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Evaluation of biochemical profile of estrus induced ewes during non breeding season

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Abstract
The present work was conducted to investigate the induction of estrus in randomly selected non lactating ewes during the non breeding season at the Govt Sheep Breeding Farm Panthal, Katra, Reasi, Jammu (J&K). Five groups (GI, GII, GIII, GIV, GV) were made from the 30 selected ewes, each group having 6 ewes (n=6), which were covered under different hormone protocols as (GI= 1/3 Norgestomet + 200 IU PMSG, GII=1/3 Norgestomet + Ram effect, GIII= P_4 sponge + PMSG 200 IU, GIV=P_4 sponge + Ram effect, GV=untreated control). All the ewes (100 %) treated with different hormonal protocols exhibited induced estrus. In conclusion, the biochemical parameters viz, Glucose, Calcium and Phosphorus levels increased significantly ($P<0.05$), while as Cholesterol, Albumin increased non significantly ($P>0.05$) and Globulin decreased significantly ($P<0.05$) at induced estrus in all the treatment groups (GI, GII, GIII, GIV). In the untreated control (GV), none of the ewes exhibited estrus, hence no sampling was done on that day.

Keywords: Biochemical profile, Crestar & P_4 Sponge, Ewe, Estrus induction, PMSG, Ram effect

Introduction
Reproductive seasonality in ewes is characterized by changes in behavioral, endocrine, and ovulatory patterns [1, 2]. Progestagens in the form of parenteral preparations, oral preparations (Melenagesterol acetate) or in the form of intra vaginal devices like CIDR (Controlled internal drug release), PRID (Progesterone releasing intravaginal device) and subcutaneous ear implants like Crestar are generally used to alter estrous cycle of domestic animals. Progestagens or its analogues along with gonadotrophins (FSH or PMSG) have been extensively used to induce estrus in anestrus ewes, although pregnancy rates are lower during anestrus than during the breeding season in progestagen-synchronized ewes. Methods for induction and synchronization of estrus in ewes during out of season breeding have revolved around the use of P_4 pessaries and PMSG [3], while as some of the protocols involve the use of intravaginal devices impregnated with progesterone or synthetic progestagen such as fluorogestone acetate (FGA) or medroxy progesterone acetate (MAP) [4]. Intravaginal sponges are usually inserted for a varying period of 6-14 days and an injection of PMSG is administered prior to or at the time of sponge removal [5, 6]. It has been found that progestagen impregnated intravaginal sponges are more effective than the natural progesterone at lower dose levels [7]. Norgestomet implants have also been incorporated for out of season breeding with pregnant mare serum gonadotropin (PMSG) [8]. Ram effect is also used to achieve breeding activity during the non-breeding season. Anestrus ewes are isolated from rams before the start of the normal breeding season, introduction of rams to ewes induces ovulation, this method is referred to as the ram effect or male effect [9]. Some changes might occur in biochemical parameters as a result of change in the behavioural and endocrine patterns. So the present study was aimed to study the alterations in the biochemical profile during the non breeding season by utilising a minimal amount of progestin, norgestomet and PMSG along with ram effect.

2. Materials and Methods
The present study was conducted on 30 randomly selected non lactating ewes during non-breeding season at the Govt Sheep Breeding Farm, Panthal, Katra, District Reasi, Jammu (J&K). The average temperature and relative humidity during the period of study were

33ºC and 58.5% respectively.The period extended from May to August 2015. Age, Body weight and Body condition score were recorded in all animals. Ewes were randomly divided into 5 groups consisting of 6 animals in each group. In group I, ewes (n=6) were treated with Crestar ear implants @ 1mg Norgestomet (1/3 of the 3mg implant used in large animals) on day 0. The implant was removed on day 12 and an injection of PMSG (200 IU) was given on the day of implant removal. In group II, ewes (n=6) were treated with Crestar ear implants @ 1mg Norgestomet (1/3 of the 3mg implant used in large animals) on day 0. The implant was removed on day 12 and a ram was introduced 72 hrs before the implant removal. In group III, ewes (n=6) were treated with conventional P_4 sponge for 12 days. An injection of PMSG (200 IU) was given on the day of P_4 sponge removal. In group IV, ewes (n=6) were treated with conventional P_4 sponge for 12 days. The sponge was removed on day 12 and a ram was introduced 72 hrs before the sponge removal. Blood samples were collected on day 0 (at the time of implant insertion), on the day of implant removal and on day of estrus in each group by venupuncture of jugular vein under aseptic conditions in dry vials containing an anticoagulant (EDTA) for biochemical examinations. Blood was centrifuged for 10-15 minutes @ 3000 rpm and plasma was harvested in sterilized vials and stored at -20 ºC for further biochemical analysis. In group V, none of the ewes showed estrus, hence blood sampling was done on the 0 day & 12th day.

3. Results & Discussion

Results pertaining to the study are presented in Table 1 and 2.

Table 1: Effect on Glucose, Cholesterol, Calcium, Phosphorus of estrus induced ewes.

Groups	Glucose (mg/dl)			Cholesterol (mg/dl)			Calcium (mg/dl)			Phosphorus (mg/dl)		
	Insertion (0 d)	Removal (12 d)	Estrus	Insertion (0 d)	Removal (12 d)	Estrus	Insertion (0 d)	Removal (12 d)	Estrus	Insertion (0 d)	Removal (12 d)	Estrus
I	41.5 ± 0.76^a	40.66 ± 2.98^a	50.5 ± 2.69^b	70.15 ± 4.24^a	74.56 ± 9.39^a	87.45 ± 4.17^a	8.20 ± 0.53^a	8.94 ± 0.83^{ab}	11.89 ± 1.67^b	2.92 ± 0.24^a	3.11 ± 0.39^a	4.29 ± 0.37^b
II	38.33 ± 1.66^a	37.83 ± 1.53^a	46.67 ± 2.84^b	68.35 ± 6.19^a	68.55 ± 6.26^a	78.21 ± 7.59^a	8.86 ± 0.58^a	9.86 ± 0.76^{ab}	11.94 ± 0.72^b	3.18 ± 0.24^a	3.36 ± 0.25^a	4.63 ± 0.30^b
III	41.16 ± 2.91^a	36.16 ± 1.47^a	49.33 ± 3.14^b	82.87 ± 3.99^a	87.79 ± 3.39^a	90. 42 ± 3.56^a	8.01 ± 0.58^a	8.29 ± 0.43^{ab}	11.48 ± 1.74^b	3.41 ± 0.24^a	3.88 ± 0.33^a	4.77 ± 0.28^b
IV	39.0 ± 2.09^a	37.5 ± 1.76^a	46.83 ± 2.35^b	73.69 ± 4.92^a	76.87 ± 5.72^a	88.79 ± 5.01^a	7.68 ± 0.22^a	8.79 ± 0.70^{ab}	9.56 ± 0.71^b	3.56 ± 0.29^a	3.68 ± 0.20^a	4.84 ± 0.31^b
V	38.16 ± 1.75^a	37.83 ± 1.49^a		69.77 ± 8.32^a	70.40 ± 8.39^a		8.48 ± 0.72^a	8.65 ± 0.44^a		3.28 ± 0.21^a	3.58 ± 0.36^a	

Means bearing different small superscripts differ significantly along the row (P<0.05) for a given parameter.

Table 2: Effect on Albumin, Globulin, Albumin/Globulin and Total Protein of estrus induced ewes.

Groups	Albumin (g/dl)			Globulin (g/dl)			Albumin/Globulin			Total Protein (g/dl)		
	Insertion (0 d)	Removal (12 d)	Estrus	Insertion (0 d)	Removal (12 d)	Estrus	Insertion (0 d)	Removal (12 d)	Estrus	Insertion (0 d)	Removal (12 d)	Estrus
I	2.70 ± 0.37^a	2.7 ± 0.17^a	3.03 ± 0.30^a	5.23 ± 0.57^a	3.76 ± 0.51^{ab}	3.45 ± 0.39^b	0.607 ± 0.19^a	0.824 ± 0.18^a	0.891 ± 0.57^a	7.93 ± 0.25^a	6.46 ± 0.54^a	6.48 ± 0.65^a
II	2.63 ± 0.14^a	2.8 ± 0.14^a	3.03 ± 0.25^a	5.15 ± 0.32^a	4.01 ± 0.18^b	3.84 ± 0.43^b	0.516 ± 0.29^a	0.707 ± 0.57^{ab}	0.882 ± 0.18^b	7.78 ± 0.41^a	6.81 ± 0.16^a	6.87 ± 0.33^a
III	2.26 ± 0.08^a	2.63 ± 0.25^a	2.82 ± 0.27^a	5.32 ± 0.58^a	4.39 ± 0.34^b	3.26 ± 0.24^b	0.457 ± 0.06^a	0.611 ± 0.06^a	0.890 ± 0.11^b	7.58 ± 0.59^a	7.02 ± 0.42^a	6.08 ± 0.39^a
IV	2.78 ± 0.20^a	3.52 ± 0.29^a	3.48 ± 0.26^a	5.42 ± 0.26^a	4.09 ± 0.52^b	3.72 ± 0.48^b	0.525 ± 0.58^a	0.934 ± 0.14^{ab}	1.040 ± 0.19^b	8.20 ± 0.27^a	7.61 ± 0.80^a	7.20 ± 0.37^a
V	2.64 ± 0.19^a	2.70 ± 0.17^a		5.28 ± 0.30^a	4.73 ± 0.49^a		0.552 ± 0.04^a	0.600 ± 0.06^a		7.96 ± 0.46^a	7.43 ± 0.54^a	

Means bearing different small superscripts differ significantly along the row (P<0.05) for a given parameter.

The mean blood glucose concentration increased significantly (P<0.05) in Group I, II, III, IV on the day of induced estrus (Table 1). Since FSH hormone is a glycoprotein, glucose is essential for biological activity of hormones [10], hence it might be the most probable cause for its increase**.** Our findings are in accordance with the results of various researchers who found that there was increased serum glucose level during follicular phase as compared to luteal phase in sheep [11-13] and in buffaloes [14] after application of progesterone vaginal sponge. However, contrary to our findings, some results suggest that glucose level was significantly lower (P<0.01) during estrus as compared to progestogenic phase (diestrus) [15], while as some results depict that serum glucose level was significantly decreased in Medroxy progesterone Acetate treated buffaloes during both follicular and luteal phases [16] which might be due to glucose induced insulin secretion from isolated rat islets, leading to decreased glucose concentration in P_4 treated animals [17]. In the control group (Group V) there was non significant variation in mean glucose value between two sampling days.

The mean cholesterol concentration increased non significantly on the day of induced estrus in Group I, II, III, IV (Table 1). This is in accordance with the results of previous report [14] who reported that cholesterol level in ewes was non significantly higher during estrus. This may be because of higher levels of cholesterol increase the estrogen level resulting in manifestation of heat [18]. However, a previous report [10] suggests that there was a significant increase in serum total cholesterol level in ewes applicated with P_4 vaginal sponge during the follicular phase. Unlike to our study, there was no change in cholesterol in cyclic and anestrus buffaloes [19] and in ewes following intravaginal P_4 treatment [20]. Some of the previous reports [21, 22, 23] found lower levels of cholesterol concentration at estrus, but in the case of cattle. In control group (Group V) there was non significant variation in mean plasma cholesterol concentration between two sampling days.

The mean calcium concentration increased significantly on

the day of induced estrus in Group I, II, III, IV (Table 1). The increase in serum calcium level might be due to the greater mobilization of calcium due to increased metabolic activity in the follicular phase. Our results are in agreement with different workers who reported that there was a significant increase in serum calcium level during the follicular phase in prepubertal heifers [24], buffaloes [15] and ewes [14,16]. In contrary to our results, various workers [25, 26, 27] found a lower level of Ca at estrus. However, few workers [28, 29] found no significant change in plasma Ca concentration. In the control group (Group V) there was non significant variation in mean plasma calcium concentration between two sampling days.

The mean phosphorus concentration increased significantly on the day of induced estrus in Group I, II, II and IV (Table 1). Our results are in complete agreement with the results of different workers [26, 30] who reported significantly higher phosphorus level in fertile and cyclic as compared to infertile and anestrus animals, respectively. Similarly, various workers found serum inorganic phosphorus increased at induced estrus in anestrus cow [20, 31] and in ewes [14]. Conversely, previous results suggested that there was no significant effect of P_4 vaginal sponge and P_4 ear implant, respectively on serum inorganic phosphorus during both phases of the estrous cycle [10, 23]. In the control group (Group V) there was non significant variation in mean plasma phosphorus concentration between two sampling days.

The mean plasma albumin concentration increased non significantly in Group I, II, III and IV on the day of induced estrus (Table 2). Similar results were published in a study [10] in which there was non-significant increase in serum albumin level of ewes applicated by P_4 vaginal sponge during both follicular phase and luteal phase. However, a previous report [14] suggested that albumin levels in ewes were significantly higher ($P<0.01$) during estrus. In the control group (Group V) there was non significant variation in mean plasma albumin concentration between two sampling days.

The mean plasma globulin concentration decreased significantly on the day of induced estrus in Group I, II, III, IV (Table 2). The significant decrease in plasma globulin concentration in ewes applicated by progesterone vaginal sponge during the follicular phase of estrous cycle has also been reported and is similar to our study's findings [10]. In the control group (Group V) there was non significant variation in mean plasma globulin concentration between two sampling days.

The mean A/G ratio increased non significantly on the day of induced estrus in Group I and significantly in Group II, III and IV (Table 2). Similarly, an earlier study reveals that A/G ratio was low in anestrus crossbred heifers than heifers at estrus [22], which is almost in similar lines with our findings. In the control group (Group V) there was non significant variation in mean plasma albumin/globulin ratio between two sampling days.

The mean plasma protein concentration decreased non significantly at induced estrus in Group I, II, III and IV (Table 2). Similar results were found earlier, in which serum total protein level showed decrease in ewes applicated with progesterone vaginal sponge during follicular phase of estrous cycle [32]. Lower protein level were found in MPA treated ewes in summer (july) compared to that of winter (January) [13]. Another study reported that the level of serum total protein was significantly higher in anestrus cows as compared to induced estrus cows [20]. In contrary to our results, several workers reported that serum total protein levels in ewes were significantly higher ($P<0.01$) during estrus [14] & some found lower level of total protein content in anestrus cows than the normal cycling cows [33]. In the control group (Group V) there was non significant variation in mean plasma protein concentration between two sampling days.

4. Conclusion

This study concludes that biochemical parameters viz, Glucose, Calcium and Phosphorus levels increased significantly ($P<0.05$), while as Cholesterol, Albumin increased non significantly ($P>0.05$) and Globulin decreased significantly ($P<0.05$) at induced estrus in the treatment groups (GI, GII, GIII, GIV) during the non breeding season.

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