



SHORT COMMUNICATION

Estrus Synchronization and Artificial Insemination in Goats during Low Breeding Season-A Preliminary Study

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ABSTRACT

A pilot project was initiated to introduce artificial insemination (AI) in goats at farmer level with chilled semen. Does (n=18) were synchronized with progesterone impregnated vaginal sponges (60 mg Medroxyprogesterone acetate; MAP) for 11 days. At 48 hrs prior to removal of the sponges, intramuscular injection of 400 IU equine chorionic gonadotropin (eCG) and cloprostenol (0.075 mg) was given. Fixed time vaginal insemination (43-45 hrs after sponge removal) was done twice (at 12 hrs interval) in 17 does with chilled Beetal buck semen (4°C) extended with Tris-citric acid (TCA) or skimmed milk (SM) based extender (75 x 10⁶ sperm/ml). Pregnancy test was performed at 45 days post insemination through ultrasonography. An overall 94.5% (17/18) of does showed heat signs and 78% of them were detected in heat between 12 - 24 hrs after sponge removal. An overall 29.4% (5/17) pregnancy rate was recorded. Higher pregnancy rate (44.4%) was obtained in does inseminated with SM extended semen as compared to 12.5% for TCA extended semen. Results were encouraging in the sense that to the best of our knowledge it was the first report of kidding through AI in heat induced does in Pakistan. Moreover, it indicated the feasibility of using synchronization and fixed time AI during low breeding season to enhance the reproductive efficiency in local goats.

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INTRODUCTION

Adaptability of the goat to wide range of harsh environment (better resistance to environmental temperature, better digestibility of pastures) allows its production in a variety of climatic conditions ranging from cold rain forest to dry desert (Shelton, 1978). Estimated goat population in Pakistan is 53.8 million heads and it is the third largest goat producing country in the world after China and India (GOP, 2006). Goats show distinctive seasonal pattern in reproductive activity in the temperate region. In tropical region, their breeding period is throughout the year and is dependent on latitude, climate, food availability, breed and breeding system (Khan *et al.*, 2008). Generally, the breeding activity of goats in Pakistan can be differentiated into low (May to August) and high (September to December) breeding season. This affects the even distribution of their production over the year, resulting in scarcity of animals on religious and social occasions.

Artificial insemination (AI) in a particular population of goats allows kidding at the desired season of the year. However, goat breeding in Pakistan is through natural mating and semen is not produced for any of the goat breeds for AI. Government livestock farms are a good source of superior males but their contribution in the development of breeds through AI is negligible (Khan *et al.*, 2008). Therefore, a preliminary study was conducted in low breeding season to assess practicability of AI in goats at farmer level.

MATERIALS AND METHODS

Animals

Non-descript, multiparous non-pregnant does (n=18) kept by common farmers around Rawat, District Rawalpindi were selected for synchronization and AI. Prior to start of the experiment, animals were dewormed with Albendazole 10% (Farbenda, Farvet, Italy) and monitored for cyclicity.

Heat synchronization

Heat synchronization treatment was started in the first week of May 2010. Experimental does were treated with progesterone impregnated vaginal sponges (Synchron, 60 mg Medroxyprogesterone acetate, Farvet, Holland) for 11 days, irrespective of estrous cycle phase. At 48 hrs prior to the removal of the sponges, the animals were injected intra muscular (I/M) with 400 IU of equine chorionic gonadotropin (eCG; Synchroject 600, Farvet, Holland) and cloprostenol (0.075 mg Dalmazin, Fatro, Italy). Animals were observed for heat signs (bleating, vigorous tail movement, mucus discharge and restlessness) for 48 hrs after removing sponges.

Extender preparation

Tris-citric acid buffer (TCA; pH 6.8) was prepared by dissolving Tris (hydroxymethyl)-aminomethane (3.03%; w/v) and citric acid (1.66%; w/v) in 74 ml Milli-Q (distilled-deionized) water. Finally, for making the extender, the buffer was supplemented with egg yolk (20%; v/v), fructose (0.2%; w/v) and antibiotics (Streptomycin sulphate 0.1 g/ml and Gentamycin sulphate 0.001 g/ml). Skimmed milk extender was prepared by dissolving 10g skimmed milk (SM) of laboratory grade (Acumedia Manufacturer Inc, USA) in 100 ml Milli-Q water. The extender was heat treated at 96°C for 10 min, cooled to room temperature and supplemented with antibiotics as mentioned above. Extenders were stored at -20°C till further use.

Semen collection and preservation

Semen was collected from one buck with artificial vagina (IMV, France) at 42°C. Semen samples were immediately brought to the laboratory and placed in a water bath at 37°C. Samples were evaluated for volume, percentage of progressively motile spermatozoa (at x400 by phase contrast microscope; Olympus, Japan) and sperm concentration (by a digital photometer at 546 nm; Minitube, Germany). The ejaculate had sperm concentration = 4.965×10^9 sperm/ml, volume 2 ml and 80% motility. Ejaculate was equally divided into two parts and diluted with TCA or SM extender at 37°C to a final concentration of 75×10^6 sperm/ml. Extended semen sample was cooled to 4°C over 2 hrs. Each of the extended semen sample was further divided into 2 aliquots to be used for AI within 24 hrs.

Artificial insemination

Fixed time vaginal AI was performed at 43-45 hrs after the removal of vaginal sponges with hit in dark method in 17 does (due to accidental death of 1 doe). Animals were inseminated twice (12 hrs interval) with 0.5 ml semen extended in TCA or SM. Pregnancy test was performed after 45 days of AI with ultrasonography (SSD 500 with 3.5 MHz probe, Aloka, Japan). Data on pregnancy rate were compared by Chi-square analysis using Minitab 12.22 (Minitab 12.22, 1996).

RESULTS AND DISCUSSION

Data on synchronization and heat incidence are presented in Table 1 indicating that 94.5% (17/18) of the does showed heat signs within 24 hrs. Majority of animals

(78%) were detected in heat between 12-24 hrs after sponge removal. Estrus response to the application of intra vaginal sponges varies greatly and depends on breed, co-treatment, management and mating system (Chemineau *et al.*, 1999). However, the data is almost consistent to the heat induction with vaginal sponges in temperate (Leboeuf *et al.*, 2003) and tropical (Freitas *et al.*, 2004) goat breeds.

Pregnancy test showed a 29.4% pregnancy rate in low breeding season of goat (Table 2). Higher pregnancy rate (44.4%) was obtained in does inseminated with SM extender as compared to TCA based extender (12.5%), but the difference was non-significant. Due to accidental death of 2 does, only 3 does gave birth to 4 live kids on the same day, after 150 days of gestation period. An earlier study showed that semen doses preserved in SM extender provided higher pregnancy rate after intra-cervical insemination compared to those in TCA extender (52% versus 42.9%; Dorado *et al.*, 2007). In France, 65% average pregnancy rate in goats was reported with AI (Leboeuf *et al.*, 2008) and this might be due to the cervical insemination using vaginal speculum.

Table 1: Efficacy of heat synchronization protocol in does during low breeding season

No. treated *	Heat within 6 hrs (%)	Heat within 6-12 hrs (%)	Heat within 12-24 hrs (%)	Overall in heat (%)
18	1 (5.5)	2 (11)	14 (78)	17 (94.5)

* Vaginal sponge from Day 1 – 11, I/M injection eCG and cloprostenol on Day 9

Table 2: Pregnancy rate during low breeding season in does inseminated with Beetal buck semen extended in Tris-citric acid or skimmed milk based extender

Group	No. inseminated *	Pregnancy rate (%)
Tris-citric acid	8	1/8 (12.5)
Skimmed milk	9	4/9 (44.4)
Overall	17	5/17 (29.4)

* First AI at 43-45 hrs after vaginal sponge removal followed by 2nd AI at 12 hrs interval; Values of pregnancy rate differ non-significantly ($\chi^2=1.17$; $P=0.28$).

To the best of our knowledge, kidding with AI in heat synchronized does was done successfully for the first time in Pakistan. The results show that fixed time AI with SM extended semen can be used effectively. However, further work is needed on method of AI (vaginal vs cervical insemination) and synchronization of estrus (hormonal treatment and buck effect) for optimal conception rate in local goats.

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