

# Comparative study of some hormonal treatment regimens for inactive ovaries in cross- breeds cows

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**Abstract:** This study was conducted to investigate the efficiency of different hormonal regimes used for treated post-partum inactive ovaries in local cross breed cows. The experimental conducted between October 2019 to April 2020. Thirty anestrous lactating cross – breed cows suffered from inactive ovaries at day 60-90 days postpartum their age was between 3-5 years old in Al-Zab city / Kirkuk province. These cows were divided randomly into three equal groups (10 cow on each one) according to the type of treatment was used, 1st group was treated by CIDR for 10 days and immediately withdrawal CIDR injected with 500 IU/ PMSG/ IM, 2nd group was treated with injected 500 IU/ PMSG/ IM, 3rd group was treated by CIDR only inserted intra-vaginal for 10 days, cows observed estrus were inseminated artificially. The reproductive performances (estrus response, estrus duration, no. of services / conception, pregnancy rates and days open) of treated cows were recorded. Plasma progesterone (P4) and estrogen concentrations were estimated from each treatment group before and after treatment. The result of present study demonstrated that the using CIDR alone or with combination with PMSG revealed high percentage estrus response (80% and 100% respectively) compared with PMSG alone (40%) and shorter duration period ( $2.4 \pm 0.63$  days,  $3.0 \pm 0.84$  days and  $4.75 \pm 0.72$  days for CIDR with PMSG, CIDR alone and PMSG alone respectively), also, the hormonal regime of CIDR with PMSG and CIDR alone showed higher pregnancy rate (90% and 87.5 % respectively) and shorter days open ( $107.8 \pm 8.36$  days and  $103.6 \pm 6.43$  days respectively) than PMSG 500 IU alone (75% and  $119.7 \pm 7.52$  days for pregnancy rate and days open respectively). higher ( $p < 0.05$ ) concentration of progesterone and estrogen hormones were recorded after treatment period in G1 ( $1.52 \pm 0.08$  ng / ml and  $15.92 \pm 3.51$  pg/ml respectively) and G3 (and  $1.21 \pm 0.03$  ng / ml and  $14.22 \pm 2.56$  pg/ml respectively) compared with G2 ( $0.77 \pm 0.02$  ng / ml and  $10.15 \pm 2.17$  pg / ml respectively). In conclusion the use of CIDR+PMSG or CIDR have more potency for improvement reproductive performance (estrus response, duration of response, pregnancy rate and days open) and each hormones of progesterone and estrogen in local cross breed cows suffering from post- partum inactive ovaries.

**Keywords:** Inactive Ovary, PMSG, CIDR, Cross breed, Days open.

## 1. Introduction

Reproductive improvement is one of the most important method which affect the evaluation of the dairy farm profitability and development of national economy (Roberts and Walter, 2007; Walsh et al., 2011). The ovaries play a main role in reproductive performance and any defect in their function mainly causes inactive ovaries (Noakes, 1996; Lucy, 2001). Anestrous was generally defined as the state of ovarian a cyclicity, reflected by complete sexual inactivity without manifestation of estrus (Khamas and Radhi, 2013; Wright and Malmo, 1992). It is associated with the presence of static ovaries, and even though there is follicular development, none of the ovarian follicles that start growing becomes mature enough to ovulate.

Various research workers have obtained satisfactory results by the use of different hormonal preparations to stimulate the hypothalamic-endocrine axis and initiate ovulation and resumption of normal cyclicity of anestrous in cows (Singh and Singh 1986; Aminudeen, 1991).

A variety of pregestational compounds have been administered (Malik,2005; Haddawi et al.,2013) to mimic the luteal function by blocking the release of gonadotrophins from pituitary, so that subsequent withdrawal of these compounds may result in release of gonadotrophins to initiate follicular activity in ovaries with establishment of estrous cycles. Recently studies depend on many programs have been used to treat inactive ovaries such as hormonal treatment include gonadotropin releasing hormone, CIDR or PRID plus eCG. The CIDR has been incorporated into a wide variety of estrus control programs in many countries and many research trials, with this insert have been performed (Kasabe,2012; Peters and Purstey, 2003). Therefor the present study was conducted to evaluate the efficiency of different hormonal regime on reproductive performance in the local cross breed cows suffering from post-partum inactive ovaries.

## **2. Materials and methods:**

### **Experimental Animals**

This study was performed on 30 lactating cross-breed cows from Oct. 2019 – April 2020. The animals aged between 3-5 years old in Al-zab city /Kirkuk province, all animals were diagnosed as inactive ovaries after 60 – 90 days from postpartum period. Cows were divided randomly into three equal groups (10 cows for each group), the 1st group (G1) treated by inserting CIDR (EaziBreed CIDR. Hamilton-Newzeland contain 1.38gm progesterone) intra-vaginal for 10 days and immediately injected with 500 IU/PMSG /IM (OVEJERO. LEON –Spain) after CIDR withdrawal in day 10, the 2nd group (G2) treated by injected with 500 IU/PMSG/IM (OVJERO. LEON –Spain), while the 3rd group treated only with CIDR (EaziBreed. Hamilton-Newzeland contain 1.38gm progesterone) inserted intra-vaginal for 10 days.Each animals failed to exhibit estrus about 60 to 90 days after parturition were examined by rectal palpation to detect cows suffered from inactive ovaries which are characterized by small smooth ovaries (about 1-2 cm) without any structures protrude from the outside of ovarian surface.

### **Estrus detection and insemination:**

Heat detection was carried by visual observation (twice daily, 6 am and 6 pm) for at least 30 minutes to detect typical signs of estrus (restlessness, bellowing, erected teats and decrease milk) and this sign confirmed by rectal examination (turgid, coiled, edematousuterus and horns besides clear vaginal mucus). Cows in all the treatment protocols that were observed in estrus were bred twice at observed estrus, with AI using fertile frozen thawed semen at 8 to 24 hours after the onset of estrus and the second AI was done 24 hours later.

**Pregnancy Diagnosis:** Pregnancy was diagnosed by rectal palpation per rectum at 60 days post AI. and the pregnancy confirmed when the present fluctuating enlargement in one horn of uterus with present CL in the same side of distended horn. Animals response (animals showed estrus after treatment), duration of response (the days from ending treatment to estrus), number of cervices/conceptions, pregnancy rate and days open (the duration between calving to first fertile estrus) are recorded for each group.

### **Blood Sampling hormones estimation:**

10 ml of blood samples from the jugular vein were collected from each cow before starting of treatment and after four days from ending treatment protocols for progesterone and estrogen concentration estimation. Serum was separated and stored at -18°C until analyzed. Serum progesterone and estrogen concentration was measured by Electrochemiluminescence immunoassay "ECLIA" with especial kit was provided by Roche Company, Germany.

### Statistical Analysis:

SAS, (2012) Statistical Analysis System- program was used to detect the effect of difference factors in study parameters. Chi-square test and Fisher's exact test in IBM SPSS Statistics version 20 were utilized to know the success rate of different treatment protocols on postpartum anestrus.

### 3. Results:

#### Reproductive performance results:

The results of the current study, as shown in table (1) revealed that the response rate to the onset of estrus was higher ( $p < 0.05$ ) in the first group of animals which was treated with CIDR for 10 days with injected the PMSG (500 IU) at the time of CIDR withdrawal (100%) and third group which was treated with CIDR alone for ten days (80%) Compared with the second group treated with only PMSG (500 IU) injection as the response rate was (40%). The duration of estrus response in the first and third group was significantly ( $P < 0.05$ ) lesser than in the second group ( $2.4 \pm 0.63$  days,  $4.75 \pm 0.72$  days and  $3.0 \pm 0.84$  days for first, second and third respectively).

Table 1: The effect of different treatment programs on animals Response and duration of response in local cross – breed cows

| Groups | No. of inseminated animals | No. of service/<br>Conception<br>M $\pm$ SE | Pregnancy rate<br>No. % | Days open M $\pm$ SE  |
|--------|----------------------------|---------------------------------------------|-------------------------|-----------------------|
| G1     | 10                         | 1.2 $\pm$ 0.3<br>a                          | 9 90<br>a               | 107.8 $\pm$ 8.36<br>b |
| G2     | 5                          | 1.54 $\pm$ 0.02<br>a                        | 3 75<br>b               | 119.7 $\pm$ 7.52<br>a |
| G3     | 8                          | 1.25 $\pm$ 0.04<br>a                        | 7 87.5<br>a             | 103.6 $\pm$ 6.43<br>b |

local cross breed cows.

\*Different small letters mean sig. differences ( $p < 0.01$ ) between groups

#### Different small letters mean sig. differences ( $p < 0.05$ ) between groups

The current study demonstrated that the average number of insemination (artificial insemination) per conception did not show any significant difference ( $p < 0.05$ ) among all treatment programs used for treated inactive ovaries in post-partum period in local cross breed cows ( $1.2 \pm 0.3$ ,  $1.25 \pm 0.04$  and  $1.54 \pm 0.02$  for first, second and third groups respectively), while the pregnancy rate was significantly higher ( $p < 0.01$ ) in the animals group treated with CIDR for 10 days with PMSG (500 I.U.) injection at CIDR withdrawal (90%) and third group treated with CIDR only for ten days (87.5 %) compared with the second group treated with injection of PMSG (500 I.U.). also, the results of current study revealed (table 2) that the treatment programs with CIDR for ten days combined with 500 iu PMSG at the time of CIDR withdrawal and CIDR alone for ten days used for treated post-partum inactive ovaries in local cross breed cows have lesser ( $p < 0.01$ ) average number of open days ( $107.8 \pm 8.36$  and  $103.6 \pm 6.43$ , respectively) compared with the group treated with an injection with 500 iu of PMSG alone ( $119.7 \pm 7.52$  days).

Table 2: the efficiency of different treatment programs on No. of service/Conception, Pregnancy rate and Days open in

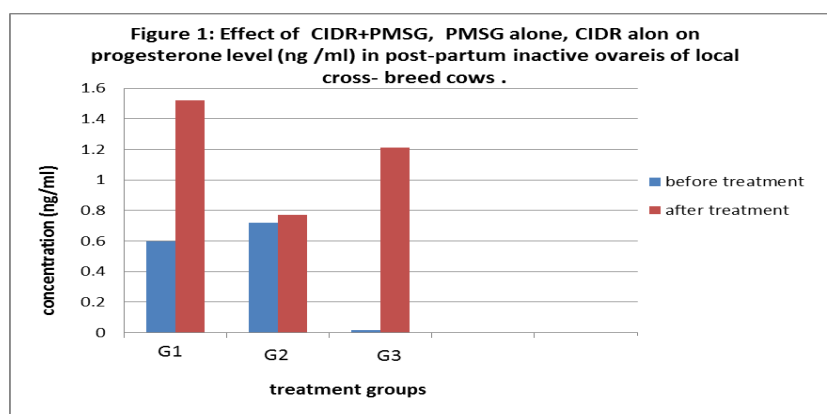
| Groups | No. of cows | Type of treatment                                        | Response animals<br>No. | %   | Duration of<br>Response(days)<br>M±SE |
|--------|-------------|----------------------------------------------------------|-------------------------|-----|---------------------------------------|
| G1     | 10          | CIDR(1.38gm)<br>intravaginal/10 days + PMSG<br>500 IU/IM | 10                      | 100 | 2.4± 0.63<br>b                        |
| G2     | 10          | PMSG 500 IU/IM                                           | 4                       | 40  | 4.75± 0.72<br>a                       |
| G3     | 10          | CIDR(1.38gm)<br>intravaginal/10 days                     | 8                       | 80  | 3.0 ± 0.84<br>b                       |

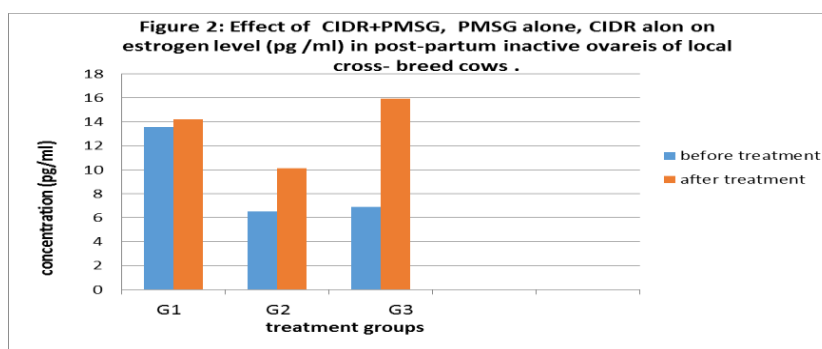
#### Progesterone and estrogen levels:

serum progesterone concentration in the present study are shown in Figure (1) progesterone concentration in cows groups treated with CIDR for 10 days with injected 500 IU of PMSG(G1) and CIDR alone (G3) were significantly higher ( $P < 0.05$ ) in the period after treatment than before treatment ( $0.60 \pm 0.01$  ng / ml and  $1.52 \pm 0.08$  ng / ml before and after treatment respectively).

Also, higher ( $p < 0.05$ ) concentration of progesterone hormone was recorded after treatment period in animals group treated with the CIDR for 10 days with 500 IU of PMSG injections immediately after CIDR withdrawal (G1) and CIDR alone (G3) for the same periods ( $1.52 \pm 0.08$  ng / ml and  $1.21 \pm 0.03$  ng / ml respectively) compared with the 2nd group treated with 500 iu of PMSG alone ( $0.77 \pm 0.02$  ng / ml).

Serum concentration of estrogen in the local cross breed cows (Figure: 2) was significantly ( $P < 0.05$ ) higher after treatment in the animals group treated with CIDR alone and CIDR combined with PMSG 500 IU at the time of CIDR withdrawal ( $15.92 \pm 3.51$  pg/ml and  $14.22 \pm 2.56$  pg/ml respectively) compared with the second group treated with injection 500 IU PMSG alone ( $10.15 \pm 2.17$  pg / ml).





#### 4. Discussion:

From the results of the current study as shown in table,(1), it was found that the treatment regime with CIDR for 10 days combined with 500 IU PMSG injected on the day of CIDR withdrawal revealed best result for the estrus response rate while the lowest response was recorded in the group treated with 500 I.U of PMSG alone (table 1), same result were reported by Naseer, et al. (2013) when used CIDR alone for 7 days in buffalos (80%), while it were better than (Al-Hamedawi, et al., 2016) when he used the same program with giving PMSG at a dose of 1000 IU which were (60%) and the results of (Ghallab and Noseir, 2016) when using the CIDR for 8 days and injecting GnRH on the day CIDR withdrawal (73%) and better than the result of (Azawi, et al., 2012) in buffalo when using the CIDR for 8 days with hormone injection GnRH (75%). The reason for the high rate of response to estrus in this study may due to the differences in the nutritional and health status of the animals in previous studies, Whereas, the current study animals were selected so that the emaciated animals or those that were suffering from chronic diseases were excluded, Ahmed, (2007) mentioned that severe underweight before birth due to diseases of the digestive system as well as chronic diseases, parasites and toxic diseases are usually associated with lack of estrus in cows. The results of estrus response by using CIDR alone for 10 days in the current study (80%) was similar to that obtained by (Mwaanga, et al., 2004) when using PRID alone for induced estrus in cattle (80.6 %) while it was higher than the results of (Ghallab and Noseir, 2016) when using the CIDR alone for induction estrus in buffalo for 7 days (41.7%), the reason for this high percentage in the present study may be due to the difference in the treatment period which used in each studies or the difference in the animals breed.

Garcia-Ispuerto et al. (2010) mentioned that that progesterone treatment during the post-partum period improved the estrous rate in cattle.

The results of duration of response showed the efficiency of both the CIDR with PMSG regime and the CIDR alone to decrease the response period compared with the regime of PMSG alone (Table: 1), These results were similar to the results which obtained by (Ghallab and Noseir , 2016) when used CIDR alone ( $2.8 \pm 0.11$  days) or in combination with GnRH injection ( $2.08 \pm 0.5$  days) and Naseer et al., (2013) who found in their study that the CIDR induced the estrus within 42 hrs after its removal with tight synchrony as compared to eCG where it 72 hrs following the injection, while the current study results were better than the results obtained by (Al-Hamedawi, et al., 2016) when they used the CIDR program with PMS at a dose of 1000 IU ( $5.37 \pm 1.14$  days) and the CIDR alone ( $29.56 \pm 2.33$  days) the reason of these difference results may due to the differences in season, geographical area, animal's health status or the breed experiment animals used among studies whereas the current study was conducted on local cross breed Frisian cows, either in the study( Al -Hamedawi, et al., 2016) was conducted on dairy Friesian cows.

Pryce et al., (2004) mentioned that the increase milk production in dairy cows it is associated with a decrease in the reproductive efficiency of cows.

The results of the current study did not show any significant difference in the number of service per conception among different treatment regime these results were in agreement with the results attained by (Al –Hamedawi, et al. , 2016 ) and with the results what they obtained by (Ghallab and Noseir, 2016) when using CIDR with GnRH and the CIDR alone for induced estrus in buffalo suffered from post-partum in active ovaries.

The highest ( $p < 0.05$ ) pregnancy rate were recorded in the G1 and G3 (90% 87.5 % respectively) compared with the G2 (75%), this result was corresponds to what was found by (Al –Hamedawi, et al., 2016) while it was higher than what was reached by (Ergene , 2012) when using PRID with GnRH injection (60%) and with what was found by (Ghallab and Noseir, 2016) when using the CIDR alone (50%) or CIDR with the hormone of GnRH (85.7%) for induced estrus in buffalos and with Naseer et al., 2013 when used 1000 iu PMSG and CIDR alone for 7 days in buffalo (50% and 60% respectively). This is consistent with (Isperto et al., 2012) which demonstrated that the addition of PMSG at a dose of 500 IU gave positive results when used in the treatment of anestrus in cows treated by PRID. Baruselli et al. (2003); Cutaia et al. (2003); Sa' Filho et al. (2011a,b) decided that the use of eCG at the time of PRID removal has been suggested to increase pregnancy rates in timed AI programs for suckled cows with a high prevalence of anestrus.

The results of the current study showed shorter ( $P < 0.01$ ) days open in the G1 and G3 compared with the G2 (Table: 2) in disagreement with our study (Al-Hamedawi, et al., 2016) found significant differences between the using CIDR for 10 days with injection PMSG 1000 iu and using CIDR alone and these differences may due to the differences of the PMSG doses between two studies.

The current study demonstrated that the level of progesterone concentration in blood serum of the cows before treated in G1 and G3 were significantly higher ( $P < 0.05$ ) than before treatment (Figure 5). This result indicates the effectiveness of both programs in inducing ovarian activity, Kindahl et al. (2004) recorded that the postpartum increase in the concentration of progesterone concentration in cows is observed after the first ovulation. Diaz et al. (1986) reported that the progesterone concentration increased gradually during estrus cycle beginning at 2 days to reach maximum level at 13th day. Where Azawi et al., 2012 mentioned that CIDR inserted intra-vaginally which are saturated with progesterone causes an increased circulatory concentration of progesterone exerted negative feedback on hypothalamus and anterior pituitary.

Estrogen concentration increasing ( $P < 0.05$ ) at the fourth day after treatment ending in G1 and G3 compared with G3 (Figure: 6). These increasing in estrogen concentration in both groups come from increasing rate of estrus response in these group (100% and 80% for G1 and G3 respectively) compared with G2 (40%),

it is an established fact that manifestation of estrus is the principal function of estrogen which acts on the tubular genital tract for its regular functionality and it also sensitizes the central nervous system for coordinating sexual behavior and receptivity to the male animals (Hafez and Hafez 2000). this result was agreed with Al-Hamedawi, et al. (2016) whose found significant increasing in estrogen concentration in both groups of CIDR alone and CIDR with 1000iu PMSG after treatment compared with its concentration before treatment in Holstein-Friesian cows and with the result of Jena et al. (2016) when they found that the treatment cow suffered from post partum an estrus with CIDR based progesterone therapy and drug combinations may affect the reproductive hormonal balance like estrogen and progesterone, which is inevitable for successful return to cyclicity and subsequent fertilization and conception. these results explained by Azawi et al. (2012) whose decided that following termination of progesterone therapy (after

CIDR withdrawal), the rapid drop in circulatory concentration of progesterone promotes the release of GnRH as the negative feedback of progesterone was abolished, followed by FSH and LH release with subsequent resumption of ovarian cyclicity. Also, Barnes et al. (1980) decided that the most obvious effect of the PRID-treatment was to restrict peak release of pituitary FSH and, hence, peak FSH of plasma until after PRID removal.

In conclusion the use of CIDR+PMSG or CIDR have more potency for improvement reproductive performance (estrus response, duration of response, pregnancy rate and days open) in local cross breed cows suffering from post-partum inactive ovaries.

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