

Estrous Response and Fertility in Postpartum Suckled Female Yaks (*Bos grunniens*) Treated with an Intravaginal Device Containing Progesterone (CIDR), Pregnant Mares' Serum Gonadotrophin and Prostaglandin Analogue

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ABSTRACT : The efficiency of a short-term treatment with an intravaginal device containing progesterone (CIDR) combined with pregnant mares' serum gonadotrophin (PMSG) and prostaglandin analogue (PGF_{2α}) was evaluated for the induction of estrus, initiation of cyclic activity, and fertility in postpartum suckled yak cows. Seventy-five postpartum suckled yak cows were assigned to three treatments: (1) insertion of a CIDR intravaginal progesterone (1.9 g) (day 0), an administration of PGF_{2α} (0.2 mg i.m.) on day 6 and PMSG (1,000 IU i.m.) at the time of CIDR withdrawal on day 7 (CPP group, n=28); (2) an administration of PGF_{2α} (0.2 mg i.m.) on day 6 and PMSG (1,000 IU i.m.) on day 7 (PP group, n=21); (3) untreated animals served as the control (CG group, n=26). Seven yak bulls were placed in pastures with the cows for natural mating. Estrus rate in the CPP group (28/28) was higher ($p < 0.01$) than in the PP group (6/21) and in the CG group (0/26) within 96 h after the end of treatment. The first service conception rate in the CPP group (21/28) was higher ($p < 0.01$) compared with in the PP group (2/9) as judged by serum P₄ concentration ≥ 2.35 ng/ml on day 21 after breeding. It is concluded that a short-term progesterone treatment combined with PMSG and prostaglandin increased the proportion of yak cows that exhibited behavioral estrus with more synchronized estrus response and satisfactory conception rate in postpartum suckled yak cows. (*Asian-Aust. J. Anim. Sci.* 2005, Vol 18, No. 4 : 470-474)

Key Words : Yak, CIDR, Prostaglandin, Estrus Induction, Pregnancy

INTRODUCTION

The yak (*Bos grunniens*) is one of the world's most remarkable domestic animals - a herbivore living on the "roof of the world", in and around the Himalayas and north, in areas of altitude ranging from 2,500 m to 5,500 m with no absolutely frost-free periods. Yak production fills an important niche in Tibetan life and in developing the prosperity of the people in the vast mountainous regions by providing food (meat and milk), transport, shelter (hair) and fuel where few other animals will survive. However, yaks (*Bos grunniens*) are seasonal breeders. The breeding season is restricted to the warm season (June to November), and the largest proportion of yak cows comes into estrus in the middle of this period (July and August) in most yak production areas. The annual reproductive rate of most yak breeds is low, i.e. 40-60% because only a small proportion of the cows return to estrus in the 1st breeding season after calving; most come into estrus in the 2nd and 3rd years under traditional management systems (Zi, 2003). For example, in Ruoergai County of Sichuan, 14.3% of yak cows return to estrus in the year of calving, 59.6% in the 2nd year and 13.9% in the 3rd year (Cai, 1979). These long postpartum intervals of anestrus eventually result in

lowered net income for yak herders, primarily because of low milk output and high replacement rates due to failure of yak cows to conceive during the breeding season. If an interval between calving is two years, the milk yield in the second year is considerably lower than in the first. Treatments that induce onset of estrous cycles in postpartum anestrus yak cows should, therefore, improve reproductive efficiency.

The efficacy of numerous hormonal treatment regimens to improve yak fertility has been evaluated in various studies. In China, triple hormones (a mixture of androgen, progesterone and estrogen) were commonly used to induce estrus in the 1980s. After treatment with triple hormones, usually more than 90% of yak cows began estrus and ovulated within 6 days. However, conception rate was variably low, and there was little benefit in terms of calving rate (Shao and Zhao, 1984; Liu et al., 1985; Shao et al., 1986). A single administration of GnRH or PGF_{2α}, or their combination induced a rise in serum LH concentration and ovulation in breeding season, and pregnancy rates at the end of breeding season were greatly improved in yak cows that calved in previous years (with or without calf) but not in the current year, however, these techniques could not improve fertility in yak cows nursing a calf born in the current year (Shao and Zhao, 1984; Shao et al., 1986; Magash et al., 1997). These results are similar to the findings in postpartum anestrus cows (*Bos taurus*) (Troxel et al., 1993; Kim et al., 2003). Recently, progesterone releasing devices and implants have been developed and their use in

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Table 1. Experimental design for allocation of postpartum suckled female yaks to treatments

Treatment	No. of cows	Day		
		0	6	7
CIDR	28	CIDR	0.2 mg Estrumate	CIDR removal, 1,000 IU PMSG
PGF _{2α}	21	-	0.2 mg Estrumate	1,000 IU PMSG
Control	26	-	-	-

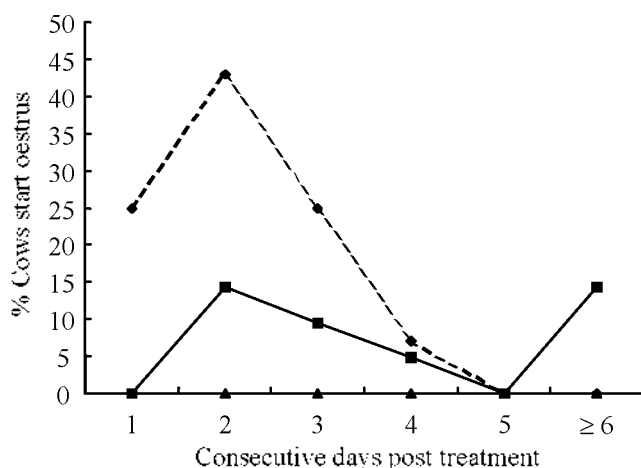


Figure 1. Effect of treatment on the percentage of postpartum suckled yak cows detected in estrus on consecutive days following treatment. CPP (♦, n=28): controlled intravaginal drug release device containing 1.9 g P₄ inserted per vagina for 7 days and 0.2 mg of Estrumate administered i.m. on day 6 and 1,000 IU of PMSG at the time of CIDR removal; PP (■, n=21): 0.2 mg of Estrumate administered i.m. on day 6 and 1,000 IU of PMSG on day 7; CG (▲, n=26): cows did not receive any treatment.

cattle demonstrates that they have some advantages in simple application and reasonable results over than the traditional methods of injections (Macmillan and Day, 1987; Macmillan and Peterson, 1993; Lucy et al., 2001; Martinez et al., 2002; Kim et al., 2003). Therefore, the objective of the study was to evaluate the effectiveness of an intravaginal progesterone-releasing insert (CIDR) in combination with pregnant mares' serum gonadotrophin and injection of prostaglandin on estrus induction and fertility in yak cows nursing a calf born in the current year.

MATERIALS AND METHODS

Animals and treatments

The trial was conducted in August in Longri Yak Farm of Sichuan, situated at 3,600 m above sea level. In the area, grass starts to grow from May, goes to seed in August and then wilts. The animals grew in body weight in the warm season (May to November) and lost weight during winter (December to April) (Zi et al., 2004). The breeding season is restricted to the warm season (June to November). Yak cows used in this experiment calved from April to May in the current year and the number of days postpartum to the hormonal treatment ranged from 60 to 110 days. All cows

were managed similarly: they were pastured on the natural grassland near the campsites for milking. Yak cows were milked in the morning once daily and their calves were kept apart from the dams for nine to twelve hours every day during the night and grazed alongside the dam for most of the day. There is not any supplementation during the winter.

Seventy-five postpartum suckled yak cows were assigned to three treatments: (1) beginning on day 0 (day of treatment initiation) yak cows were treated with an intravaginal device containing progesterone (EAZI-BREED™ CIDR[®], InterAg, Hamilton, New Zealand) for 7 days plus an administration of 0.2 mg prostaglandin F_{2α} analogue (Estrumate, Shanghai, China) on day 6 and 1,000 IU of pregnant mares' serum gonadotrophin (PMSG; Aba of Sichuan, China) at the time of CIDR withdrawal on day 7 (CPP group, n=28); (2) yak cows were administered with PGF_{2α} (0.2 mg i.m.) on day 6 and PMSG (1,000 IU i.m.) on day 7 (PP group, n=21); (3) served as controls, yak cows did not receive any hormonal treatment (CG group, n=26; Table 1).

Behavioral response to treatment and breeding

To detect onset of behavioral estrus, yak cows were observed for at least 30 min twice daily at approximately 12 h intervals from initiation of treatment (day 0) to day 22 of the experiment. A cow was considered as in estrus if she stood to be mounted or to be mated, but not in estrus even if she exhibited sexual activity but did not stand to be mounted. Mating was performed into a single group, i.e., seven yak bulls aged 4 to 6 years were placed in pastures with the cows for natural mating.

Pregnancy diagnosis

Serum progesterone (P₄) on day 21 after breeding was used as an indicator of pregnancy (Moreira et al., 2000). Blood samples were collected on day 21 after breeding via the jugular vein. Blood samples were placed on ice immediately after collection, then stored at 4°C for 24 h until centrifugation. Serum was decanted and stored at -20°C until assayed for concentrations of P₄. Progesterone assays performed by using a single antibody RIA procedure (Knickerbocker et al., 1986), had inter- and intra-assay CV less than 10 and 5%, respectively. Cows on day 21 after breeding were considered pregnant when serum P₄ concentration was equal to or higher than 2.35 ng/ml. This value used in cattle was utilized because it was found the

Table 2. Estrus induction and subsequent fertility of postpartum anoestrous yak cows by administration of PMSG and PGF_{2α} in conjunction with a short progesterone treatment

Criteria	Controls	Treatment groups	
		CIDR+PGF _{2α} +PMSG	PGF _{2α} +PMSG
No. treated	26	28	21
No. showing estrous behavior after treatment	0 (0.0%) ^c	28 (100.0%) ^a	9 (42.9%) ^b
No. showing estrous behavior within 96 h after treatment	0 (0.0%) ^c	28 (100.0%) ^a	6 (28.6%) ^b
No. pregnant	0 (0.0%) ^c	22 (78.6%) ^a	2 (22.2%) ^b

^{a, b, c} Different superscripts in a row differ significantly ($p < 0.01$).

change of serum P₄ concentration in the yak was similar to that of cattle after breeding (Yu et al., 1993).

Statistical analysis

Estrus rates and pregnancy rates among the CPP, PP and CG groups were compared by chi-square analysis as described by Cochran and Cox (1957).

RESULTS

Behavioral response to treatment

None of devices was lost during the experiment. The estrous detection rate was affected ($p < 0.01$) by the treatment employed (Figure 1 and Table 2). The estrous detection rate for yak cows in the CPP group (28/28) was greater ($p < 0.01$) than in the PP group (9/21) and in the CG group (0/26) within 15 days after treatment. Estrus was more synchronized by a short-term P₄ treatment: all yak cows in the CPP group (28/28) came into estrus within 96 h after from day 8 to 11 of the experiment, however, only 6/21 yak cows in the PP group were observed in estrus during this period and none of yak cows in the CG group came into estrus during this period.

Pregnancy rates

Pregnancy rate judged by serum P₄ concentration on day 21 after breeding for yak cows in the CPP group (21/28) was greater ($p < 0.01$) than in the PP group (2/9) (Table 2). When pregnancy rate was based on those cows presented for estrus induction programs, pregnancy rate was 21/28 and 2/21 respectively.

DISCUSSION

The annual reproductive rate of most yak breeds is low, i.e. 40-60% because only a small proportion of the cows return to estrus in the 1st breeding season after calving; most come into estrus in the 2nd and 3rd years under traditional management systems (Zi, 2003). Previous studies showed that administration of PGF_{2α} or GnRH, or combination of these two hormones could greatly improve pregnancy rates at the end of breeding season in yak cows that calved in previous years (with or without calf) but not in the current year (Shao and Zhao, 1984; Shao et al., 1986;

Magash et al., 1997). For example, Shao and Zhao (1984) reported that administration of PGF_{2α} and GnRH to yak cows that calved in the previous year (with or without calf), resulted in 73.7% of cows treated became pregnant in the following two breeding cycles compared to 38.9% of control cows. However, for yak cows nursing a calf born in the current year, only 7.0% of cows became pregnant within two breeding cycles.

The results of this study demonstrate that estrus can be effectively induced in postpartum anoestrous yak cows with a short-term progesterone treatment in conjunction with PMSG and prostaglandins. The estrous response was markedly better, in that the interval to estrus was shorter with a higher frequency of behavioral estrus; all animals came into estrus within 96 h, after treatment with P₄+PGF_{2α}+PMSG than after treatment with PGF_{2α}+PMSG. This observation was consistent with previous reports in postpartum anoestrous dairy cows (Chupin and Pelot, 1978; Roche et al., 1992; Macmillan and Peterson, 1993; Alnimer and Lubbadah, 2003), postpartum suckled beef cows (Mulvenhill and Sreenan, 1977; Petit et al., 1978; Roche et al., 1992), zebu (Rao and Suryaprakasam, 1991) and buffalo (Rao et al., 1985; Qureshi, 1998; Neglia et al., 2003). An injection of PMSG at the end of CIDR treatment appears to be necessary in yak cows because pre- and postpartum body condition at the end of winter is poor. Roche et al. (1992) reported that in beef suckler cows or in dairy cows under nutritional stress, an injection of PMSG at the end of a progesterone treatment might increase the estrus response. Macmillan and Day (1987) showed that 48% of anoestrous dairy cows treated with CIDR were inseminated within 7 days of CIDR removal compared with 22% in control anoestrous cows. Injection of 400 or 800 IU PMSG increased the estrous response to between 70 and 85% post CIDR removal.

Pregnancy rate in induced estrus was also higher ($p < 0.01$) in the CPP group than in the PP group and in the CG group. These results agree with the earlier findings in cattle and buffalos. Mulvenhill and Sreenan (1977) reported an increased rate of ovulation and a higher level of fertility in cows that received gonadotrophic stimulation, in conjunction with a short-term progestagen treatment. Chupin and Pelot (1978) achieved a high calving rate of 65.4% after two predetermined AI's in dairy cows following

P₄+PGF_{2α}+PMSG treatments, compared to 49.1% for P₄ alone. Rao et al. (1985) reported that the pregnancy rate in induced estrus was also higher (42.1%) for buffalo cows treated with P₄+PGF_{2α}+PMSG than other hormonal protocols. Qreshi (1998) reported an estrus induction rate of 91.3% and conception rate of 82.6% in dairy buffaloes getting 50 mg progesterone injections on alternate days for a total period of 12 days followed by estrus and breeding within the following three days. Although the mechanism by which treatment with progesterone improves fertility of postpartum suckled yak cows is not completely understood, most of the luteal phases that follow ovulation induced by PGF_{2α}+GnRH treatment are 6 to 12 days in cattle. When cows have been bred at an estrus with an expected subsequent short luteal phase, conception may occur but pregnancy is generally not maintained. Progesterone treatment, however, reduced the incidence of short luteal phases and improved pregnancy rates in acyclic cows (Troxel et al., 1993; Lucy et al., 2001; Martinez et al., 2002).

Numerous factors influence the interval from parturition to first estrus and ovulation in yak cows. Two major factors that affect the interval from parturition to first estrus are suckling and energy intake during late pregnancy and after parturition (Zi, 2003). Natural resumption of reproductive function in a proportion of postpartum cows occurs without signs of behavioral estrus following postpartum or seasonal anestrus, and pre- and postpartum body condition also affects signs of behavioral estrus (Long et al., 1999). Yak cows in this experiment were not supplemented during the winter and had a long period of grazing daily on poor quality pasture near the campsite after calving for milking. These might be the reasons that none of cows in the control was observed in estrus during the period of experiment.

In conclusion, this study has shown that a short-term progesterone treatment combined with PMSG and prostaglandin increase the proportion of yak cows that exhibited behavioral estrus and achieve good estrus synchronization and a higher conception rate in postpartum suckled yak cows. This technique could be possibly used to improve fertility and applied for fixed-time artificial insemination in the yak.

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