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Reproductive performance of Ossimi sheep as affected by oxytocin injection during mating season.

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ABSTRACT

Twenty adult Ossimi ewes were used to study the effect of premating oxytocin intra-muscular (IM) injection on some reproductive traits. They were randomly divided into two equal groups. Ewes in both groups were IM injected by 1 ml GnRH analogue (1st inj.) at day 0. Then at day 7, they were IM injected by 0.7ml PGF2α analogue. A second GnRH dose (1 ml analogue) was given at day 9, two days post PGF2α injection. Oxytocin (20 I.U) was IM injected to each ewe in the first group immediately premating, one day after the second injection by GnRH. Another oxytocin injection was given 12 hr. post the first one, pre-second mating by the same rams.

Results showed improvement in most reproductive traits of oxytocin injected ewes. The percentages of improvement were 33.33% either for estrus rate, non-return rate or lambing rate, 25% for twinning rate, 8.67% for litter size and 44.44% for fecundity, compared to the control group. P_4 levels was the highest ($P<0.05$) in injected group by oxytocin. While, P_4 decreased in both groups after PGF2α injection. P_4 concentration increased in both groups after the second GnRH injection. At day 20 post-mating, P_4 levels showed the highest values in both groups. It could be concluded that premating oxytocin intra-muscular injection can be used to improve reproductive performance of Ossimi ewes.

Keywords: *Oxytocin, GnRH, PGF2α, progesterone, reproductive traits, ewe.*

INTRODUCTION

Improvement of reproductive performance is one of the major sources that lead to high productivity of sheep. There are many functions of female reproductive tract. For example, some hormones secreted to control reproductive organs activity pre, during, and post pregnancy. Spermatozoa are transferred through the reproductive tract of female to the fertilization site in the oviduct, while the female uterus provides a safe environment for the embryo and fetus to grow and develop.

Oxytocin (OXY) as a hypothalamic hormone synthesized in magnocellular neurosecretory cells in the supraoptic nucleus and hypothalamus paraventricular nucleus and released from the posterior lobe of the pituitary gland into the blood. It is also a major product of corpus luteum in ruminants **(Wathes and Swann, 1982),** in cows **(Hunter *et al.*, 1989)**, but its role during the estrus period is not certain. Whereas there are good clues for participation in luteolysis **(Auletta and Flint, 1988; Wathes, 1989),** OXY levels are highest in early to mid-luteal phase **(Webb *et al.*, 1981; Schams *et al.*, 1982)** and receptor concentrations in the reproductive tract peak on the day of estrus **(Sheldrick and Flint, 1985; Ayad and Wathes, 1989; Ayad *et al.*, 1990)**. **Wathes *et al.*, (1989)** found that ewes immunized against OXY failed to establish pregnancy when mated with a fertile ram**,** and they suggested that OXY may have an uncharacterized actions in fertility regulation. Injecting 60 U OXY once daily on days 7 to 14 after ovulation induced prolonged corpus luteum function in 60 to 70 % of injected mares. Extending the treatment by OXY to 29th days can initiate CL function throughout any point in the estrus cycle with no loss in efficacy **(Vanderwall *et al.*, 2016).**

The purpose of this study was to determine the effect of OXY intra-muscular (IM) injection

premating on some reproductive traits of Ossimi ewes.

MATERIALS AND METHODS

The current study was carried out at Faculty of Agriculture Experimental Station, Minia University, with partnership of Animal Production Research Institute (APRI), Ministry of Agriculture, Egypt, throughout the period from March 2016 until September 2016.

Animals and experimental groups

Twenty adult Egyptian Ossimi ewes (averaged 42.23±3.54 kg live body weight, 2-3 years old and had 2-3 parities) were divided into two equal groups (10 ewes each) according to age, body weight and parity. In addition, four adult Ossimi rams without significant differences in semen characteristics weighed 60 kg in average were used for natural mating. All ewes were synchronized for estrus according to **Ashmawy (2012)**. Each ewe was IM injected 1ml GnRH (1st inj.) at day 0 (GnRH analogue contained 0.004 mg Buserelin, Receptal. Intervet, International BV Boxmeer-Holland). Then on day-7 each ewe was IM injected by 0.7ml PGF2α analogue (Estrumate, coopers Animal Health LTD, Berkhamsted-England). Each ml of Estrumate contained 250 µg Cloprostenol Acetate. A second injection of 1ml GnRH analogue was given two days post PGF2α injection (day 9). Oxytocin (20 I.U/IM, Adwia Company) was IM injected to each ewe in the first group immediately premating, and one day after the second injection with GnRH. Another oxytocin injection was given 12 hr. post the first injection, pre-second mating by the same rams. All animals were kept under similar environmental conditions in a semi-open shaded yard during the experimental period and were fed maintenance allowances according to **NRC (1985)**. The fresh water and salt minerals blocks were available all times of the experiment.

Estrus detection

Estrus activity was observed and detected for all ewes twice daily (8:00 am and 4:00 pm) using teaser rams. Rams were interchanged among the experimental ewes. Ewes receptive to rams and stood for mounting were considered in estrus.

Ovarian activity

Ovarian activities of ewes were monitored by using portable sonar (Ericson (*ECHOSON*) model HVat-030609F413) supplied with probe 8 MHz # 148103-3/11. They were recorded at days 7 to 12 post mating through counting different types of follicles present with different size stage. Number and diameter of corpus luteum present were also recorded for five ewes in each group (control *vs.* treated). Ewes were fasted 16 hours before examination.

Reproductive efficiency

Estrus rate (number of ewes exhibiting estrus within 72 hrs. following PGF2α injection /number of treated ewes) x 100, estrus duration (the period from onset of estrus to the end of estrus) and non-return rate were calculated. Conception rate, lambing rate and twinning rate were also recorded. Fecundity (number of born lambs/total number of treated ewes) ×100 was calculated.

Blood sampling and hormonal assay

Blood samples were collected in the morning before feeding via the jugular vein from all ewes using 10 ml heparinized tubes. Plasma was separated by centrifugation of blood at 3000 rpm for 15 min and then stored at -20 ºC until used later for P_4 assay. Blood samples represent; just pre-treatment, one day post-1st GnRH, one day post-PGF2α, one day post-2nd GnRH and day 20th post-mating (pregnancy diagnosis). Quantitative determination of plasma progesterone was carried out using radioimmuneoassay kits DSL-USA. Catalog No.3900 **(Meizger, 1992)**

Statistical analysis:

Data were statistically analyzed using the general linear model procedure **(SAS, 2002).** The differences among means were tested using Duncan's Multiple-range test **(Duncan, 1955).** The model used in statistical analysis was:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where:

Y_{ij} = an observation

μ = overall means

G_i = effect of treatment (i = control, oxytocin inj.)

e_{ij} = random error

RESULTS AND DISCUSSION

Reproductive traits of Ossimi ewes injected premating by oxytocin (OXY) are illustrated in Table (1) and Fig. (1). Results indicated that estrus duration, litter size and fecundity significantly (P<0.05) improved in treated ewes compared to the control one. Also, there were insignificant increased for treated group in values of some traits like estrus rate, non-return rate, lambing rate and twinning rate compared with untreated group. The percentages of improvement of injected ewes were 33.33% either for estrus rate, non-return rate or lambing rate, 25% for twinning rate, 8.67% for litter size and 44.44% for fecundity compared to control group.

Tahawy and Sharkawy (2014) reported that OXY increased pregnancy rate to 70% compared to control. Average days to first insemination were significantly longer for control cows than treated with OXY (90 *vs.* 77 d). Days open for cows treated by OXY was not significantly different compared to control cows. The first service to conception and all service to conception rates for control cows were significantly greater compared to treated cows (33% & 84% *vs.* 25% & 68%, respectively).

Table (1): Effect of oxytocin injection premating on some reproductive traits of Ossimi ewes.

Traits	Groups		Sig.
	Cr	Oxy	
Total No. of ewes	10	10	
No. of ewes in estrus	6	8	
Estrus rate (%)	60 (6/10)	80 (8/10)	
Estrus duration (hr)	36.90±0.76^{b}	34.40±0.76^{a}	*
Non return rate (%)	60 (6/10)	80 (8/10)	
Gestation period (day)	150.83±0.42	151.00±0.37	NS
No. of ewes lambed	6	8	
No. of ewes lambed single	3	3	
No. of ewes lambed twins	3	5	
Lambing rate (%)	60 (6/10)	80 (8/10)	
Twinning rate (%)	50 (3/6)	62.5 (5/8)	
No. of lambs born	9	13	
Litter size	1.500±0.22^{b}	1.63±0.19^{a}	*
Fecundity (%)	90.00±26.87^{b}	130.00±26.87^{a}	*

$^{a-b}$ means in the same row followed by the different superscript are significantly different (P<0.05).
Sig = Significant NS = Not significant (P > 0.05), * = (P < 0.05).

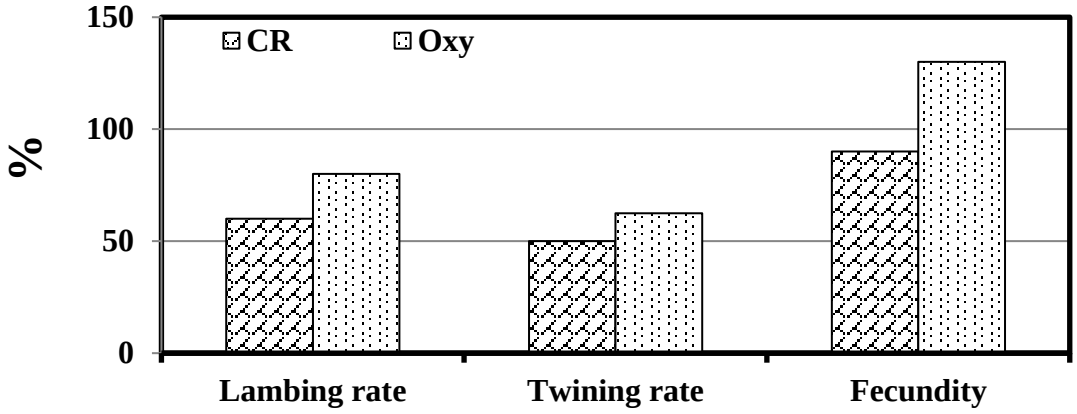

Fig (1): Lambing rate, twinning rate and fecundity of Ossimi ewes as affected by premating oxytocin injection.

Moreover, **Metwelly and EL-Bwab (1999)** recorded a significant increase in reproductive performance (calving-estrus interval, days open, services / conception and conception rate) in dairy cows treated by OXY. Also, **Hussein and Metwelly (2004)** reported that OXY injection in dairy cows immediately post parturition decreased the incidence of retained fetal membranes and improved reproductive performance.

OXY injection during estrus period increased electromyographic activity throughout the reproductive organs (oviduct, uterus and cervix) for 5-20 min **(Gilbert and Wathes, 1989)**. It is possible that OXY infusion acts on reproductive organs musculature for sperm transport to the oviduct. **Ayad *et al.*, (1990)** found that interaction with OXY receptors in the oviduct may affect rate of egg production. **Wathes *et al.*, (1989)** reported that active fortification against OXY prevented ewes from establishing pregnancy and these animals showed raise in gonadotropin concentrations. It is clear that OXY has potential to affect fertility in many ways and further work is needed to determine the most important way.

OXY dose may be acted in two ways. The first, it could have positive effect on movement speed of spermatozoa **(Watson *et al.*, 1999). Thackare *et al.* (2006)** found that paracrine action manifests itself in contractility stimulation of tubuli seminiferi, epididymis, and prostate. On the other hand, OXY stimulated the smooth uterine muscles to action **(Langendijk *et al.*, 2002).** Also, **Langendijk *et al.* (2003)** reported that intravenous or intramuscular injection of OXY during estrus influence the capacity and frequencies of uterus muscle movements, especially in animals usually characterized with weak uterine activity.

OXY concentration in the blood increased dramatically within 2 min of the onset of ejaculation **(Levis, 2000),** which increased mucosal sensitivity and endometrial results in easier and faster transport of spermatozoa to oviducts. The earlier arrival of spermatozoa and collection of the reservoir in the isthmus of the uterine tube affected the correct time of capacitating, successful fertilization of oocytes, and better embryo development **(Tur, 2012)**. This probably caused the greater number of lambs born live in treated group compared to the control group (Table 1 & Fig. 1).

Average number of different follicles diameter and corpus luteum (CL) counted at day 7th post mating for experimental ewes are shown in Table (2) and Fig. (2). There were insignificant increase (P>0.05) between the control and OXY injected group concerning number of follicles (2.8 *vs.* 3.5) and CL (1.2 *vs.* 1.5). However, dominant follicle diameter (6.8 *vs.* 6.3) and CL diameter (8.03 *vs.* 6.84) were greater (P<0.05) in oxytocin injected ewes than control ewes, respectively.

Table (2): Effect of premating IM injection of oxytocin on ovarian activity of Ossimi ewes.

Traits	Groups		Sig.
	Cr	Oxy	
No. of ewes monitored	5	5	
Ovarian follicles:			
Small (≤ 2 mm)	0.80±0.18	1.30±0.18	NS
Medium (> 2 < 4 mm)	0.80±0.23	1.00±0.23	NS
Large (≥ 4mm)	1.20±0.13	1.20±0.13	NS
Total follicles	2.80±0.32	3.50±0.32	NS
Dominant follicle diameter (mm)	6.30±0.12^{b}	6.80±0.12^{a}	*
Corpus luteum	1.20±0.15	1.50±0.15	NS
Corpus luteum diameter (mm)	6.84±0.15^{b}	8.03±0.15^{a}	**

$^{a-b}$ means in the same row followed by the different superscript are significantly different (P<0.05).
Sig = Significant NS = Not significant (P > 0.05), * = (P < 0.05).

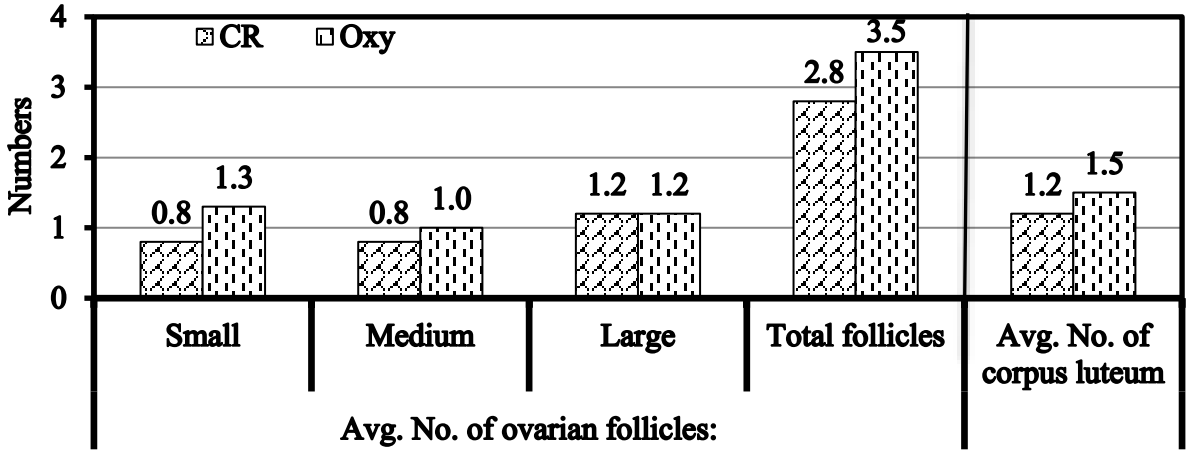

Fig (2): Average number of different follicles and corpus luteum of Ossimi ewes as affected by premating oxytocin injection.

The high levels of OXY secreted by the pumps differed according to physiological pattern of release throughout much of the cycle; long pulses of 1-3 h normally occur during luteal regression **(Flint and Sheldrick, 1983; Hooper *et al.*, 1986; Wathes *et al.*, 1986)** whereas low-amplitude, short pulses of 1-4 min occur at estrus **(Gilbert *et al.*, 1991).** Despite the high oxytocin concentrations, there was no evidence for down regulation of the oxytocin receptor during the estrous period as reported by **Flint and Sheldrick (1985).**

Lewis (2010) and **Wulster-Radcliffe *et al.* (1999)** are also consistent with the results of a study to determine whether OXY administered or intraluteally at various times during the estrous cycle or pregnancy altered luteal function in ewes**. Milvae *et al.* (1991)** found that OXY did not reduce the duration of estrous cycle in non-pregnant ewes and did not interrupt pregnancy in ewes mated before oxytocin treatments were commenced. **Hatjiminaoglou *et al.*, (1979)** reported that oxytocin administered at d 1 to d 7 of estrous cycle of ewes was luteolytic**,** while this seems to be the only report of such an effect of oxytocin. Also, **Sheldrick, (1992)** indicated that oxytocin may prolong luteal function in ewes. Thus, one should not expect oxytocin at d 7 of estrous cycle or pregnancy to induce luteolysis in ewes.

P_4 levels was the highest (P<0.05) in group injected by OXY. While, P_4 decreased in both groups after PGF2α injection. P_4 concentration increased in both groups after the second GnRH injection. At day 20 post-mating, P_4 levels showed the highest values in both groups. This finding is in agreement with **Ashmawy (2012)** and **Wulster-Radcliffe *et al.*, (1999)** who studied the effect of injecting ewes by OXY in luteal-phase**. Lewis (2010)** reported that using OXY did not reduce progesterone concentration.

Table (3): Progesterone concentration (ng/ml) in Ossimi ewes during different treatment periods, as affected by premating injection of oxytocin.

Treatment period	P_4 (ng/ml)		Sig.
	Cr	Oxy	
Pre- treatment (day 0)	1.30±0.12	1.53±0.13	NS
Post- 1st GnRH injection (day 1)	3.53±0.16^{b}	4.08±0.07^{a}	*
Post- $PGF_2\alpha$ injection (day 8)	0.49±0.04^{b}	0.71±0.07^{a}	*
Post- 2nd GnRH injection (day 10)	1.08±0.02^{b}	1.36±0.11^{a}	*
Post- mating (day 20)	4.91±0.25^{b}	6.59±0.19^{a}	**

$^{a-b}$ means in the same row followed by the different superscript are significantly different (P>0.05).
Sig = Significant NS = Not significant (P > 0.05), * = (P < 0.05).

Post-1st GnRH injection, P_4 level increased ($P<0.05$) in both groups. This result may indicate higher responses to GnRH injection for ewes in luteal phase and reflecting nearly the synchronization of ewes' reproductive status in both groups (Table, 3). These results agree with **Beck *et al.* (1996)** who reported that treatment by GnRH resulted in higher P_4 concentration.

P_4 levels decreased ($P<0.05$) to the minimal values post PGF2α injection in both groups, being less than 0.8 ng/ml. This decrease may reflect the higher response to PGF2α injection on corpus luteum regression stage induced after ovulation by the 1st GnRH injection. Post-2nd GnRH injection, P_4 levels showed again significant increase in both groups ($P<0.05$). This elevation in P_4 level was associated with the initiation of new corpus luteum in response to 2nd GnRH injection.

The pulsatile of LH release is generated in response to pulsatile GnRH release from the hypothalamus **(Levine *et al.*, 1982). Rawlings and Cook (1993)** reported that pulsatile LH release prevails at all reproductive status of ewes (before, during and after preovulatory surge of gonadotropins). Thus, the increase in P_4 level in ewes of both groups following GnRH injection may be due to the sudden release of LH, leading to ovulation or luteinization of dominant follicles of the present wave **(Örsan *et al.*, 2007).**

On day 20 post mating, P_4 level was significantly higher ($P<0.01$) in OXY injected ewes (6.59 ng/ml) than in control ewes (4.91 ng/ml) indicating the pregnancy incidence of each group (Table 3).

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الأداء التناسلي للأغنام الأوسيمي تحت تأثير الحقن بهرمون الأوكسيتوسين أثناء موسم التلقيح

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[2] قسم الانتاج الحيواني ، كلية الزراعة ، جامعة المنيا.

استخدمت عشرين نعجة أوسيمي ناضجة لدراسة تأثير الحقن العضلي لهرمون الأوكسيتوسين قبل التلقيح على بعض الصفات التناسلية. تم تقسيمها عشوائيا بالتساوي إلى مجموعتين. تم حقن جميع النعاج في كلا المجموعتين 1مل GnRH كحقنة اولى قبل بدء التجربة مباشرة . ثم في اليوم السابع تم الحقن بـ 0.7 مل PGF2α ثم تم الحقن للمره الثانية بـ 1مل من GnRH في اليوم التاسع ، وذلك بعد مرور يومين من حقن PGF2α. الأوكسيتوسين (20 وحدة دولية) تم حقنه لكل نعجة في المجموعة الأولى قبل التلقيح مباشرة، وذلك بعد يوم واحد من الحقنة الثانية للـ GnRH. ثم أعطيت حقنة أخرى من الأوكسيتوسين بعد مرور 12 ساعة من الحقنة الأولى، وذلك قبل التلقيحة التأكيدية الثانية من نفس الكباش.

أظهرت النتائج تحسن ملحوظ في معظم الصفات التناسلية للنعاج التي تم حقنها بالأوكسيتوسين. وكانت نسبة التحسن 33.33٪ في معدل حدوث الشبق، ومعدل عدم عودتهم للشياع مرة اخرى ومعدل الولادات، و 25٪ في معدل التوأمية ، 8.67 % في حجم الخلفة وكذلك 44.44% بالنسبة للـ fecundity مقارنة مع مجموعة الكنترول. تركيز هرمون البروجيستيرون كان مرتفعا بشكل معنوي في المجموعة التي تم معاملتها بحقن الأوكسيتوسين. بعد ذلك انخفض مستوي هرمون البروجيستيرون في كلا المجموعتين بعد الحقن بـ PGF2α. ثم أظهرت مستويات البروجيستيرون زيادة واضحة في كلا المجموعتين بعد الحقنة الثانية من GnRH ، كما أظهر هرمون البروجستيرون أعلى مستوي له في كلا المجموعتين في اليوم العشرين بعد التلقيح. ومن هنا نستنتج انه يمكن استخدام الحقن بهرمون الأكسيتوسين اثناء تنفيذ احد برامج تنظيم وتوحيد الشياع لتحسين الأداء التناسلي للنعاج الأوسيمي.