Kafkas Univ Vet Fak Derg
16 (1): 37-41, 2010
DOI:10.9775/kvfd.2009.354

RESEARCH ARTICLE

The Effect of Air Pressure with Cervical Artificial Insemination on the Fertility of Awassi Ewes Synchronized with PGF2α

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Makale Kodu (Article Code): KVFD-2009-354

Summary

The aim of this study was to investigate the effect of air pressure on fertility during cervical artificial insemination with fresh semen in Awassi ewes. A total of 60 reproductively normal-cycling Awassi ewes (2-5 years old) were used in the experiment during the breeding season. All ewes were treated with the PG-7 days protocol consisting of two intramuscular doses of a PGF2α analogue administered 7 days apart. Animals were divided into two groups. The first group (n=30) was cervically inseminated (CAI). The second group (n=30) was cervically inseminated by means of an air pump (APCAI). Artificial inseminations were performed 48 h after the second PGF2α injection. Pregnancy was determined using ultrasonography on d 30 after AI. The pregnancy rate was found significantly higher in the APCAI group than in the CAI group (80.0% versus 46.7%) ($P<0.01$). The lambing rate was found to be greater in the group cervically inseminated with an air pump than in the group cervically inseminated with the conventional method (70.0 versus 46.7%). The insemination method used had a significant effect on lambing rate ($P<0.05$). Prolificacy was not affected by insemination methods (APCAI, 1.09; CAI, 1.14). It was concluded that cervical insemination with an air pump in Awassi ewes might improve clinical pregnancy rates by mechanisms yet to be defined.

Keywords: *: Awassi ewe, Fertility, Artificial insemination, Air pressure*

PGF2α ile Senkronize edilen İvesi Koyunlarında Hava Basıncı ile Servikal Sun'i Tohumlamanın Fertiliteye Etkisi

Özet

Bu çalışmada, İvesi koyunlarda nativ sperma ile servikal sun'i tohumlama sırasında uygulanan hava basıncının fertilite üzerine etkisi araştırıldı. Araştırmada, aşım sezonu içerisinde bulunan ve normal siklusa sahip toplam 60 baş İvesi koyun (2-5 yaşlı) kullanıldı. Tüm koyunlara bir PGF2α analoğunun kas içi iki dozunun 7 gün arayla uygulamasını içeren PG-7 gün protokolü uygulandı. Hayvanlar iki gruba ayrıldı. Birinci gruba (n=30) sevikal sun'i tohumla yapıldı. İkinci gruba (n=30) bir hava pompası aracılığıyla servikal sun'i tohumla gerçekleştirildi. Sun'i tohumlamalar ikinci PGF2α enjeksiyonundan 48 saat sonra yapıldı. Gebelikler sun'i tohumlamayı takiben 30. günde transrektal ultrasonografi ile belirlendi. Gebelik oranı, hava pompası ile yapılan servikal sun'i tohumlamada (APCAI), servikal sun'i tohumlamadan (CAI) daha yüksek bulundu (%80.0 karşılık %46.7) ($P<0.01$). Kuzulama oranı, hava pompası ile servikal sun'i tohumlamada, servikal sun'i tohumlama grubuna göre daha yüksek bulundu (%70.0, %46.7) ve tohumlama metodu kuzulama oranı üzerinde önemli etkiye sahip oldu ($P<0.05$). Koyun başına doğan kuzu oranı tohumlama metotlarından etkilenmedi (APCAI, 1.09; CAI, 1.14). Sonuç olarak, İvesi koyunlarında hava pompası ile servikal sun'i tohumlama, mekanizması tam olarak belirlenemese de klinik yönden gebelik oranını artırdığı kanaatine varıldı.

Anahtar sözcükler: *İvesi koyunu, Fertilite, Sun'i tohumlama, Hava basıncı*

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INTRODUCTION

Vaginal, cervical, transcervical and intrauterine insemination methods can be used for the artificial insemination of ewes [1-5]. Cervical artificial insemination (CAI) is a less expensive and invasive method in comparison to the transcervical and intrauterine methods, and has been widely used for the artificial insemination of ewes. The site of deposition of frozen-thawed semen in ewes has a major effect on fertilization rate [6].

Whilst pregnancy rates in excess of 60% can be achieved with a single artificial insemination of fresh semen deposited at the external cervical opening [7], corresponding rates for frozen-thawed semen occasionally exceed 45%, with values less than 17% not rare [4]. The structure of the cervix uteri and the course of the uterine cervical canal in sheep limit the passage of AI catheters into the cervix [5,8,9]. CAI with frozen-thawed semen leads to insufficient sperm transport in the female reproductive tract and a reduction in number of sperm as well as proper traits required for good fertilization [4,5,8-10]. Thus, fresh ram semen gives very good results with CAI, but this is not an effective insemination method when used with frozen sperm. Therefore, we hypothesized that an air pump connected to the pipette sheath may serve as a useful component for the cervical AI procedure in sheep.

Laparoscopic AI is the current method of choice for frozen-thawed semen. However, it is costly, time-consuming; and requires technical proficiency and the use of anaesthetics, and therefore, can be used only on a limited scale in ewes [11,12].

Transcervical AI was tested with ram semen and for the fertility of ewes. Optimal fertility rates in trans-cervical AI depend on the fresh or frozen sperm used to inseminate the ewe, and frozen sperm must be tested to obtain maximum semen quality after thawing [1,9,12,14]. The success of artificial insemination in ewes might also depend on the structure of insemination equipment and the method used. Therefore, development of a less invasive and time consuming method than laparoscopy or transcervical AI is necessary for achieving commercially-acceptable pregnancy rates with fresh or frozen-thawed semen [7].

The use of frozen semen in CAI may cause a dis-advantage in fertility results, as reported in previous studies [1-4,10-13]. For this reason, we chose the use of fresh sperm for air pump cervical insemination (APCAI) and cervical insemination (CAI).

The aim of this study was to compare the fertility of ewes inseminated with the conventional cervical insemination and air pump cervical insemination methods. Fresh semen was deposited into the cervical opening with an air pump connected to the outer sheath of a cattle AI pipette in the APCAI group, and with a conventional ewe AI glass catheter in the CAI group. Thus, this research was conducted to develop a suitable CAI method for artificial insemination with fresh or frozen semen in ewes.

MATERIAL and METHODS

A total of 60 reproductively normal-cycling Awassi ewes (2-5 years old) were used in the experiment during the breeding season (August-November). The animals were owned by the Faculty of Agriculture of Harran University and were kept at the faculty research farm in Sanliurfa, Turkey (37.07N, 38.49E). All ewes were treated with the PG-7d protocol consisting of two intramuscular doses of a $PGF_{2\alpha}$ (250 µg Cloprestenol, Estrumate, (Estrumate® DİF Istanbul, Turkey) analogue administered 7 days apart. Animals were randomly allocated into two groups. Thirty ewes were allocated to the APCAI group, and thirty ewes were allocated to the CAI (control) group.

The rams were equally distributed within the experimental groups. Their ages ranged from 2 to 5 years. During the experiment, the rams were kept indoors in box stalls. Semen was collected from 4 adult Awassi rams of proven fertility by means of an artificial vagina. All ewes were inseminated 48 h after the second $PGF_{2\alpha}$ injection. Only ejaculates with the following characteristics were processed: volume ≥0.7 mL; sperm concentration $\geq 3 \times 10^9$ spermatozoa/mL; mass motility (10x; 0-5) ≥4; total motility (40x) ≥70%. Insemination doses contained a total of $\sim 300 \times 10^6$ spermatozoa/mL. Semen was placed in the water bath at 30°C until insemination (within 0.5 h of collection).

The insemination device used in this experiment was modified from a stainless steel outer pipette sheath of a cattle AI pipette. The air pump (2 liter/min capacity; aerator, portable battery pump) was attached to the blunt rounded end of the pipette by means of a rubber pipe with an internal diameter of 2.6 mm *(Fig 1).* A speculum was introduced into the vagina so that the external opening of the cervix could be seen in the light of the speculum lamp. Subsequently, 0.1 ml of semen was drawn into the pipette sheath through a plastic syringe, and the pipette sheath was connected to the air pump. The pipette tip was placed at the external opening of the cervix and the air pump was run to spray semen into the cervical canal. For each ewe, a different pipette sheath was used.

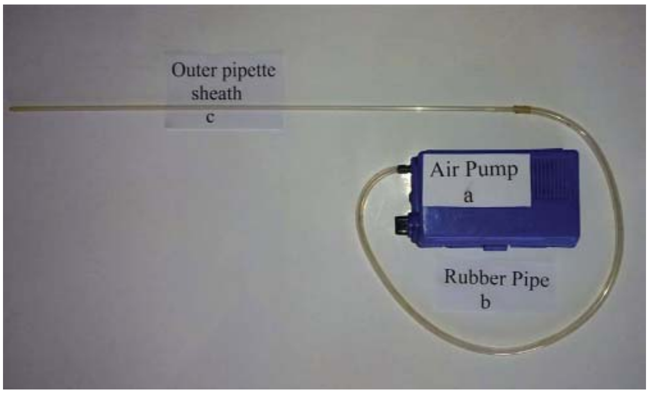

Fig 1. Artificial insemination equipment of APCAI method; **(A)** Air pump, **(B)** Rubber pipe, **(C)** a stainless steel outer pipette sheath used in cattle AI

Şekil 1. APCAI metoduyla sun'i tohumlama ekipmanı; **(A)** Hava pompası, **(B)** Plastik hortum, **(C)** Sığırlarda sun'i tohumlamada kullanılan paslanmaz çelik kateterin dış kılıfı

The ewes were allocated into two groups at the time of insemination, one for APCAI application and other CAI application group. The inseminators deposited semen into the cervix of the ewes by means of the CAI and APCAI methods.

Cervical inseminations were performed with the aid of a plexiglass speculum with an in-built light source to lighten the cervix and using a bent-tipped glass pipette. Inseminations (with 0.1 ml fresh semen) were carried out at the deepest point easily accessible without attempting to forcibly penetrate the cervical canal [7].

The following traits were evaluated in each group:

Pregnancy rate: (no. of pregnant ewes/no. of ewes inseminated) x 100

Lambing rate: (no. of ewes lambed/no. of ewes inseminated) x100

Prolificacy: no. of lambs born/ no. of ewes lambed

Pregnancy rate was determined 40 days after mating by transrectal ultrasonography using a Scanner LC 100 (Pie Medical scanner LC 100 Vet, Netherlands) provided with a 6 MHz linear probe.

Data on pregnancy rate and lambing rate were evaluated by χ^2 analysis (Minitab, State College, PA). Prolificacy was analyzed by analysis of variance using the two-sample t-test. Data are presented as mean ±sem and differences were considered significant when P<0.05.

RESULTS

Pregnancy rates for each of the two AI methods are shown in *Table 1*. Pregnancy rate was strongly influenced by the AI method used, being significantly (P<0.01) higher for APCAI (80.0%) than for CAI (46.7%).

Table 1. *Pregnancy, lambing rate, prolificacy, following different AI methods in Awassi ewes*

Tablo 1. *İvesi koyunlarda farklı suni tohumlama methodunu takiben gebelik, kuzulama ve koyun başına doğan kuzu sayısı*

Parameters		**Groups**	
		CAI	**APCAI**
Number of ewes		(n=30)	(n=30)
Pregnancy rate	(n/n) %	(14/30) 46.7 ^{a}	(24/30) 80.0 ^{b}
Lambing rate	(n/n) %	(14/30) 46.7 ^{c}	(21/30) 70.0 ^{d}
Prolificacy	(n/n)	(16/14) 1.14	(23/21) 1.09

a,b: *P<0.01;* ***c,d:*** *P<0.05*

The APCAI method (70.0%) resulted in higher rates of lambing than the CAI method (46.7%), and there was a significant difference in lambing between the two different insemination methods (P<0.05).

The CAI method had a higher prolificacy (1.14) than that of the APCAI method (1.09). However, no significant difference was found in prolificacy between the two different insemination methods.

DISCUSSION

The present study shows that the APCAI method resulted in acceptable pregnancy rates among estrus-induced ewes artificially inseminated with fresh sperm during the breeding season. The results obtained are also consistent with lambing rates of 70-87% reported by Donovan et al.[14]. The higher pregnancy rates observed with the APCAI method are likely due to use of the air pump. It is obvious that the air pump increases the passage of spermatozoa from the cervical canal into the uterus. In addition, compressed air may enhance the intracervical reservoir of spermatozoa and prolong the time during which semen can enter the cervical mucus, which may be especially important for the poor fertility of frozen sperm as opposed to fresh semen. Thus, APCAI may increase the number of spermatozoa present at the site of fertilization, consequently increasing the change of pregnancy. Secondly, we speculated that these high results may be due to a stimulator compound that may be secreted or increased in response to compressed air given into the cervix. Mizrachi and Shemesh [15] reported the presence of FSH receptors in the bovine cervix. The function of these receptors outside their previously known location in the ovaries was unknown, but it was suggested that FSH might regulate cervical function. In the cervix, the effect of FSH and oxytocin on prostaglandin levels was studied; the level of prostaglandin (PGE2) is affected by circulating FSH and oxytocin, and PGE2 has an effect on cervical relaxation. It was noted that both positive and negative PGE2 receptors are found in the cervical tract, and either a softening of the cervix, or the contraction of the cervical muscle are mediated in part by prostaglandins [15-18]. Compressed air may stimulate the cervix. We hypothesized that an air pump connected to the pipette sheath may serve as a useful component for the cervical AI procedure in sheep. Thus, the results of the experiment, which was designed to test our hypothesis, were promising.

In the present study, the effects of two insemination methods on lambing rate were investigated, and a significant effect was found. An air compressor connected to the pipette sheath had an advantage in lambing rates compared to the CAI method using a bent-tipped glass pipette, which tended to show a higher lambing rate (70.0 and 46.7%). The lambing rate (70.0%) achieved with the APCAI method was nearly comparable to the lambing rates (69.0%) obtained by Stellflug et al.[19] following the intrauterine AI of ewes. Paulenz et al.[20], after inseminating frozen-thawed ram semen into the cervical orifice, observed a lambing rate of 72%. The lambing rate achieved with the APCAI method was slightly higher than that reported (56.0-64.0%) by Fukui et al.[21] after the intrauterine insemination of Suffolk ewes with frozen-thawed semen. This was reflected in a significant difference in embryo survival rate during pregnancy after artificial insemination. Loss at pregnancy may be the major source of a lower rate of lambing. In the CAI method, loss at lambing might be due to embryo mortality or fetal loss between pregnancy and parturition [22]. Another possible explanation may be the high pregnancy rate obtained with the APCAI method. The lambing rate achieved with this method was higher compared to the rate obtained with the CAI method. These results clearly indicate that there are major qualitative differences between the APCAI and CAI methods.

With fresh semen, ewes did not show a significant decrease in prolificacy when inseminated using the APCAI method (1.09) as compared to routine cervical insemination (1.14) into the cervical opening. The reported prolificacy achieved with AI usually varied between 1.03 and 2.17[20], but could also be very low (0.22) Kareta et al.[23] and high (3.2) [14]. Prolificacy has also been evaluated in this study. We obtained a prolificacy of 1.09 with APCAI, which is comparable to that reported (1.03-1.70) by Paulenz et al.[20] and Notter and Copenhaver [24]. In this study, the air pump had a non-significant detrimental effect on litter size. The differences in the results obtained by these different researchers on prolificacy can be explained with differences in management systems, dam age, body condition, and animal breed.

In conclusion, we suggested that the use of an air pump for artificial insemination with ram semen can be an alternative to conventional cervical insemination, and may increase pregnancy rates, in particular when cryopreserved sperm is used. In the future, we are planning to work on air pump artificial insemination with cryopreserved sperm in ewes.

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