Impact of GnRH, PMSG and hCG treatments on follicular diameter, conception and lambing rates of Egyptian ewe lambs using intravaginal sponges

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ABSTRACT

The influence of gonadotropin releasing hormone (GnRH), Pregnant Mare Serum Gonadotropin (PMSG) and human Chronic Gonadotropin (hCG) on some reproductive responses of Egyptian ewe lambs was evaluated. Twenty five animals (aged 8 – 12 months and weighed 27 - 33 Kg) were divided into 5 groups (5 ewe lambs each). Group (1) served as a control, Group (2), received vaginal sponges impregnated with MAP (60 mg medroxy progesterone acetate) for 14 days, during this period, animals fed 3 kg Berseem, (*Trifolium alexandrinum*) plus 500 gm concentrate / head/ day. Group (3), received vaginal sponges impregnated with (60 mg, MAP) for 14 days, on the day of sponge removal; each animal injected by PMSG (250 IU, I/M). Group (4) animals received 1.25 ml Receptal (0.5 µg, GnRH) I/M, on day 0, seven days later, ewe lambs injected with 0.5 ml Estromate (125 µg. PGF2α) I/M, after 48 hours, animals treated with a second dose of GnRH. Group (5) received two injections of (0.5 ml Estromate) at days 0 and 7, respectively, after 72 hours from the second dose of PGF2α ewe lambs were injected 0.2 ml hCG. Heat detection was performed and trans-rectal ultrasound scanning also performed to confirm estrus, pregnancy and follicular diameter. The results revealed that, administration of PMSG (group 3) reduced the interval to estrus (the onset of heat /hours) significantly (P< 0.01) compared with control and other groups. Regarding conception and lambing rates, groups 2, 3, & 4 had affected significantly (P< 0.01) compared to groups 1 & 5. Follicular diameter (mm) tended to increase significantly in treated groups than the control one. Results suggest that, tested hormones have effect on selected reproductive responses of Egyptian ewe lambs while administration of PMSG (Group 3) has the best response compared to other hormonal treatments.

Keywords: **GnRH, PMSG, HCG, reproductive responses, Egyptian ewe lambs**

INTRODUCTION

The fertility is one of the most important parameters for sheep productivity, the number of offspring obtained per lambing is a good indicator, and according to some authors **(Petrović, 2000)** it is more important than gain of lambs. Various hormones administered in order to increase the fertility of ewes and to obtain lambs twice per year or three times in two years **(Bazer *et al.*, 2007).** However, due to a rather long anestrous period after lambing, it is usually possible for a ewe to give birth once per year **(Semra Kaya *et al.*, 2013).** Many manipulations of the normal reproductive process involve the administration of exogenous hormones that stimulate ovarian follicle development, maturation and ovulation. These stimulatory hormones, collectively referred to as gonadotropic hormone or gonadotropins, are

generally isolated from tissues like the pituitary glands of pigs and sheep (the pituitary gonadotropins follicle stimulating hormone preparations [FSH] and luteinizing hormone [LH]), the plasma of pregnant mares (equine chorionic gonadotropin, eCG), the urine of pregnant women (human chorionic gonadotropin, hCG **(Kanitz *et al.*, 2002).**

Ewe lambs have the ability to successful breeding at 7–9 months age. Early breeding of ewe lambs has a number of advantages including increased profitability and lifetime reproductive performance **(Kenyon *et al.*, 2014)**. The ewe lambs has the capability to reproduce after achieving puberty, if a ewe lamb can successfully bred to lamb at one year of age, and reared lamb(s) successfully to weaning, there is potential to increase profitability **(Young *et al.*, 2010)** and lifetime reproductive performance **(Kenyon *et al.*, 2011).** The aim of the present study was to test the hypothesis that application of GnRH, PMSG and hCG hormones before sponge removal in short-term medroxy progesterone acetate (MAP) treated ewes may improve follicular development, conception and lambing rates for Egyptian ewe lambs breed characterized by a low prolificacy rate.

MATERIALS AND METHODS

This study was carried out on 25 ewe lambs, clinically healthy. The body weight ranged between (27 - 33 Kg), with age ranged between (8 – 12 months). The ewes were housed under normal conditions and raised in the experimental sheep farm of ***Animal Reproduction Research Institute (ARRI)***, AL-Ahram, Giza. Animals fed according to **NRC (1979)** on 3.0 Kg Egyptian clover (***Trifolium alexandrinum)*** plus 250 gm concentrate in winter, while in summer the Egyptian clover was substituted by 3.0 Kg Darawa, per head, per day**.**

The animals divided into 5 groups (each has 5 ewe lambs). **Group (1**) served as control, **Group (2**) received vaginal sponges impregnated with MAP (60 mg medroxy progesterone acetate) for 14 days, during this period, animals fed 3 kg Barseem, (*Trifolium alexandrinum*) plus 500 gm concentrate per head per day (Fig.1) . **Group (3)**, received vaginal sponges impregnated with (60 mg MAP) for 14 days, on the day of sponge removal, each animal injected by PMSG (250 IU, I/M) (Fig. 2). **Group (4**) received 1.25 ml Receptal (0.5 µg, GnRH) I/M, on day 0, seven days later, ewe lambs injected 0.5 ml Estromate (125 µg PGF2α) I/M, after 48 hours animals treated with a second dose of GnRH (Fig.3). **Group (5)** received two injections of 0.5 ml PGF2α on days 0 and 7, respectively, after 72 hours from the second dose of PGF2α ewe lambs were injected 0.2 ml hCG (Fig. 4).

Heat detection performed daily by visual observation and using three fertile rams in good health condition (age, 2-3 years) for breeding during the expected days after removal of the sponges. The fertile ram introduced after 36-48 hours of sponges removal, once every 3 hours, till the end of estrus. Ewe lambs stand firmly to the ram considered in heat. Other signs also recorded. Trans-rectal ultrasound scanning also performed to confirm estrus, pregnancy and follicular diameter. Ultrasound examination of ewes performed as follow: before start of treatments for all groups to be sure that ovaries are functioning and normal to form the experimental groups, on day of treatment, on the end of treatment, after end of treatment (three day after onset of estrus) and after 30 days, once monthly, during gestation period to detect pregnancy rate by observing placentomes, fetal fluids and embryos.

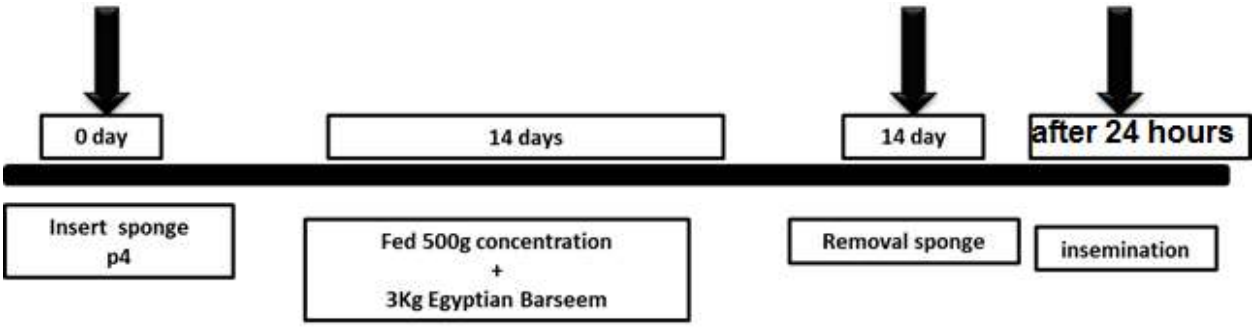

Fig.1 Flushing group (Group 2)

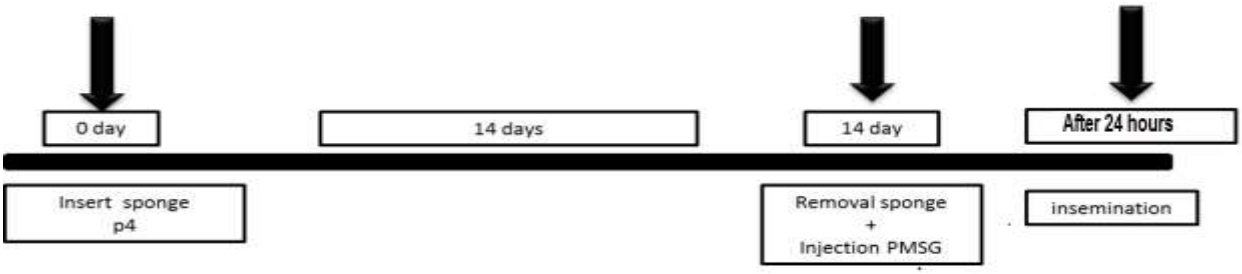

Fig.2 PMSG group (Group 3)

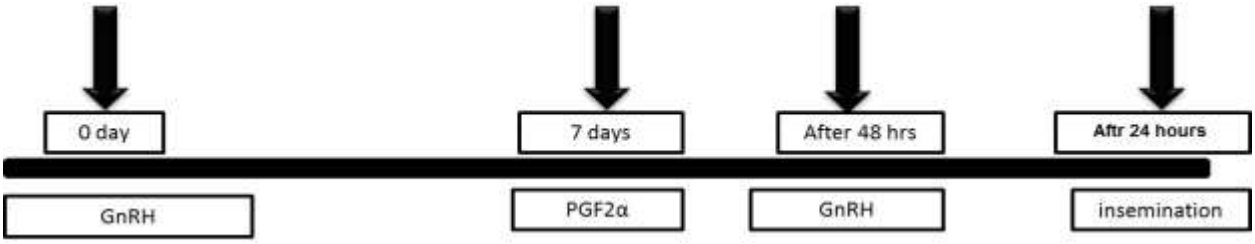

Fig.3 GPG group (Group 4)

Fig.4 PGF2α+hCG group (Group 5)

Statistical analysis

Data were statistically analyzed using general linear model (G.L.M.) procedure of **SPSS (2006).** For hormonal and flushing treatments, one-way classification used according to the following model,

$Yij = \mu + Ti + Eij$

Where; Yij = the observation. μ = General mean. Ti = Effect due to hormonal treatments. Eij = the errors related to individual observation.

RESULTS AND DISCUSSION

1. Onset of estrus and duration of estrus (h)

Estrus symptoms following ram joining earliest began in the control group (Table 1). Ewes in this group began exhibiting estrus symptoms in an average of 32.5± 3.75 h. The average estrus start times were 79.75, 39.39, 74.00 and 48.00 h in group I (flushing), group II (PMSG), group III (GPG) and group IV (PG), respectively. The statistical evaluation revealed a significant difference between group I and group III regarding start time of estrus compared with group II and group IV ($P < 0.05$). No significant difference observed between group II and group IV. In small ruminants, estrous synchronization achieved either by reducing length of the luteal phase of estrous cycle with (PGF2α) or by extending the cycle artificially with exogenous progesterone or more potent progestagens **(Kusina *et al*., 2000; Jainudeen *et al*., 2000).**

Table 1. Onset and duration of estrus in ewe lambs treated with flushing and some hormones

Treatments	Onset estrus after end of treatment / hours	Estrus duration / hours
Control (Group1)		32.50±3.75
Flushing (Group2)	79.75±3.49[a]	31.62±2.65
PMSG (Group 3)	39.39±3.23[b]	35.50±2.45
GPG(Group 4)	74.00±3.49[a]	33.75±2.65
PG (Group 5)	48.00±6.98[b]	29.00±5.31
Sig.	0.01	0.69

Values with different superscripts (a, b) in the same column are significant at (P<0.01).

2. Estrus response (ER), Conception rate (CR) and Lambing rate (LR)

Estrus response, lambing and pregnant rates for control and the experimental groups are shown in Table (2). Groups II and III exhibited significantly higher estrus response (P<0.05), fertility (P<0.0 1) and pregnancy rates (P<0.05) than control and other experimental groups. **Rekik *et al*., (2002)** found that overall fertility recorded were 80% and 93% in response to estrus when ewe lambs (aged 9 months) synchronized with intravaginal progesterone (40 mg MAP) for 14 days followed by intramuscular injection of 200 or 400 IU PMSG after sponges withdrawal. **Zeleke *et al*., (2005)** found no significant difference in percentage of ewes response to estrus (98.3%, 94.6%, 98.2%) and that time to heat onset was (39.5hr, 42.5hr, 42.0hr) for ewes synchronized by intravaginal sponges impregnated with (MAP) and injected with PMSG at 24 hr prior sponge withdrawal, at sponge withdrawal and after 24 hr of sponge withdrawal, respectively. On the other hands, **Simonetti *et al*., (2000)** found no differences between estrus response and pregnancy rate (P>0.05) for ewes synchronized with intravaginal sponges impregnated with different doses of MAP (40, 50 and 60 mg) where the percentages were 79.27%, 77.42% and 80.42% at 55.94±1.87, 56.74±1.13 and 57.70±1.02 h and (43.75%, 52.94%, 45.45%), respectively**. Gatti *et al*., (2012)** working on intravaginal sponges to synchronize estrus, using 50 mg MAP, found that ewes came into estrus between 48 and 72 h after sponges withdrawal.

Table 2. Estrus response, lambing and pregnant rates of ewe lambs treated with flushing and some hormones

Treatments	Estrus		Lambing rate		Pregnant rate	
	No	%	No	%	No	%
Control	2/5	40.0%	2/5	40.0%	2	40.0%
Flushing	4/5	80.0%	3/5	60.0%	3	60.0%
PMSG	5/5	100.0%	5/5	100.0%	5	100.0%
GPG	5/5	100.0%	5/5	100.0%	4	80.0%
PGF2α	1/5	20.0%	0/5	.0%	0	.0%
Total	17/25	68.0%	15/25	60.0%	14/25	56.0%
Sig		0.01		0.01		0.05

Values with different superscripts (a, b) in the same column are significant at (P<0.01) and (c, d,) significant at (P<0.05).

3. Follicular diameter (mm)

The follicular diameter (mm) after the end of each treatment are presented in Table (3). There was no effect of the treatment after one day of its end in comparison to the control group. It is obvious that, follicular diameter (mm) after two days in all treated groups showed higher values (P<0.01) than control group (2.76±0.23, 2.27±0.32, 3.20±0.35, 3.25±0.61 vs. 0.63±0.07, respectively). After three days, follicular diameter increased (P<0.05) for all experimental groups when compared with the control (4.35±0.36, 3.94±0.26, 3.37±0.24, 3.90±0.49 vs. 2.11±1.05). **Duggavathi *et al*. (2003)** stated that follicular diameter increased to approximately 1 to 2 mm before ovulation. In this study, the size of follicular diameter at the first day in all groups was < 1.0 mm. This result is in agreement with **Duggavathi *et al*. (2003)** who reported that the pre-ovulatory follicular diameter increased approximately to 1.0 mm. In the present study, the follicular diameter at two days after the end of treatment, in group 3 (PMSG), was 2.27±0.32 mm. This result agrees with **Tarek and Ashmawy, (2012)** who found that in sexually mature ewes, after 3–5 days from onset, a new follicle wave occurred with 2.0 to 3.0 mm diameter.

Table 3: Effect of flushing and hormonal treatments on follicular diameter (mm) and gestation period (days) for ewe lambs.

Treatments	Day after the end of treatments		
	One day	Two days	Three days
Control (Group1)	0.28±0.02	0.63±0.07^{b}	2.11±1.05d^{b}
Flushing (Group2)	0.30±0.06	3.25±0.61^{a}	4.35±0.36^{c}
PMSG (Group 3)	0.27±0.00	2.27±0.32 ^{a}	3.94±0.26^{c}
GPG (Group 4)	0.96±0.72	3.20±0.35^{a}	3.37±0.24^{c}
PG (Group 5)	0.13±0.01	2.76±0.32^{a}	3.90±0.49^{c}

Values with different superscripts (a, b) in the same column are significant at (P<0.01) and (c, d,) are significant at (P<0.05).

In addition, the follicular diameter in three days after the end of treatment in groups 2, 3, 4 and 5 were 4.35±0.36, 3.94±0.49, 3.37±0.24 and 3.90±0.26 mm, respectively (Table 3). This result is in agreement with **Contreras-Solis *et al.* (2008)** who found that follicular diameter at the end of wave 1 and 2 were 5.0±0.2 and 3.9±0.1 mm, respectively, and at wave 3 was 5.7±0.2 mm.

4. Gestation period and lambs weight

Pregnancy period and lambs weight for control and the studied groups are shown in Table 4. No statistically significant difference was observed between control and the experimental groups. The total pregnancy period was higher in flushing group (Group 2) than all other groups. Similarly, when all ewes were evaluated, the lambing weight was higher insignificantly in Group 3 (PMSG) than other studied groups. The lowest pregnancy and lambing rates obtained for ewes not received PMSG compared to those injected with 300 IU PMSG indicates the importance of administering PMSG to achieve better fertility **(Zeleke *et al*., 2005).**

CONCLUSION

We conclude due to this study that administrating PMSG and GPG together with vaginal sponges to Egyptian ewe lambs increased estrus response, fertility and the pregnancy rate, while PGF exhibited the lowest response indicating that MAP and GnRH received with vaginal sponges can used successfully to induce improvement of some reproductive performance of Egyptian ewe lambs

Table 4. Pregnancy Period and Lambs weight of ewe lambs treated with flushing and some hormones

Treatments	Pregnancy period (day)	Lambs weight (kg)
Control	152.50±1.48	2.750±0.227
Flushing	154.00±1.48	2.875±0.196
PMSG	152.42±.96	3.286±0.148
GPG	151.50±1.28	2.750±0.196
Sig.	.677	0.077

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الملخص العربى

تأثير المعاملة بكل ا من الهرمون المنبة للغدة التناسلية وهرمون الفرس الحامل وهرمون الجونادوتروفين المشيمى البشرى على قطر الحويصلات و معدلات الحمل والولادة فى الحوليات المصرية بإستخدام الاسفنجات المهبلية

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تـأثير إسـتخدام المعـاملات الهرمونيـة المنبهـة للغدد التناسلية ، هرمـون الفرس الحامـل وهرمـون الجنـادوتروفين المشـيمى البشرى تم تقييمها علـى بعض الاستجابات التناسلية . إستخدم فـى هذة الدراسـة عدد 25 حوليـة فـى حالـة صـحية جيدة تتراوح اعمارهم بين 8 الى 12 شهر بأوزان تتراوح بين 27-30 كجم . تم تقسيم الحوليات الى خمس مجاميع متماثلـة فـى العمر والـوزن. **المجموعه الاولى** اعتبرت مجموعه ضابطة ، **المجموعة الثانية** تم زرع اسفنجات مشربة بهرمون البروجستيرون لمدة 14 يوم غذيت خلال هذة الفترة على (3كجم برسيم مصرى + 500جم علف مركز 14% بروتين) / حولية / يوم ، **المجموعة الثالثة** تم زرع اسفنجات مهبليـة مشـربة بهرمـون البروجستيرون لمـدة 14 يـوم وعنـد سـحب الاسـفنجات تـم حقنهـا بهرمـون الفـرس الحامـل ،المجموعـة **الرابعة** تـم حقنهـا عضـليا فـى اليوم صـفر للمعاملـة بـالهرمون المنبـه للغدد التناسلية وفـى اليوم 7 تـم الحقن بهرمـون البروستاجلاندين وبعد 48 ساعة تم الحقن بالجرعة الثانية من الهرمون المنبة للغدد التناسلية بينما المجموعـة **الخامسـة** تـم حقنهـا بجرعتين من هرمون البروستاجلاندين بينهما 7 ايام وبعد مرور 48 ساعة تم الحقن بهرمون الغدة التناسلية المشيمية.

تتلخص أهم النتائج التى توصلت إليها الدراسة ان المعاملات الهرمونية المستخدمة كان لها تأثير معنوى على كلا من معدل الحمل ومعدل الولادة ، فقد أظهرت الدراسة ان المجموعة الثانية والثالثة والرابعة كان لهم تأثير معنوى ($P<0.01$) علـى معدل الحمل والولادة مقارنـة بالمجموعـة الاولـى والخامسـة. أظهرت الدراسـة زيـادة قطـر الحويصـلات (مم) معنويـا ($P<0.01$) فـى اليوم الثانى والثالث من المعاملات مقارنة بالمجموعة الضابطة ، وكانت زيادة الحويصلات في المجموعة الثانية فـى اليوم الثالـث اكبـر مقارنة بالمجاميع المعاملة . لايوجد تأثير معنوى على فترة الحمل والوزن عند الميلاد بين المعـاملات مقارنـة بـالكنترول، فـى حين اظهـرت المجموعـة الثالثـة زيـادة غيـر معنويـة فـى الـوزن عنـد الميـلاد مقارنـة بالمجـاميع تحـت الدراسـة. اوضـحت الدراسـة ان المعاملات الهرمونية كان لها تاثير على بعض الاستجابات التناسلية المختبرة فى الحوليات المصرية وان الاستجابة التناسلية كانت الافضل فى المعاملة الثالثة بإستخدام هرمون فرس الحامل (المجموعة الثالثة) مقارنة بالمجموعات الاخرى تحت الدراسة.