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EFFECT OF DOUBLE OR PRE-SYNCHRONIZATION OVSYNCH PROTOCOLS ON FOLLICULAR DYNAMICS, PROGESTERONE PROFILE AND FIRST SERVICE PREGNANCY RATE IN TIMED ARTIFICIAL INSEMINATION IN FRIESIAN COWS

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ABSTRACT

Aim of this study was to determine the effect of double-Ovsynch (DO) and pre-synchronization (PS) protocols on ovarian follicular dynamics, progesterone (P4) concentration and pregnancy rate at first postpartum AI in Friesian cows. Total of 44 lactating cows on 39-45 day post-partum (12 primiparous and 32 multiparous cows) were used in this study. Cows were divided into two similar groups (22 cows in each). Cows in G1 were pre-synchronized by two PGF2α (Estrumate) injections at 14 d interval (on days 42 and 56±3 post-partum), then initiate the Ovsynch-timed AI protocol after 12 days. Cows in G2 were injected on day 0 with 1st dose of GnRH (Receptal) on day 51±3 post-partum, with PGF2α on day 7 and 2nd dose of GnRH after 72 h, then initiate the Ovsynch-timed AI protocol after 7 days. Cows in both groups were treated with the same Ovsynch protocol: 1st GnRH on days 68±3 post-partum, PGF2α after 7 days, 2nd GnRH 56 h after PGF2α, and timed AI after 16 h. Cows were subjected to ultrasonography device to examine follicular number (FN) and diameter of dominant follicles (DF) as well as counting the number of corpora lutea (NCLs). Blood samples were collected to determine P4 in blood serum. Results showed that FN or NCLs/ovary on day 0 or during pre-synchronization treatment was not affected significantly by treatment. FN/ovary at 2nd GnRH of Ovsynch-breeding protocol was greater in G1 than in G2 (1.6 vs. 2.0 foll./ovary). However, NCLs and DF diameter during Ovsynch-breeding in G1 and G2 were not affected significantly by treatment. Concentration of P4 in blood serum of cows on days 0, pre-synch, 1st GnRH and PGF2 injections was not affected significantly by treatment. On day of 2nd GnRH injection, P4 concentration was higher ($P<0.01$) in G2 than in G1. Concentration of P4 was slightly higher ($P>0.05$) in pregnant than in non-pregnant cows during pre-synch protocol. Progesterone concentration on day 75 showed an opposite trend, being lower ($P<0.05$) in pregnant than in non-pregnant cows. Ovulation rate to 1st GnRH injection was insignificantly higher in G2 (88.9%) than in G1 (82.4%). While, pregnancy rate based on total number of treated and ovulated cows was insignificantly higher in G1 (45.5 and 71.4%) than in G2 (36.4 and 50.0%), respectively. Higher pregnancy rates was in association with higher P4 concentration (>3 ng/ml) on day 0 and at 1st GnRH of Ovsynch breeding protocol and lower P4 concentration (<1 ng/ml) at 2nd GnRH. Thus, pre-synchronization with Presynch-Ovsynch increased cyclicity and fertility of Frisian cows at first service postpartum in Frisian cows. However, Double-Ovsynch gave higher ovulation rate than presynch-Ovsynch.

Keywords*: Bovine, Ovsynch, pre-synchronization, pregnancy, progesterone.*

INTRODUCTION

Reproductive efficiency in lactating dairy cattle isn't optimal, encouraging the development of more different programs of reproductive management focused on developing the use of artificial insemination and conception rates in dairy herds (Thatcher *et al.,* 2006). A long standing objective of reproductive physiologists was to improve a program of hormonal synchronization that could avoid the problems and limitations related to detection of heat in dairy cows. This objective was started in 1995 with the publication of a hormonal synchronization program that combined PGF2α and GnRH to control ovarian physiology and is now usually referred to as the Ovsynch program (Pursley *et al.,* 1995).

The Ovsynch program synchronizes ovulation, luteal regression and follicular development as artificial insemination can be operated at fixed-time without the need for heat detection, usually referred to a fixed-timed artificial insemination. Pre-synchronization makes it possible to onset the Ovsynch program in many animals during early diestrus, which is the optimal stage in lactating dairy cattle (Vasconcelos *et al.,* 1999). In addition, cattle that ovulate to GnRH have shorter intervals of dominance, which refers to help with oocyte quality (ovulation of an earlier oocyte) (Cerri *et al.,* 2009). However, a mature corpus luteum induced with a pre-synchronization program assures higher circulating progesterone during follicle development, which also refers to be positive in regard to quality of oocyte, thus developing fertility (Bisinotto *et al.,* 2010; Santos *et al.,* 2010).

Aim of this study was to determine the effect of double-Ovsynch and pre-synchronization on ovarian follicular dynamics, progesterone concentration and pregnancy rate at first postpartum AI in Friesian cows.

MATERIALS AND METHODS

This study was conducted at Sakha Animal Production Research Station, belonging to Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation in co-operation with Animal Production Department, Faculty of Agriculture, Mansoura University during the period from February 2013 to March 2014.

Animals:

Total of 44 Friesian cows on days from 39 to 45 post-partum, included 12 primiparous and 32 multiparous cows) were used in this study. Animals were milked two times daily at approximately 7 a.m. and 5 p.m. Cows were chosen from Sakha herd and divided into two similar experimental groups (22 animals in each). Cows were subjected to clinical examination of the ovaries and reproductive tract before treatments. Each animal was subjected to rectal palpation to exclude any abnormalities of reproductive organs before starting

the experiment. Cows in all experimental groups were subjected to the same managerial and feeding conditions.

Experimental design:

Cows in the 1st group (G1, n=22) were treated with pre-synchronization Ovsynch protocol (Diagram 1), while cows in the 2nd group (G2, n=22) were treated with double Ovsynch protocol (Diagram 1).

Cows in G1 were pre-synchronized by injection of two administrations of PGF2α (Estrumate, containing 263 µg Cloprostenol Sodium BP (Vet) equivalent to 250µg Cloprostenol; Friesoythe, Germany) at 14 d interval (on day 42±3 and day 56±3 post-partum), then initiate the Ovsynch-timed AI protocol after 12 days.

Cows in G2 were injected on day 0 with 1st dose of GnRH (Receptal, 1ml of solution for injection contains; Buserelin acetate 4.2 Mg, corresponding to 4.0 Mg buserelin, Intervet International B.V., European Union. EU) on day 51±3 post-partum. Cows were injected with PGF2α (Estrumate) on day 7 and 2nd dose of GnRH after 72 hours, then initiate the Ovsynch-timed AI protocol after 7 days.

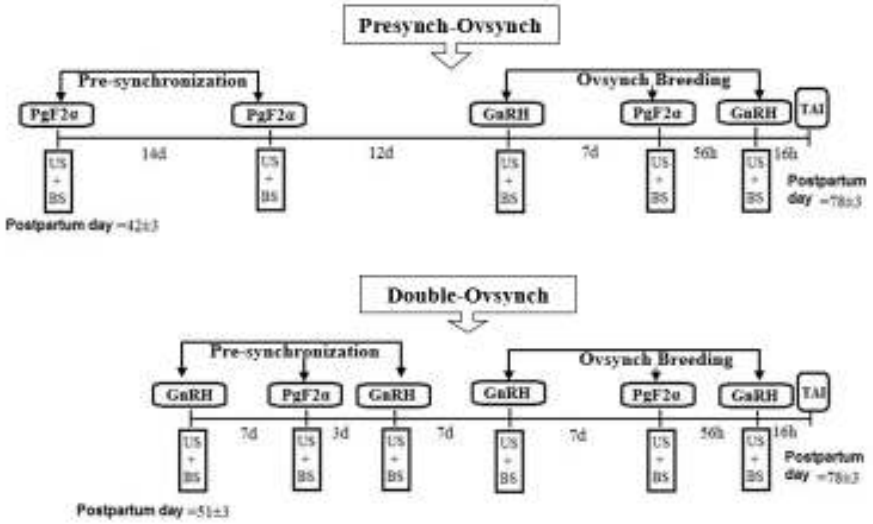

Diagram 1: Hormonal treatments, ultrasonographic exams (US), and blood samples (BS) of cows in experimental groups during post-partum.

Cows in both groups (G1 and G2) were treated with the same Ovsynch protocol: 1st-GnRH on days 68±3 post-partum, PGF2α after 7 days, 2nd-GnRH 56 hours after PGF2α, and timed AI after 16 h.

Ovarian ultrasonography and ovulatory responses:

Cows in both groups were subjected to ultrasonography device (ESAOTE Pie Medical Aquila Pro Vet + Probe 6.0/8.0 Mhz LA Rectal Veterinary Transducer) during treatment period (from the beginning treatment to TAI) to examine the ovaries in term of number and diameter of follicles as well as counting the number of corpora lutea (CLs) on the ovarian surface, then ovulation rate (OR%) was defined as the presence of a follicle at 1st-GnRH and presence of a new or an additional CL in the same location 7 d later on the day of PGF2α. Ultrasonography examination was done on post-partum days 42 (pre-treatment), 56 (pre-Ovsynch), 68 (day of 1st GnRH), 75 (day of PGF2 α) and 77 (day of 2nd GnRH) in G1 and on post-partum days 51 (pre-treatment), 58 (pre-Ovsynch), 68 (day of 1st GnRH), 75 (day of PGF2 α) and 77 (day of 2nd GnRH) in G2.

Blood collection:

Blood samples were collected from jugular vein of all cows in each group into clean dried centrifuge tubes. Samples were cooled for 2 hours at 5 °C, and then centrifuged at 3000 rpm for 20 minutes. Serum was separated into labeled glass tubes and stored at -20°C until assay of progesterone (P4) hormone. Blood samples were collected on the same days of ultrasonography examination in G1 and G2.

Statistical analysis:

Data were statistically analyzed using program of SAS (2004). Differences among means were set at $P<0.05$ using Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Ovarian structure:

Results presented in Table 1 showed that number of follicles or CLs/ovary on day 0 or during pre-synchronization treatment was not affected significantly by hormonal treatment. However, number of follicles/ovary at 2nd GnRH of Ovsynch-breeding protocol was affected significantly ($P<0.05$) by pre-synchronization treatment, being greater in G1 (P-P) than in G2 (G-P-G) (1.6 vs. 2.0 foll./ovary). However, number of CLs or dominant follicle (DF) diameter during Ovsynch-breeding protocol in G1 and G2 were not affected by pre-synchronization treatment.

The follicles observed on ovaries of cows in G1 and G2 on day 0 may indicate slight differences in reproductive status (follicular phase) of cows in both groups before starting the treatment, because treatments in G1 and G2 were initiated without any knowledge of the previous estrous cycle stage.

It has been reported that GnRH is not always successful in producing a new follicular wave in cows (Barros *et al.,* 2000; Martinez *et al.,* 1999) and that in these cows dominant follicles present on administration day continue their development and age until the last GnRH administration day, and hence

some of these aged follicles lose their ovulation or fertilization capacity (Moreira *et al.,* 2000). In the present study, diameter of DF was similar post-Ovsynch-breeding protocol regardless pre-synchronization treatment with P-P (G1) or G-P-G (G2). In this respect, Ayres *et al.* (2013) found that the size of the dominant follicle at the final GnRH treatment tended to increase in Double-Ovsynch compared with Presynch-Ovsych. A size of approximately 16 mm might be close to the optimal size for fertility (Souza *et al.,* 2007). In the present study, pre-synchronization treatment did not affect diameter of DF in a new follicular wave in G1 or G2. Such results indicated slight effect of pre-synchronization treatment only on increasing follicular number using double PGF2 before Ovsynch-breeding protocol.

Table 1: Overall mean and standard error of follicular number, CL number/ovary and dominant follicle diameter (cm) at different treatment days in the experimental groups.

Item	G1	G2	P-Value
Pre-synchronization:	(P - P)	(G–P-G)	
Follicular number pre-treatment	1.9±0.18	2.4±0.20	0.5991
CL number pre-treatment	0.3±0.08	0.2±0.08	0.3857
Follicular number pre-Ovsynch	1.9±0.19	1.7±0.11	0.0506
CL number pre-Ovsynch	0.6±0.08	0.3±0.06	0.1314
Ovsynch:	(G–P-G)	(G–P-G)	
CL number post- 1st GnRH	0.4±0.08	0.3±0.07	0.1488
Follicular number post-1st GnRH	1.9±0.19	1.6±0.16	0.1181
Diameter of DF post- 1st GnRH	1.2±0.07	1.4±0.06	0.6289
CL number post- PGF2α	0.7±0.08	0.6±0.08	0.6142
Follicular number post 2nd GnRH	2.0±0.20[a]	1.6±0.15[b]	0.0382
Diameter of DF post- 2nd GnRH	1.4±0.05	1.4±0.06	0.6786

[a and b]: **Means within the same row with different superscripts are significantly different at P<0.05. DF: Dominant follicles.**

Progesterone concentration (ng/ml):

Data in Table (2) revealed that P4 concentration in blood serum of cows on days 0, pre-Ovsynch, 1st GnRH and PGF2 injections was not affected significantly by pre-synchronization treatment. However, on day of 2nd GnRH injection, P4 concentration was significantly (P<0.01) higher in G2 than in G1. It seems likely that the double Ovsynch protocol insignificantly increased circulating P4 concentrations prior to administration of PGF2α. Indeed, Ayres *et al.* (2013) reported that pre-synchronization with double Ovsynch protocol increased the circulating P4 at PGF2α of Ovsynch compared to pre-synchronization one.

The observed similarity in P4 level at the time of PGF2α in G1 and G2 is consistent with a similar synchrony of cows at Ovsynch initiation and/or a nearly similar ovulation to the first GnRH of Ovsynch. Cows with low P4 at the time of PGF2α are likely to have undergone premature luteolysis and can have an LH surge and ovulation prior to the final GnRH treatment

(Vasconcelos *et al.,* 1999). In accordance with the present results, Herlihy *et al.* (2012) found no difference in mean P4 concentrations between pre-synchronization and double Ovsynch protocols at 1st GnRH. These findings clearly indicated that treatment with an Ovsynch protocol before beginning Ovsynch increased P4 level at 1st GnRH compared with the pre-synchronization protocol (Table 2).

Progesterone concentration during pre-synchronization treatment significantly (P<0.01) affected pregnancy incidence (Table 2). Concentration of P4 must be higher during pre-Ovsynch breeding protocol to increase the possibility of pregnancy. In the current study, P4 level was slightly higher (P>0.05) in G1 than in G2 pre-Ovsynch protocol. Progesterone concentration on day 75 showed an opposite trend, being significantly (P<0.05) lower in pregnant than in non-pregnant cows (Table 2).

These results suggested that increasing P4 level prior to pre-Ovsynch breeding protocol and decreasing its level at PGF2 treatment may result in improving pregnancy rate of cows.

Table 2: Progesterone concentration (P4) at different treatment days in the experimental groups.

Item	Pre-synchronization		Ovsynch		
	Pre-treatment	Pre-Ovsynch	D 68 (1st GnRH)	D75 (PGF2α)	D 77 (2nd GnRH)
Experimental group:					
G1	3.17±0.70	3.99±1.10	3.52±0.66	5.35±1.43	1.23±0.15^{B}
G2	4.64±1.02	3.65±1.19	4.15±0.68	5.27±0.88	1.79±0.38^{A}
Significance	NS	NS	NS	NS	P<0.01
Pregnancy status:					
Pregnant	3.84±0.80	5.60±1.66^{a}	4.37±0.67	5.01±1.16^{b}	1.55±0.41
Non-preg.	3.95±0.91	2.59±0.65^{b}	3.46±0.64	5.51±1.16^{a}	1.48±0.22
Significance	NS	P<0.01	NS	P<0.05	NS

^{a} and ^{b}: Means within the same column for each factor with different superscripts are significantly different at P<0.05.

Ovulation and pregnancy rates (%):

Results presented in Table (3) revealed that ovulation rate to 1st GnRH injection was insignificantly higher in G2 (88.9%) than in G1 (82.4%). While, pregnancy rate based on total number of treated cows or ovulated cows was insignificantly higher in G1 (45.5 and 71.4%) than in G2 (36.4 and 50.0%), respectively. Similarly, Dirandeh *et al.* (2014) found that cows in the double-ovsynch protocol had a greater ovulation rate to the first GnRH, but also had greater than presynch-Ovsynch protocol, which was in disagreement with the present results. On the other hand, Herlihy *et al.* (2012) reported that a double Ovsynch protocol was effective at inducing cyclicity in most anovular cows and improved pregnancy at the first postpartum service compared to pre-synchronization protocol (46.3 vs. 38.2%).

It is suggested that GnRH-induced turnover of follicles or induction of a new follicular wave will be most efficient if ovulation is induced in response to the first administration of GnRH (Thatcher *et al.*, 1993), and resetting follicular development could produce a new dominant follicle that contains an oocyte of

greater potential fertility (Mihm *et al.,* 1994). The cows ovulating after the first GnRH treatment of Ovsynch have higher rates of pregnancy than those of non-responders (Cirit *et al.,* 2007; Bello *et al.,* 2006).

In accordance with the present results, several authors found that the presynch-Ovsynch protocol increased fertility, as compared with the Ovsynch protocol alone (Moreira *et al.,* 2001; Navanukraw *et al.,* 2004; El-Zarkouny *et al.,* 2004; Galvao and Santos 2010).

Table 3. Ovulation and pregnancy rates of cows in the experimental groups.

	Treated cows	Responded cows		Ovulated cows		Pregnancy		
		n	%(1)	n	%(2)	n	%(3)	%(4)
G1	22	17	77.3	14	82.4	10	45.5	71.4
G2	22	18	81.8	16	88.9	8	36.4	50.0

(1): Based on presence of dominant follicles after 1st GnRH of breeding Ovsynch.
(2): Based on number of dominant follicles and CLs after 1st GnRH of breeding Ovsynch.
(3): Based on total number of treated cows following first timed artificial insemination.
(4): Based on number of ovulated cows following first timed artificial insemination.

Pregnancy outcome to Ovsynch was highly associated with the ovulatory response to initial GnRH treatment (Bello *et al.,* 2006). Similarly, Chebel *et al.* (2006) reported that pregnancy rate was 10.0% greater in cows responding to first GnRH compared to those that did not respond. Similar findings were recently reported by Dirandeh *et al.* (2014). In the present study, the higher pregnancy rate occurred in G1 in reversible situation to that ovulated to first GnRH injection of Ovsynch breeding protocol. The opposite was observed in G2. Therefore, the greater pregnancy rate obtained in cows in the pre-synchronization protocol may be attributed to a better fertilization rate in response to treatment of G1.

A lack of follicle turnover due to failure to respond to the initial GnRH administration might compromise the quality of embryos and consequently reduce pregnancy (Chebel *et al.,* 2006). Also, Dirandeh *et al.* (2014) found that the difference in pregnancy rate by ovulatory response to first GnRH depended on the timed-AI protocol. In this regard, the difference in pregnancy rate between cows that ovulated versus those that did not ovulate was similar to that reported by Chebel *et al.* (2006) and was significantly different in cows subjected to double-Ovsynch, but smaller and not significant in cows subjected to pre-synch Ovsynch.

Relationship between P4 concentration and pregnancy rate:

Results in Table 4 show higher pregnancy rates in association with higher P4 concentration (>3 ng/ml) on day 0, at 1st GnRH of Ovsynch breeding protocol and lower P4 concentration (<1 ng/ml) at 2nd GnRH.

The present results are in agreement with several authors (Chebel *et al.,* 2006; Stevenson and Pulley 2012). Also, Herlihy *et al.* (2012) found that improved fertility was observed with the greater proportion of cows with elevated P4 concentrations at the time of PGF2α treatment during double

Ovsynch protocol, as reported previously (Souza *et al.,* 2008; Wiltbank *et al.,* 2011). Moreover, Ayres *et al.* (2013) reported that pre-synchronization with double Ovsynch compared to pre-synchronization protocols increased the percentage of cows with high (>3.0 ng/ml) circulating P4 at PGF2α of Ovsynch (88.0 vs 76.3%).

Table 4. Pregnancy rate according to progesterone concentration (>3, 1-3 and <1 ng/ml) on days 0, 1st GnRH and 2nd GnRH injections.

Item	Pregnant cows	Pregnancy based on P4 level					
		High (>3 ng/ml)		Moderate (1-3 ng/ml)		Low P4 (<1 ng/ml)	
		n	%	n	%	n	%
Day 0:							
G 1	10	4	40.0	3	30.0	3	30.0
G 2	8	4	50.0	3	37.5	1	12.5
Total	18	8	44.5	6	33.3	4	22.2
On day 68 (1st GnRH of breeding Ovsynch):							
G 1	10	5	50.0	3	30.0	2	20.0
G 2	8	5	62.5	3	37.5	0	0
Total	18	10	55.6	6	33.3	2	11.1
On day 77 (2nd GnRH of breeding Ovsynch):							
G 1	10	0	0	5	50.0	5	50.0
G 2	8	1	12.5	2	25.0	5	62.5
Total	18	1	5.6	7	38.9	10	55.5

The reduction in percentage of cows with low P4 (<0.50 ng/ml) and the greater percentage of cows with high P4 (>3 ng/ ml) at the time of PGF2α were consistent with a greater synchrony of cows at Ovsynch initiation and/or a greater percentage ovulating to the first GnRH of Ovsynch. Cows with low P4 at the time of PGF2α are likely to have undergone premature luteolysis and can have an LH surge and ovulation prior to the final GnRH treatment (Vasconcelos *et al.,* 1999).

Generally, greater P4 concentrations during growth of the dominant follicle in a double Ovsynch protocol resulted in smaller ovulatory follicle size (Brusveen *et al.,* 2009) and a greater percentage of grade 1 embryos recovered on d 7 to 8 after AI (Wiltbank *et al.,* 2011). The observed increase in P4 level at 1st GnRH injection of Ovsynch breeding protocol was associated with decreased LH pulsatility and improved oocyte competence (Mihm *et al.,* 1994). Conversely, lower progesterone concentrations at initiation of Ovsynch in pre-synchronized cows resulted in lower pregnancy rate after first postpartum AI (Wiltbank et al. 2012).

Result concluded that pre-synchronization with Double-Ovsynch gave higher ovulation rate than presynch-Ovsynch. However, Presynch-Ovsynch increased cyclicity and fertility of Frisian cows at first service postpartum.

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تأثير بروتوكول تنظيم التبويض المزدوج و البروتوكول التمهيدى لبروتوكول تنظيم التبويض علي ديناميكية التطور الحويصلي و تركيز البروجيستيرون و معدل الحمل من أول تلقيحه بعد الولادة في أبقار الفريزيان.

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الهدف من هذه الدراسه هو تحديد تأثير بروتوكول تنظيم التبويض المزدوج و البروتوكول التمهيدى لبروتوكول تنظيم التبويض علي كلا من :ديناميكية التطور الحويصلى – تركيز البروجيستيرون – معدل الحمل من أول تلقيحه بعد الولادة فى أبقار الفريزيان. أستخدمت فى هذه الدراسه ٤٤ بقرة حلابة من اليوم ٣٩ الى اليوم ٤٥ بعد الولادة (١٢ بقرة موسم أول – ٣٢ بقرة متعدده المواسم). تم تقسيم الأبقار إلى مجموعتين متماثلتين (كل مجموعه ٢٢ بقرة). فى المجموعه الأولى تم حقن الأبقار بحقنتين بروستاجلاندين (PGF2α) بينهم ١٤ يوم فى يومى (٤٢ و ٥٦ ± ٣ بعد الولادة) وبعد ١٢ يوم من الحقنه الثانيه للبروستاجلاندين يبدأ بروتوكول الـ (Ovsynch Timed AI). فى المجموعه الثانيه يتم حقن الأبقار بأول جرعه من (GnRH) فى اليوم (٥١ ± ٣ بعد الولاده) وبعد ٧ أيام يتم حقن البروستاجلاندين ثم بعد ٧٢ ساعه يتم حقن الجرعه الثانيه من (GnRH) وبعدها بـ ٧ أيام يبدأ بروتوكول (Ovsynch Timed AI). تمت معاملة الأبقار فى كلا المجموعتين بنفس بروتوكول الـ (Ovsynch Timed AI) : حقنة الـ (GnRH) الأولى فى اليوم (٦٨ ± ٣ بعد الولاده), بعدها بـ ٧ أيام يتم حقن البروستاجلاندين, وبعد ٥٦ ساعه يتم حقن الحقنه الثانيه للـ (GnRH) ثم يتم التلقيح بعد ١٢ ساعه. تم فحص الأبقار بجهاز الموجات فوق الصوتيه لتحديد عدد الحويصلات المبيضيه وقياس قطر الحويصلات السائده وكذلك تحديد عدد الأجسام الصفراء. تم جمع عينات الدم لقياس تركيز البروجستيرون فى سيرم الدم. أظهرت النتائج أن عدد كل من الحويصلات والأجسام الصفراء لكل مبيض فى الأيام (صفر و أثناء معاملات الـ Presynchronization) لم تتأثر معنويا بالمعامله. عدد الحويصلات/مبيض فى يوم حقنة (GnRH) الثانيه كان أعلى فى المجموعة الأولى من المجموعة الثانية (٢ مقابل ١.٦ حويصله/مبيض). ومع ذلك عدد الأجسام الصفراء وقطر الحويصله السائده لم تتأثر معنويا بالمعامله أثناء بروتوكول (Ovsynch Timed AI). تركيز البروجستيرون فى سيرم دم الأبقار فى الأيام(صفر و أثناء Pre-synchronization و حقنة الـ GnRH الأولى و حقنة البروستاجلاندين) لم تتأثر معنويا بالمعامله . فى يوم حقنه الـ GnRH الثانيه كان تركيز البروجستيرون أعلى فى المجموعه الأولى من المجموعه الثاني (P>0.01). تركيز البروجستيرون كان أعلى نسبيا بشكل معنوى فى الأبقار العشار عن الأبقار الغير عشار (P>0.05) أثناء معاملات الـ تركيز البروجستيرون فى اليوم ٧٥ بعد الولادة أظهر إتجاه معاكس حيث كان Presynchronization. أقل معنويا فى الأبقار العشار عن الأبقار الغير عشار (P>0.05). معدل التبويض لحقنه الـ GnRH الأولى كان أعلى بشكل غير معنوى فى المجموعة الثانيه (٨٨.٩%) عن المجموعة الأولى (٨٢.٤). بينما كان معدل الحمل على أساس العدد الكلى للأبقار المعاملة و الأبقار التى حدث لها تبويض أعلى بشكل غير معنوى فى المجموعة الأولى (٤٥.٥ – ٧١.٤%) عن المجموعة الثانيه (٣٦.٤ – ٥٠%) ,على التوالى. معدلات الحمل المرتفعة كانت مرتبطة بتركيز البروجستيرون العالى (3 ng/ml<) فى الأيام (صفر – حقنة الـ GnRH الأولى) و كانت مرتبطة بتركيز البروجستيرون المنخفض (1 ng/ml<) فى يوم حقنة الـ GnRH الثانية. وبالتالى فإن بروتوكول تنظيم التبويض المزدوج أعطى معدل تبويض أعلى من البروتوكول التمهيدى لبروتوكول تنظيم التبويض. ومع ذلك, البروتوكول التمهيدى لبروتوكول تنظيم التبويض أدى إلى زيادة النشاط المبيضى و الخصوبه من أول تلقيحه بعد الولادة فى أبقار الفريزيان.