

OVARIAN ACTIVITY AND REPRODUCTIVE PERFORMANCE OF MATURE OSSIMI EWES AS AFFECTED BY PRESENCE OF RAM

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SUMMARY

Two experiments were conducted in this study to assess the role of ram effect prior to mating on ovarian follicular dynamics and reproductive performance of Ossimi ewes. In Expt. 1, fourteen Ossimi ewes 3–6 years old were used. The first group (n=7), ewes were exposed with two trained teaser rams (REG), whilst, the second group (n=7) remaining ewes were isolated of rams (CG). The number and size of all follicles ≥ 2 mm and corpus luteum were monitored by ultrasonography. Expt. 2, including two mating season, 70 Ossimi ewes (3–6 years old) were selected from 115 ewes in each mating season, multiparous, non-lactating and clinically healthy. The first mating season (n=70), ewes were kept in the presence with teaser rams (REG) during the estrus detection and mating for two consecutive estrous cycles (45 d). The second mating season (n=70), ewes were remaining isolated of rams (CG) except at the time for detection of estrus. Ewes were mated after detection of estrus. While, ewes return to estrus, were mated again. At lambing, birth dates and the lambs born number per each ewe were recorded. All ewes isolated of males for three months before the beginning of the experiments.

There were significant ($P < 0.01$) increases in the small (2–2.9 mm) and medium (3–5 mm) follicles number on ovaries of ewes in REG compared to CG. Moreover, the size of maximum ovulatory follicles and subdominant follicles (next largest follicle) were larger ($P < 0.001$) in ewes of REG compared with those of CG. In addition, the ovulation rate mean was higher ($P = 0.05$) in the REG than that of the CG (Expt. 1).

The lambing rate was higher ($P < 0.01$) in the first mating season (REG) compared to the ewes in the second mating season (CG). At the same time, the mean number of fecundity and litter size were higher ($P < 0.01$) in the REG compared to the CG. Moreover, twinning lambing rate was higher ($P < 0.01$) in the REG. by contrary, the percentage of single lambing was lower ($P < 0.01$) in the first mating season (REG). The percentage of ewes lambing at first service was higher in the REG than in CG but the difference was insignificant (Expt. 2).

In conclusion, these results show that, ram introduction prior to mating season increased the ovarian activity and reproductive performance after period of isolation of Ossimi ewes.

Keywords: Ram effect, ovarian structures, reproductive performance

INTRODUCTION

Increasing production of annual lambs can be achieved by increasing number of parturitions per year or by increasing the prolificacy. These would be achieved by control of the reproductive processes (i.e. hormones and management). Treatment by hormones of the sheep in subtropics was economically viable under prevailing production systems. Practices of management were considering the first option for lamb production increasing in subtropics such as nutritional flushing or the ram effect (Aboul-Naga, *et al.*, 1992).

Sheep lives in groups, the complex social environment immerse such as, sounds, sights and smells, mating, and lambings (Wyatt, 2009). This social interaction can be altering processes of the reproductive function (Martin *et al.*, 1986 and Ungerfeld, 2007). The sociosexual was stimuli GnRH secretion and changes in the pulsatile secretion of luteinizing hormone, its the major role effects on activity and reproductive strategy (Ungerfeld *et al.*, 2004). Phenomena termed the male effect and olfactory signals often called pheromones.

The sudden exposed of rams with seasonal anestrus ewes after period of isolation, induces

behavioral of estrus, ovulations and pregnancies (Ungerfeld *et al.*, 2004 and Delgadillo *et al.*, 2009). Responses of the reproductive processes obtained by the ram effect with anestrus ewes and the hormonal treatments were similar results (Martemucci *et al.*, 1984; Crosby and Murray, 1988 and Boly *et al.*, 2000). However, this technique (ram effect) was easy, cheap and high efficiency (Ferrería *et al.*, 2008 and Tenório Filho *et al.*, 2016). Moreover, it was non-pharmacological method, hormone free animal production for the increasing of “clean, green and ethical” productive systems (Martin, 2001 and Martin *et al.*, 2004). The “male effect” induces LH surge, estrus behavior and ovulation in anestrus ewes (Okamura *et al.*, 2010; Hawken and Martin, 2012; Jorre de St. Jorre *et al.*, 2014 and Ferreira-Silva *et al.*, 2017ab). Also, increasing LH surge in breeding season, in cyclic ewes, ram introduction was stimulated and increased secretion of the LH pulsatile, its independent of stage of the estrous cycle or ewe genotype (Hawken *et al.*, 2007). In the follicular phase, the presence of ewes with rams increases the LH surge (Lindsay *et al.*, 1975 and Contreras-Solis *et al.*, 2009), estrus onset its accelerate, and the sexual receptivity duration

reduced (Fletcher and Lindsay, 1971 and Ungerfeld and Rubianes, 1999). In the luteal phase, CL inhibits secretion of LH pulsatile by production of the progesterone (Baird, 1978), but in ovariectomized ewes treated by progesterone, the ram introduction increase the secretion of LH pulsatile in breeding season (Pearce and Oldham, 1983). The ram effect was obtained any time in the breeds less seasonal effect for example the Mediterranean regions (Folch, 1990).

If ewes remaining isolated of rams and exposed to rams in the mating season only, lambing rates, fertility increases and growing flocks faster. Thus, the aim of this study was to assess the responses of ovarian follicular development and reproductive performance of Ossimi ewes following ram introduction prior to mating after period of isolation.

MATERIALS AND METHODS

Animals and experimental design:

Two experiments were conducted on the experimental farm of Agriculture Faculty, Assiut University. In experiment 1, fourteen Ossimi ewes (3–6 years old) weighing 45.3 ± 1.2 kg were used. Ewes were randomly divided into two groups, 7 ewes each, balanced for body weight and parity. The first group, ewes were exposed with two trained teaser rams (REG), whilst the second group, remaining ewes were isolated of rams (CG). In experiment 2, including two mating seasons, 70 Ossimi ewes, 3–6 years old, were selected from 115 ewes in each mating season, multiparous, non-lactating and clinically healthy. The first mating season ($n=70$), ewes were kept in the presence with teaser rams (REG) during the estrus detection and mating for two consecutive estrous cycles (45 d). The second mating season ($n=70$), remaining ewes were isolated of rams (CG) except at the time for detection of estrus. The experiments were carried out during the August–September in the experiment 1 (2015) and for each mating season in the experiment 2 (2015/2016 – 2016/2017) and its raised in semi-open pens. All ewes isolated of males for three months before the beginning of the two experiments. The ewes were fed on the experimental diet, which formulated according to NRC (1985) for sheep. Ewes had free access to water and trace mineral salt.

Monitoring of follicular development:

The number and size of all follicles ≥ 2 mm were monitored daily at the same day of the ram introduction to the time of ovulation in the experiment 1 by Transrectal ultrasound scanning (Holland and Pie Medical, 100 LC) having a 6 to 8 MHz linear transducer. Ovulation was occurred when the largest follicles (> 5 mm) were identified and then not observed. The image of the CL was estimated as maximum diameter from the largest cross-sectional area.

Estrus observation:

Estrus behavior was detected twice daily (8.0 a.m. and 4.0 p.m.) in the experiment 2, by observing

the reaction of the ewes with the male in pen. This included seeking to the ram, restlessness, mounting the ram and immobile of standing to be mounted by the ram (Gordon, 1997). Ewes were mated after detection of estrus. While, ewes return to estrus, were mated again. At lambing, birth dates and the number of lambs born per each ewe were recorded.

Assessment of the reproductive parameters:

The following studied parameters in each mating season were evaluated:

Lambing rate or fertility (percentage of ewes lambing/ewes mated),

Fecundity (lambs born number /ewes mated),

Litter size or prolificacy (number of lambs born /ewes lambing), were recorded (Berhan and Van Arendonk, 2006).

Single lambings rate (percentage of single lambings/ewes lambing),

Twin lambings rate (percentage of twin lambings/ewes lambing),

Ewes lambing at first service rate (percentage of ewes lambing at first service /ewes mated),

Ewes lambing at second service rate (percentage of ewes lambing at second service /ewes mated).

Statistical analysis:

Statistical analyses were performed by SAS (1996). Differences of means between groups regarding ovarian follicles structure, ovulation rate, fecundity and litter size were tested by independent t-test. Results were expressed as means \pm SEM. Reproductive performance (lambing rate, single lambings, twin lambings, ewes lambing at first service and ewes lambing at second service) were statistically analyzed by Chi-square analysis (Safdarian *et al.*, 2006 and Mirzaei *et al.*, 2017). Values of probability were considered significant when less than 0.05.

RESULTS AND DISCUSSION

Follicular dynamics:

Table (1) shows, the distribution of follicular population and the patterns of follicular growth. However, the number of small (2–2.9 mm) and medium (3–5 mm) follicles on ovaries of ewes in REG (2.40 and 2.81) were significantly ($P < 0.01$) higher than that in CG (1.90 and 2.53, respectively). Moreover, the size of maximum ovulatory follicles (6.21 mm) and subdominant follicles (4.32 mm) (next largest follicle) were significantly ($P < 0.001$) larger in ewes of REG than in those of CG (5.50 mm and 3.80 mm, respectively). In addition, the ovulation rate mean was higher ($P = 0.05$) in the REG (1.43) than that of the CG (1.00). Similar results were obtained by Atkinson and Williamson (1985) and Rubianes *et al.* (1997) who observed that, the diameter and number of the larger follicles increased subjected to the presence of the rams with ewes. Moreover, it leads to larger ovulatory follicle size (Ferreira-Silva *et al.*, 2018). It could be deduced that, follicles number and size were higher of ewes in REG compared with the ewes in CG may be due to

the presence of the rams. It leads to an increase in FSH levels and the LH surge (Martin and Scaramuzzi, 1983; Ungerfeld *et al.*, 2000 and Ungerfeld, 2003). LH pulsatility increased in ewes exposed to rams that coincided with an increase the number and diameter of the largest follicle (Atkinson and Williamson, 1985 and Ungerfeld, 2003), this may be due to the preovulatory follicular emergence and development rely on pulsatility of LH (Baird and McNeilly, 1981). The continuous contact rams with ewes until the ovulation increased the LH surge and enhanced the ovarian activity (Murtagh *et al.*, 1984;

Folch, 1990 and Contreras-Solis *et al.*, 2009). After ram introduction, follicular growth and development begins immediately, this may be due to the response to the initial in the LH basal levels and pulse frequency (Atkinson and Williamson, 1985). However, the presence of ram with ewes until the ovulation leads to peaks of preovulatory LH in ewes, that acts upon this role of the growth and development of the ovarian follicles (Okamura *et al.*, 2010; Hawken and Martin, 2012; Ferreira-Silva *et al.*, 2017_{ab} and Ferreira-Silva *et al.*, 2018).

Table 1. Characteristics of follicular population in ewes with or without ram effect

Items	REG	CG	P value
Small follicles (2-2.9 mm)	2.40±0.11	1.90±0.08	0.001
Medium follicles (3-5 mm)	2.81±0.09	2.53±0.07	0.01
Subdominant follicles (mm)	4.32±0.01	3.80±0.07	0.001
Maximum ovulatory follicles (mm)	6.21±0.01	5.50±0.01	0.001
Ovulation rate	1.43±0.20	1.00±0.0	0.05

(REG): Ewes exposed with trained teaser rams, (CG): Ewes remaining isolated of rams

Reproductive performance:

It is clear from Table (2) that, the lambing rate was higher ($P < 0.01$) in the REG compared to the CG (97.14 vs 81.43 %). At the same time, the mean number of fecundity and litter size were significantly ($P < 0.01$) higher in the REG (1.37 and 1.41) than in the CG (0.97 and 1.19, respectively). Moreover, twinning lambing rate was higher ($P < 0.01$) in the REG compared to the CG (41.18 vs 19.30 %). In contrast, single lambing rate was lower ($P < 0.01$) in the REG compared to the CG (58.82 vs 80.70 %). The percentage of ewes lambing at first and second service were (88.57 and 11.43 %) in the REG and were (77.14 and 22.86 %, respectively) in the CG but the differences were not significant. These results are in agreement with those of Oldham (1980) and Knight (1983) who found that, ovulation rate of ewes mated at first estrus was higher than that of the following estrus. Whereas, ewes mated at first estrus after ram introduction produced more lambs. Furthermore, Cognie *et al.* (1980); Hudgens *et al.* (1987) and Burfening *et al.* (1989) observed that, lambing rate was higher in ewes after ram

introduction (teased ewes) compared to non teased ewes. Meanwhile, Hassan *et al.* (1988) and Aboul-Naga *et al.* (1992) reported that, after period of isolation, the numbers of ewes mated and lambing were increased following the rams exposed with ewes for one week prior to mating. Furthermore, ewes exposed to the rams, which inseminated laparoscopically showed a pregnancy rate significantly higher than recorded in control group animals (Lucidi *et al.*, 2001). The time from ram exposure to mating in ewes was reduced ($P < 0.001$) and distribution of mating and lambing more compact compared to ewes isolated (Hawken *et al.*, 2008; Ungerfeld *et al.*, 2008 and Notter, 2012). The ram effect, usually used as teasing, the ram introduction just before the onset of puberty, advanced the date of breeding and increased the rate of pregnancy and twinning lambing (Kenyon *et al.*, 2005, 2006a,b,c; Kenyon, 2012 and Cave *et al.*, 2012). Moreover, the activity of the ewe lamb-breeding onset that responses to the teasing effect is greater closer (Cave *et al.*, 2012).

Table 2, Reproductive performance in ewes with or without ram effect

Items	REG	CG	P Value
Lambing rate %	97.14 68 (70)	81.43 57 (70)	0.01
Fecundity	1.37±0.06	0.97±0.07	0.001
Litter size	1.41±0.06	1.19±0.05	0.01
Twin lambings %	41.18 28 (68)	19.30 11 (57)	0.01
Single lambings %	58.82 40 (68)	80.70 46 (57)	0.01
Ewes lambing at first service %	88.57 62 (70)	77.14 54 (70)	0.07
Ewes lambing at second service %	11.43 8 (70)	22.86 16 (70)	0.07

(REG): Ewes in first mating season were kept in the presence with rams during estrus detection and mating for two consecutive estrous cycles (45 d). (CG): Ewes in second mating season remaining isolated of rams except at the time for detection of estrus.

CONCLUSION

It can be deduced that, presence of rams with ewes during the estrus detection periods and mating enhanced the ovarian activity and reproductive performance of Ossimi ewes.

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النشاط المبيضي والأداء التناسلي للنعاج الأوسيمي الناضجة متأثراً بوجود الكباش

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تتكون هذه الدراسة من تجربتين لمعرفة تأثير وجود الذكر قبل وأثناء موسم التلقيح على نمو وتطور الحويصلات المبيضية والكفاءة التناسلية للأغنام الأوسيمي. في التجربة الأولى أجريت على أربعة عشر من النعاج الأوسيمي منتظمة دورة الشياح (3-6 سنوات). تم تقسيم هذه النعاج عشوائياً إلى مجموعتين متساويتين (n=7) ، المجموعة الأولى (REG) ويكون فيها تواجد الذكور مع الإناث حتى نهاية التجربة بينما المجموعه الثانيه (CG) تظل النعاج منعزله عن الذكور. وأثناء التجربة تم متابعة نمو وتطور الحويصلات المبيضية، حدوث التبويض وظهور الجسم الأصفر وأخذ القياسات بالموجات فوق الصوتية (السونار).

التجربة الثانية: تتكون من موسمين تلقيح كل موسم تلقيح يتكون من 70 نعجه في عمر 3-6 سنوات تم اختيارها من 110 نعجه في كل موسم. في الموسم الأول (REG) يكون فيها تواجد الذكور أثناء دورتين شياح متتاليتين (45 يوم) بينما في الموسم الثاني (CG) تظل النعاج منعزله عن الذكور ما عدا الوقت اللازم لإكتشاف الشياح. وتم تلقيح نعاج المجموعتين بعد إكتشاف الشياح لهما وتلقيح النعاج مره أخرى في حالة العوده للشياح وتم تسجيل تاريخ الولادات وعدد المواليد لكل نعجه في كل موسم. وقد تم عزل النعاج عن الذكور قبل إجراء التجربه بمده لا تقل عن 3 شهور.

أظهرت النتائج أن متوسط العدد الكلى للحويصلات المبيضية الصغيره (2 - 9 ، 2 مم) والمتوسطه (3 - 5 مم) زادت زياده معنويه (P<0.01) في نعاج المجموعه الأولى (REG) مقارنة بنعاج المجموعه الثانيه (CG). ووجد أيضاً أن قطر أكبر حويصله والحويصله التابعه لها زادت زياده معنويه (P<0.001) في المجموعه الأولى (REG) مقارنة بالمجموعه الثانيه (CG). كما أدى تواجد الذكور في المجموعه الأولى الى زيادة معدل التبويض (P=0.05).

في التجربة الثانية: أظهرت النتائج المتحصل عليها أن وجود الذكر قبل وأثناء موسم التلقيح في نعاج الموسم الأول (REG) أدى الى زيادة معنويه (P<0.01) بالنسبة لعدد النعاج الولاده من حيث عدد النعاج التي تم إكتشاف الشياح بها وتلقيحها مقارنة بنعاج الموسم الثاني (CG) والتي تم عزلها عن الذكور ما عدا وقت إكتشاف الشياح لها. ووجد أيضاً أن متوسط عدد المواليد بالنسبه للنعاج الولاده أو النعاج التي تم تلقيحها زادت زياده معنويه (P<0.01) في نعاج الموسم الأول (REG). ووجد أن معدل الولادات التوأمية زادت زياده معنويه (P<0.01) في نعاج الموسم الأول (REG). وعلى عكس ذلك وجد أن معدل الولادات الفردية منخفضة في نعاج الموسم الأول (REG) (P<0.01) مقارنة بنعاج الموسم الثاني (CG). كما أظهرت النتائج زيادة النعاج التي تمت ولادتها في الموسم الأول (REG) عند تلقيحها في أول شياح مقارنة بنعاج الموسم الثاني (CG). كما وخلصت نتائج هذه الدراسة أن تواجد الذكور مع النعاج قبل وأثناء موسم التلقيح بعد فتره من عزلها أدى الى زيادة معدل التبويض، زيادة النشاط المبيضي وتحسين الكفاءة التناسلية للنعاج الأوسيمي.