Comparison of Estrus Synchronization by $PGF_2\alpha$ and Progestagen Sponge with PMSG in Indigenous Ewes in Bangladesh

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***Abstract*- The objective of this study was to compare the efficacy of two different synchronizing agents with different doses in indigenous ewes in Bangladesh. Indigenous ewes (n=65) were allocated initially into four groups and treated with intravaginal sponges containing two doses of Flurogestone Acetate (FGA 30 and 45 mg) for 12 days and two doses of $PGF_2\alpha$ injection (Cloprostenol 100 and 175 µg) at 9 days apart. Ewes in estrus were identified using vasectomized rams. An investigation was made on vaginal smear in indigenous ewes. Fifty days after estrus and natural mating, pregnancy was determined by trans-cutaneous ultrasonography.. All the indigenous ewes used in four treatment groups exhibited signs of estrus during the observation period. Among sexual behaviors, firm standing, head-turning and soliciting were displayed more frequently. Ewes in Group I and Group II started to express receptive behavior between 48 h to70 h and in Group III and Group IV between 18-36 h following sponge removal. The time to estrus was slightly longer (58.6 ±2.64 h) in the groups treated with 175 µg Cloprostenol compared to the other groups (55.8 ±3.12, 32.9±1.60 and 30.8±2.04 h).There was significant differences (P<0.001) in terms of time to the onset between ewes treated with the two doses Cloprostenol and FGA intravaginal sponges. Using of two doses of FGA sponges may accelerate the estrus induction after sponge withdrawal. Highest number of the ewes treated with 30 and 45 mg FGA sponges showed estrus signs within 18-36 hours of sponge withdrawal. Maximum number of ewes treated with two doses of Cloprostenol (100 and 175 µg) showed estrus response within 49-60 hours of second injections. The highest percentages (68.8%) of ewes showed oestrus between 25-36h treated with 30 and 45 mg**

FGA compared with other range of time. Similarly, 52.9 and 62.5% of ewes in Cloprostenol (100 and175 µg, respectively) showed oestrus during 49 to 60 h. There was no significant difference in ewes of all treated groups (P>0.05). The estrus period was shown the predominance of superficial cells that was keratinized, largely anucleate, and had angular, folded cell margins or with pyknotic nuclei (Figures 5-6) in the vaginal smear of ewes in all the treated groups. The higher pregnancy rates observed in the 30 mg FGA based Group III (100%) compared with 100 µg Cloprostenol (88.2 %), 175 µg Cloprostenol (75%) and 45 mg FGA (93.8 %) group. It was concluded that though FGA sponge protocol presented superior results, $PGF_2\alpha$ protocol was as efficient in synchronizing estrus as the former in indigenous ewes in Bangladesh.

Keywords: Estrus response, vaginal cytology, Pregnancy rate, FGA, Cloprostenol, Ewes.

Introduction

Sheep industry is a growing activity, pointing to an increase in the number of animals in Bangladesh [1]. In livestock activity, this species is used for breeding, production and consumption, mainly because the meat of young animals has been offered and crossbreeding provides tastier meat. The breeds found in Bangladesh is easily adapted to any breeding system and pasture, is worth noting. It also presents a good carcass conformation and stands out as being very fertile, prolific, precocious, rustic and resistant to gastrointestinal parasites. The adaptability of sheep is an important feature in a country like Bangladesh. It is noteworthy that the local environmental conditions are one of the most important factors for the success of an economically viable farming system. Therefore, it becomes important to study in Bangladesh, with the purpose of obtaining a better understanding of these animal's performance in a tropical climate region, with rainy and humid summer and dry and cold winter, during which the grazing is drastically reduced.

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Reproductive management is the major problems that face the farmer which is necessitates looking for reproductive techniques and decreases the cost of production.

Estrous synchronization is a valuable management tool that has been successfully employed to enhance reproductive efficiency, particularly in ruminants [2] and can be improving sheep production systems by nutritional and\or hormonal treatments resulting in higher estrus responses and subsequent conception rates [3]. The use of estrus synchronization for small ruminant livestock production in Bangladesh is still predominantly under investigation and there is no information on estrus synchronization in sheep using different progestagen and Cloprostenol. Information regarding estrus synchronization efficiency and fertility in indigenous breed of sheep induced by hormonal treatment (progesterone and prostaglandin) will be useful in the design of an intensive and cost-effective breeding programme. The general hormonal technique for estrous synchronization in ewes is the use of progesterone, intravaginal sponge 30-45 mg of flurogestone acetate (FGA) for 11-18 days or 50-60 mg of medroxy progesterone acetate (MAP) for 15-18 days, or by using subcutaneous ear implants with a dose rate of 2-6 mg of progesterone for 9-17 days [4, 5]. As an alternative to estrus synchronization, a protocol based on prostaglandin F2a and its analogues (two injections administered 9- 11 days apart) can be used [6] and reduce cost with other managements. The objective of the present study was to compare the effectiveness of two different doses of Cloprostenol and intravaginal progestagen (Flurogestone Acetate, FGA) on estrus response and pregnancy rates in indigenous ewes in Bangladesh.

Materials and Methods

Animals and Location

The study was carried out between Januarys to December, 2011at Department of Surgery and Obstetrics, Bangladesh Agricultural University, located in Mymensingh, latitude N 24.73 and longitude, E 90.44 and elevation 9m above sea level. The average ambient temperature during experiment was 16.46 to 29.13° C and annual rainfall in this region was 174 mm, with an erratic distribution throughout the year. A total of 65 clinically healthy, 2- 3 years old and weighing between 15 to 19 kg with good body conditions (BCS: 2.00 to 5.00) indigenous ewes were used in this experiment together with 2 vasectomized ram. Indigenous experienced rams were used for natural service. The ewes were allowed to graze on natural pasture and were kept in pens overnight. Water and a mineral salt lick were provided *ad libitum.* In addition, each ewes received 300g concentrate mixture (25% Crushed Maize, 50% Wheat Bran, 20% Soyabean meal, 1% Fish meal, 2% DCP Powder,1.5% salt and 0.5% vitamin- mineral premix) daily for the entire duration of the study.

Experimental Design

Sixty five(65) adult indigenous ewes were randomly divided into four groups were synchronized by either 100µg and 175 µg Cloprostenol (Ovoprost, Bomac Laboratiries Ltd, Auckland),injecting 9 days apart in Group I (n=17) and II (n=16) or by inserting intravaginal sponges impregnated with 30 mg of FGA (Ova-Gest®, Bioniche, Australia) and 45 mg of FGA(Syncrite-45, Animal Health Supplies, Australia) for 12 days respectively for Groups III and IV (n=16). The ewes in Group III and IV were injected intramuscularly with 300IU PMSG (Intervet, The Netherlands) at the time of sponge removal from the vagina on day 12. The second injection of Cloprostenol Group I and II coincides with the injection of 300 IU PMSG at the time of sponge removal in Groups III and IV.

Estrus detection, natural service and pregnancy diagnosis

The ewes were observed twice a day (7:00 a.m. and 07:00 p.m.) for a period of sixty minutes in each observation from 12 to 66 h following sponge removal and 24hrs after Cloprostenol injection with the aid of teaser rams. Estrus was detected by observing the signs of behavioral estrus and vaginal exfoliative cytology. Each ewe was served by natural breeding twice at fixed time intervals. All ewes were tested for pregnancy 50 days following natural service with the aid of a trans-abdominal ultrasonic scanning apparatus.

Collection of vaginal smear and cytology

Vaginal smear was collected from the ewes with the aid of vaginal swabs which consisted of clean, soft and gentle pure cotton buds. The vulva and perineum were rinsed with clean water and gently wiped with a clean towel. Each ewe was well restrained in standing position by an assistant and the swab was gently inserted into the anterior vaginal with the right hand while the left thumb and fore-finger were used to expose the vulva lips. At the anterior vagina, the swab was gently and briskly rolled against the vaginal mucosa and carefully withdrawn. The swab was immediately smeared on a warm gland slide, air dried, and immediately fixed with 100% ethanol. The smears were stained with stained with Leishman stain [7]. The cells encountered in the vaginal smear were categorized as percentage of superficial, parabasal, intermediate and leucocytes. Twenty five cells were

counted from five fields of each slide and the percentage of each cell type was recorded.

Response, time of onset and duration of estrus
Estrus onset was defined as the time elapsed between sponge removal and the middle of the time interval between the last rejection to be mounted and the first tolerance [8; 9]. Estrus duration was defined as the time interval between the onset of estrus and when a ewe no longer stood to be mounted [10]. A ewe was considered to have responded to the treatment when she showed estrus during the observation periods [8].

Data and statistical analyses
The onset of estrous and duration of induced estrous periods were subjected to analyses of variance (one-way ANOVA) and the differences between means were tested for significance with the Tukey HSD. The percentages of pregnancy and exfoliative vaginal cells were compared by Chi square-test. The 95% significance level was noted. SPSS Statistics 17.0 software was used for statistical analyses.

Results

Response to Estrus induction, Onset of Estrus and Duration of Estrus
The percent of ewes response to estrus induction, time of onset of estrus following the four methods of treatment, and duration of estrus is shown in Table 1 and a summary of the distribution of animals showing estrus is set out in Table2. All the indigenous ewes used in four treatment groups exhibited signs of estrus during the observation period. The time to estrus was slightly longer (58.6 ±2.64 h) in the groups treated with 175 µg Cloprostenol compared to the other groups (55.8 ±3.12, 32.9±1.60 and 30.8±2.04 h).There was significant differences ($P<0.001$) in terms of time to the onset between ewes treated with the two doses Cloprostenol and FGA intravaginal sponges. Absence of significant differences in terms of time to the onset between ewes treated with the two different doses of FGA sponges demonstrate a similar efficiency in inducing estrus of indigenous ewes.
The distribution of ewes exhibiting oestrus following synchronization treatment for two doses of Cloprostenol (100 and175 µg) and FGA (30 mg and 45 mg) were shown in Figure 1. As mentioned in the Table 1, it is found that using of two doses of FGA sponges may accelerate the estrus induction after sponge withdrawal. Highest number of the ewes treated with 30 and 45 mg FGA sponges showed estrus signs within 18-36 hours of sponge withdrawal. Maximum number of ewes treated with two doses of Cloprostenol (100 and 175 µg) showed estrus response within 49-60 hours of second injections. The highest percentages (68.8%) of ewes showed oestrus between 25-36h treated with 30 and 45 mg FGA compared with other range of time. Similarly, 52.9 and 62.5% of ewes in Cloprostenol (100 and175 µg, respectively) showed oestrus during 49 to 60 h.

The duration of estrus was 30.5±0.66, 31.6±0.80, 33.1±1.39and 32.94±1.0 hour in two doses of Cloprostenol (100 and 175 µg) and 30 and 45 mg FGA, respectively. There was no significant difference in ewes of all treated groups ($P>0.05$).

Vaginal Cytology
The slides on vaginal cytology (Figs.3 A-F) shows variation in percentages of epithelial cells, presence or absence and degree of clumping of epithelial cells as well as presence of sperm cells observed during estrus. The observations found in the vaginal cytology during the study are presented in Table 4. The estrus period was shown the predominance of superficial cells that was keratinized, largely anucleate, and had angular, folded cell margins or with pyknotic nuclei (Fig. 3) clumping with each other in the vaginal smear of ewes in all the treated groups. Table 4 shows that there was no difference ($P>0.05$) in the percentage of epithelial vaginal cells from the vaginal smears obtained for each treatment group. However, there were highest percentages of intermediate cell found in Cloprostenol treated groups. Large number of spermatozoa was detected in all smears (Fig. 3 E) after induction of a synchronized estrus improves the accuracy of predicting ewes for mating. Concerning blood cells, very few leukocytes were found in some smears of 45 mg FGA treated groups. All vaginal swabs in FGA treated groups showed bacterial growth. Accumulation of endogenous bacteria was found distributed over the keratinized sheets of the cells (Fig. 3 F).

Signs of Estrus:
Standing to be mounted was the cardinal sign used to determine estrus response. Each ewe was considered to be in estrus when she was directly observed to accept a mount from the ram. During the observations, soliciting, sniffing scrotum, head-turning, anogenital sniffing, squatting and tail-fanning were also recorded as proceptive and standing to be mounted as receptive behavior. Swelling and redness of vulva was also remarked during observation. In the current study, ewes in all groups came on heat during the experimental period. The frequency of proceptive behavior and the number of ewes displaying proceptive behavior were highest ($P<0.01$) for FGA treated groups. Ewes in Group I and Group II started to express receptive behavior between 48 h to70 h and

in Group III and Group IV between 18-36 h following sponge removal (Table 3).

Pregnancy rate

The higher pregnancy rates observed in the 30 mg FGA based Group III (100%) compared with 100 µg Cloprostenol (88.2 %), 175 µg Cloprostenol (75%) and 45 mg FGA(93.8 %) group (Table 1).But there was no significant (P>0.05) differences among the treatment groups.

Discussion

The estrus response rates in ewes subjected to PMSG supplementation along with vaginal sponge, were in line with the studies of references [11; 12] in which the researchers used variable doses of PMSG and progesterone and different kinds of progesterone preparations in vaginal sponges. The percentage of ewes exhibiting estrus in this trial was higher from the another literature, while kor et al. [13] reported 92.89% estrus response using 30mg FGA. Öztürkler et al. [14] found 100% estrus response after double PGF2α (9 days interval), which shows agreement with the present result. Researchers have also tried to determine the optimum dose of cloprostenol for estrous synchronization. Greyling and Van der Westhuysen [15] found that with 125 µg doses of cloprostenol, only 80% of their ewes came into estrus, as compared with 100% at the 250 µg dose level or 84% at the 100 µg dose level [16]. Variation in estrus response rate might be due to differences in season, breed of sheep, type of progesterone and device [11], use of PMSG, time of PMSG injection [17], presence of ram in the herd [18], body condition and management system [19], nutritional condition, latitude [11] and length of progesterone treatment [20]. PMSG was reported to increase synchrony of estrus and ovulation with more predictability and precision [21] in breeding and nonbreeding seasons [22].

In this study the time to the oestrus onset following the withdrawal of sponge in 30 and 45 mg FGA treated group was found to be 32.9±1.60 and 30.8±2.04 h, which was lower than 40 h found [23; 24; 25] and similar to others [13]. The time of onset of estrus using two doses of Cloprostenol, 100 and 175µg, was 57.7±3.94 and 60.2±3.04h respectively and showed similarity with the findings of GREYLING and VAN DER WESTHUYSEN [26] in ewes. These differences may be explained by variation in breed, lactation, nutrition, season, use of gonadotropins and presence of males after progestagen removal [27]. The distribution of estrous in our groups was similar to that reported by Zarkawi et al. [28] and Ungerfeld and Rubianes [9].

The durations of oestrus with both the FGA protocols showed agreement with previous studies [2; 29; 30] who reported that the duration of estrus were between 18 and 72 hours with an average around 36 hours. However, the durations of oestrus observed (30.5-33.1 h) were similar to the 34.9 h in Awassi ewes [20], but longer than the 18.7 h in Dorper ewes [29]. Differences might be related mainly to the season as well as to other co-factors (e.g. protocol, feeding, etc.).

The cytology of vaginal exfoliation had been described in the sheep [31; 32]. The cytological picture of vaginal smears was greatly affected by ovarian hormones [33]. Under the influence of estrogens, the epithelial cells synthesize and accumulate glycogen as migrate toward the surface [34]. The oestrus was marked by keratinization of the squamous epithelium [35; 36]. The rate of superficial cells recorded on estrus day was similar with those obtained on mouse by Harold [37], on sow by Rodgers et al. [35; 38] and on boar by Mayor et al. [39], which ranged from 60 to 90% on estrus day. All vaginal swabs in FGA treated groups showed bacterial growth distributed over the keratinized sheets of the cells [40]. It has been reported that presence of a foreign body, such as sponge in the vagina stimulated bacterial growth and local mucous secretion during sponge treatment and these changes create a localized inflammation [41; 42].

Similar estrus signs of this study were also reported for Préalpes [43] and Merinos [44, 45]. On the other hand, BRADLEY IMWALLE and KATZ [46] suggested that tail wagging is the primary proceptive behavior expressed by female goats in estrus. Similarly, CARATY et al. [47] reported that, Ile de France ewes started to mate at 24 h following synchronization, and the mating started to decrease after 44 h and the ewes did not mate any more after 56 h. Similar to the current study, GELEZ et al. [43], who studied with Préalpes ewes, reported that all the ewes displayed receptive behavior on day 1 of estrus.

Previous studies demonstrated that the percentage of pregnancy was 80 and 75% for treatment with FGA with PMSG and Cloprostenol, respectively [26], but in this study pregnancy rates were higher because natural mating is used which is more efficient than artificial insemination. Ustuner et al. [20] found the pregnancy rates vary (20-80%) after different progestagens treatment following natural mating.

Conclusions

Both Cloprostenol and FGA sponges were equally effective for estrous synchronization in indigenous ewes in Bangladesh. Based on the results of the experiments, it can be concluded that though FGA protocol presented superior results, $PGF_2\alpha$ protocol was as efficient in synchronizing estrus as the former. However, more research on the tested parameters is required especially when dealing with larger herds of ewes.

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Table 1: Estrus response and pregnancy rate of indigenous ewes following estrus synchronization using two doses of FGA and Cloprostenol.

Groups	No. of ewes	Estrus response rate, %(n)	Mean±s.e.m time(h) of onset of estrus	Mean±s.e.m duration of estrus(h)	Pregnancy rate ,%(n)
Cloprostenol 100µg	17	100(17)	55.8 ±3.12[a]	30.5±0.66	88.2 (15)
Cloprostenol 175µg	16	100(16)	58.6 ±2.64[a]	31.6±0.804	75 (12)
FGA Sponge 30mg	16	100(16)	32.9±1.60[b]	33.1±1.39	100 (16)
FGA Sponge 45mg	16	100(16)	30.8±2.04[b]	32.94±1.33	93.8 (15)

[a,b] Values with different superscripts in the same column differ significantly at $P < 0.05$.

Table 2. Incidence of ewes marked by the teaser on treatment with PGF2a (two injections of PGF2α nine days apart) or FGA (intravaginal sponge maintained for twelve), in the intervals of estrus observation (percentage)

		Intervals of estrus observation(h)						
Groups	Ewes(n)	18-24	25-36	37-48	49-60	61-72	73-84	85-96
Cloprostenol, 100 µg, %(n/n)	17	0	0	29.4(5)	52.9(9)	5.9(1)	5.9(1)	5.9(1)
Cloprostenol, 175 µg, %(n/n)	16	0	0	6.3(1)	62.5(10)	18.8(3)	12.5(2)	0
FGA, 30 mg, %(n/n)	16	25(4)	68.8(11)	6.3(1)	0	0	0	0
FGA, 45 mg, %(n/n)	16	31.3(5)	68.8(11)	0	0	0	0	0

Table 3: The number (n) of Indigenous ewes expressed proceptive and receptive behaviors after the end of $PGF_2\alpha$ protocols (two injections of $PGF_2\alpha$ nine days apart) and FGA protocols (intravaginal sponge maintained for twelve days).

Hours	Proceptive Behavior				Receptive Behavior			
	Cloprostenol, 100 µg	Cloprostenol, 175 µg	FGA, 30 mg	FGA, 45 mg	Cloprostenol, 100 µg	Cloprostenol, 175 µg	FGA, 30 mg	FGA, 45 mg
0	0	0	0	0	0	0	0	0
12	0	0	4	5	0	0	0	0
18	0	0	4	5	0	0	4	5
24	5	1**	11**	11	0	0	4	5
36	5	1**	11**	11	0	0	11	11
48	9	10	5	4	5	1	1	4
60	5	5	3	2	9	10	0	0
72	1	3	1	0	1	3	0	0
84	1	2	0	0	1	2	0	0
96	1	1	0	0	0	0	0	0

**: According to the results of Chi-Square test differences between the values differ significantly ($P<0.01$)

Table 4. Percentage occurrence(s) of vaginal epithelial cells during induced estrus in the study.

Groups of ewes	Superficial cells (%)	Parabasal cells (%)	Intermediate cells (%)	Leukocytes (%)	Clumping	Presence of sperm cells	Presence of bacteria
Cloprostenol, 100 µg	80.0^{ns}	.0%	20.0 ^{ns}	.0	***	***	*
Cloprostenol, 175 µg	84.0 ^{ns}	.0%	16.0 ^{ns}	.0	***	***	*
FGA, 30 mg,	96.0 ^{ns}	.0%	4.0 ^{ns}	.0	***	***	***
FGA, 45 mg	92.0 ^{ns}	.0%	4.0 ^{ns}	4.0 ^{ns}	***	***	***

ns. not significant (P>0.05)

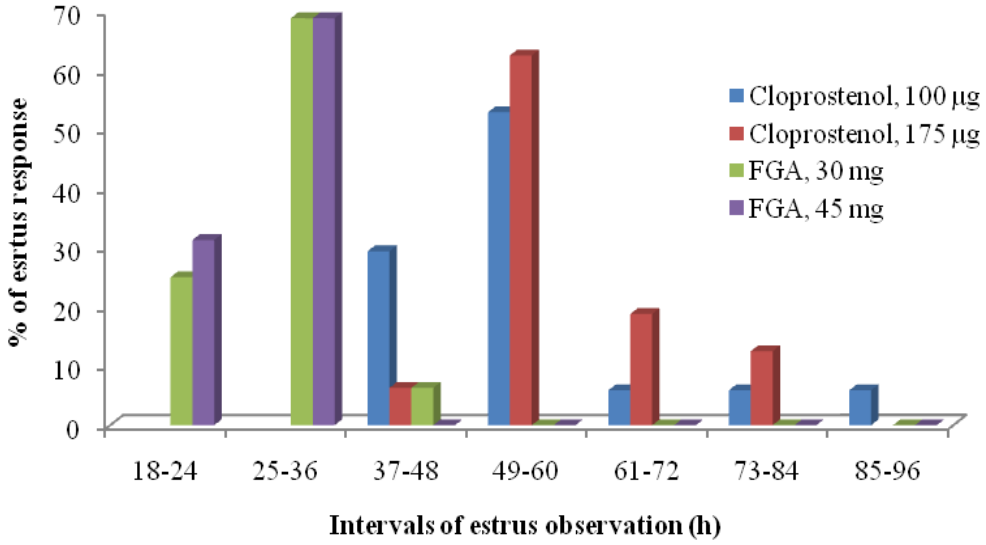

Figure 1: Accumulated data of estrus manifestation in twelve hour observation intervals from the end of $PGF_2\alpha$ protocols (two injections of $PGF_2\alpha$ nine days apart) and FGA protocols (FGA intravaginal sponge maintained for twelve days), showing the grouping of estrus manifestation at each interval until the end of the observation (percentage).

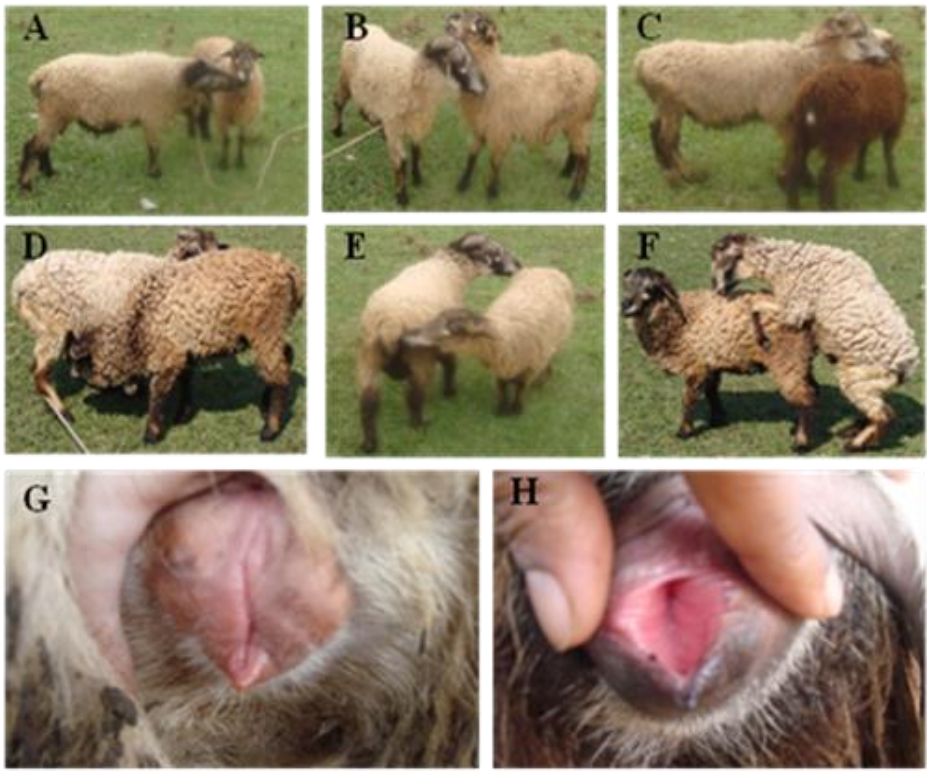

Figure 2: Signs of Estrus observed during study period: Proceptive Behavior: A & B. Soliciting C. Looking-over shoulder D &E. Anogenital and scrotal sniffing F. Receptive Behavior- standing to be mounted G &H. Swelling and redness of the vulva.

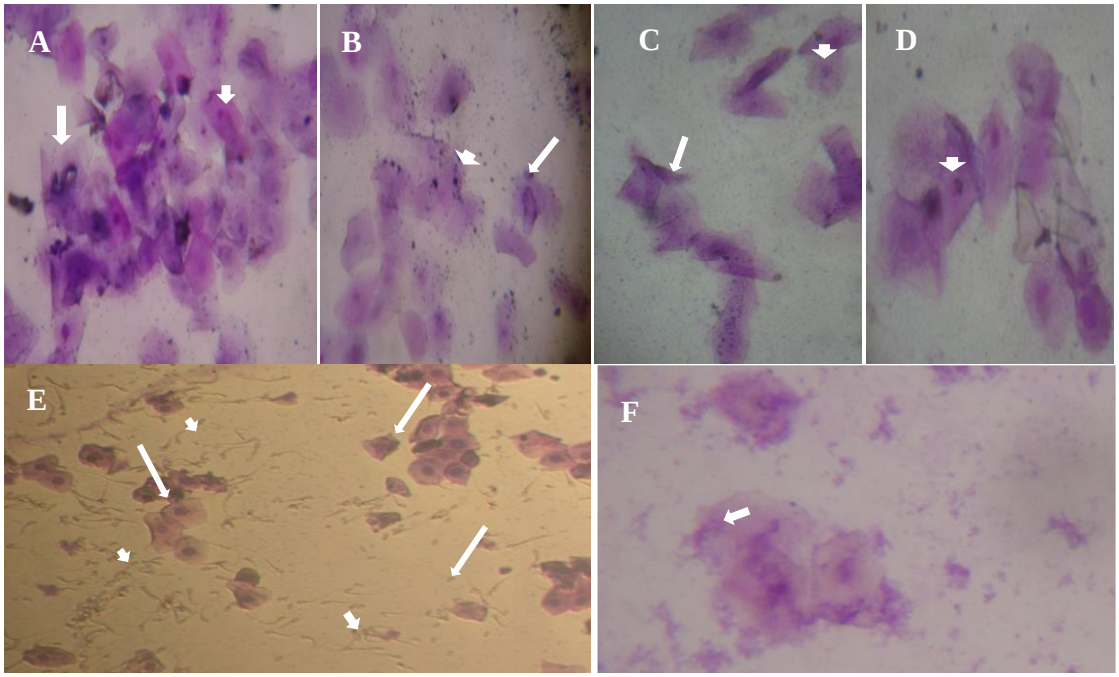

Figure 3: Vaginal smear, Estrus characterized by superficial cells with pyknotic nuclei(arrow head), keratinized (long arrow) that are largely anucleate, and have angular, folded cell margins (Leishman stain) and sheet formation of keratinized vaginal epithelial cells. 24 hours after 30 mg (A) and 45 mg (B) FGA sponge and after 100 µg(C) and 175 µg(D)of Cloprostenol injections. Nnumerous sperm cells(arrow head) mmediately after mating. (E) and keratinized exfoliated apoptotic vaginal cells invaded by endogenous bacteria (F) (arrows). X400 under normal light microscopy.

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