

## Effect of CIDR and Different Doses of PMSG on Pregnancy and Lambing Rate out of Breeding Season in Balouchi Ewes

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**Abstract:** Two blood samples, 8 days apart were taken from 150 Balouchi ewes being in their 3-5 years of age and serum Progesterone (P4) concentrations were determined. One hundred and twenty five ewes that had lower P4 concentrations have been chosen and in a completely randomized design were assigned into 5 groups of, 600 (n = 26), 500 (n = 25), 400 (n = 24), 300 (n = 26) and control (n = 24). Groups were separated just by a physical barrier. Twenty days after the second blood sampling, CIDR-G insert was applied into the vagina of all the ewes and after 12 days, inserts were removed. PMSG was immediately injected IM after CIDR removal in the rate of 600, 500, 400, 300 IU into ewes of the 600, 500, 400 and 300 groups, respectively. Ewes in the control group received an injection of normal saline solution only. Rams were introduced into the flock with the rate of 1/5 ewes the same day until a week later when they were separated from ewes. Ram effect was abolished by rotating them in the groups regularly. Ewes were monitored and in case of return to estrus, were put together with ram again. On day 50±5, after mating begun, all the ewes were checked by ultrasound for pregnancy. In case of getting negative pregnancy results and returned into estrus and having second mating, ewes were checked 50±5 days after second mating. Results showed that multiple birth and lambing rate was higher in PMSG injected ewes than the control group (p<0.05). Similarly, 600 group had the highest multiple births and 400 group had the highest lambing rate among the first 4 groups (p<0.05). There were no significant relationship between level of PMSG injection and multiple births (p>0.05). Control group had the lowest and 400 group had the highest total number of lambs and delivered ewes (p<0.05). In conclusion, use of CIDR together with PMSG in anestrus Balouchi ewes can induce and synchronize estrus and increases multiple births. Although, there were no significant difference among the PMSG injected ewes in multiple births but 400 group had the highest score in this respect.

**Key words:** CIDR, PMSG, twinning, anestrus, ewe

### INTRODUCTION

Progesterone (P4) was first used to synchronize estrus 5 decades ago (Dutt and Casida, 1948; Mary *et al.*, 1950; Robison and Smith, 1967) and fertile estrus was induced in seasonally anestrus ewes by P4 and gonadotropin (Dutt, 1953). Nowadays, induction of estrus in seasonally anestrus ewes is a routine procedure (Whitley and Jackson, 2004) but, with a lower fertility rate when using progestogens alone (Jabbar *et al.*, 1994; Safranski *et al.*, 1992; Wheaton *et al.*, 1990) or with gonadotrophins (Dutt, 1953; Hogue *et al.*, 1962) as compared with intact cycling ewes (Cardwell *et al.*, 1998; Crosby *et al.*, 1991). Increased fertility with greater concentrations of progestogen (Robinson *et al.*, 1968) probably resulted from more appropriate follicular development (Johnson *et al.*, 1996), timing of the LH

surge (Lewis *et al.*, 1974; Van Cleeff *et al.*, 1998) and sperm transport (Hawk and Conley, 1971; Quinlivan and Robinson, 1967). Ovulation rate is low during anestrus (Hall *et al.*, 1986; Hulet *et al.*, 1974; Mallampati *et al.*, 1971) but was increased by Pregnant Mare Serum Gonadotropin (PMSG) (Ainsworth *et al.*, 1985; Hackett, 1982; Hamra *et al.*, 1989) or FSH (Ahmad *et al.*, 1996) at progestogen withdrawal. In order to induce synchronized estrus and ovulation in anestrus ewes, Controlled Internal Drug Release device (CIDR-G<sup>®</sup>) (Hamra *et al.*, 1989; Welch *et al.*, 1984) have been used. P4 release from CIDR declines over time. However, treatment with progestogen for only 5 days in anestrus cows (Smith *et al.*, 1983) or 6 days in anestrus ewes (Rodriguez-Iglesias *et al.*, 1996) has been effective to induce estrus and ovulation. The original sheep CIDR contains 330 mg P4 in silicone elastomer core. Another kind of insert which contains

0.82 g of P4 has also been used (in a Polycaprolactone, PCL) in combination with FSH and sudden introduction of rams into the flock. The above method has increased ovulation rate and twinning rate in anestrus ewes (Knights *et al.*, 2001). The aim of the present study was to determine whether CIDR inserts in combination with PMSG at CIDR removal could induce fertile estrus and which doses (300, 400, 500 and 600IU) of PMSG, would induce higher rates of ovulation and increase prolificacy in anestrus Balouchi ewes.

## MATERIALS AND METHODS

The research was conducted from June to November 2006 in Abbasabad Research Station, Mashhad, Iran. Two blood samples were taken 4 days apart from a total of 150, 3-5 years old Balouchi non-lactating ewes. Serum P4 concentrations were measured by ELISA method and all the ewes with lower than 0.5 ng mL<sup>-1</sup> P4 concentrations in 2 samplings (n = 125) were considered anestrus and selected for the study. Ewes were kept in a barn and exposed to natural photoperiod. The ewes were divided into 5 groups; group 1 (n = 26) received 600 IU; group 2 (n = 25) 500 IU; group 3 (n = 24) 400 IU; group 4 (n = 26) 300 IU PMSG and group 5 (n = 24) were selected as controls in a completely randomized design. Twenty days after the second sampling, a CIDR-G was inserted into the vagina of all ewes and was removed after 12 days. The ewes in the four trial groups received 600, 500, 400 and 300 IU of PMSG (Pregnenolol®), respectively. Ewes in the control group received normal saline injection only as placebo. Rams were introduced into the flock on the same day and remained for 7 days. In order to remove the ram effect, rams were rotated in the groups regularly. Ewes were monitored constantly and in case of return to estrus, were joined with rams for the second time. Pregnancy was checked by using trans-abdominal ultrasonography 30 days after last mating. Lambing dates and numbers of lambs born were recorded.

**Statistical analysis:** The SPSS package (version 9) was used to data analysis. Paired Student's t-test was used to find out the difference between P4 levels in the two samplings. Determination of the differences in lambing rate per each individual ewe and all the ewes in different groups, total ewes giving birth in all the groups and also to find out the effects of different doses of PMSG on lambing rate, Chi square test was performed. Analysis of variance was used to determine the difference between the total lambs born in different groups. In all the tests, p<0.05 was considered significant.

## RESULTS AND DISCUSSION

Mean P4 concentrations in the plasma of the ewes in the second sampling was lower than the first sampling but the difference was not significant (p>0.05; 2.013 vs. 1.349 ng mL<sup>-1</sup> in the first and second sampling, respectively). It seems that many of the ewes were not anestrus because ewes having plasma P4 concentrations higher than 0.5 ng mL<sup>-1</sup> had not been considered anestrus by others (Jabbar *et al.*, 1994). The results showed that twinning rate was higher in the first 4 groups (PMSG groups) than the control ewes (p<0.05) but no significant difference was found among PMSG groups (p>0.05). Lambing rate was higher in the PMSG treated groups as compared with that of the control group (p<0.05). Among the PMSG groups, 400 group had the highest lambing rate (p<0.05). Highest twinning rate belonged to the ewes of 600 group as compared to the other 3 PMSG groups (p<0.05) and twinning rate was higher in the PMSG treated than the control ewes (p<0.05). Total number of lambs born was highest in the 400 group while the control ewes had the lowest number of lambs born (p<0.05). Total number of ewes giving birth was highest in the 400 group (p<0.05; Table 1).

P4 is routinely used to manipulate reproductive processes, especially in anestrus animal during the non-breeding season with or without simultaneous use of gonadotropins and/or prostaglandins or their analogues. Formerly, Norgestomet (Synchromate-B; SMB) was used in sheep. However, SMB is no longer produced for sheep, therefore, in the recent years, effectiveness of other progestines have been studied carefully and compared to SMB. CIDR-G which releases P4 upon insertion into the vagina has been used successfully (Whitley and Jackson, 2004). Researchers have applied CIDR-G to anestrus ewes and bring them into estrus in July-August (Wheaton *et al.*, 1992). In that experiment, similar to the present research, CIDR was inserted into the vagina for 12 days and before introduction of the rams into the flock, ewes were deprived of any kind of exposure (sight, smell and sound) to rams. In the present experiment, application of CIDR resulted in highest number of lambs to be born from 400 group ewes (29 lambs). However, twinning rate was different in PMSG treated ewes as compared with controls and ewes in the 600 group had the highest rate. The above data indicates that, use of progestogens along with PMSG can increase twinning rate.

An injection of PMSG after CIDR removal causes estrus signs to begin sooner, becomes more pronounced, number of ova shed is increased and enhances twinning rate, thereafter (Cognie *et al.*, 1982).

**Table 1: Data showing results of the present research: significant differences are shown by different letters (p<0.05)**

Dose of injected PMSG (IU)	600	500	400	300	(zero) control
Total number of ewes in each group	26	25	24	26	24
Mean lambing rate/pregnant ewe	1.615 <sup>b</sup>	1.452 <sup>b</sup>	1.526 <sup>b</sup>	1.583 <sup>b</sup>	1.083 <sup>a</sup>
Mean lambing rate/ewe in the group	0.875 <sup>b</sup>	0.73 <sup>b</sup>	1.2 <sup>c</sup>	0.84 <sup>b</sup>	0.5 <sup>a</sup>
Twining percentage	61.538 <sup>c</sup>	46.154 <sup>b</sup>	52.632 <sup>b</sup>	53.846 <sup>b</sup>	8.333 <sup>a</sup>
Total number of lambs born	21 <sup>b</sup>	19 <sup>b</sup>	29 <sup>c</sup>	21 <sup>b</sup>	13 <sup>a</sup>
Total number of ewes giving birth	13 <sup>a</sup>	13 <sup>a</sup>	19 <sup>b</sup>	13 <sup>a</sup>	12 <sup>a</sup>

Different doses of PMSG as low as 400IU (Jabbar *et al.*, 1994; Sifranski *et al.*, 1992), 500IU (Cardwell *et al.*, 1998; Whitley and Jackson, 2004) or as high as 700IU (Manalu *et al.*, 2000) or FSH (16; 55mg of Folltropin) have been used by researchers with similar results. If the dosage of the hormone is chosen according to body weight, large breeds should get higher and vice versa. Balouchi sheep is one of the small Iranian breeds with a mature BW of 40-45 kg for females and 55-60 kg for males. In the present study by using CIDR inserts and different doses of PMSG in non breeding season, lambing rate per ewe was higher in the hormone treated ewes than the controls, with 400 group ewes having highest lambing rate. Twinning rate was lowest in the control showing the effectiveness of hormone therapy in this respect. According to the unofficial statistics of the Department of Agriculture, mean lambing rate in Khorasan province is 0.7 lambs/ewe/year or 1.4 lambs/ewe/2years. In our experiment, lowest lambing rate belonged to the control ewes (0.5 lambs/ewe; Table 1). If the number of pregnancies could be increased in the Balouchi ewes into 3 times/2 year, i.e., two times within the breeding season in the intact ewes and one time out of season just by using CIDR, total number of lambs would increase to 1.9 lambs/2year. If the above calculations are done for PMSG treated ewes in the same way as for the controls, figures will be as follows: 2.28, 2.12, 2.6 and 2.24 lambs/2year/ewe for 600, 500, 400 and 300 groups, respectively. These figures are much above the present 1.4 lambs/2year. It should be considered that when CIDR and PMSG are used concomitantly within the breeding season, resultant lambing and twinning rate will be higher than the present figures, therefore, the above calculations are the minimum expectations for lambing and twinning rates. Wheaton *et al.* (1990) reported that use of progestogens in conjunction with sudden introduction of rams and without use of PMSG, were easier and less costly, but our results showed that concomitant use of CIDR and PMSG have improved the reproductive indices. Knights *et al.* (2001) used a device similar to CIDR, containing 0.82 g of P4 and an injection of FSH as a follicular promoting agent at the time of removal of the device and obtained higher ovulation and twinning rates. In our experiment, we obtained similar results with lower P4 concentrations in the device.

## CONCLUSION

Raising sheep in Iran is subjected to traditional rules and environmental conditions including; annual precipitation, pasture conditions, summer/winter moving of the tribes, price of the feed and other cost bearing factors. If more sophisticated methods of reproduction are to be used by the farmers, sheep are required to be merely hand fed, instead of the present method of pasturing along with feeding concentrates (mostly barley). Reasonably because lambs may be born in unfavorable seasons as far as pasture is concerned. Moreover, with higher prices of meat and the fact that in beef production, use of pasture is not a factor in establishing the price of beef in Iran, it seems that feeding sheep in the feed lot is economical because this method has been accepted and successfully applied in beef production. It can be concluded that change in the reproduction and feeding pattern in Iranian sheep industry, although it is contradictory to the traditional methods, might be economical and can be used as a solution for the overgrazing of the drought affected pastures of Iran. Application of the protocols used in this experiment is helpful to synchronize ovulation and estrus as well as increasing twinning and lambing rate in ewes out of breeding season.

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