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Comparative study of reproductive efficiency in EWES synchronized with vaginal sponges and CIDR during breeding and non-breeding seasons

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Abstract
The present study was aimed to determine the comparative study of Reproductive efficiency in ewes synchronized with Vaginal Sponges and CIDR during breeding and nonbreeding seasons. The ewes were synchronized with Vaginal Sponges containing 30 mg of Flurogestone Acetate (FGA) and controlled internal drug releasing (CIDR) device containing 300 mg of progesterone. A total of 120 postpartum, parous, healthy ewes aged about 2 to 5 years were divided into 5 groups and each group consists of 24 animals. Each group was further subdivided into 2 groups consists of 12 animals and were studied during breeding and non-breeding seasons. Group I ewes were considered as untreated control. Ewes in Group II were treated with Vaginal Sponges and CIDR were left in place for 12 days followed by intramuscular injection of 400 IU of PMSG at the time of device removal. Ewes in Group III were treated with Vaginal Sponges and CIDR and 600 IU of PMSG was given intramuscularly at the time of removal of Vaginal Sponges or CIDR. Ewes in Group IV were treated as in Group II and additionally supplementation 200 IU of hCG injection at the time of mating. Ewes in Group V were treated as in Group III and additional injection of 200 IU of hCG at the time of mating. In ewes treated with Vaginal Sponges the pregnancy rate was 50.00, 80.00, 91.67, 81.82 and 100.00 per cent in control, VS 4, VS 6, VS 4h and VS 6h, respectively during breeding season. In non-breeding season, the pregnancy rate was 66.67, 70.00, 83.33, 80.00 and 91.67 per cent in control, VS 4, VS 6, VS 4h and VS 6h, respectively. Among the CIDR treated ewes, the pregnancy rate was 50.00, 70.00, 83.33, 72.72 and 91.67 per cent in control, CIDR 4, CIDR 6, CIDR 4h and CIDR 6h, respectively in breeding season. In non-breeding season, the same values were 50.00, 60.00, 75.00, 70.00 and 83.33 per cent in control, CIDR 4, CIDR 6, CIDR 4h and CIDR 6h, respectively. The pregnancy rates were significantly (P<0.01) higher in ewes synchronized with vaginal sponge than CIDR in both seasons. Significantly (P<0.01) higher lambing rates were recorded in vaginal sponge (113.73 per cent) than the CIDR (98.04 per cent) treatment during breeding season. While in non-breeding season there was no significant difference between vaginal sponges (102.13 per cent) and CIDR (91.30 per cent) treatments. The litter size was not significantly differed in vaginal sponge (1.25±0.07 and 1.23±0.07) and CIDR (1.23±0.07 and 1.21±0.08) in breeding and non-breeding seasons, respectively. During breeding season, significantly (P<0.01) higher per cent of multiple births were recorded in VS6h/CIDR6h (34.29), followed by VS6/CIDR6 (27.59), VS4h/CIDR4h (19.05), VS4/CIDR4 (11.76) and control (0.00) group of ewes while in non-breeding season, significantly (P<0.01) higher per cent of multiple births were recorded in VS6h/CIDR6h (30.00), followed by VS6/CIDR6 (24.00), VS4h/CIDR4h (16.67), VS4/CIDR4 (13.33) and control (0.00) group of ewes.

Keywords: Reproductive efficiency, EWES synchronized, vaginal sponges, CIDR

1. Introduction
A large number of sheep remain unsettled in the farmer's field due to one or other reproductive problems and are slaughtered every year. The economically important and most commonly occurring reproductive disorder of sheep is anestrus, which causes huge economic loss to the farmers due to low fecundity and longer inter lambing period (< 1 lamb/year). Majority of the indigenous sheep breeds are mono-ovulator, which is major limitation in increase their productivity. Increasing the percentage of lamb crop and number of lambs marked are the primary two goals of sheep producers.

Different protocols have been developed for estrus induction in sheep but the information on cost effective, farmer-oriented and field applicable estrus induction protocol under semi-arid environment is scanty. Improvement of reproductive traits of sheep can be achieved by using several reproductive technologies. Since sheep breeding in India is non-controlled, an attempt to make to control the reproduction by controlling estrus cycles artificially during breeding and non-breeding seasons with the Vaginal Sponges and CIDR.

2. Materials and Methods

A total of 120 non-pregnant, healthy and parous ewes aged about 2 to 5 years (60 days postpartum) belonging to different flocks were selected. The selected ewes were studied during breeding (September to October) and nonbreeding (January to February) seasons.

The selected ewes were divided into five groups in such a manner that each major group consisting of 24 ewes. Each group of 24 ewes was divided into 2 sub-groups so that each sub group consisted of 12 ewes each. In each group 12 ewes were subjected to synchronization of estrus during the breeding season and12 ewes in non-breeding season.

Group 1 (n=24) served as controls & received no treatment.

In group 2 (n=24) The ewes were inserted with the Vaginal Sponges and CIDR for 12 days and 400 IU of PMSG was injected intramuscularly at the time of removal of sponges or CIDR.

In group 3 (n=24) the ewes were inserted with Vaginal Sponges and CIDR for 12 days and 600 IU of PMSG was administered intramuscularly at the time of removal of Vaginal Sponges or CIDR.

In group 4 (n=24) The ewes were treated with Vaginal Sponges and CIDR for 12 days, 400 IU of PMSG was injected intramuscularly at the time of removal of vaginal sponges and CIDR and injected 200 IU of hCG intramuscularly at the time of mating.

In group 5 (n=24) ewes were inserted with Vaginal Sponges and CIDR for 12 days and 600 IU of PMSG at the time of removal of sponges or CIDR and 200 IU of hCG at the time of mating was given.

Ewes of all groups were monitored for the symptoms of estrus by using a teaser ram daily 4 times with an interval of 6 hours for the duration of 30 minutes for five days after withdrawal of Vaginal Sponges or CIDR. The ewes were subjected to pregnancy diagnosis by trans-abdominal approach using real time B-mode ultrasonography (5 to 7.5 MHz).

3. Results and Discussion

The efficacy of treatment for reproductive efficiency was measured in terms of pregnancy, lambing, litter size and twinning rates and the data were presented in Table 1 and 2.

3.1 Pregnancy Rate

The comparative pregnancy rate during breeding season in vaginal sponge Vs. CIDR was 80.00 Vs. 70.00 per cent in VS4 vs. CIDR4; 91.67 Vs. 83.33 per cent in VS6 vs. CIDR6; 81.82 Vs. 72.72 per cent in VS4h Vs. CIDR4h and 100.00 Vs. 91.67 per cent in VS6h Vs. CIDR6h, respectively. Whereas in untreated control, the pregnancy rate in vaginal sponge Vs. CIDR was 50.00 vs. 50.00 per cent during breeding season. While in non-breeding season, the same were 70.00 Vs. 60.00 per cent in VS4 vs. CIDR4; 83.33 Vs. 75.00 per cent in VS6 vs. CIDR6; 80.00 Vs. 70.00 per cent in VS4h vs. CIDR4h and 91.67 Vs. 83.33 per cent in VS6h vs. CIDR6h, respectively. Whereas in untreated control, the pregnancy rate in vaginal sponge Vs. CIDR was 66.67 vs. 50.00 per cent during non-breeding season which was in alignment with the observations of Moeini *et al*. (2007) [19]. The pregnancy rate was significantly (P<0.01) higher in ewes synchronized with vaginal sponge than CIDR in both seasons. Significantly higher pregnancy rates were recorded in VS6h/CIDR6h group followed by VS6/CIDR6 and VS4h/CIDR 4h group of ewes, but there was no significant difference between VS4 and control group of ewes.

Variation in pregnancy rate with CIDR treatment might be due to differences in individual ewes body condition, breed and management systems (Yadi *et al.,* 2011) [24].

In hCG treated group of ewes, the pregnancy rates were high which might be due to increased progesterone production (Khan *et al.,* 2007) [16] and uterine secretions. This action of hCG was embryotrophic and might have resulted in stronger signal for maternal recognition of pregnancy through secreting higher quantities of interferons (Nephew *et al.,* 1994) [20] from conceptus thereby weakening the luteolytic signals. Human chorionic gonadotropin was found to be luteotrophic by converting small luteal cells to large luteal cells (Moeini *et al.,* 2009) [18] or by increasing the size of large luteal cells (Fitz *et al.,* 1982) thereby increased production of progesterone (Kelidari *et al.,* 2010) [9]. Without hCG these embryos would be degenerated (Moeini *et al.* 2009). In nutshell, the variation in pregnancy rates were also attributed to type of vaginal devices, kind of progesterone, breed (Yadi *et al.,* 2011) [24], individual animal, nutritional conditions (Nosrati *et al.,* 2011) [13], latitude and time of hormonal treatment in the year as suggested by Ozyurtlu *et al.* (2008) [21].

3.2 Lambing Rate

Comparative lambing rates of vaginal sponge and CIDR were 90.00 and 80.00 per cent in VS4 and CIDR4; 133.33 and 108.33 per cent in VS6 and CIDR6; 100.00 and 90.91 per cent in VS4h and CIDR4h; 158.33 and 133.33 per cent in VS6h and CIDR6h, respectively during breeding season. Whereas in untreated control, the lambing rate in vaginal sponge and CIDR was 50.00 and 50.00 per cent during breeding season. While in non-breeding season, the same were 80.00 and 70.00 per cent in VS4 and CIDR4; 108.33 and 100.00 per cent in VS6 and CIDR6; 100.00 and 80.00 per cent in VS4h and CIDR4h and 133.33 and 116.67 per cent in VS6h and CIDR6h, respectively. Whereas in untreated control, the lambing rate in vaginal sponge and CIDR was 33.33 and 50.00 per cent during non-breeding season. Significantly (P<0.01) higher lambing rates were recorded in vaginal sponge than the CIDR during breeding season. While in non-breeding season there was no significant difference between vaginal sponge and CIDR treatments. The result of lambing rate was in line with the studies of Moeini *et al*. (2007) [18] who observed almost similar lambing rate in both CIDR and FGA treatments.

Lower lambing rate might be also due to greater possibility of loss of single ova as compared to all multiple ova in prolific breeds (Naqvi *et al.,* 1997) [12]. However, higher lambing rate in the present study might be due to hCG injection at the time of mating (Khan *et al.,* 2003) [17].

3.3 Litter Size

Comparative litter size in vaginal sponge Vs. CIDR treatment was 1.17±0.17 Vs. 1.14±0.14 in VS4 Vs. CIDR4 group; 1.40±0.16 Vs. 1.30±0.15 in VS6 Vs. CIDR6 group; 1.13±0.13 Vs. 1.25±0.16 in VS4h Vs. CIDR4h group and 1.55±0.16 Vs. 1.45±0.16 in VS6h Vs. CIDR6h group, respectively during breeding season. Whereas in untreated control, the litter size in vaginal sponge Vs. CIDR was 1.00±0.00 vs. 1.00±0.00 during breeding season. While in non-breeding season, the same were 1.14±0.14 vs. 1.17±0.17 in VS4 vs. CIDR4 group; 1.30±0.15 Vs. 1.33±0.17 in VS6 Vs. CIDR6 group; 1.25±0.16 Vs. 1.14±0.14 in VS4h Vs. CIDR4h group and 1.45±0.16 Vs. 1.40±0.16 in VS6h Vs. CIDR6h group, respectively. Whereas

in untreated control, the litter size in vaginal sponge Vs. CIDR was 1.00±0.00 vs. 1.00±0.00 during non-breeding season. The litter size was not significantly differed in vaginal sponge and CIDR treatments in breeding and non-breeding seasons. Significantly higher litter size was obtained in VS6h/CIDR6h followed by VS6/CIDR6 groups, VS4h/CIDR4h, VS4/CIDR4 and control group of ewes in breeding and non-breeding seasons.

The increased litter size in the present study with PMSG administration was in line with the studies of Yadi *et al.* (2011) [24] who observed higher litter size might be due to PMSG injections. Contradictory to this, Moeini *et al.* (2009) [18] found no significant difference in litter size between 300, 450 and 600 IU of PMSG injections. But PMSG was believed to increase the number of follicles and therefore raised twinning and triplets (Timurkan and Yildiz, 2005) [23] which might be responsible for recording the higher twinning rate in this study.

The variation in litter size might be due to differences in the management system, age of the dam, body condition and breed of the experimental sheep (Moeini *et al.,* 2007) [18], time and dose of PMSG administration (Timurkan and Yildiz, 2005 [23] and Safdarian *et al.,* 2006) and supplementation of hCG at the time of mating (Timurkan and Yildiz, 2005) [23].

3.4 Twinning Rate

The comparative twinning rates during breeding season in vaginal sponge Vs. CIDR were 11.11 Vs. 12.50 per cent in VS4 Vs. CIDR4; 31.25 Vs. 23.08 per cent in VS6 Vs. CIDR6; 18.18 Vs. 20.00 per cent in VS4h Vs. CIDR4h and 36.84 Vs. 31.25 per cent in VS6h Vs. CIDR6h, respectively. Whereas in untreated control, the twinning rate in vaginal sponge Vs. CIDR was 0.00 vs. 0.00 per cent during breeding season. While in non-breeding season, the same were 12.50 Vs. 14.29 per cent in VS4 vs. CIDR4; 23.08 Vs. 25.00 per cent in VS6 vs. CIDR6; 20.00 Vs. 12.50 per cent in VS4h vs. CIDR4h and 31.25 Vs. 28.57 per cent in VS6h vs. CIDR6h, respectively. Whereas in untreated control, the twinning rate in vaginal sponge Vs. CIDR was 0.00 vs. 0.00 per cent during non-breeding season. There was no significant difference in twinning rates between vaginal sponge and CIDR treatments in both seasons. But, significantly (P<0.01) higher per cent of multiple births were recorded in VS6h/CIDR6h group followed by VS6/CIDR6, VS4h/CIDR4h, VS4/CIDR4 and control group of ewes in breeding and non-breeding seasons.

Administration of 600 IU of PMSG at the time of withdrawal of progesterone device in this study might be more effective which was in agreement with the studies of Timurkan and Yildiz (2005) [23] and Nosrati *et al.* (2011) [13]. But administration of 700 IU of PMSG had increased 4 to 5 ovulations but decreased pregnancy and twinning rates, which might be due to the long half-life of PMSG which causes an abnormal follicular environment and premature ageing of oocytes before ovulation. Hence, resulted in lower fertility rates (Timurkan and Yildiz, 2005) [23]. Differences in prolificacy was also attributed to body condition of the ewes (Soria-Rojas *et al.,* 2011) [14].

In the present study, the dose of 400 IU of PMSG was less effective to induce twin births. The present dose of PMSG might not be sufficient to stimulate additional follicular development or weak response of the breed to the treatment protocol as reported by Koyuncu *et al.* (2008) [10] and Moeini *et al.* (2009) [18] who suggested that response to the different PMSG doses among various breeds was also different. It was also reported that the PMSG injection at the time of removal of CIDR found to increase the ovulation rate and thereby increased multiple births (Akoz *al.,* 2006) [2], ovulation rates and litter size (Moeini *et al.,* 2009) [18].

The twinning rates in the present investigation were lower in VS4 and CIDR4 groups when compared to the hCG administered groups. This might be due to the fact that hCG was a good exogenous source of LH to accelerate ovulation rates (Akoz *et al.,* 2006) [2] and accessory corpora lutea (Khan *et al.,* 2003) [17] thereby leads to greater litter size (Moeini *et al.,* 2009) [18]. Further it was reported that hCG improves the embryo survival and increases the number of placentomes (Khan *et al.,* 2007 [16] and Moeini *et al.,* 2009) [18] through the stimulatory effect of hCG induced progesterone (Akim Cam and Kuran, 2004) [1] which might be responsible for higher twinning rates in hCG treated ewes in this study. Fertility parameters could also be influenced by different seasons such as anestrus, breeding or transitional season (Zonturlu *et al.,* 2011) [15]. The mentioned factors might have played role in bringing variation in the present study.

Variation in overall response to the different protocols in terms of pregnancy rates, lambing rate, litter size and twinning rate might be attributed to dose of PMSG (Almariol, 2010 [3], Nosrati *et al.,* 2011 [13] and Yadi *et al.,* 2011) [24]; number of PMSG injections (Hamra *et al.,* 1989); type of semen used either frozen or fresh (Nosrati *et al.,* 2011) [13]; type of progesterone content used (Husein and Kridli, 2002 [2] and Almariol, 2010) [3]; type of progesterone device (Moeini *et al.,* 2007) [19]; time of PMSG injection (Nosrati *et al.,* 2011) [13]; route of PMSG administration (Zeleke *et al.,* 2005); ram effect (Husein *et al.,* 2007) [2]; breed, age of ewes and season (Moeini *et al.,* 2007) [19]; day of treatment of estrous cycle (Husein *et al.,* 2007) [2]; mating system (Nosrati *et al.,* 2011) [13]; body condition (Yadi *et al.,* 2011) [24] and management system (Yadi *et al.,* 2011) [24].

Intravaginal progesterone devices insertion for 12 days and their removal induce estrus appearance simulating an effect of drastic reduction of progesterone levels in blood and luteolysis followed by onset of follicular activity and appearance of estrus 1 or 2 days afterwards in small ruminants (Martinez-Tinajero *et al.,* 2011) [11]. The variation in response to different protocols in this study might be attributed to different endogenous status (Das *et al.,* 2004) [4] as well as prolificacy of particular sheep breed (Moeini *et al.,* 2007) [19].

Hence, vaginal sponges and CIDR can be used interchangeably based on their availability and cost effectiveness. It was observed that the both devices are most effective in synchronizing the estrous cycle and increasing the reproductive performance in the present study.

Table 1: Comparative study of vaginal sponge with CIDR treatment on pregnancy rate, lambing rate, litter size and twinning rate during breeding season in ewes.

Sl. No	Treatment Group	Pregnancy Rate (%)			Lambing Rate (%)			Litter Size (Mean ± S.E)			Twinning Rate (%)		
		Season			Season			Season			Season		
		Vaginal sponge	CIDR	Overall	Vaginal sponge	CIDR	Overall	Vaginal sponge	CIDR	Overall	Vaginal sponge	CIDR	Overall
1	Control	50.00 (45.00)	50.00 (45.00)	50.00^{b} (45.00)	50.00 (45.00)	50.00 (45.00)	50.00^{d} (45.00)	1.00 ±0.00	1.00 ±0.00	1.00^{b} ±0.00	0.00 (0.00)	0.00 (0.00)	0.00^{c} (0.00)
2	VS4 CIDR 4	80.00 (63.44)	70.00 (56.79)	75.00^{b} (60.12)	90.00 (71.56)	80.00 (63.44)	85.00^{c} (67.50)	1.17 ±0.17	1.14 ±0.14	1.16^{b} ±0.09	11.11 (19.46)	12.50 (20.70)	11.76^{b} (20.08)
3	VS 6 CIDR 6	91.67 (73.26)	83.33 (65.88)	87.50^{ab} (69.57)	133.33 (125.24)	108.33 (106.74)	120.83^{b} (115.99)	1.40 ±0.16	1.30 ±0.15	1.35^{ab} ±0.10	31.25 (34.02)	23.08 (28.73)	27.59^{ab} (31.35)
4	VS 4h CIDR 4h	81.82 (64.75)	72.72 (60.47)	77.27^{b} (61.63)	100.00 (90.00)	90.91 (72.44)	95.46^{c} (81.22)	1.13 ±0.13	1.25 ±0.16	1.19^{b} ±0.11	18.18 (25.25)	20.00 (26.56)	19.05^{ab} (25.91)
5	VS 6h CIDR 6h	100.00 (90.00)	91.67 (73.26)	95.83^{a} (81.63)	158.33 (139.78)	133.33 (125.24)	145.83^{a} (132.51)	1.55 ±0.16	1.45 ±0.16	1.50^{a} ±0.11	36.84 (37.35)	31.25 (34.02)	34.29^{a} (35.69)
	Overall	84.31^{a} (67.29)	76.47^{b} (60.28)	80.39 (63.72)	113.73^{a} (94.36)	98.04^{b} (82.57)	105.88 (104.06)	1.25^{NS} ±0.07	1.23^{NS} ±0.07	1.32 ±0.05	25.86^{NS} (23.22)	22.45^{NS} (22.00)	24.07 (29.80)

Figures in parenthesis indicated angular values Means bearing different superscripts differed significantly ** *P*<0.01 NS: Non significant

Table 2: Comparative study of vaginal sponge with CIDR treatment on pregnancy rate, lambing rate, litter size and twinning rate during non-Breeding season in ewes.

Sl. No	Treatment Group	Pregnancy Rate (%)			Lambing Rate (%)			Litter Size (Mean ± S.E)			Twinning Rate (%)		
		Season			Season			Season			Season		
		Vaginal sponge	CIDR	Overall	Vaginal sponge	CIDR	Overall	Vaginal sponge	CIDR	Overall	Vaginal sponge	CIDR	Overall
1	Control	66.67 (54.76)	50.00 (45.00)	60.00^{c} (49.88)	33.33 (35.24)	50.00 (45.00)	40.00^{b} (40.12)	1.00 ±0.00	1.00 ±0.00	1.00^{b} ±0.00	0.00 (0.00)	0.00 (0.00)	0.00^{c} (0.00)
2	VS4 CIDR 4	70.00 (56.79)	60.00 (50.77)	65.00^{c} (53.78)	80.00 (63.44)	70.00 (56.79)	75.00^{b} (60.12)	1.14 ±0.14	1.17 ±0.17	1.16^{b} ±0.10	12.50 (20.70)	14.29 (22.22)	13.33^{b} (21.46)
3	VS 6 CIDR 6	83.33 (65.88)	75.00 (60.00)	79.17^{b} (62.94)	108.33 (106.64)	100.00 (90.00)	104.17^{a} (98.37)	1.30 ±0.15	1.33 ±0.17	1.32^{ab} ±0.11	23.08 (28.73)	25.00 (30.00)	24.00^{ab} (29.37)
4	VS 4h CIDR 4h	80.00 (63.44)	70.00 (56.79)	75.00^{b} (60.12)	100.00 (90.00)	80.00 (63.44)	90.00^{b} (76.72)	1.25 ±0.16	1.14 ±0.14	1.20^{b} ±0.11	20.00 (26.56)	12.50 (20.70)	16.67^{b} (23.63)
5	VS 6h CIDR 6h	91.67 (73.26)	83.33 (65.88)	87.50^{a} (69.57)	133.33 (125.24)	116.67 (114.20)	125.00^{a} (119.72)	1.45 ±0.16	1.40 ±0.16	1.43^{a} ±0.11	31.25 (34.02)	28.57 (32.39)	30.00^{a} (33.21)
	Overall	80.85^{a} (62.83)	71.74^{b} (55.69)	76.34 (60.87)	102.13^{NS} (84.11)	91.30^{NS} (73.89)	96.77 (79.69)	1.23^{NS} ±0.07	1.21^{NS} ±0.08	1.28^{NS} ±0.05	22.45^{NS} (22.00)	21.43^{NS} (21.06)	34.48 (35.97)

Figures in parenthesis indicated angular values Means bearing different superscripts differed significantly ** *P*<0.01NS: Non significant

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