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Comparative efficacy of three synchronization protocols in anestrous goats (*Capra hircus*)

Muhammad Shahzad[1,*], and Rehana Kausar[1]

[1]Nuclear Institute for Agriculture and Biology, Faisalabad, Punjab, Pakistan

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⊠*Corresponding author:
Muhammad Shahzad
Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.
Email: drmshahzadvet@gmail.com

Abstract

The goal of this study was to compare the usefulness of three popular synchronization protocols, viz. Ovsynch, Double Prostaglandin ($PGF_2\alpha$ /PG) injections, and MAP (Medroxyprogesterone acetate) sponges, in anestrous goats. Twenty-one non-pregnant multiparous anestrous goats with an average body condition score (BCS = 2.5) were selected. After the last PG injection, all the goats were exposed to three fertile bucks. They were observed to be in standing estrus. For further confirmation of copulation, a vaginal cytology test was performed for the presence of sperm inside the vaginal smear. Serum estradiol (E_2) peaks were also estimated by using radioimmunoassay in estrus goats. MAP sponge efficiency concerning estrus induction was found to be superior (57%) as compared to the rest (Ovsynch14 and PG 0%). Post PG standing estrus time in Ovsynch and MAP groups was recorded as 48 h and 44 ± 12 h, respectively. The double PG group failed to show standing estrus. E_2 peak levels ranging from 11-38 pg/ml in Ovsynch and 10–25 pg/ml in the MAP group were observed in estrus goats. This study found the MAP sponge protocol most efficient for inducing estrus in anestrous goats.

1. INTRODUCTION

Developing countries are home to more than 90% of the world's goat population (Akar, 2013). India, China, Pakistan, Bangladesh, Turkey, and Iran are among the leading countries in terms of goat populations (Khan *et al.*, 2003). In Pakistan, goat-rearing is mostly associated with landless and poor farmers (Iqbal *et al.*, 2008). Goat farming is primarily a low-input demanding business, making it an attractive earning source for poor men (Muhammad *et al.*, 2015). Goats have superior drought tolerance and can withstand harsh environmental conditions better than other ruminants (Lebbie, 2004), which allows them to be raised in a broader range of land in the region. In the region, goats are primarily raised for meat, with chevon (goat meat) having great demand in Asian markets (Jindal, 1984). Goat rearing for sacrificial purposes is also a lucrative source of income in Pakistan. Due to dietary awareness and cultural events, the demand for meat or its products has increased sharply in recent years (Sohaib and Jamil, 2017). Furthermore, the increasing demand for halal meat in the global market has created a great opportunity for Pakistani businessmen to fulfill the current demand-supply gap through intensive goat farming. To boost production, optimized and intensified production practices are indispensable (Bashir et al, 2015). The rhythmic reproductive behavior in goats is mainly responsible for the lean production period during different months of the year (Błaszczyk et al, 2004). The low breeding months (May-August) and high breeding months (September-December) have been observed around the year in Pakistan (Mehmood et al, 2011). So, the breeding seasonality is one of the reasons for the hike in mutton prices or its shortage in the country.

During the low breeding season, the count of anestrous goats increases in the flock which causes infertility problems. Infertility is also named as the disease of production which specifically affects cross-bred animals in the intensive farming system (Dhami *et al*, 2015). With the growing goat production business, effective and easy-to-apply estrus synchronization tools have been introduced in the field (Whitley and Jackson, 2004). These tools are playing important role in persistent and round-the-year milk and meat production (Fatet *et al*, 2011). Various methods like Ovsynch, Double prostaglandin (Double-PG), and MAP (Medroxyprogesterone acetate) sponge synchronization have been scientifically confirmed for fertility improvement in local goat breeds (Kausar *et al*, 2009; Riaz *et al*, 2012). Acquaintance with the comparative efficacy and standing estrus time related to the aforesaid synchronization protocols in anestrous goats is still scanty. The purpose of this experiment was to make breeding more consistent and efficient in the current goat

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farming system. It was done by testing and comparing the effectiveness of three different methods to bring the anestrous goats into fertile estrus and measuring the standing estrus time.

2. MATERIALS AND METHODS

2.1. Study location and animal management

This study was performed during February and March 2016 at the NIAB goat farm in Faisalabad, Pakistan (31°23'43.9"N 73°02'11.7" E). The conditions of the experiment conform to the ARRIVE guidelines and the U.K. Animals (Scientific Procedures) Act, 1986, and EU Directive 2010/63/EU for animal experiments. Twenty-one non-pregnant multiparous anestrous goats (Beetal × Dwarf goat) with average body condition score (BCS=2.5) were selected in the flock. They were randomly divided into three groups (n=7). All goats were provided with ad libitum seasonal grasses grazing and fresh clean water. Half kg (0.5 kg) concentrate was also fed per head per day.

2.2. Estrus synchronization

Three different synchronization protocols viz. Ovsynch, Double Prostaglandin $F_2\alpha$ (PG) injections, and MAP (Medroxyprogesterone acetate) sponges were applied (Figure 1). Doses of GnRH and PG hormones were injected as per the recommendation of Riaz *et al* (2012) (Tab. 1). Similarly progesterone sponges were prepared as described by Kausar *et al* (2009) and inserted for 7 days. After the last PG injection, all goats were exposed to three fertile bucks. Goats were observed for the onset of standing estrus.

Table 1: Doses of hormones administrated during the treatment

Hormone	Analogue	Dose per goat
GnRH	Lecirelin	12.5µ g
Prostaglandin	*d*- cloprostenol	37.5 µ g
Progesterone	Medroxyprogesterone acetate	60 mg

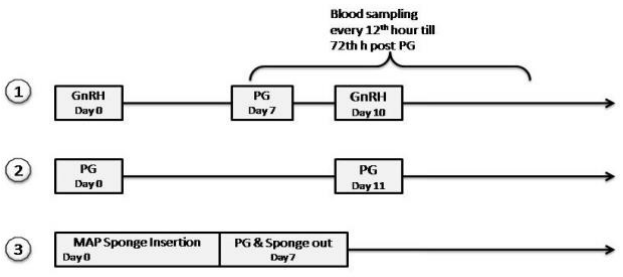

Figure 1: Time lines for hormonal therapy in three different protocols

2.3. Vaginal cytology

For confirmation of standing estrus time (mating time), vaginal cytology was performed every eighth-hour post-PG injection to confirm the presence of sperms inside the vaginal smear. For the staining of cytology slides, stock and then a working solution of Giemsa stain were prepared (Gonzales, 2016) (Figure 2). For the vaginal cytology smear, a 10 cm cotton swab soaked in normal saline was inserted in the cranial vagina and rotated 2–3 times. Then it was withdrawn and rolled on the glass slide. Smear was air-dried and stained with Giemsa stain for 10 minutes (Figure 3). After staining, slides were observed for sperm presence under the light microscope (40 x magnifications).

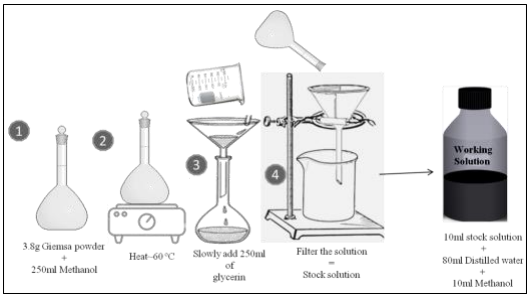

Figure 2: Preparation of Giemsa stain

Figure 3: Preparation and staining of vaginal smear slides

2.4. Hormonal analysis

For estradiol hormone (E_2) analysis, blood was collected every 12th h, starting from the last PG injection till 72 h. Blood samples were drawn in disposable syringes and serum was harvested in glass tubes. After that, the serum was stored at -20ºC till further analysis. Samples belonging to the standing estrus goats were analyzed. E_2 levels were estimated during the estrus period by solid phase radioimmunoassay method using kits (Beckmen coulter, Czech Republic). The intra- and inter-assay coefficients of variation (CV) for E_2 were ≤ 12.1% and ≤ 6.5%, respectively, whereas the analytical sensitivity was 6pg/ml.

2.5. Statistical analysis

All the data were arranged in Microsoft Excel®. Statistical software (Statistix® 8.1 version) was used for statistical analysis. Estrus response among the groups was computed by applying the Chi-square test and the significance level among different treatments was considered at $p \leq 0.05$.

3. RESULT AND DISCUSSION

The estrous cycle, the reproductive cycle of female mammals, is regulated by a complex interplay of hormones that regulate the timing of ovulation and fertility. The ability to accurately identify the optimal time for breeding in a female animal is critical for the success of a controlled breeding program. Synchronization protocols can be used to manipulate the timing of the estrous cycle,

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but the precise judgment of breeding time is essential to enhance the efficacy of these protocols and increase the chances of successful conception. (Fatet *et al*, 2011).

In the current study, the parameters exploring the efficacy and breeding time have been listed (Table 2.), which indicates that the estrus induction rate by MAP sponges was superior (57%) in comparison with the rest of the two protocols (Ovsynch 14 and PG 0%).

Standing estrus time in the MAP sponge group was recorded as 44 ± 12 h (24-72 h). Kausar *et al*, (2009) reported great variation in estrus time (65.4 ± 24.0) following the sponge removal in the same goat breed but that treatment was not supplemented with PG. The earlier onset might be due to PG-induced luteolysis. In Saanen and Black Bengal goats, estrus onset time has been reported as a range of 18-96 h (Alacam *et al*, 1985; Ishwar and Pandey, 1990). In progesterone sponge treatment variation in estrus onset time is mainly influenced by breed, co-treatment, mating scheme, and environmental factors (Chemineau *et al*, 1999).

In Ovsynch standing-estrus time was recorded as 48 h, which is quite similar to the observation of Riaz *et al* (2012) as 48.0 ± 2.6 in Beetal and Dwarf breed goats. Ovsynch-treated Boar goats also responded 49.3 ± 3.1 h after PG injection and showed standing estrus behavior in a previous study (Holtz *et al*, 2008).

Whereas in the double-PG group, none of the goats showed standing estrus in the current study. In anestrous goats due to a lack of ovulation (Pope *et al*), no corpus luteum (Pope *et al*) forms on the ovarian surface (McCracken *et al*, 1972). The main cause of anestrous is the absence of CL or no progesterone source for priming of hypothalamus E_2 receptors responsible for the expression of estrus behavior. The poor efficiency of the double-PG protocol might be due to the CL absence on ovaries. Poor response to non-progesterone protocols might also be due to suboptimal E_2 secretion by ovarian follicles (Husein *et al*, 2005).

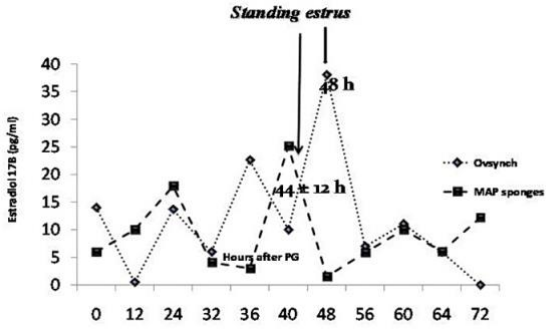
Table 2: Treatment response in different groups

Parameters	MAP sponge	Ovsynch	double PG	*p*-value 0.05
No. of goats	7	7	7	-
Goats showed Standing estrus (%)	4 / 7 (57)[a]	1 / 7 (14)[b]	0 /7 (0)[b]	Significant
Last PG to standing heat time (h)	44 ± 12 (24-72)	48	-	Significant

Values are mean ± standard deviations.

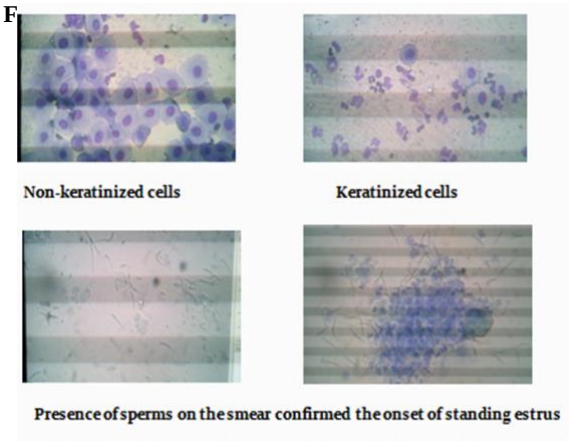
A higher concentration of E_2 is an indicator of estrus onset in animals (Błaszczyk *et al*, 2004). E_2 peaks were observed during the estrus in goats which further endorsed the estrus phase in responded animals (Figure 3). Responded goats showed E_2 peaks ranging from 11-38 pg/ml and 10-25 pg/ml in Ovsynch and MAP groups respectively. In dwarf goats, the mean serum E_2 level during estrus has been reported as 7.7 ± 1.7pg/ml (Khanum *et al*, 2008). Peaks of E_2 indicated the emergence of large ovarian follicles in response to synchronization protocol that caused estrus onset in goats (Husein and Kridli, 2003). This study is further in agreement with Billings and Katz that the progesterone treatment facilitated the induction of estrus in anestrous goats (Billings and Katz, 1999).

Figure 4: Level of E_2 concerning the standing estrus in goats



In addition to observing visual signs or measuring levels of E_2 to detect estrus in goats, vaginal cytology can also be an effective tool in the study of estrous cycles. The presence of sperm in a vaginal smear can confirm successful mating after estrus induction through synchronization protocols. This technique, known as timed vaginal smear sampling, can also be used as an alternative to continuous monitoring of mating in a farming setting. This exfoliating technique has been shown to improve reproductive performance in synchronized goats in previous studies (Leigh et al, 2010) and can be useful as a diagnostic tool to detect estrus in goats.

F smear



4. CONCLUSION

Progesterone priming with MAP sponge synchronization is an effective method for estrus synchronization in anestrous goats, and vaginal cytology can aid in detecting mating to optimize breeding.

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