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# Estrus induction and fertility response in anestrus mares with exogenous progesterone releasing device (CIDR-B) during late breeding season

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**Abstract**
The present study was conducted with 25 lactational anestrus mares during late breeding season (July/August) dividing randomly in Treatment (n=15) and Control Groups (n=10). In Treatment Group, a progesterone releasing intra-vaginal device (CIDR-B) was inserted in the vagina and placed *in situ* for a period of 12 days and on the day of removal $PGF_2\alpha$ (3 ml Ilerin i/m) was injected. In Control Group, no treatment was given. Blood samples were collected for progesterone estimation at the time of CIDR-B insertion (day 0), day 4 and 8 after CIDR-B insertion, on the day of CIDR-B removal (day 12), on the day of covering and day 12 after covering. At corresponding days, blood samples were also collected from Control Group. After CIDR-B removal, the reproductive performance of mares was recorded up to 20 days in terms of estrus induction response and pregnancy rates. In mares, which were having initial basal progesterone levels (1.64 ± 0.19 ng/ml) on the day of device insertion, the levels significantly ($p<0.01$) increased to 5.64 ± 0.89 ng/ml on day 4 and then started decreasing to 3.49 ± 0.88 and 1.33 ± 0.2 ng/ml on day 8 and 12, respectively. On the day of covering, the progesterone levels reached to base line (0.29 ± 0.02 ng/ml). However, 45.45% of these mares did not develop functional corpus luteal following covering as confirmed by progesterone estimation. An estrus induction response of 86.66% (13/15) was observed in Treatment Group which was significantly ($p< 0.05$) higher than Control Group mares (30%, 3/10). An overall conception rate of 46.15%, 6/13) was achieved in treated mares. However, only one out of 10 mares conceived in Control Group during the study period. In younger mares (4-9 years old; 48.00 ± 6.19 h) the onset time of estrus following CIDR-B removal was shorter and more synchronized compared to older mares (10-16 years old; 85.71 ± 12.6 h), however, fertility response was same in both age groups (37.5% vs 40.3%). It is concluded that CIDR-B plus PG $F_{2\alpha}$ can be effectively used for estrus induction in anoestrus mares during late breeding season. The estrus induction response was shorter and more closely synchronized in younger than older mares.

**Keywords:** Progesterone; estrus induction; lactational anestrus; mares.

## Introduction

The mare is a seasonally polyestrous animal with a distinct breeding season during spring and summer months. Most non-pregnant mares pass into a state of deep anoestrus during winter when day length decreases and in spring cyclical ovarian activity is stimulated largely by increasing daylight length. Failure of the breeding mares to develop ovulatory follicles is a common problem during the early or late breeding season and the etiology of the condition is multi-factorial but day length, nutrition, management are the main factors.

Progestagens alone or in combination have been tried extensively for induction or control of estrous cycle in farm animals including mares (Woody and Abenes, 1975; Hulet and Foote, 1967; Webel, 1977; Lubbecke *et al.,* 1994). The methods of progesterone administration in farm animals include orally, intra-muscular, sub-cutaneous injections/implants and intra-vaginal route. However, daily intra-muscular injection or progestin s/c implants, are impractical means of administering progestins to horses and gives variable results (Loy and Swan, 1966; Holten *et al.,* 1977). Synthetic oral progestagens used effectively for control of estrus in several farm species, do not have consistent effects in the mares (Loy and Swan, 1966). The use of progesterone-impregnated vaginal sponges is more practical method of administration but poor retention rates discourages its use.

Progesterone in the form of CIDR-B along with $PGF_2\alpha$ and GnRH/hCG has been used successfully for synchronization of estrus and ovulation in cyclic mares (Lubbecke *et al.,* 1994) and for induction of estrus in anestrous mares (Arbieter *et al.,* 1994; Newcombe and Wilson, 1997). However, the use of progesterone in the form of CIDR-B along with $PGF_2a\alpha$ for induction of estrus and fertility response in lactational anestrus mares particularly during late breeding season has not been studied.

**Materials and Methods**

The present study was conducted with 25 lactational anestrus mares during late breeding season (July/August) at Equine Breeding Stud, Hisar, Haryana in the age group of 4–16 years and who have already foaled 1-11 times. The mares were apparently healthy with good body condition, normal genitalia and 30-240 days post-partum. Before the start of experiment, animals were confirmed as lactational anestrus by absence of any cyclic functional structure (small and smooth ovaries without any follicular activity) on the ovaries by repeated rectal examinations at 5 days apart,. These animals were divided randomly in Treatment and Control Groups with 15 and 10 animals in each group, respectively.

In Treatment Group, a progesterone releasing intra-vaginal device (CIDR-B; Inter-AG-Hamilton, New Zealand) was inserted in the vagina and placed *in situ* for a period of 12 days and on the day of removal $PGF_2\alpha$ (3 ml Ilerin i/m; Intervet International Gmbh, Feldstrabe, Germany) was injected. In Control Group, no treatment was given.

Blood samples were collected for progesterone estimation from mares of Treatment Group at the time of CIDR-B insertion (day 0), day 4 and 8 after CIDR-B insertion, on the day of CIDR-B removal (day 12), on the day of covering and day 12 after covering. In addition to this, at corresponding days, blood samples were also collected from control mares.

Blood samples (approximately 10 ml, each) were collected by jugular venipuncture in dry heparinized vials (200 I.U. heparin / vial). Plasma was harvested by centrifugation at 3000 rpm for 20 minutes and stored at –20 $^0$C in screw capped plastic vials till progesterone estimation by radio-immunoassay.

After removal of CIDR-B, all animals were observed for external signs of estrus starting from 6 hrs following removal. Estrus detection was aided by parading all the mares to a teaser pony at 0600 and 1800 hrs. In treated mares, rectal examination was done daily for 20 days or up to covering whichever was earlier after CIDR removal. Animals showing mucous strings from vulva, frequent micturition, winking (repeated exposure of clitoris), elevation of tail head, mounting or standing to be mounted by the teaser pony, etc. along with detection of a medium to large sized soft follicle by rectal examination were confirmed to be in estrus. Animals confirmed in estrus were covered naturally with a fertile stallion/donkey at 48 hrs interval or until ovulation depending on the covering schedule undertaken at E.B.S. Hisar. However, control mares were teased and examined rectally daily during the entire experimental period. Mares, which failed to conceive at the induced estrus, were covered naturally at subsequent estruses. Pregnancy of mated animals was confirmed by rectal palpation/ultrasound after 20 days of natural mating or artificial insemination.

**Results**

Out of 15, 13 mares (86.66%) responded to the treatment and came to heat (Table 1). Mean interval from removal of the device to onset of signs of estrus was 68.30 ± 8.94 hours. In control mares, out of 10, only three mares (30%) exhibited signs of estrus during the experimental period which was significantly ($p< 0.05$) lower than treated mares (Table 1). All mares from treated and control group which exhibited signs of estrus also ovulated following covering as confirmed by rectal palpation. Mean interval to time of covering after CIDR-B removal and fertility response of the mares at induced and subsequent cycle are presented in Tables 1. When over all pregnancy rate of all treated and control mares were compared, treated mares had significantly ($p<0.05$) higher conception rate as compare to control mares (Table 1). When data on the basis of age group was analyzed, the onset time of estrus and time of covering following CIDR-B removal was shorter and more closely synchronized in younger than to older mares, however, fertility response was same in both age groups (Table 2).

Plasma progesterone profiles which were having initial basal progesterone levels (1.64 ± 0.19 ng/ml) on the day of device insertion, significantly ($p<0.01$) increased to peak concentration on day 4 following CIDR-B insertion and remained elevated on day 8 ($p<0.05$). These progesterone concentrations came down to basal levels on the day of device removal (Day 12), which were significantly lower ($p<0.05$) than Day 4 and day 8 (Fig 1). On the day of covering in all mares, the plasma progesterone concentrations were basal or below the detection limit of the assay (Fig. 1). On the day 12 post-covering, only 54.55% mares had luteal phase progesterone concentrations (4-9 ng/ml) while rest of the mares (45.45%) exhibited basal progesterone concentration.

**Table 1: Estrus induction, time of covering and fertility response of control and CIDR-B plus $PGF_{2\alpha}$-treated mares.**

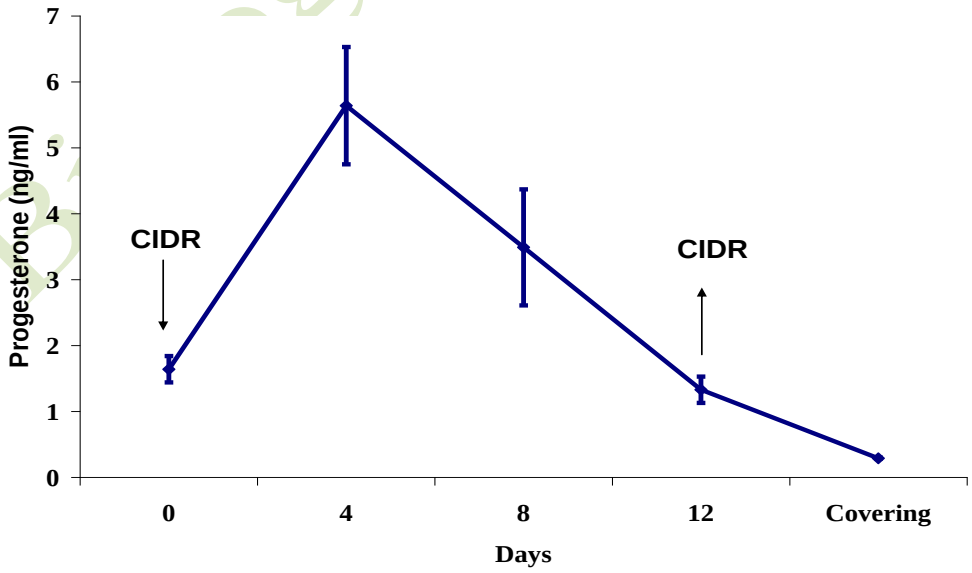
| Observations | Treatment Group | Control Group |
|---|---|---|
| Number of mares | 15 | 10 |
| Estrus response after CIDR-B removal | 13 (86.66 %)[a] | 3 (30%)[b] |
| Onset time of estrus after CIDR-B removal (hours) | 68.30 ± 8.94 | – |
| Time of covering after CIDR-B removal (days) | 5.15 ± 1.01 days (first covering) | – |
| No. of mares pregnant at induced estrus | 2 | 1 |
| No. of mares pregnant at subsequent estrus | 4 | – |
| Over all conception rate | 6/13 (46.15%)[a] | 1/3 (33.3%)[b] |

a vs b is significantly different ($p < 0.05$)

**Table 2: Estrus induction, time of covering and fertility response to CIDR-B plus PG $F_2\alpha$ treatment regimen in younger and older mares.**

| Group | Onset time of estrus after CIDR-B removal (hours) | Time of covering after CIDR-B removal (days) | Fertility response |
|---|---|---|---|
| Younger mares (4-9 years old; n=8) | 48.00 ± 6.19 | 2.83 ± 0.60 ($1^{st}$ covering) | 37.5% (3/8) |
| Older mares (10-16 years old; n=7) | 85.71 ± 12.67 | 7.14 ± 1.43 ($1^{st}$ covering) | 40.3% (3/7) |

**Fig 1: Plasma progesterone profile of mares treated with CIDR-B and $PGF_{2\alpha}$ regimen.**



**Discussion**

In the present investigation, out of 15, 13 mares (86.66%) responded to the treatment and were induced to estrus with a variable period following device removal (Mean 68.30 ± 8.94 hours). The results of the present study are in agreement with Ataman *et al.* (2000) where an estrus induction rate of 80% was achieved using the same regimen in transitional mares. However, in the latter study, the time of onset of estrus was closely synchronized (72 ± 3.1 hours) as compare to our study. The better synchrony in the latter study could be ascribed to the time of treatment (early vs late breeding season) or higher amount of progesterone (1.9 vs 1.55 gm) in the device (Dinger *et al.,* 1981). Lubbeke (1992) and Horn (1997) observed higher (95-97.5%) estrus induction rate following the same regimen as used in the present study. The better response could again be attributed to higher amount of progesterone in the device, which could have better negative effect at the hypothalamus and lead to abrupt release of gonadotrophins on its removal or use of cyclic mares in later study. An estrus induction response of 69.3% was achieved following the same regimen (CIDR-B containing 1.9 gm progesterone) in acyclic mares (Arbieter *et al.*, 1994). However, Newcombe and Wilson (1997) and Newcombe *et al.* (2002) reported an estrus induction response of 83.35% and 80.2% in lactating anestrous mares and in acyclic mares, respectively, during transition phase with the use of same device and regimen, which is well comparable with our findings. Above studies are indicative of the fact that cyclic mares respond in a better way compared to anestrus mares.

In Treated Group, an overall pregnancy rate of 46.15% was achieved. The beneficial effects of the treatment with progesterone device are clear as only one mare in the Control Group conceived during the entire study period. Similarly, a conception rate of 53 to 56% was achieved using the same treatment regimen in anoestrus mares during winter (Okolski and Tischner, 2001; Newcombe *et al.,* 2002). In cyclic mares during the breeding season where estrus was synchronized with the same treatment regimen, a very high conception rate of 81% to 88.23% at the induced estrus has been reported (Alt *et al.,* 2004; Card and Green, 2004). These studies suggest that cyclic status and season at which treatment is initiated plays a great role in resultant pregnancy rate.

From basal levels on the day of device insertion, peak values of progesterone reached on day 4 and remained elevated on day 8. These values came down to basal levels which were comparable to day 0, on the day of device removal (Day 12). In cattle, the peak values were achieved within 24 hours of insertion of the same device and the values came down steadily on day 4 and day 7 (Singh 2004) and these later observations are comparable to our study. In our study, 24 hours following CIDR-B insertion progesterone concentrations were not estimated. However, Lakra (2002) observed that in buffaloes peak progesterone values reached on day 3 following the same device insertion and came down on day 6. It indicates that there is different rate of progesterone absorption and clearance for the same device in different species (mare vs cattle and buffalo). As per our expectations, on the day of covering, the plasma progesterone concentrations were either below the detection limit of the assay or below 1 ng/ml supporting our estrus induction response.

On the day 12 post-covering, only 55.55% mares had a luteal phase concentrations of plasma progesterone giving the impression of ovulation and formation of a functional corpus luteum while rest of the five mares (45.45%) were still having the basal plasma progesterone concentrations indicating that following ovulation functional corpus luteum failed to establish. Similar observations have been reported by Johnson (1986) and Evans and Irvine (1979) where 40% and 75% mares, respectively, failed to establish a functional CL following ovulation. The failure in formation of a functional CL may be due to an inadequate LH surge at the time of the expected ovulation associated with the low pre-ovulatory estradiol-17 beta surge or these mares have already shifted to the seasonal anestrous condition.

An age related difference in onset time of estrus induction to treatment was also observed. In young mares, the onset time of estrus was significantly shorter and closely synchronized as compared to old mares following CIDR-B removal. The exact reason is not clear, however, it suggests that hypothalamo-pituitary-ovarian axis in younger mare may be more responsive than older mares to the negative feedback of progesterone. In addition, in the present investigation, progesterone at the start of experiment suggested that three mares in younger group had luteal phase concentrations of progesterone at the start of CIDR-B insertion (suggesting cyclic status but clinically anestrus; data not shown). Thus, shorter treatment to estrus interval and more

precise in its synchrony in younger mares may be due to more cyclic mares in this group. A better estrus induction response following the same regimen has been reported in cyclic mares as compared to acyclic mares (Arbieter *et al.,* 1994; Newcombe and Wilson, 1997; Newcombe *et al.,* 2002).

## Conclusion

It is concluded from the present study that CIDR-B plus PG $F_{2\alpha}$ can be effectively used for estrus induction in anestrus mares during late breeding season. The estrus induction response was shorter and more closely synchronized in younger than older mares. Lack of formation of functional corpus luteum following covering could be responsible for poor conception rate in mares induced to estrus with progesterone device.

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