

## Comparing the Efficacy of Cabergoline, Receptal and FSH in Inducing Oestrus in Anoestrous Bitches

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**Abstract:** The purpose of this work was to shorten the anestrus period and induce estrus by using dopamine agonist (Cabergoline), GnRH agonist (Receptal) and gonadotropin (FSH) and compare their efficacy in inducing fertile estrus cycle. The clinical trial involved bitches (N=21) of mixed breeds which were divided equally into three groups: group A was orally administered cabergoline (5 µg/kg) until the onset of proestrus or for maximum 40 days, group B was treated with receptal subcutaneously at a dose of 1.5 µg/kg for 11 consecutive days, followed by a dose of 0.75 µg/kg for 3 days and group C was injected with FSH at a dose of 20 IU/kg for 5 consecutive days then hCG 25IU/kg once on Day 5 by intramuscular injection. Cabergoline confirmed its efficacy for inducing fertile estrus as induced proestrus came after 32.6±5.9 days in 6 of 7 bitches and duration of proestrus was 9.6±1.6 days with ovulation rate of 100%. On the contrary, the proestrus was observed in 4 of 7 bitches in both group B and C after 21±2.21 days and 6.25±1.25 days, respectively. The results confirmed that the dopaminergic protocol is the treatment of choice and easily applied for estrus induction.

**Key words:** Bitch • Estrus Cycle • Anestrus • Gonadotropins

### INTRODUCTION

Sexual puberty of domestic dogs begins from 6 to 12 months of age except the large breeds where it may be delayed till 24 months of age [1]. Bitches have unusual estrus cycle between other domestic animals, divided into four phases as proestrus, estrus, diestrus and anestrus phases and also characterized by the prolonged follicular phases followed by the very long luteal phase with an average of 75 days [2]. The anestrus duration of fertile bitches ranges from 2 to 8 months within an average of 5 months depending on different factors between bitches but when the hypothalamus secretes GnRH, the anestrus is terminated and new follicular phase will begin [3, 4]. The end of physiological anoestrus and the beginning of a new follicular phase by endocrine changes are still not completely understood. In recent years, the veterinarian performed studies which made clear that many changes occur at the hypothalamic-pituitary-ovarian axis during late anoestrus [5]. Spattini *et al.* [6] and England *et al.* [7] demonstrated that the sensitivity of the pituitary gland to GnRH increases at the end of anestrus, this lead to an increase of the concentration of FSH, LH and estrogen and a decrease of prolactin

secretion which finally produce ovarian follicles and ovulation. Nowadays, dog owners want to shorten the inter-estrus interval in order to obtain more puppies from their bitches for many purposes. The management of breeding was tried for the termination of physiological anestrus and estrus induction [8]. Several protocols as exogenous gonadotropins, synthetic estrogens, GnRH agonists and dopamine agonists (DA) had been applied for induction of estrus in anestrus bitches [9-11]. Dopamine agonists such as cabergoline, bromocriptine or metergoline are ergot derivate alkaloids act by exerting an anti-prolactinergic effect to increase basal plasma FSH concentration so shorten the inter-estrus interval [12, 13]. Several studies used dopamine agonists or PMSG alone or compared their efficacy for estrus induction and for the treatment of primary and secondary anestrus in bitches [14, 15]. Few reports measured progesterone concentrations to confirm ovulation after cabergoline treatment in bitches [14, 16]. Therefore, the aim of this study was to use dopamine agonist (Cabergoline), GnRH agonist (Receptal) and gonadotropin (FSH) for the shortening of inter estrus interval, comparing their efficacy in inducing fertile estrus and evaluating their progesterone profiles following cycle induction in bitches.

## MATERIALS AND METHODS

Twenty-one bitches of mixed breeds (Mongrel & German shepherd) were used in this study of 10-20 Kg body weight and of 2-5 years old. They were housed from January to July months 2018 in the Clinic of Veterinary hospital (Faculty of Veterinary Medicine, Zagazig University, Egypt). Bitches were fed balanced diet and provided with water *ad libitum*. Bitches were confirmed to be in anestrus on the basis of vaginal smear, ultrasonography, hormonal assay and vaginal endoscopy. Bitches equally divided into three treatment groups. Group A was administrated dopamine agonist (Cabergoline, MJR, USA) orally at a dose of 5 µg/kg BW daily till the serosanguinous vaginal discharge appear (The sign of the beginning of proestrus) or for a maximum of 40 days. Group B was injected GnRH agonist (Receptal, Intervet, MSD) subcutaneously at a dose of 1.5 µg/kg BW for 11 consecutive days then 0.75 µg/kg BW for 3 days. Group C was injected FSH 20 IU/kg BW (SEDICO, Egypt) intramuscular for 5 consecutive days then hCG 25 IU/kg BW once on the last day with the FSH.

Vaginal smear and vaginal endoscopy were performed twice weekly until the onset of the proestrus.

Ultrasound examination: Esoate Mylap ultrasound machine (Netherlands) with linear transducer (10 MHZ) used for examination of genitalia of bitches.

**Blood Sampling and Hormone Assaying:** Blood samples were collected from the bitches by puncture of cephalic vein by using sterile plastic syringe, serum was separated by centrifugation of blood samples in clean and dry centrifuge tubes at 1000 rpm for 15 minutes then sent to laboratory (Ultra Lab, Sharkia, Egypt) at the same day for measuring estrogen and progesterone concentration by chemiluminescence assay as quantitative assay in laboratory. The interassay coefficients of progesterone varied between 10.1% and 14.2% and the intra-assay coefficients of variation ranged from 8.9% to 9.7%. Estrogen hormone measurement was measured for all the treated groups along treatment period for recording its levels. From the beginning of proestrus until the beginning of diestrus, vaginal smear and vaginal endoscopy and progesterone assay applied for detecting the optimal time of insemination and monitoring phases of estrus cycle if normal or abnormal, the ovulated cyclic bitches were mated by natural mating then examined by ultrasonography for pregnancy diagnosis 20 days after the last mating. Pregnant bitches were followed till parturition.

**Statistical Analysis:** Data characterizing the induced estrus cycle were analyzed using the SPSS program, version 22. Data are presented as the mean ± SD and compared the interval between the initiation of the treatment and the first day of proestrus, the duration of proestrus and estrus. The ovulation rate, pregnancy rate and parturition rate are presented as percentage. The average progesterone concentration for the ovulating bitches of each group and sampling day were calculated as the mean ± SD for each group, Duncan's Multiple Range test was used to separate between significant means at  $P < 0.05$ . Analyses were performed using generalized linear model. The value of  $P < 0.05$  was used to indicate statistical significance and  $P < 0.001$  indicate high significance difference.

## RESULTS

The bitches used in the current study were anestrus as confirmed by basal progesterone ( $P4 = 0.5$  ng/ml), small oval hypoechoic ovaries without any structures by ultrasonographic examination, the presence of small intermediate cells and small number of para-basal cells by making vaginal smear and by the vaginal endoscopy, vagina appeared dry, thin, reddish and without any mucosal folds with narrow vaginal lumen.

As shown in table 1, six out of seven bitches of group A came into proestrus  $32.6 \pm 5.9$  days from beginning of treatment. Four out of seven bitches of group B came into proestrus  $21 \pm 2.21$  days and four out of seven bitches of group C came into proestrus  $6.25 \pm 1.25$  days. All treated and responded bitches of group A and C and one bitch of group B ovulated. Progesterone was the confirmatory test to detect ovulation in all responded animals (four bitches which came into proestrus), progesterone did not elevate  $>1.5$  ng/ml from beginning of estrus until two months.

In group B, four out of seven bitches came into proestrus after treatment within  $21 \pm 2.21$  days; one out of the last non responding three bitches did not secrete bloody vaginal discharge absolutely in spite of presence of ovarian follicles by ultrasonographic examination, additionally the hormonal profile showed high estrogen concentration of 73.8 pg/ml and minimum progesterone concentration of 0.879 ng/ml. Those marks continued for 7 days then after that the bitch accepted the male dog for mating and its progesterone levels increased to 6.8 ng/ml on the expected day of ovulation, then continued high. All those changes indicated the normal sequence of proestrus and estrus phases, with the

absence of the bloody vaginal discharge which is considered the first sign of the proestrus onset. When laparotomy was performed for this bitch 45 days after the beginning of treatment, it revealed an enlarged, hard and closed cervix and distended enlarged uterus with blood (closed hymen).

**Progesterone Profile in Ovulatory Cycle of Responding Bitches from the Beginning of Proestrus till the Beginning of Diestrus:** Progesterone concentration during the ovulatory cycle of the three groups was relatively similar at the first day of proestrus, then on Day 3 of proestrus it significantly ( $P<0.01$ ) increased in group C compared to groups A and B then reached  $1.67\pm 0.34$ ng/ml. Progesterone concentration was significantly ( $P<0.01$ ) higher in group A ( $1.62\pm 0.09$  ng/ml) than group B ( $2.13\pm 0.12$  ng/ml). In group C, progesterone concentration reached  $1.67 \pm 0.08$  ng/ml on Day 3 from the beginning of proestrus then increased to  $3.95 \pm 0.42$  ng/ml on Day 5. At the time of expected ovulation on Day 11 for group A and B and Day 7 for group C, the average concentration reached  $5.9\pm 0.09$  ng/ml,  $6.3\pm 0.93$  ng/ml and  $7.6\pm 0.25$  ng/ml in group A, B and C, respectively. Progesterone concentration continued to increase till

reached to a peak on Day 21 from the beginning of proestrus in ovulated bitches of groups A and B and maintained high at this level until one week before birth. But the ovulated bitches of group C showed a significantly ( $P<0.01$ ) low increase of progesterone concentration compared to the other two groups where it reached a peak of  $24.5\pm 1.7$ ng/ml on Day 21 from the beginning of proestrus. Progesterone continued maintained this concentration for three weeks then decreased till reached an average of 10 ng/ml on days 35 and 38 in the two pregnant bitches only and lead to abortion.

Twenty days after the last breeding, the ultrasonographic examination of the ovulated bitches' detected early pregnancy with a pregnancy rate of 100% for group A, 66.6% for group B and 75% for group C. In group A, parturition occurred in five out of six pregnant bitches 59-63 days after breeding, only one bitch aborted on Day 50 of pregnancy. For group B, two pregnant bitches delivered 60-64 days after breeding and only one pregnant bitch gave birth 61 days after breeding of group C, but the other two pregnant bitches' had fetal death 40 days after breeding followed by abortion within few days.

Table 1: Groups of estrus induction and comparison between them

	Group A (Cabergoline)	Group B (Receptal)	Group C (FSH+hCG)
No. of treated bitches	7	7	7
No. of responding bitches	6 (85%)	4 (57%)	4 (57%)
Interval from the beginning of treatment to proestrus onset (days, mean±SD)	$32.6 \pm 5.9$	$21 \pm 2.21$	$6.25 \pm 1.25$
Duration of proestrus (days, mean±SD)	$9.6 \pm 1.6$	$8 \pm 1.6$	$5.3 \pm 0.81$
Duration of estrus (days, mean±SD)	$8.7 \pm 1.3$	$8.2 \pm 1.3$	$8.75 \pm 0.5$
Estimated ovulation rate (%)	100 (n= 6/6)	75 (n= 3/4)	100 (n= 4/4)
Pregnancy rate (%)	100 (n= 6/6)	66.6 (n= 2/3)	75 (n= 3/4)
Parturition rate (%)	83.3 (n= 5/6)	100 (n= 2/2)	33.3 (n= 1/3)

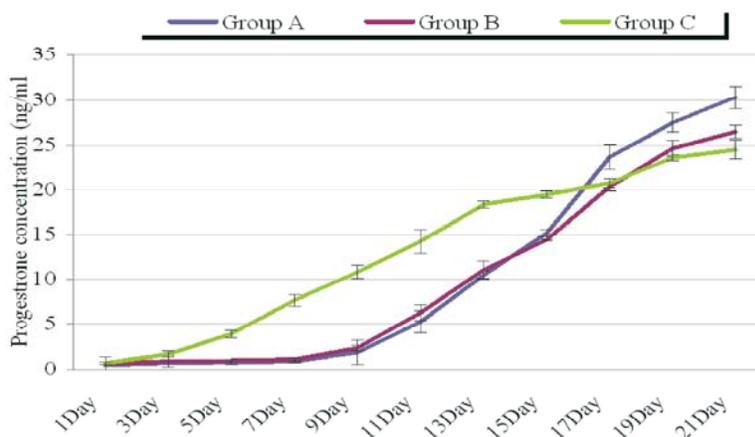


Fig. 1: Chart line of mean ±SD progesterone concentration in 3 groups from the beginning of proestrus to the beginning of diestrus

## DISCUSSION

The most important applications of estrus induction protocols in bitches used for breeding were either termination of anestrus or shortening of interval between two successive estrus cycles or for treatment of primary or secondary anestrus [17, 18]. In this study, of the use of three hormonal protocols for the termination of anestrus and estrus induction in bitches has proved the efficacy of dopamine agonist (Cabergoline) for inducing fertile and normal estrus in six bitches out of seven bitches (85%) and also included follicular development with 100% ovulation rate. Previous studies of estrus cycle stimulation with cabergoline have reported their efficacy for inducing oestrus in 60-100% with ovulation rate was 80-100% depend mainly on the anoestrus stage [14, 19]. The anoestrus stage is one of the most important factors affect the success of treatment. Although in the present study, the anoestrus stage was unknown; the obtained results correspond with those cited above. Shimatsu [20] referred that four out of five non pregnant beagle bitches had a fertile oestrus induced by cabergoline after period of time ranged from 27 to 32 days; these results are in accord with our results as proestrus induced in six bitches of cabergoline group after  $32.6 \pm 5.9$  days. Also oestrus was induced in 10 of 12 anoestrus bitches (83%) with 100 % ovulation and pregnancy rate and litter size of  $5.2 \pm 0.5$  puppies in study reported by Rota *et al.* [15] when cabergoline was used for the estrus induction in anoestrus bitches. Kutzler *et al.* [21] mentioned in their study that GnRH agonist induced oestrus within 80% of anoestrus bitches earlier ( 9-11 days of treatment) than the current results, this result not accord with our results as GnRH agonist induced oestrus in four of seven anoestrus bitches with 57% after  $21 \pm 2.21$  days from the beginning of treatment. Also Fontaine *et al.* [22] used GnRH agonist (Deslorelin implant) for oestrus induction in anoestrus bitches and reported that oestrus was induced with 100% within  $4.3 \pm 1.4$  days after implantation and achieved a pregnancy rate of 58%. This may refer to the use of different types of GnRH agonists and difference in route of administration. One non responding bitch of Receptal group did not secret bloody vaginal discharge after 25 days from start of treatment although of presence of follicles on ovaries and high estrogen level reached to 73.8 Pg/ml. Those signs appeared only in proestrus phase, but such bitch did not excrete the bloody vaginal discharge which considered the more prominent mark of proestrus signs in bitches. By laparotomy of this bitch, there was enlarged hard closed cervix with distended filled uterus with blood. The cause

of cervical closure in this bitch may be the probability of previous wound in cervix which healed by fibrous tissue and gave to cervix its hardness or persistent hymen. Different protocols used exogenous gonadotropins for estrus induction in bitches as eCG, hCG and LH [18, 23, 24]. Paisley and Fahning [25] mentioned that 7 of 10 anoestrus bitches were treated with FSH for oestrus induction. Also, Shille *et al.* [26] used FSH in anoestrus bitches for oestrus induction and mentioned that 4 of 14 bitches came into proestrus and only one bitch became pregnant. Those above cited results not correspond with our results as FSH induced oestrus in 4 of 7 anoestrus bitches (57%) and 3 bitches became pregnant. In the current study, side effects of FSH using reported as shorter proestrus duration, declining progesterone levels and premature luteal failure in pregnant bitches, these disadvantages were mentioned also in Shille *et al.* [26]. Premature luteal failure and subsequent pregnancy loss was frequently observed in bitches treated with eCG [27, 28]. The use of hCG to induce ovulation for oestrus induction protocols in bitches is quite controversial because they are spontaneous ovulators. Also, cows, ewes, mares and sows are spontaneous ovulators but a single dose of hCG could be used routinely to induce ovulation [16, 29]. The study of Wright [30] reported that ovulation occurs 26 to 30 hours after the administration of hCG in bitches, so most protocols for estrus induction in bitches preferred using a single dose of 500 IU at the last day of treatment. Progesterone secretion originating from ovarian structures may reflect ovarian morphology in both spontaneous and induced cycles and can be applied as the diagnostic tool, so progesterone could be measured during physiological cycles only [3]. The current study is one of a few reports that have evaluated the progesterone concentrations of bitches with induced cycles. In the cabergoline treated bitches, the progesterone concentration was nearly physiological and gave results as a spontaneous oestrus cycle. This result corresponds with earlier reports [14, 31, 32]. In FSH treated bitches, progesterone concentration elevated before ovulation which originated from luteinized follicles then observed significantly higher progesterone level during ovulation originated from hyperluteinized follicles. This result agreed with results from [3, 24, 33].

## CONCLUSION

Dopamine agonist cabergoline confirmed its efficacy for inducing fertile estrus in anestrus bitches with 100% ovulation rate and without side effects in bitches.

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