

## COMPARATIVE STUDY OF USE SOME TREATMENTAL REGIMES TO TREAT POSTPARTUM FUNCTIONAL INFERTILITY OF COWS IN BASRA

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### ABSTRACT

Postpartum an estrus in cows is regarded as one of the most important causes of functional infertility after parturition during reproductive lifespan. Therefore the present study was conducted to induce fertile estrus in anestrus cows which suffered from inactive ovaries (IO) or persistent corpus luteum (PCL) after parturition.

The results of current study showed the efficiency of using GnRH or FSH as a hormonal medication to induce fertile estrus with pregnancy which reach 71.42 and 72.22 % respectively, as well as decreasing the length of days open (DO) to lesser than 150 day in both programs in 70.00 and 76.92 % respectively from induced estrus cows.

Also the results revealed that the role of PGF $2\alpha$  in induction of fertile estrus with 83.33% of pregnancy in cows which suffered from postpartum PCL and decrease the length of DO in 80.00% from induced estrus cows.

The present study improved the efficacy of uterine massage (UM) through rectal palpation to treat cases of PCL in postpartum anestrus cows with 71.42% of pregnancy, and its effect to decrease the length of DO in 60.00% from induced estrus cows.

### INTRODUCTION

Normal reproduction is a complex series of events which must occur at precisely the right time. The timing mechanism is almost totally dependent on the endocrine system.

Any disturbances of the endocrine system may induce either temporary or permanent sterility.

The total system may be influenced by age, environment, or other factors. Postpartum anestrus refers to a condition where cows have not been observed or reported in estrus for several weeks after calving, often to the end of the elective waiting period (days open) in dairy cattle. DO mean the period extend from the parturition to the subsequent conception (1). A short period of ovarian inactivity during the immediate postpartum period is normal (during the normal puerperium period) which is take in average 40 day. Extended anestrus in non-suckled cattle such as dairy cows could have negative side effect on the timely reestablishment of pregnancy (1). Postpartum anestrus has been recognized as a problem by animal scientists for several decades (2). There has been significant interesting in the past two to three decades in the understanding and management of postpartum anestrus, both in beef and dairy cows. Nevertheless, postpartum anestrus continues to be a challenge to dairy practitioners and farmers alike (2).

The main cause of postpartum anestrus is PCL, the true mean of PCL is remaining of corpus luteum on the ovary beyond its normal life span without any detectable changes in the genital tract as that recorded in the following conditions: high lactating cow, hereditary deficiency of endometrial glands, marked decrease in the endometrial caruncles and marked reduction in endometrial glands due to repeated uterine infection or chronic degenerative changes in the endometrium (3).

The second cause of postpartum anestrus is IO which is usually leads to prolonged calving intervals and the resultant is an extensive economic loss. Reasons of IO have been investigated and categorized by many authors as: high lactation or extensive milking, malnutrition, puerperal and organic diseases and unfortunately due to a number of variable in age, weight, diet, and management. Hormones do not always give consistent results (4).

In this study we will focus primarily on the problems of postpartum anestrus and the best program or protocol for reestablishment the fertile estrus after parturition in cattle.

## MATERIALS AND METHODS

### Selection of animals

Postpartum (60) parous lactating cows were selected from farms and from animals that comes to clinic from owners in Basra province, which did not exhibit estrous signs more than 100 days postpartum. The incidence of anestrus was analyzed by studying the postpartum onset of estrus with the data of preceding 15 March to 15 September 2018. The hormonal induction of estrus and ovulation in anestrus cows was investigated during the months of study.

### Hormones

In current study we used some treatmental regimes (hormonal and non-hormonal) to treat some infertility causes in (60) cow after parturition (40 cow with IO and 20 cow with PCL). The cows were divided in to 4 groups as following:

In the first group (group A) includes 17 cow with IO treated with 0.5 mg of a synthetic analogous of GnRH (Fertagyl) \*. While in the second group (group B) includes 23 cow with IO treated with 1000 IU of pregnant mare serum gonadotropin (PMSG) (Folligon) \*\*

\* Intervet, International Co. Holland.

\*\* Intervet, International Co. Holland.

In the third group (group C) include 8 cows with PCL treated with 0.150 mg (150µg) of a synthetic analogous OF PGF2α (d-Cloprostenol) (Veteglan) \*. In the fourth group (group D) include 12 cow with PCL submitted for UM.

To induce ovulation in this study we are used human chorionic gonadotropin (HCG) (Chorulon) \*\* with doses of 1500 IU at fixed time of artificial insemination (AI) in all previous groups.

### Postpartum onset of estrus and ovulation induction

Among the experimental cows, forty parous cows which have not exhibited estrus with no palpable structures on either of the ovaries, flaccid tubular genitalia and free from palpable genital abnormalities, were confirmed as true anestrus IO. These parity cows were utilized for investigation of hormonal induction of estrus and ovulation by using different hormonal protocols (GnRH and FSH).

The other twenty parous cows, which have not exhibited estrus with palpable of corpus luteum structures on one or both of the ovaries, flaccid tubular genitalia and free from palpable genital abnormalities, were confirmed as true anestrus with considered as PCL and these parity cows were utilized for investigation of hormonal induction of estrus and ovulation by using different protocols (PGF $2\alpha$  and UM).

\* Calier, International Co. Barcelona, Spain.

\*\* Intervet, International Co. Holland.

### **Hormonal treatment protocols**

#### **1-Inactive ovariesgroup (40 cows)**

##### **Protocol (1): Using GnRH (Group A)**

Seventeen true anestrous cows were administered 0.5 mg of GnRH analogous on day 0 (treatment initiation day). After 3-7 days, AI was performed at observed estrus. If estrus phase occur again we were injected HCG with 1500 IU. At the time of AI, after 24 hours AI was performed again at a second observed estrus.

##### **Protocol (2): Using FSH (Group B)**

Twenty-three true anestrous cows were administered 1000 IU of PMSG on day 0 (treatment initiation day). After 3-7 days, AI was performed at observed estrus. If estrus phase occur again we were injected HCG with 1500 IU. At the time of AI, after 24 hours AI was performed again at a second observed estrus.

## **2- Persistent corpus luteum group (20 cows)**

### **Protocol (1): Using PGF2 $\alpha$ (Group C)**

Eight true anestrous cows were administered (150 $\mu$ g) of PGF2 $\alpha$  analogous on day 0 (treatment initiation day). After 3-7 days, AI was performed at observed estrus. If estrus phase occur again we were injected HCG with 1500 IU. with AI was performed at second observed estrus, after 24 hours AI was performed again at a second observed estrus.

### **Protocol (2): Using UM (Group D)**

Twelve true anestrous cow were employed UM by rectal palpation on day 0 (treatment initiation day) for 3-5 days once daily. After 3-7 days, AI was performed at observed estrus. If estrus phase occur again we were injected HCG with 1500 IU. With AI was performed at second observed estrus, after 24 hours AI was performed again at a second observed estrus.

### **Estrus response**

The estrus response was defined as the number and percentage of treated cows that exhibited estrus (5) with observable behavioral estrus symptoms. Detection of estrus during the first 7 days after the end of the treatment was taken as a response to the treatments.

### **Estrus detection**

Observation at 6 hours interval from 6 am to 6 pm in a day from owners. Standing or observable behavioral estrus was considered to have occurred when the cow stood to be mounted. Estrus was confirmed by rectal examination done after hormonal treatments. Cows were considered to be in estrus when the largest follicle showed slight fluctuation (12 to 15 mm) in diameter, uterus exhibiting strong contractility (the postpartum lactating cows were kept under observation for estrus detection using a visual tonicity) and the external orifice of the cervix was open (6 and 7).

### **Breeding**

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.

### **Artificial insemination submission rate**

AI submission rate was calculated as percentage of animals that were inseminated artificially among the animals treated.

\* Fertile frozen thawed semen (International Netherlands Co. Holland)

### **Fertility response (ovulatory response)**

Ovulatory response was studied by rectal palpation on day 10 after induced estrus for the presence of corpus luteum on the ovary post AI, and the results were expressed in per cent of the total cows which responded to treatment.

### **Conception rate at induced estrus**

First service conception rate was calculated as percentage of animals that conceived to AI at induced estrus in each protocol. Pregnancy was confirmed by rectal palpation per rectum at 60 days post AI. The influence of ovarian status was studied at induced ovulation and conception rate at induced estrus was recorded.

### **Days open**

In current study the duration of DO was estimated in all treated groups that suffered from postpartum IO and PCL according to the time of last parturition till the next pregnancy. The DO were classified into equal or less than 150 day and more than 150 days in all treated groups.

### **Overall conception rate**

Overall conception rate was calculated by percentage of cows that conceived at induced estrus and subsequent first and second estrous cycles. Pregnancy was confirmed by rectal palpation at 60 days post insemination.

### **Statistical analysis**

All collected data collected were analyzed statistically using percentage (%).

## **RESULTS**

Estrus response and fertility were studied in postpartum lactating anestrus cows by using hormonal treatment protocols (Group A) IO cows by using GnRH (Group B) IO cows by using FSH. (Group C) PCL cows by using PGF2 $\alpha$ . (Group D) PCL cows by using UM. Following estrus induction, ovulation and fertility responses were observed and recorded.

### **Incidence of anestrus**

The postpartum onset of estrus was evaluated in 45 parous lactating cow. (80%) of cows exhibited estrus in group (A,B) in cases of IO cows, and (65%) exhibited estrus in group (C, D) in cases of PCL cows. The remaining (20 and 35%) of cows did not exhibit estrus in cases of IO and PCL respectively (Table 1 and 2).

**Table 1.** Number and percentage of cows that suffer from postpartum IO which exhibited estrus and pregnancy after treatment with GnRH and FSH

Programs	Cows No.	cows showed estrus No. (%)	pregnant cows No. (%)	pregnant cows	
				From first estrus No. (%)	From second estrus No. (%)
GnRH	17	14 (82.35%)	10 (71.42%)	6 (60%)	4 (40%)
PMSG	23	18 (78.26%)	13 (72.22%)	8 (61.53%)	5 (38.47%)
<b>Total</b>	40	32 (80%)	23 (71.87%)	14 (60.86%)	9 (39.14%)

**Table 2.** Number and percentage of cows that suffer from postpartum PCL which exhibited estrus and pregnancy after treatment with PGF2 $\alpha$  and UM

Programs	Cows No.	cows showed estrus No. (%)	pregnant cows No. (%)	pregnant cows	
				From first estrus No. (%)	From second estrus No. (%)
PGF2 $\alpha$	8	6 (75%)	5 (83.33%)	3 (60%)	2 (40%)
UM	12	7 (58.33%)	5 (71.42%)	4 (80%)	1 (20%)
<b>Total</b>	20	13 (65%)	10 (76.92%)	7 (70%)	3 (30%)

### Estrus detection and response

After the hormonal treatment protocols of all groups, all the cows were observed for estrus signs in the morning and evening by owners. Estrus detection was done by observing characteristic signs like estrus discharge especially while lying down, vulval edema, congestion of vestibular mucus membrane, frequent to intermittent urination, bellowing, restlessness and alertness, sexual behavior like mounting and sniffing and

standing to be mounted by other cows. The estrus response following hormonal treatment and UM was 82.35 (14/17), 78.26 (18/23), 75 (6/8) and 58.33 (7/12) % in GnRH, FSH, PGF2 $\alpha$  and UM groups respectively (Table 1 and 2).

### **Fertility response (ovulatory response)**

The ovulatory response was calculated as percentage of animals that ovulated over the total animals that exhibited estrus. The Ovulatory response in GnRH (Group A), FSH (Group B), PGF2 $\alpha$  (Group C) and UM (Group D) was presented in Table 1 and 2. An Ovulatory response of 82.35 (14/17), 78.26 (18/23), 75.00 (6/8) and 58.33 (7/12) % observed in Group A, B, C and D respectively.

### **Conception rate at induced estrus**

The conception rate was calculated as percentage of animals that were conceived to inseminations at observed (induced) estrus in each group. The conception rates in cows of various treatment groups are given in Table 1 and 2. The conception rates were 71.42 (10/14), 72.22 (13/18), 83.33 (5/6) and 71.42 (5/7) per cent in GnRH, FSH, PGF2 $\alpha$  and UM groups respectively.

In current study the percentage of pregnant cows from the first estrus after treatment were 60.00 and 61.53% in GnRH and FSH groups consequently. While the percentage were 60.00, 80.00% in PGF2 $\alpha$  and UM consequently (Table 1,2).

### **Overall conception rate**

The overall conception rate among the anestrous animals treated was higher (71.87%) in cows with postpartum IO. While in cows with postpartum PCL luteum was (76.92 %) as presented in table (1 and 2).

### **Days open**

In current study the number and percentage of cows in all treated groups that suffered from postpartum IO and PCL were (72.72%) that lead to decrease the duration of DO less than 150 day, while (27.28%) showed DO more than 150 days (Table 3).

**Table 3. Number and percentage of pregnant cows that suffered from postpartum IO and PCL according to the length of DO after treatmental programs**

Programs	Cows No.	pregnant cows No.	pregnant cows according to the length of DO	
			DO≤150 day No.(%)	DO> 150 day No.(%)
GnRH	17	10	7 (70%)	3 (30%)
PMSG	23	13	10 (76.92%)	3 (23.08)
PGF2α	8	5	4 (80%)	1 (20%)
UM	12	5	3 (60%)	2 (40%)
Total	60	33	24 (72.72%)	9 (27.28%)

## DISCUSSION

In the present study the efficacy of four different anestrus treatmental protocols were used to treat postpartum lactating anestrus cows by using either hormonal treatment like GnRH (Group A), PMSG (Group B), PGF2α (Group C) and non-hormonal treatment like UM for enhancement fertility and decrease the period of DO in postpartum anestrus cows.

The incidence of postpartum anestrus in cows recorded by different scientists (8, 9, 10 and 11) which reported 28.42, 8.90, 17.00 and 29.12% postpartum anestrus in cows respectively. These differences in incidence of anestrus were attributed to variation in the environmental condition, management practices, milk yield and nutritional status of the animals (١٢) as well as the species (١٣).

In the present investigation, 80% and 65 % of cows exhibited estrus after postpartum anestrus which were suffered from IO (Group A, B) and that's suffered from PCL (Group C, D) respectively. The estrus response after hormonal treatment and UM was 82.35 (14/17), 78.26 (18/23), 75 (6/8) and 58.33 (7/12) % in GnRH, PMSG, PGF2α and UM groups respectively. All the responded animals were detected in estrus within three to seven days following treatmental regimes. These results came in close agreement with

many studies which conducted in anestrus cows (15, 16) and came higher than (17). The induction of estrus especially after postpartum depend completely on the hormonal protocols, body conformation, nutritional status, milk yield as well as mainly for the accurate diagnosis for the causes and suitable treatment for each case (8, 9, 15 and 16), and the period of postpartum anestrus (14). (18) showed the early diagnosis of postpartum anestrus is significantly respond to treatment.

In the current study there was no any difference in ovulation rate between GnRH and PMSG (82.35% vs. 78.26 %) which came in agreement with those reported by several authors like (28, 15 and 29) which have recorded an ovulation rate of 81.81%, 82.00% and 83.30% for the GnRH vs. 76.40, 74.14 and 72.20% for the FSH respectively. Both GnRH and FSH play an important role to stimulate the follicular development through its effect as a synergistic hormones with internal GnRH and FSH (19, 20).

The 75.00 % ovulation rate which recorded for PGF2 $\alpha$  (group C) in the present study was less than the ovulation rates reported by several authors like (25, 27 and 29) who recorded 100.00% ovulation rates. Uterine diseases and the delay in uterine involution play as a main factors for retardation the releasing of PGF2 $\alpha$  from uterus which lead to PCL. PGF2 $\alpha$  treatment act directly on PCL and induce estrus in anestrus cows (21).

Conception rate at induced estrus was calculated as percentage of animals that were conceived to inseminations performed at detected/observed estrus in each group. The conception rate at induced estrus in the present study for treatment groups involving GnRH, FSH, PGF2 $\alpha$  and UM were 71.42 (10/14), 72.22 (13/18), 83.33 (5/6) and 71.42 (5/7) %, respectively.

The conception rate at induced estrus for GnRH group recorded in the present study is 71.42%. It was higher than conception rates recorded by (14), (15) who recorded 20.00 and 33.33 % respectively.

In the present study the conception rate at induced estrus in treatmental groups is in close agreement with many reports (14, 22, and 23). However, much lower conception rates reported by (18 and 24) who reported 50.00 and 30.00% respectively. The conception rate is mainly depend on the follicular and hormonal development, while the accurate

diagnosis and the suitable selection for the treatment play an important role for those events which is accompanied by a normal hormonal control and followed by releasing the oocyte and access the pregnancy (20, 23). Also the physiological, environmental and nutritional factors contribute to increase the conception rate in induced estrus cows (12, 13).

In this study, the UM played an important role for induction of estrus in postpartum cows that suffered from PCL. This result came in close agreement with (24) who induced estrus in buffaloes that suffered from postpartum IO and also with study of (25) on mares and cows (26). Many researches referred to that the UM causes changes in ovarian blood flow or through stimulation of neural system reflex. This reflex may have resulted from activation of some intrinsic ovarian factors, also in a changes in blood ovarian circulation. This technique was regarded an economical and simple strategies that does not need to any medication or hormonal treatment as well as it considered as an efficient way to treat the anestrus animals and gives up good results for induce first fertile estrus in compared with other hormonal treatment protocols (25,27 and 28). In the present study overall conception rate among the anestrus cows treated groups was higher.

The effect of UM for induction estrus in postpartum cows due to PCL may be related to the indirect effect of UM for releasing the uterine PGF<sub>2</sub> $\alpha$  which act on PCL and automatic return to the estrus (25).

In the FSH treatment group, the estrus, ovulation and conception rates were higher in anestrus cows. The combination of FSH plus LH treatments might be sufficient to facilitate follicular growth, maturation and improved ovulation and pregnancy as reported by (28) in anestrus beef cows.

The overall conception rate recorded in the present investigation for group A, B treatment was 71.87 % and it was in close agreement with slight high with (14) it was 75.00 % while it was high compared to the earlier reports of (29 and 30) who reported 58.30 and 62.50 % respectively.

The results of AI and LH injection done at observed estrus with GnRH and FSH protocols appeared as an effective way of managing reproduction in anestrus cows with

luteal activity. The LH play an important role for ovulation, as well as its role for the maintenance of corpus luteum to secrete a sufficient amount of progesterone to maintain the early pregnancy (31, 32).

In this study, DO were shortened by using all four protocols (Group A, B, C and D). This study was in close agreement with (33, 34 and 35) and disagreement with (36 and 37). To produce a calf every year a cow has to become pregnant within 75 to 85 day after calving. Therefore, postpartum anestrus has to be shorter than 54 day for the cow to have at least two chances to breed within 85 day postpartum. For early exhibition of first postpartum estrus the involution of post gravid uterus has to be completed as early as possible.

### استخدام بعض البرامج العلاجية لعلاج حالات انعدام الشبق بعد الولادة في الأبقار في محافظة

#### البصرة

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#### الخلاصة

يعتبر انعدام الشبق في الأبقار بعد الولادة واحدة من الأسباب الرئيسية للعقم الوقتي الذي قد تصاب به الأبقار بعد الولادة خلال حياتها التناسلية . لذلك اجريت الدراسة الحالية لاستحداث الشبق الخصب في الأبقار عديمة الشبق والتي تعاني من خمول المبايض أو احتباس الجسم الاصفر بعد الولادة.

أظهرت نتائج الدراسة الحالية كفاءة استخدام GnRH أو FSH في استحداث الشبق الخصب في الأبقار التي تعاني من خمول المبايض بعد الولادة وحصول الحمل فيها بنسبة 72.22 و 71.42 % على التوالي وتقصير طول فترة الأيام المفتوحة الى اقل من 150 يوم في كلا البرنامجين بنسبة 76.92 و 70.00 % من الأبقار التي استحدثت فيها الشبق على التوالي.

كما أظهرت نتائج الدراسة الحالية كفاءة علاج  $PGF2\alpha$  في استحداث الشبق الخصب والمقرون بالحمل بنسبة 83.33 % في الأبقار والتي تعاني من انعدام الشبق واحتباس الجسم الأصفر بعد الولادة، وتقصير طول فترة الأيام المفتوحة الى اقل من 150 يوم في 80.00 % من الأبقار التي أستحدثت الشبق فيها .

كما بينت النتائج كفاءة المساج الرحمي في علاج حالات احتباس الجسم الأصفر في الأبقار عديمة الشبق بعد الولادة وحصول الحمل بنسبة 71.42% ، وتقصير طول فترة الأيام المفتوحة الى أقل من 150 يوم وبنسبة 60.00% من الأبقار التي أستحدثت الشبق فيها والتي تعاني من انعدام الشبق بسبب احتباس الجسم الأصفر.

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