Effect of time of artificial insemination on fertility of progestagen and PMSG treated indigenous Greek ewes, during non-breeding season

A. Karagiannidis\textsuperscript{a,*}, S. Varsakel\textsuperscript{b}, G. Karatzas\textsuperscript{b}, C. Brozos\textsuperscript{a}

\textsuperscript{a}Clinic of Obstetrics and AI, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 21 Kosti Palama, 551 33 Kalamaria, 540 06 Thessaloniki, Greece

\textsuperscript{b}NAGREF/Institute of Reproduction and AI of Thessaloniki, 570 08 Ionia, Greece

Accepted 17 May 2000

Abstract

A total of 2567 indigenous Greek ewes (Chios, Vlachiki and Vlachiki × Chios breeds) were used to determine the optimum time for insemination, following synchronization of oestrus with MAP-impregnated intravaginal sponges and PMSG during non-breeding season. Within each breed group, the ewes were divided into three subgroups and submitted to a double blind cervical artificial insemination 48 and 60 h (subgroup I), 60 and 72 h (subgroup II) and 48 and 72 h (subgroup III) after sponge withdrawal. From the results of the present investigation it can be concluded that the conception rate in the Chios island breed is better than that in the Vlachiki × Chios breed, with the latter being better than that in the Vlachiki breed. Using fixed time for AI, a better conception rate is obtained when applied 48 and 72 h after sponge withdrawal for Chios and Chios × Vlachiki breeds, while for Vlachiki breed a better conception rate is obtained when fixed AI is applied 48 and 60 h after sponge withdrawal. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Artificial insemination; Oestrus synchronization; Fertility; Goats

1. Introduction

Modern systems of sheep production are associated with several levels of intensification, including reproductive management. A crucial step aiming to improvement of the reproductive performance is the intensification of the rhythm of lambing, with the (3 lambings in 2 years) system being one of the most important, especially in dairy ewes. In order to achieve this goal, oestrus synchronization is probably the most popular practice, used to improve conception rates of out-of-season ewes.

* Corresponding author.
breed, nutrition and lactation of ewes, seasonal changes in ram fertility, time of insemination or a combination of all the above factors (Hulet and Stormshak, 1972; Laster and Glimp, 1976; Colas, 1979; Robinson, 1979; Echternkamp and Lunstra, 1979; Echternkamp, 1982; Romano et al., 1996). Acceptable fertility has been achieved with insemination at a predetermined time following synchronization with progestagens plus PMSG and/or hHG (Dziuk et al., 1972).

The aim of the present investigation has been to determine the optimum time of insemination in anestrus indigenous Greek ewes, following synchronization with MAP-impregnated intravaginal sponges and PMSG during the non-breeding season (April–July).

2. Materials and methods

2.1. Experimental animals and hormonal treatment

The present study has been conducted in Northern Greece during the non-breeding season (April–July) of indigenous Greek ewes over two consecutive years (1990–1991).

A total of 2567 indigenous Greek ewes, 507 of Chios breed (Group 1) 1236 of Vlachiki breed (Group 2), and 824 crossbred (Vlachiki × Chios) (Group 3) were treated with intravaginal sponges impregnated with 60 mg medroxyprogesterone acetate (MAP; Veramix, Upjohn/Veterin, Greece). The sponges remained in situ for 14 days. All ewes were checked twice daily (a.m. and p.m.) to ensure that sponges remained in place during the treatment period. Ewes that had lost their sponge were noted and the sponge was immediately replaced. All ewes were injected with 500 IU PMSG (Intergonan, Intervet) im, at the time of sponge withdrawal. Oestrus synchronization was carried out in groups of 60 ewes so that application of artificial insemination could be facilitated.

2.2. Time of the insemination

Within each group, the ewes were randomly divided into three subgroups (I–III) and submitted to a double blind artificial insemination 48 and 60 h (subgroup I), 60 and 72 h (subgroup II) and 48 and 72 h (subgroup III) after sponge withdrawal.

2.3. Semen collection and processing

Semen from Chios breed rams with excellent history of fertility was collected using an artificial vagina. Each ejaculate was evaluated for volume, wave motility and concentration. Only ejaculates with volume of more than 0.5 ml, good wave motility and a minimum concentration of $2.5 \times 10^9$ spermatozoa/ml were used. The ejaculates from each ram were mixed, pooled and maintained at 30°C. They were then diluted in one step with pasteurized skimmed cow’s milk, containing 1000 IU of sodium G penicillin and 1000 μg streptomycin sulfate per ml of diluent. The mixture was packed in 0.25 ml French mini straws, each containing $250–300 \times 10^6$ spermatozoa. After packaging, the straws were cooled at 15°C and were maintained at this temperature by means of an ampoule containing glacial acetic acid until the time of insemination.

2.4. Artificial insemination

Cervical inseminations were performed on a breeding rack by lifting the hindquarters of the ewe over the top rail while the front legs remained standing on the ground. Each ewe was inseminated within 4 h after semen preparation, with one 0.25 ml French mini straw containing $250–300 \times 10^6$ spermatozoa. The semen was deposited into the external os of the first cervical fold, using a speculum fitted with an internal light source.

2.5. Lambing records and statistical analysis

Fertility of inseminated ewes was expressed as lambing percentage. The fertility results were compared between groups using Chi-square analysis. Significance was taken at the level of $P < 0.001$ (Steel and Torrie, 1980).

3. Results

A comparison of the overall lambing percentage in the treated ewes of Chios island breed, Vlachiki breed and their crossbred (Vlachiki × Chios) is presented in Table 1. The overall lambing rate in the ewes of Chios breed (53.1%) was significantly higher ($P < 0.001$)
than that of the ewes of Vlachiki breed (38.2%) and the crossbred (Vlachiki × Chios) (47.6%).

Lambing rate results after a fixed-time double insemination in anoestrus ewes treated with MAP-impregnated sponges and PMSG at various times after sponge removal are shown in Table 2. When the fixed-time double insemination was performed 48 and 60 h after sponge withdrawal, the lambing rate in ewes of Vlachiki breed (57.0%) was significantly higher ($P < 0.001$) than that of Chios breed ewes (38.8%) and in ewes of Vlachiki × Chios breed (28.9%). On the contrary, when the fixed-time double insemination carried out at 60 and 72 h or at 48 and 72 h after sponge removal, the lambing rates in the ewes of Vlachiki breed (31.2 and 25.0%, respectively) were significantly lower ($P < 0.001$) than those of the ewes of Chios (54.5 and 63.7%, respectively) and Vlachiki × Chios breed (51.8 and 60.7%, respectively).

### Table 1

Overall lambing percentage in the ewes of Chios breed, Vlachiki breed and their crossbred (Vlachiki × Chios)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of ewes</th>
<th>Inseminated</th>
<th>Lambed</th>
<th>Lambing percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chios</td>
<td>507</td>
<td>269</td>
<td>53.1$^a$</td>
<td></td>
</tr>
<tr>
<td>Vlachiki</td>
<td>1236</td>
<td>472</td>
<td>38.2$^b$</td>
<td></td>
</tr>
<tr>
<td>Vlachiki × Chios</td>
<td>824</td>
<td>392</td>
<td>47.6$^a$</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2567</td>
<td>1133</td>
<td>44.1</td>
<td></td>
</tr>
</tbody>
</table>

$^a,b$ Values with different superscripts in the same column are different ($P < 0.001$).

### Table 2

Breed and time of artificial insemination effects on lambing rate of anoestrus ewes treated with MAP-impregnated sponges and PMSG

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Time of artificial insemination$^c$</th>
<th>48 and 60 h</th>
<th>60 and 72 h</th>
<th>48 and 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chios</td>
<td></td>
<td>38.8$^b$ (62/160)$^d$</td>
<td>54.5$^a$ (84/154)</td>
<td>63.7$^c$ (123/193)</td>
</tr>
<tr>
<td>Vlachiki</td>
<td></td>
<td>57.0$^c$ (245/430)</td>
<td>31.2$^b$ (128/410)</td>
<td>25.0$^b$ (99/396)</td>
</tr>
<tr>
<td>Vlachiki × Chios</td>
<td></td>
<td>28.9$^b$ (75/260)</td>
<td>51.8$^a$ (147/284)</td>
<td>60.7$^a$ (170/280)</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>44.9 (382/850)</td>
<td>42.3 (359/848)</td>
<td>45.1 (392/869)</td>
</tr>
</tbody>
</table>

$^a,b$ Values with different superscripts in the same column are different ($P < 0.001$).

$^c$ A double blind artificial insemination was performed at three different times after sponge removal.

$^d$ The number in parentheses represents the number of ewes lambing/number of ewes inseminated.

### 4. Discussion

The results of the present investigation indicate a marked discrepancy of the optimum time for fixed AI in Chios, Chios × Vlachiki and Vlachiki ewes, which are difficult to explain since there are contradictory references from investigators all round the world.

In anoestrus, even with a high degree of stimulation accompanied by a high ovulation rate, it is not possible to achieve an acceptable lambing rate approaching 50%. The biology of the lactating ewe and the factors — environmental, physical, physiological and endocrinological — which influence the establishment of pregnancy, are considered complicated (Robinson, 1988). The lower fertility of ewes at the first oestrus, induced with progestagen-PMSG treatment, is caused by many factors including impairment of luteinizing hormone secretion, sperm transport and fertilization (Tempest and Minter, 1987).

Seasonal effects on reproduction of progestagen-PMSG treated ewes appear to be mediated through pituitary gonadotropin secretion with breed differences as to the time and/or intensity of seasonal effects (Echternkamp, 1982). The interval from the onset of oestrus to preovulatory plasma LH-peak was shorter for the out-of-season Romney ewes than for the cyclic ones (Smith, 1977). In a study with out-of-season Merino ewes, the LH-peak concentration was measured approximately 38 h after sponge withdrawal (Robinson, 1988).

It has been previously reported that oestrus and ovulation are highly predictable within a homogeneous group of oestrus-synchronized ewes (Robinson...
and Smith, 1967; Evans and Robinson, 1980; Robinson et al., 1987), with a close relationship between the onset of oestrus and the LH-surge (Evans and Robinson, 1980; Pearce and Robinson, 1985; Robinson et al., 1987). On the other hand it has been reported that the onset of oestrus can be variable (Maxwell et al., 1986). Results of a previous investigation (Amir and Schindler, 1972) indicated that even a time interval of 40 h from insemination to the end of oestrus did not influence the conception rate. Since the end of oestrus coincides on the average with ovulation, this time interval is indicative of the duration of the fertile life of the spermatozoa.

Precise time interval of sponge withdrawal to oestrus is also difficult to determine and can be varied as in Merino ewes referred between 24 and 48 h (Maxwell and Barnes, 1986), or 59.7 h (Maxwell, 1986b) and in Galway ewes 58–78 h (Quirke et al., 1979). In Chios ewes, this interval was 45 h (Brozos et al., 1999), but all the above mentioned experiments were conducted during breeding season.

The optimum time for insemination varies among different ewe breeds. It has been reported that for Leicester × Swaledale ewes the optimum time for insemination is between 54 and 60 h after sponge withdrawal (Ffindlater et al., 1991), while for Merino ewes it was suggested to be between 60 and 72 h post sponge withdrawal, although the mean time of ovulation is the same in these two breeds (Maxwell, 1986a; Eppleton and Roberts, 1986). For Suffolk ewes, it has been reported that the optimum time for insemination is 48 h after sponge removal (Fukui et al., 1989; Ffindlater et al., 1991), while in Border Leicester × Scottish Blackface ewes, high conception rates were achieved applying AI 54–58 h after sponge withdrawal (Aitken et al., 1990).

The use of 500 IU PMSG on sponge withdrawal was reported to have improved fertility (Hackett and Wolynetz, 1982; Langford et al., 1983) but the response to this dose among different breeds can be varied. Chios breed is characterized by having ovarian activity almost all year around while Vlachiki × Chios and Vlachiki breed are seasonally breeding ewes (Vosniakou, 1983). Thus, the dose of 500 IU might not have been sufficient to stimulate follicular development in some Vlachiki and Vlachiki × Chios ewes, leading to lower overall conception rates for these two breeds.

Finally, it is well known that stress can delay or block the appearance of oestrus and even reduce fertility by impairing the cervical transport of sperm (Marinov, 1984; Ehnert and Moberg, 1991). Although all ewes of the present investigation were handled similarly, the fact that ewes of Chios breed are more accustomed and tolerant in handling than the other two breeds used, which could be of some importance.

From the results of the present investigation, it can be concluded that conception rate after progestagen treatment in anoestrus Chios ewes is better than that of Vlachiki × Chios breed with the latter being better than that of Vlachiki breed. Using fixed time for AI, better conception rate is obtained when applied 48 and 72 h after sponge withdrawal for Chios and Chios × Vlachiki breeds while for Vlachiki breed better conception rate is obtained when fixed AI is applied 48 and 60 h after sponge withdrawal.

References


