

## Induce estrus with ultrasonography examination and progesterone hormone assay for pregnancy diagnosis in Iraqi goats

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### Summary

The aim of present work is to induce ovulation in 40 female goats in non-breeding seasons and pregnancy diagnosis using RIA of progesterone serum level and trans abdominal ultrasonography with 3.5 MHz prop. The 40 Iraqi goats were naturally inseminated during estrous phase, using fertile backs, after withdrawal of intravaginal impregnated sponges with 20 mg of cronolone (Fluorogestone Acetate progestagen) kept for eleven days and 400 IU of equine chorionic gonadotrophin inject I/M 24hrs. before sponge withdraw. The results indicated that all does were showed (100%) estrous sign, the estrous time was 24-66 hrs. after sponge withdrawal and the pregnancy rate 67.5%. Blood samples were collected from the jugular vein before progesterone treatment, at the time of insemination and after 21 day of insemination. Early pregnancy diagnosis by RIA of progesterone concentration was performed and showed progesterone hormone value as were increasing significantly ( $P<0.01$ ) from  $0.26\pm 0.12$  ng/ml before insemination to  $3.29\pm 1.10$  ng/ml in 21 days after insemination. Pregnant animal was examined using transabdominal ultrasonography during 45, 60 and 90 days after insemination. In conclusion, estrus could be efficiently induced in female goats during non-breeding season using 20 mg cronolone impregnated sponge and 400 IU equine chorionic gonadotrophin I/M at 24 hrs. before spongy withdrawal. The effective of early detection of pregnancy by progesterone assay in 21 days after insemination and ultrasonography by abdominal 3.5 MHz transducer after 45 days of pregnancy.

**Keywords:** Cronolone, Vaginal sponges, Equine chorionic gonadotrophin, Ultrasonography, Progesterone, Goats.

### Introduction

Goats are one of the oldest domesticated species, and have been used for their milk, meat, hair, and skins over much of the world (1). In goat intravaginal sponge contains many synthetic preparations with progesterone activity and PMSG could be use for estrous synchronization and superovulation (2). It has been reported that PMSG could increase pregnancy and twinning rates in breeds characterized by low litter size (3) Synchronization and estrus infusion out of reproduction season creates suitable economical and managerial opportunities for producers (4). The pregnancy of goat depended on progesterone from the corpus luteum to maintain pregnancy throughout gestation period (5). So, the best use of progesterone tests is to identify nonpregnant does 3 weeks after breeding. Does with low progesterone concentrations are considered open and are scheduled for rebreeding (6). This is particularly useful for does breed out of

season, which does not reliably exhibit estrus after an infertile service. Accuracy of diagnosis from blood or milk progesterone levels, 20 to 24 days after breeding, varied from 80 to 100% for nonpregnancy and 67 to 100% for pregnancy (7 and 8). Plasma progesterone concentrations reflected more accurately the true endocrine status of does and were more accurate than milk progesterone concentrations for pregnancy diagnosis (9). Ultrasonography consider as easy simple, accurate, rapid, practical and safe technique for both operators and animals in early pregnancy diagnosis and the scanning for pregnancy could be rapidly learned and experienced examiners achieve an accuracy of 91% to 100 (10).

The aim of present study is synchronization of goat estrus using 20 mg impregnated sponges with cronolone (chronogest CR<sup>®</sup>) and 400 IU eCG during out breeding season and using natural fertilization and pregnancy

diagnoses using progesterone hormone assay and Ultrasound scanning.

### Materials and Methods

The study was carried out on forty healthy does range in age from 2-5 years old and weight  $25 \pm 2.2$  kg and four fertile bucks about 3-4 years old in age. The animals were housed semi opened, in animal place at animal farm of the College Vet. Med. / University of Baghdad from 16-Feb until 25-Jun-2014. Before progesterone treatment all experimental does were checked by male and ultrasound to confirm the presence or absence of pregnancy. Induction of estrous was achieved by insertion of intra vaginal impregnated sponge with 20mg cronolone (Fluorogestone Acetate Progestagen) (Chronogest: Intervet) for 11 days synergized with intramuscular injection of 400 IU eCG (intervet) at 24 hrs before sponge withdrawal. Blood samples were collected from the jugular vein of 40 does for early pregnancy: before the application of the vaginal sponges, at the time of insemination and after 21 day of insemination. Serum was separated by centrifugation and stored at  $-20^{\circ}\text{C}$  until assays for progesterone concentration by radioimmunoassay (RIA). Detection of pregnancy before progesterone treatment (to verification the animals nonpregnant) and after sponges withdraw at 45, 60 and 90 days post insemination based on ultrasonic examination (EDAN, China) with Prop 3.5 MHz for abdominal examination. The data were statistical analyzed by SAS program (11).

### Results and Discussion

The does not show any signs of estrous during pre-treatment period detected by buck, as well as during progesterone treatment. This result agrees with that reported by (12), which suggested that the insertion of the sponge contained progesterone has the ability to abolish the estrous as long as they exist inside the vagina and considered as artificial source of progesterone insertion. The 20 mg cronolone used in this work was enough to suppress the production of gonadotropin during non- breeding season; the does showed typical estrous signs within 24-72 hrs. after sponge removal (remove of the blockage of progesterone) lead to release of gonadotropin

and subsequent estrous and ovulation in female treated with progesterone. In Iraq Kashifalkitaa (13) recorded 100% of estrous emergence after using 40mg MAP impregnated sponge in goat. While (14) used 40 mg progesterone in does during breeding season recorded 77.55% estrous synchronization and 60 mg during non breeding season recorded 33% emergence estrous. This variation could be due to the high level of progesterone 60 mg and long treatment, which led to decrease the fertility of animals, by inhibit the activity of hypothalamus - pituitary - ovary system.

Fertility result in this study was evaluated by kidding rate and result proved that 27 does out of 40 (67.50%) gave birth. However, the percentage of nonpregnant does was 13(32.50%) which might be attributed to fertilization failure or early embryonic mortality, this finding is in agreement with (15 and 16) but disagrees with the finding of (17) (Table, 1).

**Table, 1: Distribution of animal study (does) according to pregnant and non-pregnant goats.**

Animal	Number	Percentage (%)
Pregnant	27	67.50 a
Non-pregnant	13	32.50 b
Total	40	100 %
Chi-square value ( $\chi^2$ )	---	10.215 **
P-value	---	0.0059
** (P<0.01).		

Different small letters represented significant different at the level of (P<0.01) in between group.

The present protocol was also recommended during non breeding season to improve ovulation rate by (17). Armstong (18) and Drion (19) are claimed that eCG has a long half life about 4-6 days in turn, the long action of biological activity causing to continually release few number of non ovulated immature follicles as well as the ovulated follicles caused increases in estrogen levels of uterus secretion which lead to slip-up the sperms from vagina insemination which lead to decrease pregnancy rate.

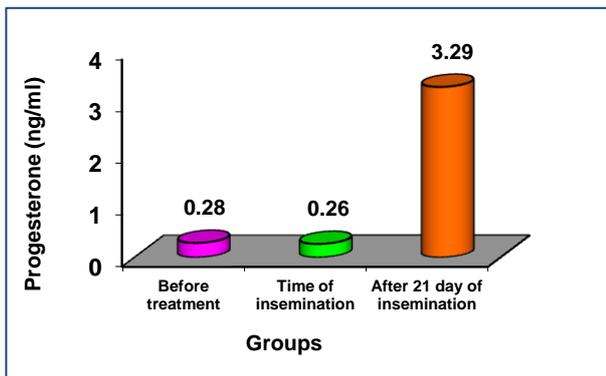
Table (2) and (Fig. 1) show the mean Values of progesterone before estrus synchronization, time of insemination and 21 days after insemination. Before estrus synchronization and at insemination time there were no differences in the mean value of

progesterone was  $0.28 \pm 0.14$  ng/ml and  $0.26 \pm 0.12$  ng/ml, respectively and there was a significant ( $P < 0.01$ ) differences in the mean value of progesterone after 21 days of insemination ( $3.29 \pm 1.10$  ng/ml) and other two time. Present result agreed with (20 and 21), how found that the mean plasma progesterone levels increased during the 20-21 days after breeding, and fluctuated around its mean level 2.5-4.5 ng/ml but the progesterone remained at basal levels 0.1-0.2 ng/ml throughout the estrus (22).

**Table, 2: Pregnancy diagnosis by level of progesterone (ng/ml) in Iraqi goats.**

Time	Mean $\pm$ SE of Progesterone (ng/ml)
Before treatment	$0.28 \pm 0.14$ b
Time of insemination	$0.26 \pm 0.12$ b
After 21 days of insemination	$3.29 \pm 1.10$ a
LSD value	0.594 **
P-value	0.0001
** ( $P < 0.01$ ): Highly significant.	

Different small letters represented significant different at the level of ( $P < 0.01$ ) within the time.

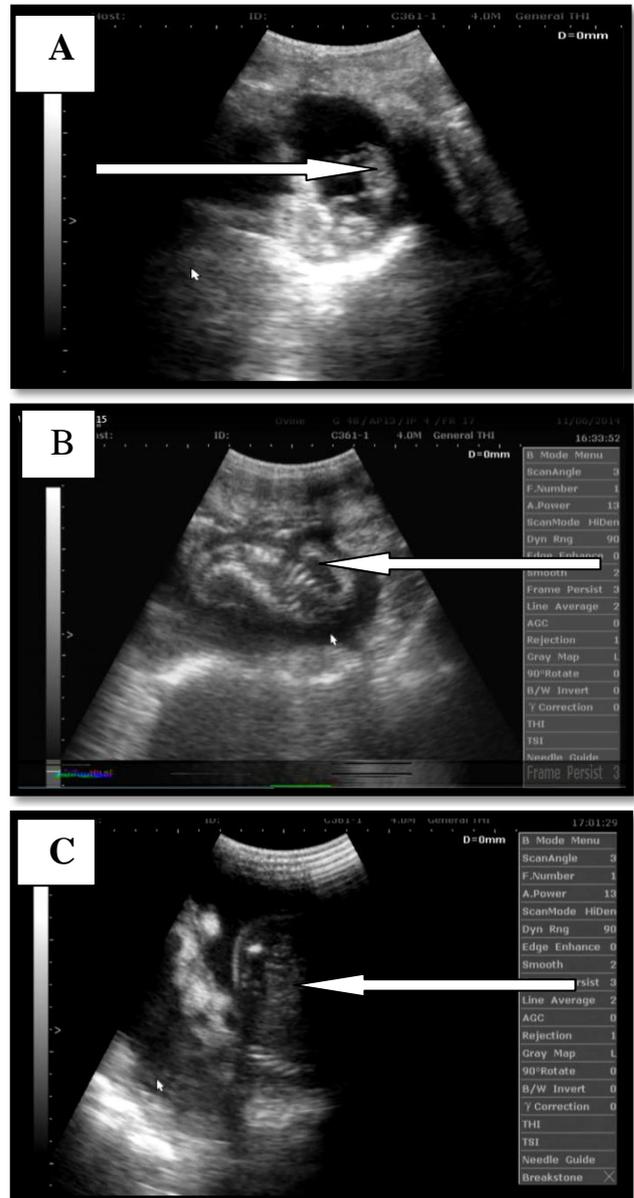


**Figure, 1: Pregnancy diagnosis by level of progesterone (ng/ml) in iraqi does**

The results of pregnancy diagnosis using 3.5 MHz prob ultrasonography, trans abdominal examination, indicated the sufficient of this method for 45 days pregnancy (Fig. 2) and could be repeated after 60-90 days for accuracy. Present work also agreed with the result that early pregnancy detection in goats was practicable and recommended as a reliable means of pregnancy detection as early as 26 days after conception and a long period of pregnancy with an abdominal 3.5 MHz transducer (23).

In conclusion, estrus could be efficiently induced in female goats during non-breeding season using 20mg cronolone impregnated sponge and 400 IU eCG The effective of early

detection of pregnancy by progesterone assay in 21 days after insemination and ultrasonography by abdominal 3.5 MHz transducer after 45 days of pregnancy. These methods of pregnancy diagnosis are simple, accurate, rapid, practical and safe for both operators and animals.



**Figure, 2: Ultrasonography pictures shows fetus in doe uterus: (A) on 45 days of gestation. (B) On 60 days of gestation. (C) on 90 days of gestation.**

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## استحداث الشبق مع الفحص بالامواج فوق الصوتية وقياس هرمون البروجستيرون لتشخيص الحمل في المعز العراقي

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### الخلاصة

استهدفت هذه الدراسة امكانية استحداث الشبق في إناث المعز خارج موسم التناسل، فضلاً عن تشخيص الحمل بقياس هرمون البروجستيرون وفحص الموجات فوق الصوتية. تم تسفيد (40) من أنثى المعز العراقية طبيعياً عند ظهور علامات الشبق والمشخصة بواسطة ذكور مؤكدة الكفاءة التناسلية بعد تزامن الشبق باستعمال الاسفنجات المهبلية المشبعة بـ 20 ملغ من كرونولون لمدة أحد عشر يوماً وحقن 400 وحدة دولية من هرمون الفرس المشيمي قبل 24 ساعة من سحب الإسفنجات المهبلية. وقد أظهرت جميع حيوانات التجربة علامات الشبق 100٪، وكان وقت ظهور الشبق 24-66 ساعة بعد سحب الإسفنجات وأن معدل الحمل 67.5٪. جُمعت عينات الدم من جميع الحيوانات من الوريد الوداجي قبل إدخال الاسفنجات المهبلية، في وقت التسفيد وبعد 21 يوماً من التسفيد. أجريت تشخيص الحمل المبكر بقياس تركيز هرمون البروجستيرون في مصل الدم خلال مدد التجربة، فضلاً عن تقييم الحمل في المعز باستعمال الموجات فوق الصوتية عبر جدار البطن باستعمال محول 3.5 ميغاهيرتز بعد 45 و60 و90 يوماً من الحمل بعد إجراء الجس عن طريق البطن أيضاً. أظهرت النتائج وجود زيادة معنوية في تركيز هرمون البروجستيرون بعد 21 يوماً من الحمل في مصل دم أنثى المعز (3.29±1.10 نانوغرام/مل) مقارنة مع مدة يوم التسفيد (0.26±0.12 نانوغرام/مل) وقبل التسفيد (0.28±0.14 نانوغرام/مل). كما تأكد الحمل بجهاز الموجات فوق الصوتية بعد 45 و60 و90 يوماً من الحمل. يتضح من هذه الدراسة إمكانية إحداث الشبق وبكفاءة في أنثى المعز العراقية خارج موسم التناسل باستعمال الاسفنجات المهبلية المشبعة بالكرونولون (20 ملغم) مع 400 وحدة دولية من هرمون الفرس المشيمي. لذا تعتبر هذه الطريقة بسيطة ودقيقة وسريعة واقتصادية في أحداث الحمل وتشخيصه في أنثى المعز العراقية.

**الكلمات المفتاحية:** كرونولون، أسفنجات مهبلية، هرمون الفرس المشيمي، الفحص بالامواج فوق الصوتية، هرمون البروجستيرون، المعز.