Factors affecting pregnancy rate after cervical insemination in dairy sheep flocks

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Abstract

The objective was to assess the factors affecting the success of cervical artificial insemination (CAI) with chilled semen in intensively reared dairy ewes in Greece. The study involved 1,242 adult ewes from 14 flocks in northern Greece (Lacaune, n=885 and Chios, n=357). A typical estrous synchronization protocol (intravaginal progestogen sponge×14 d and 400-500 IU of equine chorionic gonadotropin after sponge removal) was applied in ewes during mating period (May to November). All ewes were cervically inseminated 53-56 h after sponge removal. Semen was collected from 10 fertile (mass motility >4) adult purebred Lacaune rams using an artificial vagina. Each ejaculate was approved for CAI after evaluation of viability, sperm membrane integrity and kinematic parameters by a computer assisted sperm analyzer (CASA). Semen was diluted with skimmed milk to 1.6×10^9 spermatozoa/mL and kept at 15°C until insemination. Pregnancy diagnosis (PD) was performed by trans-dermal ultrasonography at 35-40 d after service.

The following data were available for each ewe: breed; parity; previous lambing date; body condition score at onset of synchronization (BCS_s), at the day of CAI (BCS_i) and at the day of PD (BCS_p); presence of rams during synchronization and number of previous synchronizations. Recordings during the CAI procedure included: onset of synchronization to CAI interval; semen collection to CAI interval; semen deposition depth; cervical mucus presence; duration of CAI. Housing conditions (bedding space; air volume and ventilation) and dietary management were also recorded in each farm. The association between CAI success and categorical variables was assessed with Chi-square independence test. The difference in the mean values of continuous variables between pregnant and non- pregnant ewes was tested through the application of one-way analysis of variance (SPSS v.25.0).

The results showed that breed, parity, semen deposition depth, time from semen collection to CAI and presence of rams during the synchronization period significantly affected CAI success ($P<0.05$). Pregnancy rate in farms with poor ventilation was significantly lower ($P<0.05$) compared to farms with adequate ventilation (40.0% vs. 53.4%). Ewes in medium condition at synchronization (BCS_s: 2.50-3.25) showed significantly higher ($P<0.01$) pregnancy rates compared with under-conditioned (BCS_s <2.50) and over-conditioned (BCS_s >3.50) ewes (51.4% vs. 33.0%).

Similarly, ewes at good condition at the time of CAI (BCS_i: 2.50-3.25) had a significantly higher ($P<0.01$) pregnancy rate than the rest (52.2% vs. 31.8%). Positive energy balance following the onset of synchronization seems to benefit under-conditioned and ewes at medium condition.

Instead, weight gain after sponge placement in over-conditioned ewes resulted to significantly lower (P<0.05) pregnancy rates (14.0% vs. 54.8%). In conclusion, selection of appropriate ewes, BCS recording prior to synchronization and evaluation of dietary management and housing conditions are key factors dictating pregnancy rates following CAI.

Keywords: sheep, insemination, factors, fertility.

Introduction

Assisted reproductive technologies have been implemented in livestock systems to respond to the demand for higher productivity and better quality. The use of artificial insemination (A.I.) has a significant impact on sheep breeding industry, as it enables the rapid introduction of valuable genes to improve production traits and prevents disease transmission (Faigl *et al*, 2012).

However, in comparison with other food producing animals, the implementation of A.I. in sheep is relatively limited. The only exception is France, where more than 410 000 inseminations are performed annually in both nucleus and commercial flocks of the dairy Lacaune breed (Barillet *et al*., 2001). The structural complexity of the ewe cervix prevents deep deposition of semen in uterus and leads to poor fertility rates when frozen- thawed semen is used for cervical A.I. (Salamon and Maxwell, 1995). Fertility rates can be enhanced by the application of laparoscopic insemination; however, increased cost, welfare concerns and the requirement of technical skills are some limitations that have affected negatively the demand of this procedure. Using chilled semen for cervical AI enhances fertility, but increases semen production cost, has time limitations during transportation and often gives irregular results, since the success of the method is affected by many factors. Environmental conditions, management factors, health of males and females, physiological status of ewes are among the factors that need to be controlled before AI implementation. (Donovan *et al*., 2006; David *et al*., 2008; Santolaria *et al*., 2011).

Our objective was to carry out an artificial insemination programme to study the factors affecting the success of cervical artificial insemination (CAI) with chilled semen in intensively reared dairy ewes in Greece. It is the first study that assesses the effect of female, ram, AI procedure conditions and farm nutrition program at the same time on fertility rates after AI on intensively reared dairy sheep in the area.

Materials and methods

Animals

The present trial was conducted during the usual breeding season in Greece, from May to November, for two consecutive years (2017-2018). For the purpose of the study, 1,242 adult ewes (885 Lacaune and 357 Chios) were used from 14 commercial flocks located in North and Central region of Greece. The selected ewes belong to the most common intensively reared breeds in Greece and were born and raised in the above regions. During the study, the animals were at the 5-7th month of their lactation.

Estrous synchronization and CAI procedure

Each ewe was treated with a typical estrous synchronization protocol including intravaginal placement of a sponge containing 20 mg fluogestone acetate (FGA) (Chronogest CR®, MSD Animal Health) for 14 days. At the day of sponge withdrawal,

500 IU (Lacaune) or 400 IU (Chios) of equine chorionic gonadotropin (eCG) (Gonaser®, Hipra) were intramuscularly injected to the ewes.

Semen was collected from 10 Lacaune rams that were located in the same semen collection center (Ovis PC, Thessaloniki), using an artificial vagina. Immediately after collection, motility and concentration of the undiluted semen were assessed. Only ejaculates with concentration greater than 3 x 10^9 spermatozoa/ ml and mass motility greater than 4, on the 0-5 scale described by Evans and Maxwell (1987), were used for the study. After this evaluation, semen was diluted to concentration of 1,6 x 109 spermatozoa/ ml using skimmed milk supplemented with antibiotics, gradually cooled at 15^{o}C and loaded into 0,25 ml mini straws (IMV Technologies, France) (400x106 spz/ dose). The straws were transported on farm for use inside thermos flasks with acetic acid ampoules at 15^{o}C.

Cervical fixed-time AI was performed on each farm 53-56 hours after the sponge removal. Ewes were immobilized by two assistants, with hind legs lifted. In case of mucus presence inside vagina, the animal was put again in horizontal position and the mucus was removed using a speculum. AI was performed afterwards with the help of a speculum equipped with light source and an ovine AI gun (IMV Technologies, France). All artificial inseminations were carried out by the same technician within 8 hours after semen collection. During the procedure, ewes were kept on a restrained area and released to their boxes after insemination, or they were head-locked in feed alley whenever this was applicable Ultrasonography was performed 35-40 days after AI for pregnancy diagnosis (PD) using 5MHz transducer with sector probe (Animal Profi, Draminski, Poland).

Data collection

For all inseminated ewes, data concerning breed, parity, previous lambing date and number of previous synchronizations were recorded. Body Condition Score (BCS) was assessed for each ewe at the time of sponge placement (BCS_s), CAI (BCS_i) and PD (BCS_p). BCS was assessed by palpation in the lumbar region by the same experienced evaluator. Scores assigned to the ewes were based on the existing scale of Russel *et al.* (1969) ranging from 0 to 5, according to which score (0) represents extremely emaciated animals, while the highest score (5) represents obese ones; 0.25 and 0.5 unit increments were used. Changes of BCS between sponge placement and pregnancy diagnosis (51- 56 days) were evaluated to determine whether the animals were in negative, zero or positive energy balance during that period.

At the time of AI, the following data were collected for each ewe: semen collection- AI interval, sponge removal- AI interval, time required per AI, presence of mucus in vagina, presence of rams near the females during synchronization period and semen deposition site. The latter was distinguished in 3 classes depending on the deposition depth of the catheter and retrograde flow of semen: vaginal deposition, external cervical os deposition with partial semen backflow or deeper cervical placement without semen backflow.

Housing conditions of females were assessed in each farm with the calculation of stocking density (m^2 of available floor space/ ewe), available air volume (m^3 of shed volume/ ewe) and available feed space (cm/ ewe). Quality of ventilation was subjectively distinguished in 3 classes by the same evaluator: 1) Good: absence of odour, open type building with functional characteristics that ensure adequate air renewal, side and ridge openings; 2) Moderate: not very good ventilation conditions, presence of odour at tolerable levels, constructions that couldn't always ensure the adequate air

renewal, absence of roof ridge opening; 3) Inadequate: buildings where air renewal was always inadequate or impossible, absence of ridge and side openings, odour at non- tolerant level.

Statistical analysis

The association between CAI success and categorical variables was assessed with Chi-square independence test. The difference in the mean values of continuous variables between pregnant and non- pregnant ewes was tested through the application of one-way analysis of variance (SPSS v.25.0, IBM). Significance level was set at P= 0.05.

Results and discussion

The results of the study are presented on Table 1. Ewe breed, parity, BCS at sponge placement and CAI, as well as BCS change was found to have effect on fertility of the ewes. Overall pregnancy rate was 43.4 %. Lacaune ewes had significantly higher conception rate (48.9%) than the Chios ewes (29.7%). Fertility was higher at animals on 2nd and 3rd lactation period (51.3% and 48.3%) and declined on older ewes, a finding that agrees with studies of Arranz *et al.* (2008) and Palacin *et al.* (2012). Lower conception rates of primiparous ewes could be attributed to their inclusions with older ewes that usually lamb earlier, or to nutritional deficiencies as a result to their larger requirements for growth compared with older ewes (Anel *et al.*, 2005).

Females at moderate BCS (2.5-3.25) exhibited better results in our study, as ewes in good condition have greater ovulation rate than thinner ewes. However, there seems to be a plateau on the effect of BCS on fertility as there is no benefit of increased BCS beyond a point, and conception rates decline in animals with BCS> 3.5. Our study agrees with many authors ending up that ewes should have a BCS of 2.5-3.25 at mating period (Kenyon, 2013; Fukui, 2010) The animals that retained or increased their body weight at the time around CAI, had higher pregnancy rates compared to the animals that lose weight, as it seems that low feed intake and BCS reduction at mating period, results to lower ovulation rate, decreased embryonic growth and increased fetal losses. However, the effect of BCS increase on fertility was not the same for every ewe. Animals with BCSe" 3.5 at onset of synchronization that continued to gain weight had significantly lower conception rates than thinner animals on positive energy balance (Table 2). We suggest that weight gain should be discouraged in fat sheep as can cause high ovulation rates and increase embryonic losses (Rassu *et al.*, 2004).

Regarding to the factors related to the procedure of CAI, deposition of semen inside cervix also increased significantly chances of conception compared to vaginal or external cervical os deposition (46.4% vs. 36.3 and 33.5% respectively). The deeper deposition allows more semen to reach the fertilization site and increases pregnancy chances. In our study, the site of semen placement was found to differ between the 2 breeds, as deposition inside cervix was more frequent on Lacaune than Chios ewes (80.5% vs. 61.9%) (Table 3). This must be a reason for the difference on conception rates among the breeds.

The presence of rams in the area near the synchronized ewes is a factor that could affect pregnancy rates. In farms where the rams were kept apart from the area of the ewes, pregnancy rates were higher in our study. According to Contreras- Solis *et al.* (2009), exposure of females to rams before sponge removal could reduce ecG administration- onset of estrous interval and reduce the success of classic fixed time insemination protocols. That could be overcome by inseminating the ewes earlier.

Table 1. Description of risk factors assessed in the analysis.

Risk Factors	N	Mean	Pregnancy (%)	Sig
Breed				**
Lacaune	885		48.9[a]	
Chios	357		29.7[b]	
Parity		3.11 ± 1.18		**
1	96		43.8[a,c]	
2	339		51.3[b]	
3	308		48.1[a,b]	
4	326		38.3[c]	
>4	173		28.9[d]	
Months from calving		6.02 ± 0.68		n.s.
5	272		46.3	
6	668		44.3	
7	302		38.7	
BCS_s				**
Low (<2.5)	228		35.1[a]	
Moderate (2.5-3.25)	700		51.4[b]	
High (>3.25)	314		31.5[a]	
BCS_i				**
Low (<2.5)	220		30.9[a]	
Moderate (2.5-3.25)	707		52.2[b]	
High (>3.25)	315		32.4[a]	
BCS change				**
BCS decrease	297		30.3[a]	
BCS retain	409		43.8[b]	
BCS increase	536		50.4[b]	
Semen deposition depth				**
Vagina	67		36.3[a]	
External os	242		33.5[a]	
Cervix	933		46.4[b]	
Mucus presence				n.s.
No	886		43.6	
Yes	356		43	
Previous eCG administration				n.s.
No	1179		44.0	
Yes	63		31.7	
Sponge removal to A.I. interval		54.98 ± 0.73		n.s.
Semen collection to A.I. interval		4.79 ± 1.02		**
A.I. duration (min/ animal)		1.15 ± 0.22		n.s
Presence of rams				**
No	1013		46.4[a]	
Yes	229		30.1[b]	

n.s.: Not significant *P*? 0.05; *: *P*< 0.05; **: *P*< 0.01.

Each superscript letter denotes a class of each factor that does not differ significantly from other at the 0.05 level.

Table 2. Effect of BCS increase on fertility of ewes with different BCS.

	BCS at sponge placement		
	<2.5	2.5-3.25	>3.25
BCS increase	48% (61/126) [a]	57% (201/351) [a]	14% (8/59) [b]

Different superscripts between different columns indicate significant differences (*P*< 0.05).

Table 3. Semen deposition site in Lacaune and Chios ewes.

Breed	Semen deposition site % (No. ewes)		
	Vagina	External os	Cervix
Lacaune	3.4% (30)[a]	16.2% (143)[a]	80.5% (712)[a]
Chios	10.4% (37)[b]	27.7% (99)[b]	61.9% (221)[b]

Different superscripts within same column indicate significant differences ($P < 0.05$).

Table 4. Effect of housing conditions on fertility.

Risk Factors	N	Mean	Pregnancy rates (%)	Sig
Bedding space (m2/ ewe)		1.55± 0.15		n.s.
Air volume (m3/ ewe)		9.22± 1.32		n.s.
Feed space (cm/ewe)		35.69± 6.71		n.s.
Ventilation				**
Good	238		53.4[a]	
Moderate	679		41.5[b]	
Inadequate	325		40.0[b]	

n.s.: Not significant P? 0.05; *: $P < 0.05$; **: $P < 0.01$

Each superscript letter denotes a class of each factor that does not differ significantly from other at the 0.05 level

Fertility was found to be decreased in farms where ventilation conditions were inadequate or moderate (40% and 41.5% respectively). On contrast, animals that lived in environment with good ventilation, exhibit higher pregnancy rates. This finding indicates the need of evaluating the micro- environmental conditions inside a farm, in order to achieve better productive and reproductive performance, especially in areas with high temperatures during summer. No other studied factor related to the housing of ewes was found to affect conception rates of CAI (Table 4).

Conclusion

In conclusion, ewe breed and parity, BCS and its changes and semen deposition site inside females reproductive canal, are factors affecting fertility after CAI on intensively reared dairy sheep of Greece. Conception rates can also be affected by time interval from semen collection to CAI and presence of rams near the ewes during synchronization period. Between housing parameters, ventilation seems to play crucial role in the success of the method. Targeted selection of ewes, evaluation of farm's management practices and nutrition program, as well as improvement of housing conditions, could lead to better and more consistent results of CAI, contributing to its application in greek intensive flocks in larger scale.

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