Global Journal of Pharmacology 7 (4): 383-389, 2013
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2013.7.4.8179

# Effect of CIDR -Ovsynch Regimen as a Pharmacological Agent on Fertility, Blood and Hormonal Patterns of Cross Breed Cows

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**Abstract:** The development of controlled intravaginal progesterone-releasing devices (CIDR), hastened resumption of ovarian follicular activity. Various combinations of GnRH analogues and $PGF_2\alpha$ have been used to initiate ovarian cyclicity. Another approach is the use of timed insemination protocols (The Ovsynch program), but the responses to treatments are not uniform either across herds or across cows within herds. Therefore, the present work aimed to study the pharmacological action of CIDR with $PGF_2\alpha$ and GnRH on blood and some biochemical parameters of cross breed cows. Twelve crossbreed cows (Baladi×Abundance), 8 primiparous (66.7%) and 4 pleuriparous (33.3%), their ages averaged 3.5±1.87 years, not exhibiting estrus signs for duration up to 127.33±46.98 days, were treated with CIDR-OvSynch protocol (CIDR+$PGF_2\alpha$ -GnRh). Cows were injected with 2.0 ml Estrumate® ($PGF_2\alpha$, 250 ug/ml cloprostenol) I/M. at day (8) CIDR was inserted intravaginal, with injection of Receptal® (2.5ml), CIDR was removed at day (18) with injection of 2nd dose of Estrumate® (500 ug/animal), I/M. At day (20), injection of 2.5ml Receptal®, I/M. Then at day (21), timed artificial insemination (T.A.I.). Blood samples were collected from jugular vein along the experimental period. Complete blood picture, serum progesterone and estradiol-17β were assayed. The response to treatments were determined using rectal palpation and ultrasonography (US). The results revealed that no marked changes were observed in blood parameters (RBCs, MCV, HCT, HB, WBCs, LY% & GR%) and slight fluctuations were recorded (within the normal value) after treating with CIDR, $PGF_2\alpha$ (prostaglandin F2 alpha) and GnRh protocol. A high estradiol level (38.6±1.79pg/ml) was recorded at day (21) at fixed TAI and lowest one (21.79±1.61pg/ml) was detected at day (42). A higher P4 value (10.98±3.51ng/ml) was recorded at day (16) and a significantly lowest one (1.16±0.40ng/ml) was recorded at day (21). The ovulation rate was 60%, while the pregnancy rate was 50% (6/12) in cows treated with CIDR+OvSynch protocol. It is concluded that the hormones used in CIDR-OvSynch protocol (Progesterone, $PGF_2\alpha$ &GnRh), stimulated the ovarian activity of cows and can resume the ovarian activity during summer season, improved pregnancy rate of subfertile cows and the hormones used had no adverse effect on hematological parameters and/or endogenous steroid hormones of treated animals.

**Key words:** CIDR · Gnrh · $PGF_2\alpha$ · Ovsynch · Cows · Hormonal Levels · Synchronization

## INTRODUCTION

Synchronization of estrous cycle in cows usually based on progestagens and prostaglandin (PG) or its analogues. The early methods of administrating progestins for synchronization of estrus was by daily injections [1], oral administration of melengestrol acetate (MGA) or medroxyprogesterone acetate (MPA) [2] and Progestagens had been administered intravaginally by means of impregnated sponge pessaries [3,4], which, in theory, permit a more precise treatment of individual animals or by norgestomet in silastic coated coils, the progesterone releasing intra-vaginal device [5,6] or by silicone rubber impregnated with progesterone, controlled intravaginal drug release (CIDR) [7].

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A 7-day GnRH-$PGF_2\alpha$ synchronization protocol can be effectively induces ovulation in dairy cows regardless of ovarian status [8].

In general, exogenous progestins are considered appropriate for noncyclic or anestrous postpartum cows [9]. The development of controlled intravaginal progesterone-releasing devices, or an intravaginal progesterone insert (IPI), hastened resumption of ovarian follicular activity. The IPI facilitated hormone treatments and circumvented delivery problems associated with feeding or injecting progestins. At the end of IPI treatment, hormones such as equine chorionic gonadotropin [10], estradiol, or $PGF_2\alpha$ have been given to maximize the response (resumption of follicular activity). Recently, the study of Lo´pez-Gatius *et al.* [11], 64.2% (571 of 889) of cows classified as ''subestrus or ovarian hypofunction'' group responded to 9 d of IPI treatment followed by an injection of $PGF_2\alpha$. Furthermore, estradiol or GnRH analogues have been used prior to IPI treatment [12].

Various combinations of GnRH analogues and $PGF_2\alpha$ have been used to initiate ovarian cyclicity. Another approach is the use of timed insemination protocols as the Ovsynch program [13]. In the various hormonal treatments listed above (GnRH, $PGF_2$ and Estradiol), responses to treatments are not uniform either across herds or across cows within herds; they appeared to be dependent on factors influencing the prevalence of anestrus (e.g. age, body condition and postpartum interval).

The progesterone released from the CIDR inserted was sufficient to increase and maintain a progesterone concentration in blood higher than 2.0 ng/mL in the absence of CL on the ovary [14]. Blood P4 concentrations rises rapidly to peak concentrations approximately 1h after CIDR insertion. These concentrations decline rapidly over a 12 to 24 hrs period upon CIDR removal [15,16].

Concentrations of estradiol during the Ovsynch increased with follicle diameter and were greater for Holstein cows receiving CIDR-PS protocol than Presynch [17]. Moreover Stevenson *et al.* [18] found that, heifers receiving $PGF_2\alpha$ had larger ovulatory follicles on day 7 and before ovulation, these heifers tended to have decreased concentrations of estradiol $E_2$ during proestrus. Progesterone concentration was greater in all progesterone treated heifers on day 2 and 6 than late-$PGF_2\alpha$ treated heifers on day 7 and 8 [18]. Colazo *et al.*[19] found that, plasma progesterone concentrations were lower in cows of intact ovary which received repeated GnRH treatments than in control cows.

It is important that effective estrous synchronization protocols are developed in order to increase the use of A.I. In addition, estrous synchronization protocols should be designed to reduce time and labor inputs by limiting cattle handlings and reducing or eliminating estrus detection[20].

The main purpose of this study was to evaluate the effect of OvSynch and progesterone supplementation (CIDR) in the course of OvSynch protocol on ovarian response, hematological parameters, some steroid hormones pattern (Progesterone and estradiol) and pregnancy rate in cross breed cows.

## MATERIAL AND METHODS

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

**Animals:** This study was conducted on 12 crossbreed cows (Baladi×Abundance). The age of the animals ranged from 3 to 6 years (3.5±1.87 years). 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 3.4±0.95 and scaled according to Gordon [21].

The general characteristics of the used animals and types of the applied protocol are presented in Table (1)

**Chemicals/Drugs:** Estrumate® (Synthetic prostaglandin): Each ml contains 263 µg cloprostenol sodium (BP-vet.) equivalents to 250 µg cloprostenol (Schering Plough, Essex Animal Health and Germany).

Receptal ® (Gonadotropin releasing hormone): Each ml contains 0.0042 mg buserelin acetate equivalent to 0.004 mg buserelin, 10 mg benzyl alcohol (Intervet International B.V. Boxmeer, Holland).

CIDR EAZI-BREED® (Controlled Intravaginal Drug Release device):

Each EAZI-BREED CIDR cattle insert contains 1.38 grams of progesterone in molded silicone over a flexible nylon spine accompanied with special gun for its insertion into vagina (Pharmacia & Upjohn Company Kalamazoo, Michigan, USA, Registered to and marketed by: Pfizer New Zealand Ltd).

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

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

| Variable | Cows |
|---|---|
| Animal No. | 12 |
| Breed | Crossbreed cows (Baladi×Abundance) |
| Month of experiment | Autumn (November) |
| Parity Primiparous | 8 (66.7%) |
| Pleuriparous | 4 (33.3%) |
| Average daily milk yield (kg) | 6.0 |
| B.C.S. | 3.4±0.95 |
| Interval from calving to 1st estrus (days) | 127.33±46.98 |
| Age (year) | 3.5±1.87 |
| Treatments | CIDR+ OvSynch |

## Methods

**Clinical examination:** Ovarian findings: Clinical examination was performed according to Arthur *et al.* [22]. Rectal palpation was done for detection of the ovarian activities at the beginning of treatment.

**Genital Tract:** Examination of the genital tract at the time of insemination was done as described by Arthur *et al.* [23].

**Estrus Signs:** Observations of the animals for signs of heat were done throughout the day from early morning to evening as reported by Boriek [24] and classified into strong and weak estrous signs according to the intensity of nervous manifestation exhibited by cows at estrous.

**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.* [25].

**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.

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

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

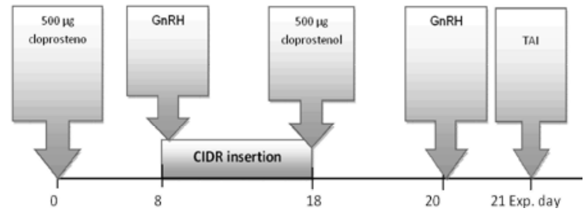


Fig. 1: CIDR Ovsynch protocol in cows

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

S.D= standard deviation se= standard error
n=number [26].

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).

**Method for Estrus Synchronization: *CIDR Ovsynch Protocol of Cows (CIDR+PG)*:** Summary of the experimental procedure was carried out according to Busch *et al.* [27]. On day (0) cows examined clinically per rectum, blood sampling and injection of 2ml Estrumate® ($PGF_2\alpha$) I/M. At day (8), CIDR was inserted intravaginal. At day (18), CIDR was removed and the 2nd injection of 2ml Estrumate® ($PGF_2\alpha$), I/M. At day (20), injection of 2.5ml Receptal®, I/M. At day (21), timed artificial insemination (T.A.I.). Pregnancy diagnosis by rectal palpation at day 60 post A.I. for the inseminated cows. Diagram of the protocol was illustrated in Fig.(1).

## RESULTS

**Hematological Parameters in Cows Subjected to Cidr-ovsynch Protocol:** The investigated blood parameters are presented in Table (2).

### Red Blood Cells (RBCs) Parameters

**Erythrocytic Count:** Our results revealed significantly higher erythrocytic counts at days 8 (6.01±0.23), 21 (7.16±0.26) and 28 (7.08±0.25 $x10^{12}$/L) than those at day (0) in both conceived (3.97±0.26) and non conceived (3.06±0.15 $x10^{12}$/L) cows.

**Mean Cell Volume (MCV):** The present study revealed no significant differences in MCV among the different treatment days in the same group. Furthermore in pregnant cows the MCV value at day 0 (48.25±1.95) was significantly higher than that recorded at the same day (40.25±0.71) in non pregnant cows.

Table 2: Some hematological parameters in cows subjected to CIDR-Ovsynch protocol

| EXP. Day | Animal condition after treatments | RBC×$10^{12}$/L (Mean±SE) | MCV FL (Mean±SE) | HCT L/L (Mean±SE) | HB g/L (Mean±SE) | WBC ×$10^9$/L (Mean±SE) | LY% (Mean±SE) | GR% (Mean±SE) |
|---|---|---|---|---|---|---|---|---|
| 0 | Pregnant | 3.97±0.26$^a$ | 48.25±1.95$^b$ | 0.2±0.020$^a$ | 125.25±5.29$^a$ | 8.59±0.51$^{ba}$ | 29.41±1.11$^a$ | 64±1.27$^c$ |
| 8 | | 6.01±0.23$^b$ | 49.13±1.11$^b$ | 0.3±0.017$^{ab}$ | 122.5±8.31$^a$ | 9.8±1.07$^{bc}$ | 36.51±1.83$^{bc}$ | 57.91±1.71$^b$ |
| 21 | | 7.17±0.26$^b$ | 48.33±1.68$^b$ | 0.35±0.017$^b$ | 148.58±10.20$^b$ | 9.24±1.13$^{bc}$ | 32.65±1.34$^{ba}$ | 60.15±1.64$^{bc}$ |
| 28 | | 7.09±0.25$^b$ | 46.9±1.59$^b$ | 0.34±0.020$^b$ | 154.5±4.62$^b$ | 7.34±0.69$^a$ | 28.88±3.88$^{ba}$ | 64.81±4.23$^c$ |
| 0 | Non Pregnant | 3.06±0.15$^a$ | 40.25±0.71$^a$ | 0.21±0.038$^a$ | 148.25±7.45$^b$ | 10.73±0.22$^c$ | 31.63±1.78$^{ba}$ | 61.72±1.72$^{bc}$ |
| 8 | | 7.64±0.36$^b$ | 47.5±1.77$^b$ | 0.36±0.029$^b$ | 125.46±10.42$^a$ | 9.26±1.04b$^c$ | 40.09±1.18$^c$ | 51.85±1.10$^a$ |
| 21 | | 7.51±0.42$^b$ | 48.5±1.55$^b$ | 0.37±0.109$^b$ | 179.75±7.91$^c$ | 9.61±1.37b$^c$ | 34.01±3.57$^b$ | 60.75±3.67$^{bc}$ |

SE= standard Error.

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

0-day: 1st dose of PGF2α; 8-day: CIDR insertion+1st dose of GnRH; 21-day: (TAI); 28-day: sampling

Table 3: Serum hormonal concentration in cows subjected to Ovsynch - protocol

| Exp. Day | Treatment (activities) | n*(12) | Estradiol( pg/ml) means ± SE** | Progesterone (ng/ml) means ± SE** |
|---|---|---|---|---|
| 0 | $PGF_2\alpha$ inj. | 12 | 24.38±3.76$^a$ | 3.49±0.97$^{ab}$ |
| 8 | CIDR insertion + GnRh inj. (1st dose) | | 28.25±3.08$^{ab}$ | 8.79±3.27$^{ab}$ |
| 16 | Sampling | | 26.54±2.65$^a$ | 10.98±3.51$^b$ |
| 18 | CIDR removal + $PGF_2\alpha$ inj. | | - | - |
| 20 | GnRh inj. (2nd dose) | | - | - |
| 21 | (TAI) | | 38.6±1.79$^b$ | 1.16±0.40$^a$ |
| 42 | Sampling | | 21.79±1.61$^a$ | 5.85±1.59$^{ab}$ |
| 53 | Sampling | | 25.32±2.23$^a$ | 8.3±1.44$^{ab}$ |

•n=number of animals

•SE = standard Error

•Values (Means) in the same column with different alphabetical are significantly different ($P < 0.05$)

Table 4: Main reproductive parameters in cows modified CIDR protocol (CIDR+PG-GnRH)

| VARIABLE | Results | |
|---|---|---|
| Cyclicity before treatment n. (%) | 50% normal cyclic<br>25% repeat breeder<br>25% non cyclic | |
| Ovulation rate | 60% | |
| Rectal findings at time of A.I. | Ovarian findings -follicular consistency | 40% turgid follicles<br>60% fluctuating follicles |
| | Consistency of uterus | 42% slightly tonic<br>58% erected |
| Pregnancy rate % (n) | 50% (6/12) | |

**Hematocrite Value (HCT):** In pregnant animals, a significantly higher HCT values were recorded at days (8, 21 and day 28) than day 0 (0.2±0.020) of experiment. In non pregnant animals the fluctuation of HCT values nearly followed the same trend of pregnant animals.

**Hemoglobin (HB):** Our results revealed no significant differences in HB values at the different experimental days in pregnant animals. Meanwhile in non pregnant animals, a significantly highest HB value (179.75±7.91) was recorded at day 21 of the experiment compared with those recorded at the rest of experimental days.

**White Blood Cells (WBCs) Parameters:** WBCs count: Our results revealed no significant differences in WBCs count at the different experimental days in non pregnant animals. Meanwhile a significantly lowest one (7.34±0.69x$10^9$/L) was detected at day (28) in pregnant animals.

**Lymphocyte percentages (LY%):** In pregnant animals, our results revealed a significantly higher LY % (36.51±1.83) at day (8) of the experiment compared with the rest of experimental days. In non pregnant cows the LY % values followed the same trend.

**Granulocyte Percentages (GR%):** In pregnant cows our results revealed, no significant difference among GR %values at different days of the experiment, meanwhile a significantly lower GR % (57.91±1.71) was recorded at day (8) compared with that recorded at day 0 (64.0±1.27). In non pregnant cows values of GR % nearly followed the same trend of pregnant cows.

## Hormones Level

**Estradiol Serum Level:** The hormonal data presented in Table (3) showed a highly significant variation (*P* value is 0.0006) in estradiol levels among the different experimental days, with a higher value (38.6±1.79 pg/ml) was recorded at day 21 and the lower one (21.79±1.61 pg/ml) was recorded at day 42.

**Progesterone Serum Level:** The obtained results revealed significant (*P* value is 0.0339) differences in P4 levels among experimental days, with a highest level (10.98±3.51 ng/ml) was recorded at day (16) and lowest one (1.16±0.40 ng/ml) was recorded at day 21.

**Main Reproductive Parameters of Cows Treated with Modified CIDR Protocol (PG-CIDR+PG-GnRH):** The results are presented in Table (4). The pregnancy rate post treatments averaged 50% of cows subjected to CIDR-OvSynch protocol.

## 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 [28, 29].

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 [30] and Victor *et al.* [31].

The results of the current study revealed a significant increase in RBCs count and MCV, 7 days after injection of GnRh; however these elevations in RBCs and MCV within normal blood value range of cattle, meanwhile GnRh had no significant effect on HCT, HB, WBCs, lymphocyte and granulocyte. These finding might attributed to GnRh, where it had no significant effect on the metabolic and/or the healthy status of treated animals as reported by Victor *et al.* [31].

Our results revealed that progesterone releasing intravaginal device or controlled intravaginal releasing progesterone (CIDR) had no significant effect on some hematological parameters such as erythrocytic parameters and WBCs at time of insertion or removal in cows, moreover the fluctuation in averages in erythocytes parameters and WBCs within normal blood values of cattle as mentioned by Nemi [30].

These findings came in agreement with Victor *et al.* [31]; they found no significant changes in the treated animals with progesterone releasing intravaginal device (PRID) both responsive and non responsive; therefore protocols depend upon exogenous progesterone doesn't affect the healthy and /or the metabolic status, The findings of erythrocyte in animals treated with CIDR ovsynch protocol showing lower erythrocytic count at the beginning of the experiment could be attributed to non cyclic condition in cross breed cows, these data were parallel to that achieved by Ijaz *et al.* [32] who also indicate that PCV, MCV, lymphocyte, monocyte and basophil percentages did not differ among cyclic, noncyclic and endometritic conditions cows however concluded that lower erythrocytic indices could be attributed to non-cyclic condition in crossbred cows.

Concerning to the effect of treatments on fertility of cows, the results revealed that pregnancy rate in cows subjected to CIDR Ovsynch protocol was 50%, these finding to some extent in agreement with data obtained by Busch *et al.*[27] who recorded that pregnancy rate after CIDR protocol were significantly greater (62%) compared to other protocols, these results can be explained in the light reports De Rensis *et al.*[33], who observed a high significant difference in conception rate when progesterone was used with the Ovsynch protocol in cyclic buffaloes. In heifers in non-cyclic cows the likelihood of conception was greater in Ovsynch protocol with progesterone supplementation compared to the standard Ovsynch protocol. It is likely that the addition of progesterone to the Ovsynch protocol may be affected by a number of variables such as age, post-partum interval and ovarian follicle development. Pregnancy rates in cows are positively associated with higher progesterone concentration in the luteal phase of the cycle preceding Al. This is substantiated by the findings of improved endometrial morphology following elevated progesterone concentrations of the preceding cycle. In addition, ovulation induced by GnRH administration results in a high proportion of pre-mature luteal regression that can be avoided by priming with exogenous progesterone.

Our findings confirm the important role of progesterone in priming the follicle to respond to the Ovsynch protocol and that progesterone supplementation to the Ovsynch protocol stimulates ovarian activity in non-cyclic animals.

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## ACKNOWLEDGEMENTS

We extend our gratitude to PIs of the US-Egypt project (BIO9-001-009) entitled: "*Improvement of fertility of cattle and buffaloes during summer season*" for the financial and technical supports to the work. The team work also thank Dr. Scott Whisnant, Department of Animal Science, College of Agriculture and life Sciences, North Carolina State University USA, for providing CIDR.

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