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Comparative efficacy of different hormonal interventions for estrus synchronization in North-Western Himalayan Gaddi ewes

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ABSTRACT

Estrus synchronization is crucial for reproductive management in seasonal breeders to achieve maximum productivity in the flock. The present study was conducted to compare different hormonal interventions for estrus synchronization in North-Western Himalayan Gaddi ewes reared at Livestock Farm Complex, CSKHPKV, Palampur, India (32.6°N, 76.3°E, altitude of 1290.8 m). A total of 36 adult, healthy, pluriparous Gaddi ewes were randomly assigned to either of three groups (n=6 each) namely progesterone-based protocol (ProEP), ovulation synchronization protocol (GPG) and control (Con) group, respectively in both breeding and non-breeding season. Synchronization protocols were compared in terms of different estrus and fertility related parameters *viz.* onset of estrus (OE), duration of estrus (DE), estrus intensity score (EIS), pregnancy rate (PR), conception rate (CR), fecundity rate (FR) and prolificacy (P). Comparatively early onset of estrus (31.6±3.1 v/s 50.3.1±2.8 hr and 26.0±1.1v/s 43.0±3.1) along with significantly higher pregnancy (66.7% v/s 33.3 % and 83.3 v/s 33.3 %) and fecundity rates (66.7 v/s 33.3 and 100.0 % v/s 66.7 %) were observed in ProEP than GPG group during both breeding and non-breeding season. The study indicated that, ProEP protocol can significantly contributes to increased flock productivity, better genetic improvement, and higher profitability for farmers involved in sheep husbandry.

Keywords: Avikesil-S, Estrus synchronization, Gaddi ewe, Gonadotropin

Small ruminants form backbone of Indian agrarian society since times immemorial and contributes around 41.67% of the total livestock population in India (223.14 million; 20th livestock census, 2019). Sheep husbandry in India is characterized by high degree of endemism and variations in agro-climatic conditions which further resulted in the development of climate specific breeds/ strains (Bhatia and Arora 2005).Gaddi sheep is a recognized breed, well known for mutton and carpet wool production, and is being reared by tribal community "Gaddi" in the North-Western Himalayan states primarily, inhabiting Kangra and Chamba district of Himachal Pradesh, hilly terrains of Uttarakhand and Kishtwar or Bhadarwah tehsils of Jammu and Kashmir (Sharma *et al.* 2014).

Proficient reproductive management of livestock is crucial for sheep husbandry to meet market demands as well due to seasonality of the estrous cycle. For economic upliftment or providing employment and income to marginal farmers estrus synchronization during both breeding and non-breeding season help in increasing year round productivity. Reproductive seasonality in sheep can be altered by administration of exogenous hormones (Khamees *et al* .2020) or change in day length or exposures to males (ram effect; Nakafeero *et al.* 2020; Jiménez-Severiano *et al.* 2023). Methods used to regulate seasonality as mentioned *vide supra* will induce and synchronize ovulations precisely, ensuring high fertility with improved reproductive efficiency in sheep. Hence, present study was envisaged to evaluate different hormonal interventions for estrus synchronization in North Western Himalayan Gaddi ewes.

MATERIALS AND METHODS

Present research was undertaken to compare different hormonal interventions for estrus synchronization during conventional non-breeding (April to June; NB) and breeding season (November to January; B), respectively in North-Western Himalayan Gaddi ewes.

Animal, localization and feeding management: Adult, healthy, pluriparous Gaddi ewes (n=36) reared at Livestock Farm Complex, CSKHPKV, Palampur, India (32.6°N, 76.3°E, altitude of 1290.8 m) were randomly allocated as per the protocol to three groups namely, progesterone-based protocol (ProEP), Ovsynch (GPG) and Control (Con), respectively, each group comprising of six animals (n=6) during NB or B season each. Average (Mean±SEM)

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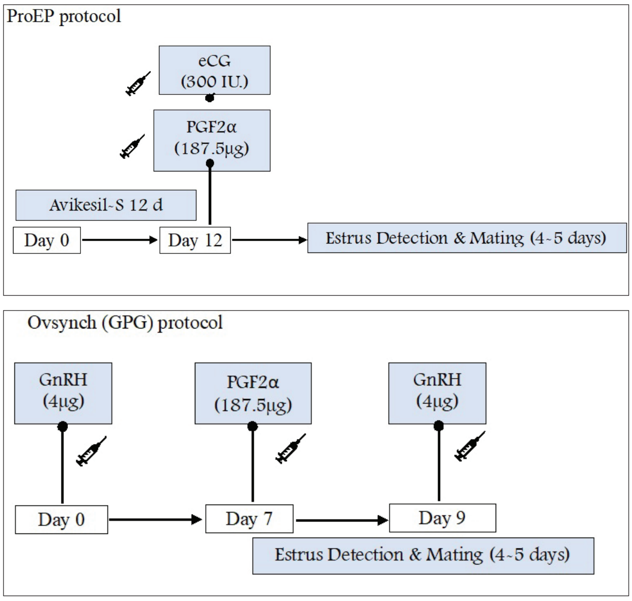

Fig. 1 Line diagram of ProEP and GPG protocol

age and bodyweight of the females during NB and B season were 1.9±0.7 and 2.4±0.2 years, and 33.4±1.6 and 36.5±1.3 kg, respectively.

Ewes were subjected to identical farm management which includes grazing (five hours) with natural photoperiodic conditions and were maintained in a semi open pen sufficient for housing 10-12 animals. Seasonal green fodder such as maize, cowpea, berseem and jowar, depending on their availability, along with concentrate mixture (@3-3.5% of body weight containing DCP-11.59% and TDN-77%) and *ad libitum* water, as per the recommended dietary requirements of Indian Council of Agricultural Research (ICAR 2013).

Following selection, each ewe assigned to progesterone-based protocol (ProEP) was inserted with an intravaginal sponge for 12 days {Avikesil-S procured from Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Rajasthan. India}. The protocol was initiated regardless of the stage of the estrous cycle. On the day of sponge removal, eCG (300 IU, i.m.; Folligon®, MSD–SP India) and $PGF_{2}\alpha$ (187.5 µg, i.m.; Pragma®, Intas Animal Health, India) were administered.

GPG assigned ewes were administered of GnRH @4µg i.m (Receptal Vet®, MSD-SP India) on Day 0, followed by injection of $PGF_{2\alpha}$ @187.5µg i.m(Pragma®, Intas Animal Health, India) on Day 7, and then a 2^{nd} shot of GnRH @4µg i.m (Receptal Vet®, MSD-SP India) on Day 9. Onset and the duration of estrus were evaluated after the administration of $PGF_{2\alpha}$ injection.

Control group (Con) ewes were routinely observed for behavioural estrus and naturally served by adult ram without any hormonal intervention.

Estrus detection was accomplished by parading adult ram (morning/evening, thirty minutes each) and visualizing behavioural changes in females. Estrus intensity was calculated using estrus score card during the period of estrus detection. Estrus response was characterized as intense, fair and weak on the basis of estrus scoring chart (Table 1) formulated for Gaddi sheep in present study. All the ewes which exhibited estrus behaviour were allowed to be tupped.

Comparative efficacy of different hormonal interventions were evaluated in terms of estrus related parameters {estrus response rate (ERR), onset of estrus (OE), duration of estrus (DE), estrus intensity score (EIS)} and fertility parameters {conception rate (CR), pregnancy rate (PR), fecundity rate (FR), prolificacy (P).

Statistical analyses were conducted using Minitab Version 18.1 and SPSS Statistics Version 25. Comparison of data between two groups were analyzed with Independent T-test and for more than two groups, one-way ANOVA test was employed. Results were presented as Mean±SEM and differences were considered significant when $p<0.05$.

RESULTS AND DISCUSSION

In this study, efficacy of different hormonal interventions following estrus synchronization in Gaddi ewes during NB and B season were evaluated on estrus and fertility related parameters. Most ProEP-treated ewes (83.3%) exhibited estrus within 48 hours, regardless of the season. Early OE in ProEP treated ewes might be hypothesized as

Table 1. Estrus score card for assessment of estrus intensity in Gaddi ewes

	Estrus Sign	Estrus Score		
		3	2	1
Ewe's Behaviour	Tail wagging	Vigorous (>3)	Intermittent (1-2)	Absent
	Moist and swollen vulva	Moist and congested vulva	Mild swelling	No swelling
	Standing estrus	Stands to mount (>2)	Present (1-2)	Absent
	Seek ram	Follow ram (>2)	Follow (1-2)	Absent
Ram's Behaviour	Flehmen's response	Present (>2)	Present (1-2)	Absent
	Approaching ewe	Present (>2)	Present (1-2)	Absent
	Mounting	Present (>2)	Present (1-2)	Absent
Cumulative Score		21	14	7

Table 2. Seasonal variation in estrus related parameters using various estrus synchronization protocols in Gaddi ewes (N=36)

Parameter	Time Interval (hr)	Non-Breeding Season (n=18)			Breeding Season (n=18)		
		ProEP (n=6)	GPG (n=6)	Con (n=6)	ProEP (n=6)	GPG (n=6)	Con (n=6)
Onset of Estrus (OE)	≤24 hr	16.7 (1/6)	0.0 (0/6)	0.0^{A} (0/6)	16.7^{x} (1/6)	0.0^{x} (0/6)	66.7^{yB} (4/6)
	24-48 hr	66.7^{a} (4/6)	16.7^{b} (1/6)	16.7^{b} (1/6)	66.7^{x} (4/6)	66.7^{x} (4/6)	0.0^{y} (0/6)
	48-72 hr	0.0^{a} (0/6)	50.0^{bA} (3/6)	16.7^{ab} (1/6)	0.0 (0/6)	0.0^{B} (0/6)	0.0 (0/6)
Average Onset of Estrus (hr)		31.6±3.1^{a}	50.3±2.8^{b}	45.0±3.0^{ab}	26.0±1.1^{x}	43.0±3.1^{y}	15.0±1.3^{z}
Duration of Estrus (DE)	≤24 hr	33.3^{ab} (2/6)	66.7^{a} (4/6)	0.0^{b} (0/6)	0.0 (0/6)	33.3 (2/6)	0.0 (0/6)
	24-48 hr	50.0^{a} (3/6)	0.0^{b} (0/6)	16.7^{ab} (1/6)	33.3 (2/6)	33.3 (2/6)	50.0 (3/6)
	48-72 hr	0.0^{A} (0/6)	0.0 (0/6)	16.7 (1/6)	50.0^{xB} (3/6)	0.0^{y} (0/6)	16.7^{xy} (1/6)
Average Duration of Estrus (hr)		25.4±2.0^{aA}	21.0±1.3^{a}	35.0±3.0^{b}	55.0±5.0^{xB}	24.3±0.8^{y}	37.5±3.6^{y}
Estrus Response (ER)	Intense	50.0 (3/6)	33.3 (2/6)	16.7 (1/6)	50.0 (3/6)	33.3 (2/6)	50.0 (3/6)
	Fair	33.3 (2/6)	33.3 (2/6)	16.7 (1/6)	33.3 (2/6)	16.7 (1/6)	16.7 (1/6)
	Weak	16.7^{a} (1/6)	33.3^{ab} (2/6)	66.6^{b} (4/6)	16.7 (1/6)	50.0 (3/6)	33.3 (2/6)
Overall Estrus Response Rate (%)		83.3 (5/6)	66.7 (4/6)	33.3 (2/6)	83.3 (5/6)	50.0 (3/6)	66.7(4/6)
Estrus Intensity Score (EIS)		12.7±1.7	11.7±1.6	9.2±1.4	13.4±1.7	11.4±2.0	12.83±1.9

Values with different superscript (a,b and x,y) within season in same row differs ($p < 0.05$); Values with different superscript (A, B) between season in same row differs ($p < 0.05$); ProEP: Progestagen + eCG + $PGF_{2\alpha}$ group; GPG: GnRH + $PGF_{2\alpha}$ + GnRH group; Con: Control group; N: Total number of animals; n: Number of animals in particular group

increased estrogen production after induced luteolysis by $PGF_{2\alpha}$ and initiation of fresh follicular wave stimulated by administration of eCG resulting in better synchrony of estrus and ovulation.These findings align with earlier observations of early onset of estrus in progesterone-based protocols in Merino (31.2±2.1 hr; Nakafeero *et al.* 2020) and Pirlak ewes (31.9±1.8 hr; Kuru *et al.* 2020). Rasool *et al.* 2020 found that eCG accelerates follicular growth and progesterone withdrawal speeds up its clearance for earlier onset of estrus in ProEP based protocol. Previous studies have reported an early onset of estrus in progesterone and eCG-treated ewes, with Corriedale and Ossimi ewes exhibiting estrus between 24-28 hours and 25-30 hours (Ezzat *et al.* 2016; Lone *et al.* 2016), respectively during the non-breeding season. During the non-breeding season, a significantly higher percentage (50.0%, p<0.05) of GPG-treated ewes showed estrus between 48 and 72 hours. Additionally, a significantly higher proportion (66.7%, p<0.05) of control ewes exhibited estrus within 24 hours compared to the other groups.

Duration of Estrus (DE): Majority of ProEP treated ewes (83.3%) exhibited DE up to 48 hrs during NB season, which was significantly higher (48-72 hr; p<0.05) during B season (Table 2). Prolonged DE was also observed in ProEP (55.0±5.0 v/s 25.4±2.0 hr; p<0.05), GPG (24.3±0.8 v/s 21.0±1.3 hr) and Con (37.5±3.6 v/s35.0±3.0) Gaddi ewes, respectively in present the study during B and NB season (Table 2).

Shorter estrus durations were observed in crossbred ewes (24.5±1.6 hr; Yadav *et al.* 2020a), and Menz (25.4± 1.5 hr; Rekik *et al.* 2016) in progesterone-based protocols during NB season. Similar, short DE were also observed in crossbred (26.4±1.6 hr; Yadav *et al.* 2020a) and Ossimi (30 and 37 hr; Ezzat *et al.* 2016) ewes, respectively while using progesterone-based protocols during B season. Yadav *et al.* (2020a, 2021) compared DE while using Avikesil-S along with eCG in crossbred ewes during B (55.0±5.0 v/s 26.4±1.6 hr) and NB (25.4±2.0 v/s 24.5±1.6 hr; NB) season, respectively.

Variable DE were observed previously while using Ovsynch protocols ranging from lower values in Menz (18.6±1.7 hr; Rekik *et al.* 2016) to higher values in Rahmani (32.0±1.6 hr; Ashmawy 2011) ewes, respectively during B season. Shorter DE in GPG protocol could possibly be due to the action of second injection of GnRH, which might have initiated the LH surge required for ovulation and leading to fading of estrus signs.

Estrus Response (ER): An intense to fair degree of estrus response was observed in ProEP treated Gaddi ewes (83.3%) during both seasons, which was non-significantly (p>0.05) higher than GPG (66.7 v/s 50.0) and Con ewes (33.3 v/s 66.7) (Table 2). Significantly lower (p<0.05) proportion of ProEP treated Gaddi ewes (16.7 v/s 66.7) exhibited weak estrus during NB season as compared to control (Table 2). Non-significant differences (p>0.05) among estrus intensity scores (EIS) have also been observed earlier in Gaddi ewes irrespective of season. Kumar *et al.* 2016 observed higher proportion (100%) of fair to intense estrus response in ewes treated with progesterone and eCG combination during NB season. Proportion of ewes exhibiting weak estrus were higher in GPG treated Gaddi ewes during both B and NB seasons (33.3 and 50.0; Table 1). Variability in estrus response depends on hormonal concentrations and can mimic the ovulatory response of ewes under different synchronization protocols.

Estrus Response Rate (ERR): Estrus response rates were statistically insignificant (p>0.05) across groups and seasons (Table 2). During non-breeding season, various researchers observed lower ERR in Lacaune (79.2-81.8; Lombardo *et al.* 2020), similar ERR in Irani (83.3; Khamees *et al.* 2020), respectively and higher ERR in Turcana (96.7; Bogdan *et al.* 2011) ewes, than in Gaddi ewes (83.3%) using progesterone-based protocols.

During B season, estrus response rate was 50.0% in GPG treated Gaddi ewes, higher than Rahmani ewes (30.0; Ashmawy 2011). Higher ERR were reported earlier in Menz (73.3; Rekik *et al.* 2016). Lower ERR in GPG treated ewes in present study might be coupled with the dominant follicle formation after prostaglandin injection which ovulates early due to LH surge stimulated by second GnRH injection (Lone *et al.* 2016).

During the NB season, ERR in GPG treated Gaddi ewe was 66.7% which was much higher than earlier reports in Suffolk (25.0; Miguel-Cruz *et al.* 2019), Corriedale (28.57; Lone *et al.* 2016) and crossbred (53.3; Yadav *et al.* 2020b) ewes, respectively.

Conception Rates (CR): Transrectal ultrasonography was done beyond Day 28 to confirm pregnancy in Gaddi ewes. Non-significantly higher CR (80.0 v/s 100.0; p<0.05) were observed in ProEP treated Gaddi ewes than other groups during NB and B season (Table 3). Better CR (100.0, 66.7 and 75.0%) were observed during the B season in ProEP, GPG and Con Gaddi ewes in the present study.

CR observed in ProEP treated Gaddi ewes in present study were better than Corriedale and Texel (60.8%; Miranda *et al.* 2017), Merino (63.2-89.5 %; Nakafeero *et al.* 2020), Awassi (66.7-75.0; Swelum*et al.* 2019) and Ossimi (71.4-85.7%; Ezzat *et al.* 2016) at different geographical locations during B season. CR in ProEP treated Gaddi ewes were 80.0 per cent during NB season in present study. The CR in GPG treated Gaddi ewes varied from 50.0-66.7 per cent in NB and B season comparatively lower CR (40.0% v/s 66.7%) were observed in Rahmani ewes (Ashmawy 2011), during B season using GPG protocol.

Embryonic Losses (EL): Sequential TRUS (14-35 day) was undertaken to characterize the presence or absence of early embryonic features in Gaddi ewes during different seasons (Table 3). During NB season, only 25 per cent of the pregnant ewes underwent EL in ProEP treated group but, significantly higher (100%, p<0.05) EL occurred in Con ewes. Yadav *et al.* (2021) observed lower percent of EL in progesterone based and control crossbred ewes (7.7 v/s 50.0). Higher EL in Con group in present study were in consonance to earlier observations of Miranda *et al.* 2017(9.7 v/s 3.7) and Yadav *et al.* 2020a (25.0 v/s 0.0). Significantly higher (p<0.05) EL were observed in Con (50.0) than GPG (0.0) treated Gaddi ewes. Zero EL was observed in GPG treated Gaddi ewes irrespective of season. Contrarily, higher EL has earlier been reported in GPG treated ewes during both NB and B season (28.57 and 22.2), respectively (Yadav *et al.* 2020b). Hormonal imbalance, poor embryo quality and environmental changes were suggested as major factors associated with EL losses in ewes (Chundekkad *et al.* 2020).

Table 3. Seasonal variation in fertility parameters using different hormonal interventions following estrus synchronization in Gaddi ewes (N=36)

Fertility Parameters	Non-Breeding Season			Breeding Season		
	ProEP (n=6)	GPG (n=6)	Con (n=6)	ProEP (n=6)	GPG (n=6)	Con (n=6)
CR (%)	80.0	50.0	50.0	100.0	66.7	75.0
PR (%)	66.7^{a}	33.3^{ab}	16.7^{b}	83.3^{x}	33.3^{y}	50.0^{xy}
EL (%)	25.0^{a}	0.0	100.0^{b}	0.0	0.0	33.3
LR (%)	60.0^{a}	50.0^{a}	0.0^{bB}	100.0^{x}	66.7^{xy}	50.0^{yA}
LS	1.3±0.6	1.0±0.0	0.0	1.2±0.2	1.0±0.0	1.0±0.0
FR (%)	66.7^{a}	33.3^{ab}	0.0^{b}	100.0^{x}	33.3^{y}	33.3^{y}
P	0.8±0.2	0.5±0.2	0.0	1.2±0.2	0.7±0.3	0.5±0.3
GL	149.3±2.2	154.0±2.0	0.0	153.5±1.9	149.0±2.0	154.33±2.7

Values with different superscript(a,b and x,y) in same row differs (p< 0.05); Values with different superscript (A,B) between season in same row differs (p< 0.05); OE:Onset of estrus; DE: Duration of estrus; ERR: Estrus Response Rate; EIS: Estrus intensity score; CR: Conception Rate; PR: Pregnancy rate; EL: Embryonic losses; LR: Lambing Rate; FR: Fecundity rate: P: Prolificacy; LS: Litter Size; GL: Gestation Length; ProEP: Progestagen + eCG + $PGF_{2\alpha}$group; GPG: GnRH + $PGF_{2\alpha}$ + GnRH group; Con: Control group; N: Total number of animals; n: Number of animals in particular group

Pregnancy rates (PR): Significantly high PR (66.7 v/s 16.7%, $p<0.05$) were recorded in ProEP treated Gaddi ewes as compared Con group during NB season. It was also significantly higher (83.3 v/s 33.3%, $p<0.05$) in ProEP than GPG treated ewes during B season. Using progesterone-based protocols lower PR than observed in the present study (66.7%) has been reported earlier in Pirlak (40.0-46.7 %; Kuru *et al.* 2020) and Turcana breeds (65.9 %; Bogdan *et al.* 2011) whereas, higher PR have been reported in Lacaune (76.5-83.3; Lombardo *et al.* 2020), Santa Ines (81.0; Silva *et al.* 2020), Assaf (83.3; Atalla 2018) and in crossbred ewes treated with Avikesil-S and eCG (92.31; Yadav *et al.* 2020a).

During B season, lower PR were recorded earlier in Awassi (15.5-65.0 %; Swelum *et al.* 2018), Corriedale and Texel (33.5%; Miranda *et al.* 2017), Lacaune (45.0-75.0%; Martinez-Ros *et al.* 2018), Segurena (68.4%; Martinez-Ros and Gonzalez-Bulnes 2019) ewes compared to ProEP treated Gaddi ewes (83.3%). Similar PR were also observed earlier in Turcana (Bogdan *et al.* 2011) and Irani ewes (Khamees *et al.* 2020) using different progesterone based protocols. Comparatively higher PR were also recorded previously in Rambouillet (91.0 %; Rassaaco *et al.* 2019) ewes.

In GPG treated Gaddi ewes during both B and NB seasons, pregnancy rates were 33.3 %. Higher PR than Gaddi ewes were observed in crossbred (90.0; Yadav *et al.* 2020b) during B season. Whereas, during NB season, higher PR among crossbred (87.5; Yadav *et al.* 2020b) were comparable to that in GPG treated Gaddi ewes.

Lambing Rates (LR): Significantly higher ($p<0.05$) LR was observed in ProEP and GPG treated Gaddi ewes (60.0 and 50.0%) during NB season whereas, during B season only ProEP treated ewes (100%) showed significantly higher ($p<0.05$) LR than. In progesterone-based protocols, lower LR were also observed by various researchers' in Malpura and Merino (52.6%; Nakafeero *et al.* 2020), Rambouillet (70.8%; Rosasco *et al.* 2019), Lacaune (75.0-90.0%; Martinez-Ros *et al.* 2018), Menz (77.8%; Besufkad 2019), and in crossbred ewes (86.6%; Yadav *et al.* 2020a), respectively. In GPG treated Gaddi ewes lambing rates in present study were 50.0 per cent during NB season which were quite lower as compared to crossbred ewes (62.4%; Yadav *et al.* 2020b). During B season, LR observations in Gaddi ewes synchronized with GPG protocol and were lower than observations in crossbred (70.0%; Yadav *et al.* 2020b) ewes.

Fecundity rate (FR): Significantly higher ($p<0.05$) FR were observed in ProEP treated Gaddi ewes in the present study during NB and B seasons (Table 3). FR observed in ProEP treated Gaddi ewes during NB season was lower than Barki (70.0; El-Mokadem *et al.* 2019). During B season, FR in ProEP treated ewes (100.0%) was higher than Awassi (61.1; Swelum *et al.* 2019), crossbred (70.0; Yadav *et al.* 2020b), Merino (78.8; Cueto *et al.* 2020) and similar to Menz ewes (100.0; Besufkad 2019). FR observed in GPG treated Gaddi ewes (33.3%) was lower than crossbred (62.5; Yadav *et al.* 2020b) during NB season and Rahmani ewes (50.0; Ashmawy 2011) during B season.

Litter size (LS): Non-significant variation in litter size were observed during nonbreeding and breeding season in Gaddi ewes. During NB season, litter size in ProEP treated Gaddi ewes was 1.3±0.6 which was higher than Pirlak (1.1; Kuru *et al.* 2020). Similar LS have earlier been reported in Assaf ewe (Atalla, 2018). Similarly, during B season LS in ProEP treated ewes (1.2±0.2) was higher than observed in Irani (1.1; Khameees*et al.* 2020) and Menz ewes (1.0; Besufkad 2019), respectively. Higher LS than present study were earlier reported in Rambouillet (1.3; Rosasco *et al.* 2019). Litter size in GPG treated Gaddi ewes (1.0±0.0) was lower than Rahmani (1.2, Ashmawy 2011; 1.6±0.15) ewes during B season.

Prolificacy (P): In all treatment groups during breeding and non-breeding seasons, prolificacy rates did not differ significantly (Table 2). During non-breeding season, prolificacy results in ProEP treated Gaddi ewes (0.8±0.2) were similar to those observed in Turcana ewes (0.8; Bogdan *et al.* 2011) and higher prolificacy rates were recorded in Barki ewes (1.5; El-MoKadem *et al.* 2019). During breeding season, prolificacy rate observed in ProEP treated Gaddi ewes was 1.2±0.2 which was higher than in Turcana ewes (1.0; Bogdan *et al.* 2011) and Merino ewes (1.0; Cueto *et al.* 2020). Prolificacy in GPG treated Gaddi ewes during NB and B season (0.5±0.2 and 0.7±0.3) were lower than earlier reports in crossbred ewes (1.0; Yadav *et al.* 2020b).

Gestation length (GL): In this present study, gestation length was not statistically significant ($p>0.05$) among different groups. The gestation length in ProEP treated Gaddi ewes during B season was reported as 153.5±1.9 days which was lower reported than that in Merino with application of CIDR and ram effect(161.4±2.9; Nakafeero *et al.* 2020) and in Rambouillet ewes administered with $PGF_{2\alpha}$ (158.9; Rosasco *et al.* 2019). In the GPG group during B season GL was 149.3±2.2 days, lower in comparison with Egyptian ewes (151.5±1.28; Daghash *et al.* 2017).

In conclusion, ProEP treated Gaddi ewes performed better with earlier onset of estrus along with significantly higher pregnancy and tecundity rate both in breeding and non-breeding season compared to GPG protocol. ProEP protocol carries significant potential to contribute to increasing flock productivity, better genetic improvement, and higher profitability for farmers involved in sheep husbandry.

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