Effect of PGF2α Double Injection on Hematological Picture, Hormonal Levels and Fertility of Cows During Summer Season

Abdel Mohsen M. Hammam[1]; El- Sayed M.M. Abdel Gawad[2]; Abdel Tawab A. Yassein[2]; Mona S. Zaki[3]; Amal H. Ali[1]

[1]Department of Animal Reproduction and A.I., Vet. Division, National Research Centre, Dokki, Cairo, Egypt.
[2]Department of Theriogenology, Faculty of Vet. Med., Beni-Suif University, Egypt
[3]Department of Hydrobiology, Vet. Division, National Research Centre, Dokki, Cairo, Egypt.
Hamam-pharma2010@hotmail.com

Abstract: Heat stress has an adverse effect on animal fertility due to direct or indirect action on hypothalamic-hypophyseal axis and/or on gonadal hormones. The present study aimed to study the pharmacological action of prostaglandin F2α injection on ovarian activity, blood picture, gonadal hormones and animal fertility during heat stress. Ten crossbred native cows (Baladi X Abundance), their ages 3-8 years, not showing estrus signs for a mean of 95.28+46.18 days(summer infertility) were treated with a regimen consisted of one or double doses of PGF2α (2.5 ml Estrumate®/animal), the second dose was injected after 9- 11 days for cows not exhibited estrus post 1st dose. The blood samples were from jugular vein, before, during and after treatment. Complete blood picture , Serum progesterone and estradiol-17β were estimated . The response to treatments were determined using rectal palpation and ultrasonography(US). The results revealed that there is no significant differences in RBCs count at different days of the experiment in non pregnant cows, whereas, a significantly lower value (6.11±0.40 vs. 7.38±0.33) was recorded at day 7 in pregnant cows .**Mean Cell Volume (MCV),** in pregnant cows was averaged (49.91 ±2.27 and 49.5 ± 1.99, $p<0.05$) at days 21 and 31, respectively, whereas, in non pregnant cows, a significantly higher value (60.5±1.45) was recorded only at day (7). **Hematocrite value (HCT),** were significantly different($p<0.05$) between pregnant and non pregnant animals. It recorded higher values ($p<0.05$) at days 7&31 in non pregnant animals than that reordered at any other day of the experiment in both pregnant and non pregnant cows. **Hemoglobin (HB): A** significantly lower HB value (104±3.58) was recorded at day (7) of pregnant cows, while the highest value (156.83±13.67) was detected at day 21. Concerning the non pregnant cows, a significantly highest HB value (157.25±8.27) was observed at day 7. However significant differences in HB values were recorded among pregnant and non pregnant animals at days 7, 21, and 31 of the experiment. **WBCs count:** In pregnant cows, a significantly higher WBCs count was recorded at day 0 (12.72±1.19) compared with values that recorded at the other days of the treatment or in non pregnant cows . **Granulocyte percentages (GR %):**A significantly higher values (63.17±0.19, 61.83±1.24) were detected at day 7 in pregnant and non pregnant cows, respectively. A significantly ($p<0.001$) highest level of estradiol was recorded at day 22 (36.64±4.26 pg/ml) and significantly lower one was measured at day 10 (18.61±0.23 pg/ml), whereas, Progesterone(P_4) was significantly decreased at day 10(1.7±0.14/ml ng). The reproductive results in cows treated with double injection of PGF2 indicated that the peak of response of cows ranged from 72 hrs to 96 hrs post inj. of 1st dose of PGF2α, it also appeared the pregnancy rate was 70% (7/10) . It is concluded that $PGF_2α$ double injection (PG-PG) was effective for resuming animal fertility and ovarian activity during summer season without adverse effect on hematological parameters and/or metabolic status of treated animals.

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1. Introduction

In the early 1970s several workers pioneered the luteolytic effect of prostaglandin F2α ($PGF_2α$) in cattle (Rowson *et al.,* 1972). Subsequent research efforts then attempted to improve the reproductive efficiency of dairy cattle by inducing estrus with $PGF_2α$ (Seguin *et al.,* 1978; Plunkett *et al.,* 1984). Several studies demonstrated the capacity of $PGF_2α$ and its synthetic analogues, alfaprostol (Randel *et al.,* 1988; Tolleson and Randel, 1988; Randel *et al.,* 1996), cloprostenol (Cooper, 1974; Cooper and Rowson, 1975), fenprostalene (Martinez and Thibier, 1984; Stotts *et al.,* 1987), luprostiol (Plata *et al.,* 1989; Plata *et al.,* 1990) and tiaprost (Schams and Karg, 1982) to trigger the regression of a mature CL in the ovary, thus provoking and synchronizing estrus

(Stevenson *et al.,* 1989; Stevenson and Pursley, 1994). When $PGF_2\alpha$ was administered to cows with a functionally mature CL, 85 to 95% reached estrus within 7 d of treatment (Armstrong *et al.,* 1989; Folman *et al.,* 1990; Rosenberg *et al.,* 1990); 70 to 90% showing signs of estrus 3 to 5 d after treatment (Ferguson and Galligan, 1993).

Prostaglandin treatment in the early stage of estrous cycle (first 5 d) was found to be ineffective in causing a luteolytic response in cattle (Cooper and Rowson, 1975; Lauderdale, 1975). Consequently, a double protocol in which $PGF_2\alpha$ was given at a 7, 11 or 14 d interval was developed so that cows at a stage in the estrous cycle other than diestrus would have a functional CL when they received the second $PGF_2\alpha$ dose (Rosenberg *et al.,* 1990; Kristula *et al.,* 1992). However, several authors report the improved reproductive efficiency of cows detected to be in estrus after the second PGF2α dose using the double regime in which $PGF_2\alpha$ doses are given 11 or 14 d apart (Ferguson and Galligan, 1993; Stevenson *et al.,* 2000). Further, an enhanced estrus response and normal fertility were reported when $PGF_2\alpha$ was given at the late, rather than early to middle stage of the luteal phase (Watts and Fuquay, 1985; Xu *et al.,* 1997). Thus, the 14 d interval double prostaglandin regimen seems to show an improved response over the 11 d protocol, since two treatments given 14 d apart ensures that most animals are in the late luteal stage (cycle Day 11 to 14) when they receive the second $PGF_2\alpha$ dose (Folman *et al.,* 1990; Rosenberg *et al.,* 1990).

Many researchers have noted normal or above normal fertility following synchronization of estrus with $PGF_2\alpha$ in cows (Lucy *et al.,* 1986; Wenzel, 1991). However, reduced conception rates due to variations in the time of ovulation have been noted after timed AI, either following single (Fetrow and Blanchard, 1987; Archbald *et al.,* 1992) or double (Waters and Ball, 1978; Stevenson *et al.,* 1987) prostaglandin administration, compared to AI at detected estrus. Reproductive performance in dairy cattle was also improved following double 14 d-$PGF_2\alpha$ treatment without assessing ovarian status when compared to a single dose based on detecting a CL by rectal palpation or by milk progesterone enzyme immunoassay (Heuwieser *et al.,* 1997).

Several studies have reported that the stage of estrous cycle at the time of prostaglandin greatly influences the conception rate in dairy cows. Armstrong (1988) reported that the conception rate among the cows treated on Day 13 (71 %) was significantly higher when compared to the cows treated on Day 8 (46 %). Larson and Ball (1992) reported that higher progesterone concentrations at the time of $PGF_2\alpha$ administration are associated with delayed onset of estrus. It has also been reported that estrus was manifested in more percentage of cows (84 %) that had high progesterone concentrations, > 3.1 ng/mL, at the day of the last $PGF_2\alpha$ injection than did cows with low progesterone levels (56 %). Folman *et al.* (1990) found that cows conceiving to AI at induced estrus had higher progesterone levels during the proceeding luteal phase than those not conceiving. However, Gyawu *et al.* (1991) showed that excessively long periods of high progesterone prior to insemination can suppress fertility. Therefore, the aim of the present work was to study the pharmacological action of double doses of $PGF_2\alpha$ on hemogram, ovarian response and some hormonal changes of mixed breed of cows .

2. Material and Methods

The present study was conducted in private sector farm in Beni-Suef Governorate, Upper Egypt, from 2010 to 2011.

1. Animals

This study was conducted on 10 crossbreed cows (Baladi×Abundance). The age of the animals ranged from 3 to 8 years and other parameters are tabulated in table(1). These animals were reared under correct management system including feeding, housing, and veterinary medical care as well as recording system. The selected animals were free from any reproductive disorders. The body condition score (BCS) of these animals was recorded and scaled according to **Gordon (1996)**.

Table 1: General reproductive and productive characteristics of cattle under experimentation.

VARIABLE		
Breed		Crossbreed cows (Baladi×Abundance)
Month of experiment		Summer (August)
Parity	Primiparous	3 (30%)
	Pleuriparous	7 (70%)
Average daily milk yield (kg)		3.5
(B.C.S.)		2.9±0.55
Interval from calving to 1st estrus (days)		95.28±46.18
Age (year)		5.35±2.17

2. Chemicals

Estrumate® (Synthetic prostaglandin)

Each ml contains 263 µg cloprostenol sodium (**BP-vet.**) equivalents to 250 µg cloprostenol (**Schering Plough, Essex Animal Health, and Germany**).

3.Semen: Bull semen no. 91, name Jiscar processed and packaged in mini straws (0.25 ml) at A.I. center , Beni-Seuf , Egypt.

Methods

1. Clinical examination

Ovarian findings:Clinical examination was performed according to **Arthur *et al.*(1982)**. Rectal palpation was done for detection of the ovarian activities at the beginning of each protocol. Follicular consistency was examined rectally at the time of insemination and classified into turgid or fluctuating follicles.

Genital tract: Examination of the genital tract at the time of insemination was done as described by **Arthur *et al.*(1989)**.

Estrus signs: Observations of the animals for signs of heat were done throughout the day from early morning to evening as reported by **Boriek (2002)** and classified into strong and weak estrous signs according to the intensity of nervous manifestation exhibited by cows at estrous.

2.Fertility indices

Rectal examination of the cows for pregnancy diagnosis was done 45-60 days and ultrasound examination at 30 days post insemination. The fertility indices were calculated as described by **Grusenmeyer *et al.* (1992)**.

3.Blood sampling and serum preparation

Blood samples were collected from the jugular vein into two test tubes; one containing anticoagulant (2-3 drops Heparin) for hematological analysis and other test tube containing no anticoagulant for serum preparation for hormonal assay.

A-Hematological examination

The blood parameters were measured by automated Animal hematology analyzer (Animal Hematology analyzer, Model XF-9080).

B- Hormonal assay

The concentrations of Estradiol (E_3) and Progesterone (P_4) in serum were measured by Radioimmunoassay technique (RIA) using the diagnostic commercial kit (Estradiol Coat-A-Count kit) and (Progesterone Coat-A-Count RIA kit) respectively from Siemens Medical Solutions Diagnosis, USA according to **Hiroshi *et al.*(2008)**, then read by γ- Counter (Berthold, Model, MAG 312, Lab of Pharmacology, NRC).

4. Protocol of synchronization for estrous :Double injection of PGF_2a .

A summary of the experimental procedure was carried out according to **Stevenson and Phatak(2005)**.

At Day (0): all cows examined clinically per rectum, blood sampling and applying of the 1st dose of 2.5 ml Estrumate® (I/M). Insemination of cows which detected in heat (2 to 5 days after the 1st dose of Estrumate®). At day (10) of experiment, injection of 2.5 ml Estrumate® (2nd dose) for the not inseminated cows. Two days after 2nd dose heat detection (HD) and insemination (AI) of cows were done. Pregnancy diagnosis by rectal palpation at day 72 of experiment for the inseminated cows. Blood sampling was performed at days 0, 4, 7, 10, 22, 32 and 72 for measuring some hematological parameters and hormones.

Statistical analysis:

Probabilities of the different fertility levels were calculated as expansion of binomial distribution according to the following equation:(mean)

$$\bar{X} = \sum \frac{fx}{\sum f} \quad , \quad S.D. = \sqrt{\frac{\sum fx^2}{n} - \bar{x}^2}$$

$$se = \frac{sd}{\sqrt{n}}$$

S.D= standard deviation se= standard error

n=number **(Thirkettle, 1981).**

The obtained probabilities were multiplied by 100% to obtain the probability %. Analysis of variance was done by calculation of the LSD using the **PC-STAT (1985).**

3. Results

I. **Hematological Parameters.** The investigated blood parameters are presented in table (2).

Red Blood Cells (RBCs) Parameters

Erythrocytes count: Our results revealed no significant differences in RBCs count at different days of the experiment in non pregnant cows. Concerning the pregnant cows, a significantly lower value (6.11±0.40) was recorded at day 7 compared with that recorded at day 0 (7.38±0.33).

Mean Cell Volume (MCV): In pregnant cows, a significantly higher values (49.91 ±2.27) and (49.5 ± 1.99) were recorded at days 21 and 31 respectively compared with values at day 0 (44.46±1.78) and day 7 (41.5±7.07). In non pregnant cows, a significantly higher value (60.5±1.45) was recorded at day (7) compared with those recorded at any other day of the protocol.

Hematocrite value (HCT): Regarding the pregnant animals, a significantly lowest value (0.25±0.003) was detected at day (7) compared with that recorded at other days of the experiment. Meanwhile a significantly higher values (0.38±0.032) and (0.38±0.016) were recorded at days 21and 31, respectively. Moreover there were significant differences in (HCT) values between pregnant and non pregnant animals. In non pregnant animals the (HCT) values at days 7 and 31 were significantly higher than that reordered at any other day of the experiment in both pregnant and non pregnant cows.

Hemoglobin (HB): In pregnant cows, a significantly lower HB value (104±3.58) was recorded at day (7), while the highest value (156.83±13.67) was detected at day 21.Concerning the non pregnant cows, a significantly highest HB value (157.25±8.27) was observed at day 7. However significant differences in HB values were recorded among pregnant and non pregnant animals at days 7, 21, and 31 of the experiment.

White Blood Cells (WBCs) Parameters

WBCs count: In pregnant cows, a significantly higher WBCs count was recorded at day 0 (12.72±1.19) compared with values that recorded at the other days of the treatment. Regarding the non pregnant cows no significant differences in WBCs count were observed among the different experimental days.

Lymphocyte percentages (LY %): The results indicated that no clear significant differences among values at different days of experiment in both pregnant and non pregnant animals.

Granulocyte percentages (GR %): In pregnant cows, a significantly higher value (63.17±0.19) was detected at day 7 compared with those recorded at the other days of the experiment. While in non pregnant cows a significantly highest value (61.83±1.24) was recorded at day (7).

Table 2: Some hematological parameters in cows subjected to $PGF_2\alpha$ double injection regimen.

EXP. Day	Response to treat.	RBC $\times 10^{12}$/L (Mean±SE)	MCV FL (Mean±SE)	HCT L/L (Mean±SE)	HB g/L (Mean±SE)	WBC$\times 10^9$/L (Mean±SE)	LY% (Mean±SE)	GR% (Mean±SE)
0	Pregnant	7.38 ± 0.33^{ba}	44.46 ± 1.78^{a}	0.33 ± 0.025^{b}	133.8 ± 10.69^{bc}	12.72 ± 1.19^{b}	32.4 ± 3.29^{a}	61.58 ± 3.03^{c}
7		6.11 ± 0.40^{a}	41.5 ± 2.24^{a}	0.25 ± 0.003^{a}	104 ± 3.58^{a}	6.59 ± 0.47^{a}	40.6 ± 5.05^{ba}	63.18 ± 0.19^{cd}
21		7.67 ± 0.51^{b}	49.91 ± 2.27^{b}	0.38 ± 0.032^{c}	156.83 ± 13.67^{d}	9.12 ± 1.35^{a}	49.61 ± 19.29^{b}	50.9 ± 1.64^{b}
31		$7.64\pm0.29b^{a}$	49.5 ± 1.99^{b}	0.38 ± 0.016^{c}	118.5 ± 7.24^{ab}	8.05 ± 0.70^{a}	39.17 ± 3.67^{ba}	42.9 ± 1.82^{a}
0	Non Pregnant	6.45 ± 0.15^{ba}	$46.5\pm0.99b^{a}$	$0.31\pm0.013b$	117.16 ± 5.84^{ab}	8.83 ± 0.41^{a}	31.8 ± 1.33^{a}	61.83 ± 1.24^{c}
7		7.495 ± 0.13^{ba}	60.5 ± 1.45^{c}	0.46 ± 0.044^{d}	157.25 ± 8.27^{d}	9.06 ± 0.13^{a}	46.22 ± 2.05^{ba}	74.85 ± 1.34^{d}
21		6.35 ± 0.04^{ba}	47.5 ± 1.12^{b}	0.37 ± 0.025^{c}	141 ± 2.68^{c}	8.87 ± 0.83^{a}	45.9 ± 6.93^{ba}	57.17 ± 2.62^{cb}
31		7.39 ± 0.37^{ba}	51.16 ± 1.46^{b}	0.47 ± 0.041^{d}	149.66 ± 13.76^{c}	9.63 ± 0.71^{a}	32.93 ± 2.41^{ba}	61.53 ± 2.89^{cb}

SE = Standard Error

Values in the same column with different letter are significantly different ($P < 0.05$)

0-day:1st dose of PGF2 &; 7-day: sampling; 21-day: sampling, 31-day:sampling

Table (3): Serum hormonal levels in cows assigned to $PGF_2\alpha$ double inj. Protocol.

Exp. Day	Treatment (activities)	n* (10)	Estradiol (pg/ml) Mean ± SE**	Progesterone (ng/ml) Mean ± SE**
0	$PGF_2\alpha$ 1st dose + sampling	10	29.7 ± 3.67^{ab}	2.06 ± 0.46^{b}
3	Heat detection +Insemination	5	-	-
4	Sampling	10	26.76 ± 3.47^{ab}	3.24 ± 0.89^{b}
5	Heat detection +Insemination	2	-	-
7	Sampling	10	18.72 ± 0.40^{a}	2.3 ± 0.58^{b}
10	Pgf2α 2nd dose + sampling	3	18.61 ± 0.23^{a}	1.7 ± 0.14^{a}
12	Heat detection +Insemination	3	-	-
22	Sampling	10	36.64 ± 4.26^{b}	2.10 ± 0.88^{b}
32	Sampling	10	19.40 ± 0.98^{a}	3.83 ± 1.01^{b}
72	Pregnancy diag. + sampling	10	19.78 ± 1.33^{a}	2.27 ± 0.15^{b}

n=number of animals SE = standard Error

Values in the same column with different letter are significantly different ($P < 0.05$)

Table 4: Response and fertility results of cows subjected to PGF2α double inj. protocol

VARIABLE	Results	
Cyclicity before treatment n. (%)	39.6% normal cyclic	
	20.4% repeat breeder	
	40% non cyclic	
Distribution of estrus RESPECTIVELY onse in cows treated with $PGF_2\alpha$ inj.	Post 1st dose	20% after120hrs (2/10) 50% after72hrs (5/10)
	Post 2nd dose	20% after 48hrs2/10
Rectal findings at time of A.I.	ovarian findings -follicular consistency	20% turgid follicles
		80% fluctuating follicles
	consistency of uterus	20% slightly tonic

		80% erected
Pregnancy rate % (n)	70% (7/10)	

4. Discussion

To synchronize the estrous cycle, ovarian activity is manipulated so that the time of ovulation can be predicted. This is achieved by (1) controlling the luteal phase of the cycle through the administration of prostaglandins or progesterone analogues or (2) controlling follicle development and ovulation using different combinations of prostaglandins, progesterone or gonadotrophin releasing hormone (GnRH).

Modern estrus synchronization protocols involve either lengthening or shortening the animal's estrous cycle to achieve synchrony. A variety of techniques are available for producers to utilize and all are based on several strategies of hormonal supplementation including progestin, $PGF_2\alpha$ and gonadotropins (Odde and Holland, 1994; Ryan *et al.,* 1995).

The results of the present study revealed significant changes in some erythrocytic parameters including (RBCs count, MCV, HCT and HB) but these changes within the normal blood value of cattle according to Nemi (1986) and Victor *et al.*(2000) .

Many protocols involving exogenous administration of $PGF_2\alpha$ have been developed and the normal treatment regimen for $PGF_2\alpha$ consists of two injections spaced 10 to 14 d apart (MARK, 2002).

The results of the current study revealed that, cows synchronized with $PGF_2\alpha$ varied in response to treatment according to stage of cycle at the beginning of experiment as reported by King *et al.* (1982); Tanabe and Hann (1984).who reported that, The main factors affecting synchrony when using $PGF_2\alpha$ treatment was the stage of the estrous cycle and the sensitivity of the CL to $PGF_2\alpha$ administration, it was greatest on d 10 of cycle, also MARK (2002) added the theory behind this treatment is at least one injection will be administered during the middle stage of the estrous cycle, and in theory, all cattle should be responsive to $PGF_2\alpha$ at this time. When cattle were injected with $PGF_2\alpha$ during d 5-16 of the estrous cycle a return to estrus was observed within 2 to 4 d.

Our results reveal that the peak of response of cows after 1st injection ranged from 2-5 days, where 70% of responsive animals exhibited estrous within that period and the rest of responsive animals come in heat 48hrs post 2nd dose of $PGF_2\alpha$. These findings may be attributed to the type of follicular structure present at time of injection, such findings coincides with that obtained by De Rensis and Lopez (2007) , who recorded that the effect of $PGF_2\alpha$ administration is very similar to that observed in cattle and the time interval between $PGF_2\alpha$ treatment and the onset of estrus in buffaloes varies according to the stage of follicular development at the time of PGF2 alpha administration. Animals treated when follicles are in the pre-dominance stage (DF) of development display estrus 4-6 days later, whereas animals treated in the presence of a DF display estrus 23 days after $PGF_2\alpha$ administration. Thus, since the intervals between treatment, estrus, and ovulation vary after PGF2alpha administration, a timed artificial insemination protocol cannot be applied. The author of this latter study reported that the mean interval from $PGF_2\alpha$ to estrus was 88hrs (range 48-144hrs), $PGF_2\alpha$ induced estrus and ovulation in about 60-80% of treated animals.

The results of our study revealed that 10% of treated animals not respond for the treatment, these results can be explained in the light of published report by Kojima *et al.(*2000) who indicated that, cattle that failed to respond to the 2nd $PGF_2\alpha$ injection had either a cystic follicle or early developing CL.

Our results revealed overall pregnancy rates was 70%, these results are higher than those found by De Rensis and Lopez (2007) who indicated that, pregnancy rates following prostaglandin treatment are 45-50% on average and appear to be similar to those obtained after natural estrus.

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Corresponding author:
Abdel Mohsen M. Hammam
Department of Animal Reproduction and A.I., Veterinary Division, National Research Centre, Dokki, Cairo, Egypt
e-mail:hamam-pharma2010@hotmail.com

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