Studies on Estrus Induction in Ewes during Non-Breeding Season

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Abstract

The present study was aimed to study estrus induction in 30 randomly selected ewes during a non-breeding season, at government Sheep Breeding Farm Panthal, Katra, Reasi, Jammu and Kashmir. Ewes selected were divided into 5 groups (GI, GII, GIII, GIV & GV), each group consisting of 6 ewes (n = 6), which were treated with different hormonal protocols as (GI = 1/3 Norgestomet + 200 IU PMSG, GII = 1/3 Norgestomet + Ram effect, GIII = P4 sponge + PMSG 200 IU, GIV = P4 sponge + Ram effect, GV = untreated control). All the ewes (100 %) covered under hormone protocols exhibited induced estrus with intense, fair or weak estrus signs within mean onset of 25.83 ± 1.49 hrs, 44.16 ± 3.97 hrs, 29.16 ± 1.62 hrs and 54.83 ± 1.95 hrs and mean duration of estrus 28.83 ± 1.81 hrs, 36.33 ± 2.75 hrs, 25.66 ± 0.71 and 28.33 ± 2.89 hrs in GI, GII, GIII and GIV, respectively. The conception rate was similar in GI, GII, GIII (50%), while GIV showed lowest conception rate among treated groups (16.66%). The lambing rate was similar in all the groups (100%). In untreated control (GV), none of the ewes exhibited estrus.

Keywords
Ewe, Estrus induction, P4 Sponge, PMSG, Ram effect.

Introduction

The control of estrus and ovulation in sheep with progesterone and its analogues has been extensively evaluated, applied and accepted in sheep breeding programmes (Robinson, 1967; van Niekerk and Belonje, 1970; Boshoff et al., 1973; Haresign, 1978; Hunter, 1980). Reproductive seasonality in ewes is characterized by changes in behavioral, endocrine, and ovulatory patterns (Epstein, 1985; Rosa and Bryant, 2003). The majority of sheep breeds are anestrous for at least some proportion of the year (Rekwot et al., 2001) with the degree and depth of seasonality dependent upon breed and climate. In Jammu and Kashmir non breeding season of sheep falls mostly between April and September. The methods for induction of estrus out of season breeding in ewes have revolved around the use of P4 pessaries and PMSG (Mcleod and Haresign, 1984). Progestagens are administered as daily injections, orally, intra vaginal devices like CIDR, PRID and
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subcutaneous ear implants like Crestar. Progestagens or its analogues along with gonadotrophin have been extensively used to induce estrus in anestrus ewes, although pregnancy rates of progestagen-synchronized ewes are lower during anestrus than during the breeding season. Intravaginal sponges are usually inserted over periods of 6-14 days and an injection of PMSG is administered prior or at time of sponge removal (Wildeus, 2000 and Ustuner et al., 2007). Many studies have incorporated the use of norgestomet implants for out of season breeding with pregnant mare serum gonadotropin (PMSG) (Fitch et al., 1986 and Yelich et al., 1992). Ram effect is also used to achieve breeding activity during the non-breeding season. Anestrus ewes are isolated from rams before the start of normal breeding season, introduction of rams to ewes induces ovulation, and this method is referred to as the ram effect or male effect (Jordan, 2005). The ram effect allows induction of breeding during anestrus and produces some synchrony in the cycle among the ewes in flock (Chanvallon et al., 2008). The aim of this study was to evaluate the effect of crestar and intra vaginal progesterone sponge in combination with ram effect or Equine Chorionic Gonadotropin (eCG) on the reproductive efficiency of ewes during non-breeding season.

Materials and Methods

The present study was conducted on 30 ewes during non-breeding season at government Sheep Breeding Farm, Panthal, Katra, District Reasi, Jammu (J&K). The average temperature and relative humidity during the period of study were 33ºC and 58.5% respectively. The period extended from May to August, 2015. Age, Body weight and Body condition score were recorded in all animals. Ewes were randomly divided into 5 groups consisting of 6 animals in each group. In group I, ewes (n = 6) were treated with Crestar ear implants @ 1mg Norgestomet (1/3 of the 3mg implant used in large animals) on day 0 (Fig. 1b). The implant was removed on day 12 and an injection of PMSG (200 IU) was given on the day of implant removal. In group II, ewes (n = 6) were treated with Crestar ear implants @ 1mg Norgestomet (1/3 of the 3mg implant used in large animals) on day 0. The implant was removed on day 12 and a ram was introduced 72hrs before the implant removal. In group III, ewes (n = 6) were treated with conventional P₄ sponge for 12 days (Fig. 1a). An injection of PMSG (200 IU) was given on the day of P₄ sponge removal. In group IV, ewes (n = 6) were treated with conventional P₄ sponge for 12 days. The sponge was removed on day 12 and a ram was introduced 72hrs before the sponge removal. In group V, ewes (n = 6) were kept without any treatment and were sampled on the same days as in treatment groups.

Results and Discussion

The efficacy of all the protocols was studied regarding estrus induction response, onset of estrus, duration of estrus, estrus intensity, conception rate and lambing rate. The estrus intensity was described as intense fair and weak on the basis of signs of estrus. The data regarding all the parameters in all the five groups is shown in Table 1.

All the ewes (100.00%) in Group I, II, III and IV responded to treatment and exhibited estrus, whereas, in Group V (Control), none of ewes exhibited estrus during the period of study. Our findings were in complete agreement with the work done by various workers in which estrus induction rate was 100% on treating with exogenous progestagen for a specific time period or using a combination of progestagen and gonadotropins (Juma, 2010; Das et al., 1999; Kashifalkita, 2003; Alwan, 2012; Kohno et al., 2005). Likewise, our results in PMSG
treated ewes (Group I, Group III) are in close agreement with results of Boland et al., (1979), Carpenter et al., (1981), Alifakiotis (1985), Tritschler et al., (1991), Amer and Hazzaa (2009), Kor et al., (2012). Differing from our results, lower estrus induction rates ranging from 46% to 93% were reported by Redmer et al., (1998), Kusina et al., (2000), Mellado et al., (2000), Das et al., (2004), Ataman et al., (2006), Dogan and Nur (2006), Ali (2007), Abu Gazal (2010), Bogdan et al., (2011), Santos et al., (2011), Sarminejad et al., (2014).

Our results of Group II and IV (ram effect groups) are almost in close proximity with results of Mellado et al., (2000) who found an estrus induction rate of 92% when bucks were exposed to goats 2 days before the end of Synchromate-B (SMB) treatment. Chanvallon et al., (2008) also observed that ram effect allows induction of breeding during anestrus and produce some synchrony in the cycle among the ewes in flock. Unlike to our results, Ungerfeld et al., (2005) found induction rate of 71% when ram was introduced to ewes primed with MAP for 6 days during non-breeding season. Iida et al., (2004) found that ram presence resulted in higher ovulation rate than without rams but there was no significant (p<0.05) difference. Higher estrus induction rate in our study might be due to less seasonal breeding pattern of ewes, good body condition, good ram to ewe ratio, better management and feeding of ewes. Other factors like age, parity, breed of ewe, lambing to induction interval, breed and percentage of rams used, depth of anestrus, geographical location might have lead to variation in response.

Estrus onset was earlier in groups treated with PMSG (Group I and III). Our results are in complete agreement with earlier results of Botha et al., (1975), Dogan and Nur (2006), Omontese et al., (2012), Cline et al., (2001), Redmer et al., (1998) and Gardon et al., (2015) who reported that time to estrus was shorter in eCG treated ewes than eCG untreated ewes.

In contradiction, higher estrus onset interval were also reported by Marco-Jimenez et al., (2014) (73.2 ± 86.7 h), Abu Gazal (2010) (60.7 ± 20.3 h), Ali (2007) (69 ± 9.9 h). Estrus onset was delayed in groups exposed to ram prior to P₄ withdrawal. Our results are in agreement with earlier reports of Ungerfeld et al., (2005) and Jarquin et al., (2014). Unlike to our study, a shorter estrus onset interval of 21 h was reported by Iida et al., (2004). Between the ram exposed groups, estrus onset was more delayed in Group IV. This might be due to failure in absorption of P₄ from Intravaginal sponge, due to which there would have been some P₄ residue which would have lead to prolonged negative feedback of P₄ on cyclicity and hence delayed onset of estrus (residual effect). Besides it, ram effect might not be adequate to increase the LH pulse frequency. Similarly, Iida et al., (2004) reported that absorption of P₄ is important for proper estrus behavior and ovulation. There might be also low circulating testosterone level in the ram used, which might have led to delay in LH surge, hence delayed estrus onset.

Similar results were earlier reported by Perkins and Fitzgerald (1994) who observed that factors affecting the circulating levels of testosterone will affect the quality and thus efficacy of the ram stimulus. Besides it, other factors like quality, type and duration of ram stimulus are critical to extent of ovulatory response to the ram effect (Walkden-Brown et al., 1999), which might have lead to delayed estrus onset in our study.
**Fig.1** (a) Conventional sponge protocol, (b) Crestar protocol

| Treatment Groups | No. of ewes treated | No. of animals responded (%) | Estrus onset (hr) (Mean ± SE) | Estrus Duration (hr) (Mean ± SE) | Total Conception Rate | Lambing Rate (%) |
|------------------|---------------------|-----------------------------|-----------------------------|----------------------------------|----------------------|------------------|
| Group I          | 6                   | 6 (100)                     | 25.83 ± 1.49<sup>a</sup>     | 28.83 ± 1.81<sup>a</sup>        | 50.00%               | 2/2 (100.00%)    |
| Group II         | 6                   | 6 (100)                     | 44.16 ± 3.97<sup>b</sup>     | 36.33 ± 2.75<sup>b</sup>        | 50.00%               | 3/3 (100.00%)    |
| Group III        | 6                   | 6 (100)                     | 29.16 ± 1.62<sup>a</sup>     | 25.66 ± 0.71<sup>a</sup>        | 50.00%               | 3/3 (100.00%)    |
| Group IV         | 6                   | 6 (100)                     | 54.83 ± 1.95<sup>c</sup>     | 28.33 ± 2.89<sup>a</sup>        | 16.66%               | 1/1 (100.00%)    |
| Group V          | 6                   | 0 (0.00)                    |                             |                                  | 0.00%                | 0/6 (0.00%)      |

Means bearing different superscripts down a column differ significantly (P<0.05)

**Table.2** Data regarding estrus induction response, onset of estrus, duration of estrus, conception rate and lambing rate in all the five groups

| Treatment Groups | Intense | Fair | Weak |
|------------------|---------|------|------|
| Group I          | 4 (66.66%) | 1 (16.66%) | 1 (16.66%) |
| Group II         | 4 (66.66%) | 2 (33.33%) | 0 (0.00%) |
| Group III        | 4 (66.66%) | 2 (33.33%) | 0 (0.00%) |
| Group IV         | 3 (50.00%) | 1 (16.66%) | 2 (33.33%) |

Figures in parenthesis represent the percentage
The duration of estrus was longest in Group II and differed significantly (P<0.05), compared to other groups which differed non-significantly (P>0.05). Estrus duration was less in Group I, III and IV ewes. In previous studies, and in accordance with current study, it was reported that mating decreased the duration of estrus (Romano, 1993). One service reduced the duration of estrus by 45%, but the response was not affected (Romano, 1994a). Authors have suggested that penile intromission stimulates mechanisms involved in estrus shortening (penile effect) (Romano, 1994b). But in Group II of our study, ram was introduced close to onset of breeding season in ewes that might have lead to increased response of ewes, resulting in prolonged estrus duration. Likewise, Cushwa et al., (1992) and Oldham et al., (1984) also observed that ewes are more receptive to the ram stimulus when rams are introduced close to the spontaneous onset of the breeding season. In contradiction to our results, shorter estrus duration were reported by Sareminejad et al., (2014) (14.77 ± 1.33 h), Abu Gazal (2010) (12.1 ± 7.3 h, 12.8 ± 8.3 h and 13.7 ± 11.3 h w.r.t. different dosage rates of P4 and PMSG), Ekiz et al., (2006) (18.0 ± 2.86 h).

Intensity of estrus in Group I ewes was good in 4 ewes (66.66%), fair in 1 ewe (16.66%) and weak in 1 ewe (16.66%). This is in accordance with the findings of Bhoraniya et al., (2012) who reported that cows treated with CIDR protocol, showed prominent (66.66%), moderate (16.66%) and weak (16.66%) estrus signs. In Group II and III ewes, intensity of estrus was intense in 4 ewes (66.66%), fair in 2 ewes (33.33%) and weak in none of the ewes (0.00%). This is in close concurrence with result values of Amle et al., (2012) who studied the effect of Ovsynch plus CIDR protocol in postpartum crossbred cows and found the intensity of estrus was 70% intense, 30% intermediate and 0% weak. In Group IV ewes, intensity of estrus was intense in 3 ewes (50%), fair in 1 ewe (16.66%) and weak in 2 ewes (33.33%). This is in close concurrence with result values of Ungerfeld et al., (2005) who found that % of ewes in estrus was 71% approximately, with 35.5% (approx.) ewes showing overt signs and 35.5% (approx.) showing silent estrus.

Conception rate was equal to 50% in Group I, II and III. Our results were in close concurrence with earlier results of Carpenter et al., (1981), Husein and Kridli (2002), Kohno et al., (2005), Amer and Hazzaa (2009), Garoussi et al., (2012), Sareminejad et al., (2014) who reported conception rates in the range of 50% to 55%. Unlike to our study, higher conception rates ranging from 71.42% to 100% were reported by Das et al., (1999), Dogan and Nur (2006), Awel et al., (2009), Bogdan et al., (2011), Zonturlu et al., (2011), Taher (2014). Likewise lower conception rates rate of 10% was reported by Taher (2014) using FGA sponge; 44.3% by Yelich et al., (1992) using norgestomet+ PMSG-hCG combination; 44.4% by Dogan and Nur (2006) using MAP alone; 42.85% by Awel et al., (2009) using full dose of norgestomet implant + injectable + eCG; 40.7% by Kohno et al., (2005) using intravaginal P4 cream + eCG. Conception rate was lowest in Group IV, equal to 16.66%. This low conception rate in current study might be due to poor estrus intensity of Group IV ewes (fair=16.66% and weak =33.33%) and delayed estrus onset which might have lead to poor LH surge and ovulation, hence conception was lowest (Table 2).

Lambing rate was 100.00% in all the groups i.e., Group I, II, III, IV. In Group I ewes, only 2 ewes lambed and 1 ewe died before lambing out of the 3 conceived ewes and this dead one was excluded from the experiment, thus the total lambing rate was 2/2 (100.00%). In Groups II, III & IV ewes, all conceived ewes lambed (3/3 ewes in Group II & III; 1/1 ewes
in Group IV). None of ewes in treatment group showed twinning or triplet births. In Group V, none of the ewes were observed in estrus and conceived, therefore there was no lambing. In norgestomet groups (Group I and Group II), our results are in complete agreement with earlier results of Alifakiotis (1985) and Awel et al., (2009). In sponge groups (Group III and Group IV), our results are in complete agreement with earlier results of Zonturlu et al., (2011) and Taher (2014). On the other hand, a lower lambing rate of 62%, 39%, 71%, 28% were reported by Alifakiotis (1985).

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