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Follicular diameters and progesterone level in Egyptian ewe lambs using flushing and some hormonal treatments

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Abstract

The objectives of this study were to explore the influence of flushing and hormonal treatments on the follicular diameters and progesterone level during estrus and pregnancy rate in Egyptian ewe lambs. This study was carried out on 25 ewe lambs clinically healthy (body weight 27-33 Kg. and age 8-12 months). The animals divided into 5 groups; group 1: served as a control, group 2: received vaginal sponges impregnated medroxyprogesterone acetate (MAP) for 14 days, group 3: received vaginal sponges impregnated with MAP for 14 days, on day of sponge removal each animal injected with pregnant mare serum gonadotropin (PMSG), group 4: received gonadotrophin releasing hormone (GnRH) on day 0, and injected with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on day 7, after 48 hours, animals treated with the second dose of GnRH and group 5: injected with $PGF_{2\alpha}$ 7 days and after 72 hours the animals injected with *h*CG during this period. Animals fed 3 kg. *trifolium alexandrinum* plus 500 gm concentrate diet. The results revealed that the follicular diameters (mm) showed higher ($P<0.05$) values after two days of the treatments than control group, and all treatment groups were increased ($P<0.05$) in the follicular diameters (mm) on both ovaries after the end of treatments within three days. In addition, during the first month of gestation period concentrations of serum progesterone were significantly ($P<0.05$) higher in groups 2 and 3 compared to groups 1 and 4. From this study we concluded that, using intra-vaginal sponges impregnated with medroxy-progesterone acetate plus pregnant mar serum gonadotropin (PMSG) and gonadotropin releasing hormone plus prostaglandin F2α (GPG) protocol, may be improved reproductive performance in native ewe lambs.

Keywords: MAP, PMSG, GnRH, PGF2α, flushing, reproductive performance, Egyptian ewe lambs.

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1. Introduction

There are about 4.6 million heads of sheep in Egypt (FAO, 2008), which are mainly used for lamb and mutton production. The Ewe lambs can be successfully bred at 7–9 months of age, breeding ewe lambs has several advantages including increased profitability and lifetime reproductive performance (Kenyon *et al.*, 2014). Ovarian follicles of ewe lambs increased in number and size in response to exogenous gonadotropin stimulus, efficiency of gonadotropin releasing hormone (GnRH) in synchronizing ovulation, increasing the ovulation rate and improving fertilization (Facciolongo *et al.*, 1994). However, Rutigliano (2010) stated that that the level of progesterone concentration was only less than 1.0 ng/ml, when synchronized ewes by two injections of $PGF_{2\alpha}$. Weems *et al.* (2007) stated that the corpus luteum (CL) must be maintained and continue to produce high concentrations of progesterone at least until the developing placenta can assume responsibility for progesterone production. In sheep this transition from CL dependent to placenta-dependent progesterone production occurs as early as 55-90 d after conception.

2. Materials and methods

2.1 Experimental animals

This study was carried out on 25 native ewe lambs. The body weight was ranged between 27 and 33 Kg with an average age ranged between 8 and 12 months. The ewe lambs were housed under the experimental sheep farm condition of Animal Reproduction Research Institute (ARRI), Al-Ahram, Giza, Egypt. The animals divided into 5 groups, each group has 5 ewe lambs. Each group was containing 2 ewe lambs from 8 to10 months of age and weighing from 27 to 30 kg and 3 ewe lambs from 10 to 12 months of age and from 30 to 33 kg body weight. Group 1: served as a control without any treatment. Group 2: (flushing), animals received vaginal sponges impregnated with 60 mg medroxy progesterone acetate (MAP, Pfizar manufactured, NV/SA, Puurs, Belgium) for 14 days. Group 3: (PMSG), animals received vaginal sponges impregnated with 60 mg MAP for 14 days. On the day of sponge removal; each animal injected with 250 IU, I/M, pregnant mare serum gonadotropin (PMSG, Syncropart. PMSG 600, Ceva, Sante Animale, Liboume, France). Group 4: (GPG), each animal received 1.25 ml, (0.5 μg I/M, GnRH, Receptal, MSD, Intervet, International, GmbH, Germany), on day 0. Seven days later ewe lambs injected with 0.5 ml (125 μg I/M, $PGF_{2\alpha}$, Estromate, equivalent to cloprostenol, Schering, Plough animal health, Germany). After 48 hours, animals treated with the second dose of GnRH. Group 5: ($PGF_{2\alpha}$+*h*CG), each animal received two injections of 0.5 ml (125 μg. I/M, $PGF_{2\alpha}$, Estromate, equivalent to cloprostenol, Schering, Plough animal health, Germany) on days 0 and 7, respectively. After 72 hours from the second dose of $PGF_{2\alpha}$ ewe lambs were injected 0.2 ml *h*CG. During the experimental trials animals fed 3 kg *trifolium alexandrinum* plus 500 gm concentrate diet.

2.2 The ultrasound examination and blood samples

Ultrasound examination (U/S) of ewes was performed all over the time as follow, at 14 days before the experiment, to be sure that ovaries and the reproductive tract of the ewe lambs are normal and functioning, and after the end of treatment (for three days) to follow up the follicle size. The blood samples were collected at intervals; on day of treatment, at the end of treatment and once daily during the three days after 24 hours from the end of treatment. Animal which detected pregnant and confirmed by ultrasonography; blood sample was taken once per month from each ewe lamb till parturition. The serum progesterone level was assayed by ELISA kit (Enzyme immunoassay for the quantitative determination of progesterone concentration, Biocheck, Inc. Foster City, CCA 94404 U.S.A.).

2.3 Statistical analysis

Statistical analysis of the data obtained in the study was performed by using the SPSS computer programs (2006), method of analysis (Snedecor and Cochran, 1982). For hormonal and flushing treatments, one-way classification was used as the following model:

$$Yij = \mu + Ai + Bj + Eij$$

Where, Yij = the trait of study. μ = the overall mean. Ai = the effect of age of dam (j = 1 and 2). Where, (1= 8-10 and 2= 10-12 months). Bkj= the effect of body weight of dam (k = 1 and 2) where (1=27-30 and 2 = 30-33 Kg).

3. Results and Discussion

3.1 Follicular diameters

There were no statistical differences after one day from the end of treatments between groups. It is obvious that, the follicular diameters (mm) after two and three days from the end of the treatment in all treated groups showed higher values ($P<0.05$) than that of a control group (Table 1). In the present study, the follicular diameter in two days after the end of treatment in group 3 (PMSG) was (2.27±0.32) mm. This result agrees with Pawel *et al.* (2006) and Ashmawy (2012). They found that in sexually mature ewes, follicles after 3–5 days from onset a new wave was from 2.0 to 3.0 mm. The marked increase in serum concentrations of estradiol during the follicular phase induces the LH surge in sheep. The surge of LH reaches a peak 4-12 hours after the beginning of estrus which results in ovulation of the dominant follicle at approximately 24 to 28 hours later. Viñoles *et al.* (2004) stated that the follicular diameter increased to an approximately 1to 2 mm before ovulation. They added that the ovarian ultrasonography becomes a useful tool in tracking the progression of antral follicular kinetics, ovulation, and corpus luteum (CL) formation. In this study, the size of follicular diameter on the first day after the end of treatment in all groups was < 1.0 mm. This result agreed with those of Duggavathi *et al.* (2003) who reported that the pre-ovulatory follicular diameter increased to an approximately 1.0 mm.

Table (1): Effect of different nutritional and hormonal treatments on follicular diameter (mm) in ewe lambs by Ultrasonography.

Treatment	Days after the end of treatment		
	One day	Two days	Three days
Control	0.28±0.02	0.63±0.07[a]	2.11±1.05[a]
Flushing	0.30±0.06	3.25±0.61[b]	4.35±0.36[b]
PMSG	0.27±0.00	2.27±0.32[b]	3.94±0.26[b]
GPG	0.96±0.72	3.20±0.35[b]	3.37±0.24[b]
PGF2α+*h*CG	0.13±0.01	2.76±0.23[b]	3.90±0.49[b]
P	(NS)	0.05	0.05

Different superscript letters indicate significance within the same column ($p<0.05$).

3.2 Serum progesterone concentration (ng/ml) in ewe lambs

3.2.1 Serum progesterone level around the time of treatment

Obviously, group 2 (flushing) had higher ($P<0.01$) value of serum progesterone concentration (ng/ml) at the end day of treatment compared to groups 3 (PMSG), 4 (GPG) and 5 ($PGF_{2\alpha}$+*h*CG) (Figure 1). The corresponding values were 0.30±0.03 vs 0.12±0.04, 0.14±0.02 and 0.04±0.02, respectively. In the present study, the Serum progesterone concentrations (ng/ml) of flushing and PMSG groups at start day of treatment were 0.12±0.04 and 0.60±0.16, respectively. However, the Serum progesterone concentrations (ng/ml) of flushing and PMSG groups at end day of treatment were 0.30±0.03 and 0.12±0.04, respectively. These results were in accordance with D'Souza (2013) they reported that the progesterone concentration at the time of CIDR insert was 0.6 ng/ml, and at the time of the removal of the CIDR was 0.4 ng/ml. In this study, in group 4 (GPG) and group 5 ($PGF_{2\alpha}$+*h*CG) the serum progesterone concentration (ng/ml) at start day of treatment were 0.56±0.25 and 0.76±0.37, respectively, and at the end day of treatment were 0.14±0.02 and 0.04±0.02, respectively. Similar results were found by Rutigliano (2010) stated that that the level of progesterone concentration was only less than 1.0 ng/ml, when synchronized ewes by two injections of $PGF_{2\alpha}$. However, Hashem *et al.* (2015) reported that the serum progesterone concentrations at the end of treatment were 6.50 and 5.62 ng/ml in GPG protocol and duple injections with $PGF_{2\alpha}$, respectively. These differences may be due to difference dose of $PGF_{2\alpha}$ and GnRH. Also, Khan *et al.* (2007) found that low progesterone concentration in ewe lambs after GnRH or *h*CG treatment due to that the CL in ewe lambs was less responsive to these treatments. In our study, in group 2 (flushing) and group 3 (PMSG), the serum progesterone concentrations (ng/ml) on day 0 estrus were 0.12±0.02 and 0.18±0.05 ng/ml, respectively, on day 1 of estrus were 0.14±0.04 and 0.58±0.20 ng/ml, respectively, and on day 2 of estrus were 0.24±0.09 and 0.80±0.19 ng/ml, respectively. These results agreed with Ayman *et al.* (2015) who reported that the levels progesterone concentrations were 0.2, 0.4 and 1.2 ng/ml at the CIDR withdrawal, after 24 hours and 48 hours. Also, Beard *et al.* (1991) found that the

level of progesterone concentration at 24 hours before estrus was 0.5 ng/ml. On other hand, this result was lower than that recorded by D'Souza (2013) who reported that the levels of plasma progesterone concentration between 3rd and 4th days of the treatment end were 2.8 and 3.4 ng/ml, respectively, when synchronized ewes with CIDR and CIDR + FSH, respectively.

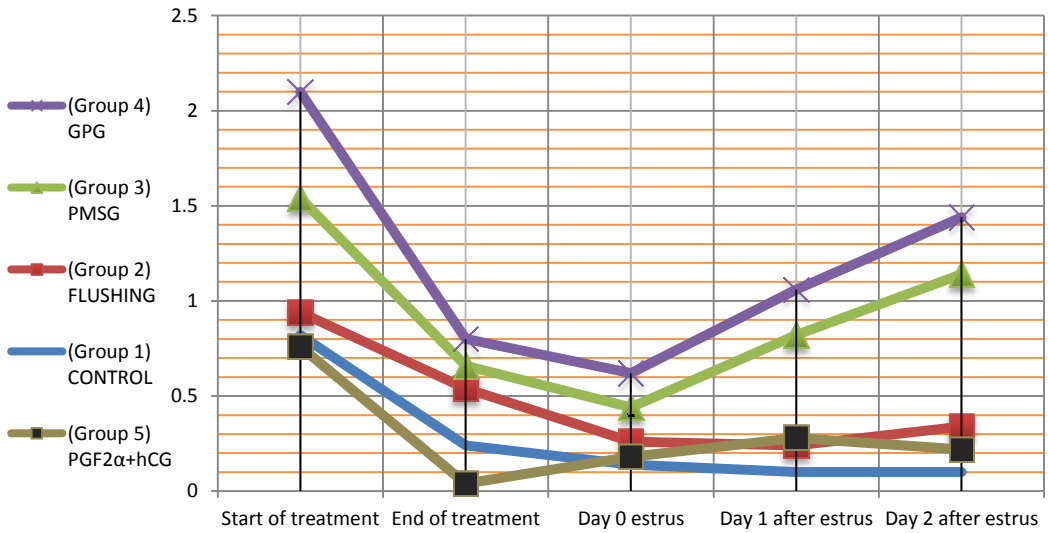

Figure (1): Effect of different nutritional and hormonal treatments on Serum progesterone concentrations (ng/ml) in ewe lambs around the time of treatments. Control, without any treatment; Flushing, animals received 60 mg medroxy progesterone acetate for 14 days and 500 gm concentrate diet; PMSG, animals received 60 mg medroxy progesterone acetate for 14 days and injected with PMSG; GPG, animals received GnRH and $PGF_{2\alpha}$ on day 7 and GnRH on day 9; $PGF_{2\alpha}$+*h*CG, received two injections of $PGF_{2\alpha}$ and *h*CG.

3.2 Serum progesterone level during gestation period in ewe lambs

It was clear that the serum progesterone concentrations around first month of gestation period were significantly ($P<0.05$) higher in group 2 (Flushing) and 3 (PMSG) compared to group 1 (control) and group 4 (GPG), the corresponding values were 3.87±0.78 and 3.78±1.05 vs 2.00±.30 and 1.40±0.54, respectively. On the other hand, there were no-significant changes in serum progesterone level among groups during 2nd, 3rd, 4th and 5th months of gestation period in ewe lambs, as shown in Figure (2). In this study, there was an increase in the progesterone concentration during gestation period, from the first month until the end of gestation, in all groups. Similar result was found by Alwan *et al*. (2010). They reported the progesterone concentrations during gestation period were 6.70±1.01, 7.75±0.75, 15.71±1.53, 24.91±2.53 and 11.70±1.23 ng/ml, at 1st, 2nd, 3rd, 4th and 5th months of gestation, respectively. Similarly, Ranilla *et al.* (1994) reported that, the progesterone concentrations

during gestation period were 4.0, 5.5, 5.0 to 6.0, 8.0 to 10.0 and 10.0 to 12.0 (ng/ml) at first, second, third, fourth and the end month, respectively, after synchronized ewes by 60 mg MAP for 14 days, and injection with 300 IU PMSG at the removal sponges. The progesterone levels were significantly higher in groups 2 (flushing) and 3 (PMSG) than groups 1 (control) and 4 (GPG), this strongly affirmed the role of progesterone in enhancing the embryo survival which leads to good conception values in such groups, this in accordance with Mulvaney (2011). Increasing of progesterone level during early pregnancy reduce embryonic losses and increase pregnancy rate and fertility (Ataman *et al.*, 2013).

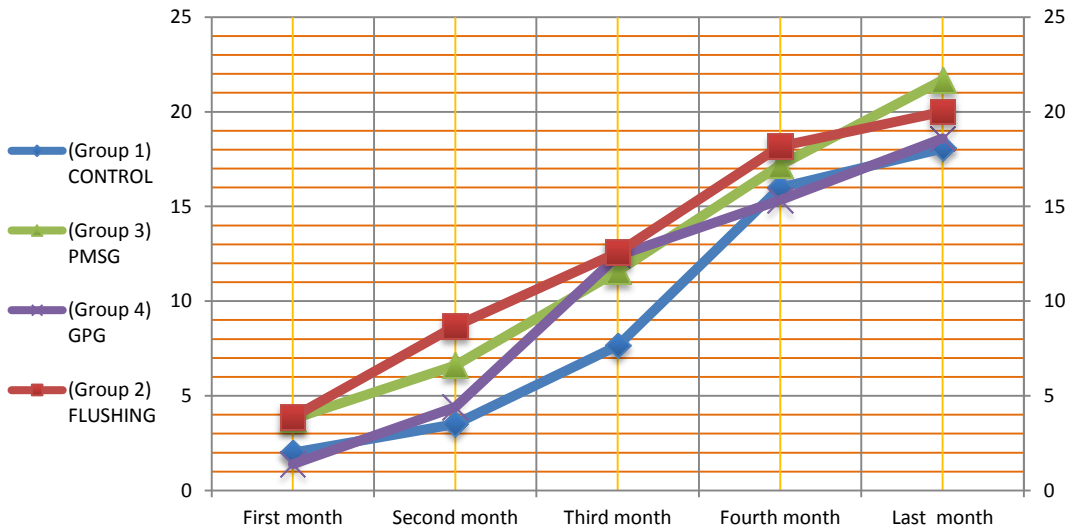

Figure (2): Serum progesterone concentration (ng/ml) as affected by different nutritional and hormonal treatments* during gestation period in ewe lams. Control, without any treatment; Flushing, animals received 60 mg medroxy progesterone acetate for 14days and 500 gm concentrate diet; PMSG, animals received 60 mg medroxy progesterone acetate for 14days and injected with PMSG; GPG, animals received GnRH and $PGF_{2\alpha}$ on day 7 and GnRH on day 9; $PGF_{2\alpha}$+*h*CG, received two injections of $PGF_{2\alpha}$ and *h*CG.

However, this result was lower than that recorded by Youtov *et al.* (2007) they reported that the serum progesterone concentration was 11.1±3.8, 15.2±4.1 and 20.1±3.0 (ng/ml) after 20, 40 and 60 days of insemination, respectively, when synchronized ewes by intra-vaginal sponge. Also, Campbell (1992) reported that the progesterone levels on days 73, 94 and 116 of gestation period were 5.6, 11.1 and 18.6 (ng/ml), respectively, when treated ewes with $PGF_{2\alpha}$. Furthermore, Abu Gazal (2010) found that the progesterone concentration after 15 days from sponges removal was 2.7 ng/ml, when synchronized ewes by 60 mg MAP and injection with 300 IU PMSG at the sponge removal. Weems *et al.* (2007) stated that the CL must be maintained and continue to produce high concentrations

of progesterone at least until the developing placenta can assume responsibility for progesterone production. In sheep this transition from CL dependent to placenta-dependent progesterone production occurs as early as 55-90 d after conception.

4. Conclusions

Using intra-vaginal sponges impregnated with medroxy-progesterone acetate plus pregnant mar serum gonadotropin (PMSG) and gonadotropin releasing hormone plus prostaglandin $F_{2\alpha}$ (GPG) protocol, may be improved reproductive performance in native ewe lambs.

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