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Study of progesterone concentration in EWES synchronized with vaginal sponges

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Abstract
The present study was aimed to determine the progesterone profiles in ewes synchronization with Vaginal Sponges. The ewes were synchronized with Vaginal Sponges containing 30 mg of Flurogestone Acetate (FGA). 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 nonbreeding seasons. Group I ewes were considered as untreated control. Ewes in Group II were treated with Vaginal Sponges and 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 600 IU of PMSG was given intramuscularly at the time of removal of Vaginal Sponges. 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. Plasma progesterone concentrations of experimental ewes were measured by enzyme-linked immunosorbant assay (ELISA). The progesterone levels before insertion were 1.23±0.12 ng/ml and 2.46±0.11, 3.40±0.13 and 4.71±0.14 ng/ml on day 3, 6 and 9 of treatment, respectively in breeding season. In nonbreeding season, the progesterone levels were 0.96±0.12 ng/ml before insertion of vaginal sponges. During treatment, the progesterone levels were 1.62±0.13 ng/ml on day 3, 2.32±0.11 ng/ml on day 6, 3.35 ±0.14 ng/ml on day 9 and 3.19±0.12 ng/ml on day 12 of treatment (at the time of removal). The progesterone levels were significantly (P<0.01) increased from day 0 to day 9 of treatment and thereafter it was significantly (P<0.01) decreased on day 12 of treatment. Significantly, higher progesterone levels were recorded in all groups of ewes inserted with vaginal sponges (2.76±0.06 to 2.84±0.07 ng/ml) compared to control group of ewes (1.67±0.08 ng/ml).

Keywords: Progesterone concentration, EWS synchronized, vaginal sponges

1. Introduction
Sheep husbandry plays a significant role in sustaining the rural economy of arid and semi-arid regions of the country. The number of offspring per female is a major determinant of sustainability of sheep industry in general and meat production in particular. Sheep farming is generally carried out as a dry land enterprise under adverse environmental conditions. In this kind of harsh environment, the sheep develop better survival character at the expense of their ability to produce. Hence, the reproductive efficiency of most of the flocks is relatively low (Mittal *et al.,* 2004) [10]. Therefore assisted reproduction technology such as estrus synchronization is considered to be a useful tool to enhance reproductive efficiency
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Of local sheep by enhancing pregnancy rates with high prolificacy in shorter duration during nonbreeding season.
Estrus synchronization in sheep is achieved by control of the luteal phase of estrous cycle, either by providing exogenous progesterone or by inducing premature lute lysis. The latter approach is not applicable during seasonal anestrus, whereas exogenous progesterone in combination with gonadotropin can be used to induce and synchronize estrus in an ovular ewes and does. Exogenous hormonal regimen used to induce fertility in anestrus ewes consists of 12–16 day progesterone treatment followed by injection of gonadotropin.

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 for 12 days and 400 IU of PMSG was injected intramuscularly at the time of removal of sponges.

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

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

In group 5 (n=24) ewes were inserted with Vaginal Sponges for 12 days and 600 IU of PMSG at the time of removal of sponges 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 intravaginal Vaginal Sponges. The plasma progesterone profiles were studied on day 0, 3, 6, 9 and 12 of treatment. The Plasma progesterone concentrations of experimental ewes were measured by enzyme-linked immunosorbant assay (ELISA).

3. Results and Discussion

3.1 Progesterone Profiles

The progesterone levels in ewes synchronized with Vaginal Sponges were presented in Table 1. The progesterone levels were 1.05±0.13, 1.94±0.19, 2.53±0.26, 3.81±0.43 and 2.64±0.47 ng/ml on day 0, 3, 6, 9 and 12th day of observation, respectively in control group of ewes. While in nonbreeding season, the progesterone levels were 0.98±0.08, 0.87±0.04, 1.01±0.06, 0.96±0.11 and 0.91±0.07 ng/ml on day 0, 3, 6, 9 and 12th day of observation, respectively in control group of ewes.

Similar progesterone levels were also recorded in untreated ewes by Sudhir Chandra Reddy *et al.* (1989) [16] and Hussein *et al.* (1998). Higher levels of progesterone were reported by Ezzo *et al.* (1992) [2] and lower levels of progesterone were recorded by Vinoles *et al.* (2001) [19] and Hussein and Kridli (2002) [2] in untreated ewes. Contrary to this, Murray *et al.* (1994) [12] reported that progesterone levels were undetectable during seasonal anestrus in ewes. Overall progesterone levels in ewes synchronized with vaginal sponge were 1.67±0.08, 2.84±0.10, 2.78±0.09, 2.76±0.06 and 2.84±0.07 ng/ml in control, VS4, VS6, VS4h and VS6h groups, respectively. The progesterone levels were significantly (P<0.01) higher than the control group of ewes.

Overall progesterone levels were 1.23±0.12 ng/ml prior to the treatment, 2.46±0.11, 3.40±0.13 and 4.71±0.14 ng/ml on day 3, 6 and 9 of treatment, respectively and 2.56±0.16 ng/ml at the time of removal of vaginal sponge during breeding season. The same for nonbreeding season was 0.96±0.12 ng/ml prior to treatment, 1.62±0.13, 2.32±0.11 and 3.35±0.14 ng/ml on day 3, 6 and 9, respectively and 3.19±0.12 ng/ml on day 12 of treatment.

Similar studies were also carried with respect to vaginal sponge and PMSG with different kinds of progesterone preparations and variable doses of progesterone and PMSG by Todini *et al.* (2007) [18], Ralchev *et al.* (2008) [14], Takada *et al.* (2009) [17], Letelier *et al.* (2009) [8] and Naderipour *et al.* (2012) [13]. Progesterone levels recorded in the present study were in accordance with findings of Moeini *et al.* (2009) [11].

In the present study, the progesterone levels were gradually increased and reached peak levels on day 9 of insertion of vaginal sponge and later significantly decreased on the day of sponge removal (day 12) in breeding season. Similar trend was also noticed in ewes during nonbreeding season but the progesterone levels were significantly (P<0.01) lower than the progesterone levels recorded during breeding season. Similar trend of increasing progesterone levels after 6 to 10 days of insertion of vaginal sponge were also reported by Husein and Ababneh (2008) [5] and Naderipour *et al.* (2012) [13].

Increasing and decreasing trends of progesterone levels in ewes inserted with vaginal sponges was in corroboration with the studies of Hamra *et al.* (1986) [4] who stated that progesterone concentration started to increase within 24 h to near maximum levels, reached highest levels on day 4 and then declined. But in the present study, the progesterone levels reached maximum levels on day 9 and started to decline on day 12 (at the time of removal of sponges). But in previous experiment, Hamra *et al.* (1986) [4] had utilized ovariectomized ewes in his experiment which might be the reason for variation in the progesterone levels. However, low progesterone levels were recorded in overiectomized ewes than the present study during the period the vaginal sponge kept in place. The progesterone levels during the period of vaginal sponge kept in place were also comparable with progesterone levels recorded by Schoombee *et al.* (1989) [15] where the vaginal sponges were inserted during different days of estrous cycle i.e. on day 0, 2, 6 and 12 of estrous cycle. The present findings were also in corroboration with the findings of Naderipour *et al.* (2012) [13] with little variation in progesterone levels.

The progesterone levels were significantly (P<0.01) at lower levels during nonbreeding season than breeding season in the present study which was in line with the studies of Ezzo *et al.* (1992) [2] who reported that season had a significant influence on concentration of progesterone in ewes. The progesterone concentration at the time of removal of sponges were significantly (P<0.01) higher compared to that of control group which was in corroboration with the studies of Husein and Kridli (2002) [2] and might be due to exogenous treatment. The ewes administered with PMSG in conjunction with vaginal sponge, the progesterone levels were believed to be increased upto 8 days after ovulation, maintained relatively constant and reached the highest concentration after 13 days of ovulation as observed by Levya *et al.* (1998a). Perhaps the same mechanism might have existed in the present study in setting of good fertility rates when compared with untreated ewes. Thus the vaginal sponge inserted in ewes during breeding and nonbreeding season, did not inhibit the luteolytic process but inhibit estrus in ewes might be the contributory factor for the variation of progesterone levels as reported by Ahmad and Cooke (1994) [1]. Schoombee *et al.* (1989) [15] was also in the same opinion and concluded that progesterone administration had no effect on the development and degeneration of corpus luteum and its progesterone production.

The variation of progesterone levels might be due to type of progesterone used in this study. But Husein and Kridli (2002) [2] stated that progesterone levels were similar in MAP or FGA treated ewes. However, the concentration of

progesterone in vaginal sponge had no significant effect on serum progesterone levels as evidenced by the studies of Greyling *et al.* (1994) [3] who studied during outside the breeding season in Merino breed of ewes.

Table 1: Progesterone profile (ng/ml) in ewes synchronized with vaginal sponges during breeding and nonbreeding season

Sl. No	Group	Season										Overall Mean
		Breeding					Non breeding					
		Day of treatment					Day of treatment					
		0	3	6	9	12	0	3	6	9	12	
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
1.	Control	1.05 ±0.13	1.94 ±0.19	2.53 ±0.26	3.81 ±0.43	2.64 ±0.47	0.98 ±0.08	0.87 ±0.04	1.01 ±0.06	0.96 ±0.11	0.91 ±0.07	1.67^{b} ±0.08
2.	VS 4	1.24 ±0.10	2.77 ±0.25	3.84 ±0.50	4.82 ±0.56	2.76 ±0.34	0.87 ±0.06	1.72 ±0.14	2.70 ±0.23	3.98 ±0.29	3.75 ±0.23	2.84^{a} ±0.10
3.	VS 6	1.29 ±0.09	2.38 ±0.17	3.42 ±0.29	4.86 ±0.29	2.37 ±0.24	1.06 ±0.09	1.87 ±0.16	2.74 ±0.12	3.96 ±0.17	3.85 ±0.19	2.78^{a} ±0.09
4.	VS 4h	1.29 ±0.10	2.57 ±0.24	3.54 ±0.30	5.02 ±0.39	2.54 ±0.21	0.95 ±0.12	1.70 ±0.25	2.35 ±0.18	3.94 ±0.54	3.71 ±0.35	2.76^{a} ±0.06
5.	VS 6h	1.28 ±0.08	2.63 ±0.15	3.65 ±0.21	5.03 ±0.38	2.46 ±0.22	0.93 ±0.10	1.96 ±0.17	2.77 ±0.22	3.90 ±0.32	3.77 ±0.32	2.84^{a} ±0.07
6.	Overall Mean	1.23^{e} ±0.12	2.46^{c} ±0.11	3.40^{b} ±0.13	4.71^{a} ±0.14	2.56^{c} ±0.16	0.96^{e} ±0.12	1.62^{d} ±0.13	2.32^{c} ±0.11	3.35^{b} ±0.14	3.19^{b} ±0.12	2.58 ±0.08

Means bearing different superscripts differed significantly

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