



Effect of Dopamine antagonist (Domperidone) treatment on induction of ovulation in anestrus mares

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Abstract

The current study is an endeavor for profound understanding some hormonal and biochemical constituents during induction of ovulation in anestrus mares by using domperidone (Dopamine antagonist). Fifteen anestrus pure Arabian mares divided randomly into 2 groups, group (1) five mares and serve as a control, while Group (2) ten mares treated with 1.1 mg/kg domperidone for 25 days. Ultrasonographic scan was performed twice per week for detection of ovarian activity and follicular growth. Blood samples were collected one month before the treatment for 3 times with 10 days intervals for detection of progesterone, and twice per week after treatment till 10 days after the end of treatment for determination of progesterone, estrogen and prolactin hormones in addition to total protein, total cholesterol and triglycerides, before and after response for treatment. Eight mares (80%) showed ovulation and CL formation from the domperidone treated group while 2 mares (20%) showed no large follicles formation till the end of the trial period. The obtained results showed a significant increase in progesterone, estradiol and prolactin concentration in treated group compared to control one at 3rd and 4th week from treatment, while cholesterol level showed significant increase in treated group compared to control at 3rd week of treatment. And non significance change was recorded in protein and triglycerides levels. We can conclude that using Dopamine antagonist (Domperidone) is effective alternative for induction of ovulation in anestrus Arabian mares under Egyptian circumstances.

Key words: anestrus mares, domperidone, progesterone, prolactin, ultrasonography, ovulation, Arabian mares.

Introduction

Although, mares are a seasonally polyestrus with ovulatory activity being related to long days and light. Mares are often presented with a history of anestrus during the breeding season. The confusion associated with the long and variable transition from anestrus to cyclicity in mares greatly magnifies the complexity of efficient reproductive management for this category of mares **Burns et al. (2007)**. For the reason horse breeders prefer to breed mares as early as possible in the year, outside the physiological breeding season. Attempts must be made to stimulate follicular development and ovulation using exogenous hormones **Medan and Al-Daek (2014)**. Several pharmacological protocols, including pituitary extracts, GnRH, progestogens, and dopamine antagonists, have been designed to reduce the length of treatment **Nagy et al. (1999)**. A few studies investigated the role of dopamine in the control of reproduction in mares, but it has been recorded that dopamine concentration in cerebrospinal fluid is higher during the anovulatory than during the breeding season (**Melrose et al. 1990**), and it is inversely related to prolactin (PRL) plasma levels (**Johnson, 1986**). Moreover, dopamine receptors have been recently observed on both theca and granulosa cells, as well as a higher PRL level in fluid of follicles >20 mm of

diameter, correlated with dopamine seasonal variations (**King et al. 2008**).

Donald et al. (2015) showing that prolactin treatment, or inducement of high prolactin secretion by means of antidopaminergic agents, in winter can induce ovarian activity and ovulation in seasonally anovulatory mares. Beginning with dopamine antagonist treatment when follicles were 25 mm in diameter resulted in a significant advancement of cyclicity in non-phto-stimulated mares. Pregnancy rates after artificial insemination of treated mares were similar to those of untreated animals (**Panzani, et al. 2011**).

Using of a dopamine antagonist, such as domperidone or sulpiride, stimulates an increase in plasma prolactin concentrations. Prolactin acts directly on the ovary to stimulate expression of gonadotropin receptors. Pituitary gonadotropin (FSH and LH) secretion is not altered by dopamine antagonist therapy, but treated mares are more sensitive to endogenous gonadotropins due to increased numbers of gonadotropin receptors which may stimulate follicular development (**McCue and Patrick, 2014**). Administration of domperidone (1.1 mg/kg) to seasonally anestrus mares maintained under natural photoperiod stimulated follicular development within 14 days of treatment and hastened mean date of ovulation by 78 days (**Brendemuehl and Cross, 2000**).

Sulpiride is effective in advancing the beginning of transition period and the first ovulation whereas domperidone is successful only in some mares (Mari, et al. 2009).

Domperidone also used in the prevention of reproductive complications in mares Crossa et al. (2012). Administration of domperidone Brendemuehl and Cross (2000), has been demonstrated to stimulate follicular growth and/or ovulation in seasonally anovulatory mares. However, the effects of dopamine antagonists on the onset of reproductive activity conflict McCue et al. (1999) between experiments; in addition, a high variability among individuals has been observed Nagy et al., (1999). Changes in biochemical constituents of blood are important indicators of physiological state of an animal Perveen and Usmani (1993). Metabolic changes in the blood serum may be reflected in the biochemical composition of follicular fluid and can indirectly influence reproduction efficiency O'Callaghan and Boland (1999).

The aim of this work is study the effect of using domperidone in the induction of cyclicity in anestrus mares under Egyptian circumstances.

Materials And Methods

1.1. Experimental animals and housing:

The current study was performed on (fifteen anestrus pure Arabian mares) located in different private stud farms in El-haram, Giza, Egypt. Mares were 3-15 years old, with an average weight 400 kg. Mares were under ambient lighting before and during the study. They were kept in boxes with possibility of access into paddocks during the day. Mares were found free from pathological conditions after a complete breeding soundness evaluation and were considered in deep anestrus on the basis of the following criteria: (a) plasma progesterone (P4) concentration below 1 ng/ml in three samples taken at 10 days intervals, (b) uterine and cervical tone, detected by clinical and ultrasonographic examinations, consistent with anestrus condition and divided randomly into 2 groups: Group (1): five mares and serve as control. Group (2): ten mares treated with domperidone "treated group".

2. 2. Nutrition and treatment:

The animals were fed Egyptian clover (*Trifolium alexandrinum*) and legume hay.

The animals were provided with concentrated ration and wheat straw in amounts sufficient to maintain body weight and had free access to water and mineralized salt. Domperidone treated mares (Group 2), (n = 10) dosed daily with 1.1 mg/kg domperidone (Motilium, 10 mg tablets, Janssen-Cilag S.p.A., Egypt) for 25 days.; while control group (Group 1), (n = 5) received no treatment.

2. 3. Ovarian examination:

Ultrasonography was performed using real time B-mode scanners (Esaote Mylab30-Netherlands) equipped with 5-7.5 MHz frequency LV513 linear-array rectal transducer, The scanner have a built-in electronic caliper system for measuring distance, area and circumference, angle and auto follow measurements. Ultrasonic gel (Carboxymethylcellulose) was used as a lubricant during scanning. Ultrasonographic scan was performed once every 10 days for detection of anestrus then twice per week for detection of ovarian activity and follicular growth after beginning of treatment.

2.4. Blood sampling and hormonal assay:

As mentioned above, in order to assess the reproductive status and to confirm the anestrus condition, one month before the beginning of the treatment three blood samples were collected at 10 days interval for progesterone determination. During the treatment, and until 10 days after the end of treatment, blood samples were collected twice per week. Blood was collected by jugular venipuncture followed by centrifugation at 3000 rpm for 10 minutes to obtain serum then kept in deep freeze at (-18° C) for hormonal assay and biochemical analysis. Blood samples were collected during anestrus phase and after response of treatment with appearance of mature follicle on the ovary.

2.5. Biochemical and hormonal assay:

Serum samples were used for determination of progesterone, estrogen and prolactin hormones according to [Tietz (1995); Stabenfeldt, and Hughes (1986) and Uotila et al. (1981)] before and after response for treatment respectively, in addition to total protein, total cholesterol and triglycerides according to [Henery (1968);Watson (1960) and Zollner and Kirsch (1962)] before and after response for treatment respectively, the average result /week in each parameter was prepared for

statistical analysis. Days of ovulation were recorded as the first day when a corpus luteum (CL) was detected by ultrasonography and confirmed by plasma progesterone concentrations.

2.6. Statistical analysis:

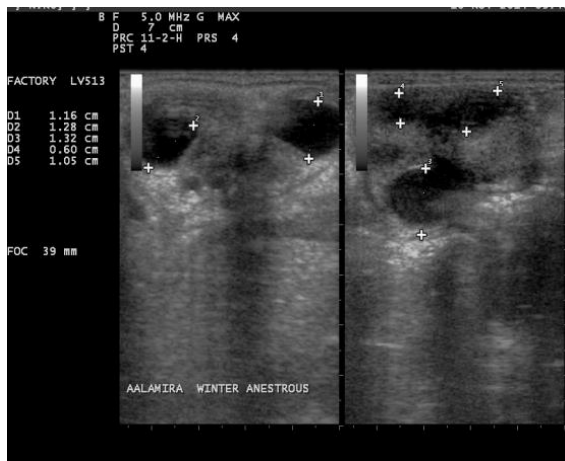
Data were subjected to statistical analysis using SPSS / PC using independent samples T test according to **Snedecor and Cochran, (1982)**.

Results

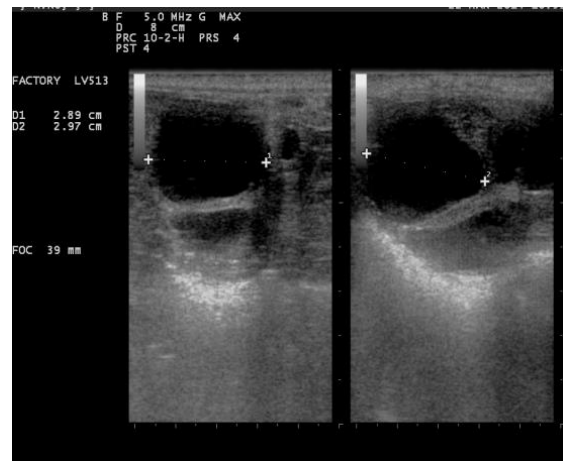
1.2. Ovarian examination:

Each mare in the groups considered as anestrus according to the results of serum progesterone level (<1.0 ng/ml in all mares) and diameter of the largest follicle at the start of the treatment (11.50 ± 0.34 mm for treatment

group and 11.50 ± 0.35 mm for control group). Ultrasonographic scanning revealed presence of small follicles on both ovaries with diameters less than 20 mm (Fig. 1-A). Ultrasonography was performed twice per week to find out the progress of follicular growth concurrently with administration of domperidone. The response for domperidone treatment was evaluated through detection of growing follicles that reached to over 25 mm in diameters (Fig. 1-B) and large follicles over 35 mm (Fig. 1-C) then detection of ovulation and CL formation (Fig. 1-D). The response for treatment was recorded in 8 mares while 2 mares showed no large follicles formation till the end of the trial period.



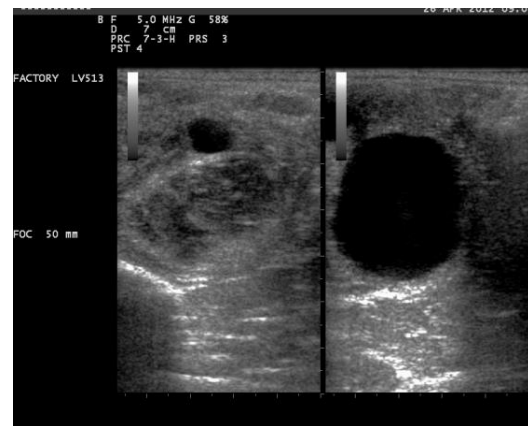
(A)



(B)



(C)



(D)

Fig. 1: (A) Small follicles on both ovaries of anestrus mares less than 20 mm in diameters. (B) Growing follicles over 25 mm in diameter. (C) Large follicle over 35 mm in diameter. (D) Evidence of CL formed after ovulation.

3.2. Biochemical and hormonal Assay:

The animal chosen for the study subjected to progesterone analysis for 3 successive blood samples 10 days apart the obtained results were 0.45 ± 0.06 , 0.52 ± 0.04 and 0.47 ± 0.05 ng/ml, respectively as shown in table (1).

Table (1): Progesterone level (ng/ml) in the samples taken from studied mares. (Mean + SEM)

| Studied mares | Sample (1) | Sample (2) | Sample (3) |
|--------------------------|-----------------|-----------------|-----------------|
| Progesterone level ng/ml | 0.45 ± 0.06 | 0.52 ± 0.04 | 0.47 ± 0.05 |

Serum estradiol concentration increased gradually after 1 week from the beginning of domperidone treatment and showed significant increase ($P < 0.001$) at 3rd and 4th week compared to control group, as seen in (table 2).

Table (2): Serum estradiol (pg/ml) concentration for control and treated groups. (mean+SEM)

| Parameters | | before | 1 st week | 2 nd week | 3 rd week | 4 th week | 5 th week |
|------------|-----------|---------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Estradiol | Group (1) | 11.60 | 12.20 | 12.20 | 13.40 | 11.60 | 13.00 |
| | | \pm 2.14 | \pm 2.13 | \pm 1.39 | \pm 1.43 | \pm 1.44 | \pm 1.70 |
| | Group (2) | 11.90 | 12.10 | 17.30 | 27.60 | 23.20 | 16.10 |
| | | \pm 1.27 | \pm 1.08 | \pm 2.80 | \pm 2.37** | \pm 2.33 ** | \pm 2.08 |

** : ($P < 0.001$)

Serum progesterone level increased significantly ($P < 0.001$) over 1 ng/ml in the 4th week from the beginning of treatment as shown in table (3).

Table (3): Serum progesterone concentration (ng/ml) for control and treated groups.

| Parameters | | before | 1 st week | 2 nd week | 3 rd week | 4 th week | 5 th week |
|--------------|-----------|-----------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Progesterone | Group (1) | 0.46 | 0.49 | 0.57 | 0.57 | 0.52 | 0.58 |
| | | \pm 0.05 | \pm 0.05 | \pm 0.05 | \pm 0.05 | \pm 0.04 | \pm 0.06 |
| | Group (2) | 0.46 ± 0.04 | 0.51 | 0.51 | 0.62 | 1.16 | 1.21 |
| | | \pm 0.05 | \pm 0.05 | \pm 0.03 | \pm 0.05 | \pm 0.17 ** | \pm 0.21 |

** : ($P < 0.001$).

In treatment group, serum prolactin concentration began to increase from the first week of treatment and was significant at 3rd week ($P < 0.05$) and 4th week ($P < 0.001$); of treatment, while in control group there were no significant increase in prolactin hormone as seen in (table 4).

Table (4): Serum prolactin concentration (ng/ml) for control and treated groups. (mean+SEM)

| Parameters | | before | 1 st week | 2 nd week | 3 rd week | 4 th week | 5 th week |
|------------|-----------|---------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Prolactin | Group (1) | 6.80 | 7.40 | 9.00 | 10.80 | 12.40 | 13.60 |
| | | \pm 1.07 | \pm 1.03 | \pm 1.92 | \pm 1.74 | \pm 1.86 | \pm 1.50 |
| | Group (2) | 5.90 | 16.10 | 16.60 | 20.00 | 23.90 | 16.00 |
| | | \pm 0.62 | \pm 1.43 | \pm 1.78 | \pm 2.07 * | \pm 2.26** | \pm 0.75 |

** : ($P < 0.001$); * : ($P < 0.05$).

The serum total protein showed non significant difference between treated and control groups as seen in table (5).

Table (5): Serum total protein (gm/dl) for control and treated groups. (mean+SEM)

| Parameters | | before | 1 st week | 2 nd week | 3 rd week | 4 th week | 5 th week |
|---------------|-----------|---------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Total protein | Group (1) | 6.28 | 6.59 | 6.60 | 6.99 | 6.67 | 6.58 |
| | | \pm 0.26 | \pm 0.23 | \pm 0.35 | \pm 0.12 | \pm 0.27 | \pm 0.33 |
| | Group (2) | 6.53 | 6.53 | 6.52 | 6.63 | 6.83 | 6.63 |
| | | \pm 0.18 | \pm 0.16 | \pm 0.19 | \pm 0.14 | \pm 0.13 | \pm 0.15 |

The serum total cholesterol showed significant increase ($P < 0.001$) and ($P < 0.05$) in the 2nd and 3rd weeks respectively, as seen in table (6).

Table (6): Total cholesterol (mg/dl) patterns for control and treated groups. (mean+SEM)

| Parameter | | before | 1st week | 2nd week | 3rd week | 4th week | 5th week |
|---------------|-----------|--------|----------|----------|----------|----------|----------|
| T.Cholesterol | Group (1) | 155.20 | 159.20 | 158.60 | 161.00 | 167.00 | 169.00 |
| | | ± 3.06 | ± 4.57 | ± 4.34 | ± 1.87 | ± 3.81 | ± 3.08 |
| | Group (2) | 154.00 | 159.60 | 169.00 | 174.00 | 179.00 | 176.00 |
| | | ± 2.13 | ± 1.68 | ± 2.85* | ± 2.99** | ± 2.34 | ± 2.45 |

**:(P < 0.001); *(P < 0.05).

Triglycerides showed non significant difference between treated and control groups as seen in table (7).

Table (7): Triglycerides patterns for control and treated groups.

| Parameters | | before | 1st week | 2nd week | 3rd week | 4th week | 5th week |
|---------------|-----------|--------|----------|----------|----------|----------|----------|
| Triglycerides | Group (1) | 64.90 | 65.55 | 63.27 | 62.93 | 66.69 | 66.93 |
| | | ± 1.83 | ± 1.51 | ± 1.87 | ± 3.09 | ± 2.19 | ± 3.38 |
| | Group (2) | 62.83 | 64.54 | 62.99 | 64.86 | 65.54 | 66.55 |
| | | ± 1.17 | ± 1.23 | ± 1.52 | ± 1.28 | ± 1.55 | ± 1.54 |

Discussion

The current study was applied for using a non-traditional treatment for anestrus in Arabian mares in Egypt. It included using of dopamine antagonist (domperidone) in mares showed small follicles on the ovaries that were less than 20 mm in diameters with absence of CL, and serum progesterone level was less than 1 ng/ml. the obtained results demonstrated that, the majority of mares (8 mares) showed a response to treatment through appearance of larger follicles, mature follicles followed by ovulation, through the treatment period, while 2 mares showed no response till the end of treatment. These results similar to that obtained by (Panzani et al. 2011) who has have used dopamine antagonist (sulpiride). The same results were mentioned by (Brendemuehl and Cross, 2000) who have used domperidone treatment which resulted in development of follicular growth that happened 14 days from the beginning of treatment. The exact mechanism of action of catecholamines, dopamine in particular, on follicular dynamics is not known but is believed to involve regulation of prolactin as pituitary production of prolactin which primarily regulated through inhibition by the neurotransmitter dopamine (Cross et al., 1995).

The explanation of the response to the treatment with dopamine antagonist varied in the hypothesis, in the current study, prolactin and estradiol serum levels began to increase after the first week of treatment that indicated follicular development as a result of inhibition

of dopamine and increased stimulation of gonadotrophin receptors on the ovary, these results were potentiated by the results of (Freedman et al., 1979; Schally et al., 1973; Duittoz and Batailler, 2000; Clarke and Cummins 1982; Mari et al., 2009 and McCue and Patrick; 2014) Also our finding supported by Hiroshi-kato et al., (2016), who found that, the effects of domperidone, a dopamine antagonist, on prolactin release in female rats were studied. Panzani et al. (2011) recorded that, beginning with dopamine antagonist treatment when follicles were 25 mm in diameter resulted in a significant advancement of cyclicity in non-photo-stimulated mares. The serum total cholesterol showed significant increase (P < 0.001) and (P < 0.05) in the 2nd and 3rd weeks respectively may be due to the need for dramatic changes in the hormones levels and as known the cholesterol is the precursor of many hormones. Oral administration of domperidone for 14 days caused a significant increase in serum prolactin levels in mature female rats. Kelly et al. (2006) who clearly showed a positive effect of estradiol pretreatment on the efficacy of dopamine antagonist therapy in anestrus mares in addition to Mitcham et al. (2014) recorded that, the pretreatment with estradiol benzoate prior to the administration of dopamine antagonist lead to enhancement of prolactin secretion and superior advancement of first ovulation. On the other hand, (Mari et al. 2009) who founded good results with sulpiride and poor results with domperidone, in

inducing ovulation in anestrus mares, this finding may be referred to the environmental conditions in Egypt and breed of Arabian mares. The serum prolactin concentration in treatment group in the current results, began to increase from the first week of treatment and was significant at 3rd week ($P < 0.05$) and 4th week ($P < 0.001$); of treatment, while in control group there were no significant increase in prolactin hormone. This result coincides with the findings recorded by **Donald et al. (2015)** who found raising prolactin concentrations in blood with the onset of ovarian activity and ovulation in seasonally anovulatory mares. The increase in serum progesterone concentration significantly ($P < 0.001$) over 1 ng/ml in the 4th week from the beginning of treatment in current results agreed with that mentioned by **Vizuete et al. (2013)** who concluded that the treatments with progesterone may be effective

for oestrous induction in mares during the late phase of the seasonally anovulatory period. Also **Polo et al. (2016)** concluded that, increase progesterone concentration by two administrations of P4 affected ovarian cyclicity in anestrus mares, and synchronized follicular waves and ovulation. The obtained results showed non-significant difference in serum total protein and Triglycerides levels between treated and control groups; this may be due to balanced ration that matches with the requirement that offers to the mares in both groups **Benjamin (1979)**. However, **Ali et al. (2004)** reported that the level of serum total protein was comparatively lower in infertile mares when compared with those in estrus and regular breeder mares. We can conclude that using Dopamine antagonist (Domperidone) is an effective alternative for induction of ovulation in anestrus Arabian mares under Egyptian circumstances.

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الملخص العربي

تأثير استخدام العلاج بمضادات الدوبامين (دومبيريدون) على حث التبويض في حالة انعدام الشبق في الأفراس
د. طه عبد القادر غطاس- د. محمد كمال درباله

معهد بحوث التناسليات الحيوانية بالهرم- مركز البحوث الزراعية

تمت هذه الدراسة لمعرفة تأثير استخدام مادة الدومبيريدون على حث التبويض في الأفراس التي تعاني من انعدام الشبق وكذلك فهم التغيرات الهرمونية والبيوكيميائية المصاحبة. أجريت هذه الدراسة على عدد 15 فرس عربي أصيل مصري وتم تقسيمها الى مجموعتين، الأولى (5 أفراس) كمجموعة قياسية (كنترول) بينما المجموعة الثانية (10 أفراس) تم معالجتها بمادة الدومبيريدون 1.1 مجم/كجم عن طريق الفم ولمدة 25 يوم، تم الفحص بالموجات فوق الصوتية مرتين في الأسبوع لمتابعة نمو الجريبات على المبايض وتم تجميع عينات الدم من الأفراس محل الدراسة قبل بدأ العلاج بشهر ثلاث مرات بواقع 10 أيام بين كل عينه، ثم مرتين كل أسبوع مع الفحص بالسونار لقياس مستوى البروجستيرون والأستراديول والبرولاكتين والبروتين الكلي والكوليستيرول الكلي والتراي جلسريد. ومن النتائج التي تم الحصول عليها هو 8 أفراس (80%) حدث فيها تبويض وتكوين جسم أصفر في المجموعة التي تمت معالجتها بالدومبيريدون بينما عدد 2 فرس (20%) لم يظهر فيها تكوين جريبات حتى نهاية التجربة، ومن النتائج أيضا هو زيادة ملحوظة في مستوى البروجستيرون والأستراديول والبرولاكتين مقارنة بمجموعة القياس عند الأسبوع الثالث والرابع من بداية العلاج، ولم يتم تسجيل تغيرات جوهرية في مستوى البروتين والتراي جلسريد، فمن خلال هذه الدراسة نستطيع أن نستنبط أن استخدام مادة الدومبيريدون هو بديل مؤثر في الحث على التبويض في الأفراس العربية التي تعاني من انعدام الشباع تحت ظروف البيئة المصرية.

الكلمات الدالة: دومبيريدون- أفراس- موجات فوق صوتية - تبويض - هرمون بروجيستيرون- برولاكتين