Induction of estrus in suckled female yaks (*Bos grunniens*) and synchronization of ovulation in the non-sucklers for timed artificial insemination using progesterone treatments and Co-Synch regimens

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Abstract

The objectives of the present study were to evaluate the induction of estrus and fertility in yak cows treated with Co-Synch regimens or progesterone (P4). In Experiment 1, postpartum suckled yaks were assigned to three treatments: (1) A (n = 28), insertion of an intravaginal device containing P4 (CIDR) on Day 0, PGF2α (i.m.) on Day 6 and PMSG (i.m.) at the time of CIDR removal on Day 7 (P4-PGF2α-PMSG); (2) B (n = 21), PGF2α (i.m.) on Day 6 and PMSG on Day 7; (3) C (n = 26), control group. Seven yak bulls were grazed with the cows for natural breeding. Rate of estrus within 96 h of the end of treatment was greater (*P* < 0.05) in A (100.0%) than in B (28.6%) or C (0.0%). First service conception rate (CR) determined by serum P4 on Day 21 after breeding was greater (*P* < 0.05) in A (78.6%) than in B (22.2%). Also, pregnancy rate (PR) during the breeding season was greater (*P* < 0.05) in A (82.1%) than in B (19.0%) and C (7.7%). In Experiment 2, non-suckled yaks that calved in previous years but not in the current year were assigned to three treatments: (1) A (n = 31), GnRH (i.m.) on Day 0, followed by PGF2α on Day 7 and timed artificial insemination (TAI) concurrently with GnRH treatment on Day 9 (Co-Synch regimen); (2) B (n = 50), a CIDR device for 7 days plus PGF2α and PMSG at the time of CIDR withdrawal on Day 7 and TAI on Day
9 (P$_4$-PGF$_{2\alpha}$-PMSG); (3) C ($n = 50$), yak cows were artificially inseminated at spontaneous estrus. Frozen semen of Holstein and Jersey were used for insemination in Experiment 2. The CR assessed by rectal palpation 35 days after TAI was not different in A (22.6%), B (30.0%) and C (33.3%), but PR was greater in A and B than in C, when based on those cows presented for estrous synchronization programs. It is concluded that P$_4$-PGF$_{2\alpha}$-PMSG protocol could efficiently induce estrus and result in an acceptable pregnancy rate in postpartum suckled yak cows. This technique and Co-Synch regimen can be applied successfully for TAI of non-suckled yak cows.

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**Keywords:** Yak-CIDR; Co-Synch; Estrous induction; Timed artificial insemination; Pregnancy rate

1. Introduction

The yak (*Bos grunniens*) is a seasonal breeder with the breeding season in the northern hemisphere being from June to November. In China, the largest proportion of yak cows comes into estrus in the middle of this period (July and August). The annual pregnancy rate of most yak breeds is 40–60% because only a small proportion of the cows return to estrus in the first breeding season after calving; most come into estrus in the second and third years under traditional management systems (Zi, 2003). These long postpartum intervals of anestrus eventually result in lowered net income for yak producers. If an interval between calving is 2 years, the milk yield in the second year is considerably less than in the first (Cai and Wiener, 1995; Zi et al., 1997).

Artificial insemination (AI) has made a significant contribution to genetic improvement in cattle and has potential to do likewise in yaks. In addition, slaughter weights and milk yields of hybrids of yak with improved breeds of cattle (*Bos taurus*) were 20–70 and 100–300% greater, respectively, than in the pure yaks (Cai and Wiener, 1995). However, AI is necessary because bulls of improved cattle breeds do not adapt to the harsh conditions and high altitudes typical of where yak are managed. The application of AI is made difficult in yaks undergoing spontaneous estrus and ovulation due to the minimal intensity of estrous behavior, variable duration of estrus and difficulty in observing estrus and predicting the time of ovulation (Katzina and Maturova, 1989; Cai and Wiener, 1995).

Accordingly, the efficacy of numerous hormonal treatment regimens to improve yak fertility and various protocols to synchronize estrus and ovulation have been evaluated in yaks in an attempt to overcome postpartum anestrus and the difficulty of applying AI in spontaneously ovulating animals. A single treatment with either GnRH or PGF$_{2\alpha}$+GnRH can successfully induce estrus in yak cows that calved in previous years but did not calve in the current year, however it has little effect in cows nursing a calf born in the current year (Shao and Zhao, 1984; Shao et al., 1986; Magash et al., 1997). There was no estrous synchronization program, which consistently synchronized estrus in yak cows, allowing fixed-time AI.

Recently, progesterone releasing devices (e.g. CIDR®; PRID) and Co-Synch synchronization regimens have been developed and use in cattle demonstrates some advantages over traditional methods in terms of simple application, synchrony of ovulation and acceptable pregnancy rates (approximately 50–70%) following AI (Macmillan and Peterson, 1993;
Martinez et al., 2002; Kim et al., 2003; Mialot et al., 2003). Therefore, the objectives of the present study were: (1) to evaluate the effectiveness of a CIDR in combination with pregnant mare serum gonadotropin and injection of prostaglandin on estrous induction and fertility in yak cows nursing a calf born in the current year and (2) to compare the relative efficiencies of the Co-Synch regimen of hormonal treatment and CIDR protocols for estrous synchronization and AI using frozen semen of Holstein and Jersey in non-suckled yak cows.

2. Materials and methods

2.1. Animals

The trial was conducted in August on yak farms located in Hongyuan County, Sichuan Province, in Southwestern China. The area is situated at 3600–3700 m above sea level and the pasture growing season is from May to August. Generally, the animals grow in body weight in the warm season (May–November) and lose weight during winter (December–April) (Zi et al., 2004). Animals in this study were pastured on the natural grassland without any supplementation. Yak cows used in Experiment 1 calved from April to June in the year the study was conducted and the number of days postpartum to the hormonal treatment ranged from 60 to 110 days. All cows were pastured near the campsites for milking. They were milked once daily in the morning and their calves were kept apart from the dams for 9–12 h during the night. Yak cows used in Experiment 2 calved in previous years (without calf) but not during the year the study was conducted. They were not milked and kept apart from yak bulls.

2.2. Experiment 1—Induction of estrus in suckled female yaks for natural breeding

Seventy-five postpartum suckled yak cows were assigned to three treatments: (1) Group A (n = 28)—yak cows were treated with an intravaginal device containing progesterone (EAZI-BREED™ CIDR®, InterAg, Hamilton, New Zealand) for 7 days (Day 0: day of initiation of treatment) and administered (i.m.) 200 μg prostaglandin analogue (PGF2α, Estrumate, Shanghai, China) on Day 6 and 1000 IU of pregnant mare serum gonadotropin (PMSG; Aba of Sichuan, China) on Day 7 coincident with the time of CIDR withdrawal (P4-PGF2α-PMSG); (2) Group B (n = 21)—yak cows were treated as Group A but without CIDR device insertion (PGF2α-PMSG); (3) Group C (n = 26)—yak cows did not receive any hormonal treatment (control group).

To detect onset of behavioral estrus, yak cows were observed for at least 30 min twice daily at approximately 12 h intervals from Day 0 to Day 22. A cow was considered in estrus if she stood to be mounted or to be mated. Seven yak bulls were pastured with these cows for natural breeding from August (start of treatment) to October (end of breeding season). Serum progesterone (P4) concentrations on Day 21 after breeding were used as an indicator of pregnancy (Moreira et al., 2000). Blood samples were collected on Day 21 via the jugular vein, placed on ice immediately after collection and then stored at 4 °C for 24 h until centrifugation. Serum was decanted and stored at −20 °C until assayed. The assay used a single antibody RIA procedure (Knickerbocker et al., 1986) and had inter- and intra-assay
CV of less than 10 and 5%, respectively. First service conception rate from Day 0 to Day 22 of the experiment was based on serum P₄ concentration on Day 21 after breeding; cows were considered pregnant when the P₄ concentration was ≥2.35 ng/ml. This value, used in cattle, was utilized because the change in serum P₄ concentration in the yak was similar to that found in cattle after breeding (Yu et al., 1993). Pregnancy data during the breeding season were based on calving data and postmortem examination for those that died during the winter.

2.3. Experiment 2—Synchronization of ovulation in the non-suckled yak cows for timed AI

One hundred and thirty-one non-suckled yak cows were assigned to three treatments: (1) Group A (n = 31)—yak cows were given (i.m.) 25 μg LRH-A₃ (GnRH, Nanjing Longyao, China) on Day 0 (start of treatment) and 200 μg PGF₂α analogue on Day 7 followed by timed AI (TAI) concurrent with LRH-A₃ treatment on Day 9 (approximately 48 h after PGF; Co-Synch program); (2) Group B (n = 50)—yak cows were treated with an CIDR device for 7 days followed by the administration (i.m.) of 200 μg PGF₂α analogue and 500 IU PMSG at the time of CIDR withdrawal on Day 7 and TAI on Day 9 (approximately 48 h after PGF; P₄-PGF₂α-PMSG); (3) Group C (n = 50)—yak cows were artificially inseminated at spontaneous estrus (AIE; control group).

Frozen semen of Holstein and Jersey were used for AI. Semen straws (0.25 ml) were thawed simultaneously in groups of 31 in Group A and 50 in Group B and it took about 5 h for inseminations. Artificial inseminations were performed by the same operator, and animals were palpated per rectum to assess estrous status (presence of a pre-ovulatory follicle, in presence or absence of CL or mucous vaginal discharge) at the time of AI in Group A and Group B. Yak cows were considered to be in premature estrus if they displayed estrus prior to PGF₂α treatment. Yak cows were considered to have undergone incomplete luteal regression if there was a CL at TAI. Yak cows in Group C were inseminated twice at an interval of 12 h once they were detected in estrus. Pregnancy diagnosis was assessed by an experienced veterinarian by rectal palpation of the uterine contents 35 days after AI.

2.4. Statistical analysis

Differences between treatments in incidence of estrus, pregnancy rate and calving rate were analyzed by Chi-square analysis as described by Cochran and Cox (1957).

3. Results

3.1. Experiment 1—Induction of estrus in suckled female yaks for natural breeding

None of devices was lost during the experiment. The incidence of estrus within 15 days of treatment was affected (P < 0.05) by the treatment (Table 1). This figure was greater in Group A (100.0%, 28/28) compared with either Group B (42.9%, 9/21) or Group C (0.0%, 0/26). Estrus was more synchronized by a short-term P₄ treatment; all yak cows in Group A
Table 1
Estrous induction and subsequent fertility of postpartum suckled yak cows after administration of PMSG and PGF2α, in conjunction with a short progesterone treatment

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of yak cows</td>
<td>28</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>Estrous rate (%) within 15 days after treatment</td>
<td>100.0 (28/28)b</td>
<td>42.9 (9/21)h</td>
<td>0.0 (0/26)i</td>
</tr>
<tr>
<td>Estrous rate (%) within 96 h after treatment</td>
<td>100.0 (28/28)b</td>
<td>28.6 (6/21)h</td>
<td>0.0 (0/26)i</td>
</tr>
<tr>
<td>First service conception rate (%) d</td>
<td>78.6 (22/28)b</td>
<td>22.2 (2/9)b</td>
<td>–</td>
</tr>
<tr>
<td>Pregnancy rate in the breeding season (%) e</td>
<td>82.1 (23/28)b</td>
<td>19.0 (4/21)h</td>
<td>7.7 (2/26)i</td>
</tr>
<tr>
<td>Percentage of cows in fact conceived that were initially detected pregnant using P4 f</td>
<td>90.9 (20/22)b</td>
<td>50.0 (1/2)b</td>
<td>–</td>
</tr>
</tbody>
</table>

Values within rows with different superscripts (g–i) differ ($P < 0.05$).

* Cows in Group A received a CIDR device on Day 0, an injection of PGF on Day 6 and PMSG on Day 7 at the time of CIDR removal and natural breeding with yak bulls.

b Cows in Group B were treated as in Group A but without CIDR device insertion.

c Cows in Group C did not receive any hormonal treatment.

d Cows were considered pregnant when the P4 concentration was $\geq 2.35$ ng/ml on Day 21 after breeding.

e Pregnancy rate = cows calved + pregnant cows observed by postmortem examination/cows joined. Four cows that were initially detected pregnant using P4 died in Group A, two bearing triplets and two bearing twins observed by postmortem examination and these were included in the data set as pregnant cows.

f Cows conceiving were determined by number of cows calving and by postmortem examination.

(100%, 28/28) came into estrus between Day 8 and Day 11 (a 96 h period) compared with only 28.6% (6/21) of yak cows in Group B and none in Group C.

The first service conception rate, as assessed by serum P4 concentration $\geq 2.35$ ng/ml on Day 21 after breeding, was greater ($P < 0.05$) in Group A (78.6%, 22/28) than in Group B (22.2%, 2/9) (Table 1). Pregnancy rates after estrous induction were 78.6% (22/28) and 9.5% (2/21), respectively, when based on those cows presented for estrous induction programs. Also, pregnancy rate during the breeding season in Group A (82.1%, 23/28) was greater ($P < 0.05$) than in Group B (19.0%, 4/21) and in Group C (7.7%, 2/26). Four cows in Group A that were initially detected pregnant died between 180 and 220 days of gestation at the end of winter, two bearing triplets and two bearing twins observed by postmortem examination and these were included in the data set as pregnant cows because conception had occurred in these cows after estrous induction rather than to a bull later in the breeding season, because multiple fetuses should be as a result of PMSG induction. In addition, two died in Group B and two died in the Group C but none of these animals were pregnant. Seventeen out of 24 yaks that were initially detected pregnant using P4 concentration calved in Group A and Group B within a duration of 20 days, but only one of other cows calved within this period.

3.2. Experiment 2—Synchronization of ovulation in the non-suckled yak cows for TAI

None of devices was lost during the experiment. Results (Table 2) indicate that follicular development, luteal regression and mucous vaginal discharge on the day of TAI were enhanced ($P < 0.05$) in Group B as compared with Group A. The percentages of yak cows that had an incomplete CL regression and premature estrus were greater ($P < 0.05$) in Group A (41.9 and 9.7%) than in Group B (2.0 and 0.0%). The conception rates, after TAI, were
Table 2
Estrous status at the time of AI (approximately 48 h after PGF) and pregnancy rates following Co-Synch or CIDR protocol in non-suckled yak cows

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Group A(^\text{a})</th>
<th>Group B(^\text{b})</th>
<th>Group C(^\text{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of yak cows</td>
<td>31</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Percentage of yak cows that</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had a pre-ovulatory follicle at TAI</td>
<td>38.7 (12)(^\text{d})</td>
<td>70.0 (35)(^\text{e})</td>
<td>–</td>
</tr>
<tr>
<td>Ovulated at TAI</td>
<td>9.7 (3)(^\text{d})</td>
<td>22.0 (11)(^\text{d})</td>
<td>–</td>
</tr>
<tr>
<td>Had a mucous vaginal discharge at TAI</td>
<td>16.1 (5)(^\text{d})</td>
<td>46.0 (23)(^\text{e})</td>
<td>–</td>
</tr>
<tr>
<td>Had incomplete CL function at TAI</td>
<td>41.9 (13)(^\text{d})</td>
<td>2.0 (1)(^\text{e})</td>
<td>–</td>
</tr>
<tr>
<td>Were observed in estrus from Day 0 to Day 7</td>
<td>9.7 (3)(^\text{d})</td>
<td>0.0 (0)(^\text{e})</td>
<td>20.0 (10)(^\text{d})</td>
</tr>
<tr>
<td>Yak cows inseminated from Day 0 to Day 9</td>
<td>100.0 (31)(^\text{d})</td>
<td>100.0 (50)(^\text{d})</td>
<td>24.0 (12)(^\text{e})</td>
</tr>
<tr>
<td>First service conception rate (%)</td>
<td>22.6 (7)(^\text{d})</td>
<td>30.0 (15)(^\text{d})</td>
<td>33.3 (4)(^\text{d})</td>
</tr>
</tbody>
</table>

Values within rows with different superscripts (d and e) differ \(P < 0.05\).

\(^\text{a}\) Cows in Group A were treated with GnRH and 7 days later with PGF. They were inseminated and received a second injection of GnRH 48 h later.

\(^\text{b}\) Cows in Group B received a CIDR device on Day 0, an injection of PGF, PMSG on Day 7 at the time of CIDR removal and were inseminated 48 h later.

\(^\text{c}\) Cows in Group C did not receive any hormonal treatment and were inseminated at spontaneous estrus.

4. Discussion

The annual pregnancy rate of most yak breeds is 40–60% because only a small proportion of the cows return to estrus in the first breeding season after calving with most coming into estrus in the second and third years under traditional management systems (Cai, 1979; Zi, 2003). For example, in Ruoergai County of Sichuan Province, 14.3% of yak cows return to estrus in the year of calving, 59.6% in the second year and 13.9% in the third year (Cai, 1979). Previous studies showed that administration of PGF\(_{2\alpha}\) or GnRH, or a 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 year when calving occurred (Shao and Zhao, 1984; Shao et al., 1986; Magash et al., 1997). Shao and Zhao (1984) reported that the administration of PGF\(_{2\alpha}\) and GnRH to yak cows that calved in the previous years (with or without calf) resulted in 89.5% of yak cows ovulating within 7 days after treatment; 73.7% of cows became pregnant in the following two breeding cycles compared with 38.9% of control cows. However, for yak cows nursing a calf born during the treatment period, only 7.0% became pregnant within two breeding cycles.
The results of in Experiment 1 demonstrate that estrus can be effectively induced in postpartum suckled yak cows with the P₄-PGF₂α-PMSG protocol. The estrous response was markedly enhanced, in that the interval to estrus was shorter with a greater frequency of behavioral estrus; all animals came into estrus within 96 h, and both the first service conception rate after estrous induction and pregnancy rate during the breeding season were also greater \((P < 0.05)\) after treatment with P₄-PGF₂α-PMSG than after treatment with PGF₂α-PMSG or in the control group. The observations in Experiment 1 were consistent with previous reports in postpartum anestrous dairy cows (Roche et al., 1992; Macmillan and Peterson, 1993), beef cows (Roche et al., 1992; Troxel et al., 1993), zebu (Rao and Suryaprakasam, 1991) and buffalo (Rao et al., 1985). Although the mechanism by which treatment with progesterone improves fertility of postpartum suckled yak cows is not completely understood, Troxel et al. (1993) reported that most luteal phases (78.0%) in beef cattle that follow ovulation induced by PGF₂α-GnRH treatment were 6–12 days in duration. When cows were bred at an estrus with an expected subsequent short luteal phase, conception might occur but pregnancy was generally not maintained (Kesler and Troxel, 1984). Progesterone treatment, however, reduced the incidence of short luteal phases and improved pregnancy rates in otherwise anestrous cows (Kesler and Troxel, 1984; Troxel et al., 1993).

Administration 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 marginal. Roche et al. (1992) reported that in beef cows suckling calves or in dairy cows under nutritional stress, an administration of PMSG at the end of a progesterone treatment may increase the estrous response. However, 1000 IU of PMSG appears to be too great given the occurrence of some twin and triplet bearing pregnancies. Serum P₄ concentrations on Day 21 after breeding appears to be an adequate indicator of pregnancy in the yak with an 87.5% (21/24) accuracy for positive diagnoses.

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). Long et al. (1999) reported that natural resumption of reproductive function in a proportion of postpartum cows occurred without signs of behavioral estrus following postpartum or seasonal anestrus with pre- and postpartum body condition affecting the signs of behavioral estrus. Yak cows in Experiment 1 were not nutritionally supplemented during the winter and had a long period of grazing on poor quality pasture despite having calved and being suckled by calves and milked daily. This management procedure might be the reason why none of the cows in Group C displayed overt estrus during the observation period. However, after the end of August, milking was stopped and the animals grazed alpine pasture that enabled them to recover their body condition. A small proportion of these animals would, therefore, be expected to naturally come into estrus in September and October. A pregnancy rate of 7.7% was observed in Group C which is similar to the average (6–12%) recorded on the Longri Yak Farm in previous years.

Meat and milk production of yaks is greatly improved by crossing with improved breeds of cattle (Bos taurus) but AI is necessary because bulls of improved cattle breeds can not adapt to the harsh conditions and high altitudes typical for yak (Cai and Wiener, 1995). The results of Experiment 2 show that the proportion of yak cows conceived after TAI based on yak cows joined during the period of experiment was greatly increased by estrous synchronization protocols, although the difference was not significant between Co-Synch
regimens and control. The conception rate after synchronization of estrus with the P₄-
PGF₂α-PMSG protocol (30.0%) was similar to that after AIE (33.3%), but it was greater
than that after synchronization with Co-Synch regimens (22.6%), although the difference
was not significant. Estrous cycling status of the yaks was not determined prior to estrous
synchronization, but previous studies showed that approximately 30–50% non-lactating
yaks were still in anestrous in August (Cai and Wiener, 1995). The ovulation rate and preg-
nancy rate were less with the Ovsynch protocol than with P₄-PGF₂α-PMSG protocol in
anestrous beef cows and buffalo, but there was no significant difference in cows that had
initiated estrous cycles at the time of treatment (Rao et al., 1985; Mialot et al., 2003). Sarkar
and Prakash (2005) observed that seven out of eight non-lactating yaks that had initiated
estrus cycles ovulated in response to Ovsynch treatment and the interval from the second
GnRH injection to ovulation was 20–32 h which is similar to 24–32 h in cattle reported by
Pursley et al. (1995). Cattle displaying estrus prior to PGF₂α treatment (premature estrus)
in Ovsynch and Co-Synch regimens are unlikely to become pregnant after TAI (Kim et al.,
2003). In Experiment 2, approximately 9.7% of cows treated with the Co-Synch regimen
underwent premature estrus before administration of PGF₂α, while none of the P₄-PGF₂α-
PMSG treated yaks displayed a premature estrus. Similar to the findings in cattle (Moreira
et al., 2000; Kim et al., 2003), maintenance of progesterone concentrations until administra-
tion of PGF₂α in the P₄-PGF₂α-PMSG group prevented the premature occurrence of estrus,
which may have contributed to the greater pregnancy rate than was observed in the Co-
Synch regimen group. Incomplete luteal regression following the administration of PGF₂α
with the Ovsynch and Co-Synch protocols has also been associated with lesser conception
rates (Moreira et al., 2000; Kim et al., 2003). In the present study, 41.9% of the Co-Synch
regimen group underwent incomplete luteal regression, based on palpation per rectum 48 h
after PGF₂α, while only 2.0% of P₄-PGF₂α-PMSG treated yaks had luteal regression during
this period. Handling and thawing of frozen semen before AI might be another constraint.
Howard and Pace (1988) reported that semen thawed at 37°C should be deposited in the
animal within 15 min after thawing. Semen straws were thawed simultaneously in groups
of 31 in Group A and 50 in Group B and it took about 5 h to conduct all inseminations.
Temperature changes during this period might have affected the motility of spermatozoa
and hence reduced the conception rate.

There is a marked difference between pregnancy rates resulting from pure breeding and
those from hybridization. When the yak female is naturally or artificially inseminated
with semen of another species of cattle, the conception rate is less (e.g. 18–35%; Cai, 1979;
Cai and Wiener, 1995). Conception rates were 78.6% after breeding with yak
bulls in P₄-PGF₂α-PMSG treated female yaks in Experiment 1 but only 30.0% after TAI and
33.3% after AIE with cattle breeds in Experiment 2. In addition to handling and thawing
of frozen semen, He et al. (2004) reported that female yaks after repeated insemination
with semen from cattle breeds produced sperm antibodies which reduced conception rate.
Pregnancy rate of female yak was significantly improved by immunization with sperm
antibodies (55.6%) compared with control (27.6%).

In conclusion, the present study has provided information that a short-term progesterone
treatment combined with PMSG and prostaglandin increased the proportion of yak cows
that exhibited behavioral estrus, improved the degree of estrous synchronization as well as
improving the conception rate in postpartum suckled yak cows. This technique and the Co-
Synch regimen of hormonal treatment can be applied successfully for TAI in non-suckled yak cows.

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