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Oestrus Synchronization with Ovuprost® and Prostenol® in the Indigenous Ewes of Bangladesh

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ABSTRACT

The present study was designed to observe the oestrus responses in the indigenous ewe induced by cloprostenol sodium manufactured by two different companies (Ovuprost®, BOMAC, Newzealandand Prostenol®, Techno, Bangladesh). Twelve local ewes were divided into 3 groups (n= 8). The ewes in Group I and II were induced by intramuscular injection of 100 µg (0.4 ml) of cloprostenol sodium (Ovuprost® and Prostenol®), respectively. The 2nd injection in each group was given at 9 days apart. The ewes in Group III were kept as control for observing natural oestrus characteristics and comparing the responses with induced oestrus. Hundred and 75% ewes showed oestrus following 2nd injection of Ovuprost® and Prostenol®, respectively. The average time of onset of oestrus following 1st and 2nd injection of Ovuprost® and Prostenol® were 50.5 ± 3.5 vs 48.0 ± 0.0 h and 49.9 ± 1.9 vs 49.5 ± 1.7 h, respectively. There was no significant difference between the two types of cloprostenol sodium group on the onset of oestrus. The average duration of oestrus was 27.5 ± 0.7 vs 27.5 ± 0.0 h and 25.9 ± 3.3 vs 24.2 ± 0.3 h in Ovuprost® and Prostenol® treated ewes, respectively. For natural oestrus, the duration of oestrus was 25.2 ± 3.3 h. There was no significant difference among the cloprostenol sodium produced by two different companies and natural oestrous ewes on the duration of oestrus. The higher percentages of cornified cells were present in induced oestrus (90 and 85%) compared with natural oestrus (80%), although there was no significant difference among them. The pregnancy rates were 75, 66.7 and 100% in Ovuprost®, Prostenol® and natural oestrous ewes, respectively. The above results indicate the suitability of using cloprostenol sodium for synchronization of oestrus in indigenous ewes in Bangladesh.

(Key words: oestrus, synchronization, vaginal cytology, pregnancy rates, ewes, Bangladesh)

INTRODUCTION

Sub-tropical weather in Bangladesh is favorable for sheep breeding. The few numbers of non-descriptive called indigenous sheep are sparsely distributed throughout the country. However, the productivity of this sheep is low due to poor genetic merit (Alam *et al.*, 1989a; Rahman *et al.*, 2005), poor nutrition (Alam *et al.*, 1989b) and management.

Oestrus is usually manipulated to ensure optimum production or convenient for the owner, to facilitate the use of AI under extensive farm condition, to detect oestrus in case of weak oestrus symptoms. Continued research has been conducted to establish optimal doses and agents to use for favorable synchrony of oestrus and fertility (Whitley and Jackson, 2004). Issues involving oestrus synchronization include the fact that new product development and inclusion of sheep in labeling for hormone products used in synchronization has not been so much coincided with the user interest. Many researchers have been conducted for synchronization of oestrus in sheep either by reducing the length of luteal phase of the oestrous cycle with $PGF_{2\alpha}$ or by extending the cycle artificially with exogenous progesterone or more potential progestagens (Evans and Maxwell 1986; Jainudeen *et al.*, 2000). Henderson *et al.* (1984) studied about the effect of double injections of different doses of $PGF_{2\alpha}$ at 9 day intervals along with the administration of progesterone impregnated vaginal sponges. They reported that there were no differences in the lambing rates of ewes treated with different doses of $PGF_{2\alpha}$. Beck *et al.* (1987) reported that

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the oestrous rates during 2 consecutive breeding seasons were greater in ewes treated with double injections of $PGF_{2\alpha}$ at 11 days intervals than in those treated with a single injection only. In a subsequent study, they reported that there was no difference between the lambing rates of ewes injected with 125 μg of cloprostenol at 11 day intervals and in the lambing rates of those injected with a combination of $PGF_{2\alpha}$ and GnRH (Beck *et al.*, 1996). The grazing land in Bangladesh is becoming less. Ultimately in future we have to depend on farming system where synchronization of oestrus is essential for breeding purpose. However, there are few studies involving oestrous synchronization in ewes in Bangladesh. Therefore, the present study was designed to compare the effectiveness of cloprostenol, produced by two different companies (home and abroad). Finally their effects were also compared with the natural oestrus for improved productive adaptation in the breeding purposes.

MATERIALS AND METHODS

1. Animals and Management

A total of 24 indigenous ewes, 2～3 years old and 15～20 kg body weight were used for this study. A well ventilated house containing concrete-floor, tin roofed shed and brick made wall was provided to the ewes. The ewes had the access for free outside movement during daylong for grazing and social interactions to each other. They were maintained with pasture grazing and concentrate mixture.

2. Synchronization of Oestrus

The ewes were randomly divided into 3 groups. Each group consisted of 4 ewes. Synthetic cloprostenol produced by two different companies (home and abroad) were selected for inducing oestrous ewes in this study. They were namely, Ovuprost®; BOMAC, Newzealand and Prostenol®; Techno, Bangladesh. The ewes in Group I (Ovuprost®) and II (Prostenol®) were induced by intramuscular injection of 100 μg (0.4 ml) of cloprostenol sodium, respectively. The first injection was given ignoring the stage of the oestrus cycle. After 9 days the same doses were injected to all ewes of Group I and Group II, regardless of oestrus. The ewes in Group III were selected as natural oestrus group.

3. Detection of Oestrus, Onset, Duration and Intensity of Oestrous Signs

The ewes were observed closely for detecting the signs of oestrus at 4 hours interval after Day 1 of injection in treated group. The oestrous ewes were detected by using teasur ram for inducing male effect. Ewes were inspected for 4 hours intervals after 1 day of injection. The onset and sings of oestrus were recorded on the basis of clinical signs and symptoms. Duration of oestrus was calculated from the time of onset of oestrus to the end of oestrus as rejection of female to the male. Detected oestrus was also confirmed by observing the cornified vaginal epithelial cells. In natural oestrus group, the day of onset of oestrus was suspected from counting the end of previous cycle. The oestrus was detected in the same process as did for induced groups.

4. Measurement of Vaginal Cytology during Oestrus

During oestrus vaginal fluid was collected from ewes in all groups to determine the characteristic of vaginal epithelial cells. The fluid was placed in the marked slides and the types of epithelial cells were observed under microscope at the 10X power. The percentage of cornified epithelial cells were counted under microscope at the 10x and compared among two induced cloprostenol drug and natural oestrus.

5. Natural Service

Natural service was given about 12 hours after the onset of oestrus by using a proven ram.

6. Pregnancy Rate

Pregnancy was determined by ultra-sound scanning on 50 days after service and the rate was calculated.

7. Statistical Analysis

The data was entered in the Excel sheet. Results were calculated as mean ± standard deviation (S.D) by using SPSS software (SPSS® version 16.0). Comparison between two induced drugs of cloprostenol on the percentage of ewes showed oestrus, time of onset of oestrus, duration of oestrus were determined by using student *t*-test. Comparison among two induced oestrus and natural oestrus on the percentage of ewes showed oestrus, duration of oestrus and pregnancy rates were determined by using one way ANOVA.

RESULTS

1. Time of Onset of Oestrus and Duration of Oestrus

The effects of Ovuprost® and Prostenol® on time of onset and duration of oestrus in ewes are shown in Table 1. The percent of ewes showed oestrus following 1st injection of Ovuprost® and Prostenol® were 50 and 25%, respectively (Table 1). However, the percentage was 100 and 75% following 2nd injection (Table 1). The range of time of onset of oestrus in both groups of ewes were similar (45～54 and 45～50 h, respectively). The mean time of onset of oestrus following 1st and 2nd injection of Ovuprost® and Prostenol® were 50.5 ± 3.5 vs 48.0 ± 0.0 h and 49.9 ± 1.9 vs 49.5 ± 1.7 h, respectively (Table 1). There was no significant difference between the two types of cloprostenol on the onset of oestrus.

Like the onset of oestrus, the range of duration of oestrus in ewes was similar in both types of cloprostenol group (24～33 and 24～30 h). The mean durations of oestrus were 27.5 ± 0.7 vs 27.5 ± 0.0 h and 25.9 ± 3.3 vs 24.2 ± 0.3 h, respectively (Table 1). There was no significant difference between two cloprotenol groups produced by two different companies.

The comparison of duration of oestrus is shown in Table 2. The duration of natural oestrus varied from 24～36 hrs. There was no significant difference between the induced and natural oestrus on the duration of time.

2. Oestrous Signs and Its Intensity

The signs of oestrus and its comparison among natural and induced oestrous ewes are shown in Table 3. The most specific signs of oestrus in all groups were, stand to be mounted, head turning towards ram and sniffing of vulva by the ram, sniffing of scrotum by the ewe. They wagged their tails vigorously and urinate frequently. Reddening of the vulva and vaginal discharges were observed in most of the ewes in all groups (Fig. 5). However, signs in induced oestrous ewes were more severe in intensity compared with natural oestrous ewes.

Table 2. Comparison of duration of oestrus among natural and induced oestrus

Type of oestrus	Duration of oestus (mean± SD) in hours	Level of significant
Ovoprost® (n=8)	26.4 ± 2.7	NS
Prostenol® (n= 8)	25.0 ± 1.7	NS
Natural oestrus (n=8)	25.2 ± 3.3	NS

NS = not significant.

3. Vaginal Cytology during Oestrus

The comparison of vaginal cytology during oestrus has been shown in Table 4. Vaginal cytology was observed in all induced and natural heated ewes. Cornified epithelial cells were observed in the vaginal smear of all groups. Oestrus was dominated by the cornified epithelial cells compared with nucleated or white blood cells. The higher percentages of cornified cells were present in induced oestrus compared with natural oestrus. However, there was no significant difference.

4. Pregnancy Rates

The pregnancy rates following service in induced and natural oestrus has been shown in Table 5. The pregnancy rates were 100% following natural oestrus. However, it was 75 and 66.7% following insemination in oestrus induced by Ovuprost® and Prostenol®, respectively. However, there was no significant difference among the natural and induced oestrus on the pregnancy rates.

Table 1. Effect of different types of cloprostenol on induction and duration of oestrus

Types of cloprostenol sodium	No. and percentage of ewes came in oestrus (response)		Time of onset of oestrus (Mean ± SD) (hours)		Duration of oestrus (Mean ± SD) (hours)	
	1st injection	2nd injection	1st injection	2nd injection	1st injection	2nd injection
Ovuprost® (100 µg) (n= 8)	2(50)	4(100)	50.5 ± 3.5	49.9 ± 1.9	27.5 ± 0.7	25.9 ± 3.3
Prostenol® (100 µg) (n= 8)	1(25)	3(75)	48.0 ± 0.0	49.5 ± 1.7	27.5 ± 0.0	24.2 ± 0.3
Level of sign.	NS	NS	NS	NS	NS	NS

NS = not significant.

Table 3. Comparison among natural and induced oestrous signs measured by its intensity

Oestrous signs	Intensity of oestrous signs		
	Ovoprost® (n=8)	Prostenol® (n=8)	Natural oestrus (n=8)
The female stands and allows the male to mount	****	****	****
Affinity to stay with male	****	****	***
Head turning towards ram and sniffing of vulva by the ram	***	***	**
Sniffing of scrotum by ewes	***	***	**
They wagged their tails vigorously and urinate frequently	****	***	*
Swelling and reddening of the vulva	***	***	**
Vaginal discharges	**	*	*

Number of *(1～4) indicates the intensity of the oestrous sign.

Table 4. Comparison of vaginal cytology among natural and induced oestrus

Type of oestrus	Total no. of cells	Cornified cell	Nucleated cell
Natural oestrus (n=8)	100	80%	20%
Ovoprost® (n=8)	100	90%	10%
Prostenol® (n=8)	100	85%	15%
Level of significant		NS	NS

NS = not significant.

Table 5. Comparison among types of oestrus on pregnancy rates after service

Treatment	Served following induced oestrus	Ultrason-graphic scanning	Pregnancy rate (%)
Natural	8	8	100
Ovuprost® 100 μg	8	6	75
Prostenol® 100 μg	6	4	66.7
Level of significant			NS

NS = not significant.

Discussion

Oestrus detection in indigenous ewes is very difficult because of their weak oestrus signs. So, oestrus synchronization is necessary for controlled breeding in ewes. In Bangladesh, it is more difficult to detect oestrus accurately in ewes as they are reared on traditional breeding system. For successful breeding management oestrus synchronization is essential in indigenous ewes.

The objectives of present study were to observe the effects of Ovuprost® and Prostenol® on percentage of ewes come in oestrus, time of onset and duration of oestrus, vaginal cytology during oestrus and pregnancy rates following insemination in induced oestrus.

Prostaglandin creates hypoxia within active sensitive corpus luteum and regresses it. It also interferes the binding of LH hormone in the cell surface receptors and thus induce oestrus in the synchronized animals (Nett *et al.*, 1976). The success of oestrus results and conception can be affected by the quality and dose of synthetic product of prostaglandin and time of injection (Virakul *et al.*, 1981; Jindal *et al.*, 1990).

In the present study, cloprostenol sodium of two different companies in abroad and home (Ovuprost® & Prostenol®) were used to observe their efficacy in oestrus responses and pregnancy rates in local ewes. In the present study, higher percentages of ewes showed heat following induction with Ovuprost® compared with prostenol. Ovuprost® is the product of newzealand and Prostenol® is the product of home country. The fineness in the production of this prostaglandin in abroad may be the explanation for this difference in oestrus response between the two products. Seventy five to 100% local ewes responded by induction with 100 μg cloprostenol sodium. This indicates that 100 μg cloprostenol sodium may be the suitable dose rate for synchronization of oestrus in the indigenous ewes.

The induced ewes showed oestrus within 45～50 h of injection of both Ovuprost® & Prostenol®. This result is similar

to results of Trounson *et al.* (1975) where 84% of ewes were detected in oestrus 29～48 hours later of injection of cloprostenol. The mean time of onset of oestrus after injection of 1st and 2nd injection of 100 μg Ovuprost® & Prostenol® were 50.5 ± 3.5 vs 48.0 ± 0.0 h and 49.9 ± 1.9 vs 49.5 ± 1.7 h, respectively. There was no significant (p>0.05) difference on the mean time of onset of oestrus following injection of Ovuprost® and Prostenol®. Similar mean time of onset of oestrus was also observed in the work of Nuti *et al.* (1992) in which the mean time from injection to behavioral oestrus was 46 to 48 hrs. A slight difference was observed in the work of Perera *et al.* (1978) who reported that single and double injection of cloprostenol sodium (125 μg) IM at 10 days interval showed 84% of ewes in oestrus within 32 hrs. This could be due to the differences of breed and place of work (environment).

The range of duration of oestrus was 24～30 vs 24～33 h following induction with Ovuprost® and Prostenol®. Following 1st and 2nd injection of cloprostenol sodium (100 μg Ovuprost® and Prostenol®), the duration of oestrus were 27.5 ± 0.7 hrs. vs 27.5 ± 0.0 hrs. and 25.9 ± 3.3 vs 24.2 ± 0.3h, respectively. Similar, duration of oestrus was also observed during natural oestrus 25.15 ± 3.25h. The results showed some difference with Perera *et al.* (1978) who reported the duration of oestrus 20 hrs. on average. The quality of synthetic prostaglandins, dose, individual response or frequency of observation may have an effect on the differences in response between the experiments. However, the ewes used for this experiment were indigenous type and numbers of ewes used were of very small size. Therefore, a large number of ewes are required to establish a definite conclusion.

The range of duration of natural oestrus was 24～36 h. The average duration was 26.4 ± 2.7, 25.0 ± 1.7 and 25.2 ± 3.3 hrs. in Ovuprost®, Prostenol® and natural oestrus group, respectively. There was no significant (p>0.05) difference on the duration of oestrus among the groups. The results showed some difference with Perera *et al.* (1978) who reported the duration of oestrus 20 hrs. on average.

Normally ewes do not exhibit heat symptoms unless the presence of rams. In this experiment, ewes were introduced to the vasectomized ram 24 hrs. after injection of both types of cloprostenol. The most specific signs of oestrus in all groups were, stand to be mounted, head turning towards ram and sniffing of vulva by the ram, sniffing of scrotum by ewes. They wagged their tails vigorously and urinate frequently. Reddening of the valva and vaginal discharges were observed in most of the ewes in all groups. These symptoms were similar to other breeds of sheep observed by other scientist (Dalton, 2008; Susan, 2012). Intensity of oestrus signs were more prominent in indused oestrus than natural oestrus, in respect of the female stands and allows the male to mount, affinity to stay with male, head turning towards ram and They wagged their tails vigorously and urinate frequently. These symptoms were similar to other breeds of sheep observed by other scientist (Dalton, 2008; Susan, 2012). This more intensity of oestrus in induced groups compared with natural oestrus could be due to increase concentration of oestrogen activity, induced with regression of corpus luteum by cloprostenol.

In the present study of cornified vaginal epithelium cells were dominant (80～90%) in the vaginal flushing fluid. This cornified epithelium cells could be due to the effect of high concentration of estrogen level during oestrus as observed in other species (Bishnoi *et al.*, 1980). The higher number of cornified epithelial cells were present in the Ovuprost® induced group compared with Prostenol® group. Again this indicates the effectiveness of abroad product compared with home product of prostaglandin. The higher percentages of cornified cells were present in induced oestrus compared with natural oestrus. This indicates the severity of action and or concentration of induced oestrogen hormone followed by regression of CL compared with oestrogen which could be produced for natural oestrus.

In the present study, there was no significant difference on pregnancy rates (100, 75 and 66.7%, respectively) following insemination in natural and induced oestrus, although the rate higher in natural oestrus. Risvanli *et al.* (2010) achieved a pregnancy rate of 70% in ewes which had received a single dose of cloprostenol sodium. This rate of pregnancy was higher compared with some other published works using other products of $PGF_{2\alpha}$ (Üleri, 1985; Tekeli *et al.*, 1997; Ozt.rkler *et al.*, 2003) or same product as cloprostenol (Horoz *et al.*, 1997; Gonzalez *et al.*, 2005). The pregnancy rates following insemination in the naural oestrus was higher compared with induced oestrus. This result is similar with other published work (Üleri, 1985; Horoz *et al.*, 1997; Tekeli *et al.*, 1997; Ozt.rkler *et al.*, 2003; Gonzalez *et al.*, 2005). The high oestrugenic effects on the mucus secretion and genital tract contraction following induction with cloprostenol may compromise the sperm transport through cervix, resulting in lower fertilization rates and hence pregnancy rates (Üleri, 1985; Horoz *et al.*, 1997; Tekeli *et al.*,

1997; Ozt.rkler *et al.*, 2003; Gonzalez *et al.*, 2005). The present work was conducted on small number of ewes. Further work is continuing in the same Dept. to establish the present results (oestrus response and pregnancy rates in controlled breeding programme) in local ewes.

CONCLUSIONS

The results of the present study lead to the conclusions that 100 µg cloprostenol (Ovuprost® and Prostenol®), may be suitable for oestrus synchronization in indigenous ewes in respect of percent of ewes heat, time of onset, duration of oestrus, vaginal cytology during oestrus and pregnancy rates. The Ovuprost® may be more effective compared with Prostenol® for in duction of oestrus in indigenous ewes. Further work is going on in the same laboratory to establish the present results involving induction of oestrus using cloprostenol sodium.

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