SCIENCE REVIEWS JOURNALS

Volume 7, 2020

Fertility Improvement on Repeat Breeder Friesian Heifers with Hormonal Treatments

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Abstract: The objective of this study was to evaluate some hormonal treatment protocols to fertility improvement on repeat breeder Frisian heifers. A total of 36 Frisian heifers failed to conceive after 3-4 services (repeat breeder heifers) was used in this study. Results showed that pregnancy rate was higher significantly in G3, 83.3% than in G1, 50% and G2, 58% in repeat breeder heifers. Progesterone (P4) concentration in G1 or G2 was significantly ($P<0.01$) lower at artificial insemination (estrus) in pregnant heifers than in non-pregnant, while it was higher post-treatment 5, 10, 12 and 24 days post-artificial insemination in pregnant than in non-pregnant repeat breeder heifers. But, P4 concentration not significant during pre-treatment in all animals. At estrus concentration of P4 were lower significantly in pregnant 0.284, 0.392 and 0.246 ng/ml in G1, G2 and G3, respectively than in non pregnant in 1.356, 1.635 and 1.835 ng/ml, respectively. In G3, P4 concentration pre- and during treatment tended to be similar in pregnant and non-pregnant repeat breeder heifers. The P4 concentration on day 5, 10, 12 and 24 post-mating was significantly ($P<0.001$) higher in pregnant than in non-pregnant heifers in G3. The economic evaluation indicated that hCG at insemination and at 5 days post- insemination protocols had the cheapest cost (L.E 22/animal), while hCG-$PGF_2\alpha$-hCG timed AI protocol showed the highest cost (L.E 64/animal).

Keywords: Friesian heifers, repeat breeder, hCG, pregnant rate and progesterone concentration.

1. Introduction

Repeat breeding (BR), which occurs in 10–25% for cows (Sharma *et al.,* 1983 and Bartlett *et al.,* 1986), is one of the major gynecological problems affecting reproductive efficiency and profitability of dairy farms.

Repeat breeding is a substantial problem in cattle breeding. It leads to large economic loss for the dairy producer due to additional inseminations, increased calving intervals, and higher culling rates. Repeat breeding has been defined as failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities (Gustafsson and Emanuelson, 2002).

Reproductive efficiency is the core of dairy farms profitability (Nebel and Jobst, 1998). Maximum economic return could be achieved when females conceive early with fewer services (less than three inseminations).

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Follicular dynamics and pituitary-ovarian axis (Båge *et al*., 1997) are the main reasons for repeat-breeding phenomenon. Within the six days post estrus, repeat breeders have lower progesterone concentrations relative to normal ones (Båge, 2002). Treatment with human chorionic gonadotropin (hCG) between days 4 and 7 post-insemination stimulates ovulation of the first wave dominant follicle and the formation of an additional corpus luteum, which results increased progesterone level (Price and Webb 1989). The response to treatment with hCG five days post-insemination has been inconsistent (Nascimento *et al*., 2013). Santos *et al*. (2001) found that treated first service cows with 3300 IU of hCG resulting in an increase in progesterone concentration and higher pregnancy rate. Moreover, in cows hCG injection increased pregnancy rate (Nascimento *et al* 2013). In contrast, Kendall *et al* (2009) using the same treatment protocol showed increased pregnancy rate in cows. Nonetheless, other studies with first service cows (Schmitt *et al* 1996a, Hanlon *et al* 2005) and with repeat breeders (Walton et al 1990) have failed to show the beneficial effect of hCG administration.

To overcome this inconvenient phenomenon in dairy farms prostaglandin F2α (PGF2α) have been used in different combinations to improve the reproductive efficiency in lactating dairy animals (Yaniz *et al.,* 2004).

Therefore, the aim of the current study is to test the more efficient hormonal protocols to improve reproductive performance of repeated breeder Friesian cows.

2. Materials and Methods

This study was carried out at the Animal Production Experimental Station, Sakha, Kafer El-sheikh Govemorate (located in the north of the Nile Delta), during the period from May 2014 to September 2015.

Animals:

A total of 36 Friesian heifers those failed to conceive after 3 services (repeat breeders) the used in this study. The experimental heifers had 18-24 month old and body weight of 380-460 kg. Animals were fed on concentrate feed mixture, maize silage, rice straw, and berseem (*Trifolium alexandrinum)* hay according to the systems adopted by Animal Production Research Institute (APRI). Fresh water was available all times since, heifers were housed loose in semi-open sheds.

Treatment protocols:

Animals were divided into three groups. Repeat breeder heifers in the first group (G1, n=12) were injected intramuscularly with a single dose of 3000 IU hCG at the time of service (artificial insemination by frozen semen).

Heifers repeat breeder in the second group (G2, n=12) were intramuscularly injected with a single dose of 3000 IU hCG at 5 days post service (artificial insemination by frozen semen).

Repeat breeder heifers in the third group (G3, n=12) were given 3000 IU Human chorine gonadotrpin (hCG) as intramuscular injection (day 0) followed 7 days later by injection with 3 ml $PGF_2\alpha$ analogue (Synchromate Bremer Pharma 27540 Bremerhaven Germany) containing (0.750 µg cloprostenol), followed 2 days later by injection with 3000 IU hCG as intramuscular injection (day 9) followed **by** 16-20 h later with timed artificial insemination by frozen semen (on day 10).

Estrous detection, service and pregnancy diagnosis:

Estrus was detected visually two times daily at 8 a.m and 7 p.m. Heifers in heat (standing to mount) in all groups were inseminated by artificial insemination, excepted the third group was inseminated binned by artificial insemination at day 10 of protocol.

Pregnancy diagnosis was confirmed by P4 concentration on day 24 post-mating and rectal palpation on day 60 post-mating.

Blood sampling:

Blood samples were collected from the jugular vein for progesterone determination. Blood samples were collected 3-4 d pre-treatment in all treatment groups and post- treatment based on the planned protocol for each treated group. Samples were collected on day of estrus in G1, G2 and G3 groups after every treatment dose. Thereafter, samples were collected on day 5, 10, 12 and 24 post-mating. Blood samples were centrifuged for 15 minutes at 3000 rpm for serum separation, which stored at -20 ºC till the P4 assay.

Progesterone assay:

Direct radioimmunoassay technique (RIA) was performed for determination of P4 concentration in blood serum using ready antibody coated tubes kit (Diagnosis Systems Laboratories, Texas, USA) according to the procedure outlined by the manufacturer.

According to the manufacture's information, the cross reaction of progesterone antibody (at 50% binding), was 100% with progesterone while was 6.00, 2.50, 1.20, 0.80, 0.48, and 0.10% with 5α-pregnone-3, 20-dione 11-Deoxycorticosterone, 17α-Hydroxyprogesterone, 5β-pregnone-3, 20-dione 11-Deoxycortisol, and 20α-Dihydroxyprogesterone, respectively and less than 0.1% with any of the other steroids.

The standard curve of P4 ranged from 0.0 to 60.0 ng /ml. The sensitivity value was reported to be 0.12 ng /ml. The intra and inter-assay coefficients of variation were 8.0% and 13.1%.

Statistical Analysis:

To compare between both conceived and non-conceived heifers within each treatment group, results of P4 concentration were statistically analyzed according to Snedecor and Cocharn (1982) and the statistical model was:

$Y_{ij} = U + A_i + e_{ij}$.

Where:

Y_{ij} = Observed values.

U = Overall mean.

A_i = Animals (conceived and non- conceived).

e_{ij} = Random error.

Chi-square was used to test the differences in conception rate among treatment protocols. Duncan Multiple Range test (Duncan, 1955) was used to get the mean separations among treatment protocols for total cost/animal.

3. Results and Discussion

Estrus and pregnancy rate:

Results in Table 1 shown that use of the hCG-$PGF_2\alpha$-hCG protocols (G3) appears to be effective an increasing estrus response 91.67%. However, average ages at 1st AI and days from 1st AI to treatment were not significant in all groups.

Results of this treatment revealed that 6, 7 and 10 treated heifers were pregnant rate out to 12 heifers in each group in G1 (50%), G2 (58.3%) and G3 (83.3%), respectively (Table 1). In similar pattern to the obtained results, Bhattacharyya and Hafiz (2009) found that conception rate for cows treated with hCG was higher (66.67%) than those treated with placentrex (33.33%). The hCG mimics the effects of pituitary luteinising hormone causing ovulation. Continuous action of hCG due to its long half life resulted in more proliferation and differentiation of luteal tissue by recruiting more granulose cells (Abhilas *et al*., 2006).

Therapeutic use of PGF2α for repeat breeders has been demonstrated with some improvement in pregnancy rate compared to untreated controls during the last two decades (Peters, 2005; Ahuja *et al.,* 2005).

Table (1): Reproductive evaluation on Friesian heifers of different treatments.

Item	Hormonal protocol		
	G1(1)	G2(2)	G3(3)
Treated animals (n)	12	12	12
Age at 1st AI (month)	16.4±2.4	16.7±1.9	16.6±2.2
average days from 1st AI to treated	105±6.8	111±11.3	109±10.6
Estrus rate (%) after treatment	100	100	91.67
Pregnant animals (n)	6	7	10
a and b: Pregnancy rate (%)	50	58.34	83.33

(1): hCG at service, (2): hCG at 12 days post-service, (3): hCG-PGF$_2$α-hCG
Means within the same row with different superscripts are significantly different at ($P<0.05$).

The administration of hCG during the early luteal phase induces ovulation of the first-wave dominant follicle and formation of a functional accessory CL. Schmitt *et al*. (1996a) observed that most of the increase of progesterone production after hCG injection was due to the formation of accessory CL. Furthermore, cows treated with hCG on d 5 after estrus had a greater increase in plasma progesterone from d 6 to 13 and greater circulating progesterone on d 13 of the estrous cycle. Therefore, it is possible that hCG induction of an accessory CL on d 5 of the estrous cycle may increase plasma progesterone and enhance embryo survival, which would improve conception rates in dairy cows.

In addition, hCG treatment increases the occurrence of three-wave follicular cycles, in which the emergence of the third-wave dominant follicle is delayed (Schmitt *et al*., 1996b). Such an alteration in follicular dynamics may enhance conception rate because a greater number of heifers with three follicular waves after insemination conceived compared with heifers having two follicular waves (Ahmad *et al*., 1997).

The administration of hCG in the early luteal stage induces the formation of accessory corpora lutea, increases the volume of the CL, encourages luteal cells to become larger and rise plasma P4 concentrations. This rise was mainly due to secretion by accessory CL besides the stimulation of the spontaneous CL (Schmitt *et al*., 1996a).

Progesterone concentration:

Concentrations of P4 in blood serum of repeat breeder heifers (pregnant and non-pregnant) treated with hCG on the day 0 and 5 of artificial insemination are presented in Table (2) and Figure (1 and 2). Results show that P4 concentrations in G1 or G2 was significantly ($P<0.01$) lower at artificial insemination (estrus) in pregnant heifers than in non-pregnant, while it was higher post-treatment 5, 10, 12 and 24 days post-artificial insemination in pregnant than in non-pregnant repeat breeder heifers. But, P4 concentration has not significant during pre-treatment in all animals.

Inspite the observed hCG injection at insemination pronouncedly increased P4 concentration in pregnant than in non-pregnant at days 5, 10, 12 and 24 post-treatment (Table 2). The incidence of pregnancy in repeat breeder heifers were indicated on day 24 post-insemination, whereas the P4 concentration was 10.598 and 12.536 ng/ml in pregnant versus 3.562 and 4.935 ng/ml in non-pregnant repeat breeder heifers in G1 and G2, respectively. The present results regarding hCG protocol at insemination indicated better, which may indicate the main problem in incidence of repeat breeder cases as a result of distrusting the time between onset of estrus, ovulation and insemination.

In similarity with the present results, El-Moghazy (2003) mentioned that P4 concentration was almost higher than 1 ng/ml in pregnant and less than 1 ng/ml in those failed to conceive, which might be one of the causes of the increased conception rates in these cows.

The sharp reduction in P4 concentration in non-return/non-conceived cows post-treatment may be related to failure in embryo implantation or early embryonic mortality. In this respect, Moore *et al.* (2005) observed lower concentrations of serum P4 coincident with embryo loss between day 24 and 28.

The present results regarding hCG protocol at day 5 post-insemination indicated that P4 concentration was recorded the highest value at days 10 to 12 in heifers. These results agreement with Urzúa *et al*. (2017) who found that progesterone

concentration was higher at days 11 and 15 in cows treated with hCG. Mann and Lamming (2001), Stronge *et al*. (2005) and Mann *et al*. (2006) noted that augmented progesterone concentration in blood on days 5 to 9 post-insemination increased trophoblast size and interferon-τ concentrations. It is therefore plausible to suggest that in the present study, the higher progesterone concentration observed in heifers treated with hCG could have favor embryonic development, which increased embryonic competence to establish the maternal pregnancy recognition.

Table (2). Progesterone levels (ng/ml±SEM) of the pregnant and non- pregnant animals in the different treatments groups

Time	Items	Treatments		
		G1[(1)]	G2[(2)]	G3[(3)]
Pre-treatments:-				
	Pregnant	3.568±0.3	4.625±0.4	3.158±0.3
	Non-pregnant	2.985±0.3	3.426±0.5	2.329±0.2
During treatments: -				
Day -10	Pregnant	-	-	6.238±0.5
	Non-pregnant	-	-	5.328±0.3
Day -3	Pregnant	-	-	4.175±0.6
	Non-pregnant	-	-	3.965±0.2
Day -1	Pregnant	-	-	1.235±0.4
	Non-pregnant	-	-	3.024±0.3
At artificial insemination:-				
At oestrus	Pregnant	0.284±0.1^{b}	0.392±0.1^{b}	0.246±0.1^{b}
	Non-pregnant	1.356±0.2^{a}	1.635±0.2^{a}	1.835±0.3^{a}
Post-treatments:-				
Day 5	Pregnant	8.586±0.5^{a}	8.852±0.7^{a}	8.235±0.4^{a}
	Non-pregnant	3.985±0.4^{b}	5.021±0.4^{b}	4.635±0.5^{b}
Day 10	Pregnant	9.235±0.9^{b}	10.235±0.9^{b}	9.827±0.8^{b}
	Non-pregnant	2.695±0.4^{b}	4.256±0.5^{b}	3.215±0.4^{b}
Day 12	Pregnant	9.645±0.7^{a}	12.896±0.9^{a}	11.532±0.8^{a}
	Non-pregnant	3.485±0.6^{bc}	4.235±0.4	3.025±0.3^{b}
Day 24	Pregnant	10.598±0.7^{a}	12.536±0.8^{a}	12.135±0.9^{a}
	Non-pregnant	3.562±0.5^{c}	4.935±0.5^{b}	1.985±0.5^{b}

(1): hCG at service, (2): hCG at12 days pot-service and (3): hCG-PGF$_2$α-hCG protocols. a, b and c: Means within the same column with different superscripts are significantly different at (P<0.05).

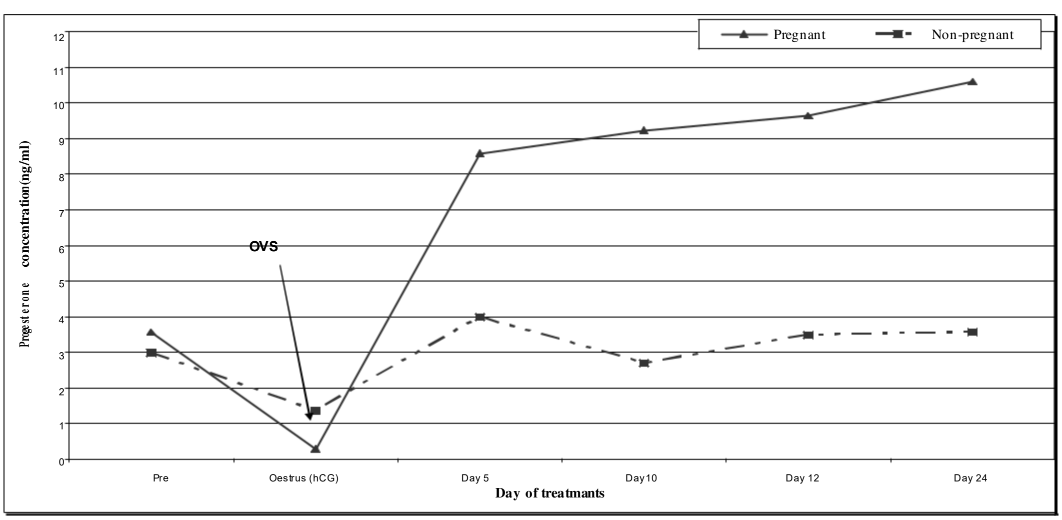

Fig. (1): Progesterone profile pre-treatment, estrus and post- hCG treatment in pregnant and non-pregnant Friesian heifers. (O: estrus, V: ovulation and S: service)

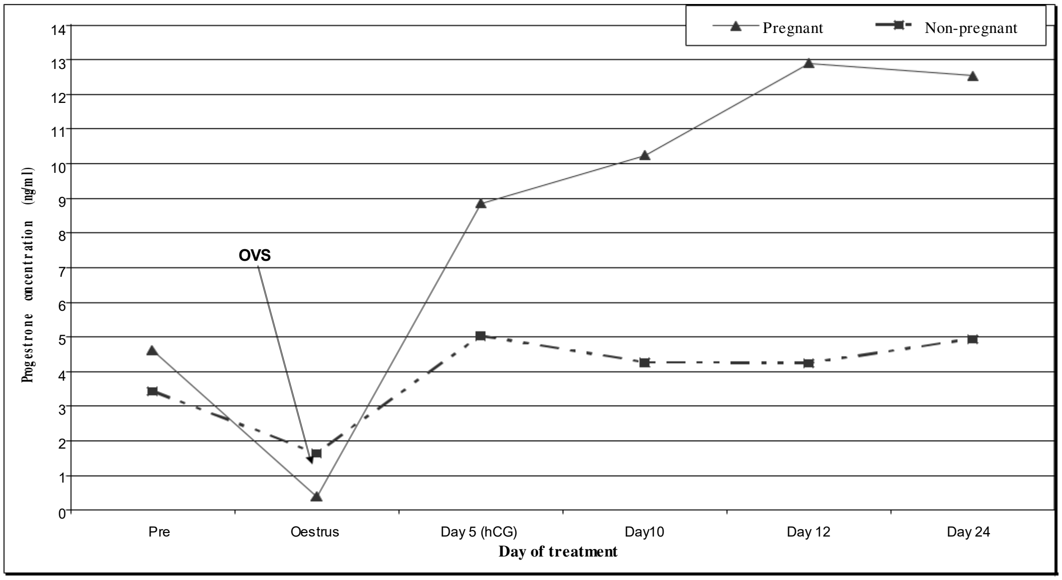

Figure (2): Progesterone profile pre-, estrus and post- hCG treatment in pregnant and non-pregnant Friesian heifers. (O: estrus, V: ovulation and S: service)

Concerning P4 profile pre-, during and post-treatments of hCG- $PGF_2\alpha$-hCG timed AI results are presented in Table (2), P4 concentration pre-treatment tended to be similar in pregnant and non-pregnant repeat breeder heifers. However, the 1st hCG injections was insignificantly increased P4 concentration in pregnant and non-pregnant repeat breeder heifers.

After $PGF_2\alpha$ injection, a regression of the functional CL occurred and then concentration of P4 sharply reduced in pregnant and non-pregnant heifers. These values were insignificantly lower in non-pregnant than in pregnant heifers (Table 2 and Fig. 3 and 4).

Progesterone concentration at 2nd hCG injections was significantly lower ($P<0.05$) in pregnant heifers than in non pregnant heifers in G3 group.

Post-2nd $PGF_2\alpha$ or hCG injections induced growing of dominant follicle, which ovulated within 24 hours and subsequent rise in P4 concentrations by an average of 24 h relative to animals just receiving a GnRH-$PGF_2\alpha$ regimen (Peters *et al.,* 1999).

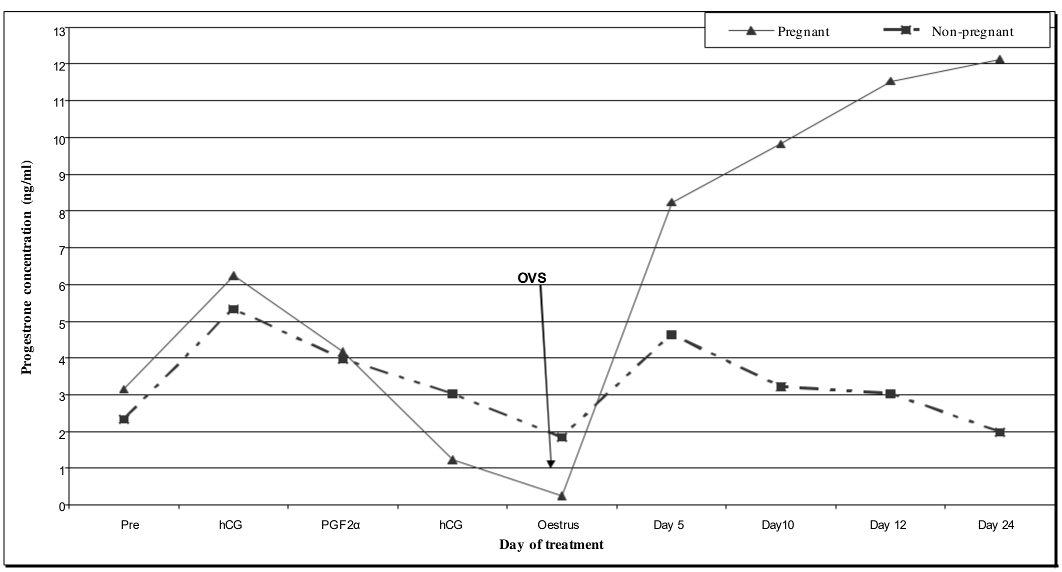

Figure (3): Progesterone profile pre-, during and post- hCG-$PGF_2\alpha$-hCG treatment in pregnant and non-pregnant cows. (O: estrus, V: ovulation and S: service)

At estrus concentration of P4 was lower significantly in pregnant heifers 0.284, 0.392 and 0.246 ng/ml in G1, G2 and G3, respectively than in non-pregnant heifers 1.356, 1.635 and 1.835 ng/ml, respectively (Table, 2).

Generally, the obtained high pregnancy rate in this study may be attributed to an appropriate time of estrus incidence and consequently good time of insemination.

Additionally, pregnancy of heifers was indicated by P4 profile on day 5, 10, 12 and 24 post-mating, being significantly ($P<0.001$) higher (8.235, 9.827, 11.532 and 12.135 ng/ml in G3, respectively) in pregnant than in non-pregnant heifers in G3 (4.635, 3.215, 3.025 and 1.985 ng/ml, respectively, Table 2). The pregnant animals had blood serum P4 concentrations of about 9 to 11.5 ng/ml during luteal phase, which were comparable to luteal concentrations as reported by Foster *et al.* (1997) from 11-18 day of estrous cycle.

As known as progesterone is an essential hormone in the maintenance of pregnancy in cows. The higher levels of progesterone concentration the early pregnancy are related to the embryonic development and increase in interferon-τ production and pregnancy rates (Beltman, *et al.*, 2009). Low systemic progesterone concentrations on day 5 post-ovulation or delay in normal rise in progesterone between days 4 and 5 post-ovulation have been related with reduced pregnancy rates (Shams-Esfanabad and Shıraz 2006 and Larson, *et al.*, 2007).

Comparison among protocols:

From the reproductive point of view, 23 out of 36 treated Friesian repeat breeder heifers (60.89%) were pregnant using all hormonal protocols, being the highest was shown in hCG-$PGF_2\alpha$-hCG timed AI protocols (83.34%, while it was moderate (58.34%) in hCG at day 5 post insemination and the lowest (50%) in hCG at insemination protocol. Also, the economic evaluation indicated that injection with hCG at insemination and at day 5 post insemination protocols had the cheapest cost (L.E 22/animal), followed by hCG-$PGF_2\alpha$-hCG timed AI protocol showed the highest cost (L.E 64/animal, Table 3).

Table (3): Reproductive evaluation and economic efficiency of different hormonal treatments.

Time	Treatments		
	G1[1]	**G2[2]**	**G3[3]**
Reproductive evaluation of treatment:			
Treated animals (n)	12	12	12
Conceived animals (n)	6	7	10
Non conceived (n)	6	5	2
Conception rate (%)	50	58.34	83.34
Economic efficiency of treatment:			
Treatment period (day)	0	0	9
Price of 1st injection (L.E)	22	22	22
Price of 2nd injection (L.E)	-	-	20
Price of 3rd injection (L.E)	-	-	22
Total cost of protocol (L.E/animal)	22 ± 2.1^b	22 ± 2.1^b	64 ± 2.1^a

Price of each injection from hCG and $PGF_2\alpha$ was L.E 22 and 20, respectively. [a, b and c:] Means within the same row with different superscripts are significantly different at ($P<0.05$).

Conclusion

This study demonstrated that the use of hCG-$PGF_{2\alpha}$-hCG timed AI protocol (G3) increased blood serum progesterone level and improves the pregnancy rate in repeat breeder Friesian heifers when compared with other protocols in G1 and G2 under Egyptian conditions.

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