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THE EFFECT OF *TRIGONELLA-FONUM-GRAECUM* ON REPRODUCTIVE HORMONES IN SUDANESE DESERT SHEEP

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ABSTRACT

Fourteen non pregnant ewes were synchronized and divided into two groups. The animals in group1as control(N1) and group2(N2) were given *Trigonella foneum* at dose of 2.5 g/kg bwt for 6 weeks. Blood samples were obtained every four hours for 3 days (D1, D2 and D3) during the heat signs was appeared for three consecutive cycles for detection of progesterone levels. All groups showed response to heat after synchronized with CIDR and PMSG protocol. Regular oestrus cycles were obtained the result showed gradual significant decrease ($P<0.05$) in progesterone profile levels in three consecutive cycles.

KEYWORD: *Trigonella*, Reproductive Hormones, Sheep.

INTRODUCTION

Fenugreek (*Trigonella Foenum-graecum* L.) belongs to the family leguminosae, sub-family papilionaceae, tribe-trifolreae and sub-tribe trigonellinae (Nagakura, 1975). Fenugreek is an annual herb belonging to the family leguminous; its an erect, hared annual plant, brownish in colour with a deep furrow dividing them into two equal part, its branched at the base growing to a height of 0.3-0.8m it has a sharp, spicy aroma (Grieve, 1970).

There are several used of Fenugreek as common feed, flavoring, spice, and also as traditional remedy for the treatment of different disease (Flammang *et al*., 2004). The uses of the seed for other purposes are still under search.

Willard (1991) reported that Fenugreek extracts have an oxytocic effect in guinea pig uterus tissue. In rats the seed lowered the concentration of testosterone and luteinizing hormones (Mokhtari, 2008). Rabbits fed fenugreek as 30% of their diet had increased progesterone levels, antifertility effects in females, and reduced fetal and placental weights. In male rabbits, damage to seminiferous tubules and reduced testis weight was observed by Kassem (2006). In premenopausal women, 6g per day of fenugreek powder decreased hot flashes and vasomotor symptoms but with a less pronounced effect than hormone replacement therapy (HRT conjugated estrogen and medroxyp rogesterone acetate) (Hakami, 2006).

Aboel-Nor (1999), reported that in buffaloes fenugreek feeding increased plasma levels of prolactin, however, the role of this hormone in lactating ruminant is not clear and for away from understanding, Forsyth (1993). Fenugreek seeds contain flavonoids (phytoestrogens), whose action in regulating the hormonal production facilitates the development of the mammary glands which feed on estrogens and a source of diosgenin, which is used in the synthesis of steroid hormones.

Ponda (1999) showed that administration of Fenugreek seed extract (0.11g kg body wt. (-1) for 15 days to both mice and rats significantly decreases serum triiodothyronine T3 concentration and T_3/T_4 ratio, but increases thyronine T_4 levels and body weight. Although administration of fenugreek seed (220µg/kg/day) and A uiumsativum (500mg/kg/day) extract in hyperthyroid animals decreased the serum glucose concentration as well as serum thyroid hormones (Tahiliani, 2003).

The majority of circulating progestesterone is bound to albumin and corticosteroid globulin (CBG). The bioactive free hormone represents only 2.5-3% of the total progesterone (IA EA, 1984).

In both goat and sheep, pregnancy is maintained by progesterone (P4). P4 in the ewe is produced first by the corpus lutum (a structure formed in the ovary following shedding of ovum) and then by the placenta in last-third of pregnancy. However, in goats the sale source of P4 throughout pregnancy is the corpus luteum (Thornburn and Schneider, 1972).

Vasconcelos (2003) mentioned that progesterone is critical for maintenance of pregnancy. Plane of nutrition combined with high clearance rate of P_4 in the liver has been found to have an inverse relationship with circulating P_4 in ewes and gilts (Parr *et al.*, 1993; Prime, 1993). Measurement of serum progesterone is of diagnostic value for reproduction disorder antinfertility. Plasma P_4, at milk P_4 and Fat free milk P_4 have been examined and high correlation was found between their values (Heapand Holdswarth, 1981).

The aim of the experiments presented here was to study the effects of *Trigonella Foneum-graecum* on oestrus cycle and to estimate the progesterone profile levels in ewes.

MATERIAL AND METHODS

The animals were individually housed within the premises of the Central Veterinary Research Laboratories (CVRL) at Soba. All animal were adapted to feed green forage and concentrate ration and provided drinking water *ad libitum*.

Experimental animals

At the end of preliminary period and after clinical examination, 14 non pregnant female, healthy sheep were selected, aged 2-3 years, weighing 32-37 kg used in this study. The animals were put in pens (3x3m) and fed with balanced feed (concentrate and forage) and water was supplied *ad libidum* after the animal were synchronized then they were divided randomly into two groups (group 1($N_{1)}$ and group 2(N_2).

Group 1 (N_1)

Undosed and serve as a control.

Group 2 (N_2)

Before the experiment was performed the animals weight were measured and the doses were calculated then the treatment was began on day 4 (Day 0) after the signs of the oestrus were disappeared. The animals were given *Trigonella foneum* at dose of 2.5g/kg bodyweight in water orally for 6 weeks. Blood samples were obtained from the jugular vein puncture from each ewe every four hours for 3 days (D_1 & D_2 and D_3) for the detection of progesterone level, after 3 weeks and during the heat signs was appeared, serum samples were collected from the animals for hormonal assays. The treatment was continued until another oestrus cycle began; serum samples were obtained and stored at 0^oC until analyzed.

Synchronization of Oestrous cycle

To induce oestrus cycle in all experimental animals at the same time, synchronization was performed by insertation of intra-vaginal drug release (CIDR) device that contains 0.3gm slow release progesterone (inter Ag, Hamiton, Netherlands). The CIDR remained in situ for 14 days (Ritar *et al*., 1984) at the time of CIDR withdrawal, the ewes received an intramuscular injection of 400-500iu of PMSG (intervet, UK).

Detection of oestrus cycles.

Vasectomized ram was selected on the basis of their sexual desire to detect oestrus 24-72 hours following the removal of CIDR and injection of PMSG. Heat signs which appear in synchronized ewes raised tail, red vulva with mucus discharge, nervousness, reduced appetite and milk production.

To estimate actual progesterone profile for each ewe, progesterone level in serum was determined by Radio-immuno assay technique.

The radioimmunoassay method depends on the completion between progesterone in samples and 125_{1-} labeled progesterone for a limited number of binding sites on progesterone specific antibody. The separating agent is micro particles coupling with second antibody. Separation of the antibody bound fraction is effected by centrifugation and decantation of the supernatant by measuring, the proportion of 125_{1-} labeled progesterone bound in presence of reference standard sera containing known amounts of progesterone. Then the concentration of progesterone present in samples can be determined.

- One bottle of 125_{1-} labeled progesterone solution (22ml) with preservative agent.
- One bottle containing progesterone rabbit antibody solution (11ml) with preservative agent.
- Seven reference standard of progesterone in lyophilized forms with preservative agent:
  A : o ng/ml B: 0.1 ng/ml C. 0.3 ng/ml D: 2.1 ng/ml E: 6.1 ng/ml
  F: 19.2 ng/ml G: 75 ng/ml.
- One bottle separating agent containing solid phase second antibody microparticles suspension (22ml) with preservative agent.

Serum progesterone assay procedure

All reagents were allowed to equilibrate to room temperature prior to use in the assay. Duplicate tubes were labeled and arranged for total count (T), non-specific binding (NSB),

zero standard (standard = B0), standards (2-6) and sample(s) 0.5ml saline each of standards were dissolved (except A standard with 1ml saline). 100µl of each standard, control and serum sample were pipette to the appropriate labeled tubes. Zero standard A used for NSB reagent tubes. 200 µl of 125_{1-} progesterone trace solution were added and mixed with vortex to each tube. Then 100 µl progesterone antiserum (NSB) were added to the appropriate tubes and mixed briefly with vortex, 200 µl suspension of separating agent was then added to each tubes all tubes were thoroughly mixed with and were then incubated for 3 hours at 37^{o}C. After that all tubes were centrifuge for 20 minutes at 1500xg then the supernatant was discord by suction. The radioactivity of the precipitate remaining in the tubes was determined in the RIA multi-channel gamma counter. (Oak field instrument Ltd., Oak Field industrial estate Eynsham Oxon. 0X81JA, U.K.).

Calculation of Results

The average counts per minute (CPM) for each pair of duplicates tubes were determined and the percent B/B0 for each standard, control and sample were derived and calculated by using the following equation:

$$\frac{B}{B0} = \frac{B - NSB}{B0 - NSB}$$

Semi-logarithmic paper was used to construct a standard curve by plotting the B/B0 of each standard against its concentration in ng/ µl. progesterone serum concentration was obtained by reference to standard curve.

Statistical analysis

Statistical analysis of the data obtained was carried out using ANOVA with a significance difference $P<0.05$.

RESULTS

Oestrus cycle's data were recorded for two months during the experimental period, all groups showed response to heat after synchronized with CIDR and PMSG protocol. Regular oestrus cycles were obtained every 3-4 weeks and the signs of heat were observed after 48-72 hours post injection of PMSG. The serum progesterone profile was obtained during the signs of oestrus every 4 hours in three consecutive cycles for three days (D1, D2 and D3). The progesterone profile levels were determined for up to 72 h. Table (1). Day 1 in three consecutive cycles showed slightly lower than that of N1 (Day1 before treatment) at all time ($P<0.05$). The lowest levels were evaluated 0.94±0.153, 0.92±4.163, 0.91±2.309 and

0.89±0.292 (ng/µl) at times 10 am, 2 am, 6 pm and 10 pm/h respectively. After 6 weeks of the treatment, whereas, the difference was insignificant (P>0.05) between hours in GN2. Table (2) Fig. (2).

Table 1: Mean ± SEM of progesterone (ng/µL) levels in serum sheep dosed orally with 2.5g/kg body weight in three consecutive cycles (Day 1).

Animal	Day one				P-value
	10 Am	2 Am	6 PM	10pm	
Before treatment	1.09±0.103[a]	1.04±4.163[ab]	0.98±6.429[a]	1.11±0.254[a]	0.703
After treatment (1 month)	1.07±0.14[a]	0.98±2.000a	0.95±4.582[b]	0.92±2.646[b]	0.164
After other cycle	0.94±0.153[b]	0.92±4.163	0.91±4.163[b]	0.89±9.292[b]	0.929
P-value	0.393	0.018	0.221	0.264	-----

Values with different small superscripts within same rows were significantly different at (P<0.05).

Values with different capital superscripts within same columns were significantly different at P<0.05).

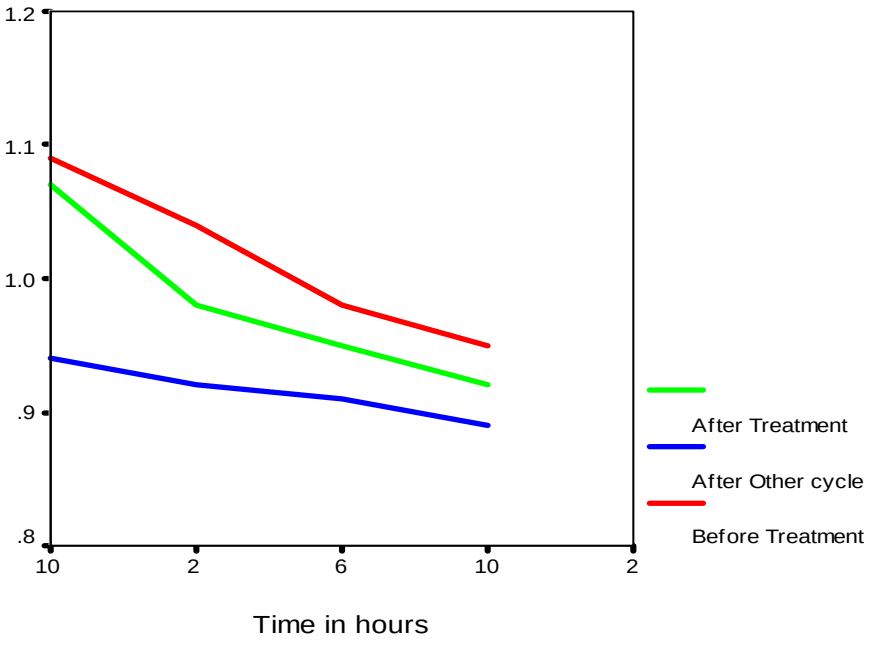

Fig. 1: Mean ±SEM of progesterone (ng/µL) levels in serum sheep dosed orally with 2.5 /Kg Bwt in three consecutive cycles (Day 1)

The progesterone profile was continuing to decline gradually in day 2 Table (2). Moreover, the levels were noted to decrease significantly (P<0.05) at all times, the lowest values were observed after 6 weeks of treatment in GN2 were 0.88±0.11, 0.79±0.87, 0.76±6.25 and 0.71±5.69 ng/µl at times 2 am, 6 am, 10 pm and 6 pm respectively Table (2) Fig. (2).

Table 2: Mean ± SEM of progesterone (ng/µL) levels in serum sheep dosed orally with 2.5g/kg body weight in three consecutive cycles (Day 2).

Animal	Day two				P-value
	2 Am	6 Am	10 PM	6pm	
Before treatment	0.95±7.77[a]	0.92±4.04[a]	0.86±4.04[a]	0.81±6.03[a]	0.068
After treatment (1 month)	0.85±3.64[b]	0.81±1.53[b]	0.95±4.582[b]	0.75±3.51[b]	0.041
After other cycle	0.83±0.11[b]	0.79±9.87[b]	0.76±6.25[b]	0.71±5.69[b]	0.306
P-value	0.298	0.141	0.076	0.164	-----

Values with different small superscripts within same rows were significantly different at (P<0.05).

Values with different capital superscripts within same columns were significantly different at (P<0.05).

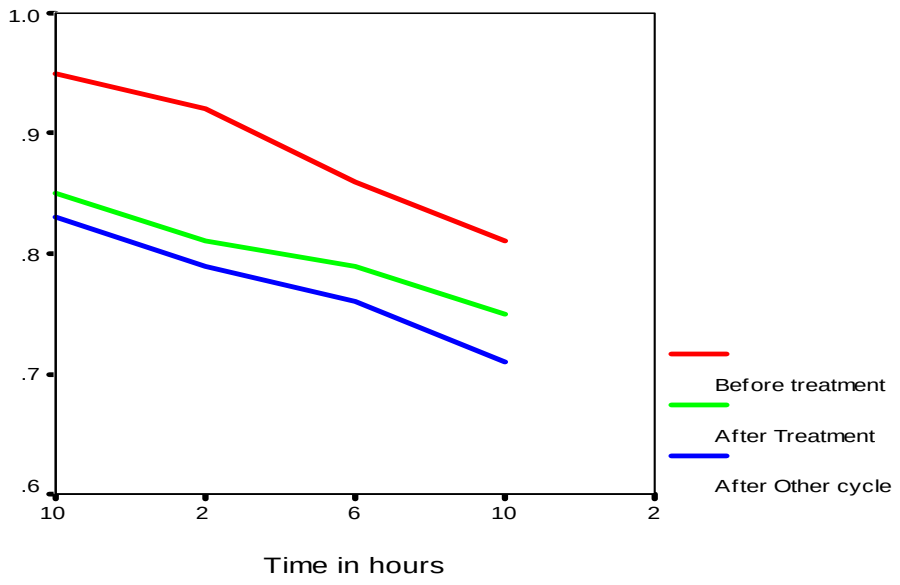

Fig. 2: Mean ±SEM of progesterone (ng/µL) levels in serum sheep dosed orally with 2.5 /Kg Bwt in three consecutive cycles (Day2)

Table (3) showed the detectable progesterone profile levels in D3, the levels were found to be significantly decreased. The difference was significantly in all times (P<0.05) minimum

levels were evaluated 0.69±3.21, 0.71±3.61, 0.72±2.52 and 0.75±3.61 ng/µl at times 10 am, 2am, 6pm and 10 pm respectively, Table (3) Fig. (3).

Table 3: Mean ± SEM of progesterone (ng/µL) levels in serum sheep dosed orally with 2.5g/kg body weight in three consecutive cycles (Day 3).

Animal	Day three				P-value
	2 Am	6 Am	10 PM	6pm	
Before treatment	0.78±2.31[a]	0.76±3.00[a]	0.77±2.52[a]	0.79±4.58[a]	0.698
After treatment (1 month)	0.75±3.22[a]	0.72±4.73[a]	0.77±5.86[a]	0.79±4.04[a]	0.434
After other cycle	0.69±3.21[ab]	0.71±3.61[a]	0.72±2.52[a]	0.75±3.61[a]	0.266
P-value	0.025	0.326	0.239	0.462	-----

Values with different small superscripts within same rows were significantly different at (P<0.05).

Values with different capital superscripts within same columns were significantly different at (P<0.05).

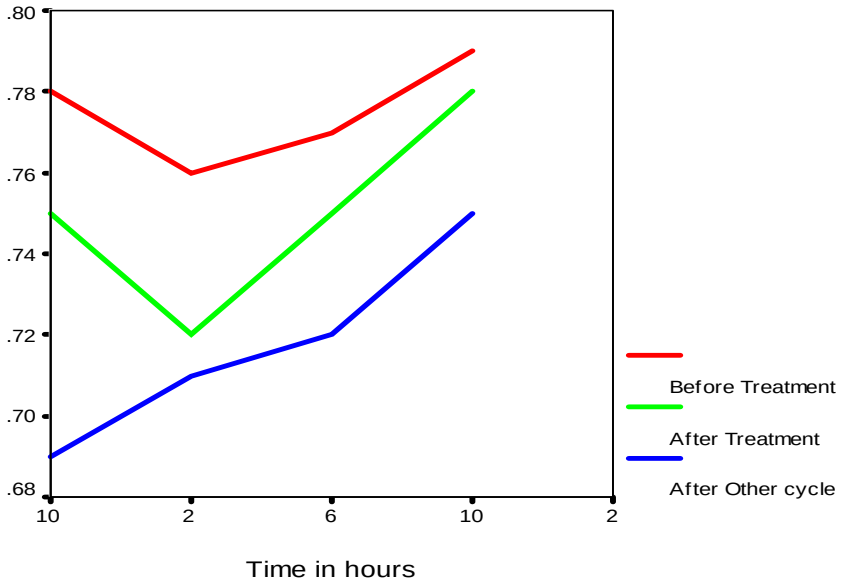

Fig. 3: Mean ±SEM of progesterone (ng/µL) levels in serum sheep dosed orally with 2.5 /Kg Bwt in three consecutive cycles (Day3)

DISCUSSION

The results found in this study showed that estrus in sheep can be effectively induced and synchronized by using different hormonal protocols. This is in agreement with what has been

previously reported (Langford *et al*., 1983) and (Leboeuf *et al*., 2000). The response by showing estrus following CIDR and PMSG treatment in this experiment showed that 91.71% of the treated animals exhibited estrus within 72 hr. after the second dose which is comparable to the result reported by Randall *et al*. (1980) and Corteel (1975).

The oestrus behavior observed was closely similar to those signs reported by Evans and Maxwell (1987). However, the oestrus duration resulting from the hormonally treated groups was (48-72hr) which was nearly similar to that reported by Evans and Maxwell (1987). The monitoring of progesterone concentration in plasma produces comparable profiles in each treated animals and these can be used for determining the reproductive status of ewes. The gradual decline of progesterone profile in treated ewes indicated regular oestrus cycles with slight signs throughout the sampling of three consecutive cycles. This may be an effect of supplementation of *Trigonella foneum* which have high level of crude protein on the onset of oestrus.

This finding agrees with that of Kusina *et al*. (2001) in goats where dietary energy restriction was found to decrease the proportion of does showing onset of oestrus and delay the appearance of oestrus signs, also agree with Mansour *et al.* (2000) who conducted that energy level had a significant effect on onset and duration of oestrus, ovarian activity and quality of embryos and this in contrast with Mani *et al.* (1992) who found that low level of feeding results in delay and suppression of oestrus. Similar results have been mentioned in sheep by (Gunn and Doney (1975) and in pig Beltramea *et al*. (1991) our study conducted that the detectable progesterone profile levels in D_1, D_2 and D_3 in three consecutive cycles were found to be significantly decrease.

This is probably attributed to the enhancement effect of *Trigonella foneum* administration on the pituitary gland to secrete gonadotrophinses which suppress progesterone secretion. This is agree with that of Shesworth and Esdon (1983) and Sabra (1994) who reported that under Feeding in sheep tends to increase their plasma progesterone which leads to an oestrus while flushing with soyabean decreases progesterone secretion. The same observations mentioned by Blauwickel (1986) who found that dietary protein can alter the secretion of progesterone and LH in cattle.

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