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NON-SURGICAL INTRAUTERINE INSEMINATION IN EWES USING FROZEN SEMEN

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ABSTRACT

This study was carried out at Sakha Research Station.Sixty six crossbred (Finn x Rahmani) ewes aging 3-5 year and weighing 50.94 kg were used to study the effect of site of semen deposition (vaginal *vs.* cervical) and the number of inseminated spermatozoa on conception rate. Sixty-three of them exhibited estrous after injection of 187.5 µg cloprostenol intramuscularly per ewe 9-12 days post onset of natural heat. Overall mean time at which estrous has occurred post hormonal injection was 33.50±0.65 hrs. and average heat duration was 46.30±1.17 hrs. They were distributed equally over 3 groups. Animals in the first group (control, group 1) were inseminated at Os cervix with a dose of 200 x 10^6 post thaw motile sperm. Animals in the other two groups were injected intravenously with 20 IU oxytocin/ewe to dilate the cervix and were intrauterine trnascervical inseminated within 8 min. of injection. Doses of 100 x 10^6 (group 2) and 200 x 10^6 post thaw motile sperm (group 3) were used for insemination. Conception rate increased as site of semen deposition was deeper in the reproductive tract. All ewes (n=21) in group one with 0.0 IU oxytocin was inseminated vaginally; their conception rate was 42.86%. Ewes in groups 2 and 3 (21 ewes each) were similar in cervical dilation, in response to oxytocin (20 IU) injection. Percentage of ewes didn't responded to oxytocin and were vaginally inseminated was 4.76% (2 ewes). They failed to conceive. Those of cervix partially dilated allowing semen deposition in the cervix (2-4 cm depth) were 9.52% (4 ewes, 2 ewes each), their conception rate was 50%, while those of cervix completely dilated and intrauterine transcervically were 85.71% (36 ewes, 18 ewes each). Their conception rates were 55.6 and 61.11%, respectively. Conception rate was significantly higher between group 3 (61.11%) and group 1 (42.96%).

It could be concluded that non surgical intrauterine insemination in ewes using frogen thawed semen increased conception rates as site of semen deposition was deeper in the reproductive tract.

Keywords: Non-surgical, Insemination, Frozen semen, Ewes.

INTRODUCTION

The availability of an effective artificial insemination (AI) procedure utilizing frozen-thawed semen is the essential key to the widespread use of AI in animal breeding programmes (Donovan *et al.*, 2001). AI in sheep has been poorly implemented and is carried out mainly with chilled semen because of the low fertility results obtained when using frozen-thawed semen (Salamon and Maxwell, 2000). The site of deposition of frozen-thawed semen has a major effect on fertilization rate. A better understanding of the reasons for the generally low conception rate achieved following cervical deposition of frozen-thawed semen is still an important objective towards the goal of establishing an effective and widely applicable AI procedure for achieving acceptable pregnancy rate in sheep.

There are two major barriers in sheep: size and shape of the external cervical Os and eccentric nature of the cervical canal (Dun, 1955 and Halbert *et al.*, 1990a).

There are three obvious methods for reducing the physical effects of the cervix. The first is physically by attaching a hemostat to the external cervical Os and retracting the cervix to align the cervical Os and decrease obstructions to the uterine lumen (Halbert *et al.*, 1990b), mechanically. The second, by designing appropriate transcervical device to overcome the physical difficulties associated with the ovine cervix (Wulster-Radcliffe and Lewis, 2002). The third, hormonally by dilating the cervix with $PGF_{2\alpha}$ or oxytocin (Barry *et al.*, 1990 and Wulster-Radcliffe and Lewis, 2002).

Successful attempts to dilate the cervix in ewes were reported (Khalifa *et al.*, 1992 ; Khallifa, 1993 and Fateh El-Bab *et al.*, 2000) to facilitate the passage of inseminating catheter by use of oxytocin. Therefore, it was the objective of this study to learn more about the effect of oxytocin in cervical dilation, effect of site of semen deposition and the number of inseminated spermatozoa on conception rate. Time of insemination related to the onset and duration of the synchronized estrus has been assessed.

MATERIALS AND METHODS

This study was carried out at Sakha Research Station, belonging to the Animal Production Research Institute, Ministry of Agriculture, from September to February.

1-Animals and management:

Sixty six mature Finn. crossbred ewes (1/2 Finn x 1/2 Rahmani) of 3-5 years of age and average body weight of 50.84 kg were used in this study. Five mature rams on the same crossbred group aging 2-3 years and weighing 60 kg were used for semen collection and processing.

All animals were kept under equal management conditions. They were fed peleted concentrate mixture plus clover according NRC allowances (1985). Drinking water was allowed all time.

3- Estrous synchronization:

Estrous:synchronization was carried out by giving 0.75 ml Estrumate (187.5 μg colprostenol, Agropharm Inc., Willowdale. Ontario Canada) and 200 IU PMSG (Folligon, Intervet International B.V. Boxmeer. Holland) intramuscularly per ewe 9-12 days post onset of natural heat (Sallam, 1999). Starting from twenty-four hours post injection, estrus was detected by teaser rams at 4 hr interval and onset and duration of estrus were recorded.

4- Semen processing and insemination:

Semen extender:

A Tris-based extender was used according to Evans and Maxwell (1987). It consisted of 3.634 g Tris (Hydroxymethyl amino methane), 0.50 g glucose, 1.99 g citric acid monohydrate, 5 ml glycerol, 15 ml fresh egg yolk, 100000 IU Penicillin, 10000 μg Streptomycin and distilled water up to 100 ml.

Freezing method:

Semen was collected from the allocated rams for the experiment using ewes in estrous and artificial vagina. Initial examination was carried out and only ejaculates of 70 to 80% initial motility were diluted (1 part semen to 4 parts extender resulting in 300 x 10^6 sperm/ml) at 30°C. The extended semen was cooled to 5°C after 1.5-2 hours (Maxwell *et al.*, 1995). The cooled

semen was frozen in pelleted form (0.30 ml/pellet) on a fluorethene plate with holes engraved in the surface and cooled to -79°C, then to -140°C by immersion in liquid nitrogen (Evans and Maxwell, 1987).After 2 to 3 minutes, the frozen pellets were transferred into liquid nitrogen and stored for use.

Post-thaw motility evaluation:

Before motility evaluation, frozen pellets were withdrawn from the liquid nitrogen container and two to three pellets were dropped into a pre-warmed glass test tube containing 0.50 ml of thawing solution held in a water bath at 40°C. Using a phase-contrast microscope (400 X) equipped with a thermal stage at 37°C, sperm progressive motility was assessed immediately after post-thawing. An inseminate dose was 1.1 ml was adjusted as to be containing 200 x 10^6 progressively motile spermatozoa.

5- Experimental design:

A total of 63 out of the 66 experimental ewes exhibited estrus after cloprostenol treatment. These animals were assigned into 3 equal groups (21 each). Animals in the first group were artificially inseminated by the conventional method (at Os cervix) with a dose of 200 x 10^6 post-thaw motile sperm. Animals in the other two groups were injected intravenously with 20 IU oxytocin/ewe to dilate the cervix and were intrauterine transcervically inseminated within 8 min of injection. Doses of 100 x 10^6 (group 2) and 200 x 10^6 post thaw motile sperm (group 3) were used for insemination. Artificial insemination was carried out 44 and 52 hr post-Estrumate injection in all groups. The rate of cervical penetration in groups 2 and 3 was similar between the two times. The insemination tube penetrated all the cervical canal into uterus in 18 ewes of each of those of groups 2 and 3. In the rest of inseminated animals (4 ewes) has been deposited in the cervix (2-4 cm deep) in 2 animals in each group, and at Os cervix in one of each. Data were recorded and analyzed for the effect of site of semen deposition (at Os cervix vs. intrauterine) and effect of number of motile sperm /dose on conception rate. Pregnancy rate was calculated for each insemination dose and deposition category and Chi square (x^2) of independence was used to compare pregnancy rates between groups (Duncan, 1955).

RESULTS AND DISCUSSION

1- Onset of estrous:

The results of this study revealed that the majority of ewes (n=35, 55.56%) were observed in heat after 34.08 hrs post hormone injection. Such category was closed to average (33.5 h). Ewes which came in estrous earlier represented 30.16%, while those came later were only 14.28% (Table 1).

Table (1): Distribution of ewes according to onset of estrous in response to treatment (Cloprostenol & PMSG) in ewes.

Ewes came in estrous		Onset of estrous (hr)	
No	%	Mean±SE	Range
19	30.16	28.10±0.54	24-30
35	55.56	34.08±0.08	>30-36
7	11.11	39.71±0.28	>36-42
2	3.17	50.00±0.00	50-50
63	100	33.50±0.65	24-50

2- Estrous duration:

Ewes varied in their duration of estrous post hormonal treatment (Table 2) making a normal distribution-like pattern. The majority of the ewes (52.38%) stayed in heat for a period of >36-46 hrs, while 33.3% stayed in heat for ≥47 hrs.

Table (2): Distribution of ewes according to duration of estrous in response to treatment (Cloprostenol & PMSG) in ewes.

Experimental ewes		Duration of estrous (hr)	
No	%	Mean±SE	Range
9	14.29	29.75±1.22	28-36
33	52.38	43.35±1.33	37-46
21	33.33	55.13±1.51	≥47
63	100	46.30±1.17	28-70

Average heat duration (Table 2) for the 63 synchronized ewes was 46.30±1.17 with a range from 28-70 hrs.

It has been reported that synchronized estrous was detected when double dose of 175 μg cloprostenol was given to ewes of different breed groups at 11 days interval (Meinecke-Tillmann and Meinecke, 1984) or given to Merino ewes at 9 days interval (Wolf *et al.*, 1991). Estrous occurred after 24-96 hr and 45-51 hr, respectively, following hormonal treatment in the two studies.

Vesa Rainio (1992) reported that response of Finn sheep to cloprostenol treatment did not differ due to level of dose (250 or 125 μg) with respect to incidence of estrus (94%) or time of onset of estrus (43 and 41 hr post treatment, respectively). The majority of ewes (69%) showed estrous between 30-47 hr post hormonal treatment. Mansour (1993) gave a low dose of cloprostenol (125 μg to Rahmani ewes at 5 to 13 days of the estrous cycle, 77% of the injected ewes showed estrous within 55.2 hr (33-84 hr) and estrous duration was 27.0 hr (12-48 hr).

Higher incidence of estrous (88-95%) was observed when cloprostenol was given in double injections at interval of 7-13 days (Boland *et al.*, 1978 ; Acritopoulu-Foucroy *et al.*, 1982 and Zanwar and Agrowal, 1983).

The present results confirm the finding of previous studies (Vesa Raino, 1992 and Sallam, 1999), that most ewes responding to estrous synchronization within 30-42 hr following treatment.

El-Maghraby (2003) reported that response of ewes to cloprostenol treatment when gave double dose of 175 μg at 8 days a part or one dose of 175 μg to cyclic ewes, 100% of ewes showed estrous during 18-32 hr and 22-70 hr post treatment and styed in heat for 26-50 and 26-50 hr, respectively.

Sallam (1999) found that 55% of the ewes stayed in heat for a period of 28-48 hrs while 21.7% and 23.3% stayed in heat for 24-28 and >48 hr, respectively, following El-Shamaa *et al.* (2003) stated that percentage of ewes exhibited estrous within 23 to 32.7 hrs following the application of prostaglandin (100 μg cloprostenol) via interavulva-submucosa (IVSM) or intravenous (IV) were equal (100%) and higher than those injected

intramuscularly (i.m.) with 250 μg cloprostenol (83.7%), (DC) deep cervically (50%) or untreated ewes (28.6%). The time elapsed from treatment to estrous was shorter (P<0.05) in IVSM ewes (36.9 hrs) compared with 40.6 hrs in IV; 44.7 hrs in DC and 47.7 hrs in IM group. The corresponding duration of the induced estrous were 23.1, 32.7, 41.3 and 34 hrs, respectively. Conception rate was highly significant (P<0.01) in group 3 (61.11%) compared to 42.86% in group 1.

3. Effect of site of semen deposition and sperm dose on conception rate:

Results of this study (Table 3) showed that conception rate increased as site of semen deposition was deeper in the reproductive tract. All ewes (n=21) in group one with 0.0 IU oxytocin were inseminated vaginally, their conception rate was 42.86%. Ewes in groups 2 and 3 (21 ewes each) were similar in cervical dilation, in response to oxytocin (20 IU) injection. Percentage of ewes didn't responded to oxytocin and were vaginally inseminated was 4.76% (2 ewes). They failed to conceive. Those of cervix partially dilated allowing semen deposition in the cervix (2-4 cm depth) were 9.52% (4 ewes, 2 ewes each), their conception rate was 50%, while those of cervix completely dilated and intrauterine transcervically were 85.71% (36 ewes, 18 ewes each) . Their conception rates were 55.6 and 61.11%, respectively.Conception rate was highly significant(p<0.01) in group 3 (61.11%) compared to (42.86%) in group 1 .

Table (3): Effect of site of semen deposition and seminal dose on conception percentage.

Items	Group 1 Sperm high dose*	Uterine transcervical			
		Group 2 Sperm low dose**		Group 3 Sperm high dose	
	Vaginal	Uterine	Cervical	Uterine	Cervical
Ewes inseminated	21	18	2	18	2
Ewes lambed	9	10	1	11	1
Conception rate	42.86[b]	55.6	50.0	61.11	50.00

* high dose = 200 x 10^6 motile sperm ** low dose = 100 x 10^6 motile sperm

a, b: Conception rate with different litters in the same raw was significantly different (P<0.01)

It was reported that the deeper the site of semen deposition in the reproductive tract of the ewe, the higher the conception rates achieved (Saab and Slieman, 1987 ; Halbert *et al.*, 1990a ; Machado and Simplico, 1990 ; Schakell *et al.*, 1990 and Fateh El-Bab *et al.*, 2000).

It has been reported (Khalifa *et al.*, 1992) that the inseminating catheter passed into the uterus of 77% of intravenously injected ewes with 200, 400 and 600 USP oxytocin compared to 0.0% in the non treated ewes. However, the study lacked any conception evaluation.

Fateh El-Bab *et al.* (2000) found that conception rates of ewes inseminated vaginally, trans-cervically and laparoscopically (Intra-uterine) were 43.8, 53.1 and 64.5%, respectively. All ewes with 0.0 IU oxytocin inseminated vaginally while incidence of intra-uterine insemination was 68.5% and 78.9% for those injected with 10 and 20IU oxytocin, respectively (Fateh El-Bab *et al.*, 2000).

Anel *et al.* (2005) recorded that AI using laparoscopy (AIL) showed significantly higher fertility results than AI via vagina (44.89% vs. 31.25%), despite using frozen semen in AIL and chilled semen (with higher number of spermatozoa) in VAI.

The high conception rate in the intra-uterine inseminated ewes is believed to be due to the availability of high sperm concentration close to the site of fertilization. The cervix constitutes a barrier to the passage of the spermatozoa. It causes a limitation in the availability of sperm and hence reduces conception percentage. This might explain the need of ewes to high dose of motile sperm not less than 150-200 x 10^6/vaginal insemination as compared to 5-10 x 10^6 motile sperm/cervical or uterine insemination in cattle. After vaginal insemination, semen drainage from the vagina to the exterior accounts for the great loss of spermatozoa from the ewe reproductive tract (Hawk and Conly, 1971 and 1972 and Fateh El-Bab, 1975). It was reported that only less than 3% of the inseminated spermatozoa was recovered from the ewe genital tract one hour after insemination (Fateh El-Bab, 1975). It is believed that lower number of motile sperm for intrauterine insemination in ewes could yield acceptable conception percentage. Hence, increases the usability of the ram, increases the efficiency of ram selection and maximizes genetic improvement through artificial insemination in sheep.

In conclusion, intrauterine trans cervical insemination using 20IU oxytocin compared with the traditional vaginal insemination could yield acceptable conception rates using the same number (200 x 10^6) of motile sperm or lower (100 x 10^6) depending on the value of sperm source and/or semen cost.

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التلقيح غير الجراحى داخل الرحم فى النعاج باستخدام سائل منوى مجمد

بدير السيد الصعيدى ، عبد العزيز عبد العظيم سلام ، محمد جبر خليل جبر
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اجريت هذه الدراسة بمحطة بحوث الانتاج الحيوانى بسخا – التابعة لمعهد بحوث الانتاج الحيوانى – وزارة الزراعة فى الفترة من سبتمبر الى فبراير. استخدمت فى هذه الدراسة ٦٦ نعجة خليط فلندى × رحمانى عمرها ٣–٥ سنوات ووزنها ٥٠,٩٤ كجم لدراسة تأثير مكان وضع السائل المنوى (فى المهبل مقارنة بداخل عنق الرحم) ، وعدد الحيوانات المنوية التى تم التلقيح بها على معدل الخصوبة. اظهرت ٦٣ نعجة علامات الشياع بعد الحقن بـــ ١٨٧,٥ ميكروجرام كلوبرستينول فى العضل /نعجة بعد ٩ – ١٢ يوم من بداية الشياع الطبيعى . كان المتوسط العام لوقت ظهور الشياع بعد الحقن بالهرمون ٣٣,٥ ساعة ومتوسط فترة الشياع للنعاج ٤٦,٣ ساعة ، وقد وزعت هذه النعاج على ثلاثة مجاميع متساوية وتم تلقيح المجموعة الاولى (كنترول – مجموعة ١) عند بداية عنق الرحم بسائل منوى تركيزة ٢٠٠ × ١٠ ٦ حيوان منوى متحرك بعد الاسالة. وتم حقن حيوانات المجموعتين (٢ ، ٣) فى الوريد بـــ ٢٠ وحدة دولية اوكسيتوسين/نعجة لتوسيع عنق الرحم وتم تلقيحهم داخل الرحم من خلال عنق الرحم بعد ٨ دقائق من الحقن بالاوكستوسين بجرعة سائل منوى تركيزها ١٠٠ مليون حيوان منوى متحرك بعد الاسالة (مجموعة ٢) أو ٢٠٠ مليون حيوان منوى متحرك بعد الاسالة (مجموعة ٣) .

اظهرت الدراسة زيادة معدل الخصوبة كلما وضع السائل المنوى اعمق داخل الجهاز التناسلى. نعاج المجموعة الاولى (٢١ نعجة) والتى لم تحقن بالاوكستوسين تم تلقيحهم داخل المهبل وكان معدل الخصوبة ٤٢,٨٦%. وقد تساوت نعاج المجموعتين ٢ ، ٣ فى درجة الاستجابة لتوسيع عنق الرحم عند الحقن بـــ ٢٠ وحدة دولية اوكستوسين/نعجة حيث لم تستجيب ٤,٧% من النعاج (٢ نعجة – ١ نعجة/مجموعة) للحقن بالاوكستوسين وتم تلقيحهم داخل المهبل ولم تخصبا. اظهرت نسبة ٩,٥٢% (٤ نعاج – ٢ نعجة /مجموعة) استجابة جزئية لتوسيع عنق الرحم لتمكين وضع السائل المنوى بعنق الرحم (بعمق ٢–٤ سم) وكان معدل الخصوبة لهما ٥٠% ، بينما اظهرت نسبة ٨٥,٧١% (٣٦ ، ١٨ نعجة لكل مجموعة) من النعاج استجابة كاملة للحقن بالاوكستوسين وتم تلقيحهم داخل الرحم وكان معدل الخصوبة لهما ٥٥,٦ ، ٦١,١١% للمجموعتين ٢ ، ٣ على الترتيب.

يستنتج من هذة الدراسة أن التلقيح غير الجراحى داخل الرحم فى النعاج بستخدام سائل منوى بعد إسالتة من التجميد قد أدى إلى زيادة معدل الخصوبة كلما وضع السائل المنوى اعمق داخل الجهاز التناسلى للنعاج.