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Fertility rate of short-term progestagen pretreated ewes in relation to breed: A field study

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[+]This manuscript is dedicated to the memory of Dr. Thomas Lainas, for his great and valuable offer to the Greek livestock development. His love and passion for the veterinary science and the selflessness, the modesty and the integrity of his character are still an inspiration to many people who were fortunate to know and work with him.

Επίδραση της μικρής-διάρκειας αγωγής με προγεσταγόνα στη γονιμότητα προβατίνων σε σχέση με τη φυλή: Μελέτη σε επίπεδο εκτροφής

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ABSTRACT. The effect of short-term instead of long-term progestagen treatment on fertility of Karagouniko and Chios ewes, after natural mating or artificial insemination, was investigated. Two experiments were performed during the transition period from anoestrous to the breeding season. In the 1st experiment (natural mating, NM), Karagouniko and Chios ewes were randomly allocated into 3 groups, that were KLM (long-term progestagen treatment; n=35), KSM (short-term progestagen treatment; n=34), KSP (short-term progestagen treatment; prostaglandin; n=35) and CLM (n=40), CSM (n=35), CSP (n=38), respectively. In the 2nd experiment (intracervical artificial insemination, AI) Karagouniko and Chios ewes were randomly allocated into 3 groups, that were KLA (long-term progestagen treatment; AI at 54h; n=50), KSA1 (short-term progestagen treatment; AI at 54h; n=20), KSA2 (short-term progestagen treatment; AI at 48h; n=28) and CLA (n=40), CSA1 (n=16), CSA2 (n=20), respectively. At sponges' removal (d0) all ewes received 400 IU eCG. Ten rams served NM, while for AI fresh diluted semen was used. Pregnancy diagnosis was performed, 45-50 days later. In the 1st experiment, blood samples were collected, daily for 5 days, starting on d0, for serum progesterone assessment. Conception rate in Karagouniko ewes after NM was higher ($P<0.05$) in KSM (35.29%) compared to KLM (17.14%) group, but did not differ with KSP (28.57%) group, while after AI it was higher ($P<0.05$) in KLA (42.00%) or KSA1 (40.00%) compared to KSA2 (14.29%) group. In Chios ewes no significant differences were observed between groups either after NM [CLM (45.00%), CSM (36.84%), CSP (34.29%)] or after AI [CSA1 (50.00%), CSA2 (50.00%), CLA (45.00%)]. No significant differences were observed after NM or after AI in the litter size in both breeds. These results indicate that short-term progestagen treatment for oestrus synchronization could be applied in indigenous Greek sheep breeds, resulting in equal (Chios) or improved fertility (Karagouniko) than the common long-term one. Also, the fertility rate in ewes subjected to short-term progestagen treatment depends on the time of AI in relation to breed.
Keywords: *Artificial insemination; Chios breed; Karagouniko breed; Oestrus synchronization; Fertility*

ΠΕΡΙΛΗΨΗ. Στην παρούσα έρευνα μελετήθηκε η επίδραση της μικρής-διάρκειας αγωγής με σπόγγους προγεσταγόνων σε σύγκριση με την αντίστοιχη μακράς-διάρκειας αγωγή στη γονιμότητα προβατίνων Καραγκούνικης φυλής και φυλής Χίου, μετά από φυσική οχεία ή τεχνητή σπερματέγχυση. Διενεργήθηκαν 2 πειραματισμοί κατά τη μεταβατική περίοδο από την άνοιστρη στην ένοιστρη περίοδο. Στον 1ο πειραματισμό (φυσική οχεία, NM), οι προβατίνες Καραγκούνικης φυλής και φυλής Χίου χωρίστηκαν σε 3 ομάδες που ήταν οι KLM (μακράς διάρκειας αγωγή, n=35), KSM (μικρής διάρκειας αγωγή, n=34), KSP (μικρής διάρκειας αγωγή, προσταγλανδίνη, n=35) και CLM (n=40), CSM (n=35), CSP (n=38), αντίστοιχα. Στον 2ο πειραματισμό (ενδοτραχηλική τεχνητή σπερματέγχυση, AI), οι προβατίνες Καραγκούνικης φυλής Χίου χωρίστηκαν τυχαία σε 3 ομάδες που ήταν οι KLA (μακράς διάρκειας αγωγή, AI:54h, n=50), KSA1 (μικρής διάρκειας αγωγή, AI:54h, n=20), KSA2 (μικρής διάρκειας αγωγή, AI:48h, n=28) και CLA (n=40), CSA1 (n=16), CSA2 (n=20), αντίστοιχα. Την ημέρα αφαίρεσης των σπόγγων (ημέρα 0) έγινε έγχυση 400 IU eCG. Για τη φυσική οχεία συμμετείχαν δέκα κριάρια ενώ για την εφαρμογή της AI χρησιμοποιήθηκε νωπό σπέρμα. Διάγνωση κυοφορίας πραγματοποιήθηκε 40-45 ημέρες αργότερα. Στον 1ο πειραματισμό, γίνονταν καθημερινά αιμοληψίες για 5 ημέρες ξεκινώντας την ημέρα 0, με σκοπό τον προσδιορισμό της συγκέντρωσης της προγεστερόνης. Στην Καραγκούνικη φυλή μετά τη NM το ποσοστό εγκυμοσύνης ήταν μεγαλύτερο ($P<0.05$) στην ομάδα KSM (35,29%) σε σύγκριση με την KLM (17,14%), αλλά δεν διέφερε από την KSP (28,57%), ενώ μετά την AI ήταν μεγαλύτερο ($P<0.05$) στην ομάδα KLA (42,00%) ή στην KSA1 (40,00%) σε σύγκριση με την KSA2 (14,29%). Καμία σημαντική διαφορά στο ποσοστό εγκυμοσύνης δεν διαπιστώθηκε μεταξύ των ομάδων στη φυλή Χίου μετά τη NM [CLM (45,00%), CSM (36,84%), CSP (34,29%)] ή την AI [CSA1 (50,00%), CSA2 (50,00%), CLA (45,00%)]. Ο δείκτης πολυδυμίας δεν διέφερε μεταξύ των ομάδων των δύο φυλών. Συμπερασματικά, η εφαρμογή μικρής-διάρκειας αγωγή με προγεσταγόνα μπορεί να βελτιώσει (Καραγκούνικη) ή να μην επηρεάσει (Χίου) τη γονιμότητα των προβατίνων σε σχέση με την αντίστοιχη αγωγή μακράς-διάρκειας. Επίσης, η γονιμότητα των προβατίνων μετά από τεχνητή σπερματέγχυση εξαρτάται από το χρόνο εφαρμογής της σε σχέση με τη φυλή.
Λέξεις ευρετηρίασης: *Τεχνητή σπερματέγχυση, Καραγκούνικη φυλή, φυλή Χίου, Συγχρονισμός οίστρου, Γονιμότητα*

The most common method for accelerating the rate of genetic improvement in sheep is the artificial insemination (Faigl et al., 2012). Artificial insemination (AI) is an easily applied method under field conditions. The absence of typical clinical signs of oestruses in conjunction with the use of fresh semen for AI and the need for inseminating a large number of ewes have led to the development of programs for fixed time artificial insemination (TAI) (Menchaca and Rubianes, 2004). TAI is performed at certain time points after hormonal treatment without oestrus detection. The common practice for TAI in sheep flocks is comprised firstly by oestrous synchronization, using progestagen sponges, remained in situ for 14 days, followed by an eCG intramuscularly injection at sponges' removal. TAI is usually performed intracervically once at 54-56 h or 15-17 hours after the detected oestrus, even twice at 48-50 and 58-60 h after sponges' removal (Evans and Maxwell, 1987;Cseh et al., 2012). Pregnancy rate varies from 45 to 78%, depending on the hormonal treatment, the breed, the season, the method of AI and the number of inseminations performed (Evans and Maxwell, 1987; Chemineau and Congié, 1991; Menchaca and Rubianes, 2004; Cseh et al., 2012). State of the art in methodology and technology currently applied in sheep artificial insemination had been reviewed by Cseh and coworkers (2012).

The common applied progestagen treatment for oestrous synchronization in ewes lasts 12-14 days, a time period similar to the lifespan of a cyclic corpus luteum (Abecia et al., 2012). However, this common treatment has been found to have detrimental effects on conception rate due to ovulation of aged follicles (Johnson et al., 1996; Viñoles et al., 2001). After sponges' insertion progesterone profile shows the opposite pattern compared with that of the normal oestrus cycle; for the first 4 days blood serum progesterone is higher than this observed during the physiological luteal phase of the cycle and then after the 6th day declines to subluteal levels until sponges' removal (Greyling et al., 1994). This fact in conjunction with the positive impact of high progesterone concentrations on follicular health status (Rubianes et al., 1996) has led to practice alternative high-level short-term progestagen treatments instead of long-term progestagen ones.

A thorough study performed in Greek sheep breeds (Chios, Karagouniki, Serres), during the breeding season, indicated that AI should be performed 17h before ovulation or at least about 35h after a 14-days treatment with progestagen impregnated sponges (Menegatos, 1990). The timing of AI varies according to the AI method used, the nature of oestrus (spontaneous or induced), the type of semen (fresh diluted / undiluted or frozen), the age and the breed of the ewes, and whether single or double AI is to be performed (Faigl et al., 2012). According to our previous study (Valasi et al., 2005), good oestrous synchronization in cycling Karagouniko ewes may be achieved by short-lasting progestagen priming in conjunction with prostaglandins, showing oestrus onset 48 to 60h after sponges' removal. So far, it has not been tested the effectiveness of short-term progestagen pretreatment in indigenous Greek breed ewes on their fertility and pregnancy rate.

Thus, the present study was designed to evaluate in Karagouniko and Chios breed ewes, during the transition period from anoestrous to breeding season, the effect of duration of progestagen pre-treatment combinedcombines with eCG and/or prostaglandin on fertility and pregnancy rate after natural mating or fixed-time intracervical artificial insemination.

MATERIALS AND METHODS

Two experiments were carried out during two successive years 2013-2014 in two different flocks in the region of Thessaly, the first in Karagouniko and the second in Chios breed ewes. All the experimental procedures were in compliance with guidelines provident by the competent Veterinary Authority according to the National legislation (Presidential Degree 56/2013 on harmonization of the Directive 2010/63/EU on the protection of animals used for scientific purposes).

Animals

In total, 202 dairy ewes of Karagouniko breed and 189 dairy ewes of Chios breed, 3-4 years old, weighted 55-65kg were included in this study, after the end of the lactation period. The study was conducted during the transition period from anoestrous to the breeding season (June). The ewes of each breed were housed in different farms (latitude: 39º 26΄ N) and fed with alfalfa hay and a concentrate compound feed; water was available to all ewes *ad libitum*. The selected ewes in both farms were in good clinical health status. Ewes in Karagouniko herd had received eCG twice or thrice at the previous breeding seasons, in contrast to Chios ewes which had never received eCG for oestrus synchronization.

1st experiment (natural mating)

The ewes of each breed were randomly allocated into 3 groups; that were for Karagouniko breed the group KLM (long-progestagen treatment; n=35), the group KSM (short-progestagen treatment; n=34) and the group KSP (short-progestagen treatment and prostaglandin, n=35). Accordingly, the Chios breed groups were characterized as CLM (n=40), CSM (n=35) and CSP (n=38). The oestruses of all ewes were synchronized using intravaginal sponges contains 60 mg medroxyprogestrone acetate (Veramix, Pfizer, Hellas), which remained *in situ* for 14 days (KLM and CLM groups) or for 6 days (KSM, KSP, CSM and CSP groups). In groups KSP and CSP an intramuscularly injection of 125 μg of prostaglandin (Estrumat, Schering-Plough Animal Health, USA) concomitantly with sponges' insertion was performed. At sponges' removal (day 0), all ewes received intramuscularly 400IU equine chorionic gonadotrophin, (eCG, Intergonan, Intervet, Hellas). One day later, 10 fertile rams of each breed were introduced in each flock for natural mating. Pregnancy diagnosis was performed by transabdominal ultrasonography (4.5-6 MHz convex trans-ducer; SonoVet 2000; Medison CO, Seoul, Korea) 45-50 days later. Pregnancy rate (based on detection of live fetus/es), date of parturition, lambing rate and litter size were recorded.

Blood sampling

Progesterone concentration was measured in blood samples collected from the jugular vein early in the morning for 5 days, starting on the day of sponges' removal (day 0), for the estimation of the onset of oestrus of each animal. After clotting, blood samples were centrifuged (1100 x g; 20 min; 4ºC); serum was aspirated and stored at – 20 oC until assayed.Onset of oestrus was estimated as the time, expressed in days after sponges' removal, when progesterone concentration in each animal decrease to <0.5 ng mL-1.

Progesterone assay

Progesterone concentration was determined in extracted blood serum samples, in duplicate, by radioimmunoassay (RIA), as described by Martin et al. (1987), with minor modifications (Rekkas et al., 1991). Radiolabelled solutions of progesterone [(1,2,6,7–3H) Progesterone] was purchased by Amersham Biotech (Buckinghamshire, UK) and progesterone antiserum, raised in rabbits against a progesterone-11-α-hemisuccinate-bovine serum albumin conjugate, by the Institute of Molecular Biology (Heraklion, Crete, Hellas) (Theodosiadou et al., 2004). The lower limit of sensitivity was 0.019 ng mL-1, the intra-assay variability was 2.0-3.2% (n=8), the inter-assay variability was 6.8% (n=56) and the recovery rate was 95.8±2.2% (mean ± sd; n=56).

2nd experiment (fixed-time intacervical AI)

The ewes of each breed were randomly allocated into 3 groups; that were for Karagouniko breed the group KLA (long-progestagen treatment; AI at 54h; n=50), the group KSA1 (short-progestagen treatment; AI at 54h; n=20) and the group KSA2 (short-progestagen treatment; AI at 48h; n=28). Accordingly, the Chios breed groups were characterized as CLA (n=40), CSA1 (n=16) and CSA2 (n=20). The oestruses of all ewes were synchronized as in the 1st experiment; using intravaginal sponges which remained *in situ* for 14 days (KLA and CLA groups) or for 6 days (KSA1, KSA2, CSA1 and CSA2 groups) and an intramuscular injection of eCG at sponges' removal. Fixed-time intracervical AI was performed, 54h (KLA, KSA1, CLA and CSA1 groups) or 48h (KSA2 and CSA2 groups) after sponges' removal (day 0), using fresh diluted semen (300 x 106 spermatozoa/dose) from 6 rams of known and proven fertility of each breed. One week later, fertile rams were introduced in the latter groups for natural mating of recycling ewes. Pregnancy diagnosis was performed by trans-abdominal ultrasonography (4.5-6 MHz convex trans-ducer; SonoVet 2000; Medison CO, Seoul, Korea) 45-50 days later. Pregnancy rate (based on detection of live fetus/es), date of parturition, lambing rate and litter size were recorded.

Data management and analysis

All analyses were performed by means of the statistical program SPSS, v.17 for Windows (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P<0.05$. Comparisons were made between groups within each group and each experiment. In both breeds, all ewes that conceived after natural mating or TAI (conception rate) and were diagnosed pregnant by ultrasonographic examination, retained their pregnancy to term and lambed healthy offspring. Thus, pregnancy rate was equal to lambing rate, and only pregnancy rate were compared between groups.

1st experiment (natural mating)

Progesterone concentration in time-series blood samples were compared within each group and between groups of each breed (between KLM, KSM and KSP or between CLM, CSM and CSP) by using the general linear model for repeated

Table 1. Number of pregnant ewes and number of lambs born per ewe in each group of the 1st (mating) and the 2nd experiment (artificial insemination).

Experiment	Breed	Group	Number (%) of pregnant ewes after treatment	Number of lambs born per ewe (mean ± sd)
1st *Mating*	Karagouniko	KLM	17.14%[a]	1.50 ± 0.84
		KSM	35.29%[a]	1.58 ± 0.67
		KSP	28.57%	1.60 ± 0.97
	Chios	CLM	45.00%	2.00 ± 0.69 [a, b]
		CSM	36.84%	2.54 ± 0.66 [a]
		CSP	34.29%	2.62 ± 0.65 [b]
2nd *Artificial Insemination*	Karagouniko	KLA	42.00%[c]	2.00 ± 0.55 [c]
		KSA1	40.00%[d]	1.25 ± 0.46 [c]
		KSA2	14.29% [c, d]	1.50 ± 0.58
	Chios	CLA	45.00%	2.50 ± 0.61
		CSA1	50.00%	2.00 ± 0.53
		CSA2	50.00%	2.33 ± 0.50

Same superscript between different rows in the same column indicates statistically significant difference at P<0.05.

Figure 1. Mean progesterone concentration (ng mL-1) in daily blood samples collected from groups KLM, KSM and KSP after sponges' removal (d 0).

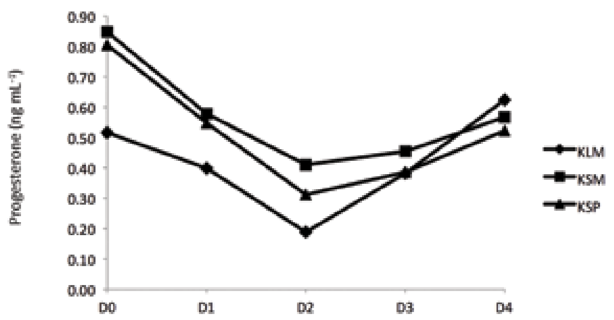
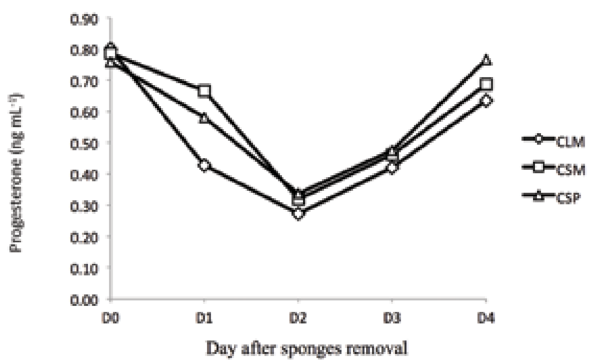


Figure 2. Mean progesterone concentration (ng mL-1) in daily blood samples collected from groups CLM, CSM and CSP after sponges' removal (d 0).



measurements. Each outcome was evaluated in an Analysis of Variance with time of blood sampling as a within-subjects factor and group as a between-subjects factor. Pregnancy rate between natural mating groups, within each breed was evaluated by means of the Pearson's chi-square test. Mean time of the onset of oestruses and litter size was compared between groups in each breed by a one-way Analysis of Variance. All multiple comparisons were done by means of the Duncan test.

2nd experiment (fixed-time intacervical AI)

Pregnancy rate and litter size were compared between groups in each breed by using the same statistical methods as in 1st experiment.

RESULTS

1st experiment (natural mating)

Progesterone assessment

Fluctuation of progesterone concentration within groups varied within time ($P<0.05$), as it is presented in *Figure 1 and 2*. Onset of oestrus (mean ± sd) was estimated at the time when progesterone concentration decrease to <0.5 ng mL-1 after sponges removal (day 0). For Karagouniko ewes, onset of oestrus was estimated at 2.00 ± 0.00, 2.39 ± 0.99 and 2.06 ± 0.24 days after sponges removal for KLM, KSM and KSP groups, respectively ($P>0.05$) *Figure 1*. For Chios ewes, onset of oestrus was estimated at 1.80 ± 0.52, 2.20 ± 0.58 and 2.05 ± 0.46 days (mean ± sd) after sponges' removal for CLM, CSM and CSP groups, respectively ($P>0.05$) *Figure 2*.

Pregnancy rate

In Karagouniko breed ewes the pregnancy rate was higher ($P<0.05$) in KSM (35.29%) compared to KLM (17.14%) group, but did not differ between KSM and KSP (28.57%) groups ($P>0.05$), *Table 1*. Litter size did not differ between KLM, KSM and KSP groups ($P>0.05$), *Table 1*. In Chios breed ewes the pregnancy rate did not differ between CLM (45.00%), CSM (36.84%) and CSP (34.29%) groups ($P>0.05$), *Table 1*. Liter size was lower in CLM group compared with CSM or CSP group ($P<0.05$), *Table 1*.

2nd experiment (fixed-time intacervical AI)

In Karagouniko breed ewes the pregnancy rate was higher ($P<0.05$) in KLA (42.00%) or KSA1 (40.00%) compared to KSA2 (14.29%) group, *Table 1*. Litter size was greater in group KLA compared with KSA1 ($P<0.05$), but did not differ between KSA2 and KSA1 or KLA ($P>0.05$), *Table 1*. In Chios breed ewes the pregnancy rate was numerically higher ($P>0.05$) in CSA1 (50.00%) or CSA2 (50.00%) compared to CLA (45.00%) group, *Table 1*. Litter size did not differ between CLA, CSA1 and CSA2 groups ($P>0.05$), *Table 1*. All Karagouniko and Chios ewes that failed to conceive at induced oestrus after natural mating or intracervical AI conceived at the subsequent oestrous cycles.

DISCUSSION

The present study indicates that short-term progestagen treatment for oestrus synchronization could be applied in indigenous Greek sheep breeds, resulting in equal or improved fertility than the common long-term progestagen treatment. Indeed, the pregnancy rate was improved after natural mating in Karagouniko short-treated ewes, but remained unaffected by the duration of progestagen treatment in artificially inseminated Karagouniko ewes at 54h after the end of the treatment. Accordingly, in Chios short-progestagen treated ewes, pregnancy rate remained unaffected either in naturally or artificially fertilized ewes, irrespective of the time of AI. Thus, these results show that fertility rate in ewes subjected to short-term progestagen treatment is breed-dependant and varies according to the time point at which fixed-time AI is performed.

In this study natural mating after long-term exposure to progestagen sponges resulted in low pregnancy rate (17.14%) in Karagouniko ewes, in contrast to others showing better outcome (56-70%) (Deligiorgis et al., 1996). In the same breed, pregnancy rate was improved after short-term exposure to progestagens compared to long-term exposure. Our results are in accordance with others, where short treatments combined with natural mating resulted in higher (Viñoles et al., 1999) or equal (Ungerfeld and Rubianes, 1999; Ataman et al., 2006; Housein et al., 2007) pregnancy rate, during the breeding or anoestrous season, respectively, compared to long treatment. Recently, Martemucci and D'Alessandro (2011), shown that FGA-PGF2α-eCG short-term (5-days) treatments could be used as a valid alternative to FGA-eCG long-term protocol for synchronization of oestrus after natural mating during the breeding season. The reasons for this might be attributable to the sub-luteal serum progesterone concentration observed at the end of the long-term treatment with progesterone (Gaston-Parry et al., 1988; Viñoles et al., 1999). This was associated with the persistence of the largest follicle (Viñoles et al., 1999) and the presence of aged follicles (Johnson et al., 1996), with subsequent detrimental effects on pregnancy rate (Viñoles et al., 2001). However, it is difficult to compare fertility results of different studies performed 'in season' or 'out-of-season' using nulli- or pluriparous ewes of various breeds with different fertility.

Likely, hormonal treatment and environmental factors, e.g. high temperature during summer (Marai et al., 2007), could be some of the factors that negatively affect animals' fertility in the present study. Almost all of the ewes that had not been fertilized or had failed to retain their pregnancy after treatment were fertilized later at subsequent oestrous cycles and gave birth to healthy offspring. Hormonal treatment may unfavorably impact on survival of sperm inside the female genital tract, as well as, to quality status of oocyte and formed corpus luteum (Chemineau and Cognié, 1991). Also, the administration of eCG has been associated with negative effects on pregnancy rates due to its immunogenic action, especially in repeatedly treated animals (Menchaca and Rubianes, 2004).

The repeated use of eCG treatments for the induction of ovulation is generally followed by a decrease in fertility from 60% to 40% (Baril et al., 1992, 1993). This phenomenon has been explained by unwanted immunological responses (Roy et al., 1999a). Indeed, the presence of anti-eCG antibodies (anti-eCG Abs) in the plasma of eCG-treated goats and ewes has been demonstrated (Baril et al., 1993; Roy et al., 1999b). All Karagouniko ewes participated in the first experiment had previously received eCG twice or thrice, in contrast to Chios ewes, which had never before received this hormone (data obtained from farms' archives).

The detrimental effects of eCG were not so evident for short-term progestagen treated Karagouniko ewes. It seems that this treatment improved the health status of follicles with the potential to ovulate (Menchaca και Rubianes, 2004). This is indicated in the present study by the greater litter size in short treated Chios ewes compared with long treated Chios ewes in the 1st experiment (Table 1). The effect of progestagen pretreatment on ovulation rate seems to be more evident in the high prolific Chios breed compared with the low prolific Karagouniko one. In the latter breed there was no difference in the litter size between long or short treated ewes. This effect is further supported by our previous results where the short-term exposure to progestagens improved the meiotic competence of oocytes collected from superovulated ewes either in the breeding or in anoestrous period (Valasi et al., 2006). With respect to KSP and CSP groups (short-term progestagen treatment and prostaglandin injection at sponges' insertion), conception rate were not different to KSM and CSM groups (without prostaglandin). Although, it has been proposed the use of prostaglandin in short-term progestagen treatments to induce luteal regression in breeding season (Menchaca and Rubianes, 2004; Abecia et al., 2012), in our study was not requisite due to the period studied, which was the transitory period. Some ewes during the transitory period may present luteal activity but others may not; those that had not received prostaglandin shown delayed oestrus probably due to delayed luteal regression.

Given the uncertain use of prostaglandins during the transitory period, this hormone was not used in TAI groups. According to previous studies, the optimal time in long progestagen TAI protocols is 54 h after sponges' withdrawal (Gordon, 1983), when one AI is to be performed. Based on physiology of oestrus characteristics (Menegatos, 1990) and on progesterone profile of first experiment (onset of oestrus), only two time points for AI were tested. The best pregnancy rate in Karagouniko ewes was achieved at 54h, while for Chios ewes the two time points (48 or 54h) were equally effective.

The lack of synchronization between the time of ovulation and the application of AI was suggested as the most common cause of AI failure (Jabbour and Evans, 1991). Time of ovulation depends on protocol applied for oestrus synchronization, breed, animal, age, flock and season of the year (Evans, 1988; Walker et al., 1989). In the present study, the onset of oestrus, as evaluated by progesterone concentrations, was not different between the two breeds, although it was presented a little earlier in Chios than in Karagouniko ewes. This earlier onset of oestrus may support the better pregnancy rate in Chios ewes, irrespective of the time of AI. Likely in short-term progestagen treated Chios ewes oestrus duration seemed to be shorter and thus LH peak and ovulation were evoked earlier than in Karagouniko ones.

Moreover, it should be considered that ewes of both breeds, characterized by different ovulation rate, received the same dose of eCG (400 IU), implicating the role of eCG treatment not only in increasing the litter size but, also, in hastening the oestrus characteristics (e.g. duration, time of LH peak) in Chios breed compared with Karagouniko one. In addition, hormonal treatment used for estrus synchronization has been associated with aberrations in hormonal profile of animals, alterations of cervical mucus characteristics and reduced transport and poor survival of sperm in the female reproductive tract (Hawk, 1983; Stefanakis, 1988). In this respect, electrical resistance of cervical mucus may be used as a reliable tool to increase the likelihood to pregnancy at fixed time AIs (Theodosiadou et al., 2014).

This study indicates an impact of breed in the success of cervical AI using fresh semen, which is in accordance with previous studies using fresh or frozen-thawed semen (Donovan et al., 1998; 1999; 2000). Breed effects on the timing of ovulation, and hence inappropriate timing of AI, may not be the explanation for the breed differences considering the lack of significant differences among foreign (Donovan et al., 2000) or indigenous breeds (Menegatos, 1990) for the intervals from progestagen withdrawal to either the preovulatory surge or to ovulation. The large differences among ewe breeds provide a lever for evaluating the importance of variation in transport through the cervical barrier, survival in the uterus and physiological mechanisms governing the milieu at the site of fertilization.

These results indicate that short-term treatment with progestagens instead to long-term treatment could be an alternative method for effective oestrus synchronization in Greek breeds of sheep. Based on the present studied short-term progestagen protocols, the most appropriate time for TAI is 54h after sponges' removal either in Karagouniko or Chios ewes.

Finally, it is given evidence that fertility and pregnancy rate in ewes subjected to short-term progestagen treatment is breed-dependant and varies according to the time point at which fixed-time AI is performed. Further research is underway to define the best fixed-time AI protocol in different flocks of Karagouniko or Chios ewes subjected to short-term progestagen treatment for improving their fertility throughout the year.

Concluding remarks

Conclusively, the short-term progestagen treatment for oestrus synchronization could be applied in Karagouniko and Chios breed ewes, during the transition period between anoestrous and breeding season, offering equal (Chios) or even better (Karagouniko) fertility, either after natural mating or AI, than the common long-term one. The most appropriate time for fixed intracervical AI in short-term progestagen treatment protocol is 54 h after sponges' removal, either in Karagouniko or Chios breed ewes, during the transition period. Finally, the fertility rate in ewes subjected to short-term progestagen treatment seems to depend on the time of fixed-time AI in relation to breed.

CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare.

REFERENCES

Abecia JA, Forcada F, Gonzalez-Bulnes A (2012) Hormonal control of reproduction in small ruminants. Anim Reprod Sci 130: 173-179.

Ataman MB, Aköz M, Akman O (2006) Induction of synchronized oestrus in Akkaraman cross-bred ewes during breeding and anestrus seasons: the use of short-term and long-term progesterone treatments. Revista de Medicina Veterinária 157: 257-260.

Baril G, Remy B, Leboeuf B, Beckers JF, Saumande J (1993) Synchronization of estrus in goats: the relationship between eCG binding in plasma, time of occurrence of estrus and fertility following artificial insemination. Theriogenology 40:621–628.

Baril G, Remy B, Vallet JC, Beckers JF (1992) Effect of repeated use of progestagen- PMSG treatment for estrus control in dairy goats out of breeding season. Reprod Dom Anim 27:161–168.

Chemineau P, Cognié Y (1991) Training manual on artificial insemination in sheep and goats. Food and Agriculture Organization on the United Nations, Rome, Italy.

Cseh S, Faigl V, Amiridis GS (2012) Semen processing and artificial insemination in health management of small ruminants. Ani Reprod Sci 130: 187-192.

Deligiorgis SG, Apostolopoulos KD, Deligiannis C, Rogdakis E (1996) Economic evaluation of the effects of mating season on the breeding of sheep (in Greek). Animal Science Review 22: 37-48.

Donovan A, Hanrahan JP, Duffy P, Boland MP (2000) AI in sheep: breed differences in timing of ovulation. Irish J Agr Food Res 39: 464-465.

Donovan A, Hanrahan JP, Duffy P, Byrne GP, Boland MP (1999) Cervical AI with fresh or frozen-thawed semen: effect of ewe breed. Irish J Agr Food Res 38: 267.

Donovan, A, Hanrahan JP, Kummen E, Duffy P, Boland MP (1998) Ewe fertility following cervical AI of fresh or frozen-thawed semen at a natural or synchronized oestrus. Irish J Agr Food Res 37: 108-109.

Evans G (1988) Current topics in artificial insemination of sheep. Aust J Biol Sci 41: 103-116.

Evans G, Maxwell WMC (1987) Salamon's Artificial Insemination of Sheep and Goats. Butterworths Pty Limited.

Faigl V, Vass N, Javor A, Kuscsar M, Solti L, Amiridis GS, Cseh S (2012) Artificial insemination of small ruminants-A review. Acta Vet Hung 60: 115-129.

Gaston-Parry O, Heasman K, Nemorin JKE, Robinson TJ (1988) A radioimmunoassay for flurogestone acetate (FGA) and its application to the measurement of plasma FGA and progesterone in ewes treated with FGA-impregnated intravaginal sponges. Aust J Biol Sci 41: 57-67.

Gordon I (1983) Fixed-timed sheep artificial insemination. In (Ed.: Gordon I) Controlled Breeding in Farm Animals, Pergamon Press, Oxford, UK, pp. 197-208.

Greyling JPC, Kotze WF, Taylor GJ, Hagendijk WJ, Cloete F (1994) Synchronization of oestrus in sheep: use of different doses of progestagen outside the normal breeding season. S Afr J Anim Sci 24: 33-37.

Hawk HW (1983) Sperm survival and transport in the female reproductive tract. J Dairy Sci 66: 2645-60.

Housein MQ, Ababneh, MM, Abu-Ruman DS (2007) The effects of short or long term FGA treatment with or without eCG on reproductive performance of ewes bred out-of-season. American J Anim Vet Sci 2: 23-28.

Jabbour HN, Evans G (1991) Fertility of superovulated ewes following intrauterine or oviductal insemination with fresh or frozen-thawed semen. Reprod Fert Develop 3: 1-7.

Johnson SK, Dailey RA, Inskeep EK, Lewis PE. (1996) Effect of peripheral concentrations of progesterone on follicular growth and fertility in ewes. Domest Anim Endocrinol 13: 69-79.

Marai IFM, El-Darawany AA, Fadiel A. Abdel-Hafez MAM (2007) Physiological traits as affected by heat stress in sheep - a review. Small Rumin Res 71: 1-12.

Martemucci G, D'Alessandro AG (2011) Synchronization of oestrus and ovulation by short time combined FGA, PGF2α, GnRH, eCG treatments for natural service or AI fixed-time. Anim Reprod Sci 123: 32-39.

Martin G, Sutherland SRD, Lindsay DR (1987) Effects of nutritional supplements on testicular size and the secretion of LH and testosterone in Merino and Booroola rams. Anim Reprod Sci 12: 267-281.

Menchaca A and Rubianes E (2004) New treatments associated with timed artificial insemination in small ruminants. Reprod Fert Develop 16: 403-414.

Menegatos I (1990) Endocrinological changes in ewes after hormonal treatment for oestrus synchronization with progestagen (MAP) and PMSG. Ph.D. Thesis. Agricultural University of Athens. Department of Animal Production.

Rekkas C., Bellibasaki S., Taitzoglou I., Kokolis N., Smokovitis A. (1991) Increased plasminogen activator activity and plasminogen activator inhibition in spermatozoa and seminal plasma of the ram after serum gonadotrophin (PMSG) administration. Correlation with the increased level of testosterone in the blood. Andrologia 23: 273-278.

Roy F, Maurel MC, Combes B, Vaiman D, Cribiu EP, Lantier L, Pobel T, Dele´tang F, Combarnous Y, Guillou F (1999a) The negative effect of repeated equine chorionic gonadotropin treatment on subsequent fertility in alpine goats is due to a humoral immune response involving the major histocompatibility complex. Biol Reprod 60:805–813.

Roy F, Combes B, Vaiman D, Cribiu EP, Pobel T, Dele´tang F, Combarnous Y, Guillou F, Maurel MC (1999b) Humoral immune response to equine chorionic gonadotropin in ewes: association with major histocompatibility complex and interference with subsequent fertility. Biol Reprod 61:209–218.

Rubianes E, de Castro T, Carbajal B (1996) Effect of high progesterone levels during the growing phase of the dominant follicle of wave 1 in ultrasonically monitored ewes. Can J Anim Sci 76: 473-475.

Stefanakis AE (1988) Changes in the properties of cervical mucus of sheep after estrus synchronization and their influence on spermatozoa behaviour. Ph.D. Thesis, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Theodosiadou E, Amiridis GS, Tsiligianni Th (2014) Relationship between electrical resistance of cervical mucus and ovarian steroid concentration at the time of artificial insemination in ewes. Reprod Biol 14: 234-237, Short Communication.

Theodosiadou E, Goulas P, Kouskoura T, Smokovitis A (2004) Oestrogen and progesterone concentrations in plasma and oviductal tissue of ewes exhibiting a natural or induced oestrus. Anim Reprod Sci 80: 59-67.

Ungerfeld R, Rubianes E (1999) Effectiveness of short-term progestagen priming for the induction of fertile oestrus with eCG in ewes during late seasonal anoestrus. Anim Sci 68: 349-353.

Valasi I, Papanikolaou T, Deligiannis C, Theodosiadou E, Amiridis GS (2005) Effects of follicular aspiration on the number and the quality of collected oocytes from superovulated Karagouniko ewes. Proceedings of 6th International Sheep Veterinary Congress, Hersonissos, Crete, Greece, p. 373-374.

Valasi I, Papanikolaou Th, Goulas P, Deligiannis C, Amiridis GS (2006) Effect of progestagen pretreatment on meiotic competence of oocytes collected from superovulated ewes. 10th Annual Congress of the European Society for Domestic Animal Reproduction (Portoroz-Lipica, Slovenia), Reprod Dom Anim 41(4): 370.

Viñoles C, Forsberg M, Banchero G, Rubianes E (2001) Effect of long-term and short-term progestagen treatment on follicular development and pregnancy rate in cyclic ewes. Theriogenology 55: 993-1004.

Viñoles C, Meikle A, Forsberg M, Rubianes E (1999) The effect of subluteal levels of exogenous progesterone on follicular dynamics and endocrine patterns during early luteal phase of the ewe. Theriogenology 51: 1351-1361.

Walker SK, Smith DH, Godfrey B, Seamark RF (1989) Time of ovulation in the South Australian Merino ewe following synchronisation of oestrus. 1. Variation within and between flocks. Theriogenology 31: 545-553.