

## **USE OF OXYTOCIN COMPARED WITH PROSTAGLANDIN TREATMENT FOR OESTROUS SYNCHRONIZATION IN SHEEP AND GOATS**

Ashmawy, T. A. M. ; B. E. El-Saidy, and M. G. Gabr

Animal Production Research Institute, Agricultural Research Center

### **ABSTRACT**

The current study was carried out at Sakha Animal Production Research Station, belonging to Animal Production Research Institute, during September breeding season of ewes and does. The aim of this study was to compare the effects of use of oxytocin and PG hormonal treatments in synchronization of oestrus and subsequent fertility of ewes and does during the breeding season.

A total of 30 mature crossbred ewes (1/2 Finnish Landrace . 1/2 Rahmani) of 3-4 years of age and 45-50 kg body weight, and 30 does (1/2 Damascus . 1/2 Baladi) of similar age and 35-40 kg body weight were used. Animals of each species were divided into 3 similar groups (10 animals each). In each species, animals were intramuscularly injected with double doses 11 days apart of 25 I.U. oxytocin, for the 1<sup>st</sup> group (25 Ox) ; 50 I.U. oxytocin for the 2<sup>nd</sup> group (50 Ox) and 0.7 ml Cloprostenol for the 3<sup>rd</sup> group (PG). In both species, all females were detected for the heat occurrence starting from 24 hrs post-treatment. Females came in estrus following 2<sup>nd</sup> injection were artificially inseminated with fresh semen twice, 24 and 36 h after the onset of oestrus. Blood samples were collected from all treated females for determination of plasma progesterone concentration.

Full response of both species to treatment with 50 Ox or PG, while 25 Ox group resulted in low rates of the occurrence of estrus. The earliest time of oestrous response was detected in treated ewes and does in PG group, while the moderate time in ewes and does of Ox-treatments (50 Ox and 25 Ox). After day 11 progesterone level dropped sharply within 24 hr in response to different treatment in both sheep and goats. Post insemination, no clear differences occurred between treatment groups in progesterone profile in pregnant ewes and does up to day 30 when progesterone level was markedly lower in Ox groups than in PG one and lower in group 25 Ox than in 50 Ox. Ewes of the low oxytocin dose (25 Ox) had similar fertility rate to that for those treated with PG. In does, fertility was significantly higher in the group of 25 Ox than in the other two groups. It is of interest to observe that the hormonal treatment affect markedly on litter size of does and ewes.

In conclusion, oxytocin in the low dose (25 IU) is not recommended and that in dose of 50 IU may be used in some cases. More studies in this field are needed.

**Keywords:** Sheep, Goats, Oestrous, Synchronization, Hormone

### **INTRODUCTION**

Sheep and goats are very important domestic animals in tropical and subtropical regions. Improved oestrous detection and control methods are the major means achieving improved reproductive performance.

Oestrous control measures are likely to be of good practical interest as means of facilitating the application of artificial insemination (AI) in sheep and goats.

PGF<sub>2α</sub> (or its analogues) has been employed as a practical mean of synchronizing oestrus in cyclic ewes and does. Regression of CL in the cyclic cows is thought to result from an interaction between oxytocin, released by

CL and PGF<sub>2α</sub> secreted by the uterus (Lafrance and Goff, 1988). Oxytocin is able to stimulate the secretion of PGF<sub>2α</sub> around the time of luteolysis (Flint *et al.* 1994 and Bainbridge *et al.*, 1996). There is a positive correlation between oestradiol/progesterone ratio and the PGF<sub>2α</sub> metabolite (PGFM) in response to oxytocin late in the luteal phase (Silvia and Taylor, 1989). Although oxytocin receptors are present in bovine and ovine luteal cells (Okuda *et al.*, 1992), oxytocin bindings dramatically increase in membrane from the late luteal phase compared with the early or mid luteal phase (Fuchs *et al.*, 1990). Exogenous oxytocin may involve in luteolysis during the luteal phase in cattle (Zollers *et al.*, 1989).

The aim of this study was to compare the effects of use of oxytocin and PG hormonal treatments in synchronization of oestrus and subsequent fertility of ewes and does during the breeding season.

### **MATERIALS AND METHODS**

The current study was carried out at Sakha Animal Production Research Station, Kafr El-Sheikh Governorate, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, during September breeding season of ewes and does.

A total of 30 mature crossbred ewes (1/2 Finnish Landrace . 1/2 Rahmani) of 3-4 years of age and 45-50 kg body weight, and 30 does (1/2 Damascus . 1/2 Baladi) of similar age and 35-40 kg body weight were used. The experimental animals were housed in semi-open sheds and fed berseem hay and concentrate feed mixture (14% CP) according to NRC (1984). Trace mineralized saltlick blocks and water were available all day times.

Animals of each species were divided into 3 similar groups (10 animals each). In each species, animals were intramuscularly injected with double doses 11 days apart of 25 I.U. oxytocin (Oxytocin synth., Richter, LTD), for the 1<sup>st</sup> group (25 Ox) ; 50 I.U. oxytocin for the 2<sup>nd</sup> group (50 Ox) and 0.7 ml PGF<sub>2α</sub> (Estrumate, Coopers Animal Health LTD, Berkhamsted-England) for the 3<sup>rd</sup> group (PG). Each ml of Estrumate contained 263 µg Cloprostenol Sodium equivalent to 250 µg Cloprostenol.

In both species, all females were detected for the heat occurrence four times daily (30 min each) using aproned intact fertile male (ram/buck) starting from 24 h post-treatment.

#### **Insemination:**

Females came in estrus following 2<sup>nd</sup> injection were artificially inseminated with fresh semen twice, 24 and 36 h after the onset of oestrus. Semen was collected from mature males (rams /bucks) and only ejaculates of 80-90% initial motility were diluted (1 part semen : 4 part extender) at 37°C. The used extender contained 2.9 g sodium citrate, 0.75 g glucose, 5 ml egg yolk, 100.000 IU Penicillin and 100.000 mg Streptomycin and distilled water up to 100 ml. Insemination was carried out using a simple inseminating pipette with fine blunt bent end and a vaginal speculum. Semen (about 1 cm) was deposited into the cervix as far as possible.

#### **Progesterone hormone assay:**

Blood samples were collected from all treated females on days -1, 0, 3, 7, 10, 11, 12, 13, 14, 15, 23 and 30 of the 1<sup>st</sup> injection. Blood plasma was

separated by centrifugation of blood samples at 3000 rpm for 15 min and stored at -20°C until analysis for determination of plasma progesterone concentration.

Progesterone hormone concentration was determined by Radioimmunoassay procedure in samples of 5 selected animals (3 lambed/kidded and 2 non-lambed/kidded females) from each group. Quantitative determination of progesterone in plasma samples was carried out using progesterone radioimmunoassay kit (catalog No. 1188 manufactured by Immunotech, France).

**Statistical analysis:**

Data were analyzed using SAS (1999), analysis of variance for onset of oestrus and litter size and Chi-squares for estrous occurrence and fertility rate. Duncan Multiple Range test (Duncan, 1955) was used to get the mean separations among the effects of treatment on the studied traits.

**RESULTS**

**Occurrence of estrus:**

Data presented in table (1) revealed full response of both ewes and goats to treatment with 50 IU oxytocin or PG. On the other hand, the low dose of oxytocin (25 Ox) resulted in low rates of the occurrence of estrus in both species.

**Table (1): Response of the experimental females to different hormonal treatments.**

Treatment group	Total number	Animals in estrus	
		n	%
<b>Ewes</b>			
25 OX	10	6	60 <sup>b</sup>
50 OX	10	10	100 <sup>a</sup>
PG	10	10	100 <sup>a</sup>
<b>Does</b>			
25 OX	10	5	50 <sup>b</sup>
50 OX	10	10	100 <sup>a</sup>
PG	10	10	100 <sup>a</sup>

a and b: Means denoted within the same column for each species with different superscripts are significantly different at P<0.05.

**Onset of oestrus (h):**

Data in table (2) show that all animals exhibited estrus in response to PG treatment within 56 hr from 2<sup>nd</sup> injection. In comparison only 60% of each of ewes and does in group 50 Ox and 3 out of 6 (50%) and 3 out of 5 (60%) in group 25 Ox came in estrus within the same period. The rest of animals (both species) in 25 Ox and 50 Ox came in estrus later with considerable parts showing estrus behind 72 hr from treatment. These results indicate wide dispersion in onset of estrus following oxytocin treatment.

**Progesterone profile:**

Progesterone profiles around times of different treatments in the selected pregnant and non-pregnant ewes and does are illustrated in Fig. 1.

Before and at time of 1<sup>st</sup> injection of different treatments, animals of both species were at different phases of the ovarian cycle. By day 7,

progesterone level in all animals exceeded 0.5 ng/ml indicating initiation of synchronized luteal phase. After day 11 (2<sup>nd</sup> injection), progesterone level dropped sharply within 24 hr in response to different treatments in both sheep and goats. In ewes, this drop was of less speed in lower oxytocin treated groups than in PG group since progesterone reached the basal level in PG group after 24 hr, the time at which progesterone level was still above basal in groups 25 Ox and 50 Ox. Basal progesterone level has been reached 24 hr later in the two Ox treatment groups. In goats, the drop in progesterone was less sharp in the oxytocin than in PG treated animals, as in sheep but in all treatment groups of does, the basal level have been reached after 48 h post treatment.

Post insemination, no clear differences occurred between treatment groups in progesterone profile in pregnant ewes and does up to day 30 when progesterone level was markedly lower in Ox groups than in PG one and lower in group 25 Ox than in 50 Ox. Progesterone level maintained basal up to day 30 in the non-pregnant goats of all treated groups and ewes of only PG group indicating absence of CL. In the non-pregnant ewes of groups 25 Ox and 50 Ox, progesterone fluctuated around low levels but above basal indicating presence of interrupted or inadequate CL.

**Table (2): Frequency distribution time of oestrus post 2<sup>nd</sup> injection of hormonal treatments in the experimental ewes and does.**

Treatment group	N	Onset of estrus post treatment (h)							
		Category							
		<36		36-56		>56-72		>72	
n	%	n	%	n	%	n	%		
<b>Ewes</b>									
25 OX	6	-	-	3	50	1	17	2	33
50 OX	10	1	10 <sup>b</sup>	5	50	2	20	2	20
PG	10	6	60 <sup>a</sup>	4	40	-	-	-	-
<b>Does</b>									
25 OX	5	1	20 <sup>b</sup>	2	40	2	40 <sup>a</sup>	-	-
50 OX	10	3	30 <sup>ab</sup>	3	30	1	10 <sup>b</sup>	3	30
PG	10	7	70 <sup>a</sup>	3	30	-	-	-	-

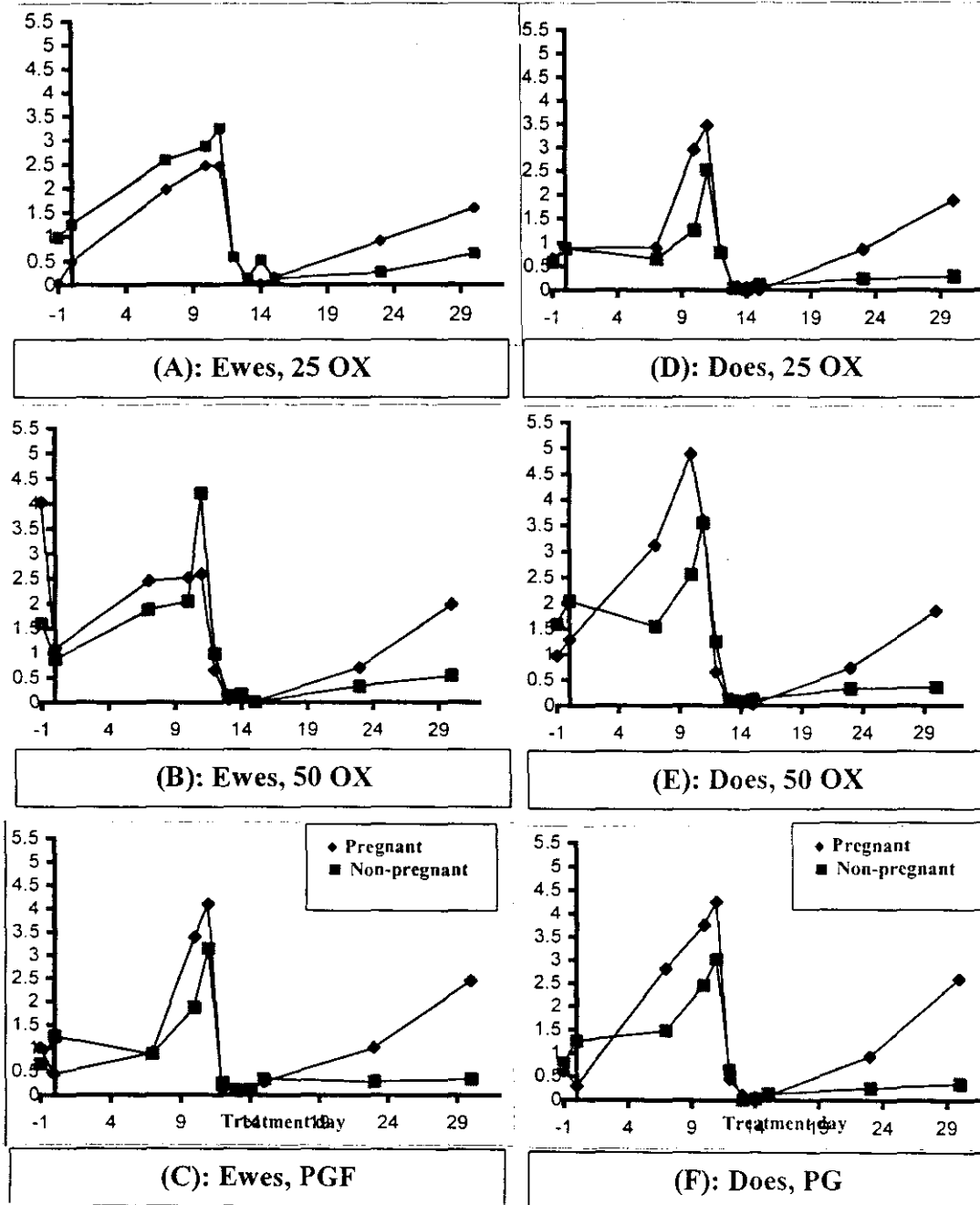
a and b: Means denoted within the same column for each species with different superscripts are significantly different at P<0.05.

N: Number of females came in oestrus

**Fertility and litter size:**

The conception rate as the number of ewes (does) lambed (kidded) per ewes (does) inseminated was different among hormonal treatment groups in both species (Table 3). Ewes of the low oxytocin dose (25 Ox) had similar fertility rate to that for those treated with PG and insignificantly higher value than that for ewes treated with the high dose of oxytocin (50 Ox). In does, fertility was significantly higher in the group of 25 Ox than in the other two groups.

When fertility rate was considered at the basis of total number of animals, ewes and does in 25 Ox treatment groups had the lowest fertility rates (40%) significantly (P<0.05) in ewes and insignificantly in does. Fertility in animals received the high oxytocin dose did not differ significantly from those in PG and 25 Ox groups in either cases.



**Fig. (1): Changes in progesterone concentration around the treatment days in ewes and does of different treatment groups.**

**Table (3): Effect of hormonal treatment on lambing/kidding rate and litter size of treated ewes and does.**

Treatment group	Total No.	Inseminated animals	Lambing/Kidding			Number of lambs/kids born	Litter size
			n	CR	FR		
<b>Ewes</b>							
25 OX	10	6	4	66.7	40 <sup>b</sup>	7	1.75 <sup>a</sup>
50 OX	10	10	6	60	60 <sup>ab</sup>	9	1.50 <sup>ab</sup>
PG	10	10	7	70	70 <sup>a</sup>	8	1.14 <sup>b</sup>
<b>Does</b>							
25 OX	10	5	4	80 <sup>a</sup>	40 <sup>b</sup>	5	1.25
50 OX	10	10	6	60 <sup>b</sup>	60 <sup>a</sup>	8	1.33
PG	10	10	6	60 <sup>b</sup>	60 <sup>a</sup>	7	1.16

a and b: Means denoted within the same column for each species with different superscripts are significantly different at P<0.05.

CR= number of females lambing (kidding) /number of inseminated animals x 100

FR= number of females lambing (kidding) /total number x 100

Concerning the results of litter size (Table 3), it is of interest to observe that the hormonal treatment affect markedly litter size of does and ewes. However, pronounced significant differences were found in litter size of ewes, being the highest in 25 Ox treatment group (1.75), followed by 50 Ox treatment group (1.50) and the lowest in PG treatment group (1.14).

## DISCUSSION

In both ewes and does, estrus occurred in full response to treatment with a dose of 50 IU oxytocin as well as with PG. The low level of oxytocin (25 IU) resulted in considerable low response in both species. Zollers *et al.* (1989) reported the probability of exogenous oxytocin to be involved in luteolysis during the luteal phase in cattle.

Regression of CL in the cyclic cow is through to results from an interaction between oxytocin, released by CL, and the uterine PGF<sub>2α</sub> (Lafrance and Goff, 1988). Moreover, physiologically surges of PGF<sub>2α</sub> released from the uterus were associated with episodes of release of ovarian oxytocin (Flint and Sheldrick, 1983). Oxytocin is also able to stimulate the secretion of PGF<sub>2α</sub> around the time of luteolysis (Flint *et al.*, 1994 and Bainbridge *et al.*, 1996). Oxytocin and progesterone play role as an autocrine/paracrine regulator in the bovine CL during luteal phase, as well as play some roles in regulating the functionality of PGF<sub>2α</sub> receptors (Kimura *et al.*, 1992 and Ito *et al.*, 1994). During late luteal phase in cattle, treatment with 50 IU of oxytocin increased oxytocin in blood, which increased PGFM in blood plasma (Kotwica *et al.*, 1998).

The low response to treatment 25 Ox in the present study may be attributed to probable wide variation in CL stage following 1<sup>st</sup> injection and/or the low hormone dose itself at 2<sup>nd</sup> injection. However, rates of responded buffaloes injected with 50 or 100 IU oxytocin at late luteal phase compared with PG were high and did not differ markedly (Daghash, 2001).

Onset of estrus in the oxytocin treatment groups was much variable and late compared with PG treatment. This is consistent with results of Daghash (2001) on buffaloes supporting the hypothesis of the indirect effect

of oxytocin through stimulating the episodic release of  $\text{PGF}_{2\alpha}$  during luteolysis (Asslin *et al.*, 1996). Treatment difference in the onset of estrus was consequent to the slower decline of progesterone level at luteolysis and later attainment of basal level due to 2<sup>nd</sup> injection of oxytocin compared with PG treatment which was in agreement with Daghash (2001).

In conclusion, treatment with oxytocin resulted in variable response in terms of either occurrence of estrus depending on dose or onset of estrus irrespective the dose. However, the dose of 25 IU oxytocin has been proven as low and of weak effect and is not recommended to be used for synchronization of estrus.

Double dose of 50 IU oxytocin 11 days apart resulted in full occurrence of estrus, but the wide variation in onset of estrus doesn't allow for promising AI. It can be used with natural mating in the case of the availability of males in some commercial flocks.

The oxytocin dose 50 IU, if to be used with AI time based on estrus observation will result in acceptable fertility rates for sheep costs (6 L.E./head for oxytocin vs. 9 L.E./head for PG).

More studies are needed on oxytocin dose-response and side effects a meaning at finding optimum oxytocin-ovulation synchronization protocol, which allows maximum fertility rates.

## REFERENCES

- Asselin, E. ; A. K. Golf ; H. Bergeron and M. A. Fortier (1996). Influence of sex steroids on the production of prostaglandin  $\text{F}_{2\alpha}$  and E2 and response to oxytocin in cultured epithelial and stromal cells of the bovine endometrium. *Biology Reprod.* 54(2): 371-379.
- Bainbridge, D. R. J. ; M. Davies ; R. J. Scaramuzzi and H. N. Jabbour (1996). Exogenous interferon delays luteal regression in red deer hinds (*Cervus elaphus*) by suppressing steroid induced endometrial oxytocin sensitivity. *Biology Reprod.* 55: 883-888.
- Duncan, D. B. (1955). T-test and interval for comparison suggested by the date. *Biometrics*, 31: 339-359.
- Flint, A. P. F. ; H. N. Jabbour and A. S. I. Loudon (1994). Oxytocin stimulates uterine prostaglandin  $\text{F}_{2\alpha}$  secretion in red deer (*Cervus elaphus*). *Reproduction and Fertility Development* 6: 269-271.
- Fuchs, A. R. ; O. Behrens ; H. Helmer ; A. Vangsted ; M. Ivanisek ; J. Grifo ; C. Barros and M. Fields (1990). Oxytocin and vasopressin binding sites in human and bovine ovaries. *American J. of Obstetric Gynaecology* 163: 961-967.
- Ito, S. ; K. Sakamoto ; N. Machizuki-Oda ; T. Ezashi ; K. Miwa. ; E. Okuda-Ashitaka ; V. I. Shevchenko and O. Hayaishi (1994).  $\text{PGF}_{2\alpha}$  receptor is coupled to Gq in cDNA-transfected Chinese hamster ovary cells. *Biochemical Biophysiology Res. Communication* 200: 736-762.
- Kimura, T. ; O. Tanizawa ; K. Mori ; M. J. Brownstein and H. Okayama (1992). Structure and expression of human oxytocin receptor. *Nature* 356: 526-529.

- Kotwica, J.; D. Skarzynski and G. Miszkiel (1998). Oxytocin modulated the pulsatile secretion of PGF<sub>2α</sub> in initiated luteolysis in cattle. Res. Vet. Sci., 66: 1-5.
- Lafrance, M. and A. K. Goff (1988). Effect of progesterone and oestradiol-17β on oxytocin induced release of prostaglandin F<sub>2α</sub> in heifers. J. of Reprod. and Fert. 82: 429-436.
- Okuda, K.; A. Miyamoto ; H. Sauerwein ; F. J. Schweigert and D. Schams (1992). Evidence for oxytocin receptors in cultures bovine luteal cells. Biology Reprod. 46: 1001-1006.
- Silvia, W. J. and M. L. Taylor (1989). Relationship between uterine secretion of prostaglandin F<sub>2α</sub> induced by oxytocin and endogenous concentrations of estradiol and progesterone at three stages of the bovine estrous cycle. J. Anim. Sci., 67: 2347-2353.
- NRC (1984). Nutrient Requirements of Sheep and Goats. National Academy press, Washington, DC.
- SAS/STAT (1999). Guide for personal computers. Version 8 Ed. Cary NC, USA, SAS Institute.
- Zollers, W. G.; H. A. Garverick and M. F. Smith (1989). Oxytocin-induced release of prostaglandin F<sub>2α</sub> in postpartum beef cows: comparison of short versus normal luteal phases. Biolgy Reprod. 41: 262,267.

## مقارنة استخدام المعاملة الاوكستوسين بالبروستاجلاندين لتنظيم الشبق فى الاغنام والماعز

طارق ع شماوى محمود ع شماوى ، بدير السيد الصعيدى ، محمد جبر خليل  
معهد بحوث الانتاج الحيوانى ، مركز البحوث الزراعية

اجرى هذا البحث بمحطة بحوث الانتاج الحيوانى بسخا التابعة لمعهد بحوث الانتاج الحيوانى خلال موسم تناسل سبتمبر فى الاغنام والماعز. كان الهدف من هذا البحث هو المقارنة بين تأثير استخدام هرموني الاوكستوسين والبروستاجلاندين لتنظيم الشبق فى الاغنام والماعز. استخدم فى هذه الدراسة ٣٠ نعجة خليط (٢/١ فنلندى × ١/١ رحمانى) عمرها ٣-٤ سنوات ووزن جسم ٤٥-٥٠ كجم وكذا ٣٠ معزة خليط (٢/١ دمشقى × ٢/١ بلدى) عمرها ٣-٤ سنوات ووزن جسم ٣٥-٤٠ كجم. وقسمت الحيوانات فى كل نوع الى ثلاثة مجاميع لكل منهما (١٠ حيوانات فى كل مجموعة). تم حقن الحيوانات بحقنيتين فى العضل بفاصل ١١ يوم بـ ٢٥ وحدة دولية للمجموعة الاولى و ٥٠ وحدة دولية للمجموعة الثانية و ١٠٧ مل كلوبيرستينول (بروستاجلاندين) للمجموعة الثالثة. تم تسجيل استجابة الحيوانات للمعاملة ووقت حدوث الشبق بعد الحقنة الثانية التى تم التلقيح صناعيا بعد ٢٤ ، ٣٦ ساعة من بدء ظهور الشبق. تم اخذ عينات دم لتقدير تركيز هرمون البروجسترون.

اظهرت النتائج حدوث استجابة كاملة فى كلا من النعاج والماعز وحدث الشبق لها استجابة للمعاملة بـ ٥٠ وحدة دولية اوكستوسين او بروتاجلاندين بينما كانت المجموعة المعاملة بـ ٢٥ وحدة دولية اوكستوسين هى الاقل فى الاستجابة.

كما اظهرت الحيوانات التى عولمت بالبروستاجلاندين مظاهر شياخ مبكرا فى كل من النعاج والماعز عن المعاملة بالاوكستوسين سواء بـ ٢٥ او ٥٠ وحدة دولية.

بعد اليوم ١١ من الحقنة الهرمونية الثانية تناقص تركيز البروجسترون خلال ٢٤ ساعة استجابة للمعاملة الهرمونية فى كل من الاغنام والماعز وبعد التلقيح لم يكن هناك فروق واضحة فى تركيز البروجسترون بين النعاج المعاز حتى اليوم ٣٠ بينما كان تركيز البروجسترون منخفضا فى الحيوانات المعاملة بالاوكستوسين عن المعاملة بالبروستاجلاندين وكان تركيز البروجسترون اقل فى المعاملة بـ ٢٥ عن تلك المعاملة بـ ٥٠ وحدة دولية.

تساوى معدل الخصوبة تقريبا فى النعاج التى عولمت بـ ٢٥ وحدة اوكستوسين مع تلك التى عولمت بالبروستاجلاندين وكانت فى الماعز التى عولمت بـ ٢٥ وحدة اوكستوسين اعلى من المعاملتين الاخرين واسو حظ تأثير المعاملة على معدل التوائم فى النعاج والماعز المعاملة.

نوصى هذه الدراسة بعدم استخدام الحقن بـ ٢٥ وحدة دولية اوكستوسين فى الاغنام والماعز وتستخدم جرعة ٥٠ وحدة دولية فى بعض الحالات والاحتياج الى دراسات اخرى فى هذا المجال.