Slovak J. Anim. Sci., 42, 2009 SUPPLEMENT 1: 51-55

ISSN 1337-9984

Scientific paper formerly presented

EFFECT OF ASSISTED OESTRUS ON THE OVULATION RATE AND REPRODUCTIVE PERFORMANCE OF TSIGAI SHEEP

I. MARAČEK[1*], R. VLČKOVÁ[1], J. KAĽATOVÁ[1], D. SOPKOVÁ[1], K. KLAPÁČOVÁ[1], I. VALOCKÝ[2], J. POŠIVÁK[2]

[1]Institute of Physiology at the Department of Anatomy, Histology and Physiology,
[2]Clinic of horses, University of Veterinary Medicine in Košice

ABSTRACT

The aim of the work was to observe the effect of innovated and new methods of assisted oestrus of sheep on basic parameters of fertility and reproductive performance of the ewes raised for the production of slaughter milk lambs and sheep milk for market utilisation. Verifying observation was carried out in the Tsigai sheep in one control flock (n=355) with a traditional Carpathian system of mating, and in 6 flocks with assisted oestrus, where 1528 animals were treated and prepared for mating. Of them the assisted oestrus was used in three flocks for earlier starting of the mating period according to the need of earlier preparation of lambs for an Easter' market. In the other three flocks the assisted oestrus was verified at the preparation of slaughter milk lambs for a Christmas' market and subsequent milking for production of sheep milk and its products for a winter tourist season. At the assisted oestrus, of 1528 ewes 1285 ones lambed and 1510 live-born lambs were obtained, which represents 118.2 % natality in conditions of breeding, where in the control flock the average natality was 111.9 %. Valuable information was gained when at assisted oestrus in spring at the preparation for production of slaughter milk lamb for Christmas market the preparation was divided into two stages. Using this method a significantly higher occurrence of twin parturitions and subsequent natality (133.2 %) in comparison with the second stage (102.9 %; P<0.001) was reached. The innovated methods are favourable regarding the environmental and ecological aspects.

Key words: ewe; assisted oestrus; cronolon; OvSynch; fertility; fecundity; prolificacy

INTRODUCTION

An important component of assisted reproduction in sheep is assisted oestrus that represents a complex system of methodological procedures and measures for reaching the fertile oestrus in a certain period for productive use according to requirements of a breeder and market for the main products of the given flock.

The term 'assisted reproduction' means the system of continuous control and positive intervention into the sex functions of ewes and ewe hog and goal-directed influencing of their course during the whole reproductive cycle. The intention and purpose is to ensure a reproductively active state of a determinant majority of ewes and breeding ewe hogs in the flock and to limit to the minimum an occurrence of the disorders of fertility and sterility. Stimulation, induction and synchronisation of the oestrus, as well as influencing the ovulation rate (OR), represent significant biotechnical methods, which after addition of the integrated complex of breeding, veterinary, and managing measures, respecting requirements of the market, result in its own assisted oestrus ensuring an optimal reproduction of the sheep flock with economical profit (Bartlewski, 2001; Bartlewski et al., 1999; Maraček et al., 2004; 2005; 2009).

***Correspondence:** E-mail: maracek@uvm.sk
Imrich Maraček, DVM, (MVDr), DrSc., prof., University of veterinary medicine, Komenského 73, Košice, Slovak Republic, Phone:+421 915 984 701

This work was supported by the grant AV 4/0113/06.

Intensification of the milking sheep reproduction means an increase in the fertility by a rise of the mean natality through the increase in multi-foetus (especially twins). This can be reached by increasing the whole life fertility of ewes and regarding health by a reduction of ewe losses, occurrence of organisational and pathological sterility, embryonic losses, abortions, diseases of the genital system, dead-born lambs and decreasing of lamb death rate at growing (Gamčík and Hartwig, 1988; Maraček et al., 1995; Ptaszynska, 2002).

This work was aimed at the study of influence of innovated method of assisted oestrus and ovulation of sheep by the observation of the flocks fertility bred for a production of slaughter milk lambs and subsequently sheep milk for market use. The goal was to obtain objective data about fertility, fecundity, natality (prolificacy), an occurrence of twin parturitions and a sex ratio of born lambs after application of verified methods of assisted oestrus.

MATERIAL AND METHODS

The experiments were carried out in a farm of Tsigai sheep. The control flock (n=355) of the breeding was mated traditionally according to the Carpathian system of breeding. The experimental flocks (n=1528) were kept under conditions of productive breeding and raised traditionally in sheep-folds on a pasture, and in winter in sheep-cote. For induction and synchronisation of oestrus the **Chrono-gest®** preparation, containing 40 mg FGA (fluorogestonacetate) in a sponge of Intervet Co., and later since 2007 an innovated preparation on the basis of FGA (cronolon) **- Chronogest ® CR** (Intervet), containing 20 mg FGA in a sponge were used. At the time of the FGA withdrawal, serum equine chorionic gonadotropin (eCG) was injected i.m. in a mixture with the **Sergon inj. a. u. v. (Bioveta Inc)** preparation; on day 2 after the eCG administration the mating was started (Tab. 1). According to the Ovsynch protocol following preparations were used:

(1) Supergestran (GnRH) inj. a.u.v. (Ferring-Léčiva, Inc., CR) – with prolonged effect. The application triggers LH and an increase in the blood circulation can be detected after 30 minutes and it persisted for 240 min.

(2) Remophan (a $PGF_{2\alpha}$ analogue) inj. a.u.v. (Bioveta, Inc., CR) – active substance D-cloprostenol 0.25 $mg.ml^{-1}$. After the dose 2 of GnRH, on the next day a mating was started and lasted for 48 hours. After 14 days, the stage 2 of mating followed when the rams were admitted into the flock.

Definition of the parameters observed, their finding, analysis and evaluation were performed according to Ptaszynska (2002). The differences between two experiments were statistically evaluated using χ^2 – test.

Table 1: Observed and evaluated flocks of Tsigai sheep

Sheep flock	Number of animals	Procedure of assisted oestrus	Preparation	Dose
control	355	Without assistance, traditional mating started 2.9.2006	Test rams from 21.8.2006	–
assisted oestrus	180	FGA vaginal sponges 21.8. for 12 days and at withdrawal of eCG	Chrono-gest Sergon	40 mg FGA 500 IU eCG
assisted oestrus in - stage I - stage II Total	 197 213 410	In stage I, 27. 4. 2006 FGA vaginal sponges at withdrawal of 9.5. i.m. eCG and start of stage II. as treatment in stage I.	Chrono-gest Sergon in stage I Sergon in stage II	40 mg FGA 1000 IU eCG 500 IU eCG
assisted oestrus in - stage I - stage II Total	 193 215 408	In stage I 4.5.2007 FGA vaginal sponges for 12 days at withdrawal 16.5. i.m. eCG and start of stage II as treatment in stage I	Chrono-gest Sergon	40 mg FGA 500 IU eCG
assisted oestrus in - stage I - stage II. Total	 200 180 380	In stage I 29.4.2008 FGA vaginal sponges for 12 days at withdrawal 11.5. i.m. eCG and start of stage II as treatment in stage I.	Chronogest® CR Sergon	20 mg FGA 500 IU eCG
early start of mating season	100	First week in August FGA for 12 dayd at their withdrawal i.m. eCG	Chronogest® CR Sergon	20 mg FGA 500 IU eCG
according to OvSynch protocol	50	First application of GnRH i. m. after 5 days i. m. administration of Remophan and after 48 h second injection of GnRH	Supergestran inj. Remophan	0.125 mg 0.125 mg

RESULTS

The results of the fertility analysis in the flocks, observed in the production breeding of milk Tsigai sheep (T) for slaughter milk lambs for both the Easter's and Christmas' markets, as well as a subsequent production of sheep milk, are summarized in tables 2 and 3.

The data in table 2 shows significant differences in natality between individual flocks or between the first and second stage of assisted oestrus in given flocks (flocks 3, 4, and 5). Very valuable is a finding that the natality after assisted oestrus has a tendency to raise in comparison with the first flock prepared and mated traditionally. Significantly higher values (P<0.05) were found only in

Table 2: Basic parameters of fertility in Tsigai sheep after traditional Carpathian system of mating and after assisted oestrus determined by the requirements of production and market

Flock	Mated		Lambed		Number of lambs (n)	Fecundity		Prolificacy (Natality) %
	n	%	n	%		% of selected	% of mated	
traditionally mated number of animals (n=355)	330	93.0	303	91.8	339	95.5	102.7	111.9a
assisted oestrus in August (n=180)	150	83.3	137	91.3	167	92.8	111.3	121.9
assisted oestrus in:								
- stage I (n=197)	186	94.4	175	94.1	254	128.9	136.6	145.1b
- stage II (n=213)	191	89.7	155	81.2	162	76.1	84.8	104.5 b
Total (n=410)	377	91.9	331	87.5	416	101.5	123.4	26.1a
assisted oestrus in:								
- stage I (n=193)	181	93.8	170	93.9	212	109.8	117.1	124.7c
- stage II (n=215)	208	96.7	202	97.1	204	94.8	98,1	101.0c
Total (n=408)	389	95.3	372	95.6	416	102.0	106.9	111.8
assisted oestrus in:								
- stage I (n=200)	192	96.0	170	88.5	220	110.0	114.6	129.4d
- stage II (n=180)	173	96.1	161	93.1	167	92.7	96.5	103.7d
Total (n=380)	365	96.1	331	90.7	387	101.8	106.0	116.9
early mating n=100	92	92.0	90	97.8	103	103.0	111.9	114.1
ovsynch protocol n=50	45	90.0	24	53.3	29	58.0	64.4	120.8

[a]P<0.05 - χ^2=4.099; [b]P<0.001 - χ^2=39.309; [c]P<0.01 - χ^2=10.048; [d]P<0.001 - χ^2=12.706

Table 3: Occurrence of twin parturitions and division of born lambs by sex after assisted oestrus according to market requirement for slaughter milk Tsigai lambs

Flock of lambed	Ewes that lambed								Sex			
	1 lamb		2 lambs		3 lambs		4 lambs		Ram lamb		Ewe lamb	
	n	%	n	%	n	%	n	%	n	%	n	%
traditional n=303	260	85.8	43	14.2a,c	–	–	–	–	157	46.3	182	53.6
assisted oestrus August n=137	96	70.1	41	29.9a	–	–	–	–	84	50.3	83	49.7
assisted oestrus in:												
- stage I (n=176)	100	56.8	75	42.6b	–	–	1	0.6				
- stage II (n=155)	146	94.2	9	5.8b	–	–	–	–				
Total (n=331)	246	74.3	84	25.4c	–	–	1	0.3	211	50.7	205	49.3
assisted oestrus in:												
- stage I (n=170)	129	75.9	40	23.5d	1	0.6	–	–				
- stage II (n=202)	200	99.0	2	1.0d	–	–	–	–				
Total (n=372)	329	88.4	42	11.3	1	0.3	–	–	224	53.8	192	46.2
assisted oestrus in:												
- stage I (n=170)	124	72.9	42	24.7e	3	1.8	1	0.6				
- stage II (n=161)	158	98.1	3	1.9e	–	–	–	–				
Total (n=331)	282	85.2	45	13.6	3	0.9	1	0.3	195	50.4	192	49.6
early mating n=90	77	85.5	13	14.5	–	–	–	–	54	52.4	49	47.6
ovsynch proto n=29	19	79.2	5	20.8	–	–	–	–	13	44.8	16	55.2

[a]P<0.01- χ^2=7.17; [b]P<0.001- χ^2=36.91; [c]P<0. 05- χ^2=3.95; [d]P<0.001- χ^2=23.55;[e]P<0.001- χ^2=22.79

the flock 3 after higher dose of eCG (1000 IU).

Findings summarised in table 3 – number of twin parturitions, indicate that assisted oestrus increases occurrence of parturition of 2 lambs and facilitates an ovulation rate (OR).

DISCUSSION

Based on the results of verifying operational observations with assisted fertile oestrus better and comparable results were obtained than those in the control flock 1, raised using traditional technology of the Carpathian system under the same conditions of breeding. The number of lambs born per a hundred lambed ewes in the fertility analysis and evaluation represents a natality expressed in a percentage that is limited by the occurrence of twins, or multiple litters (Mucsi et al., 1998; Ptaszynska, 2002; Maraček et al., 2004, 2005). This is also supported by the results obtained from an assisted oestrus.

Management of twin parturitions is a promising reserve. Capability of double or multiple ovulation is genetically determined, e.g. dominantly and autosomally to the Booroola (FecB) gene bound breeding characteristics, which is at present well detectable by molecular-biological methods (Montgomery et al., 2001; Mulsant et al., 2001; Novotni-Dankó et al. 2002). The occurrence of the ewes with two or more ovulating follicles in a flock (OR) also depends on a body condition score (BCS) and, to a lower extent, on the actual state of the energy metabolic balance (Wilkins, 1997).

The effect of flushing is multi-factorial: besides an increase in the glucose metabolism in GnRH-producing neurons it increases the aromatase activity of follicular granulosa cells of the antral ovarian follicles. Increased intra-follicular IGF-I concentrations, as well as an increase in biological availability of IGF-1, were recorded (Maraček et al., 2004; Vlčková et al., 2006). The increased number of ovulation (OR) can also be reached using the preparations with FSH effect, such as eCG after i.m. administration (Maraček et al., 2004; 2005), or by the treatment with androgens, that are products of the internal follicular theca as an androgen offer for the system of estrogens, which increases OR.

The treatment according to the Ovsynch protocol can induce fertile oestrus in sheep (Evans et al., 2004; Davis, 2004). Deligianis et al. (2005) verified a success of this method in sheep (n=48). The first treatment with GnRH (day 0) induced the onset of ovulation and the development of corpus luteum or intra-follicular luteinization. On day 5, administration of $PGF_{2\alpha}$ caused a luteolysis and 48 hours after its application repeated administration of GnRH induced ovulation. After 36–62 hours from the second GnRH injection the ewes were intra-uterinely inseminated with fresh diluted semen by the laparoscopic technique. The half of animals (n=24) was pregnant.

This finding supports our results: 53 % of ewes after assisted oestrus lambed according to the Ovsynch protocol. Our finding is noteworthy that after assisted oestrus using the innovated preparation on the basis of fluorogestonacetate (FGA, cronolon) there is a tendency of higher occurrence of ram lambs, whilst after application of the Ovsynch protocol occurrence of ewe lambs is a significantly higher than it was observed in the flock with a traditional system of breeding.

CONCLUSION

Our results reveal the potential of a wider application of verifying methods of assisted oestrus for optimization of the quality and weight of slaughtered lambs for the market in required time in balanced turnovers with subsequent production of sheep milk with positive economical effect. Differences in the results are compared after using the innovated methods on the basis of a new preparation since 2007, as well as using the procedure according to the OvSynch protocol, that are favourable regarding the environmental and ecological aspects. Very interesting was the finding that after assisted oestrus using the innovated preparation on the basis of fluorogestonacetate (FGA, cronolon) there is a tendency of higher occurrence of ram lambs, whilst after application of OvSynch protocol there is a significantly higher occurrence of ewe lambs than it was observed in the flock with traditional system of breeding.

REFERENCES

BARTLEWSKI, P. M. 2001. The relationships between ovarian antral follicle dynamics, luteal function and endocrine variables in ewes. A thesis submitted to the College of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of philosophy, University of Saskatchewan, Saskatoon, Canada, 2001, 270 p.

BARTLEWSKI, P. M. – BEARD, A. P. – COOK, S. J. – CHANDOLAI, R. K. – HONARAMOOZ, A. – RAVLINGS, N. C. 1999. Ovarian antral follicular dynamics and their relationships with endocrine variables throughout the estrous cycle in breeds of sheep differing in prolificacy. *J. Reprod. Fert.,* 115, 1999, p. 111-124.

DAVIS, G. H. 2004. Fecundity genes in sheep. *Anim. Reprod. Sci.,* 82-83, 2004, p. 247-254.

DELIGIANNIS, C. – VALASI, I. – REKKAS, C. A. – GOULAS, P. – THEODOLOSIAU, E. – LAINAS, T. – AMIRIDIS, G. S. 2005. Synchronization of ovulation and fixed time intrauterine inseminations in ewes. *Reprod. Dom. Anim.* vol. 40, 2005, p. 6-10.

EVANS, A. C. – DUFFY, P. – CROSBY, T. F. – HAWKEN, P. A. – BOLAND, M. P. – BEARD, A. P. 2004. Effect of ram exposure at the end of progestagen treatment on oestrus

synchronization and fertility during the breeding season in ewes. *Anim. Reprod. Sci.,* vol. 84, 2004, p. 349-358.

GAMČÍK, P. – HARTWIG, W. 1988. Veterinárno – chovateľská kontrola reprodukcie oviec. In: P. GAMČÍK, W. BUSCH, E. KUDLÁČ*: Veterinárno – chovateľská kontrola reprodukcie úžitkových zvierat.* Bratislava : Príroda, 1988, p. 209-262. 6513 – SÚKK 121/I-88

MARAČEK, I. – HENDRICHOVSKÝ, V. – KRAJNIČÁKOVÁ, M. 1995. Súčasné problémy reprodukcie oviec v podmienkach Slovenska. *Slov. veter. čas.*, vol. 20, 1995, p. 60-66.

MARAČEK, I. – VLČKOVÁ, R. – DANKO, J. 2004. Effectiveness of biotechnical reproduction control program in dairy ewes of improved Wallachian sheep. In: Proc. of 5th Middle-European Buiatrics Congress *"The effect of herd health of cattle, sheep and goat production efficiency"*, Hajdúszoboszló : A/3 Nyomdaipari és Kiadói szolgáltató Kft. Budapest, 2004, p. 739-743. ISBN 963 216 200 5

MARAČEK, I. – KRAJNIČÁKOVÁ, M. – KOSTECKÝ, M. 2005. Biotechnical control of oestrus in Slovak Tsigaya sheep during anoestrus phase of reproductive cycle. *Agriculture (Poľnohospodárstvo),* vol. 51, 2005, p. 185-192.

MARAČEK, I. – VLČKOVÁ, R. – SOPKOVÁ, D. – JANKUROVÁ, J. – KLAPÁČOVÁ, K. – ŠVANTNER, R. 2009. Efekt asistovaného estru a ovulácie v príprave stáda dojných oviec pre produkciu jatočných mliečnych jahniat na skorý veľkonočný trh. *Slov. Vet. Čas.*, vol. 34, no. 2, p. 96-99.

MONTGOMERY, G.W. – GALLOWAY, S. M. – DAVIS, G. H. – MCNATTY, K. P. 2001. Ganes controlling ovulation rate in sheep. *Reproduction*, 121, p. 843-852.

MUCSI, I. 1997. *Juhtenyésztés és tartás.* 2nd ed., Budapest : Mezőgazda. Kiadó, 1997. p. 411. ISBN 963 9121. 24 X

MULSANT, P. – LECERF, F. – FABRE, S. – SCHILBER, L. – MONGET, P. – LANNELUC, I. – PISSELET, C. –RIQUET, J. – MONNIAUX, D. – CALLEBAUT, I. – CRIBIU, E. – THIMONIER, J. – TEYSSIER, J. – BODIN, L. – COGNIE, Y. – CHITOUR, N. – ELSEN, J. M. 2001. Mutation in bone morphogenic protein receptor-IB is associated with increase ovulation rate in Booroola Merino ewes. *Proc. Natl. Acad. Sci. USA*. vol. 98. no. 9, 2001, p. 5104-5109.

NOVOTNI-DANKÓ, G. – KULCSÁR, M. – MAGYAR, K. – NIKOLIC, A. J. – KÁTAI. L. – DOMBÓVÁRI, E. – HUSYENICZA, Gy. 2002. Som metabolic aspects of ovarian activity in out-of-season Prolific Merinoewes. Allattenyésztés és takarmányozás. *Hung. J. Anim. Prod*., vol. 51, 2002, p. 79-84.

PTASZYNSKA, M. 2002: Ovine reproduction. In: *Ptaszynska, M. (Ed): Compendium of Animal Reproduction.* 5-th revised ed. Boxmeer, The Netherlands : Publ. Intervet International BV, 2002, p. 125-148. ISBN 90-801886 – 6 -2

VLČKOVÁ, R. – KOSTECKÁ, Z. – FAIGL, V. – MARTON, A. – KERESZTES, M. – ÁRNYASI, M. – KULCSÁR, M. – DANKÓ, G. – ŠVANTNER, R. – NAGY, S. – CSATÁRI, G. – CSEH, S. – SOLTI, L. – HUSZANICZA, G. – MARAČEK, I. 2006. Recent progress in endocrine, nutritional and genetic aspects of ovine reproduction. *Folia Veterinaria*, vol. 50, no. 3, 2006, p. 157-166.

WILKINS, J. F. 1997. Methods of stimulating ovulation rate in Merino ewes may affect conception but not embryo survival. *Anim. Reprod. Sci.*, vol. 47, 1997, p. 31-41.