follicular development and ovulation, GnRH or GnRH agonists like gonadorelin, buserelin, or lecirelin are frequently utilized. At any stage of the estrous cycle, GnRH and its agonists induce surges in LH, FSH, and estradiol. This promotes the ovulation of a DF or the luteinization and/or atresia of pre-dominant follicles. The follicular diameter needed for ovulation in response to GnRH appears variable (**Campanile *et al.*, 2008**). Follicles measuring (6.7± 2.4) mm in diameter in one research study failed to ovulate (**Baruselli, 2001**). In other studies, ovulation occurred in follicles with a diameter of 4 to 12 mm (**Rastegarnia *et al.*, 2004; Campanile *et al.*, 2008**). The optimal follicular size required for a response to GnRH in buffalo may be better understood by studies that focus on follicular LH and FSH receptors. The next follicular wave emerges within 2 or 3 days after GnRH administration (**Pursley *et al.*, 1995**).

Color Doppler ultrasound is a real-time imaging technique of any structure's vascular architecture that enables the estimation of the amount of blood flow into the tissue and the assessment of the presence, direction, and intensity of vascularization (**Lüttgenau and Bollwein, 2014**). Throughout the estrous cycle, the pattern of CL vascularization fluctuates, with an increase in blood flow at the start of the formation and a decrease during its regression

(**Lüttgenau and Bollwein, 2014; Scully *et al.*, 2014**). According to previous studies, CL function can be evaluated based on blood perfusion throughout the estrous cycle (**Herzog *et al.*, 2010**) and echotexture (**Siqueira *et al.*, 2009**). CL area and the percentage of CL vascularization are positively correlated with serum $P_4$ concentrations (**Vrisman *et al.*, 2018**).

With these considerations, the objectives of the current study were to evaluate the fertility outcomes after the blind application of standard Ovsynch and G6G-Ovsynch in Egyptian buffalo during the breeding season and the effect of both synchronization protocols on luteal and follicular hemodynamics .

## MATERIALS AND METHODS

### Animal Ethics

The study protocol was reviewed and approved by the Animal Care and Ethical Use Committee of the Faculty of Veterinary Medicine at Cairo University (Approval ID: ***Vet CU2305 2022457).***

### 2.1. Animals and Husbandry Practices

The current study was performed in Giza governorate **(**30.01222°N,31.20889°E), Egypt during the breeding season (from January 15, 2022, to April 19, 2022) **(Ali, 2015)**. Fifty multiparous Egyptian buffalo cows ***(Bubalus bubalis)*** were used. They were physically and reproductively healthy based on clinical and breeding soundness examinations**.** Buffalo cows were housed in free-stall barns at the faculty of Agriculture, Cairo University, varying from 4 to 8 years old (first to third lactations*),* with moderate body weight (512 to 620) and body condition score (2.5 to 3 points; 1-5 scale). The buffaloes were ~90–100 days in milk, and calves were weaned after 4 weeks of calving. According to National Research Council (**NRC, 2001**) recommendations, the animals were fed on a total mixed ration (TMR). Fresh and clean water was accessible 24h to each animal. Milking was practiced using a machine twice daily.

### 2.2. Experimental Design

Buffalo cows used in the current study (n=50) were randomly allocated into two groups (n=25 each) blindly without any previous knowledge of the stage of the estrous cycle. The Ovsynch group animals (group 1) were intramuscularly injected on D 0 with the first GnRH (G1; Buserelin acetate,10 µg, Receptal inj.®; Intervet, Angers, France), $PGF_{2\alpha}$ (25 mg of Dinoprost tromethamine, Lutalyse; Pfizer Animal Health, New York, USA) on d7, and then the second dose of GnRH (G2) 56 hr after $PGF_{2\alpha}$ (**Pursley *et al.*, 1995**). In the G6G-Ovsynch group (group 2), cows were injected with $PGF_{2\alpha}$ followed in 2 days with GnRH, then 6 days later cows were started OV-synch (G1at D0, $PGF_{2\alpha}$ at D7, G2 56 hr later**)** (**Waqas *et al.*, 2016**). All buffaloes were artificially inseminated at a fixed time 16 and 40 hours after the second GnRH of the protocol using frozen-thawed semen from proven fertile bulls ($30\times10^6$ sperm cell per 0.25ml straw, 40-45% post-thawing motility**;** Semen Cryopreservation Station, Abbasia, Cairo, Egypt).

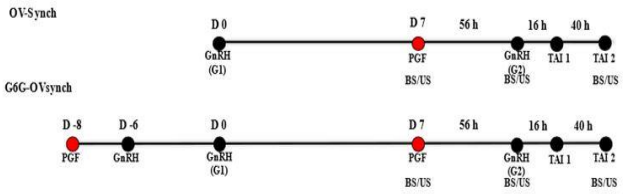


Fig .1: Fig .1: Schematic diagram of experimental design. Buffalo cows were assigned randomly to be treated with Ovsynch, or G6G-Ovsynch. Transrectal ultrasonography (US), collection of blood samples (BS), and timed artificial insemination (TAI) were performed as indicated.

All settings for the US system (brightness, depth, and contrast) were standardized and fixed uniformly and all examinations were conducted by the same operator throughout the study. The number and average of vertical and horizontal diameters of all follicles ≥ 4 mm were recorded. The preovulatory follicles were defined as dominant follicles (DF) measured and mapped at the time of the final GnRH that were not present 2 days later.

The vertical and horizontal diameters of the CL were recorded on day 7. Besides, a frozen picture of the CLs was saved on the US device for assessment of the luteal echogenicity (pixel intensity) using the computer-assisted image analysis (CAIA) with the Image-J program. The volume and area of luteal tissue were determined with these formulas: $[4/3 \times \pi \times (0.5 \times \text{diameter})^3]$ and $[\pi \times (0.5\times\text{diameter})^2]$, respectively (**Moore *et al.*, 2014**). In the cavitary corpus luteum (CCL), the volume and area of the cavity were calculated and subtracted from the total volume and area of the CCL, respectively. The luteal and follicular Doppler ultrasonography was conducted on d7 and the day of the final GnRH injection, respectively. The pregnancy diagnosis was performed on day 35 post-TAI by the transrectal US and confirmed on day 45.

The synchronization rate was defined as the percentage of cows that responded to both $PGF_{2\alpha}$ by regression of a functional CL and the final GnRH of the protocol by ovulation (**Pursley *et al.*, 1995**). Pregnancy per AI (P/AI) was estimated by dividing the number of pregnant cows by the number of cows subjected to TAI, which was multiplied by 100 (**Allahyari *et al.*, 2023**).

## 2.3. Blood sampling and progesterone determination

Blood samples were collected from the median coccygeal vein of each animal at three time points, PGF2α, G2, and2 days after G2. Samples were collected using tubes without anticoagulant (BD Vacutainer, Preanalytical Solutions, Franklin Lakes, NJ). Samples were refrigerated for 30 min and centrifuged at 3,000 r.p.m for 20 min. The serum was collected and stored frozen at −20°C until $P_4$ was assayed. Concentrations of $P_4$ (ng/mL) in serum were determined using a commercially available solid-phase radioimmunoassay (Coat-A-Count Progesterone, Diagnostic Products Corporation, Canoga Park, CA, USA). The intra- and inter-assay coefficients of variation were 1.79 and 3.13%, respectively and the assay sensitivity was 0.033 ng/ml.

## 2.4. Statistical analysis

All results were expressed as mean± SEM and $p < 0.05$ was considered statistically significant. Data were analyzed using statistical software (SPSS version 16.0, IBM Corp., Armonk, NY, USA). Normality was visually assessed and confirmed by the Kolmogorov-Smirnov method. The chi-square test was used to compare the binomial variables (CL presence at the time of $PGF_{2\alpha}$ injection, synchronization, and P/AI rates), and follicular and luteal sonographic parameters while, the general linear model for repeated measures accompanied Tukey's post-hoc test was used to compare protocol, time effects as well as time × protocol interactions on contentious variables i.e., $P_4$ concentrations.

At the time of $PGF_{2\alpha}$ injection (Table 1), the serum $P_4$ (ng/mL) concentrations were significantly higher in G6G-Ovsynch than in the Ovsynch group. At the time of G2 injection, the serum $P_4$ concentrations in the G6G-Ovsynch group were lower ($P < 0.05$) compared to OV-synch (1.8±0.4 vs 3.1±1.1, respectively). Two days after the G2, serum $P_4$ concentrations in G6G-Ovsynch were higher ($P < 0.05$) than in the Ovsynch group (3.9±0.7 vs 2.5±0.7, respectively). Within groups, the fluctuations in $P_4$ concentrations at the 3-time points of blood analysis were pronounced ($P < 0.05$) in the G6G-Ovsynch group.

Table 1: Serum progesterone concentrations (mean ± SEM) of buffalo cows subjected to Ovsynch and G6G-Ovsynch protocol.

| | Ovsynch (n=25) | G6G-Ovsynch (n=25) | *p-value* |
|---|---|---|---|
| $P_4$ at $PGF_{2\alpha}$ (ng/mL) | $4.9\pm0.9^{a}$* | $7.85\pm0.8^{b}$* | 0.02 |
| $P_4$ at G2 (ng/mL) | $3.1\pm1.1^{a}$ | $1.8\pm0.4^{b}$** | 0.03 |
| $P_4$ 40 h after G2 (ng/mL) | $2.5\pm0.7^{a}$ | $3.9\pm0.7^{b}$ | 0.04 |

Progesterone concentrations were measured at the time of $PGF_{2\alpha}$, at the second GnRH treatment(G2), and 40 h after the G2 of Ovsynch. Means with different superscripts differ (at least at $P < 0.05$) i.e., * difference within the same group (columns) at different time points, $^{a\text{-}c}$ difference between groups (rows) at the same time point.

The luteal sonographic parameters at the time of $PGF_{2\alpha}$ administration (Table 2) i.e., the luteal diameter (mm), luteal area $(mm)^2$, and luteal volume $(mm)^3$ showed no significant difference between G6G-Ovsynch and Ovsynch group. The Luteal echogenicity in the G6G-Ovsynch group was higher ($P < 0.05$) than OV-synch (82.6±1.5 vs 65.8±0.5, respectively). The total-colored area $(mm)^2$ was significantly lower in Ovsynch than in the G6G-Ovsynch group. However, the vascularity index (%) in the G6G-Ovsynch group was higher ($P < 0.05$) than in the Ovsynch group (36.6±1.5 vs 25.6±0.6, respectively).

Table 2: Luteal sonographic parameters (mean ± SEM) of buffalo cows at the time of $PGF_{2\alpha}$ injection.

| | Ovsynch (n=25) | G6G-Ovsynch (n=25) | *p-value* |
|---|---|---|---|
| Luteal diameter (mm) | 18.2±1.9 | 18.5±2.1 | 0.09 |
| Luteal area $(mm)^2$ | 306.2±2.2 | 313.4±1.5 | 0.06 |
| Luteal Volume $(mm)^3$ | 4204.6±0.8 | 4268.9±1.1 | 0.07 |
| Luteal Echogenicity | $65.8\pm0.5^{a}$ | $82.6\pm1.5^{b}$ | 0.04 |
| Total colored area $(mm)^2$ | $80.2\pm2.3^{a}$ | $112.2\pm1.2^{b}$ | 0.03 |
| Vascularity index (%) | $25.6\pm0.6^{a}$ | $36.6\pm1.5^{b}$ | 0.04 |

mm=millimeter. Means in the same raw with different superscripts differ (at least at $P < 0.05$)
n=number of animals

Regarding the follicular sonographic parameters at the time of G2 administration (Table 3) i.e., the antral follicular count (AFC), DF diameter (mm), and total colored area of DF wall $(mm)^2$

exhibited no significant difference between both groups. Vascularity index of the DF wall (%) in the G6G-Ovsynch group was higher (P< 0.05) than in the Ovsynch group (15.2±1.9 vs 5.8±3.1, respectively).

Table 3: Follicular sonographic parameters (mean ± SEM) of buffalo cows at the time of final GnRH injection.

| | Ovsynch (n=25) | G6G-Ovsynch (n=25) | *p-value* |
|---|---|---|---|
| AFC | 5.6±1.6 | 4.2±1.3 | 0.07 |
| DF diameter (mm) | 12.5±2.6 | 12.4±1.6 | 0.07 |
| Colored area of DF wall (mm)2 | 10.2±2.8 | 11.6±1.5 | 0.06 |
| Vascularity index of DF wall (%) | 5.8±3.1 [a] | 15.2±1.9 [b] | 0.03 |

AFC= antral follicular count, DF= dominant follicle. Means in the same raw with different superscripts differ (at least at $P < 0.05$). n=number of animals

The ovulatory response of buffalo cows after G1, synchronization, and pregnancy rates after Ovsynch and G6G-Ovsynch protocols were depicted in Fig. 2. The ovulatory response at the beginning of the synchronization protocol indicated by the presence of CL at the $PGF_{2\alpha}$ administration time point was higher (P < 0.05) in G6G-Ovsynch than in the Ovsynch group (73 vs 51%, respectively). However, the synchronization rate 2 days after the G2 administration time point was comparable between both groups. Also, the pregnancy rate in the G6G-Ovsynch was higher (P < 0.05) than in the Ovsynch group (61 vs 33%, respectively).

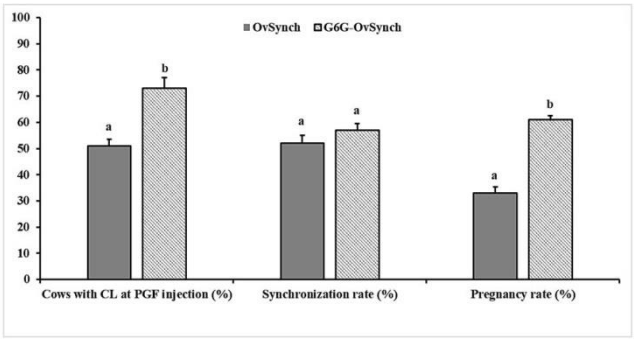


Fig.2. Buffalo cows with CL at PGF2α PGF injection, synchronization, and pregnancy rates after Ovsynch and G6G-Ovsynch protocols. Means with different superscripts for each parameter differed (at least P < 0.05).

## DISCUSSION

To the best of our knowledge, this is the first study to examine the effect of the standard Ovsynch and G6G-Ovsynch protocols on follicular and luteal hemodynamics in Egyptian Buffaloes during the breeding season. According to the results of the present study, applying the G6G-Ovsynch blindly to buffalo cows cause a significant rise in the vascularity index of the corpus luteum (%), the vascularity index of the DF wall (%), the ovulatory response at the start of the protocol, and the pregnancy rate.

Buffalo cows with the greatest fertility to timed AI have mid-level $P_4$ concentrations at the first GnRH treatment, high $P_4$ at the $PGF_{2\alpha}$ treatment, and low $P_4$ at the last GnRH treatment of the Ovsynch protocol (**Fricke, 2020**). In the current study, serum progesterone concentrations were increased in the G6G-Ovsynch group at the time of $PGF_{2\alpha}$ injection (d7) compared to the Ovsynch group. In agreement, higher $P_4$ concentrations in G6G-Ovsynch in comparison with Ovsynch were previously observed in Nili-Ravi buffalo (**Waqas *et al.*, 2016**) and dairy cows (**Bello *et al.*, 2006**). In the G6G-Ovsynch group, the circulating $P_4$ concentration had a similar pattern to that previously reported in Yak (**Sarkar *et al.*, 2009**) and Holstein cows (**Dirandeh *et al.*, 2015**). Increased $P_4$ levels at the $PGF_{2\alpha}$ injection time point are primarily due to synchronized ovulatory response to the first GnRH of Ovsynch in the G6G-Ovsynch group. Moreover, the incidence of double functional CL on either or both ovaries at the time of $PGF_{2\alpha}$ injection was higher in the G6G-Ovsynch group as indicated in a previous study (**Waqas *et al.*, 2016**). In the G6G-Ovsynch group, serum $P_4$ levels were significantly decreased at the time of the final GnRH injection and followed by a significant increase 2 days later. This might attribute to a higher ovulation rate in response to the first GnRH (84 vs 56% in G6G-Ovsynch and Ovsynch, respectively) which was considered the fundamental event for coordination of next follicular wave emergence and formation of functional CL as indicated in a previous study (**Waqas *et al.*, 2016**). Regardless of treatment, the CL in cows that ovulated after the first GnRH of Ovsynch were more likely to experience luteolysis in response to $PGF_{2\alpha}$ of Ovsynch as indicated in earlier studies (**Bello *et al.*, 2006**).

The mean of CL diameters reported in the present study was similar to those previously reported in buffalo (**Baruselli *et al.*, 1997; Yindee *et al.*, 2011**). The results of this study revealed no significant differences in luteal diameters between groups contradicting the results reported in a previous study on Nili-Ravi buffalo (**Waqas *et al.*, 2016**). This could be due to breed variation and different field conditions. Upon these results, the calculation of luteal area $(mm)^2$ and luteal volume $(mm)^3$ had no significant difference between groups. The presynchronization with $PGF_{2\alpha}$ and GnRH, respectively, 8 and 6 days before the start of the standard Ovsynch-TAI protocol increased

vascularization of CL on D7, reflected by an increment of total colored area $(mm)^2$ and vascularity index (%). In the present study, the $P_4$ level in the G6G-Ovsynch group was higher than the Ovsynch group. According to **Lüttgenau and Bollwein, (2014),** the correlations between luteal blood flow (LBF) and cyclic changes in the luteal size (LS) or between $P_4$ and LS during the estrous cycle was lower than a correlation between $P_4$ and LBF. The correlation between $P_4$ and LBF makes sense given that luteal blood vessels supply the CL with steroid precursors (**Janson *et al.,* 1981**) and adequate LBF is needed to release $P_4$ in general circulation (**Acosta *et al.,* 2002**).

In contrast, previous studies reported either a moderate correlation between $P_4$ and LBF (**Herzog *et al.,* 2010**) or no significant correlation (**Lüttgenau *et al.,* 2011**). Luteal echogenicity (pixel intensity) in the G6G-Ovsynch group was higher than in the Ovsynch group. In agreement with our results, the positive association between Luteal volume (LV) and plasma $P_4$ concentrations was previously recorded (**Vasconcelos *et al.,* 2001**). Therefore, low $P_4$ levels in the Ovsynch group reflect lower echogenicity than in the G6G-Ovsynch group. Since the number of luteal cells determines the amount of $P_4$ produced by CL.

In the present study, AFC had no significant difference between groups, this could be due to tight ovulation at the beginning of the Ovsynch protocol which synchronize follicular wave emergence (**Pursley *et al.,* 1995**). However, AFC In G6G-Ovsynch was numerically lower than in the Ovsynch group, which seems reasonable since Ovsynch protocol was applied randomly throughout the estrous cycle. In contrast to synchronized follicular wave emergence under the effect of Pre synch $PGF_{2\alpha}$ and GnRH, respectively, 8 and 6 days before the start of the breeding Ovsynch-TAI protocol was recorded. The DF size at the time of the final GnRH injection in the current study was nearly similar to those previously reported in Nili-Ravi buffalo (**Waqas *et al.,* 2016**) and smaller than those observed in lactating dairy cows (**Bello *et al.,* 2006**). Results of the present study had shown no significant difference in a colored area of DF wall $(mm)^2$ between groups. However, a greater vascularity index of DF wall (%) was seen in the G6G-Ovsynch in comparison to the Ovsynch group. In agreement with our results, a weak positive association between preovulatory follicle (POF) blood flow and follicular size was previously recorded in dairy buffalo (**Varughese *et al.,* 2014**), where larger POF (>16mm) had lower follicular wall vascularization than smaller follicles. Moreover, the latter author reported a greater positive association between plasma and intrafollicular concentrations of estradiol and POF blood flow. Unfortunately, serum estradiol concentrations did not analyze in the present work. In contrast, a strong positive association between DF size, vascular area, and intrafollicular estradiol was observed in dairy cattle (**Mattioli *et al.,* 2001**).

In the current study, ovulatory response to the first GnRH of Ovsynch indicated by the presence of CL on day 7 in the G6G-Ovsynch group was lower than those observed in Nili-Ravi buffalo and nearly similar to Ovsynch group (**Waqas *et al.,* 2016**). Higher ovulatory response in the G6G group attributed to pre-synchronization with $PGF_{2\alpha}$ and GnRH, respectively, 8 and 6 days before the start of the standard Ovsynch-TAI protocol. This includes non-cycling animals that responded only to Pre GnRH, animals in the last stage of the estrous cycle in which regression of corpus luteum had occurred, and cycling animals that either responded to pre-synch injections (**Bello *et al.,* 2006**). In contrast to the random distribution of animals throughout the estrous cycle in the Ovsynch group. However, the synchronization rate (%) represented by the number of buffalo cows that responded to both $PGF_{2\alpha}$ by (regression of CL) and final GnRH by ovulation had no significant difference between groups. In contrast, the high number of pregnant cows on day 45 post-TAI in the G6G-Ovsynch group compared to Ovsynch.

A similar pregnancy rate was observed in Nili-Ravi buffalo (**Waqas *et al.,* 2016**) and lactating dairy cows (**Bello *et al.,* 2006**). However, the synchronization rate in the same previous studies was higher compared to results of the present work. The low synchronization rate in the G6G-Ovsynch group could be due to the low number of buffalo cows in the current study, different field conditions, physiological status of buffalo cows, and breed. Preovulatory follicle size, $E_2$ concentrations, and $P_4$ concentration at the time of $PGF_{2\alpha}$ injection (d7) were significant predictors of fertility as indicated in earlier studies (**Bello *et al.,* 2006**). The high pregnancy rate in the G6G-Ovsynch group, despite the low synchronization rate could be due to high $P_4$ levels on day 7. Previous studies have established that a higher pregnancy rate was associated with greater blood flow of POF in mares (**Silva *et al.,* 2018**) and in Holstein heifers (**Siddiqui *et al.,* 2009**).

## CONCLUSION

Based upon results of the present study, it was concluded that the G6G-Ovsynch protocol enhanced ovulatory response at the time of first GnRH treatment, serum $P_4$ concentrations on day 7 in Egyptian buffaloes. Moreover, G6G-Ovsynch

increased vascularization of CL and DF wall (%) and pregnancy rate. G6G-Ovsynch was an effective protocol to improve luteal and follicular functions to improve fertility in Egyptian Buffaloes.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## REFERENCES

**ACOSTA, T. J., YOSHIZWA, N., OHTANI, M., and MIYAMOTO, A., 2002.** Local Changes in Blood Flow Within the Early and Midcycle Corpus Luteum after Prostaglandin F2α Injection in the Cow1. Biology of Reproduction, 66(3), 651-658. https://doi.org/10.1095/biolreprod66.3.651

**Ali, A., 2015.** Seasonal variations of the ovarian activity and pregnancy rate in the Egyptian buffalo cows (Bubalus bubalis). Tropical animal health and production, *47*(5), 815-818. https://doi.org/10.1007/s11250-015-0793-8

**ALLAHYARI, I., GHARAGOZLOU, F., VOJGANI, M., POOLADZADEH, P., MOBEDI, E., and AKBARINEJAD, V., 2023.** Replacement of the first GnRH by estradiol in the breeding Ovsynch of Double Ovsynch protocol could improve fertility in Holstein dairy cows. Animal Reproduction Science, *252*, 107228. https://doi.org/10.1016/j.anireprosci.2023.107228

**BARUSELLI, P., MUCCIOLO, R., VISINTIN, J., VIANA, W., ARRUDA, R., MADUREIRA, E., OLIVEIRA, C., and MOLERO-FILHO, J., 1997.** Ovarian follicular dynamics during the estrous cycle in buffalo (Bubalus bubalis). Theriogenology, *47*(8), 1531-1547. https://doi.org/10.1016/S0093-691X(97)00159-3

**BARUSELLI, P. S., 2001.** Control of follicular development applied to reproduction biotechnologies in buffalo. In: Proceedings of the I Congresso Nazionale sull'allevamento del Bufalo; 2001. Book of the Congress, p. 128–46.

**BELLO, N. M., STEIBEL, J., and PURSLEY, J., 2006.** Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. Journal of dairy science, *89*(9), 3413-3424. https://doi.org/10.3168/jds.S0022-0302(06)72378-5

**BÓ, G. A., DE LA MATA, J. J., BARUSELLI, P. S., and MENCHACA, A., 2016.** Alternative programs for synchronizing and resynchronizing ovulation in beef cattle. Theriogenology, *86*(1), 388-396. https://doi.org/10.1016/j.theriogenology.2016.04.053

**BRIDGES, G., and LAKE, S., 2011.** Comparison of the CIDR Select and 5-day Select Synch+ CIDR protocols that included limited estrus detection and timed insemination for synchronizing estrus in beef heifers. The Professional Animal Scientist, *27*(2), 141-146. https://doi.org/10.15232/S1080-7446(15)30461-7

**CAMPANILE, G., VECCHIO, D., NEGLIA, G., DI PALO, R., PRANDI, A., and D'OCCHIO, M., 2008.** Progesterone and pregnancy status of buffaloes treated with a GnRH agonist. Livestock science, *115*(2-3), 242-248. https://doi.org/10.1016/j.livsci.2007.08.001

**CARAVIELLO, D., WEIGEL, K., FRICKE, P., WILTBANK, M., FLORENT, M., COOK, N., NORDLUND, K., ZWALD, N., and RAWSON, C., 2006.** Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. Journal of dairy science, *89*(12), 4723-4735. https://doi.org/10.3168/jds.S0022-0302(06)72522-X

**DE RENSIS, F., and LOPEZ-GATIUS, F., 2007.** Protocols for synchronizing estrus and ovulation in buffalo (Bubalus bubalis): A review. Theriogenology, *67*(2), 209-216. https://doi.org/10.1016/j.theriogenology.2006.09.039

**DIRANDEH, E., ROODBARI, A. R., GHOLIZADEH, M., DELDAR, H., MASOUMI, R., KAZEMIFARD, M., and COLAZO, M., 2015.** Administration of prostaglandin F2α 14 d before initiating a G6G or a G7G timed artificial insemination protocol increased circulating progesterone prior to artificial insemination and reduced pregnancy loss in multiparous Holstein cows. Journal of dairy science, *98*(8), 5414-5421. https://doi.org/10.3168/jds.2015-9417

**FAOSTAT, F., 2019.** Food and agriculture data. Crop Statistics. Available online: http://www. fao. org/faostat (accessed on 14 August 2020).

**FRICKE, P. 2020.** The evolution of fertility programs for lactating dairy cows. American Association of Bovine Practitioners Conference Proceedings (pp 104-112). https://doi.org/10.21423/aabppro20207979

**GALVÃO, K., FEDERICO, P., DE VRIES, A., and SCHUENEMANN, G. M., 2013.** Economic comparison of reproductive programs for dairy herds using estrus detection, timed artificial insemination, or a combination. Journal of dairy science, *96*(4), 2681-2693. https://doi.org/10.3168/jds.2012-5982

**HERZOG, K., BROCKHAN-LÜDEMANN, M., KASKE, M., BEINDORFF, N., PAUL, V., NIEMANN, H., and BOLLWEIN, H., 2010.** Luteal blood flow is a more appropriate indicator for luteal function during the bovine estrous cycle than luteal size. Theriogenology, *73*(5), 691-697. https://doi.org/10.1016/j.theriogenology.2009.11.016

**JANSON, P., DAMBER, J.-E., and AXEN, C., 1981.** Luteal blood flow and progesterone secretion in pseudopregnant rabbits. Reproduction, *63*(2), 491-497. https://doi.org/10.1530/jrf.0.0630491

**KHEDKAR, C., KHEDKAR, G., PATIL, M., and KALYANKAR, S., 2003.** Encyclopedia of Food Sciences and nutrition(pp. 705-709). Maryland: Academic Press. In.

**LÓPEZ-GATIUS, F., SANTOLARIA, P., YÁNIZ, J., GARBAYO, J., and HUNTER, R., 2004.** Timing of early foetal loss for single and twin pregnancies in dairy cattle. Reproduction in Domestic Animals, *39*(6), 429-433. https://doi.org/10.1111/j.1439-0531.2004.00533.x

**LOPEZ, H., SATTER, L., and WILTBANK, M., 2004.** Relationship between level of milk production and estrous behavior of lactating dairy cows. Animal Reproduction Science, *81*(3-4), 209-223. https://doi.org/10.1016/j.anireprosci.2003.10.009

**LÜTTGENAU, J., and BOLLWEIN, H., 2014.** Evaluation of bovine luteal blood flow by using color Doppler ultrasonography. Reproductive biology, *14*(2), 103-109. https://doi.org/10.1016/j.repbio.2014.03.003

**LÜTTGENAU, J., ULBRICH, S. E., BEINDORFF, N., HONNENS, A., HERZOG, K., and BOLLWEIN, H., 2011.** Plasma progesterone concentrations in the mid-luteal phase are dependent on luteal size, but independent of luteal blood flow and gene expression in lactating dairy cows. Animal Reproduction Science, *125*(1-4), 20-29. https://doi.org/10.1016/j.anireprosci.2011.02.002

**MATTIOLI, M., BARBONI, B., TURRIANI, M., GALEATI, G., ZANNONI, A., CASTELLANI, G., BERARDINELLI, P., and SCAPOLO, P. A., 2001.** Follicle activation involves vascular endothelial growth factor production and increased blood vessel extension. Biology of Reproduction, *65*(4), 1014-1019. https://doi.org/10.1095/biolreprod65.4.1014

**MOORE, S., SCULLY, S., BROWNE, J., FAIR, T., and BUTLER, S., 2014.** Genetic merit for fertility traits in Holstein cows: V. Factors affecting circulating progesterone concentrations. Journal of dairy science, *97*(9), 5543-5557. https://doi.org/10.3168/jds.2014-8133

**NATIONAL RESEARCH COUNCIL (NRC), 2001.** Nutrient requirements of dairy cattle. National Research, *319*.

**NEGLIA, G., DE NICOLA, D., ESPOSITO, L., SALZANO, A., D'OCCHIO, M. J., and FATONE, G., 2020.** Reproductive management in buffalo by artificial insemination. Theriogenology, *150*, 166-172. https://doi.org/10.1016/j.theriogenology.2020.01.016

**PERERA, B. 2008.** Reproduction in domestic buffalo. Reproduction in Domestic Animals, *43*, 200-206. https://doi.org/10.1111/j.1439-0531.2008.01162.x

**PERERA, B., 2011.** Reproductive cycles of buffalo. Animal Reproduction Science, *124*(3-4), 194-199. https://doi.org/10.1016/j.anireprosci.2010.08.022

**PURSLEY, J., MEE, M., and WILTBANK, M., 1995.** Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology, *44*(7), 915-923. https://doi.org/10.1016/0093-691X(95)00279-H

**RASTEGARNIA, A., NIASARI-NASLAJI, A., HOVARESHTI, P., SARHADDI, F., and SAFAEI, M., 2004.** The effect of different doses of Gonadorelin on ovarian follicle dynamics in river buffalo (Bubalus bubalis). Theriogenology, *62*(7), 1283-1291. https://doi.org/10.1016/j.theriogenology.2004.01.014

**ROY, K., and PRAKASH, B., 2009.** Changes in endocrine profiles during ovsynch and ovsynch plus norprolac treatment in Murrah buffalo heifers at hot summer season. Tropical animal health and production, *41*(4), 677-687. https://doi.org/10.1007/s11250-008-9241-3

**SARKAR, M., BORAH, B. D., and PRAKASH, B., 2009.** Strategies to optimize reproductive efficiency by regulation of ovarian function in yak (Poephagus grunniens L.). Animal Reproduction Science, *113*(1-4), 205-211. https://doi.org/10.1016/j.anireprosci.2008.07.005

**SCHALLENBERGER, E., SCHAMS, D., BULLERMANN, B., and WALTERS, D.L., 1984.** Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. J Reprod Fertil, *71*(2). https://doi.org/10.1530/jrf.0.0710493

**SCULLY, S., EVANS, A. C., DUFFY, P., and CROWE, M. A., 2014.** Characterization of follicle and CL development in beef heifers using high resolution three-dimensional ultrasonography. Theriogenology, *81*(3), 407-418. https://doi.org/10.1016/j.theriogenology.2013.10.012

**SHAH, K. B., TRIPATHY, S., SUGANTHI, H., and RUDRAIAH, M., 2014.** Profiling of luteal transcriptome during prostaglandin F2-alpha treatment in buffalo cows: analysis of signaling pathways associated with luteolysis. PLoS One, *9*(8), e104127. https://doi.org/10.1371/journal.pone.0104127

**SHARIFUDDIN, W., and JAINUDEEN, M., 1983.** The accuracy of rectal diagnosis of corpora lutea in water buffalo (Bubalus bubalis). Animal Reproduction Science, *6*(3), 185-189. https://doi.org/10.1016/0378-4320(83)90036-2

**SIDDIQUI, M., ALMAMUN, M., and GINTHER, O., 2009.** Blood flow in the wall of the preovulatory follicle and its relationship to pregnancy establishment in heifers. Animal Reproduction Science, *113*(1-4), 287-292. https://doi.org/10.1016/j.anireprosci.2008.07.008

**SILVA, L., GASTAL, E., GASTAL, M., BEG, M., and GINTHER, O., 2018.** Relationship between vascularity of the preovulatory follicle and establishment of pregnancy in mares. Animal Reproduction (AR), *3*(3), 339-346. https://www.animal-reproduction.org/journal/animreprod/article/5b5a6080f7783717068b47cd

**SINGH, I., and BALHARA, A., 2016.** New approaches in buffalo artificial insemination programs with special reference to India. Theriogenology, *86*(1), 194-199. https://doi.org/10.1016/j.theriogenology.2016.04.031

**SIQUEIRA, L. G. B., TORRES, C. A., AMORIM, L. S., SOUZA, E. D., CAMARGO, L. S. A.,**

**FERNANDES, C. A., and VIANA, J. H. M., 2009.** Interrelationships among morphology, echotexture, and function of the bovine corpus luteum during the estrous cycle. Animal Reproduction Science, *115*(1-4), 18-28. https://doi.org/10.1016/j.anireprosci.2008.11.009

**TENHAGEN, B.-A., DRILLICH, M., SURHOLT, R., and HEUWIESER, W., 2004.** Comparison of timed AI after synchronized ovulation to AI at estrus : Reproductive and economic considerations. Journal of dairy science, *87*(1), 85-94. https://doi.org/10.3168/jds.S0022-0302(04)73145-8

**VARUGHESE, E., BRAR, P., HONPARKHE, M., and GHUMAN, S., 2014.** Correlation of blood flow of the preovulatory follicle to its diameter and endocrine profile in dairy buffalo. Reproduction in Domestic Animals, *49*(1), 140-144. https://doi.org/10.1111/rda.12242

**VASCONCELOS, J., SARTORI, R., OLIVEIRA, H., GUENTHER, J., and WILTBANK, M., 2001.** Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology, *56*(2), 307-314. https://doi.org/10.1016/S0093-691X(01)00565-9

**VRISMAN, D. P., BASTOS, N. M., ROSSI, G. F., RODRIGUES, N. N., BORGES, L. P. B., TAIRA, A. R., DE PAZ, C. C. P., DE PAULA NOGUEIRA, G., TEIXEIRA, P. P. M., and MONTEIRO, F. M., 2018.** Corpus luteum dynamics after ovulation induction with or without previous exposure to progesterone in prepubertal Nellore heifers. Theriogenology, *106*, 60-68. https://doi.org/10.1016/j.theriogenology.2017.10.018

**WAQAS, M., MEHMOOD, M., SHAHZAD, Q., KAUSAR, R., SATTAR, A., and NASEER, Z., 2016.** Comparative efficacy of G6G and Ovsynch protocols on synchronization and pregnancy rate in Nili–Ravi buffalo. Animal Reproduction Science, *166*, 9-14. https://doi.org/10.1016/j.anireprosci.2015.12.006

**YINDEE, M., TECHAKUMPHU, M., LOHACHIT, C., SIRIVAIDYAPONG, S., NA-CHIANGMAI, A., RODRIGUEZ-MARTINEZ, H., VAN DER WEYDEN, G., and COLENBRANDER, B., 2011.** Follicular dynamics and oestrous detection in Thai postpartum swamp buffaloes (Bubalus bubalis). Reproduction in Domestic Animals, *46*(1), e91-e96. https://doi.org/10.1111/j.1439-0531.2010.01647.x

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