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APPLICABILITY OF ASSISTED REPRODUCTION TECHNIQUES IN CONTEMPORARY SMALL RUMINANT FARMING

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ABSTRACT

Sheep and goat breeding has long tradition in the Balkan countries, making it leading trade in animal husbandry. Dynamic changes in global agriculture production from traditional to industrial livestock technologies, has also impacted the small ruminant farming systems. The reproduction management is considered to be a crucial point for good farming practice among other animal husbandry factors (housing, nutrition, selection, healthcare, etc.). In the last few decades, our sheep and goat farming systems have introduced various assisted reproduction techniques, such as estrous and ovulation synchronization, laparoscopic intrauterine insemination, embryo production (MOET and IVF), semen cryoconservation, photoperiod manipulation etc. This article reviews the current novelties in this field, presenting worldwide scientific reports and our personal experiences in research and translation to everyday farm practice.

Ovine and caprine species have been considered as typical seasonal breeders, becoming sexually active as result of pineal gland and day-length alterations in late summer/early autumn. Lambing/kidding and milk production follow established seasonal patterns. In order for farmers to yield these productive traits in various seasons of the year, they are able to use different hormonal combination of progestagens, prostaglandins, exogenous gonadotropins (eCG) or "natural" methods: light control or exposure to a male after period of isolation ("ram/buck effect"). The hormonal treatment by vaginal sponges is applicable throughout the year, resulting in pregnancy and higher lambing rates compared to seasonal breeding (see review Dovenski and Gvozdic 2012).

The implementation of most recent Artificial Insemination techniques in small ruminants, suggests that the transcervical insemination success rates could be improved by intracornual deposition of semen (laparoscopy), bypassing the "cervical barrier". Our results indicate high pregnancy rates could be obtained by Intrauterine Laparascopic Insemination in sheep (45% out of season, 60% during the breeding season) and even higher in goats 70-80% (Dovenski at al. 2012). Some attempts for pharmacological relaxation of uterine cervix in sheep with unsatisfactory success have been also reported by Candappa et al (2009).

Survival rate of buck's spermatozoa has been dramatically improved by using the "egg-yolk free" extenders, based of soya lecithin as cryoprotectant. Conversely, ram semen cryopreservation did not make substantial progress in past decades, despite the great research work in testing of novel media supplemented by various antioxidants (oxidized glutathione, reduced glutathione, cysteine), and the peculiar in-vivo trials which has shown optimistic results.

Key words: assisted reproduction, sheep, goats, artificial insemination, estrous synchronization.

Introduction

The small ruminants are seasonally polyestrous animals and they show signs of sexual activity at regular intervals during the breeding season. The season of sexual activity in a moderate climate zone lasts from mid-summer to mid-winter, i.e. in the period when duration of the day light become shorter, therefore they are called "short day breeders". The whole process is dependent upon the excretion of melatonin from epiphysis during night time, which is under the influence of

genetic and environmental factors. The season of kidding and lambing lasts from January until early summer, with the most prominent period in spring when climate and nutrition conditions are optimal for surviving of offspring.

According to the study of Abecia et al. (2012), the economic impact may be influenced by several factors, such us the breeding models, reproduction management and reproductive efficiency at the farm. Apart of these basic factors, the author suggests several other means by which the economic outcomecould be altered,

- Providing continuous supply of markets with lamb meat throughout the year
- Providing a greater exploitation of facilities and equipment used for sheep farming
- Even distribution and utilization of the labour force during the year
- Reducing the risks of adverse conditions of production and products sales, which could appear in some period of the year
- Providing a stable source of income to producers throughout the year

The reproductive management on small ruminant farms is based on application ofhormonal and non-hormonal methods.

Hormonal methods for oestrus synchronization Applying progestagen impregnated vaginal sponges is the most common method which needs to be used in strictly defined protocols.

The protocol for goats involves the application and maintaining ofvaginal sponges for 11 days. Forty-eight hours prior to the sponge removal, the veterinarian should administer IM prostaglandin injection (PG) to ensure luteolysis of existing corpus luteum on the ovaries, as well as injection of equine chorionic gonadotropin (eCG), which will initiate the final follicular development and ovulation (Gonzalez de Bulnes et al. 1999).

In sheep, the protocol takes 12–14 days and the eCG is applied on the day of the sponge removal. Dose of the eCG depends on the period of the year, the level of milk production and the parity of the animal (nulliparous or multiparous) (350–600 IU).

The heat detection should be performed 24–30h after the sponge removal and in goats insemination should be carried out around 43 h (36-47 h) after sponge removal (in sheep 55 h, 52 h – ewe lambs). The success of the method is around 70% of kidding/lambing. Goats in which oestrus was not positively detected 24–30 h after sponge removal, have fertility of only 30% after the AI. It should be noted that the occurrence of vaginal discharge following sponge removal does not adversely affect the results.

If this protocol is frequently used, it becomes less and less effective because of the creation of eCG antibodies that reduce the effectiveness of the protocol and the female does not ovulate at the expected time. Furthermore, the European Union regulative is becoming more strict regarding maximum allowed levels of hormone residues (MRLs) in milk (96/22/EC) (Grizelj et al. 2008).

Non-hormonal methods are becoming more interesting for modern sheep and goat breeders, especially when farmers desire to follow modern market trends for organic food production and increasingly stringent legislation imposed by the European Commission. These methods include the "photoperiodic treatments" and the "male effect".

By applying the photoperiodic treatments (manipulation of the day length, aiming to change the period of sexual activity), it is possible to control the sexual activity of sheep and goats and cause the occurrence of sexual activity during the non-breeding season (spring) or transition period (so-called "breeding season advancement").

The "buck effect" is a phenomenon based on the fact that females in the transitional period, could be provoked, synchronized and introduced into sexual activity by presenting males among

the group of receptive females, which have been previously isolated for 2 months. The males should be housed at the remote objects, at proper distance, thus preventing olfactory, visual and/or acoustic contact (total isolation). The deliberate introduction of male among the group of females (to assure permanent contact and ratio 1 male to 10 females) will result in synchronous appearance of fertile oestrus peaks in the goats 7–11 and then 27–35 days after introduction of the male. In sheep this effect appears 20–25 days following the introduction of the ram (Grizelj et al. 2014).

The "ram effect" has been studied and observed in a large number of breeds. However, the efficiency of the method looks to be influenced by the "depth" of anoestrus. Thus, the Merino sheep in which anoestrusis relatively shallow, reactbetter to the deliberate introduction of male then breeds with deepanoestrus. But, even within Merino breed all females will not ovulate after the introduction of the ram; the exact percentage could vary from 40–90% (Martin et al., 1983).

Responsiveness to the "ram effect" differs significantly among various breeds, depending on theseason in which the stimulation is performed. Merino sheep react at any time of the year, while the Romney breed react only if the stimulation is performed in the late anoestrus period. In our climatic conditions, the ram effect during the early anoestrus (February to May) has better response in crossbred Tsigai x Wurttenberg (27.1% in oestrus) than in Tsigai breed (13.3%) (Sahinovic and Stancic, 1991).

A large percentage of corpora lutea formed by oestrus synchronization and "male effect" introduction may be insufficiently functional and could regress within the first 6 days (up to 50%). This effect can be avoided by single dose injection of progesterone (20 mg) prior to introducing of the males among the females.

Flock synchronization with non-hormonal methods is most commonly performed by combining the afore mentioned methods. After submitting the animals (males and females) to the long-daylight period (70–90 d, 16 h light/day), a short-day-light treatment needs to be applied (8–12 h of continuous lighting or melatonin subcutaneous implant application). During this time males should be isolated from females. Sixty days after the "short daylight treatment", the introduction of males among females is performed. This introduction among the receptive females will provoke synchronous appearance of fertile estruses.

Artificial Insemination

Crucially important method in assisted reproduction is Artificial Insemination (AI). It is usually applied in selection programs for farm animals. In sheep satisfactory results were not obtained by using transcervical insemination, especially during non-breeding season and using deep frozen-thawed semen. Low fertilization rates are probably due to difficult transport of spermatozoathrough the cervix (Boland et al., 1983). However, this difficulty could be resolved by using laparoscopic intra-uterine AI, where semen is deposited directly into the uterine horns (Dovenski et al., 2012). Significantly higher pregnancy and lambing rates are achieved by IUAI in compared to the intracervical AI (80.95% vs. 67.48% in goats; 61.36% vs. 45.24% in sheep).

Artificial insemination is a routine practice among goat breeders in some countries (out of 1.300.000 goats in France 400.000 are under AI program). The cervix does not present a major barrier during the semen deposition in goats, so the use of straws with frozen semen is also possible. Freezing resistance of buck spermatozoa is much higher than the rams' spermatozoa. However, prior to dilution with Tris-egg-yolk extender, the spermatozoa should be washed with a sodium Ringer lactate solution and subjected to the centrifugation process for removal of the seminal

plasma (Mukul et al., 2017). This procedure is necessary to prevent reaction of Egg yolk with some bulbourethral gland's glycoproteins that have triacylglycerol hydrolase activity, resulting in decreased sperm motility and disruption of the cell membrane (Pellicer-Rubio and Combarnous, 1998). Recently, Chelucci et al. (2015) reported that soy-lecithin can be a suitable alternative to egg yolk extender for goat semen cryopreservation, due to its cryoprotective effects on the sperm membrane which obtains higher fertilization rates after AI.

Results of induction and synchronization of oestrus in a large sheep flock

Application of hormonal reproduction control method by induction and synchronization of oestrus outside the breeding season, has yielded significant results on a large size farm in Macedonia (>5000 sheep). Induction and synchronization of oestrus was performed byapplying intravaginal sponges impregnated with a synthetic progestagen. They were removed12 days following the application, and an eCG dose of 500 IU/sheep was applied. Table 1. shows the results of induction and synchronization of oestrus during 2008.

Table 1: Results of the implementation of the hormonal control of reproduction through the induction and synchronization of oestrus outside breeding season on a large farm in the Republic of Macedonia in 2008 (Dovenski and Gvozdic, 2012).

Date of sponge application	Inserted sponges Sheep No	Removed sponges d. 12.		Pregnancy rate		No of lambs	Lambing rate	Twining	Dead born		Abortus	
	N	N	%	N	%	N	%	%	N	%	N	%
25.Jan.	300	298	99.33	225	75.50	326	144.89	33.89	4	1.3	10	3.4
09.Feb.	300	297	99.00	218	73.40	329	150.92	37.37	3	1.0	11	3.7
08.March	300	300	100.00	209	69.67	354	169.38	48.33	3	1.0	8	2.7
04.May	300	300	100.00	244	81.33	345	141.39	33.67	5	1.7	12	4.0
05.0 May	300	300	100.00	232	77.33	369	159.05	45.67	2	0.7	5	1.7
12. May	300	299	99.67	251	83.95	407	162.15	52.17	0	0	4	1.3
13. May	300	298	99.33	233	78.19	372	159.66	46.64	4	1.3	10	3.4
20. May	300	300	100.00	247	82.33	387	156.68	46.67	4	1.3	7	2.3
Total	**2400**	**2392**	**99.67**	**1859**	**77.71**	**2889**	**155.51**	**43.06**	**3.1**	**1.1**	**8.38**	**2.8**
Jan.-March	**900**	**895**	**-**	**652**	**72.86**	**1009**	**155.06**	**-**	**-**	**-**	**-**	**-**
May	**1500**	**1497**	**-**	**1207**	**80.63**	**1880**	**155.79**	**-**	**-**	**-**	**-**	**-**
Breeding season	**5120**	**5120**	**-**	**4528**	**88.44**	**5343**	**104.36**	**15.92**	**38**	**0.7**	**77**	**1.5**

Out of 2.400 ewes included in the program of oestrus induction and synchronisation, out of the breeding season, 1.500 ewes have been included in May, 2008, whereas 900 ewes were included in the period between January and March, 2008. Average lambing rate in sheep synchronized outside of the breeding season has beenhigher (155%) then in ewes during the breeding season (104%), although pregnancy rate has been insignificantly lower (77.7% vs. 88.4% respectively).

Results of the systematic application of hormonal reproduction control on the farm during the period 2008–2011 are shown in Table 2. These data indicate that the systematic implementation of this method on a farm with over 5.000 sheep could yield more than 7.900 lambs per year, which in 4-years period of continuous trend, can reach up to 31.000 lambs. Probably the best indicators of the success are the in-season and out-of-season-breeding lambing rates which range 118–137% and 155–162%, respectively. Induction and synchronization of oestrus outside the breeding season

promote the occurrence of twinning in sheep; the percentage of twinning outside the breeding season varied between 43–48%, while in the breeding season the twinning rate was significantly lower (16–21%).

Table 2: Results of the systematic implementation of the hormonal control of reproduction through the induction and synchronization of oestrus outside breeding season on a large farm in the Republic of Macedonia during the period 2008–2011 (Dovenski and Gvozdic, 2012).

Year	Season	Sponges Sheep No	Removed sponges d. 12.		Pregnancy rate		No of lambs	Lambing rate	Twining	Dead born	Abortus
		N	N	%	N	%	N	%	%	%	%
2008	Non-breeding	2400	2392	99.7	1859	77.7	2889	155	43	1.0	2.8
	Breeding	-	5120	-	4528	88.4	5343	118	16	0.7	1.5
2009	Non-breeding	1800	1797	99.8	1385	77.1	2256	162	48	1.1	2.3
	Breeding	-	4578	-	4138	90.4	5109	123	21	0.5	1.2
2010	Non-breeding	1500	1495	99.7	1195	79.9	1867	156	45	0.9	2.1
	Breeding	-	4835	-	4312	89.2	5139	119	17	0.7	1.3
2011	Non-breeding	1800	1800	100.00	1390	77.2	2251	162	48	0.8	2.2
	Breeding	-	4356	-	3561	81.8	4892	137	-	0.5	1.3

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