Pakistan J. Zool., pp 1-7, 2022. DOI: https://dx.doi.org/10.17582/journal.pjz/20211115171134

Improving Fertility in Friesian Cows by Synchronization of Fixed Time Insemination

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Article Information
Received 15 November 2021
Revised 18 February 2021
Accepted 03 March 2022
Available online 08 August 2022 (early access)

Authors' Contribution
MAAE, AMM and ZRG presented the idea of research and designed the experiments. WAE and MAE performed statistical and chemical analysis. WAE conducted the experiments. MAAE and AMM wrote and revised the manuscript.

Key words
Friesian cows, High or low fertility, GnRH, $PGF_2\alpha$, Pregnant rate

ABSTRACT

This study aimed to examine several hormonal treatments approaches for improving fertility in Friesian cows with high and low fertility. This study used a total of 40 Friesian cows, 20 of which were unable to conceive after a single service (high fertility), and 20 of which were unable to conceive after three services (low fertility). Animals were classified into four groups (n=10 /group) for two hormonal protocols. Cows in the first and second groups high or low-fertility (G1 and G2, respectively) were treated with $PGF_2\alpha$-GnRH protocol. Cows in the third and forth groups high or low-fertility (G3 and G4, respectively) were treated with OvSynch protocol. Results showed effective increasing oestrus responses 90, 70, 100 and 80% in G1, G2, G3 and G4, respectively. Pregnancy rate was significantly higher in G1 (80%) and G2 (60%) than in G3 (50%) and G4 (40%) in cows. The concentration of progesterone (P4) significantly decreased in pregnant cows compared with non-pregnant in G2 and G3 at artificial insemination (AI), while it was higher at days 7, 14 and 24 post-AI in pregnant than in non-pregnant cows. At oestrus, the P4 concentration was significantly decreased in G1, G2, G3 and G4, in pregnant cow than in non-pregnant of high or low fertility. Economic analysis revealed that the PGF2-GnRH protocols were the least expensive (L.E. 65/animal), while the OvSynch treatment was the most expensive (L.E 95/animal).

INTRODUCTION

The core of dairy farm success is reproductive efficiency (Nebel and Jobst, 1998). Reproductive decline is a significant financial loss in the dairy sector for various causes, including environment, nutrition, and management. Although many factors influence fertility, one of the most common causes of lower fertility is an increase in early embryonic mortality, which occurs between days 8 and 17 of pregnancy due to the embryo's failure to support the maintenance of the corpus luteum (CL).

Management of reproductive protocols has been emphasized on synchronizing estrus with PGF2α. When cows had bred following a detected estrus, these were exceedingly successful. To increase heat detection rates, different methods of artificial insemination (AI) management were used. AI becomes more efficiency when estrus synchronized with PGF2α (Stevenson and Pursley, 1994).

The classic ovisynch protocols by GnRH-$PGF_2\alpha$ are influenced by the follicular stage at the initial GnRH injection time in the synchronization program (Vasconcelos *et al*., 1999). Pre-synchronization with use dabbles injections of $PGF_2\alpha$ 12-14 d apart, regresses CL in the luteal phase of the estrous cycle in cows (early to mid-luteal phase) before the start of OvSynch by 14 days and this approach consequently leads to higher pregnancy rate (Moreira *et al*., 2000, 2001).

Follicular dynamics and pituitary-ovarian axis (Båge *et al*., 1997) are the main reasons for repeat-breeding phenomenon. Within the six days post oestrus, repeat breeders have lower progesterone concentrations relative to normal ones (Båge, 2002). The repeat breeder was decreasing in dairy farms when we used combination between GnRH and prostaglandin F2α (PGF2α) so, it become efficient way to improve the reproductive performance in dairy animals (Yaniz *et al*., 2004).

Thus, the pressent study aimed to evaluate more efficient hormonal treatments to improve reproductive performance of high and low fertility Friesian cows.

MATERIALS AND METHODS

The current study was conducted at the Animal Production Experimental Station, Sakha, Kafer El-Sheikh Govemorate (located in the north of the Nile Delta) from

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0030-9923/2022/0001-0001 $ 9.00/0

May to September 2020. This study was conducted after an agreement from the Animal Care and Ethics Committee of Kafrelsheikh University, Egypt.

Animals

A total of 40 Friesian cows, 20 cows were unable to conceive after 1 service only (high-fertility) or 20 cows were failed to conceive after 3 services (low-fertility) were used. Cows had 46 to 86 months old and body weight of 460-580 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). Cows were housed unrestrained in semi-open barns, fresh water was always available.

Animals were divided into four groups, cows in G1 and G3 were high-fertility, however, cows in G2 and G4 were low-fertility.

Treatment protocols

Cows in the first and second groups high or low-fertility (G1 and G2 respectively, n=10) were intramuscularly (i.m.) injected with 3 ml $PGF_2\alpha$ analogue containing 0.750 µg cloprostenol (Synchromate, Bremer Pharma 27540 Bremerhaven, Germany) at day 0, 5 ml GnRH analogue (Receptal, Hoechst, Germany) containing 20 µg Buserelin GnRH at day 14, 3 ml $PGF_2\alpha$ analogue containing 0.750 µg cloprostenol (Synchromate, Bremer Pharma 27540 Bremerhaven, Germany) at day 21, and 5 ml of Receptal two days later (day 23), followed by 16-20 h later with timed artificial insemination by frozen semen on day 24 (protocol 1, Fig. 1).

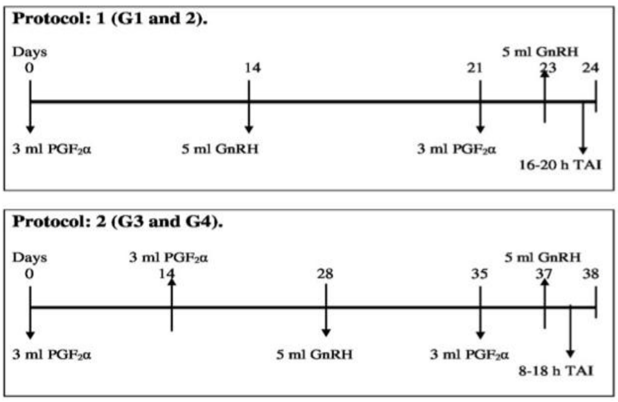

Fig. 1. Diagrams of activities treatments during the study, Friesian cows were randomly assigned timed artificial insemination (TAI) in 1 and 2 protocols.

Cows in the third and four groups high or low-fertility (G3 and G4, respectively) were i.m. injected with 3 ml $PGF_2\alpha$ analogue containing (0.750 µg cloprostenol**)** (Synchromate Bremer Pharma 27540 Bremerhaven Germany) at days of 0 and 14 (Pre-Synchronization), 5 ml Receptal at day 28, 3 ml $PGF_2\alpha$ analogue containing 0.750 µg cloprostenol (Synchromate, Bremer Pharma 27540 Bremerhaven, Germany) at day 35, and 5 ml of Receptal two days later (day 37), followed by 8-18 h later with timed artificial insemination by frozen semen on day 38 (protocol 2, Fig. 1).

Oestrous detection, service, and pregnancy diagnosis

Oestrus has detected visually three times daily between 8 a.m and 6 p.m. A teaser bull supplied with paint has used for each treatment group during the night (from 7 p.m up to 7 a.m) to detect the oestral animals.

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

Progesterone assay

Progesterone levels were determined using blood samples taken from the jugular vein. 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 have collected on day of oestrus in and after every treatment dose. Thereafter, samples have collected on day 7, 14 and 24 post-mating. In order to separate the serum, the blood samples were centrifuged at 3000 rpm for 15 min. Serum was then stored at -20 ºC till the P4 assay.

By following the manufacturer's instructions using a ready antibody-coated tubes kit of the radioimmunoassay technique (RIA) (Diagnosis Systems Laboratories, Texas, USA), the progesterone antibody has a 100% cross-reaction (at 50% binding). On the other hand, with 5-pregnone-3, 20-dione 11-Deoxycorticosterone, 5-pregnone-3, 20-dione 11-Deoxycortisol, 17-Hydroxyprogesterone, and 20-Dihydroxyprogesterone, it becomes 6.00, 2.50, 1.20, 0.80, 0.48, and 0.10%, respectively, and less than 0.1% with any of the other steroids.

According to reports, the sensitivity value is 0.12 ng/ml. The coefficients of variance within and between assays were 8.0 and 13.1%, respectively.

Statistical analysis

To compare between both conceived and non-conceived heifers within each treatment group, results of P4 concentration had statistically analyzed according to Snedecor and Cocharn (1980) and statistical model:

$Y_{ij} = A + B_i + e_{ij}$.

Where; Y_{ij} is Observed values; A is Overall mean; B_i is Cows (conceived and non-conceived); e_{ij} is Random error.

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

RESULTS AND DISCUSSION

Oestrus and pregnant rate

Results in Table I showed that use of the $PG_2\alpha$-GnRH and OvSynch protocols in high or low fertility cows regimen were effective in increasing oestrus response 90, 70, 100 and 80% in G1, G2, G3 and G4, respectively. However, average days from calving to 1st AI and treatment were not significant in all groups.

Table I. Reproductive evaluation and economic efficiency of different hormonal treatments.

Item	Hormonal protocol			
	G1[1]	G2[2]	G3[3]	G4[4]
Treated animals (n)	10	10	10	10
Average days from calving to 1st AI	69.6±6.4	79.8±7.1	69.5±7.6	78.8±6.2
Estrus rate (%)	90	70	100	80
Pregnant animals (n)	8	6	5	6
Pregnancy rate (%)	80[a]	60[b]	50[b]	60[b]

[1 and 2]: $PGF_2\alpha$-GnRH protocol of high or low fertility cows, respectively. [3 and 4]: OvSynch protocol of high or low fertility cows, respectively. [a and b]: Within a single row, the means with varied superscripts differ considerably at (P0.05).

Results of this treatment revealed that 8, 6, 5 and 4 treated cows out of 10 cows were pregnant with the following rates 80%, 60%, 50% and 40%, in G1, G2, G3, and G4, respectively (Table I). In similar pattern to the obtained results, the GnRH injection at oestrus increased conception rate (CR) from 41.3 to 55.5% compared to the control and this effect could be attributed to prevention of ovulation failure (Kaim *et al*., 2003; Nakao *et al*., 1984). The combined treatment with GnRH and PGF2α improved pregnancy rates in poor fertility cows when compared to untreated controls (Peters, 2005; Ahuja *et al*., 2005). Santos et al. (2008) found that, more than 90% of cycling cows responded positively to the second $PGF_2\alpha$ treatment, when two injections of $PGF_2\alpha$ have given 10 to 14 days apart. The use of PGF2 can shorten the time between observed estrous cycles and increase the accuracy of estrus detection (Pursley et al., 2001).

Among the numerous dairy farmers who employ the OvSynch procedure followed by a fixed time of AI at 16 h following the second GnRH injection, about 87% of GnRh treated cows induced ovulation after the second injection of GnRH (Martel, 2008). The OvSynch protocol promotes the release of LH following the 1st and 2nd injections of GnRH, and the response rate of ovulation was 64% and 92%, respectively, when GnRH was delivered at various stages of the estrus cycle (Vasconcelos *et al*., 1999). The ideal outcome of the OvSynch regimen is somewhat dictated by the estrous cycle stage at which therapy is commenced (Cartmill *et al*., 2001). The increase of CR resulted to GnRH treatment was favorable (Kaim *et al*., 2003). The second LH peak did not increase the spontaneously when GnRH injected at AI (Ryan *et al*., 1994). Pregnancy rates were significantly greater in OvSynch protocols compared to cows treated with PGF2 14 days apart (Momcilovic *et al*., 1998; Moreira *et al*., 2001).

Progesterone concentration

Concentrations of P4 in serum of high or low fertility cows (pregnant and non-pregnant) treated with GnRH protocol have presented in Table II. At AI (oestrus), the P4 content in G1 or G2 was considerably (P0.05) lower in pregnant cows than in non-pregnant cows but was significantly higher in pregnant cows at 7, 14, and 24 days-post-AI. Nonetheless, during pre-treatment, P4 concentrations were not significant in any animals. In spite the observed GnRH injections pronouncedly were increased P4 concentration in pregnant than in non-pregnant at days 7, 14 and 24 post-treatment (Table II). The incidence of pregnancy in pregnant cows were indicated on day 24 post-insemination, whereas P4 concentration was 9.723 and 11.503 ng/ml in pregnant versus P4 than 2.014 and 4.399 ng/ml in non-pregnant cows in G1 and G2, respectively. In accordance with increase P4 level of G1 and G2 at oestrus in non-pregnant cows than in pregnant cows.

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. Similarly, Mee *et al*. (1993) reported that GnRH raised blood P4 levels in repeat breeder cows, which could be a factor in these cows' increased conception rates. The sharp reduction in P4 concentration in non-return/non-conceived cows' post-treatment may be relate to failure in embryo implantation or early embryonic mortality. In this respect, Moore *et al*. (2005) discovered reduced serum P4 concentrations in conjunction with embryo loss between days 24 and 28.

The present results regarding OvSynch protocol indicated better in high fertility cows and similar results of treatment with GnRH protocol, which may indicate the main problem in incidence of low fertility cases as result of distrusting the time between onset of oestrus, ovulation, and insemination. In this respect, many authors have GnRH-induced ovulation, it has been proposed, may give better synchronization between insemination and ovulation (Stevenson, *et al*., 1988; Abu El-Hamd *et al*., 2014). In this respect, Peters (2005) proposed utilizing GnRH as a 'holding' hormone on the day of insemination to increase the probability of a successful pregnancy, particularly in cows.

Concerning P4 profile pre- during and post-treatment of protocol 1 and 2 were results presented in Table II show that pre-treatment P4 concentrations in pregnant and non-pregnant tended to be similar in high or low fertility cows. However, the 1st GnRH injections insignificantly increased P4 concentration in pregnant and non-pregnant high or low fertility cows. After $PGF_2\alpha$ injection, a regression of the functional CL occurred and then P4 sharply reduced in pregnant and non-pregnant cows. These levels were just marginally lower in non-pregnant cows than pregnant cows (Table II). In all groups, at the second GnRH injection, pregnant cows had non-significantly lower progesterone levels than non-pregnant cows. Post-2nd GnRH injections induced growing of dominant follicle, which ovulated within 24 h, and resulted in an average 24-h increase in P4 concentrations compared to mice receiving only a GnRH-PGF2 regimen (Peters *et al*., 1999). At oestrus P4 concentration P4 was decreased significantly in pregnant 0.250, 0.383, 0.213 and 0.208 in G1, G2, G3 and G4, respectively than non-pregnant of high or low fertility in 1.611, 1.225, 1.426 and 2.624, respectively (Table II). Generally, the obtained high pregnancy rate in this study has attributed to an appropriate time of oestrus incidence and consequently good time of insemination and fertilization.

Table II. Progesterone levels (ng/ml±SEM) of the pregnant and non-pregnant animals in the treated and control groups.

Time	Items	Treatments			
		G1[1]	G2[2]	G3[3]	G4[4]
Pre-treatments					
	Pregnant	2.542±0.4	3.235±0.5	2.441±03	2.625±0.4
	Non-pregnant	2.754±0.2	2.426±0.4	2.895±0.4	2.545±0.3
During treatments					
Day -37	Pregnant	-	-	3.751±0.5	4.245±0.6
	Non-pregnant	-	-	3.021±0.6	3.526±0.4
Day -21	Pregnant	2.536±0.4	2.865±0.3	3.265±0.5	3.254±0.7
	Non-pregnant	3.254±0.2	2.965±0.4	2.365±0.3	2.315±0.3
Day -14	Pregnant	3.254±0.3	2.789±0.3	2.985±0.4	3.265±0.6
	Non-pregnant	3.658±0.5	2.874±0.5	3.124±0.5	3.965±0.5
Day -7	Pregnant	2.643±0.6	2.547±0.4	2.403±0.5	2.821±0.7
	Non-pregnant	3.542±0.4	4.340±0.5	3.208±0.4	2.285±0.3
Day -1	Pregnant	1.432±0.5	1.041±0.3	1.081±0.5	0.987±0.5
	Non-pregnant	3.243±0.6	3.483±0.5	3.684±0.3	3.281±0.4
At artificial insemination					
At oestrus	Pregnant	0.250±0.1[b]	0.383±0.1[b]	0.213±0.2[b]	0.208±0.1[b]
	Non-pregnant	1.611±0.2[a]	1.225±0.2[a]	1.426±0.3[a]	2.624±0.3[a]
Post-treatments					
Day 7	Pregnant	9.858±0.6[a]	10.715±0.6[a]	11.013±0.8[a]	9.839±0.5[a]
	Non-pregnant	4.246±0.5[b]	5.021±0.5[b]	3.167±0.7[bc]	3.103±0.4[b]
Day 14	Pregnant	9.875±0.4[a]	11.207±0.6[a]	10.573±0.6[a]	10.855±0.5[a]
	Non-pregnant	3.178±0.6[bc]	3.980±0.5	3.928±0.7[b]	2.591±0.4[b]
Day 24	Pregnant	11.230±0.8[a]	11.103±0.5[a]	11.350±0.6[a]	11.250±0.7[a]
	Non-pregnant	1.814±0.6[c]	3.959±0.6[b]	2.628±0.5[c]	2.672±0.5[b]

For explanation of superscripts, see Table I.

Generally, pregnancy of cows had indicated by P4 profile on day 7, 14 and 24 post-mating being significantly (P<0.001) highest (11.013, 10.573 and 11.350 ng/ml in G3 and 9.839, 10.855 and 11.250 in G4, respectively) in pregnant than non-pregnant cows in G3 (3.176, 3.928 and 2.628 ng/ml and G4 (3.103, 2.591 and 2.672 ng/ml, respectively, Table II). The pregnant animals had serum P4 concentrations of about 9 to 11.5 ng/ml during luteal phase, which were comparable to luteal concentrations reported by Foster *et al*. (1997) from 11-18 day of oestrous cycle. The use of one more protocol of estrus synchronization plays a positive role in boosting ovulation synchrony. However, the second GnRH injection has a synchronization influence, whereas the 1st GnRH injection has the effect of lengthening the luteal phase in late-cycle treated animals (Peters, 2005). Finally, the preovulatory LH surge that initiates ovulation occurs within an 8-h window beginning around 24 h after the injection, 48 h after the $PGF_2\alpha$ injection, a second GnRH injection is performed (Pursley *et al*., 1995). Progesterone is a critical hormone in maintaining pregnancy in cows throughout early pregnancy, therefore higher levels of progesterone are linked to embryo development, increased interferon production, and higher pregnancy rates (Beltman *et al*., 2009). On day 5 post-ovulation, low systemic progesterone levels had a detrimental effect on pregnancy rates (Shams-Esfanabad and Shıraz, 2006; Larson *et al*., 2007).

Comparison among protocols

From the reproductive point of view, 14 out of 20 treated Friesian high fertility cows (70%) and 11 out of 20 treated Friesian low fertility cows (55%) were pregnant using all hormonal protocols, being the highest in $PGF_2\alpha$-GnRH timed AI protocols (80%) in high fertility cows, moderate (60%) in OvSynch protocol of high or low fertility cows and the lowest (50%) in $PGF_2\alpha$-GnRH protocol of low fertility cows. In addition, the economic evaluation indicated that $PGF_2\alpha$-GnRH timed AI protocols were the least expensive (L.E. 65/animal), whereas the OvSynch protocol was the most expensive (L.E 95/animal) (Table III).

CONCLUSION

The current study indicated that some differences between protocols and average P4 concentration during oestrous cycle, being with higher values in pregnancy rate in high fertility cows. Repeat breeder cows treated by $PGF_{2\alpha}$-GnRH- $PGF_{2\alpha}$-GnRH timed AI protocols improved their reproductive performance. From the economic point of view, $PGF_{2\alpha}$-GnRH- $PGF_{2\alpha}$-GnRH timed AI protocol showed the best results (pregnancy rate and moderate costs) compared to the OvSynch protocol.

Table III. Reproductive evaluation and economic efficiency of different protocols treatment.

Time	Treatments			
	G1[1]	G2[2]	G3[3]	G4[4]
Reproductive evaluation of treatment				
Treated animals (n)	10	10	10	10
Conceived animals (n)	6	5	7	8
Non conceived (n)	4	5	3	2
Conception rate (%)	40	50	70	80
Economic efficiency of treatment				
Treatment period (day)	28	28	37	37
Price of 1st injection (L.E)	25	25	15	15
Price of 2nd injection (L.E)	15	15	15	15
Price of 3rd injection (L.E)	25	25	25	25
Price of 4rd injection (L.E)	-	-	15	15
Price of 5rd injection (L.E)	-	-	25	25
Total cost of protocol (L.E/ animal)	65 ± 1.1^{b}	65 ± 2.1^{b}	95 ± 1.1^{a}	95 ± 1.1^{a}

For explanation of superscripts, see **Table I**.

ACKNOWLEDGEMENTS

We thank lab members of Sakha Animal Produc tion Research Station, Animal Production Research Institute, Agricultural Research center, Ministry of Agriculture for helping us during sampling and evaluation of blood parameters.

Statement of conflict of interest

The authors have declared no conflict of interest.

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