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Assessment of NC synch and other hormonal protocols for oestrus synchronization and fertility enhancement in Sirohi does

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ABSTRACT

The Sirohi goat, a dual-purpose breed native to Rajasthan, India, faces challenges in reproductive efficiency due to seasonal breeding and management constraints. This study evaluated the efficacy of four oestrus synchronization protocols—GnRH-$PGF_2\alpha$-GnRH, 7-day vaginal sponge plus GnRH, 11-day vaginal sponge plus GnRH and North Carolina State University Sync (NC Sync) protocol on reproductive performance, vaginal cytology, serum progesterone profiles and conception rates following artificial insemination with frozen semen in Sirohi does. Twenty-four healthy, non-pregnant goats were allocated equally into four treatment groups. Oestrus response, vaginal cytology and serum progesterone concentrations were monitored before, during and after hormonal treatments. The NC Synch protocol achieved the highest oestrus detection (100%) and conception rate (67%), significantly outperforming the other groups. Vaginal cytology revealed increased cornified superficial cells at 24–48 hours post-treatment, correlating with optimal breeding time. Progesterone levels increased significantly during treatment in vaginal sponge groups, indicating enhanced luteal activity, while other protocols showed gradual declines. These results demonstrated that the NC Synch protocol offered superior synchronization and fertility outcome in Sirohi goats, providing a viable strategy to improve reproductive efficiency and genetic advancement through timed artificial insemination.

Keywords: NC Synch protocol, Oestrus synchronization, Sirohi goat, Vaginal cytology

The Sirohi goat, native to the Sirohi district of Rajasthan, India, is a dual-purpose breed valued for its meat and milk. It thrives well in dry tropical climates and exhibits strong resistance to diseases and environmental stress (NABARD, 2020). Despite its adaptability, Sirohi goats suffer from low reproductive efficiency due to seasonal breeding patterns, poor genetic selection and uncontrolled reproduction (Kanduri *et al.* 2022). India ranks second globally in goat population, yet its reproductive productivity remains below potential (20th Livestock Census, 2019). Oestrus synchronization has emerged as a pivotal tool to regulate breeding cycles, improve conception rates and allow for fixed-time artificial insemination (Stevenson and Britt, 2017; Parmar *et al.* 2020).

Several hormonal protocols are available, including progestagen-releasing intravaginal devices, prostaglandin F2α (PGF2α), GnRH and eCG. These can be applied in short-term (5–7 days) or long-term (12–14 days) treatments to manipulate luteal activity and induce synchronized ovulation (Endo *et al.* 2020; Hasani *et al.* 2018). The Ovsynch protocol, which combines GnRH and PGF2α, effectively coordinates follicular growth and luteolysis, facilitating timed AI (Holtz *et al.* 2008). Studies show that conception rates following oestrus synchronization vary with the protocol used. In goats, the Ovsynch (GnRH–PGF2α–GnRH) protocol yields an overall kidding rate of about 58%, which can increase to nearly 88% in animals that undergo proper luteolysis (Holtz *et al.* 2008). In sheep, Ovsynch produces pregnancy rates of 46–66% after fixed-time AI, increasing to 85–88% when followed by a short mating period (Endo *et al.* 2020). Progesterone sponge protocols combined with eCG are widely used in goats, typically achieving fertility rates of 60–65%, though success depends on management and season (Leboeuf *et al.* 2003; Swelum *et al.* 2015). Among intravaginal devices for ewes, CIDR has been shown to provide higher pregnancy, fertility and fecundity than FGA sponges (Hasani *et al.* 2018). Prostaglandin-based (PGF2α) treatment alone gives a moderate pregnancy rate, but combining them with eCG

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significantly improves both overall pregnancy and lambing performance (Endo *et al.* 2020). Comparisons suggest that Ovsynch can match or even surpass sponge-based protocols in goats when luteolysis is reliable (Holtz *et al.* 2008), while in sheep, the addition of eCG consistently enhances outcomes across sponge and PGF2α regimens (Hasani *et al.* 2018). Overall, protocol choice, luteal response, device type, and eCG supplementation strongly influence conception success, with Ovsynch and CIDR systems offering the most consistent results.Vaginal sponges also serve as an alternative, releasing synthetic progestins that trigger oestrus upon withdrawal (Leboeuf *et al.* 2003; Swelum *et al.* 2015).

NC Synch, developed at North Carolina State University, follows a structured schedule of PGF2α and GnRH to synchronize oestrus in cycling goats over 17 days (Farooqi *et al.* 2021). Bowdridge *et al*. (2013) achieved a 68% conception rate (45/66 goats) using the NC-Synch protocol, while Parmar *et al*. (2020) reported 55.55% (10/18 goats) in Surti goats. This method simplifies AI without the requirement of oestrus detection.

Given the limited data on oestrus synchronization in Sirohi goats (Sharma and Purohit, 2009; Inwati, 2016; Shakya, 2017), this study was undertaken to evaluate and compare the reproductive efficacy of various synchronization protocols. The main objectives were to assess the fertility response of Sirohi goats to different oestrus synchronization protocols and to evaluate changes in serum progesterone levels before, during and after hormonal treatment.

MATERIALS AND METHODS

Location and place of work: The study was conducted at Amanala goat farms, Jabalpur district, Madhya Pradesh and at the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur after approval of the institutional ethical committee (No. 55/IAEC/Vety./2024).

Selection of experimental animals: Twenty-four healthy, non-pregnant Sirohi does were selected based on a minimum of 120 days since last kidding and confirmed non-pregnancy status through two ultrasonographic exams 30 days apart.

Reproductive hormones for oestrus synchronization: The protocols involved administration of several reproductive hormones, including Gonadotropin-Releasing Hormone (GnRH) in the form of buserelin acetate at a concentration of 0.0042 mg/ml, Prostaglandin F2 alpha (PGF2α) as dinoprost tromethamine at 5 mg/ml and vaginal sponges impregnated with 300 mg of Medroxyprogesterone acetate (MAP) to simulate the luteal phase by releasing progesterone.

Intravaginal sponge insertion: Sponges were inserted using a sterilized, lubricated speculum; positioned deep in the vagina, with the string secured inside the vulva to prevent displacement (Menchaca *et al.* 2017).

Experimental design: The goats were divided into four groups of six animals each, with each group subjected to a different synchronization protocol. Group 1 (GPG) received buserelin acetate on day 0, PGF2α on day 7 and buserelin again on day 9, followed by artificial insemination (AI). Group 2 was treated with a progesterone vaginal sponge for 7 days, followed by buserelin injection at sponge removal and AI. Group 3 underwent a similar treatment but with the progesterone sponge left in place for 11 days before buserelin administration and AI. Group 4 (NC Synch) received PGF2α on day 0, GnRH on day 8, PGF2α again on day 15 and GnRH on day 18, with AI conducted afterward.

Vaginal cytology: Samples were collected pre- and post-treatment by vaginal swab, stained with Giemsa and examined microscopically to classify cells as parabasal, intermediate, superficial and cornified, reflecting the reproductive stage (Salvetti *et al.* 2007).

Serum progesterone profiling: Blood samples (2 ml) were collected aseptically before, during and after treatment. Serum was separated and stored at -20°C. Progesterone levels were measured using a DRG ELISA kit (Martemucci and D'Alessandro, 2011).

Artificial Insemination (AI) and ultrasonography: Artificial insemination was performed 20–24 hours after oestrus detection using 0.25 mL frozen–thawed semen containing approximately 200 million spermatozoa per straw, deposited with a French AI gun. Transabdominal ultrasonography (5–7.5 MHz, B-mode) was used on day 0 to confirm non-pregnancy and on day 45 post-AI to confirm pregnancy.

RESULTS AND DISCUSSION

The oestrus detection rate varied among the four synchronization groups, reflecting the effectiveness of different protocols in inducing heat (Table 1). In Group 1, five out of six goats exhibited oestrus, resulting in a

Table 1. Serum progesterone concentration before, during and after synchronization among different groups

Treatment groups	Progesterone (ng/ml)			p-value
	Before treatment	During treatment	After treatment (24–48 hours post-final injection)	
G1	3.58 ± 0.77	2.63 ± 0.66	1.54 ± 0.51	0.0001***
G2	3.91 ± 0.81	6.58 ± 1.05	1.91 ± 0.56	0.0001***
G3	3.74 ± 0.79	6.53 ± 1.04	1.47 ± 0.49	0.0001***
G4	4.00 ± 0.82	3.44 ± 0.76	1.28 ± 0.46	0.0001***

*Mean ± SE values within the row with $p<0.05$ differ significantly.

detection rate of 83.33%. Group 2 showed a lower rate, with only four out of six animals (66.66%) coming into heat. Group 3 matched Group 1 with 83.33% detection, but the highest rate was recorded in Group 4, where all six goats (100%) exhibited oestrus, indicating superior protocol efficiency. These results aligned with previous studies, such as Kanduri *et al*. (2022), who reported similar success with GPG protocols and Teleb and Ashmawy (2007), who observed comparable rates using vaginal sponges combined with buserelin acetate. Longer sponge application also appeared to improve outcomes, and were consistent with the findings by Saribay *et al*. (2011). The 100% response in Group 4 is supported by Parmar *et al*. (2020). Variations in heat detection may be influenced by factors such as silent oestrus, breed differences, environmental conditions, nutrition and stress and protocol consistency. Overall, the study confirmed that hormonal synchronization protocols varied in success but could achieve better oestrus detection with appropriate management.

The study examined the effects of different synchronization protocols on vaginal cytology across four groups of Sirohi does, focusing on changes in exfoliative vaginal cells before and after treatment. In Group 1 (GPG protocol), there was a significant reduction in immature parabasal cells from 66.00% to 12.00% and intermediate cells decreased from 29.33% to 11.66%. Conversely, mature superficial cells increased markedly from 2.83% to 16.33% and cornified cells surged from 1.83% to 60.00%, indicating a physiological shift towards oestrus. Similarly, Group 2 (7-day vaginal sponge plus GnRH) showed significant decrease in parabasal (61.66% to 23.5%) and intermediate cells (34% to 15.66%), alongside increase in superficial (3% to 13.33%) and cornified cells (1.33% to 47.5%). Group 3 (11-day vaginal sponge plus GnRH) demonstrated comparable trends with parabasal cells dropping from 66% to 13.16%, intermediate cells from 30.66% to 11.33% and marked rises in superficial and cornified cells. Group 4 (NC Synch) exhibited the most dramatic changes, with parabasal cells decreasing from 65.33% to 2.16%, intermediate cells from 31% to 8.16%, while superficial cells increased from 2.33% to 19% and cornified cells rose sharply from 1.33% to 70.66%. These results strongly suggested that synchronization treatments promote epithelial maturation, reducing immature cell types and increasing mature and cornified cells associated with the oestrus phase. The findings aligned with earlier studies indicating that the rise in superficial and cornified cells reflects increased estrogenic activity preparing the reproductive tract for copulation and fertilization (Indhumathi *et al.* 2020; Sitaresmi *et al.* 2019). Similar trends were observed by Dogra *et al*. (2017) and Patel *et al*. (2023), who reported enhanced superficial and cornified cell populations during oestrus synchronization in goats. Furthermore, Pretorius (1977) and Leigh *et al*. (2010) noted that superficial cells dominated during oestrus and synchronization protocols amplify this effect, consistent with the present study. Additionally, Noakes *et al*. (2009) and Hafez and Hafez (2000) described distinct exfoliative cell variations during the oestrus cycle, with decreased parabasal and intermediate cells and increased superficial cells, confirming the hormonal influence on epithelial stratification observed in the present study. Overall, the vaginal cytology changes observed validate the effectiveness of the applied synchronization protocols in advancing the reproductive cycle toward oestrus, supporting their practical application in goat reproductive management.

All changes observed in vaginal cytology and serum progesterone levels among the treatment groups were statistically significant ($p < 0.001$). These results suggested that Groups 2 and 3, which received vaginal sponges plus GnRH, had an exogenous progesterone-induced elevation during treatment, followed by a decline as the hormone was withdrawn, a pattern consistent with effective synchronization. Groups 1 and 4, with other protocols, showed gradual progesterone reductions, reflecting different modes of hormonal control. These observed progesterone profiles aligned with the prior findings in goat reproductive physiology and synchronization protocols. Menchaca and Rubianes (2001) reported variable durations of progesterone elevation depending on treatment and cycle stage, with controlled internal drug release (CIDR) and sponge devices inducing sustained progesterone release. Saravy *et al*. (2011) and Marei *et al*. (2012) documented progesterone levels rising to approximately 3.0 ng/ml during vaginal sponge treatments, consistent with the mid-treatment elevations observed in our Groups 2 and 3. Additionally, the elevated progesterone levels during treatment in Groups 2 and 3 (6.53–6.58 ng/ml) mirrored values reported by Balaganur *et al*. (2024), who found the levels to be 6.28 ng/ml during functional corpus luteum or silent heat phases in Sirohi goats, suggesting these levels were physiologically relevant for maintaining the reproductive cycle. Agarwal *et al*. (2015) also reported peaks near 6.0 ng/ml with CIDR use, supporting the efficacy of progesterone-releasing devices in synchronization. Natural progesterone fluctuations rise to 6.0–11.39 ng/ml during the luteal phase during the estrous cycle, described by Medan *et al*. (2004), Bauernfeind and Holtz (1991) and Debbarma *et al*. (2013), a closely resembled our findings during the treatment phase in Groups 2 and 3. This confirmed that the synchronization treatments effectively mimicked the natural luteal phase progesterone dynamics to promote timely oestrus induction.

The conception rates obtained post-artificial insemination (AI) varied notably across the four treatment groups (Table 1). Group 1 (GPG protocol) and Group 3 (VS for 11 days + GnRH) both achieved a conception rate of 33%, Group 2 (VS for 7 days + GnRH) showed the lowest rate at 16%, while Group 4 (NC Synchronization protocol) demonstrated the highest conception rate of 67%. Oestrus detection rates also varied, with Group 4 showing 100% of animals exhibiting oestrus, whereas Groups 1 and 3 had 83% (5/6 animals) and Group 2 had

Table 2. Heat detection and conception rate (%)

Group (n=6)	Animals in Heat	Oestrus Detection Rate (%)	Pregnant Animals	Conception rate (After 1st service)
G1	5	83.33	2	33
G2	4	66.66	1	16
G3	5	83.33	2	33
G4	6	100	4	67

67% (4/6 animals). Artificial insemination was performed on all animals regardless of oestrus signs, with those not detected in oestrus inseminated 24–48 hours after the last synchronization treatment to optimize fertility chances. Pregnancy confirmation via ultrasonography at 45 days post-AI revealed viable pregnancies, characterized by the presence of concave cotyledons and gestational sacs (Plate 08). The conception rate of 67% in Group 4 alignsedclosely with Gore *et al*. (2020), who reported a 71.42% conception rate using a short-term progesterone protocol in Toggenburg goats. This suggests that the NC Synch protocol employed in Group 4 might offer improved timing for ovulation and insemination, leading to higher fertility success. Conversely, Group 2's lower conception rate (16%) might be due to the shorter duration of progesterone exposure (7 days), potentially resulting in less optimal follicular development and synchronization. Previous studies also present a broad range of conception rates with frozen semen in goats. Ranjan *et al*. (2020) recorded a 37.57% conception rate in Sirohi and other breeds inseminated during natural oestrus, while Khadse *et al*. (2019) and Gibbons *et al*. (1992) documented still lower rates of 26.04% and 28.5%, respectively. Kanduri *et al*. (2022) reported an 80% conception rate using the GPG protocol, which is notably higher than the 33% found in Group 1 of the current study, potentially reflecting differences in management, semen quality, or animal physiological status. Studies specifically evaluating the NC Synch protocol also support its efficacy, with Bowdridge *et al*. (2013) and Parmar *et al*. (2020) reporting conception rates of 68% and 55.55%, respectively, which are comparable to the 67% observed in this study. The enhanced success in Group 4 may be attributed to better synchronization of follicular waves and luteolysis, ensuring optimal ovulation timing and uterine receptivity at insemination. Overall, the variation in conception rates among groups may be influenced by multiple factors including the length of progestogen exposure, hormonal combinations, insemination timing, animal physiological responses and management practices. The higher conception rate in Group 4 suggested that the NC Synch protocol could be a promising approach to improve fertility outcomes in Sirohi goats under similar field conditions.

Both vaginal sponge–based and NC-Synch synchronization protocols were found to be effective in inducing oestrus and improving fertility in goats. Vaginal sponge protocols effectively synchronized oestrus; however, their use involves additional costs related to intravaginal devices. Longer sponge retention also extends treatment duration, affecting practical field application. The NC-Synch protocol eliminates the need for intravaginal devices, thereby reducing treatment cost and handling stress. Although the duration of the NC-Synch protocol is slightly longer, it requires fewer interventions and has lower labour input. The higher oestrus response and conception rate achieved with NC-Synch compensated for the extended duration. Overall, NC-Synch provides the best balance between, treatment duration and reproductive performance. Therefore, it is considered more practical for large-scale field application under diverse management conditions.

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