MendelNet

Conference Brno 2021

Editors:

Radim Cerkal

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Lenka Prokešová

Proceedings of 28th International PhD Students Conference

10 November 2021, Brno, Czech Republic

Mendel University in Brno
Faculty of AgriSciences

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PREFACE

Each year, the editors of the volume you are about to read are tasked with the responsibility of putting a coherent form to the proceedings from MendelNet, the international PhD Students Conference of the Faculty of AgriSciences of Mendel University in Brno.

The event which reached, this year, on November 10, 2021, its 28th edition, is traditionally aimed at both under and postgraduate students from the Czech Republic, Europe and beyond, and proudly welcomes the participants of various professional and cultural backgrounds. And while this time the people could not gather on-site due to globally-imposed covid-19 restrictions, the conference swiftly transformed itself into a virtual and fascinating beehive of results, opinions and brand new research paths and ideas.

Here in Brno, under the spell of great genetician G. J. Mendel and the guidance of skilled senior researchers and supervisors, students can introduce, defend and discuss their scientific results while those who do not feel confident enough to present and pen their paper in English are invited to join as spectators and follow-up discussion participants.

The best submissions are, after rigorous peer-review process, collected here and range from plant and animal production to fisheries and hydrobiology to wildlife research while agroecology and rural development, food technology, plant and animal biology, techniques and technology and applied chemistry and biochemistry also belong to the core areas being investigated.

The collection as varied and huge as this can succeed only as a team effort, both on authors' and editors' side, so we would like to express our thanks and gratitude to all committees and reviewers both for their outstanding work and invaluable comments and advice.

The Editors

TABLE OF CONTENTS

PLANT PRODUCTION

ANIMAL PRODUCTION

FISHERIES AND HYDROBIOLOGY

WILDLIFE RESEARCH

AGROECOLOGY AND RURAL DEVELOPMENT

FOOD TECHNOLOGY

PLANT BIOLOGY

ANIMAL BIOLOGY

TECHNIQUES AND TECHNOLOGY

APPLIED CHEMISTRY AND BIOCHEMISTRY

Determination of optimal insemination time in sheep by assessing cervical mucus arborization

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Abstract: Insemination and estrus synchronization are one of the most used biotechnological reproductive methods used in sheep breeding, but also in most other livestock species. Subsequent insemination values are influenced by several factors such as age, condition, health, hereditary establishment of the animals, quality of the insemination doses and the quality of the performance of the insemination itself. Another way to optimise the insemination values is to determine the optimal time for insemination, which varies between livestock species but may also vary slightly within the individuality of the individuals within the breed. One way of determining the optimal time for insemination is to assess the arborisation of cervical mucus, which forms different types of structures after crystallisation at different stages of estrus. Changes in the arborisation structures are influenced by endocrine changes, by the action of oestrogen on the cervical glands, where electrolytes are concentrated and which, after association with mucin and subsequent crystallisation, form typical structures. The experiment took place from September 2020 (synchronization of estrus and insemination) to March 2021 (end of lambing). In this experiment, it was found that the type of crystallization structures at the time of insemination has a conclusive effect on the subsequent pregnancy rate. Animals with "V" type crystallization achieved the best pregnancy rate (80%).

Key Words: arborization, cervical mucus, insemination, sheep, Zwartbles

INTRODUCTION

Small ruminants are generally seasonally polyestrous animals. Sexual activity is influenced by the length of the light day and its shortening affects the onset of the reproductive season. In tropical animals, reproductive activity is not affected by seasonal variation and estrus can occur throughout the calendar year. The methods used to synchronize estrus or induce estrus depend on the season: ither breeding season, transitional period and nonbreeding season (Romano 2021).

Synchronisation of estrus (whether using natural or artificial methods) is used mainly to save time, reduce labour intensity and the associated economic demands of breeding, because of more accurate recording of animals, facilitating the assessment of health status and the possibility of scheduling births into a targeted period, which is associated, for example, with greater seasonal sales of lambs or dairy or meat products. The synchronisation of estrus results in turntable lambing, which allows group weaning and subsequent rearing in larger and more balanced groups of lambs (Štolc et al. 2007, Whitley and Jackson 2004).

Thanks to the subsequent breeding of the sheep (natural breeding, insemination or a combination of both, for example by forming harems after insemination) we are able to select the best males, shorten the period of breeding and know the approximate date of birth, thanks to the synchronisation of estrus, we can situate it in a suitable period to be able to produce lambs at Easter and Christmas, when demand is highest, or to focus on off-season production of lambs for slaughter (Kuchtík et al., 2007; Štolc et al., 2007).

After the synchronization of estrus, the choice of the method of insemination is important. Due to the lower labour, time and economic requirements (compared to the laparoscopic method) and the good results of insemination, which are around 60%, vaginal insemination with vaginal speculum and intracervical deposition is mainly used (Kuchtík et al. 2007). Due to the very complex structure of the cervical septum, intracervical deposition of the insemination dose is only possible to a depth of one to three centimetres (Leethongdee 2010). The success rate of insemination increases significantly with greater depth of penetration. However, this is influenced by cervical openness, breed affiliation and age of the ewe (Eppleston and Maxwell 1995). (Eppleston and Maxwell (1995) reported that, on average, it is possible to insert the insemination pipette to a cervical depth of 1 cm in 31% of animals, 2 cm in 31% of animals, 3 cm in 30% of animals and only 8% of inseminated animals to a depth exceeding 4 cm.

Therefore, determining the optimal stage of estrus and the optimal time of insemination is essential for insemination. The activity of the females, changes in the genital organs (swelling, redness, quantity and quality of cervical mucus, openness of the cervix, etc.) are monitored.

The average length of an estrus cycle is 17 days and estrus lasts 24 to 36 hours in ewes. Ovulation occurs towards the end of estrus (24 to 27 hours after the start of estrus). The ovulation rate is between 1 and 3 eggs per cycle and increases with age, peaking between 3 and 6 years of age and then gradually decreasing. After ovulation, the egg is capable of fertilization for 10 to 25 hours (Gimenez and Rodning 2007). Čunát et al. (2013) state that in ewes with natural onset of estrus, insemination is best performed 12 to 18 hours after the onset of estrus and in ewes synchronized with intravaginal sponges 50 to 60 hours after removal.

One of the methods of determining the optimal insemination time is to assess the arborization (crystallization) of cervical mucus, which forms different types of structures after crystallization in the different stages of estrus. Changes in arborization structures are influenced by endocrine changes, namely the action of estrogen on the cervical glands/epithelium, where electrolytes are concentrated and form typical structures after association with mucin and subsequent crystallization (Cortés et al. 2014).

Several authors have addressed this issue in cattle. There are not many publications with results of arborization evaluation in sheep (or goats), especially studies related directly to the determination of the appropriate time of admission/insemination. Recent studies include, for example, the evaluation of cervical mucus in goats – Fonseca et al. (2017), Maddison et al. (2017), or the evaluation of crystallization forms from saliva smears in sheep – Gonçalves et al. (2020).

MATERIAL AND METHODS

A total of 51 Zwartbles sheep were monitored in this experiment. The age of the sheep ranged from 2 to 8 years with a BCS (body condition score) of 3. The sheep were housed on the family farm of Mr. Ing. Martin Hošek Ph.D. in Mohelno. The experiment itself was conducted from September 2020, when estrus synchronization was performed using intravaginal sponges, until March 2021, when the lambing of the group of animals under study ended. Before the actual insemination (mid-October 2020), the collection of the rams' ejaculate, its evaluation and dilution were carried out. Pregnancy diagnosis was carried out in mid-December 2020.

Inseminated ewes and breeding rams used for ejaculate collection and insemination doses were fed on silage from stale forage and hay ad libitum. As flushing, 200 g of fodder potatoes and 400 g of cereals per head were used one month before insemination and one and a half months after insemination. Flushing was mainly used to modify the condition of the animals, improve ejaculate quality, accentuate estrus symptoms and improve pregnancy rates.

To synchronise the estrus, which took place at the end of September and the beginning of October 2020, intravaginal sponges (tampons) (Ovigest - medroxyprogesterone acetate 60 mg, Laboratorios Hipra, Spain) were used, which were injected into the sheep vagina for 14 days. After their subsequent removal, sheep were injected intramuscularly with lyophilized serum gonadotropin (PMSG – 0.2 ml/sheep = Sergon 200 IU, Bioveta Ivanovice, CZ).

Ejaculate was collected from rams into an artificial vagina on the day of insemination (Minitübe, Germany) after the ejection on an ewe in heat (fixed on a fixation pad). In case of a smaller quantity of collected ejaculate, a second jump was performed. The ejaculate was then macroscopically and microscopically evaluated and diluted to the required amount according to sperm concentration and motility. The diluted ejaculate ready for insemination was stored in plastic containers cooled to 3 °C in a refrigerated box until the time of insemination.

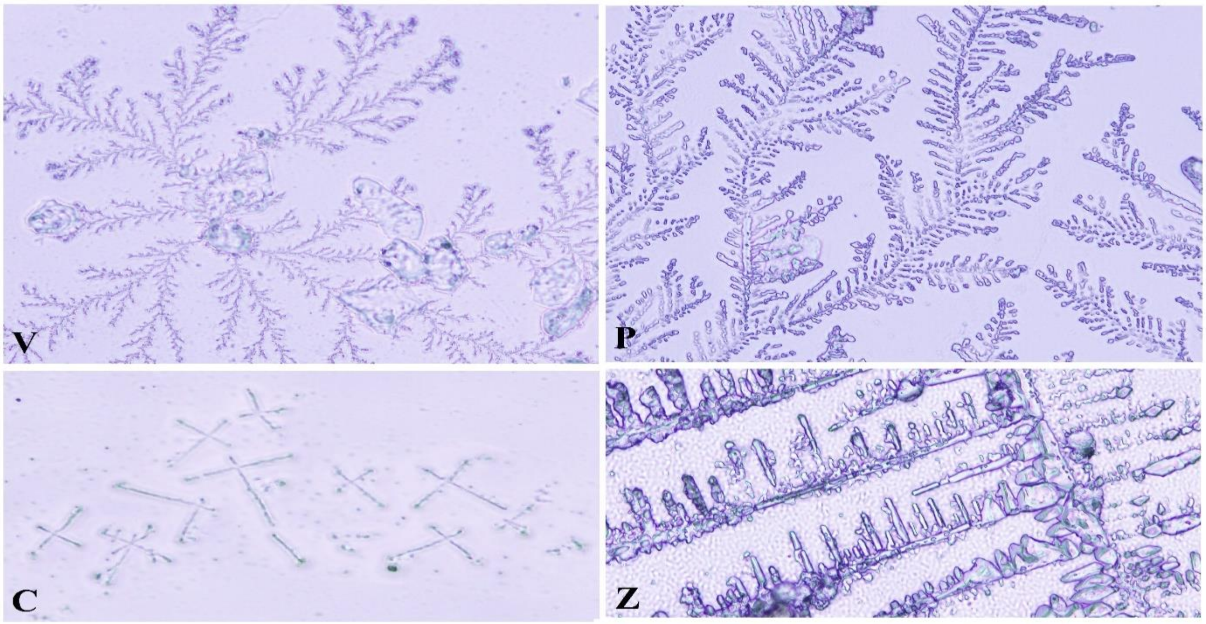
For each inseminated ewe, several parameters (colour and amount of cervical mucus, cervical openness, genital blood supply and behavioural activity – willingness to mate) were monitored before insemination and a cervical mucus sample was taken for arborization examination. Prior to insemination, the external genitalia were cleaned and disinfected, and the vagina was inspected using an ovine vaginal speculum. Each insemination dose of cooled dilute ejaculate was injected in a quantity of 0.4 ml through the vaginal speculum using a plastic loader into the cervix to a depth of approximately 1 to 2 centimetres.

The cervical mucus sample was collected using a plastic loader through the vaginal speculum before insemination. This sample was transferred to a microscope slide immediately after collection and then smeared at an angle of 45 °. Once the cervical mucus had sufficiently dried on the slide, it was labelled (date and time of collection, ewe number) and placed in a plastic box. The smears were then examined microscopically (Olympus BX51TF; Olympus Optical CO., LTD, Tokyo, Japan). At 200× magnification and their crystallization type were evaluated (examples of crystallization types in Figure 1). The procedure of smear formation, their evaluation and crystallization types were determined according to standard methodologies for assessing cervical mucus arborization in cattle (Cortés et al. 2014, Stádník et al. 2013). The individual samples were divided into different groups according to the type of crystallization (V: twigg-shaped, V + P: twigg-shaped-clubmosses, P: clubmosses, P + K: clubmosses-fern frond, K: fern frond, Z: swollen, C: cellularization).

Sequence of crystallization types during estrus is: Type "V" (pre-estrus period), type "V+P", type "P" (beginning of estrus), type "P+K" (half of estrus), type "K" (second half of estrus). The undesirable structures include type "Z" (swollen crystallization - post-estrus period) and "C" (cellularization - occurring during inflammation) (Stádník et al. 2013).

Type "V" is characterised by fine structures that resemble twigs, type "P" is characterised by branching structures that resemble plants of the genus Lycopodium (clubmosses), type "K" is characterised by a shape that resembles palm leaves or fern frond. The swollen crystallisation of type "Z" produces enlarged branched crystals. Type "C" crystallizes into simple forms composed of several short segments.

Figure 1 Examples of crystallization types



In Figure 1 are the examples of crystallization types: type V (twigg-shaped: pre-estrus), type P (clubmosses: beginning of estrus), type C (cellularization: occurring during inflammation) and type Z (swollen: post-estrus).

Preliminarily, the value of pregnancy rate was determined during pregnancy diagnosis and control, which took place on days 43 and 83 after insemination using OVI-SCAN ultrasound (BCF technology, Scotland. The exact value of the pregnancy rate was determined by recording the date and time of insemination and insemination. Subsequently, trimming values were compared with each type of cervical mucus crystallisation: post-estrus period).

STATISTICA 12.0 was used for statistical evaluation of the results.

RESULTS AND DISCUSSION

The cervical mucus samples were divided into several groups according to the type of crystallization. These types of crystallization structures change during estrus.

Table 1 Total frequency of crystallization structures

Type of crystallisation	Number of samples (n)	Frequency (%)	Sx	Vx
V	15	29.4	10.05181023	0.66067239
V+P	16	31.4		
P	7	13.7		
P+K	6	11.8		
Z	3	5.9		
C	4	7.8		

Legend: Sx – Standard deviation, Vx – Coefficient of variation

Table 1 shows the frequency of the individual crystallization structures. Most of the inseminated sheep (60.8%) had "V" type crystallization (29.4%) and a mixed form of "V"+"P" type crystallization (31.4%). In 25.5% of inseminated sheep, "P" and mixed forms of "P"+"K" crystallization occurred. Crystallisation of the "K" type did not occur in the animals studied. In 5.9% of the inseminated sheep, a swollen crystallization of the "Z" type occurred, and in 7.8% of the animals, a crystallization of the "C" type occurred.

Table 2 Influence of crystallization type on pregnancy rate

Type of crystallisation	Number of samples (n)	Number of pregnant sheep (n)	Pregnancy rate (%)
V	15	12	80.0
V+P	16	7	43.8
P	7	4	57.1
P+K	6	3	50.0
Z	3	2	66.7
C	4	0	0.0
			P = 0.002858
∑	51	28	54.9

Legend: $P < 0.01$ = high statistically significat differences

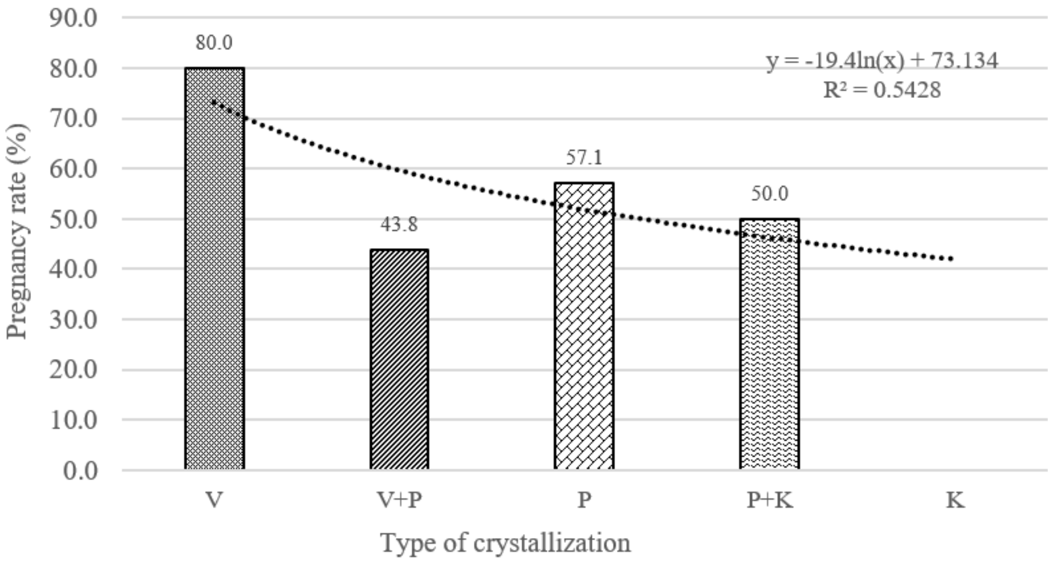
According to Stádník et al. (2013), the optimal form of crystallization structures (suitable time for insemination) is the "P"+"K" and "K" type, which occurs in cattle in the second half of estrus. According to the results shown in Table 1, it can be assumed that most of the inseminated animals were inseminated prematurely around the beginning of estrus (type "V", "V"+"P", "P").

Table 2 and Figure 2 show the effect of crystallization types on the pregnancy rate. The highest value of pregnancy (80.0%) was found in animals that had a "V" type crystallization pattern. For the "V+P", "P" and "P+K" types, a total of 48.3% (14 pregnancies out of 29 inseminated) were crystallized. The type of crystallization structures "V", "V"+"P", "P" and "P"+"K" had a highly statistically significant effect ($P = 0.002858$) on the pregnancy rates.

In cattle, the highest values of pregnancy at insemination are reached at the time of crystallization structures "P" to "K". On the contrary, the lowest values of pregnancy are achieved by animals with atypical crystallization (Ježková et al. 2007, Ježková et al. 2008, Stádník et al. 2013).

The value of pregnancy in inseminated animals with "Z" type crystallization was 66.7%. As this is a structure occurring in cattle at the end of estrus/postpartum, the higher values (compared to similar observations - see Stádník et al. (2013): 20.0%) are probably due to the low number of animals with this type of crystallisation. The lowest values of pregnancy were found for crystallization type "C" (0.0%), which occurs in inflammation.

Figure 2 Influence of crystallization type on pregnancy rate



The overall rate of pregnancy rate after insemination was 54.9% (28 pregnancies out of 51 inseminated). This is below average, compared to the average of 60% reported by Kuchtík et al. (2007).

CONCLUSION

In this research, it was found that the type of crystallization of cervical mucus at the time of insemination had a conclusive effect on the subsequent pregnancy values. Animals with type "V" crystallization had the highest values of pregnancy and animals with atypical type "C" crystallization had the lowest values.

The most common types of crystallization in the animals studied were the "V" type and the mixed "V"+"P" form. These forms occur in cattle in the pre-estrus and early estrus. In relation to the temporal sequence of changes in crystallization patterns reported in cattle, these values are opposite, with a decreasing trend in the values of pregnancy.

The resulting values for the different types of crystallisations differ from those reported for cattle. To obtain more accurate results, it is necessary to repeat the experiment on a larger number of animals and to determine at what times during estrus the changes in each type of crystallisation occur and, in particular, whether the sequence of changes in crystallisation structures during estrus is identical to the results reported for cattle.

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