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# Effect of Using Low Doses of $PGF_2\alpha$ and GnRH Hormones on Reproduction of Dairy Cows

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THE research aims to improve reproductive performance of repeat breeder or anestrous cows using PGF2 α and GnRH through in intra vulva injection. In this study, 30 Friesian dairy cows were not conceiving for a period ranging from 120 to 250 days after calving. Cows divided into three groups, 10 cows in each group and were subjected to the following protocols. Group1 (G1): was injected with 50 µg GnRH on Day 0 and 5 mg PGF2α on Day 7 then the oestrus cycle was monitored. Group 2 (G2): cows were injected twice 5 mg PGF2α, 11 days apart. Group 3 (G3): was injected with 50 µg GnRH at Day 0, 5 mg PGF2α, at Day 7 and then injected with 50 µg GnRH at day 9 and fixed timed artificial insemination (TAI) was performed 16 to 18 h. The results showed that, exhibited oestrus 100, 90 and 80% in G3, G2 and G1, respectively. Pregnancy rates were significantly highest in G3 and G2 than in G1. The progesterone (P4) concentration on day 24 post-insemination was 10.253, 11.235 and 11.567 ng/ml in pregnant versus were 3.245, 1.985 and 2.036 ng/ml in non- pregnant cows in G1, G2 and G3, respectively. Progesterone concentration at $2^{nd}$ GnRH or $PGF_2\alpha$ injections were significantly lower in pregnant cows than in non-pregnant cows in G1, G2 and G3 groups. The economic evaluation was shown that 2 $PGF_{2\alpha}$ protocols had the cheapest cost (L.E 21/animal) highest pregnant cows.

**Keywords:** Friesian cows, reproductive performance, PGF2, GnRH, progesterone

## Introduction

Dairy herd profitability heavily influenced by reproductive success. Several variables, including oestrus detection efficiency, day’s open and calving interval, discovered to have an impact on reproductive success [1].

Regular dairy herd monitoring in the context of reproduction management is the key to evaluating the dairy herd's reproduction performance, which is a single index that offers an overall evaluation of the reproductive and takes into consideration many different factors. In all systems, reproductive performance is an increased priority, but in seasonal calving systems, it is even greater because to ensure that each cow has a calf every year, the possibility for a cow to calve and get pregnant is restricted [2]. Ovulation synchronisation is a common reproductive control strategy in the dairy industry, and hormones play an essential role in cattle reproduction [3].

The GnRH treatment after calving period was improved reproductive performance in cows with an abnormal before calving and perhaps reduced the number of days to first estrus, but had no effect on future reproductive performance [4]. hese treatments improved estrus response [5, 6], and on the other hand, GnRH-treated animals had a lower pregnancy rate, as well as embryonic and foetal losses [7].

In Iran, Honaramooz and Fazelie [8] reported that in comparison with intramuscularly (IM) injection, the intra-vulval-vaginal submucosal route of administration permitted markedly lower doses (25% of IM dose) of prostaglandin to be used in estrus synchronization.

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When compared to cyclic cows, the Ovsynch (GnRH on day 0, PGF2 on day 7, GnRH on day 9, and insemination on day 10) has shown to function less well in anovular cows. Numerous Ovsynch modifications have suggested as a result, including starting the Ovsynch with PGF2 plus GnRH and switching to PGF2 14 days later.

The research aims to improve reproductive performance of repeat breeder or anestrous cows with PGF2 α and GnRH in intra vulva injection with low doses of hormones.

## Material and Methods

The current study was conducted between November 2020 and March 2021 at the Sakha Station of Animal Production Researches, which is a part of the Animal Production Research Institute, Agricultural Research Centre, Ministry of Agriculture, in collaboration with the Animal Production Department, Faculty of Agriculture, KafrelShakh University.

*Experimental cows:*

A total of 30 Friesian dairy cows were used in this study. All cows were apparently considered, since they did not conceive for a period ranging from 120 to 250 days post-partum. When the experimental work first began, cows were 3-5 years old, 440 to 640 kg live body weight (LBW), 2.5 to 3.5 body conditions score (BCS) and were between first to 5$^{th}$ parity.

*Management and feeding*

At 7 a.m. and 5 p.m. every day, all cows were mechanically milked. All cows were kept untied outside of milking time in a partially open, shaded yard. Throughout the experiment, all animals were fed in groups based on their body weight, milk production, and reproductive status. The experimental cows were fed a concentrate feed mixture (CFM), maize silage (MS), berseem hay, and rice straw from March to the end of December. Animals were fed fresh berseem from the months of January to the end of April. At around 8:00 a.m. and 3:00 p.m. each day, the concentrate feed mixture was provided, while rice straw was accessible all day. All the cows had access to the water three times per day. *Experimental design*

The study excluded cows with uterine-ovarian adhesions, metritis, pyometra, and other reproductive disorders. Animals (n = 30) were randomly divided into by parity and calving date three groups which were subjected to one of the following treatment protocols.

*Group1 (G1): SelectSynch protocol*

Ten cows were injection with 50 µg GnRH (Receptal IM on Day 0) and 5 mg PGF2α, (inter vulva submucosal, 0.4 ml of Cloprostenol) (Estrumate, Synthetic prostaglandin Jorox, Australian) on Day 7 and showing estrus cycle and inseminations within 72 and 96 hours (Figure 1).

*Group 2 (G2): PGF2α - PGF2α:*

Ten postpartum lactating cows in estrus were injected twice with 5 mg PGF2α, (inter vulva submucosal, 0.4 ml of Cloprostenol, (Estrumate, Synthetic prostaglandin Jorox, Australian) 11 days apart. artificial insemination, was done between 72 and 96 hours (Figure 2).

*Group 3 (G3): OvSynch protocol*

Animals of this group were subjected to OvSynch protocol in which Ten cows in estrus cycles were injected with 50 µg GnRH (Receptal i.m. on Day 0), 5 mg PGF2α, (inter vulva submucosal, 0.4 ml of Cloprostenol, (Estrumate, Synthetic prostaglandin Jorox, Australian) on Day 7 and then injected with 50 µg GnRH on day 9 and FTAI was performed 16-18 h after the second GnRH administration (Figure 3).

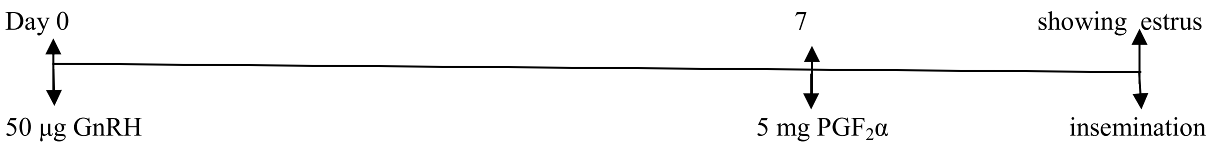

**Fig. 1. SelectSynch protocol**

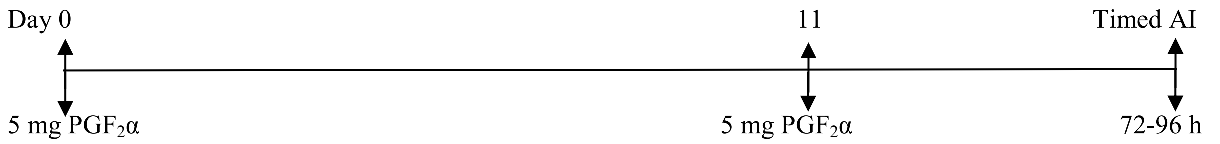


**Fig. 2. PGF$_2\alpha$ - PGF$_2\alpha$ protocol**

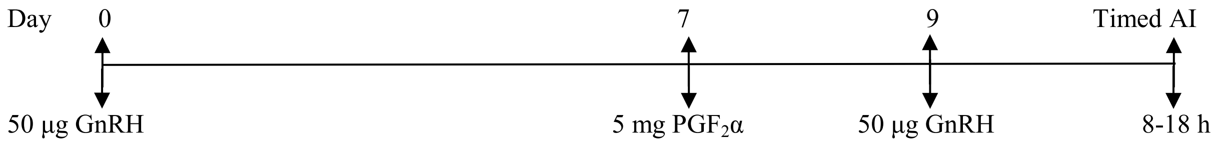


**Fig. 3. OvSynch protocol**

*Detection of estrus and insemination*

After the end of hormonal protocols cows returned to estrus were Animals of this group were subjected to OvSynch protocol in which signs of estrus 3 times daily for the end of all protocols using as a teaser bull for 30 min during each time. Animals observed in heat were AI by frozen semen.

About 60 days following artificial insemination, the presence of pregnancy was determined by rectal uterine palpation.

*Blood sampling*

Blood samples were taken from all animals in each group in the first experiment. Blood sample were collected from the jugular vein and were allowed to clot, centrifuged (3000 xg for 20 min), and serum was collected and stored at -20 c$^o$ until assayed for progesterone (P4) concentrations. Samples were collected once every week and at the 1$^{st}$ GnRH; 1$^{st}$ PGF2α; 2$^{nd}$ PGF2α; 2$^{nd}$ GnRH injection, 7, 14, and 24 days after AI.

*Progesterone assay*

The plasma P4 concentration was determined using a direct radioimmunoassay method (RIA) utilising diagnosis Systems Laboratories Texas, USA, ready antibody coated tubes kit by the manufacturer's instructions.

According to information from the manufacturer, the cross reaction of P4 antibody (at 50% binding) was 100% with P4, while it was 6.00, 2.50, 1.20, 0.80, 0.48, and 0.10% with 5-pregnone-3, 20-dione 11-Deoxycorticosterone, 17-Hydroxyprogesterone, 5-pregnone-3, 20-dione 11-Deoxycortisol, and 20α-Dihydroxy-P4, respectively and less than 0.1% while using additional steroids.

*Data recorded*

Friesian dairy cows were divided according to their conception rate, days open, postpartum cyclic and acyclic cows.

*Statistical analysis*

The statistical analysis of data obtained was performed by S.A.S® statistical software [9]. Prior to chi square analysis, the arcsine of the square root of some variable was employed to convert all percentages. The one-way ANOVA analysis was performed to analyze the effect of different hormonal protocols and levels alone. The following model was used:

$Yij = M + Ti + eij$

Where: Yij = observed values. M= Means Ti = effect of hormonal protocols. eij = the stander error.

Multiple range tests were used to determine the significant differences among mean [10].

## Results and Discussion

*Reproductive traits:*

Result in Table (1) showed oestrus rates as affected by different four hormone protocols. Results cleared that all cows treated with OvSynch (G3) exhibited oestrus with rate of 100% followed by cows treated with 2 PGF2α protocol (G2, 90%) while the cows injected with Select Synch protocol (G1) gave the lowest value (80%). Concerning the effect of different hormonal protocols on pregnancy rate in treated animals our results informed that, 5, 7 and 8 of treated cows were pregnant with a rate of 50%, 70% and 80% in groups 1, 2 and 3, respectively (Table 1), with a significant highest conception rate in group 3 than in group 1.

In the absence of the corpus luteum, exogenous PGF2 may have improves on the uterus during the post calving period, and therapy with PGF2α on 15 days post calving reduced NS/C considerably [11]. Liu *et al*. [12] found that an s.c. GnRH implant on 14 days post calving triggered ovulation in 100 percent of nursing Holstein cows due to its capacity to release luteinizing hormone (LH). Following full luteolysis caused by two modest doses of PGF2, GnRH treatment improved ovulation rate. As a result, it appears that GnRH's capacity to ovulation and CL function in post calving dairy cows are induced will be highest in cows that successfully limit the severity and length of negative energy balance issues [13]. Cows with the most negative energy balance and post-calving ovarian inactivity, on the other hand, would benefit the most from a prospective medication such as GnRH to induce ovulation and start the estrous cycle [14].

**TABLE 1. Evaluation of several hormonal treatment options for reproduction.**

| Item | Hormonal protocol | | |
|---|---|---|---|
| | **G1$^{(1)}$** | **G2$^{(2)}$** | **G3$^{(3)}$** |
| Animals (n) | 10 | 10 | 10 |
| Estrus exhibited (n) | 9 | 9 | 10 |
| Estrus rate (%) | 80 | 90 | 100 |
| Pregnant animals (n) | 5 | 7 | 8 |
| Pregnancy rate (%) | **50$^{b}$** | **70$^{ab}$** | **80$^{a}$** |

$^{(1)}$: SelectSynch, $^{(2)}$: $PGF_2\alpha$- $PGF_2\alpha$ and $^{(3)}$: OvSynch protocols.
$^{a\ and\ b}$: With different superscripts, the means of the same row differ significantly at (P<0.05).

The PGF2α promotes the production of LH in a variety of species, including estrous cyclic or anovular cows [15], and it may function as an ovulatory stimulant in pre pubertal heifers [16] and postpartum beef cows receiving timed AI [17]. Improved luteolysis by successive PGF2α dosages has been shown to enhance P/AI in dairy cows treated to timed AI procedures [18]. This reaction is most commonly seen in estrous cycle cows with a newly established CL. Recently; Pereira *et al.* [19] found that anovular dairy cows getting two consecutive PGF2α injections during a scheduled AI programmer had higher P/AI than those treated a single PGF2α injection. Overall, it's possible that pre-ovulatory PGF2α therapy enhances dairy cow fertility in addition to its direct effects on luteal regression. It needs to be seen if the putative processes are connected to gonadotropin release or other impacts on the follicle itself. Furthermore, because hyperthermia damages follicles and oocytes in dairy cattle, increases in fertility by altering the Fertility detection is more likely in pre-ovulatory follicles in cows not subjected to heat stress [20, 21].

*Progesterone concentration:*

The P4 concentrations in blood serum of Friesian cows (pregnant and non-pregnant) treated with different hormonal protocols are presented in Table 2.

The P4 concentration in pregnant Friesian cows treated with different hormonal protocols (G1, G2, and G3) was significantly (P<0.01) decreased at AI (oestrus) than in non-pregnant Friesian cows, while it was increased post-treatment 7-, 14-, and 24-days post-AI in pregnant Friesian cows than in non-pregnant Friesian cows. However, in all animals, P4 concentration was not significant during pre-treatment (Table 2).

The prevalence of pregnancy in pregnant cows was observed on day 24 post-insemination, with P4 concentrations in pregnant cows in groups G1, G2, and G3 being 10.253, 11.235, and 11.567 ng/ml, respectively, compared to 3.245, 1.985, and 2.036 ng/ml in non-pregnant cows. El-Moghazy [22] and Abu El-Hamd et al. [23] reported that P4 concentrations were almost higher than 1 ng/ml in pregnant and less than 1 ng/ml in those who were unable to conceive, which is comparable to the results of the current study. Additionally, Mee et al. [24] reported that repeat breeder cows' blood P4 levels were boosted by GnRH, which may have contributed to these cows' higher conception rates.

The current findings regarding the GnRH-GnRH protocol at insemination suggested better or similar results of treatment with PGF2-PGF2 protocols at the time of insemination by about 10%, which may indicate the primary issue in incidence of repeat breeder cases as a result of mistrusting the interval between the onset of oestrus, ovulation, and insemination. In this regard, it has been suggested by numerous writers that GnRH-induced ovulation may provide better synchronisation between insemination and ovulation [25].

According to results reported in Table 2, the concentration of P4 pre-treatment tended to be similar in pregnant and non-pregnant Friesian cows. This is in relation to P4 profile pre-, during, and post-treatment with GnRH-PGF2–GnRH (G2) and PGF2–PGF2– (G3) timed AI. First injections of GnRH in G1 and G2 or first injections of PGF2 in G2 and G3 did not significantly change the P4 concentration in either pregnant or non-pregnant Friesian cows.

Following PGF2 injection, both pregnant and non-pregnant cows saw a dramatic reduction in P4 and a regression of the functional CL. Table 2 shows that these values were not statistically different between pregnant and non-pregnant cows.

Pregnant cows in the G1, G2, and G3 groups had significantly lower progesterone concentrations at the second GnRH or PGF2 injections than non-pregnant cows (P 0.05).

Following post-secondary GnRH or PGF2 injections, dominant follicles may grow and ovulate within 24 hours, causing a rise in P4 concentrations of an average of 24 hours in comparison to animals only receiving a GnRH-PGF2 regimen [26, 27, 23]. The findings of this study demonstrated that the P4 concentration at estrus was considerably lower in pregnant women in G1, G2, and G3 (i.e., 0.21, 0.285, and 0.235 ng/ml, respectively) than in non-pregnant women (i.e., 1.214, 0.857, and 1.386 ng/ml, respectively) (Table 2). In this study, a favourable time for oestrus occurrence and, as a result, a good time for insemination and fertilisation, may be responsible for the high pregnancy rate that was observed.

**TABLE 2. Progesterone levels (ng/ml) in the treatment groups' pregnant and non-pregnant animals**

| Time | Items | Treatments | | |
|---|---|---|---|---|
| | | G1$^{(1)}$ | G2$^{(3)}$ | G3$^{(4)}$ |
| **Pre-treatments:-** | | | | |
| | Pregnant | 3.895±0.34 | 3.568±0.23 | 3.024±0.33 |
| | Non-pregnant | 3.548±0.37 | 3.028±0.46 | 2.857±0.25 |
| **During treatments** | | | | |
| **Day -14*** | Pregnant | - | 4.235±0.47$^{a}$ | 3.856±0.56$^{a}$ |
| | Non-pregnant | - | 3.254±0.54$^{a}$ | 3.025±0.44$^{a}$ |
| **Day -7** | Pregnant | 3.657±0.57$^{a}$ | 4.235±0.55$^{a}$ | 4.235±0.67$^{a}$ |
| | Non-pregnant | 3.457±0.51$^{a}$ | 3.147±0.48$^{a}$ | 3.567±0.36$^{a}$ |
| **Day -1** | Pregnant | 1.514±0.31$^{b}$ | 1.856±0.29$^{b}$ | 1.789±0.35$^{\mathbf{b}}$ |
| | Non-pregnant | 3.254±0.37$^{a}$ | 2.985±0.27$^{a}$ | 3.142±0.36$^{a}$ |
| **At oestrus** | Pregnant | 0.210±0.11$^{b}$ | 0.285±0.15$^{b}$ | 0.235±0.14$^{b}$ |
| | Non-pregnant | 1.214±0.26$^{a}$ | 0.857±0.34$^{a}$ | 1.386±0.25$^{a}$ |
| **Post-insemination** | | | | |
| **Day 7** | Pregnant | 8.598±0.65$^{a}$ | 9.235±0.8$^{a}$ | 10.245±0.4$^{a}$ |
| | Non-pregnant | 3.532±0.56$^{b}$ | 3.425±0.6$^{b}$ | 3.532±0.6$^{b}$ |
| **Day 14** | Pregnant | 9.568±0.43$^{a}$ | 10.356±0.5$^{a}$ | 10.685±0.2$^{a}$ |
| | Non-pregnant | 4.532±0.46$^{b}$ | 4.025±0.8$^{b}$ | 3.024±0.3$^{b}$ |
| **Day 24** | Pregnant | 10.253±0.67$^{a}$ | 11.235±0.4$^{a}$ | 11.568±0.6$^{a}$ |
| | Non-pregnant | 3.245±0.35$^{b}$ | 1.985±0.6$^{b}$ | 2.036±0.5$^{b}$ |

$^{(1)}$: SelectSynch, $^{(2)}$: $PGF_2\alpha$- $PGF_2\alpha$ and $^{(3)}$: OvSynch protocols. $^{a,\ b\ \text{and}\ c}$ With different superscripts, the means of the same row differ significantly at (P<0.05). * At first injection of GrRH and $PGF_2\alpha$ in G2 and G3 protocols (0 time),

Generally, pregnancy of cows was indicated by P4 profile on day 7, 14 and 24 post- oestrus, being significantly (P<0.001) increased (8.598 to 10.253 ng/ml in G1, 9.235 to 11.235 ng /ml in G2 and 10.245 to 11.568 ng /ml in G3, respectively) in pregnant than in non-pregnant cows in G1 3.253 to 4.532 ng/ml, in G2 1.985 to 4.025 ng/ml and in G3 .036 to 3.532 ng /ml, respectively Table 2. During the luteal phase, the pregnant animals' serum P4 concentrations ranged from 9 to 11.5 ng/ml, which was equivalent to the luteal concentrations from day 5 to day 24 of the estrous cycle [28]. GnRH would be administered 72, 48, and 24 hours after PGF2 in cows bearing small, medium, and large follicles at the time of initial treatment in all experimental cows by the end of the dominant follicle's growth phase or the start of its static phase when GnRH would be administered 72, 48, and 24 hours after PGF2 in cows bearing small, medium, and large follicles at the time of initial treatment.

In controlled breeding programmes, the GnRH–PG2–GnRH regimen appears to be beneficial in improving ovulation synchronization [23]. The major synchronizing impact, however, appears to be in the second GnRH injection, whereas the first is important for extending the luteal phase in animals treated late in the cycle [30]. Finally, 48 hours after PGF2, a second GnRH injection is given to produce a preovulatory LH surge that initiates ovulation within an 8-hour period, starting about 24 hours after the injection [31]. Furthermore, greater progesterone levels in early pregnancy are linked to embryonic development, increased interferon production, and higher conception rates [32].

*Comparison among protocols:*

From the reproductive point of view, 20 out of 30 treated Friesian cows (70%) were pregnant using all hormonal protocols, being the highest in OvSynch protocols (80%, moderate (70%) in 2 $PGF_{2\alpha}$ and the lowest (50%) in Select Synch protocols. Also, the economic evaluation indicated that 2 $PGF_{2\alpha}$ protocols had the cheapest cost (L.E 21/animal), followed by Select Synch had maturate cost (L.E 51.75/animal) but lower pregnant cows. However, OvSynch showed the highest cost (L.E 63/animal, Table 3) and highest pregnant cows (Table 3).

**Table 3. Evaluation of various hormonal therapies' effects on reproduction and their financial viability**

| Item | Hormonal protocol | | |
|---|---|---|---|
| | Select Synch | 2 $PGF_{2\alpha}$ | OvSynch |
| Treated animals (n) | 10 | 10 | 10 |
| Cows of conceived (n) | 5 | 7 | 8 |
| Cows of non-conceived (n) | 5 | 3 | 2 |
| Pregnancy rate (%) | $50^{b}$ | $70^{ab}$ | $80^{a}$ |
| Period of treatments (days) | 7 | 10 | 13 |
| The 1st injection price (L.E) | 41.25 | 10.5 | 41.25 |
| Price of 2nd injection (L.E) | 10.5 | 10.5 | 10.5 |
| The 3rd injection price (L.E) | - | - | 41.25 |
| Protocol cost (L.E/cow) | $51.75^{b}$ | $21^{c}$ | $93^{a}$ |

a, b and c With different superscripts, the means of the same row differ significantly at (P<0.05).

## Conclusion

Results are possible to deduce from these findings that hormonal protocol in Friesian cows improved reproductive performance and progesterone levels. As wall as, evaluation indicated that 2 $PGF_{2\alpha}$ protocols (G2) had the cheapest cost (L.E 21/animal).

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The authors declare that the present study has no financial issues to disclose.

***Conflict of interest***

None

***Author's contributions***

All authors contributed to the study's conception, and design. Data collection, examination and experimental study were performed by ZEE, AMM, YME and MAA. All biochemical analysis and data analysis were performed by ZRG, MMH, YME and AMM. All authors drafted and corrected the manuscript; MAA, MMH and YME revised the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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## تـأثير اسـتخدام جرعـات منخفضـة مـن هرمـوني GnRH و $PGF_2\alpha$ علـي التنـاسـل فـي الأبقار الحلابة

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يهدف البحث إلى تحسين الأداء التناسلي للأبقار متكررة الشياع أو الأبقار التى لم تشبع باستخدام PGF2α و GnRH والحقن داخل الفرج في الطبقة تحت المخاطية. واستخدم في هذه الدراسة 30 بقرة فريزيان حلابة غير عشارلفترة تتراوح من 120 إلى 250 يومًا بعد الولادة. قسمت الأبقار إلى ثلاث مجموعات ، 10 أبقار في كل مجموعة. المجموعة 1 (G1): تم استخدام بروتوكول SelectSynch بمقدار 50 ميكروحرام من GnRH في اليوم 0 و 5 ملجم من PGF2α في اليوم السابع تم مراقبة الشياع والتلقيح. والبروتكول الثاني (PGF2α - PGF2α :(G2 ، كانت الأبقار حقنتين من 5 ملجم من PGF2α ، بفارق 11 يومًا. أما البرتوكول الثالث (G3): OvSynch تم حقن 50 ميكروجرام من GnRH في اليوم 0 و5 مجم PGF2α في اليوم 7 وحقن 50 ميكروجرام من GnRH في اليوم 9 وتم إجراء التلقيح الاصطناعي المحدد (TAI) من 72 إلى 96 ساعة. أظهرت النتائج ظهول الشياع بمعدل 100 و 90 و 80٪ في G3 و G2 و G1 على التوالي. كانت معدلات الحمل أعلى بشكل ملحوظ في G3 و G2 منها في G1. كان تركيز P4 في اليوم 24 بعد التلقيح 10.253 و 11.235 و 11.567 نانوجرام / مل في الابقار العشار مقابل 3.245 و 1.985 و 2.036 نانوجرام / مل في الأبقار غير عشار في G1 و G2 و G3 على التوالي. كان تركيز البروجسترون في الحقن الثاني GnRH أو PGF2α أقل بشكل ملحوظ في الأبقار العشار عنها في الأبقار غير العشار في مجموعات G1 و G2 و G3. أظهر التقييم الاقتصادي أن بروتوكول الثاني من PGF2α كان أرخص تكلفة (21 جنيهاً للحيوان) وأعلى في الأبقار العشار.

**الكلمات الدالة:** أبقار الفريزيان والكفاءة التناسلية وGnRH وPGF2α والبروجستيرون.