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**Research Article**

# THE EFFECT OF hCG ADMINISTRATION ON CONCEPTION RATE FOLLOWING INDUCED OESTRUS BY EXOGENOUS PROGESTERONE IN MARES

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## ABSTRACT

The present study was conducted in 25 lactational anoestrus mares (4–17 years) during late breeding season (July/August). The mares were randomly divided in group A (n=8), B (n=7) and C (n=10). In group A and B, mares received progesterone releasing intravaginal device (CIDR-B) which was kept *in situ* for 12 days. At the time of CIDR-B removal (on day 12), mares were injected with prostaglandin $F_2$ alpha (3 ml Iliren, i/m. containing 0.196 mg of tiaprost trometamol). In group B, mares also received hCG (Chorulon 3000 IU, i/v.) at the time of covering. In group C, no treatment was given and served as controls. After removal of CIDR-B, all animals were observed for external signs of oestrus aided by parading a teaser pony and rectal examination for detection of a medium to large sized soft follicle on ovaries. Animals confirmed in oestrus were either artificially inseminated or covered naturally with a fertile stallion. Pregnancy of mated animals was confirmed by rectal palpation after 20 days of natural mating or artificial insemination. In group A, out of 8 mares, 6 mares (75%) and in group B, all 7 mares exhibited signs of oestrus following treatment. Similarly, one mare each in group A and B conceived during first oestrus and two mares each in both groups conceived during second oestrus cycle following treatment. Thus, out of 15 mares, 13 mares (86.66%) responded to the treatment and came to heat. Mean interval from removal of the device to onset of signs of oestrus was 68.30 ± 8.94 h. In control mares, out of 10, only three mares (30%) exhibited signs of oestrus during the experimental period which was significantly ($p< 0.05$) lower than treated mares. An overall pregnancy rate of 46.15% (6/13) was achieved in mares exhibiting oestrus which was significantly higher ($p<0.05$) than control mares (30%; 1/3). In younger mares (4-9 years), the onset time of oestrus was significantly shorter and closely synchronized (48.00 ± 6.19 h) compared to older mares (10-17 years, 85.71 ± 12.67 h) following CIDR-B removal ($p<0.01$). It can be concluded that in lactational anoestrus mares during the late breeding season, oestrus can be induced with the help of intravaginal progesterone releasing device plus PG $F_2$ alpha regimen. The response is much more synchronized and quicker in younger mares as compared to older mares. However, hCG treatment at the time of mating/A.I. following induced oestrus did not improve the conception rate.

**Key words:** Progesterone, anoestrus, mares

Progestagens alone or in combination with other hormonal preparations have been tried extensively for induction or control of oestrous cycle in farm animals including mares (Hulet and Foote, 1967, Woody and Abenes, 1975, Webel, 1977, Lubbecke *et al.*, 1994, Glazar *et al.*, 2004). The routes of progesterone administration in farm animals include oral, 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 even when given orally (Loy and Swan, 1966, Holten *et al.*, 1977). The use of progesterone-impregnated vaginal sponges is more practical method of administration but poor retention rates (Dinger *et al.*, 1981).

Progesterone in the form of CIDR-B along with $PGF_2\alpha$ and GnRH/hCG has been used successfully for synchronization of oestrus and ovulation in cyclic mares (Lubecke *et al.*, 1994) and for induction of oestrus in anoestrous 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 oestrus and ovulation in lactational anoestrus mares particularly during late breeding season has not been studied. Thus, in the present study, attempts have been made to induce oestrus and ovulation in lactational anoestrus mares using this regimen and to know the efficacy of hCG administration at the time of mating on conception rate.

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## MATERIALS AND METHODS

The present study was conducted in 25 lactational anoestrus mares aged 4-17 years during late breeding season (July/August) at Equine Breeding Stud, Hisar, Haryana. These mares had 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 anoestrus by absence of any cyclic functional structure on the ovaries by repeated rectal examinations at 5 days apart. The mares were randomly divided in group A (n=8), B (n=7) and C (n=10). In group A and B, mares received progesterone releasing intravaginal device (CIDR-B, Inter-AG-Hamilton, New Zealand) which was kept *in situ* for 12 days. At the time of CIDR-B removal, mares were injected with prostaglandin $F_2$ alpha (3 ml Iliren, i/m., Intervet International Gmbh, Feldstrabe, Germany). In group B, mares also received hCG (Chorulon 3000 IU, i/v. Intervet India Ltd.) at the time of covering. In group C, no treatment was given and served as controls.

All animals were observed for external signs of oestrus starting from 6 h following CIDR-B removal. Oestrus detection was aided by parading all the mares to a teaser pony at 06 and 18 h. In treated mares, rectal examination was done daily for 20 days or up to covering whichever was earlier after CIDR-B removal. Animals showing mucous strings from vulva, frequent micturition, winking, elevation of tail head, mounting or standing to be mounted by the teaser pony along with detection of a medium to large sized soft follicle by rectal examination, were confirmed to be in oestrus. Animals confirmed in oestrus were either artificially inseminated using fresh semen or covered naturally with a fertile stallion/donkey at 48 h 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 oestrus, were remated naturally at subsequent oestrus. Pregnancy was confirmed by rectal palpation after 20 days of natural mating. Oestrus induction response was defined as number of mares manifesting oestrus out of total treated/control mares. First-service conception rate was designated as number of mares pregnant at the induced oestrus out of total artificially inseminated or covered naturally. Overall conception rate was calculated as total number of females pregnant at induced or subsequent oestrus out of total artificially inseminated or covered.

## RESULTS AND DISCUSSION

In group A, 6 mares (75%) responded to the treatment and in group B, all 7 mares exhibited signs of oestrus following treatment (Table 1). Similarly, one mare each in group A and B conceived during first oestrus and two mares each in both groups conceived during second cycle following treatment (Table 1). The oestrus induction response and conception rate in group A and group B, was similar indicating that hCG treatment did not increase the conception rate. Thus, out of 15 mares, 13 mares (86.66%) responded to the treatment and came to heat while in controls, out of 10 only three mares (30%) exhibited signs of oestrus which was significantly lower ($p<0.05$) than treated mares (Table 1). Mean interval from removal of the device to onset of signs of oestrus was 68.30±8.94 h. An overall pregnancy rate of 46.15% (6/13) was

**Table 1**

**Oestrus induction and fertility response to CIDR-B plus PG $F_2\alpha$ treatment in mares**

| Observations | Treated groups | | Total treated mares | Control group (Group C) |
|---|---|---|---|---|
| | Group A | Group B | | |
| No. of mares | 8 | 7 | 15 | 10 |
| No. exhibited oestrus after CIDR-B removal | 6 (75)[a] | 7 (100)[a] | 13 (68.30)[a] | 3 (30)[b] |
| No. pregnant at induced oestrus | 1 | 1 | 2 | 1 |
| No. pregnant at two subsequent oestrus | 2 | 2 | 4 | - |
| Over all pregnancy rate | 3/6 (50) | 3/7 (42.8) | 6/13 (46.15)[a] | 1/3 (33.3)[b] |

a vs b between the different groups differ significantly ($p<0.05$), figures in paranthesis indicates percentage

achieved in the inseminated mares. However, out of 10 mares in controls, three exhibited signs of oestrus and only one mare conceived during the entire period of investigation (Table 1). The treated mares had significantly ($p<0.05$) higher conception rate (46.15%) as compared to control mares (33.33%, Table 1). The results of the present study are in agreement with others where an oestrus induction rate of 80% and 69.3% was recorded using the same regimen in transitional mares (Ataman *et al.*, 2000) and in acyclic mares (Arbieter *et al.*, 1994) respectively. Lubbecke (1992) and Horn (1997) observed higher (95-97.5%) oestrus induction rate following the same regimen as used in the present study. The better response in later studies could be attributed to higher amount of progesterone (1.9 g) in their CIDR compared to present study (1.55 g) which could have better negative effect at the hypothalamus and lead to abrupt release of gonadotrophins on its removal. Dinger *et al.* (1981) also reported high synchronization rates with the use of intra-vaginal sponges containing 2 g progesterone as compared to sponges containing 1 g progesterone in mares. Alternately, these investigations have been carried out in cyclic mares for synchronization of oestrus which could be responsible for better oestrus induction response (Arbieter *et al.*, 1994, Bergfelt *et al.*, 2007). However, Newcombe and Wilson (1997) and Newcombe *et al.* (2002) reported an oestrus induction response of 83.35% and 80.2% in lactating anoestrous and acyclic mares respectively, during transition phase with the use of same device and regimen which is well comparable with present findings. Above studies are indicative of the fact that cyclic mares respond in a better way as compared to anoestrus mares.

An age related difference in oestrus induction response to treatment was also observed. In young mares (4-9 years old) the onset time of oestrus was significantly shorter and closely synchronized (48.00 ± 6.19 h) as compared to old mares (10-17 years old, 85.71 ± 12.67 h, $p<0.01$) following CIDR-B removal (Table 2). The exact reason is not clear, however, it suggests that hypothalamo-pituitatry-ovarian axis in younger mare may be more responsive than older mares to the negative feedback of progesterone. In

**Table 2**

**Oestrus induction and time of covering in response to CIDR-B plus PG $F_2\alpha$ treatment in younger and older mares**

| Group | Onset time of oestrus after CIDR-B removal (h) | Time of covering after CIDR-B removal. (days) |
|---|---|---|
| Younger mares (4-9 years) | 48.00±6.19 | 2.83±0.60 (1st covering) |
| Older mares (10-17 years) | 85.71±12.67 | 7.14±1.43 (1st covering) |

addition, in the present investigation, progesterone in 11 treated mares randomly at the start of experiment was also estimated and four mares had luteal phase concentrations of progesterone (suggesting cyclic status), three of them belonged to younger group and seven mares which had basal progesterone, five of them belongs to older group. Thus, shorter treatment to oestrus interval and more precise in its synchrony in younger mares may be due to more cyclic mares in this group. A better oestrus 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).

In treated mares, two mares conceived at the induced oestrus and four mares conceived during the subsequent oestrus. Thus, an overall pregnancy rate of 46.15% was achieved in the treated mares which was significantly ($p<0.05$) higher as compared to controls. 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).

An oestrus induction response and conception rate in group A and group B, was similar thus, administration of hCG at the time of covering/A.I. to ensure ovulation did not improve the conception rate in the present study. The effect of hCG for ovulation induction depends upon the size of the existing follicle at the time of treatment, with results being poor if follicle size is less than 33 mm (Laing *et al.*, 1998). In present study, hCG has been given at the time of covering on the basis of rectal palpations without measurement of the follicular size by ultrasound. The unenhanced conception rates even after hCG

administration may be attributed to the time of treatment, when follicular size was inadequate to respond. In addition, a seasonal effect of hCG on ovulation rate has also been reported (Webel *et al.*, 1977, Handler *et al.*, 2006) with poor results during early or late breeding seasons as compared to breeding season.

It can be concluded that in lactational anoestrus mares during the late breeding season, oestrus can be induced with the help of intravaginal progesterone releasing device plus $PGF_2$ alpha regimen. The response is much more synchronized and quicker in younger mares as compared to older mares. However, hCG treatment did not improve the conception rate.

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