

NONSURGICAL INTRAUTERINE ARTIFICIAL INSEMINATION IN SHEEP USING EXOGENOUS OXYTOCIN

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SUMMARY

This experiment was conducted on Egyptian Rahmani sheep in early summer season to develop AI by an easy intrauterine insemination method using different doses of exogenous oxytocin. Two injections of 15 mg Prostaglandin F₂ Alfa (PGF_{2α}) at 12 days interval (23 ewes) or progestogenated vaginal pessaries (PRVP) for 13 days (21 ewes) were used to synchronize estrus. The synchronized ewes were injected intravenous with 0 (saline), 100 or 200 USP units of oxytocin. Immediately after saline or oxytocin treatment, one ml of fresh extended semen (3×10^8 motile sperm cells/ml of tris buffer) in standard pipette (5 mm diameter) was gently placed into uterus (if the pipette was easily penetrated the cervix) or on the os cervix. These treatments were repeated again 8 h later and in unconceived ewes during the following estrus. Conception rate (CR) was 69.6% of ewes treated by PGF_{2α} during the induced estrus (16/23) compared to 28.6% (6/21) in PRVP treated ewes (P<0.01). In the second service, CR was 68.2%, while in first service (synchronized estrus) CR was 50%. Oxytocin treatments increased (P<0.01) the number of intrauterine inseminated ewes. All ewes injected by 200 USP units of oxytocin (21 ewes) and 13 of 23 ewes injected by 100 usp units of oxytocin were easily intrauterine inseminated, while in saline group none of cervixes (22 ewes) were easily penetrated. Regardless of first or second service, PGF_{2α} or PRVP, CR was 66.7, 56.5 and 45.5% in ewes treated by 200, 100 and 0 USP units of oxytocin, respectively. There were no significant effects of oxytocin, PGF_{2α} or PRVP on percentage of lambed ewes and lambing rates. These results may be important to improve nonsurgical intrauterine AI techniques in Rahmani ewes.

Keywords: Sheep, Intrauterine, AI, Oxytocin

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INTRODUCTION

The commercial use of artificial insemination (AI) in sheep is still limited because of the low fertility rates. One of the major factors that has contributed most to developing AI in sheep is the anatomical nature of sheep cervical canal (Dun, 1955, Halbert et al., 1990), which prevents easy intrauterine AI. The successful intrauterine has been conducted surgically in superovulation ewes followed by embryo transfer. Exogenous oxytocin given at 44 and 52 h after removal of progestogen pessaries has been reported to dilate the cervix of ewes and allowing passage of a stainless steel rod deeply in the uterus (Khalifa et al. 1990, 1992).

This experiment was conducted in summer on Rahmani ewes to determine the effect of A) prostaglandin F₂ alfa or progestogenated vaginal pessaries on conception rate b) exogenous oxytocin given during synchronized or natural estrus on nonsurgical intrauterine AI C) exogenous oxytocin treatment followed by AI on conception or lambing rate.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS: In June - July 1991, forty four Rahmani ewes 2 to 4 years of age at the Faculty of Agriculture experimental farm, Ismailia, Egypt were used in this study. The animals were synchronized for estrus by two intramuscular injections of 15 mg prostaglandin F_{2α} (PGF_{2α}) (Lutylase, Sigma co.) at 12-day intervals (23 ewes) or by progestogenated vaginal pessaries (PRVP) (6-methyl-17-hydroxy-progesterone acetate; MPA, 50mg/pessary) for 13 days (21 ewes). The animals were weighed during natural or induced estrus. Body weights ranged from 37 to 54 kg. The animals were fed by 0.5 kg concentrate mixture, 0.5 kg clover hay/head/day. Rice straw was continuously presented from June to October. Starting in late October to lambing period, the animals were fed 3/4 kg concentrate mixture and 5 kg berseem /head/day. The ewes were reared in an open pens.

ESTRUS DETECTION: Estrus was detected by an aproned teaser ram two times daily (7 am and 4 pm). Estrus ewes were given oxytocin treatment and artificially inseminated. Ewes that did not exhibit signs of estrus during the next two estrous cycles post-insemination was considered pregnant.

Oxytocin treatments and AI: The estrus-induced ewes were divided randomly into three groups and injected intravenously with either 0 (saline), 100 or 200 usp units of oxytocin (VEDCO, inc. Joseph, MO, 64504) just before each insemination. These doses of

oxytocin were chosen to evaluate the minimum dose needed for cervix dilation. Khalifa et al. (1990, 1992) did not detect any significant differences on cervix dilation with either 200, 400 or 600 USP units of oxytocin injection during synchronized estrus for Hampshire x Suffolk ewes. Ewes were restrained with the "over the rail method" (Evans and Maxwell, 1987). Just before each saline or oxytocin injection, a speculum was inserted into the vagina of each ewe and the standard 5 ml classic glass pipette (5 mm diameter) contained 1 ml fresh extended semen (approximately 3×10^8 motile sperm cells) was inserted into the cervical os. If this inseminating pipette passed easily through the cervix within 10 minutes after saline or oxytocin injection, intrauterine insemination was recorded, if not the semen was deposited on the os cervix. Within 5-6 minutes of oxytocin injection were enough to dilate the cervix (Khalifa et al. 1992). This procedure was repeated after 8 hours. The unconceived ewes (second service) were treated by the same doses of oxytocin and artificially inseminated using the same methods used for synchronized ewes at the first service. These ewes were observed throughout pregnancy and each lambing was recorded.

Semen processes: Semen was collected from two fertile rams by artificial vagina just before artificial insemination. The ejaculated semen samples were separately evaluated for volume, progressive motility and sperm concentration, then extended at room temperature by tris buffer solution to approximately 3×10^8 motile sperm cells/ml.

Statistical analysis: Analysis of variance was used to analyze the body weight data of oxytocin treatment groups. Chi square test (χ^2) of independence was used to compare the percentage values of first service with that of second service. The test was used also to compare $\text{PGF}_{2\alpha}$ treatment values with those of PRVP. However Chi square test of homogeneity was used to determine whether the effects of dose of oxytocin on the number of successful uterine entries differed between treatments during the induced estrus and estrus followed. The same test was used to analyze the pregnancy result (Steel and Torrie 1984). Percentage values of Group 1 (saline group) was considered the expected values.

RESULTS AND DISCUSSION

As shown in table 1 ewes treated with PGF_{α} had a greater conception rate ($P < 0.01$) during the induced estrus (16 of 23 ewes or 69.6%) than ewes synchronized with PRVP (6 of 21 ewes or 28.6% CR). Many investigators had studied the CR producing from the synchronized ewes. As there is wide variation in the experimental designs and results, it is difficult to compare the findings. However, Allison

and Kelly (1978) reported 45.7% CR with two injections of 10 mg of prostaglandin 10 days apart, Haresign and Acrotipoulou (1978) had reported 68% CR. Conception rate of 33% was found for ewes artificially inseminated during the synchronized estrus by progesterone pessaries (Wani et al., 1987). Regardless of estrus induction method, CR producing from the second service (followed estrus) was 68.2% higher than that of first-service (50%). These result support the conclusion of others that a higher CR from synchronized ewes is expected at the second estrus (El-Alamy et al., 1977, wani et al., 1987 and Mathur et al., 1987). Quinlivan and Robinson (1967) suggested that, progesterone treatment may be followed by an abnormal pattern of sperm transport. According to this suggestion the low conception rates obtained from the first insemination could be explained.

Table 1. Artificial insemination results of ewes shynchronized to estrus by Prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$) or Progesterogenated Vaginal Pessaries (PRVP).

Item	First service *			Second service *			Overall
	PGF $_{2\alpha}$	PRVP	Total	PGF $_{2\alpha}$	PRVP	Total	
No. of ewes used	23	21	44	7	15	22	66
No. of inseminations	23	21	44	7	15	22	66
No. of ewes conceived	16	6	22	5	10	15	37
Conception rate (%)	69.6 ^a	28.6 ^b	50.0	71.4	66.7	68.2	56.1
No. of ewes lambed	14	3	17	4	7	11	28
% of ewes lambed	87.5	50	77.3	80	70	73.3	75.7
No. of lambs born	15	3	18	4	7	11	29
Lambing rate (%)	107.1	100	105.9	100	100	100	103.6

* The ewes were inseminated during the induced estrus (First service) however the unconceived ewes were inseminated during the following estrus (second service).

a,b Values with different superscripts in a row differed at $b(P<0.01)$, using Chi square test.

Regardless of the estrus induction methods or number of services, dose of oxytocin increased ($P<0.01$) the number of ewes with intrauterine insemination (Table 2). All the ewes injected by 200 USP units of oxytocin (Group 3) and 13 of 23 ewes treated by 100 USP units of oxytocin (Group 2) were easily intrauterine inseminated. None of cervixes of ewes in Group 1 (saline treated ewes) could be easily penetrated, consequently the semen was deposited near the os cervix, The results of the present experiment indicate that oxytocin effect on the penetrationability of the cervix are in agreement with results of Khalifa et al. (1990,1998). In the latter reports higher doses of oxytocin were used on heavier body weight ewes (Hampshire X Suffolk) but did not study the oxytocin effects on conception or subsequent lambing rates. In the present study, the oxytocin treatments increased conception rates ($P<0.01$). Conception rates were 45.5, 56.5

and 66.7% for Group 1,2 and 3, respectively. These results may indicate that the doses of oxytocin used in the present study had no harmful effects on sperm transport. From these data, it could be concluded that the sperm transport may not be the major factor which depressed the fertility in estrus induced ewes, especially in those ewes inseminated via deep intrauterine insemination. Ovulation disorders may be one of factors depressing fertility. While all ewes administered by 200 USP units of oxytocin were intrauterine inseminated, CR was 60% only during the synchronized estrus and 50% of ewes intrauterine inseminated (100 USP units of oxytocin) conceived. Oxytocin treatments had no effects on percentage of ewes lambing or lambing rates.

Table 2. Some reproductive aspects of ewes artificially inseminated followed oxytocin injection during synchronization (induced esturs) and natural second estrus.

Item	0 Saline-Group 1			Oxytocin treatment by USP units					
	induced	second	total	100-Group 2			200 Group 3		
				induced	second	total	induced	second	total
No. of inseminations	14	8	22	15	8	23	15	6	21
No. of conceived ewes	6	4	10	7	6	13	9	5	14
Conception rate (%)	42.9	50	45.5	46.7	75.0	56.5	60	83.3	66.7
Chi-square test a	---	---	---	---	**	---	**	**	**
No. of intrauterine inseminated ewes	0	0	0	8	5	13	15	6	21
% of intrauterine inseminated ewes	0	0	0	53.3	62.5	100	100	100	100
Chi-square test a	--	--	--	**	**	**	**	**	**
CR of intrauterine inseminated ewes(%)*	0	0	0	50	80	61.5	60	83.3	66.7
No. of ewes lambed	5	2	7	5	5	10	7	4	11
% of ewes lambed	83.3	50	70	71.4	83.3	76.9	77.8	80.0	78.6
No. of lambs born	6	2	8	5	5	10	7	4	11
Lambing rate(%)	120	100	114.3	100	100	100	100	100	100

* =No. of intrauterine insemination and conceived ewes x100
No. of intrauterine insemination ewes

a Percentage values of group 1 was considered the expected values

** Significant at P<0.01

In Conclusion, 200 USP units of exogenous oxytocin injection allowed transcervical, intrauterine AI of Rahmani ewes nonsurgically. The dose of 200 USP units of oxytocin injection followed by intrauterine AI quietly increased CR. Other experiments are suggested to study, effect of oxytocin treatments on uterine contractions and sperm transport.

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تلقيح صناعي داخل رحم الأغنام لاجراحيا باستخدام أوكسي توسين خارجي

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نفذت هذه التجربه علي اغنام الرحمانى المصرىه فى اوائل فصل الصيف لتطوير التلقيح الصناعى بطريقه سهله للتلقيح الصناعى داخل الرحم وذلك باستخدام جرعات مختلفه من الأوكس توسين الخارجى. حقنتين من ١٥ ملجم من البروستاجلاندين F₂ ينهما ١٢ يوم (٢٣ نعهه) أو الأسفنجيات المهبلية المعامله بالبروجسترون لمده ١٣ يوم (٢١ نعهه) أستخدمتا لأحداث الشياح. النعاج المتزامنه الشياح حقنت فى الوريد بصفر (محلول ملحي) أو ١٠٠ أو ٢٠٠ USP وحده من الأوكس توسين. مباشره بعد الحقن بالمحلول الملحي أو الأوكس توسين فرغ ١ مل من السائل المنوي الطازج المخفف (٣ × ١٠ حيوان منوي متحرك/ مل محلول منظم من الترس) الموضوع فى ماصه زجاجيه (قطر ها ٥ ملم) داخل الرحم (اذا أخترقت الماصه قناه عنق الرحم بسهوله) أو علي عنق الرحم. وكررت المعامله السابقه بعد ٨ ساعات وأيضاً خلال الشياح التالي للأغنام التي لم تخصب. كان معدل الخصوبه ٦٩.٦% فى النعاج المعامله بالببروستاجلاندين F₂ أثناء الشياح المحدث (٢٣/١٦) مقارنة ٢٨.٦% (٢١/٦) خصوبه للنعاج المعامله بالأسفنجيات (P < 0.01) بينما فى التلقيحه الثانيه معدل الحمل كان ٦٨.٢% بينما فى التلقيحه الأولي كان ٥٠% (الشياح المحدث) ولم توجد اختلافات فى معدل الحمل بين النعاج المعامله بالببروستاجلاندين والنعاج المعامله بالأسفنجيات فى التلقيحه الثانيه.

معاملات الأوكس توسين أزادت (P < 0.01) عدد نعاج التي لهدت داخل الرحم. كل النعاج التي حقنت بـ ٢٠٠ USP وحده من الأوكس توسين (٢١ تلقيحه) و ١٣ من ٢٣ نعهه حقنت ١٠٠ USP وحده من الأوكس توسين لهدت داخل الرحم بسهوله بينما فى المجموعه بالمحلول الملحي ولا عنق رحم (٢٢ نعهه) أمكن أختراقها. بغض النظر عن التلقيحه الأولي أو الثانيه، المعامله بالببروستاجلاندين أو الاسفنجيات المهبلية فأن معدل الحمل كان ٤٥.٥ ، ٥٦.٥ ، ٦٦.٧% فى النعاج المعامله ٢٠٠ ، ١٠٠ ، صفر USP وحده من الأوكس توسين علي الترتيب. لم يوجد للأوكس توسين أو البروستاجلاندين F₂ أو الأسفنجيات المهبلية علي نسبه النعاج التي ولدت أو نسبه الحملان المولوده. هذه النتائج ربما تكون هامه لتطوير التلقيح الصناعى داخل الرحم فى نعاج الرحمانى لاجراحيا.