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Studies on Estrus Induction in Ewes during Non-Breeding Season

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A B S T R A C T

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, Jaamu 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 = P_4 sponge + PMSG 200 IU, GIV = P_4 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.

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 anestrus 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 P_4 pessaries and PMSG (Mcleod and Haresign, 1984). Progestagens are administered as daily injections, orally, intra vaginal devices like CIDR, PRID and

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 P_4 sponge for 12 days (Fig. 1a). An injection of PMSG (200 IU) was given on the day of P_4 sponge removal. In group IV, ewes (n = 6) were treated with conventional P_4 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 P_4 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 P_4 from Intravaginal sponge, due to which there would have been some P_4 residue which would have lead to prolonged negative feedback of P_4 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 P_4 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

Table.1 Data regarding estrus induction response, onset of estrus, duration of estrus, conception rate and lambing rate in all the five groups

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^{a}	28.83 ± 1.81^{a}	50.00%	2/2 (100.00%)
Group II	6	6(100)	44.16 ± 3.97^{b}	36.33 ± 2.75^{b}	50.00%	3/3 (100.00%)
Group III	6	6(100)	29.16 ± 1.62^{a}	25.66 ± 0.71^{a}	50.00%	3/3 (100.00%)
Group IV	6	6(100)	54.83 ± 1.95^{c}	28.33 ± 2.89^{a}	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	Intensity of Estrus		
	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 P_4 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 P_4 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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