Pharmacodynamics of gonadotrophin releasing hormone (Receptal®) and prostaglandine (Estrumate®) on ovarian activity, hematological picture and some steroid hormones of cows during summer season

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Abstract: Treatment of infertility and induction of estrous cycle in cows is usually based on **progestagens, prostaglandin** and **gonadotropin releasing hormone** or its analogues. gonadotropin-releasing hormone from hypothalamus a polypeptide hormone was found to regulate the secretion of luteinizing and follicle stimulating hormones. The present study aimed to study the pharmacodynamics of GnRH and PGF2α (OvSynch protocol) treatment on cattle fertility and some biochemical and hormonal status during summer season. Fifteen cross breed cows (Baladi X Abundance), 9 primiparous and 6 pleuriparous cows, their ages (4.84±1.91) years, not exhibiting estrus signs for duration of 108±45.52 days, were treated with OvSynch protocol (GnRh-PGF2α-GnRh). Cows were injected with 2.5ml Receptal® (GnRH) I/M. Seven days later, 2ml Estrumate® ($PGF_2α$)was injected I/M., followed 48 hours later with injection of 2nd dose of GnRH (2.5ml Receptal®), I/M .,then 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 occurred in blood parameters(RBCs, MCV, HCT , HB, WBCs, LY%& GR%), after treating with the drugs, Receptal®-Estrumate®-Receptal® (OvSynch protocol) . But it is clear that, these fluctuations in blood parameters lie within the normal blood value range of cattle. A high estradiol level (35.9±3.62pg/ml) was recorded at day (10) at fixed TAI and lowest one (21.03±0.79pg/ml) was detected at day (73). A higher P4 value (17.05±5.23ng/ml) was recorded at day (12) and a significantly lowest one (0.29±0.05ng/ml) was recorded at day (73). The ovulation rate was 72%, while the pregnancy rate was 40% in cows treated with GPG protocol. It is concluded that the hormones used in OvSynch protocol (GnRH, PGF2α &GnRh), stimulated the ovarian activity of cows and can overcome summer infertility and the hormones used had no adverse effect on hematological parameters and/or metabolic status of treated animals.

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1. Introduction

It was reported that a polypeptide namely, gonadotropin-releasing hormone from hypothalamus, was found to regulate the secretion of luteinizing and follicle stimulating hormones (Schally *et al.,* 1973).

In cycling cows, administration of GnRH or a derivative induces a gonadotropin surge (Chenault *et al.,* 2003) with peak LH within 2 to 3 h (Williams *et al.*,1982) and alters the pattern of follicle growth (Thatcher *et al.*, 1989; Wolfenson *et al.*,1994). Administration of GnRH induces a LH surge with similar maximum LH concentrations (McDougall *et al.*,1995) but with approximately half the duration (Chenault *et al.*,2003), when compared to the endogenous LH release during the normal estrous cycle at the time of ovulation (Rahe *et al.*, 1980; Chenault *et al.*, 2003). A single injection of GnRH or an agonist is sufficient to induce ovulation or atresia of a dominant follicle (Crowe *et al.*, 1993; Twagiramungu *et al.*, 1995). Several reports demonstrated that growing follicles greater than 10 mm in diameter ovulate after GnRH injection (Pursley *et al.*, 1997; Silcox *et al.*, 1995; Martinez *et al.*, 2000)

The Induction of ovulation of ovarian follicles was demonstrated in milked (Britt, *et al.*, 1974) and suckled cows (Schams *et al.*, 1977) following an injection of Gonadotropin-releasing hormone. GnRH induced effects is indirect (Chenault *et al.*, 2003) through their induced release of LH (Britt *et al.*,1974) and follicle stimulating hormone (FSH) from anterior pituitary gland. Later, GnRH analogues and agonist

were developed, which were more potent than native GnRH (Thatcher *et al.*, 1989).

Synchronization of follicular waves and selection of new large follicle following GnRH at any stage of the estrous cycle was used as a tool to further develop estrous synchronization programs for fixed timed AI (Twagiramungu *et al.*, 1995).

To improve the estrus synchrony exogenous GnRH, which controls the developmental stage of the preovulatory follicle has been included with prostaglandin for synchronization of estrus in dairy cows (Stevenson and Pursley, 1994; Thatcher *et al.*, 2001). The random administration of GnRH during the estrous cycle results in LH release (Chenault *et al.,* 2003), causes ovulation or luteinization of large follicles present in the ovary, synchronizes the recruitment of a new follicular wave (Thatcher *et al.*,1989; Martinez *et al.*, 2000), and equalizes follicle development waves (Twagiramungu *et al.,* 1995; Schmitt *et al.*, 1996). Subsequent administration of PGF2α induces the regression of an original or GnRH-induced CL, and allows final maturation of the synchronized dominant follicle (Schmitt *et al.,* 1996). Further, there is no apparent detrimental effect of GnRH on the responsiveness of GnRH-induced CL or spontaneous CL to prostaglandin (Twagiramungu *et al.,* 1995).

Recently, 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 (Larson *et al.,* 2006).

The main purpose of this study was to study the pharmacodynamics of Receptal®(GnRH) and Estrumate® (PGF2α) injection on animal fertility , hematological and steroid hormone pattern and pregnancy rate in cows .

2. Material and Methods

The present study was conducted in private sector farms in Beni-Suef Governorate, Upper Egypt, during 2010 to August/2011.

1. Animals

This study was conducted on 15 crossbreed cows (Baladi×Abundance). The age of the animals ranged from 3 to 8 years (4.84±1.91 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 recorded(2.7±0.57) and scaled according to **Gordon (1996)**.

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

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

VARIABLE		Cows
Breed		Crossbreed cows (Baladi×Abundance)
Month of experiment		Summer (August)
Parity	Primiparous	9 (60%)
	Pleuriparous	6 (40%)
Average daily milk yield (kg)		3.5
(B.C.S.)		2.7±0.57
Interval from calving to 1st estrus (days)		108±45.52
Age (year)		4.84±1.91

2. Chemicals

A. Estrumate® (Synthetic prostaglandin)

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

B. Receptal ® (Gonadotropin releasing hormone)

Each ml contains 0.0042 mg buserelin acetate equivalent to 0.004 mg buserelin, 10mg benzyl alcohol (**Intervet International B.V. Boxmeer, Holland**).

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.* (1989)**. 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 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.

4.Hematological examination

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

5. OvSynch Protocols of synchronization of estrous (GPG)

Summary of the protocol procedure was carried out according to **Mialot *et al.* (2003).** On day (0) cows examined clinically per rectum, blood sampling and injection of 2.5ml Receptal® (GnRH) I/M. Seven days later, injection of 2ml Estrumate® ($PGF_2\alpha$) I/M. After 48 hours, animals were injected I/M with 2nd dose (2.5ml) of GnRH. Timed artificial insemination (T.A.I.) 24 hours after the 2nd dose of GnRH. Pregnancy diagnosis by rectal palpation at day 73 of experiment for the inseminated cows (Fig.1).

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 illustrated in table (2).

Erythrocytic count: The results of the current study revealed that a significantly higher value ($7.26\pm0.32x10^{12}$/L) was detected at day (7) in pregnant animals. Meanwhile, no significant differences in erythrocytic count were detected among other days of experiment in non pregnant animals.

Mean Cell Volume (MCV): A significantly higher value (48.75±0.09 FL) was recorded at day (7) in conceived (pregnant) animals compared with other days of experiment. It was also appeared that, in non pregnant animals a higher MCV value (46.42±0.67FL) was recorded at day 21 in comparison with that recorded at the rest of the experimental days.

Hematocrite value (HCT): The findings of the present study revealed no significant differences in HCT values among the different days of the experiment within the same group. However a significantly higher value (0.36±0.015 L/L) was detected at day 7 in pregnant cows compared with other values in non pregnant cows.

Hemoglobin (HB): A significantly lowest value (76.58±6.51 g/L) was recorded at day 31of the experiment in pregnant cows compared with other days of experiment. On the other hand in non pregnant animals no clear significant differences in HB values were recorded among the different experimental days.

White Blood cells (WBCs) Parameters

WBCs count: The results of the current study revealed that, a significantly lowest value (6.45±0.58 $x10^9$/L) was detected at day (10) compared with other days of the experiment in conceived /pregnant cows. Significantly higher values (10.36±0.61 and 9.39±0.54 $x10^9$/L) were recorded at days (7and 31) in pregnant cows compared with that (6.41±0.46 and 6.65±0.35 $x10^9$/L) recorded at the same days in non pregnant cows. No significant differences were observed in WBCs count among different days of the experiment in non pregnant cows.

Lymphocyte percentages (LY %): In pregnant animal, our results revealed no significant differences among values of LY % at different days of experiment. Meanwhile in non pregnant animals, a significantly lower value (21.85±1.49) was recorded at day (10) compared with values that recorded at days 7 (36.19±1.46) and 31(36.7±1.41).

Granulocyte percentages (GR %): Our results proved that treatment had no significant effect on GR % at different days of experiment in pregnant animals. Meanwhile in non pregnant animals, a significantly highest value (72.25±1.61) was recorded at day 10 compared with that (55.80±1.58 and 56.99±1.47) recorded at days (7 and 31) respectively. Moreover no significant differences in GR % were detected between the pregnant and non pregnant cows.

Hormonal patterns :

Estradiol level

Although hormonal data presented in table (3) showed no significant (P = 0.0295) differences in estradiol level among the different experimental days, a high estradiol level (35.9±3.62 pg/ml) was recorded at day (10) at fixed TAI and lowest one (21.03±0.79 pg/ml) was detected at day (73).

Progesterone serum level

A highely significant (P < 0.0001) variation in (p4) levels among different days of the protocol was obserevd, with a higher value (17.05±5.23 ng/ml) was recorded at day (12). Meanwhile a significant lowest one (0.29±0.05ng/ml) was recorded at day (73).

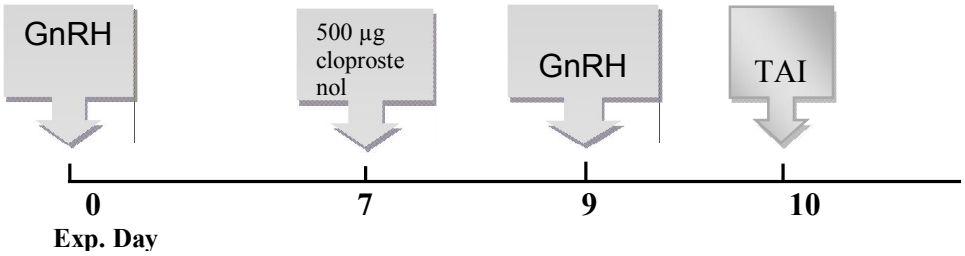

Table 2:- Some hematological parameters in cows subjected to Ovsynch protocol (GPG).

EXP. Day	Response to treatment	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	6.25±0.31^{a}	42.6±1.82^{a}	0.28±0.021^{ab}	109.9±11.70^{bc}	10.316±0.72^{b}	26.51±2.90ab	63.54±2.49^{abc}
7		7.27±0.32^{b}	48.75±0.09^{b}	0.36±0.015^{b}	125±12.41^{bc}	10.36±0.61^{b}	29.43±2.97^{bac}	64.13±3.17^{abc}
21		6.19±0.39^{a}	46.5±1.27^{b}	0.30±0.023^{ab}	127.62±14.64^{c}	6.46±0.58^{a}	29.27±1.58^{bac}	68.59±0.88^{cb}
31		6.92±0.24^{a}	43.08±0.90^{c}	0.30±0.013^{ab}	76.58±6.51^{a}	9.39±0.54^{b}	35.98±1.95^{cb}	57.95±2.06^{ab}
0	Non Pregnant	6.25±0.10^{a}	41.75±1.12^{a}	0.26±0.008^{a}	94.16±4.65^{ab}	8.85±0.81^{ab}	29.74±2.24^{bac}	64.03±2.72^{abc}
7		6.14±0.19^{a}	42.08±1.03^{a}	0.27±0.013^{a}	113.83±7.82^{bc}	6.41±0.46^{a}	36.19±1.46^{cb}	55.80±1.58^{a}
21		5.71±0.19^{a}	46.42±0.67^{b}	0.27±0.005^{a}	108.21±5.52^{bc}	6.77±0.40^{a}	21.85±1.49^{a}	72.25±1.61^{c}
31		6.013±0.33^{a}	44.61±0.99^{ba}	0.30±0.026^{ab}	93.5±8.55^{ab}	6.65±.0.35^{a}	36.7±1.41^{c}	56.99±1.47^{ab}

SE = standard Error

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

0-day: 1st dose of GnRH; 7-day: $PGF_2\alpha$; 10-day: timed A.I.; 31-day:sampling.

Table 3: Serum hormonal concentration in cows subjected to Ovsynch – protocol (GPG)

Exp. Day	Treatment (activities)	n*(15)	Estradiol (pg/ml) means ± SE	Progesterone (ng/ml) means ± SE
0	GnRh (1st dose)	15	31.07±3.79^{a}	1.86±0.44^{a}
4	Sampling		33.7±3.38^{a}	1.05±0.20^{a}
7	$PGF_2\alpha$		24.71±2.53^{a}	8.88±2.62^{ab}
9	GnRh 2nd dose		-	-
10	TAI		35.9±3.62^{a}	1.17±0.29^{a}
12	Sampling		33.9±3.15^{a}	17.05±5.23^{b}
21	Sampling		32.58±4.82^{a}	2.7±0.53^{a}
31	Sampling		26.88±3.74^{a}	0.4±0.09^{a}
73	Pregnancy diagnosis		21.03±0.79^{a}	0.29±0.05^{a}

n=number of animals

SE = standard Error

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

II. Main reproductive results of cows subjected to GPG (OvSynch protocol).

Table 4. Main reproductive results in cows subjected to OvSynch (GPG) protocol.

VARIABLE	Results	
Cyclicity before treatment n. (%)	35.75% normal cyclic	
	29.25% repeat breeder	
	35% non cyclic	
Ovulation rate	72%	
Rectal findings at time of A.I.	ovarian findings -follicular consistency	30% turgid follicles
		70% fluctuating follicles
	consistency of uterus	28% slightly tonic
		72% erected
Pregnancy rate % (n)	40% (6/15)	

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).**

The data of the current study revealed a significant increase in RBCs count and MCV, 7 days after injection of GnRh in group (2); 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.*(2000).**

Our results in cows revealed that the average animals that responded to the treatment was 72%, these finding were lower than those obtained by **Pursley *et al.*(1997)** whom found that the second GnRH injection induces ovulation in 87 to 100% of cows, which occurs 24 to 32 h after GnRH was administered.

The decrease in response to exogenous GnRH and PGF2 was reported previously by many authors, **Stevens *et al.* (1995)** reported that administration of **GnRH** and prostaglandin simultaneously on Day 8 or 10 of estrous cycle does not improve the synchrony of estrus and ovulation (luteolysis in only 6 of 16 animals) because **GnRH** disrupts follicular dynamics and induces premature ovulation or delays the normal return to estrus. **Birnie *et al.* (1997)** treated heifers with **GnRH** injections every 24 or 48 h from Day 3 until Day 17 of estrous cycle and administered prostaglandin on Day 13 of estrous cycle to study the luteal response of **GnRH** treated animals to a physiological dose of prostaglandin. They observed the luteolytic activity by using ultrasonography only in seven of 16 animals in **GnRH** treated group. **Birnie *et al.* (1997)** reported that the luteolytic activity **PGF2α** analogue is reduced when it is administered in combination with the **GnRH** agonist. They reported that reduced luteolytic effect of prostaglandin when given simultaneously with **GnRH** may be due to a luteotropic protection of **GnRH** on the **CL**, thus preventing the usual cascade of oxytocin stimulation and progesterone inhibition that occurs until completion of luteolysis. In cattle, administration of **GnRH** during the early or mid luteal phase causes an alteration of follicular distribution in the ovary by increasing the number of medium sized follicle and decreasing the number of large follicles by inducing luteinization and or atresia (**McNatty *et al.*, 1981; Thatcher *et al.*, 1989; Guilbault , *et al.*, 1990**). **GnRH** administered on Day 11 to 13 of estrous cycle alters the ovarian follicular dynamics (**Skaggs *et al.*, 1986**) since the dominant follicle either luteinizes (**Thatcher *et al.*, 1989**) or develops into a secondary **CL** following ovulation (**Stevenson *et al.*, 1993**). **Wolfenson *et al.* (1994)** studied the dynamics of follicular development by ultrasonography in cows following administration of a single dose of **GnRH** in the mid luteal phase (Day 12) of the estrous cycle. They reported the preovulatory follicles in cows following the injection of **GnRH** during the luteal phase were more homogeneous (belonging to the same follicular wave), more estrogen-active, probably due to preovulatory follicles being recruited and selected close to the time of estrus, and more dominant. **GnRH** induced ovulation or atresia of dominant follicle is followed by a new wave emergence within 3 to 4 d of treatment at any stage of estrous cycle (**Twagiramungu *et al.*, 1995**). Administration of **GnRH** induces a **FSH** increase at any stage of the estrous cycle (**Ryan *et al.*, 1994**). Thus, in cows treated with **GnRH** after the selection of a dominant follicle, gonadotropin surge is followed by a transient **FSH** increase, that is associated with the emergence of a new follicle wave. When **GnRH** treatment is applied before the selection of the dominant follicle, follicular growth is not affected **(Ryan *et al.*, 1994**).

In addition, the results of the current study revealed that overall pregnancy rate were 40% . These data are in agreement with the finding of **Pursley *et al.*(1997 ; 1998),** they found that pregnancy rates resulting from fixed time AI in lactating dairy cows vary from 32 to 45% following administration of Ovsynch.

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