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Fertility response using Ovsynch based fixed time insemination protocols in goats

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Abstract
Fertility response following fixed time insemination was studied in 48 Sirohi goats, randomly divided into 4 groups, each comprising 12 animals (n=12). Animals of group l received Ovsynch protocol; in group II (IMOvP-1), treatment was similar to group-I plus additional 3-doses of long-acting insulin @ 0.14 IU/ kg b. wt. subcutaneously on day 4, 5 and 6. In group-III (IMOvP-2), treatment was similar to group-I plus 3 additional doses of long-acting insulin @ 0.14 IU/ kg b. wt. subcutaneous on day 8, 9 and 10. However, animals of group–IV were kept as control. All the animals were inseminated once at fixed time *i.e.* 16 hrs after second GnRH injection using fresh semen and pregnancy was confirmed by ultrasonography at 30 days post insemination.

The results of fertility response using fixed time insemination protocol in goat shows significantly higher synchronization of oestrus in treatment groups (83.33%-GI; 91.66%-GII and 100%-GIII) as compared to control group (8.33%-GIV). The conception rate at fixed time insemination was also recorded higher in treatment groups (58.33%-GI, 83.33%-GII and G-III) as compared to control group (8.33%-GIV). The analysis of results revealed higher conception rate in insulin modified Ovsynch protocols where the intensity of oestrus was better during fixed time insemination.

Keywords: Oestrus synchronization, Ovsynch, Sirohi goat, insulin, pessaries

Introduction
Artificial insemination is commonly used in goats as it significantly speeds up progression of genetic merit. Fixed-time artificial insemination (TAI) is helpful especially when oestrus detection is not very efficient or due to lack of time to carefully perform it. An additional bonus of TAI is that it synchronizes ovulation and thus, kidding and the lactation period of groups of does which can later be managed as a batch. In goats, oestrus control is generally brought about by the use of progestagon-impregnated vaginal pessaries with limited success (Corteel *et al.*, 1988; Leboeuf *et al.*, 1998 and Holtz, 2005) [3, 10, 7]. Ovsynch is one of the "classical" and widely known ovulation synchronization systems. The main advantage of Ovsynch protocol is oestrus synchronization which can be done at all stages of the cycle. It ensures a homogenous ovarian follicular status at induction of luteolysis. As a result, precision of oestrus after prostaglandin induced luteolysis and synchrony of the LH surge can be improved. Both follicular development and regression of the corpus luteum (CL) can thus be synchronized. The technique was successfully used for synchronization of ovulation and fixed time artificial insemination in goats during breeding season and may serve as an alternative to vaginal pessaries (Holtz *et al.*, 2008) [8]. Efficacy of the Ovsynch protocol is dependent on the stage of follicular development at the time of initial GnRH injection (Renesis *et al.*, 2005) [18]. Fertility obtained with Ovsynch protocol is highest, when animal ovulate to the first GnRH injection (Pursley *et al.*, 1997) [16]. Still, there is a need to improve the efficacy of Ovsynch for better fertility and applicability at field level. Several studies have demonstrated that treatment with metabolic hormones and growth stimulants, such as somatotrophin, insulin and insulin like growth factor (IGF-I), may improve the reproductive performance of the farm animals (Gong *et al.*, 1994) [4]. The administration of exogenous insulin may influence follicular development, favoring gonadotrophic-dependent follicle recruitment and increasing the ovulatory follicle diameter and the ovulation rate (Ramoun *et al.*, 2007 and Sarath *et al.*, 2008) [17, 19] also stimulate a greater follicular estradiol production in ruminants (Butler *et al.*, 2004 and Ramoun *et al.*, 2007) [2, 17]. Insulin based Ovsynch protocols were also found effective in buffaloes (Singh *et al*, 2014 and Gupta *et al*, 2015) [26]. Insulin administered goats with progesterone based intravaginal sponge showed large follicles and a higher ovulation rate as well as more efficient synchronization of ovulation and larger size corpus luteum (Pinheiro *et al.*, 2012) [14].

Materials and Methods

Animals: The Sirohi goats used for the present study were reared under semi-intensive housing conditions. Does grazed on natural pastures during the day and were penned at night, because of the presence of predators. When penned, all animals received 250 g/head of a concentrate containing 18% crude protein. Animals of all the goat farms were fed commercially available concentrate mixture along with wheat straw and seasonally available green fodder like berseem. They were also given additional supplement of mineral mixture, jaggery and mustard oil, time to time. Deworming was practiced 2 times in a year in different farms. Clean drinking water was made available to the animals *ad lib*. All the farms included in the present study maintained almost optimal and nearly identical conditions of feeding and management. However, none of the goat farms used in the study qualifies for receiving optimal standards of managemental practices.

Buck Semen: Semen was collected from breeding bucks of the Sirohi breed by AV method and evaluated using standard methods of macroscopic (colour, volume and consistency) and microscopic (abnormal percentage, live percentage and sperm concentration) tests. The excellent to good quality semen was diluted ten times in egg yolk citrate extender and stored at 5 ºC and used within 3 days for insemination. The minimum standards taken for the semen are as follows-

Macroscopic-
Colour- pale yellow to cream
Volume- 0.5 to 1.5 ml
Microscopic -
Mass motility- 80-90%
Progressive forward motility – 50-60%
Abnormal percentage- <5%
Live percentage - >90%
Sperm concentration- 1800-2000×10^6/ejaculate

Technical programme

This study was carried out systematically in Sirohi goats and described under the following sub-headings.

Selection of animals: The study was conducted in 48 apparently healthy Sirohi goats maintained at Amanala goat farm, NDVSU, Jabalpur. The selection of animals was done on the basis of history of kidding at least 40-50 days before, with no apparent gynaecological disorders and non-pregnant on two consecutive ultrasonographical examinations at 14 days interval.

Experimental design

The selected postpartum Sirohi goats were randomly divided into four groups, each comprising 12 animals (n=12) and treatments were given as per the protocol summarized in the treatment schedule (table 1).

Table 1: Group wise treatment protocol for oestrus synchronization and artificial insemination in goats

Groups	Treatment Protocol
G-I	GnRH (day-0) + $PGF_2\alpha$ (day-7) + GnRH (day-9) + TAI
G-II	GnRH (day-0) + Insulin (day- 4, 5 and 6) + $PGF_2\alpha$ (day-7) + GnRH (day-9) + TAI
G-III	GnRH (day-0) + $PGF_2\alpha$ (day-7) + Insulin on day 8, 9 and 10 + GnRH (day-9) + TAI
G-IV	Control

GnRH: Buserelin acetate 0.004 mg I/M (Holtz *et al.*, 2008) [8]
Insulin: Long acting biphasic insulin @ 0.14 lU/Kg b.wt, (Pinheiro *et al.*, 2012) [14]
$PGF_2\alpha$: Dinoprost @ 5.00 mg I/M or cloprostenol @ 100 mcg I/M
TAI: Fixed time single AI after 16 hrs of last GnRH injection using fresh semen (Holtz *et al.*, 2008) [8]

Monitoring of animals and fertility response

All the animals were inseminated once at fixed time *i.e.*, 16 hrs after second GnRH injection using liquid semen of Sirohi buck. Fertility response in terms of oestrus synchronization and conception rate was studied. The synchronization rate was assessed by visual observation for oestrus signs at the time of artificial insemination and grades were awarded as per the criteria given in table-2. All the animals were examined ultrasonographically for confirmation of pregnancy at 30 days post insemination and conception rate was calculated.

Table 2: Criteria used for grading of oestrus synchronization

Parameters during TAI	Grades of oestrus synchronization				
	Excellent	Good	Fair	Poor	Nil
Vulvar lips swollen and edematous	Yes	Yes	Yes	Yes	No
Vulvar mucus membrane moist and congested	Clear	Moist, Less Congestion	Moist and Pale	Slight Moist and Pale	Dry and Pale
Cervico-vaginal mucus in vagina	Copious	Scant	Scant	Not Seen	Not Seen
Lubrication of vaginal lumen	Good	Good	Slight	Slight	Absent
External Os of cervix opened	Complete	Complete	Partial	Partial	Close
Does posturing	Present	Present	Present	Absent	Absent
Tail flagging	Present	Present	Present	Absent	Absent

Results and Discussion

In the present study significantly higher oestrus synchronization was recorded in all the treatment groups *i.e.*, Ovsynch (83.33%-GI), insulin modified Ovsynch-I (91.66%-GII), insulin modified Ovsynch-Il (100%-GIII) as compared to control (8.33%-GI) (p<0.05).

The results of oestrus synchronization using Ovsynch protocol in this study are comparatively lower than the findings of Holtz *et al.* (2008) [8] where 96% Boer goats in Germany were induced in oestrus 49 hours after $PGF_2\alpha$ injection. Whereas, synchronization of oestrus in the present study was higher than Panicker *et al.* (2015b) [13], who

recorded 75% synchronization using Ovsynch protocol in Malabari goats in Kerala. The variation in synchronization rate may be due to difference of breed, managemental and geographical conditions.

The oestrus synchronization was graded as excellent, good, fair, poor and nil based on intensity of oestrus signs observed during buck parading and artificial insemination. The analysis of results revealed that all the treatment groups (original and insulin modified ovsynch protocol) have comparatively more excellent and good grades of the synchronization as compared to control group, but higher excellent percent in insulin treated group–II followed by group-I.

In the present study, conception rate using fixed time insemination was significantly higher in the Ovsynch (58.33%) and modified Ovsynch protocol (83.33%- GII and GIII) as compared to control (8.33%-GIV). However, the trend of better conception (83.33%-GII and GIII) was recorded in insulin modified Ovsynch followed by Original (58.33%-GI). The conception rate using Ovsynch protocol in Sirohi goat recorded in the present study are in fair agreement to Holtz *et al.* (2008) [8] and Paniker *et al.* (2015) who reported a conception of 58% in each. However, comparable and non-significantly higher oestrus synchronization and conception rate in insulin modified Ovsynch as compared to the original Ovsynch protocol was recorded in the present study which may be due to beneficial effect of insulin on folliculogenesis and maturation of follicle.

The literature is lacking regarding the use of proposed modified Ovsynch protocol with insulin for fixed time AI in goats. Pinherio *et al.* (2012) also recorded large follicles at 0, 12 and 18 hours of oestrus period, a more efficient synchronization of ovulation and larger size corpus luteum using insulin (0.14 IU and 0.20 IU s/c for 3 consecutive days) along with intravaginal sponge (60mg MAP) for 10 days and 50 µg cloprostenol in nulliparous goat. They also found that the reproductive performance appears to be independent of the increase of the insulin dosage.

However, in one study Singh (2014) [26] in postpartum buffaloes found highest conception rate using insulin modified Ovsynch protocol (66.66%) where second dose of GnRH was completely replaced by insulin on day 8, 9 and 10 followed by Ovsynch protocol using half dose of GnRH plus additional administration of insulin on day 8, 9 and 10 (58.33%); lowest in the modified Ovsynch protocol where additional insulin was administrated on day 8, 9 and 10. In their study 50% conception was found using Ovsynch protocol without any effect of reducing second dose of GnRH. In another study, Singh (2013) found better conception (75-88.89%) using such modified Ovsynch protocol with insulin in postpartum anoestrus buffaloes.

The beneficial effects of insulin alone and in various combinations using GnRH and PMSG in anoestrus cattle and buffaloes were also reported by Shukla *et al.*(2005) [23], Ramoun *et al.* (2007) [17], Gupta *et al.* (2010) [5], Purkayastha *et al.* (2015) [15], Kumar *et al.* (2013) [9] and Singh (2013) [24] which supports the present study.

Insulin has a direct stimulatory effect on estradiol production (Bucholtz *et al.*, 2000; Butler *et al.*, 2004) [1, 2]. This action can be ascribed to the effect of insulin on steroidogenesis (Selvaraju *et al*., 2003) [20]. A higher percent of induced oestrus has been reported in cows, buffaloes and goats treated with exogenous insulin (Ramoun *et al*., 2007; Sarath *et al*., 2008) [17, 19]. Similar reports in goats have shown an increase in the ovulation rate after insulin treatment (Selvaraju *et al*., 2003) [20]. In goats and ewes, insulin induces a reduction in follicular atresia and an increase in the number of gonadotrophin-dependent follicles (Gong *et al*., 1994; Majumdar *et al*., 1997) [4, 11]. Many researchers have stated that the ovulation rate may be manipulated by direct action of insulin on the ovary, stimulation of activity of the modulators, such as IGF-I, through changes in the insulin–glucose system (Meza-Herrera *et al*., 2008) [12].

The use of insulin in the modified protocols may be one of the reasons of better conception as reported by Sharma *et al.* (2011 and 2012) in repeat breeder cows. The beneficial effects of insulin on resumption of ovarian cyclicity and fertility may be due to its effects on the folliculogenesis and steroidogenesis (Gong *et al.*, 1994 and Stewart *et al.*, 1995) [4, 28]. Insulin enhances growth and proliferation of granulosa, theca and luteal cells present in the ovary (Spicer *et al.*, 1993 and Stewart *et al.*, 1995) [27, 28]. This enhances folliculogenesis either acting through specific insulin receptor and IGF-1 or both type of the receptors. Insulin also induces LH pulse secretion and thus maturation of follicles (Tanaka *et al.*, 2000) [29].

As the insulin is non-steroidal metabolic hormone, cheap, easily available and has beneficial effect in the modified Ovsynch based protocol thus can be utilized for a better fertility response in goats.

Conclusions

Oestrus synchronization and conception were recorded higher in insulin modified protocols and comparable to original Ovsynch protocol in Sirohi goats.

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