

THE CLINICAL APPLICATIONS OF SOME REPRODUCTIVE HORMONES IN TREATMENT OF SOME REPRODUCTIVE DISORDERS AND THE EFFECT OF THIS TREATMENT ON THE ESTRUS ONSET IN THE COWS IN IRAQ

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ABSTRACT : This study was designed to evaluate efficacy of different therapeutic protocols of hormones and their influence on the reproductive performance in cows. The current study was conducted in the College of Vet. Medicine, University of Diyala, and extended from 1/10/2018 until 1/2/2019 and includes 32 multiparous cows. The number of cows suffering inactive ovaries was 16(50%) (Gr-1) were allocated to two equal subgroups (Gr- A and Gr- B), one of which was applied Folligon for one time and other Receptal treatment. The time of estrus onset for each subgroup were (18-23)day and (28-32)day, respectively. There was no significant difference for these two groups of hormonal treatment in terms of estrus onset at ($P<0.05$). Seven cows (21.88%) (Gr-2) suffering ovarian follicular cysts were treated with Chorulon, for one time and the estrus onset and inseminated artificially about 23-28 days after treatment. Two cows(6.25%) (Gr-3) experiencing ovarian luteal cyst treated with Estrumate ($PGF^2\alpha$), for one time and came to estrus and inseminated artificially after 29-32 days of treatment, without significant difference for the two categories of hormonal treatment in terms of estrus onset at ($P<0.05$). Five cows (15.63%) (Gr-4) have retained placenta and were injected with Estrumate for one time, three cows were recovered after 3-5 days of treatment and came to estrus and artificially inseminated about 50-60 days of treatment. Whilst other two cows did not respond to Estrumate and treated manually removal of fetal membranes and insertion intrauterine tablets of oxytetracycline, with oxytetracycline solution (20%) injection in addition to estradiol (estrogen) intramuscularly for one time after manual removal, those cows came to estrus and with complete recovery and inseminated artificially after 72 days of treatment. There was no significant difference for these hormonal treated cows in terms of the onset of estrus at ($P<0.05$). Two cows (6.25%) (Gr-5) had pyometra and treated with Estrumate for one time with intrauterine therapy of oxytetracycline (10%), three times with 7 days apart. These came to estrus with good recovery and inseminated artificially about 55-60 days of treatment and not differed significantly from the retained placenta treated cows in terms of the onset of estrus. There was significant difference among the groups of treated cows with Folligon, Receptal, Chorulon, Estrumate and groups of treated cows with retained placenta, pyometra in terms of the onset of estrus at ($P<0.05$). It can be concluded that these hormones investigated in the study were effective to treat the reproductive disorders encountered with various results, besides to a variation in the onset of estrus. This variation may be attributed to many reasons like the origin of pharmacologic product and the variable responses of treated cows involved in this study to a pharmacologic product.

Key words : Reproductive disorders, hormonal therapy, multiparous cows, ultrasound.

INTRODUCTION

Gonadorelin (GnRH), which is produced endogenously by the hypothalamus and causes the release of FSH and LH by anterior pituitary. GnRH is prepared synthetically. Gonadorelin is used to treat ovarian follicular cyst and inactive ovaries in dairy cattle. It has also been used in protocols of Estrous synchronization (Wanamaker, 2004; Hafez and Hafez, 2000).

FSH (Follicle Stimulating Hormone), which is released endogenously by the anterior pituitary gland causes growth and maturation of the ovarian follicles in females. FSH may be obtained from pituitary glands of slaughtered

animals (FSH-p) and from the serum of pregnant mares (PMSG) (FSH like hormone) between 40th and 140th day of pregnancy. FSH is used in veterinary medicine to treat inactive ovaries and to induce superovulation and for out-of season breeding (in seasonal breeders) (Noakes *et al*, 2001; Andrews *et al*, 2004).

LH (luteinizing hormone) is also released by anterior pituitary, and causes ovulation in females. LH may be prepared from the pituitary glands of slaughtered animals or obtained from urine of pregnant women in the form of human chorionic gonadotropin (hCG), hCG is used to treat ovarian follicular cyst (nymphomania) and also ovulation

failure in dairy cattle (Wanamaker, 2004; Dothwaite and Dobson, 2000; Dobson *et al*, 2001a).

Prostaglandins are a group of naturally occurring of the six classes (A, B, C, D, E, F), only prostaglandin F2alpha has significant clinical application in the reproductive system. Prostaglandin F2 alpha causes lysis of the corpus luteum, contraction of uterine muscles and relaxation of the cervix (Wanamaker, 2004; Ptaszynska, 2010).

Estrogens are a group of steroid hormones synthesized by the ovaries and to a lesser extent by the adrenal cortex and placenta. Estrogens are necessary for normal growth and development of the female gonads. They cause secondary female characteristics and these hormones increase uterine tone, and increase the duct system development in mammary gland. In cattle, estrogens are used to expel purulent material from uterus, and to expel retained placentas (Ambrose *et al*, 2004; Cavalieri *et al*, 2008).

MATERIALS AND METHODS

This study was conducted in the college of Veterinary Medicine in Diyala province and included thirty two multiparous cows suffering from various reproductive disorders and coming to the farm of the College of Veterinary Medicine for treatment purpose and all these cows were bred in farmers houses or in small farms. All cows were fed on green fodder and concentrated food besides to the roughage food.

After the taking of case history of these cows, and making rectal palpation to diagnose the type of reproductive disorder, Ultrasonography Real-time ultrasound scanning of uterus and ovaries was facilitated via the transrectal route to confirmed these conditions, with a 5-7.5 MHz linear array transducer (WellD ultrasound, Shenzhen well. D. Medical Electronics Co. LTD., China). Light wave record and play video, USB 2.0 TV BOX (Stroud BK. Clinical applications of bovine reproductive ultrasonography. *Comp ContEducPrac Vet* 1994; 16: 1085–1097). According to the treatment, the animals allocated to the following groups: First group (n = 16) cows were suffered inactive ovaries (Fig. 1) were allocated to two equal subgroup (Gr- A and Gr- B), one of which was applied Folligon (PMSG) 1000 IU, Intervet, Holland) for one time and other Receptal 20µg (Buserelin acetate 4.2 µg corresponding to 4.0 µg Buserelin (Intervet, Holland) treatment. The time of estrus onset for each subgroup were (18-23) day and (28-32) day, respectively.

Second group (n = 7) cows experiencing ovarian follicular cysts (Fig. 2) and treated hormonally with chorulon (Gonadotrophine, human chorionic

gonadotrophine (hCG) 1000 IU, Intervet, Holland) for one time.

Third group (n = 2) cows with luteal cyst (Fig. 3) and treated with 500µg of Estrumate (solution contains 263µg cloprostenol sodium BP(vet) equivalent to 250 micrograms cloprostenol sodium (Schering- Plough Animal Health) USA) intramuscularly for one time.

Fourth group (n = 5) cows have retained placenta, were treated with 500 µg Estrumate for one time, three cows responded to treatment, while other two cows did not respond to Estrumate and were treated by manual remove of retained placenta, with insertion of 5 uterine tablets of oxytetracycline (each tablet contains 500 mg oxytetracycline HCL, ALSHARK Veterinary products, Syria) intrauterinely and Vetasterol (injectable oily solution, each ml contains 2mg Oestradiol Benzoate(Base), AburairhanPharma Co. Iran) and oxytetracycline (20%) (4gms) (20 ml) (Imoly 20% (solution, each ml contains 200 mg oxytetracyclin, Intracin Co LTD, India) injection intramuscularly for one time.

Fifth group (n = 2) cows suffered pyometra (Fig. 4) and treated with 500 µg of Estrumate injection intramuscularly for one time, and intrauterine treatment of oxytetracycline(10%) (100 mg/ml Oxytetracycline HCL, ALFASAN, Holland).

2 gms (20 ml) of oxytetracycline solution diluted with 30 ml normal saline (sodium chloride 0.9% w/v, each 100 ml contains sodium chloride USP 0.9 gm, PIONEER for pharmaceuticals industries, Iraq for three times with 7 days apart.

The statistical analyses were carried out to know the significant statistical differences among these groups of cows by using (Chi-square) (X²) (SPSS) software (version 18.0, IBM Inc., Chicago, USA).

RESULTS AND DISCUSSION

Table 1 shows the number of cows have inactive ovaries which was 16(50%). The results in this study are in disagreement with many previous studies (Bloch *et al*, 2006; Diskin *et al*, 2002; Kharche and Srivastava, 2007) and this may be due to the higher number cases of inactive ovaries included in this study. And this highly occurrence of inactive ovaries may attributed to the insufficient or mal nutrition of these cows. Eight inactive ovaries cows were treated with Folligon and all these cows came to estrus and were inseminated artificially within interval 18-23 days after treatment, compare to other 8 cows that were treated with Receptal and came to estrus and inseminated artificially within interval 28-32 days after treatment. There is no significant difference between two

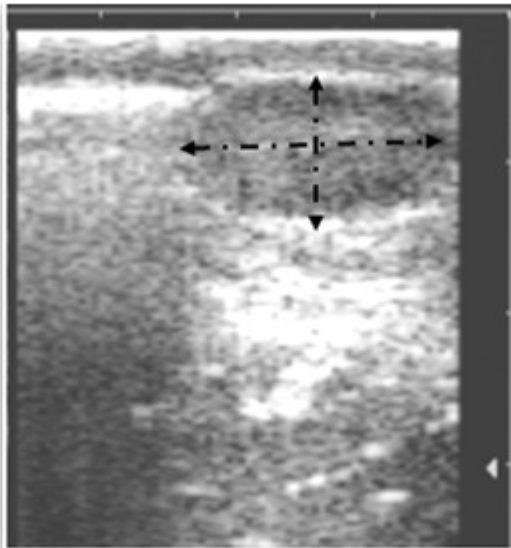


Fig. 1 : Ultrasonographic images of an inactive ovary, characterized by almond - shaped and firm and has a uniform echogenicity that equals to or is slightly greater than the cervix without functional structures (6.5MHz linear probe), the ovary delineated by a black lines.



Fig. 2 : Ultrasonographic images of a follicular cyst (29mm) in diameter, characterized by the persistence of large anovulatory structures for various periods of time in the absence of corpora lutea, thin-walled (6.5MHz linear probe). F.cys t= follicular cyst; u.b = urinary bladder.

protocols of hormonal therapy of inactive ovaries and this may be related to the similar pharmacologic role of both Folligon (PMSG) and Receptal (GnRH) in treating of inactive ovaries, despite of the fact that Folligon hormone has direct action on ovaries whilst the Receptal has direct action on anterior pituitary endogenously (Noakes *et al*, 2009; Ball and Peters, 2004; Youngquist and Therlfall, 2007).



Fig. 3 : Ultrasonographic images of a Luteal cyst (20mm) in diameter, characterized by thick walled (3-4mm). (6.5MHz linear probe). Arrows: Edge of the luteal cyst.



Fig. 4 : Ultrasonography of a pyometra, Arrow: Uterine wall; F: Lumen contents with highly echogenic particles. The typical pyometra sonogram shows the accumulation of a large quantity of an echogenic liquid with echoic particles and a thickened uterine wall (6.5MHz linear probe).

The number of cows have follicular ovarian cysts were 7(21.88%) and this percentage of follicular cysts is similar to other studies (Hafez and Hafez, 2000; Noakes, *et al*, 2001; Rosenberg, 2010). These cows were treated with Chorulon and came to estrus and inseminated artificially about 23-28 days after treatment. The results of this study are in accord with many studies revealed

that Chorulon containing LH like hormone (hCG) has good efficacy in rupturing the follicular cyst in one or both cow ovaries and make the affected cow to come to estrus and inseminated with conception rates relatively higher within short time after treatment (Noakes *et al*, 2001; Braw-Tal *et al*, 2009).

The number of cows suffered luteal ovarian cyst was 2(6.25%) in this table and this percentage is agreed with other studies and researchers (Wanamaker, 2004; Hafez and Hafez, 2000; Noakes *et al*, 2001). These cows were treated with Estrumate contains cloprostenol sodium which is synthetic analogue of Prostaglandin $F_2\alpha$ and have recovered and came to estrus and inseminated artificially within interval 29-32 days. These results reveal that the Prostaglandin $F_2\alpha$ is a hormone of choice in treating luteal cyst and is very successful in destruction of luteal cyst and make the treated cows resuming estrus cycle with accepted conception rates (Wanamaker, 2004; Noakes *et al*, 2001; DeVries *et al*, 2006). Both categories of cows either suffering follicular cysts and treated with Chorulon, or experiencing luteal cyst and treated with Estrumate returned to estrus in the same time nearly, and without significant difference ($P < 0.05$) between two categories of treated cows and this indicate that the protocols of hormonal therapy for both types of cystic ovaries were highly effective in treating these reproductive abnormalities and successful in terminating them and bringing these cows to estrus in a relatively short time.

The cows have suffered retained placenta in this table were (5) (15.63%) and have received Estrumate treatment and for one time, 3 cows recovered after 3 to 5 days and came to estrus and inseminated after 50-60 days of treatment. These results reflexed the efficacy of Prostaglandin $F_2\alpha$ (Estrumate) in treating the cases of retained placenta due to uterine atony, and it is action in uterine contractions and facilitating the expulsion of retained fetal membranes was well known (Hafez and Hafez, 2000; Andrews *et al*, 2004; Tucho, 2017). Two cows did not respond to treatment of Estrumate and the manual removing of retained fetal membranes with uterine tablets of oxytetracycline intrauterinely and intramuscular injection of 20% oxytetracycline was done, those cows came to estrus with complete recovery and inseminated artificially after 72 days of treatment. The manual removal of retained placenta as a method of treating is a controversial subject, many theriogenologists don't prefer this type of treatment in accounting for the detrimental effect of manual removing on the uterine tissue in addition to the weakening of uterine defense mechanism (Andrews *et al*, 2004; Noakes *et al*, 2009; Bolinder *et*

Table 1 : The influence of different treatment on the onset of estrus.

Groups	No	Treatment(no)	Time of estrus onset (days)
Gr-1	16	Subgroup-A(8) PMSG	18-23 ^a
		Subgroup-B (8) Buserelin	28-32 ^a
Gr-2	7	HCG	23-28 ^a
Gr-3	2	Estromate	29-32 ^a
Gr-4	5	Estromate (3)	50-60 ^b
		Manualremoval(2)	70 ^b
Gr-5	2	Estromate+Oxytetracyline	55-60 ^b

^{a,b}different superscripts letters within columns indicate a significant difference ($P < 0.05$).

al, 1988). However, the hanging fetal membranes from vulva is uncomfortable sight and it is preferable to eliminate these membranes manually (Andrews *et al*, 2004; Drillich *et al*, 2005; Drillich *et al*, 2006b). Both types of treatment of retained placenta, either hormonally or by manual removal didn't influence the return of treated cows to estrus, and this may attributed to the impacts of postpartum period and its own time taken to elapsed which is relatively long (Andrews *et al*, 2004; Noakes *et al*, 2009).

Table 1 shows 2(6.25%) cows had pyometra and treated with Estrumate for one time and received 2 grams intrauterine oxytetracycline (10%) for three times with interval seven days among these three times. These cows came to estrus with good recovery and inseminated artificially about 55-60 days of treatment. The results of this study agreed with many previous studies have shown that Prostaglandin $F_2\alpha$ (Estrumate) has higher efficiency in treating pyometra, it is role seems to it is efficacy in regressing of the corpus luteum associated with pyometra and expulsion of purulent material present in uterus (Bolinder *et al*, 1988; Szenci, 2009; Stevens *et al*, 1995) besides to the intrauterine injection of oxytetracycline that it is effective against the causative agents of uterine inflammation (Szenci, 2009; KACAR and KAYA, 2014). There is complete recovery for these cases of pyometra, which indicated that the method of treatment was very effective in treating these cases. The return to estrus in this group of cows that have pyometra seems to be long and it lasts nearly two months, this may be related regardless of the type of treatment to the huge detrimental effect of pyometra on the uterus, and ovaries consequently and the reproductive organs needed long time to be recovered and to resume their physiologic role (Youngquist and Therlfall, 2007; Tucho, 2017; Szenci, 2009).

In the comparison among the groups of Folligon,

Receptal, Chorulon, Estrumate treated cows and groups of treated cows have retained placenta and pyometra, in terms of the estrus onset, it is obviously that there is significant difference ($P < 0.05$) in term of return to estrus, since the return to estrus was shorter in the treated cows with Folligon, Receptal, Chorulon, Estrumate, while it was longer in treated cows have retained placenta and pyometra. This difference may related to that retained placenta and pyometra may need time to recovery longer than time elapsed for inactive ovaries and cystic ovarian disease which lead eventually to delay the return to estrus in treated cows (Noakes *et al*, 2009; De Vries *et al*, 2006; Tucho, 2017; Drillich *et al*, 2005).

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