

## Induction of estrus using human menopausal gonadotrophin in Iraqi Awassi ewes

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### Article information

#### Article history:

Received May 17, 2020  
Accepted July 14, 2020  
Available online June 24, 2021

#### Keywords:

HMG  
PMSG  
Hormone  
Awassi ewes  
Estrus synchronization

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### Abstract

This study aimed to induce estrus by using Human Menopausal Gonadotrophin (HMG) in Iraqi Awassi Ewes and comparison its action with Pregnant Mare Serum Gonadotrophin (PMSG). The study was carried out between first of June 2018(non or earlier-breeding season) until all ewes lambing in the middle of November 2018. Sixty Iraqi Awassi ewes aged 2-4 years old and weight 35-45 kg were used in this study. Ewes were divided randomly into three groups, (20 ewes per each). All ewes comprised of intra-vaginal sponge impregnated by progesterone for 12 days. Control ewes (C) were injected intramuscularly with 2ml of sterile saline solution, HMG treatment group (HMG) were injected intramuscularly with 300 IU of HMG hormone while PMSG treatment Group (PMSG) were injected intramuscularly with 500 IU of PMSG at the same day of sponge removal. Estrus response percentages were 40% (8/20), 85% (17/20), 90% (18/20) in control (which significantly change  $P<0.05$ ), HMG and PMSG groups, respectively. Estrus onset time was  $71\pm 1.9$ ,  $72\pm 1.9$ , and  $71\pm 1.0$  hours, respectively, also the data records no significant changes in estrus duration means which were  $31\pm 0.5$ ,  $31\pm 0.7$  and  $31.0\pm 0.5$  hours, respectively in all groups. The pregnancy rate was 30% (6/20), 85% (17/20), 90% (18/20), respectively (control group significantly change  $P<0.05$ ). The gestational period was  $155\pm 1.4$ ,  $155\pm 1.3$  and  $155\pm 1.6$  days respectively in all the groups and the twinning rate were  $1.1\pm 0.3$ ,  $1.1\pm 0.1$  in both HMG and PMSG groups respectively without significant changes ( $P<0.05$ ) while control group record no twinning data. The result of this study recorded rise of dystocia and pregnancy toxemia which were higher in the PMSG group when compare with other groups. We can conclude that using HMG and PMSG hormones show a high rate of successful when they used in estrus synchronization programs but the birth problems need more investigation.

DOI: [10.33899/ijvs.2020.127132.1466](https://doi.org/10.33899/ijvs.2020.127132.1466), ©2021, College of Veterinary Medicine, University of Mosul.  
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### Introduction

Awassi sheep is regarded as highly productive native dairy breed as well as producing wool and meat, which play an important role in the economic life of a farmer and their families (1,2). Previous studies have reported that synchronization in sheep is achieved by control of the luteal phase of the estrus cycle, either by providing exogenous progesterone or by inducing premature luteolysis and extend

the luteal phase or shorten this phase by prematurely regressing existing corpora lutea (3). The success of hormonal treatment for estrus synchronization depends on the productive status of ewes (4), the season of the year (5), the type of hormonal protocol and the method of administration (3,6). Multiple ovulation programs are also possible with the use of estrus synchronization for improving herd productivity (7). Gonadotrophin hormones are essential for growth, maturation and ovulation were a complete

successful estrus cycle in ewes (8). Various protocols have been developed for estrus induction, superovulation and to increase the fertility in the ewes such as Follicle Stimulating Hormone (FSH), equine Chorionic Gonadotropin (eCG) or Pregnant Mare Serum Gonadotropin (PMSG), Gonadotropin hormone (GnRH), Melatonin, immunized against synthetic inhibin- $\alpha$  and Prostaglandin (PGF $2\alpha$ ) with or without using Progesterone, in or out of reproductive seasons with varying degrees of success (9,10). The variation among using these hormones and its results, may be related to the variation in sheep breed, doses of hormones, seasons, mating system and types of hormones (3). Human Menopausal gonadotropins or HMG is one of the exogenous gonadotropins hormone which have been used for superovulation and for the treatment of low fertility in women and men with more acceptable of successful, which contain an equal ratio of FSH and LH become available in the late 1960s, and it's also known for using in superovulation and embryo transfer in cattle, mare, and swine (11). Pregnant Mare Serum Gonadotropins or PMSG hormone is one of the Gonadotropins hormones which have been used widely as an exogenous hormone for estrus synchronization programs and increase fertility in ewes due to its FSH- and LH-like actions hormone (12), leading to ovulation and luteinizing of granulosa and theca cells to form luteal cells for subsequent progesterone secretion. This protocol ensures appropriate progesterone concentration to induce turnover and ovulation of a non-persist follicle (13). A high percentage of estrus successful rate has been recorded following the application of intra-vaginal sponges contain 30-40 mg of progesterone hormone for 12-14 d with different doses of PMSG (14). Controlled internal drug release (CIDR) is effective when used with PMSG in ewes (15). PMSG hormone is required to stimulate follicle growth, leading to high ovulation rate by an estrus animal out of the breeding season (16).

It is necessary to applied superovulation programs to increase total numbers of animals, twins percentage with all available methods, so the study was designed to compare between using HMG and PMSG in estrus synchronization programs and normal gestation period with recorded parturition with/or without problems.

## **Materials and methods**

### **Location of the study**

The study was carried out at a farm located in Kokagli district, Mosul city, Nineveh province at Northern of Iraq its located 36.34 Latitude and 43.13 Longitude and it's situated at elevation 288 meter above sea level. It carried out earlier to breeding season started from the first of June 2018 until all ewes lambing in the middle of November 2018.

### **Animal of the study**

Sixty Iraqi Awassi ewes aged 2-4 years and weighted 35-45 kg were used in this study. animals were fed on 1.5-2 kg

of mixture food during the entire period of study, in addition, the ewes grazed daily for 6-8h on pasture with water freely in all the day. All animals were healthy and had good body condition. Animals were treated against sheep disease like sheep pox, enterotoxaemia, and external and internal parasite infection during the period of study. Animals were examined for pregnancy diagnosis or reproductive disorders by using B mode ultrasonography. All groups of ewes were served naturally by using fifteen proven fertile rams (five males for each group) which were freely grazed and kept with the ewes in the same condition. Detection of estrus cycle was depend on the signs of estrus including restlessness, seeking for rams and rubbing their body and neck against the ram, stand still during mounting, were recording for each ewe.

### **Experimental design**

The ewes were divided randomly into three groups (n=20 ewes for each group). all ewes comprised of intra-vaginal sponge impregnated by progesterone (Synncropart 40 mg sheep sponge, Ceva Sante Animal, France) for 12 days. Group I: Control Group (C): ewes were injected intramuscularly with 2ml of sterile saline solution on the same day of sponge withdrawal. Group II: HMG Treatment Group (HMG): ewes were injected intramuscularly with 300 IU of HMG (Pergonal 150 IU for each vial, production of Serum Institute of India Ltd. India) on the same day of sponge withdrawal. Group III: PMSG Treatment Group (PMSG): ewes were injected intramuscularly with 500 IU of PMSG (Folligon; Intervet International B.V., Boxmeer, Holland) on the same day of sponge withdrawal. Ewes were kept monitoring till estrus signs were showed, all ewes showed signs of estrus after 72 h after sponge withdrawal. Natural mating for all ewes in estrus also recorded as summarized in Figure 1. All ewes were examined monthly by using B mode ultrasonography for pregnancy diagnosis and kept under monitoring during the gestational period. Lambing of pregnant ewes and twinning rate (number of twins), fetal abnormalities and dystocia were recorded for all studied groups. The criteria which considered are: clinical examination to detection of ring womb cases and failure of ewes to get parturition after 6 hours from starting labor signs and treated under authors supervising every 30 minute from beginning of labor signs.

### **Statistical analysis**

Data of the study were expressed as Mean + SE. Normally distributed data were compared by ANOVA 1 (One-Way Analysis of Variance). Significant differences were determined by Duncan's Multiple Range Test. statistical analyses were performed by Sigma Stat (Jandel scientific software V3.1) and (P<0.05) was consider as statistically significant.

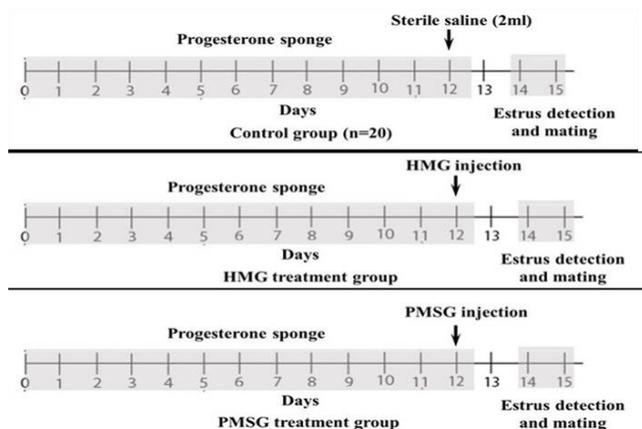


Figure 1: Time scale of hormones injection and estrus detection in the studied groups.

### Results

Data of this study were summarized in table 1 for all three groups. Estrus response percentages were 40% (8/20), 85%

(17/20) and 90% (18/20) in control group (C), HMG and PMSG groups ( $P<0.05$ ), respectively (control group significantly change  $P<0.05$ ); while Estrus response times show changes in its means which were  $71\pm1.9$ ,  $72\pm0.9$  and  $71\pm1.0$  hours, respectively with no significant changes between control and PMSG groups. Estrus durations means were  $31\pm0.5$ ,  $31\pm0.7$  and  $31.0\pm0.5$  hours in all groups, respectively without significant changes.

Pregnancy data were estimated in table 2 for all studied groups. The pregnancy rates were 30% (6/20), 85% (17/20) and 90% (18/20) in the studied groups (control group significantly change  $P<0.05$ ), respectively. The Gestational periods were  $155\pm 1.4$ ,  $155\pm1.3$ ,  $155\pm1.6$  days in the studied groups, respectively. The twinning rate were  $1.0\pm0.3$  and  $1.1\pm0.1$  in both HMG and PMSG groups, respectively without significant changes ( $P<0.05$ ), while the control group recorded no twinning data. The result of this study recorded a rise of some birth problems which were dystocia and pregnancy Toxemia (according to the clinical signs) in four cases which recorded in the PMSG groups and one case of dystocia in the HMG group with mild of severity of each one.

Table 1: Shows Estrus values differences (%) between the treatment's groups

Groups	N	Estrus Response %	Onset of Estrus(Hours) mean± SE	Duration of Estrus(hours) mean± SE
Control	20	A 40 (8/20)	$71\pm1.9$ A	$31.0\pm0.5$ A
HMG	20	B 85 (17/20)	$72\pm0.9$ A	$31.0\pm0.7$ A
PMSG	20	B 90 (18/20)	$71\pm1.0$ A	$31.0\pm0.5$ A

Values are expressed as Means ± SE, Different letters denote significantly different at ( $P<0.05$ ).

Table 2: Show pregnancy values differences (%) between the treatments group

Groups	Pregnancy%	Gestational period (days) mean± SE	Twinning rate mean± SE	Lambing problems	
				Dystocia	Pregnancy toxemia
Control	30 (6/20) A	$155\pm1.4$ A	$0.0\pm0.0$ A	-	-
HMG	85 (17/20) B	$155\pm1.3$ A	$1.1\pm0.3$ B	1	-
PMSG	90 (18/20) B	$155\pm1.6$ A	$1.0\pm0.1$ B	3	1

Values are expressed as Means ± SE, Different letters mean significantly different at ( $P<0.05$ ).

### Discussion

The result of this study recorded high percentage of successful rate when using Human Menopausal Gonadotropin in estrus synchronization protocol with progesterone as intra-vaginal sponges during non-breeding season in Iraqi Awassi ewes with similar or slightly effects compared with PMSG but without significantly changes. Both hormones showed similar results for estrus response percentages, estrus response times and estrus durations time with no significant changes in its means but control groups recorded lower data in estrus response percentage when comparing HMG and PMSG groups. The present results were in agreement with the previous reports that referred to

using PMSG hormone as a most successful hormone for estrus synchronization program in or out of breeding season (18,19). This effect may be due to the action of PMSG hormone as gonadotropins which increase the number of the growing follicle, ovulation rate, litter size, and its effect on IGF-1 which plays an important role in reproductive metabolic signals of reproductive system and increase numbers of medium estrogenic follicles (20,21).

The effect of HMG may be due to its FSH- and LH-like action to stimulate ovaries to produce follicle and show estrus signs with a high rate of pregnancy (14). The requirement for using HMG (Pergonal) for estrus synchronization program are many, first, it is successfully proven effect in cattle and camel and swine and secondly, its

action has been proven in human, where used for treated of subfertility in men and women couples (22). The other advantage for using HMG in estrus synchronization instead of some Gonadotropins or Gonadotropin like is due to its save usage without any obstetric problems which were reported by the use of PMSG (23). The long half-life of PMSG can disadvantage since it may cause over and/or prolonged stimulation, leading to a second wave of follicular development after ovulation, so PMSG treatment needs to be neutralized by PMSG antiserum to prevent the secondary development of ovarian follicles (24).

The percentage of pregnancy rate showed highly significant changes between control group in comparison with HMG and PMSG groups and the differentiation related to the change in the percentage of estrus synchronization response. The gestational period in the three groups show no significant changes. Twining rate also show no significant changes between HMG and PMSG groups. In addition, a rise of some labor problems were recorded in PMSG treated group, such as dystocia (three cases) and pregnancy toxemia (one case), which not record in HMG group except in the case of dystocia (one case). There was no clear correlation between the recorded birth disorder with using gonadotropin during estrus synchronization programs, but some previous reports by Park *et al.* (23) and Summon *et al.* (24) were suggest that the excess gonadotropin stimulation may cause side effect, Such as impairment of reproductive function and maternal environment. The changing in uterus environment and impairment of reproductive function may be lead to difficult birth or dystocia (25). The mechanism of parturition depended up on hormones or tissue response to hormonal secretion, Calcium, Magnesium, Sodium, Vitamin A and other contribute with this mechanisms (26), any disturbance may be leading to dystocia if discard other mechanical causes of dystocia (27). Although the current study recorded the emergence of dystocia and pregnancy toxemia after the use of the hormones, but It is not possible to say for sure that their use led to the emergence of these cases, and therefore we recommend more studies to investigate the topic.

## Conclusion

It can be concluded that Estrus synchronization in Iraqi Awassi ewes by using HMG or PMSG in does (300 IU), (500 IU) respectively have been indicated, in the present study, with a good estrus response and pregnancy rate during the breeding season. Human Menopausal Gonadotropin (HMG) reveled good result in estrus synchronization, pregnancy, lambing and time of parturition. Dystocia, pregnancy toxemia and other birth problems (like hypocalcemia) need more investigations to find out if there is a correlation between the type of hormones used for estrus synchronization program and the incidence of these cases.

## Acknowledgments

The authors are very grateful to the university of Mosul/ college of veterinary medicine for their a provide facilities, which helped to improve quality of this work, and also the authors very thankful to animal's owners who helped us to complete this study.

## Conflict of Interest

No conflict

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## إحداث الشبق باستخدام هرمون محرض القند الحيضي البشري في النعاج العواسية العراقية

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فرع الجراحة وعلم تناسل الحيوان، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

### الخلاصة

إن الهدف من الدراسة هو أحداث الشبق في النعاج العواسية العراقية باستخدام هرمون محرض القند الحيضي البشري ومقارنة تأثيره مع هرمون مصل دم الفرس الحامل. أجريت الدراسة مابين الأول من حزيران ٢٠١٨ (خارج موسم التناسل) ولغاية ولادة جميع النعاج في منتصف تشرين الثاني ٢٠١٨. استخدم في الدراسة ستون نعجة عواسية عراقية تراوحت أعمارها ما بين ٤-٢ سنة وأوزانها ٣٥-٤٥ كغم. قسمت النعاج إلى ثلاثة مجاميع (عشرون نعجة لكل مجموعة). أدخلت الإسفنجات المهبلية المشبعة بهرمون البروجستيرون إلى جميع النعاج ولمدة اثنا عشر يوماً. حققت مجموعة السيطرة بمحلول الملح الفسيولوجي وبواقع ٢ مل بالعضل وحققت مجموعة هرمون محرض القند الحيضي البشري وبواقع ٣٠٠ وحدة دولية بالعضل بينما تم حقن مجموعة هرمون مصل دم الفرس الحامل بالهرمون وبواقع ٥٠٠ وحدة دولية بالعضل أيضاً في نفس اليوم الذي تم إزالة فيه الإسفنجات المهبلية من النعاج. النسبة المئوية لحصول الشبق كانت ٤٠% (٢٠/٨)، ٨٥% (٢٠/١٧)، ٩٠% (٢٠/١٨) لكل من مجموعة السيطرة، مجموعة هرمون ومجموعة هرمون على التوالي، أما مدة الشبق فكانت ١,٩±٧١، ١,٩±٧٢ و ١,٠±٧١ ساعة على التوالي، ولم تسجل الدراسة فروقات معنوية في مدة الشبق حيث كانت ٣١±٠,٥، ٣١±٠,٧ و ٣١±٠,٥ ساعة على التوالي في جميع المجاميع. كانت النسبة المئوية لحصول الحمل في الحيوانات ٣٠% (٢٠/٦)، ٨٥% (٢٠/١٧) و ٩٠% (٢٠/١٨) على التوالي. بلغت فترة الحمل ١٥٥±١,٤، ١٥٥±١,٣ و ١٥٥±١,٦ يوم للمجاميع الثلاثة، وبلغ معدل حصول التوائم ١,١±٠,٣ و ١,١±٠,١ في مجموعتي هرمون محرض القند المشيمي البشري و هرمون مصل دم الفرس الحامل تالياً دون وجود فروقات معنوية بينهما، ولم تسجل مجموعة السيطرة حصول حالة توائم. سجلت الدراسة حصول حالة عسر الولادة وحالة تسمم الحمل في مجموعة هرمون مصل دم الفرس الحامل وبمعدل اعلى عند مقارنتها ببقية المجاميع. يمكن الاستنتاج ان استخدام هرمون محرض القند المشيمي البشري وهرمون مصل دم الفرس الحامل أدى إلى حصول معدلات نجاح عالية عند استخدامها في برامج توحيد الشبق لكن أدى استخدامها إلى حصول بعض مشاكل الولادة والتي بحاجة إلى مزيد من الدراسة والاستقصاء مستقبلاً.