Research article

A new approach for estrus induction in Arabian mares suffering from ovarian inactivity

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(Received 20/7/2017, Accepted 26/11/2017)

Abstract

Twenty-six Arabian mares affected with ovarian inactivity, as monitored by the clinical examination and ultrasonographical scanning, were selected during December and January. These animals were subjected to a new protocol of sequential hormonal injections as a trial for estrus induction, progesterone (300 mg/day) was injected on days 1, 4, 7, 10 and 14, Estradiol benzoate (15mg/day) on days 6 and 9, and GnRH (0.01mg/day) on days 18, 20, and 22 of the treatment regimen. An estrus induction response of 80.77% (21/26) was observed following hormonal treatment. The onset time of post treatment was short (66.48 hours) and more synchronized. The responded group showed significant (P<0.05) increase in follicular size from day -3 (3.26 cm) until reaching its maximum size at the pre-ovulatory day (4.48cm). This was accompanied by continuous significant (P<0.05)decrease in the serum concentrations of progesterone and increase in the levels of serum estradiol-17β from day -3 (0.42 ng/ml and 56.48 pg/ml, respectively). The minimum level of progesterone (0.28 ng/ml) was detected at day 0 of ovulation, whereas the maximum level of estradiol-17β was observed at the preovulatory day (89.22 pg/ml). However, 28.57% (6/21) of the responded mares did not develop functional corpus luteum so confirmed by functional progesterone estimation. Reasons for failure of 19.23% mares (5/26) to respond to hormonal treatment could be due to the small size of emerging follicles at the end of treatment (1.05 vs 1.82 cm ) as well as the older ages of these animals (15.36 vs 8.87 years old) compared to the responded group.

Keywords: Arabian mares, Estrus induction, Inactive ovary, Synchronization.

Introduction

Arabian mares are considered a national wealth in the Arab world and a major pure breed worldwide. Anoestrum is one of the main problems affecting mare’s reproduction by decreasing life span of producing new off spring (13, 17). Progesterone impregnated vaginal sponges (CIDR-B or PRID) alone or with PGF2α and GnRH has been used extensively for synchronization of estrus and ovulation in cyclic mares (14, 16). (5) And for induction of estrus in anestrous mares (3, 16, 13). However, the use of vaginal devices are impractical means of administrating progestin to horses owing to their retention rates (13). Moreover, the use of progesterone in serial injections at 3 days intervals along with estrogens and GnRH injections for induction of estrus in anestrous Arabian mares particularly during December and January has not been studied. The present work aimed to (1) evaluate the efficacy of using progesterone in combination with estrogens
and GnRH injections for induction of estrus in anestrus Arabian mares during the breeding season, and (2) predict ovulation following estrus induction using clinical and ultrasonographical examinations as well as serial measurements of peripheral progesterone and estrogen concentrations.

Materials and Methods

Ethical approval
The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 423.

Animals and treatment
The present study was conducted on 26 anestrus Arabian mares during the breeding season. These animals were selected randomly from different governmental and private equine farms, aged between 6 and 18 years old and have already foaled 1-9 times. The mares were apparently healthy with good body condition, normal genitalia and 60-240 days postpartum. Before the start of treatment, animals were confirmed as anestrus by absence of any cyclical functional structure (small and smooth ovaries without any follicular activity) on the ovaries by repeat rectal examination and ultrasonographical scanning at 5 days intervals. These animals were subjected to sequential hormonal injections for 22 days. Progesterone (300 mg/day) was injected on days 1, 4, 7, 10 and 14, estradiol benzoate (15mg/day) on days 6 and 9 and finally GnRH (0.01 mg/day) on days 18, 20 and 22 of the experiment.

Clinical examination
After the end of the treatment (day-4), all mares were observed for external signs of estrus and estrus detection was aided by parading, all mares to a teaser stallion two times daily. Animals showing mucous strings from the vulva, frequent micturition, winking (repeated exposure of clitoris), and elevation of tail head, mounting or standing to be mounted by the teaser stallion were confirmed to be in estrus. Follicular development was assessed by daily examination of both ovaries using rectal examination and ultrasonographical scanning starting from the last day of treatment (day-4) until 4 days post ovulation (day0).

Blood sampling and hormonal assays
Blood samples were collected daily at corresponding days of clinical examination via jugular venipuncture into vacutainer tubes held at room temperature for 2 hours, and then refrigerated overnight. Samples were centrifuged at 3000 rpm for one hour and serum were harvested and stored frozen pending hormonal analysis. Measurement of serum progesterone (15) and estradiol-17β (18) was done by quantitative enzyme immunoassays.

Statistical analysis
The changes in follicular development concentrations of serum progesterone and estradiol 17β relative to day 0 of ovulation were examined, using MIXED procedure of SAS with repeated measure statement. Differences between responding and non–responding groups concerning follicular diameter and hormonal concentrations at different days of the experiment were under taken using student ”t” test.

Results
The follicular diameter and hormonal concentration were expressed as mean± SEM. out of 26 anestrus mares, 21(80.77%) responded to the treatment and came to heat. Mean interval from the last day of treatment to the onset of signs of estrus was 66.48±2.31 hours. All responded mares had ovulated as confirmed by rectal examination and ultrasonographical scanning, whereas none of the remaining five mares, which did not respond to the treatment, had ovulated and their follicles regressed precipitously within
3-5 days without reaching preovulatory size after the end of treatment Figure (1).

![Figure (1): Follicular diameter in responding and non-responding Arabian mares](image1)

In the responded group, the continuous significant (P<0.05) increase in follicular size from day-3 (3.26±0.26 cm) until reaching its target size at the preovulatory day (4.48±0.45 cm) was accompanied by decrease concentrations of serum progesterone Figure (2) and increase in the concentrations of serum estradiol-17β (Fig.3) from day -3 (0.42±0.05 ng/ml and 56.48±6.75 pg/ml, respectively). The minimum level of progesterone (0.28±0.02 ng/ml) was detected at day 0 of ovulation, whereas the maximum level of estradiol-17β was observed on day -1 (89.22±9.33pg/ml). Following ovulation, the serum levels of progesterone showed a continuous increase (P<0.01) in 71.43% (15/21) of the responded group reaching 5.81±0.91 ng/ml on day 4 post ovulation, while the other six mares (28.57%) exhibited basal progesterone concentrations Figure (2).

In the non-responded groups, the limited influence of treatment regimen on follicular size enhancement was associated with non-significant changes in both progesterone and estradiol-17β concentrations throughout the experimental days (Figures (2, 3)).

![Figure (2): Progesterone concentrations in responding and non-responding Arabian mares](image2)

![Figure (3): Estradiol-17β concentrations in responding and non-responding Arabian mares](image3)

**Discussion**

In the present study, out of 26 anestrus mares, 21(80.77%) responded following the end of treatment. These findings were in agreement with (4, 13, 16, 17) where an induction rate of 70-77% was achieved using progesterone intra vagina device (CIDR-B) for a period of 12 days. However, in the present experiment, the time interval of onset of estrus after end of treatment was shorten and closely synchronized (66.48±2.31 hours) compared with the previously mentioned authors where mares were responded to estrus with a variable period following device removal (ranged between 72 and 81 hours). The better synchrony in our study could be ascribed to the inclusion of both estradiol benzoate (15mg/day) on day 6 and 9 and GnRH (0.01 mg/day) on day 18,20 and 22 of the treatment regimen. Administration of estradiol benzoate was coincided with the
mid interval of sequential progesterone administration between days 1 and 14 of the treatment regimen that mimic estradiol-17\beta elevation during the mid-luteal phase in cycling mares (8). This increase in exogenous or endogenous during mid-luteal period is important for initiating a second ovulatory follicular wave in the mare (8, 10). In addition, successful estrus induction and synchrony with GnRH analogues every 48 hours, as was done in the present study, was reported in seasonally anovulatory mare (7). The present findings demonstrated that the follicular status of the mare following treatment could affect the outcome of both estrus induction and synchrony. Mares in the non-responded group having smaller follicles at the end of treatment compared to responding group (1.05± 0.10 vs 1.82±0.15 cm) were less likely to show an ovulatory response to exogenous GnRH. These results confirm the conclusion of (8, 11) that mares in shallow anestrus are more likely to respond to the treatment than are mares in deep anestrus (non-responding group in our study). Moreover, four of the five non responding mares were over 15 years old suggesting that hypothalamo- pituitary – ovarian axis in younger mares (responded group) may be more sensitive than older mares (non-responding group) to the negative feed act of exogenous progesterone (13). On day 4 postovulation, 71.43% (15/21) of the responded group had luteal phase serum progesterone giving the impression of ovulation and formation of functional corpus luteum, while rest of the five (28.57%) mares were still having the basal progesterone concentrations denoting that following ovulation functional corpus luteum failed to establish. Similar observations have been reported by (6, 12, 8) where 75%, 40% and 45% of mares, respectively, failed to establish a functional corpus luteum following ovulation in progesterone treated anestrus mares. The failure in the formation of a functional corpus luteum may be due to an inadequate LH surge at the time of the expected ovulation associated with preovulatory estradiol-17\beta surge (1, 2, 13).

Conclusion

It is concluded from the present study that progesterone in combination with estradiol benzoate and GnRH injections can be effectively used for estrus induction in anestrus mares. The estrus induction response was higher and closely synchronized in younger than older mares. Moreover, the ultrasonography accompanied with estimation of progesterone and estradiol -17\beta serum levels have a predictive value for assessment of follicular sizes, ovulation time and establishment of functional corpus luteum post ovulation.

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