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# THE EFFECT OF USING DIFFERENT PERIODS OF ESTRUS SYNCHRONIZATION ON THE REPRODUCTIVE PERFORMANCE & SUBSEQUENT HAEMATOLOGICAL VALUES BEFORE & DURING PREGNANCY IN HOLSTEIN HEIFERS

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**ABSTRACT**
This study was conducted to investigate the effect of using double prostaglandin F2α with different injection intervals on reproductive performance and hematological parameters before and during pregnancy in Holstein heifers. Twenty nine Holstein heifers were used and divided randomly into three groups G1= 10 heifers treated with double I.M. injection regimen of PGF2α analogue Estrumate (Cloprostenol 500 µg) at 10 days apart, G2 = 11heifers got the same treatment at 11days apart, G3 = 8 heifers got the same treatment at 12 days apart. Results revealed that estrus rate was similar in G1 and G3 (100%) and significantly (P<0.01) higher than G2 (90.9%). The duration of response was lower in G2 (48.4±3.22) hours (P< 0.01) than G1 (53.3±614) hrs and G3 (56.8±4, 16) hrs. On the other hand, services per conception was greater (P<0.01) in G3 (1.8±0.52) than G1 (1.3±0.25) and G2(1.6±0.17) . According to the results of this research we can conclude that the treatment of Holstein heifers with double I.M. injection of 500µg PGF2α, at 10 days apart obtained better reproductive performance before and during pregnancy. The significant differences in the values of the hematological picture before & during pregnancy are still within the recommended or acceptable range mentioned by several authors, which indicate that there is no tremendous effect of PGF2α analogue (Estrumate) treatment on the hematological values before & during pregnancy.

**KEY WORD:** Estrus synchronization, Reproductive performance, Hematological values.

## INTRODUCTION

The role of PGF2α in estrus synchronization of cattle has been first experienced by (1). Then it was widely used in many aspects of bovine reproduction & obstetrics (2 &3). Double injection of PGF2α was also implemented to improve conception rate after getting successful estrus synchronization as well as combating silent heat (4, 5 & 6). The haematological values of the animals may show variations according to changes in the normal physiological activities of the body (7&8) or due to pathological & metabolic disorders (9&10). Some of those disorders may lead to a heavy economic loss & may also affect on the reproductive performance of the animal (11&12). Other factors such as management , feeding level , age , sex , breed health status , method of blood collection , haematological technique used , diurnal & seasonal variations, ambient temperature & physiological status of the animal were also incriminated in changing the blood parameters (13&14) .The variations in the biochemical & hematological features values may reflect the metabolic disturbance which arises as a result of imbalanced & under feeding which influences on the production & reproduction of the animals (12,15&16) . The effect of giving exotic hormones in heifers before pregnancy on the hematological values has not been experienced before. Thus we planned to investigate the effect of giving double injection of PGF2α at a different three periods on the timing & rate of estrus synchronization , pregnancy rate, No. of services / conception & the subsequent hematological values before & during pregnancy.

## MATERIALS & METHODS

Twenty nine Holstein heifers aged 18-24 months were used in this study in the farms of Agriculture & Veterinary medicine colleges during the years 2010-2011. Estrus synchronization was achieved by using double I.M. injection regimen of PGF2α analogue Estrumate (Cloprostenol 500 µg). The animals were randomly divided in to 3 groups: The 10 animals of the first group (G1) were injected twice with 2ml Estrumate (Cloprostenol 500 µg) i.m. at 10 days apart, the same doses were repeated on 11 heifers (G2) at 11 days apart, while the 8 heifers of the third group (G3) were given the same doses at 12 days apart. Duration of response after the second injection were recorded when signs of standing estrus have been seen. The heifers were then transferred with bulls to ensure mating. Pregnancy was then diagnosed after 2-3 months. 2 ml of blood samples were taken in tubes containing anticoagulant (EDTA) from the jugular vein of all heifers before treatment (Estumate) & used as control then other samples were taken at each trimester of pregnancy. Total RBCs count was determined by using the (Improved Neubaur Haemocytometer)

according to (16). Haemoglobin was estimated by using (Spectrophotometric method, PCV was achieved by using (Microhaematocrit), WBCs was counted by using (Meander system count or Improved Neubaur haemocytometer ), MCV , MCH , MCHC were counted by applying statistical equations. Data were analyzed using one way analysis of variance. The statistical analysis, which includes the mean, standard deviation, Q-square, F-test & the analysis of variance were used according to (17).

## RESULTS

Results of table 1- shows that the of animals in (G1) responded were 10 (100%) , Duration of response was 53.3 ± 6.14 days & the number of services / conception was 1.3 ± o.23 .The number of animals responded in (G2) were 10(90.9%), Duration of response was 48.4 ± 3.22 days & the number of services / conception was 1.6 ±0.17.In (G3) the number of animals responded were 8(100%), Duration of response was 56.8 ± 4.16 days & the number of services / conception was 1.8 ±0.52.Table 2- shows that the number of animals in (G1) became pregnant were 9 (90%), one of them was suffering from difficult birth, 4 of them were male & all of them were alive. In (G2) the number of animals became pregnant were 9 (90%) , 2 of them suffered from dystocia , 5 of them were male & one of them were died . While the number of animals became pregnant in (G3) were 8 (100%) one of them suffered from dystocia , 5 of them were male & all of them were alive.

**TABLE 1:** The response of Holstein heifers to 3 regimens of Cloprostenol double injections

| Group | No of animals | Hormonal treatment | Animals responded | Duration of response (days) | No of services per conception |
|---|---|---|---|---|---|
| 1 | 10 | 2 ml (500 µg) cloprostenol | 10 (100%) | 53.3±6.14 | 1.3±0.35 |
| | | 10 days apart | A | A | A |
| | 11 | 2 ml (500 µg) cloprostenol | 10 (90.9%) | 48.4±3.22 | 1.6±0.17 |
| 2 | | 11 days apart | B | B | A |
| 3 | 8 | 2 ml (500 µg) cloprostenol | 8 (100%) | 56.8±4.16 | 1.8±0.52 |
| | | 12 days apart | A | A | A |
| Total | 29 | | 28/29 (96.55%) | | |

Different letters indicate significant differences between the groups at ($p<0.01$)

**TABLE 2:** Results of pregnancy rate, nature of calving, birth sex and viability in Holstein heifers treated with different injection interval of PGF2α.

| Group No. | No of animals used | Animals responded | Pregnancy No. & Rate | Nature of birth | | Sex of off sprigs | | Viability of off springs | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Norm. | Dyst. | M | F | A | D |
| 1 | 10 | 10(100%) | 9(90%) | 8 | 1 | 4 | 5 | 9 | 0 |
| 2 | 11 | 10(90.9%) | 9(90%) | 7 | 2 | 5 | 4 | 8 | 1 |
| 3 | 8 | 8(100%) | 8(100%) | 7 | 1 | 5 | 3 | 8 | 0 |
| Total | 29 | 28/29 | 26/28 | 22/26 | 4/26 | 14/26 | 12/26 | 25/26 | 1/26 |

**TABLE 3:** Hematological values in Holstein heifers before & during the three trimesters of pregnancy

| Parameters Examined | Non-pregnant M ± S.D | First trimester | Second trimester | Third trimester |
|---|---|---|---|---|
| RBCs | 6.24 ± 0.13 | 5.92 ± 0.08 | 5.72 ± 0.16 | 5.89 ± 0.06 |
| 1000000/ µl | A | B | B | B |
| WBCs | 7.25 ± 0.16 | 6.87 ± 0.15 | 7.33 ± 0.8 | 8.21 ± 0.12 |
| 1000/ µl | A | B | A | c |
| Hb | 10.86 ± 0.11 | 10.24 ± 0.07 | 9.14 ± 0.5 | 9.65 ± 0.3 |
| g/100 ml | A | A | B | B |
| PCV | 34.06 ± 0.44 | 30.13 ± 0.19 | 29.22 ± 0.05 | 34.6 ± 0.83 |
| % | A | B | B | A |
| MCV | 54.58 ± 3.38 | 50.89 ± 2.37 | 51.08 ± 0.31 | 58.74 ± 1.38 |
| FI | A | B | B | C |
| MCH | 17.40 ± 0.84 | 17.29 ± 0.87 | 15.97 ± 3.12 | 16.38 ± 0.5 |
| Pg | A | A | B | B |
| MCHC | 31.36 ± 0.4 | 29.42 ± 2.71 | 31.96 ± 0.10 | 35 ± 2.76 |
| g/100 ml | A | A | A | B |

Different letters indicate significant differences between the groups at ($P<0.01$)

Table 3- shows the hematological values of heifers before & during pregnancy, in which RBCs count, WBCs count, Hb concentration, PCV percent., MCV, MCH, & MCHC before pregnancy were 6.24±0.13, 7.25±0.16, 10.86±0.11, 34.06± 0.44, 54.86±3.38 , 17.40±0.84 & 31.36±0.4 respectively . Whereas RBCs count in the first , second & third trimester of gestation was 5.92±0.08, 5.72±0.16 & 5.89±0.06 respectively. WBCs count was 6.87±0.15,

7.33±0.8 & 8.21±0.12. Hb concentration was 10.24±0.07, 9.14±0.5 & 9.65±0.3. PCV percent was 30.13±0.19, 29.22±0.05 & 34.6±0.83. MCV value was 50.89±2.37, 51.08±0.31 & 58.74±138. MCH value was 17.29±0.87, 15.97±3.12 & 16.38±0.5. MCHC value was 29.42±2.71, 31.96±0.10 & 35 ±2.76 respectively.

Table 4- represents the mean hematological values before & during pregnancy. The values before pregnancy are the same as mentioned in table 3 but the mean values during pregnancy for RBCs count, WBCs count, Hb concentration, PCV percent., MCV, MCH and MCHC was 5.84±0.3, 7.47±1.07, 9.67±0.87, 34.0.6±0.44, 53.57±4.06 , 16.54±4.49 & 32.41±5.57 respectively.

**TABLE 4:** The mean hematological values before & during pregnancy

| Parameters Examined | Values before pregnancy M ± S.D | Values during pregnancy M ± S.D |
|---|---|---|
| RBCs | 6.24 ± 0.13 | 5.84 ± 0.3 |
| 1000000/ µL | A | B |
| WBCs | 7.25 ± 0.6 | 7.47 ± 1.07 |
| 1000/ µL | A | A |
| Hb | 10.68 ± 0.11 | 9.67 ± 0.87 |
| g/ 100 ml | A | B |
| PCV | 34.06 ± 0.44 | 31.31 ± 1.07 |
| % | A | B |
| MCV | 54.58 ± 3.38 | 53.57 ± 4.06 |
| FI | A | A |
| MCH | 17.40 ± 0.84 | 16.54 ± 4.49 |
| Pg | A | B |
| MCHC | 31.36 ± 0.4 | 32.41 ± 5.57 |
| g/100 ml | A | B |

Different letters indicate significant differences between the groups at ($P<0.01$)

**DISCUSSION**

From table -1, estrus manifestation after the 2nd injection was recorded as 100% in G1 & G3 compared to 90.9% in G2 , this may indicate that there is a structural or functional abnormalities in the un-responded heifer.(6) . However the duration of response to double injection of PGF2α was relatively & significantly shorter in G2 and agrees with the findings of (18). Never the less G3 needed 1.8 ± 0.52 services per conception compared to 1.3 ± 0.35 in G1 which is significantly lower and makes 10 days apart of G1 a recommended period of double PGF2α injection. On the other hand results of table 2 shows that pregnancy rate was significantly higher (100%)in G3 compared to( 90%) in both G1 & G2 which may referred to the probability of un seen embryonic loss & agreed with the findings of (19). Dystocia was recorded in all groups as 1/9 , 2/9 & 1/8 in G1,G2 & G3 respectively. This result is not uncommon in heifers due to early sexual puberty & un-proportional pelvic size of the heifers (20). In table 3, parameters of hematology showed a significant increase in the RBCs in the non pregnant heifers compared to pregnant heifers along the course of pregnancy which may be attributed to the high demand of O2 & hemo-dilution during pregnancy as a result of increased water flow to the uterus and agrees with the findings of (21).

WBCs were significantly increased in the third trimester which agreed with (22 & 23) & significantly decreased In the first trimester which may be attributed to increase progesterone level in blood since it will decrease the amount of blood supply to the uterus (24 &16). Hb showed a significant decrease at the last 2 trimesters which is coincident with RBCs decrease since RBCs are usually rich in Hb & agreed with the findings of (23). PCV is significantly decreased during the 1st& 2nd trimesters of pregnancy which agrees with (25) & goes equivalent to RBCs count since PCV depends mainly on the number of cells in the sample , while it showed a significant increase at the last trimester which agreed with (26). MCV is significantly increased at the last trimester of pregnancy due to increased blood volume & osmotic pressure of RBCs (27 &21) .MCH is significantly decreased at the last 2 trimesters of pregnancy which did not agrees with the findings of (23) who did not find significant difference . MCHC is significantly increased at the last trimester of pregnancy, which agrees with the records obtained by (28) & disagreed with (23). In table- 4, RBCs count during pregnancy is lower than the range mentioned by (29) & higher than the range indicated by (30) but it is still within the range mentioned by (31), However the significant decrease of RBCs count during pregnancy is due to hemo-dilution which has a compromising effect to the increase of volume & number of RBCs & to compensate the increased demand of O2. (32). Hb concentration is significantly decreased during pregnancy which is in turn following up the level of RBCs count. PCV showed a significant decrease during pregnancy compared to non-pregnant heifers , however it is still regarded as a recommended acceptable range mentioned by (33) .MCV did not show significant differences in its value compared to non-pregnant heifers perhaps due to the lower number of population in this study . MCH showed a significant decrease during pregnancy compared to non pregnant heifers & this would agreed with the findings of (23) MCHC showed a significant increase during pregnancy which agreed with the records obtained by (8 &26)& disagreed with (14). In conclusion, the higher percentage

(100%) of pregnancy in heifers treated at 12 days apart is associated with the higher number of services / conception 1.8 ± 0.25 compared to 90% pregnancy & 1.3 ± 0.35 services / conception in heifers treated at 10 days apart . However heifers treated at 11 days apart showed pregnancy at 90% & number of services / conception at 1.6 ± 0.17. So we recommend the treatment of 10 days apart since we got the best reproductive performance before and during pregnancy. Concerning the hematological values , it seems that there is no tremendous significant variation due to hormonal estrus synchronization since they remains within the recommended values mentioned by several authors.

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