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Lambing rate as a function of artificial insemination depth in ewe lambs, primiparous and multiparous ewes

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ABSTRACT

The objective of this study was to survey the depth of penetration of a helicoid type catheter for cervico-uterinal insemination in breeding season.
This study evaluated:
- *the differences in the penetration depths of the catheter in ewe lambs, primiparous and multiparous ewes.*
- *the differences in the penetration depths between the morning and afternoon inseminations.*
- *the differences in the penetration depths between the ewes that lambed and ewes that failed to lamb after AI.*
- *relationship between the depth of semen deposit with this catheter and the lambing rate.*

Lacaune ewe lambs (n=204), primiparous ewes (n=215) and multiparous ewes (n=227) were artificially inseminated with 2 to 4ºC cooled, diluted, short-time preserved ram semen. Depth of penetration examinations was conducted with modified Milovanov catheter. Depth of penetration of the catheter into the cervix was different (P<0.01) in ewe lambs, primiparous and multiparous ewes. Penetration depth differed significantly (P<0.01) between ewes that lambed and failed to lamb. There was a statistically significant positive correlation between insemination depth and lambing rate. Nevertheless, there were no significant differences in penetration depth between the morning and afternoon inseminations. Analysis of the data indicated that adequate fertility results could be obtained with deep cervical deposit of 2 to 4ºC cooled, diluted ram semen.
(Keywords: sheep, artificial insemination, transcervical, penetration depth, lambing rate)

ÖSSZEFOGLALÁS

A termékenyítési mélység és az ellési arány közötti összefüggés vizsgálata jerkék, egyszer és többször ellett anyajuhokban

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Cervicouterinális inszeminálások során egy helikoid típusú katéter cervixbe vezethetőségének mértékét vizsgáltuk juhokban, a tenyésztési szezonban. A kísérletek során
- *a katéter cervixbe vezethetőségének mértékét jerkékben, egyszer és többször ellett anyajuhokban,*
- *a behatolási mélységben adódó különbségeket a délelőtti és a délutáni termékenyítések során,*

– *a termékenyítési mélységet az ellett és nem ellett anyákban,*
– *a termékenyítési mélység és az ellési arány közötti összefüggést vizsgáltuk.*

204 lacaune jerkét, 215 egyszer és 227 többször elett anyajuhot termékenyítettünk 2-4ºC-ra hűtött, hígított, rövid időre tartósított kosspermával. A termékenyítéseket módosított Milovanov-féle katéterrel végeztük. A katéter cervixbe vezethetőségének mértéke jerkék, egyszer, illetve többször ellett anyák között szignifikánsan eltért (P<0,01). Ugyancsak szignifikánsan különbözött (P<0,01) a termékenyítési mélység az ellett és nem ellett állatok között. A délelőtti és a délutáni termékenyítések során kapott behatolási mélységek között azonban nem kaptunk szignifikáns eltérést. Az adatok elemzése alapján megállapítható, hogy 2-4ºC-ra hűtött, hígított kossperma használatakor a megfelelő termékenyítési eredmények eléréséhez mélycervikális spermadeponációra kell törekedni.

(Kulcsszavak: juh, mesterséges termékenyítés, transzcervikális, termékenyítési mélység, ellési arány)

INTRODUCTION

The cervix of the ewes, which has been examined by numerous authors (*Dun,* 1955; *Stojanov,* 1980; *Kristinsson et al.,* 1985; *Reinhold et al.,* 1987; *Halbert et al.,* 1990a) is a major barrier when one wants to deposit semen into the uterus. The caudal part of the cervix is a little widened and mildly compressed in dorsoventral direction. It is approximately 6.7 cm in length and contains funnel-shaped rings. The average number of the rings is 4.9 and some of them take up places eccentrically. The narrowest part of the cervix is at the second and the third rings. The wider openings of the rings can be found in cranial direction so the cervix of the ewes becomes convoluted in structure. The anatomical structure of the cervix is not influenced significantly with stage of estrous or age of the ewe. The results of the spontaneous motility examinations of the cervix have shown that the largest frequency can be measured during time of ovulation (*Garcia et al.,* 1982).

The inseminating devices varied from the simple, bent-tipped pipette (*Salamon et al.,* 1967) to the helicoid type (*Milovanov et al.,* 1978) and the semiflexible-ending (*Wulster-Radcliffe et al.,* 2002) catheters. The insemination techniques also had a great variety. Some authors called the attention to the risk of injury of the cervical canal during intrauterine insemination (*Fukui et al.,* 1977; *Campbell et al.,* 1996). Pulling out the entrance of the cervix into the vagina with surgical forceps increased the depth semen could be deposited. Semen could be deposited 2 to 5 cm deep into the cervix or transcervically (*Halbert et al.,* 1990b; *Windsor,* 1995; *Naqvi et al.,* 1998; *Cappai et al.,* 1998) with this technique. Some authors reported lower pregnancy rates when the semen was deposited into the mid-cervix (2.5 to 4 cm) rather than in the external opening of the cervical canal (*Andersen et al.,* 1973). *Paulenz e*t *al.* (2002) did not find significant differences in the non-return rate between cervical and vaginal inseminations. *Pau et al*. (1998) reported attempts to surgically remove some rings from the cervical canal and introduce the catheter into the uterus. Others gave hormones to dilate the cervical canal. Relaxin injected 12 hours before insemination had no relaxation effect on the cervix and did not influence the depth of semen deposit (*Salamon et al.,* 1967). *Croy et al*. (1999) obtained similar results after human interleukin 8 treatment because it was not sufficient to relax the cervix. Exogenous oxytocin dilated the lumen of cervix and made it possible to introduce the catheter into the uterus (*Khalifa et al.,* 1992; *Sayre et al.,* 1996; *Stellflug et al.,* 2001). In the review of *Salamon et al.* (1995), a great number of data could be found about the semen deposit depth results, which were done before 1995. It could be ascertained from these data that increasing the depth of semen deposit increased the pregnancy rate.

The aim of this study was to measure the depth of penetration of a special, spiral-ending catheter into the cervix in ewe lambs and ewes during the breeding season. It was examined whether any differences existed in the depth of penetration in sheep varying in parity (Trial 1). It was surveyed whether there were any differences in the penetration depth between the morning and afternoon insemination times (Trial 2). Comparisons were made in the depth of semen deposit between the ewes that lambed and the ewes that failed to lamb after AI group (Trial 3). A correlation between the depth of penetration of the catheter and lambing rate after AI was evaluated (Trial 4).

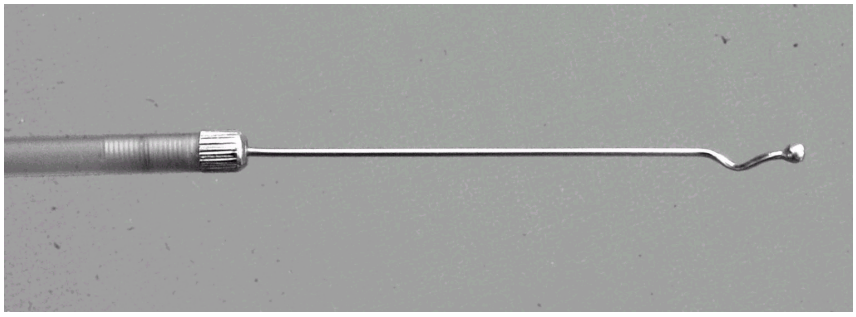
MATERIALS AND METHODS

The study was carried out in the northwest of Hungary in continental climate from September to November in 2000 and 2001. Lacaune and lacaune crossed ewes were analysed during the main estrous period. Ewe lambs from 18 to 30 months of age (n=204; Group A), primiparous ewes from 30 to 42 months of age (n=215; Group B) and multiparous ewes from 42 to 96 months of age (n=227; Group C) were used in this study. A total of 1292 depth of penetration examinations was completed.

In addition to native pasture, the animals were given meadow or lucerne hay (2 kg/ewe/day), concentrate (0.2 kg/ewe/day) and mineral salt ad lib. Temperate, automatic waterers provided water supply ad lib. Daily in the early AM hours, vasectomized teaser rams were used to check for signs of estrous. Ewes that exhibited a standing response were considered to be in estrous. After this selection, double AI was done at 09.00 and 15.00 with 2 to 4ºC cooled, diluted ram semen. Insemination dose was 0.2 to 0.3 cm^3 that contained 50 million live, motile spermatozoa. Degree of dilution was determined by colorimeter. Inseminations were done by modified Milovanov catheter (*Picture* 1).

Picture 1

Modified Milovanov catheter



1. kép: Módosított Milovanov katéter

The insemination device was a stainless steel needle, 80 mm long and had a winding end with a 3 mm bulb attached to the end. The steel edge was connected by threads to a transparent plastic tube, which was 250 mm long and 4 mm in diameter. A syringe was connected to the plastic tube to help dispense semen from the catheter.

Catheters were marked with coloured stripes at every 0.5 cm in order to determine the depth of semen deposit precisely. The vaginal speculum contained an incandescent

lamp to light the vagina and make it possible to read the colour stripes, which quantified the depth of deposit of semen.

The ewes were positioned for AI with the assistance of one or two co-workers. The animals were manually lifted to a stand and restrained. After cleaning the vulva, the inseminator introduced the vaginal speculum into the vagina then searched for the entrance of the cervix with the catheter and threaded it into the cervical canal with a turning motion. The next movement was to inject the semen and then remove the catheter and the vaginal speculum. After cleaning the insemination devices, the next ewe that was lifted into position could be inseminated. The whole insemination procedure required less than one minute for each ewe.

Statistical analysis

Analysis of variance was used for comparison of penetration depth values in each group in *Trial 1, 2*, and *3*. Penetration depth values were divide into eight stages (every stage indicated 0.5 cm increase in depth of insemination). The main effect in Trial 1 was groups of ewes related to parity. The main effects in Trial 2 were groups of ewes, time of day of insemination and the interaction. The main effects for Trial 3 were groups of ewes and depth of semen deposit and the interaction. Differences among groups were compared using F-test and two-sample t-test (significance at $P<0.01$) [26, 27]. In Trial 4 relationship between the penetration depths and the lambing rates was analysed using test correlation coefficient (*Sváb,* 1973; *Précsényi et al.,* 2000).

RESULTS

Trial 1: Penetration depths of the catheter in ewe lambs, primiparous and multiparous ewes

Table 1 shows the depth of penetration of the catheter in ewe lambs (Group A), primiparous ewes (Group B) and multiparous ewes (Group C). There were significant differences ($P<0.01$) in the penetration depth of the catheter among the three groups. The shortest penetration depth occurred in ewe lambs and increased gradually in primiparous and multiparous ewes. Twenty % of the ewe lambs had less than 1.0 cm penetration depth compared to only 3% in the primiparous and multiparous ewes. The catheter could not be introduced deeper than 4.0 cm in any ewe lambs.

The frequency data among the groups is depicted in *Figure 1* indicating that the depth of penetration is gradually increasing from ewe lambs (Group A) to primiparous (Group B) and multiparous ewes (Group C).

Trial 2: Surveying of penetration depth values in AM versus PM insemination

Penetration depths values of modified Milovanov catheter did not differ significantly between the morning and afternoon inseminations. The average results in AM vs PM inseminations were 2.79±0.10 vs 2.89±0.09 (1.5 to 2.0 cm penetration depth) in ewe lambs, 3.53±0.10 vs 3.74±0.11 (2.0 to 2.5 cm penetration depth) in primiparous ewes and 4.28±0.11 vs 4.37±0.11 (2.5 to 3.0 cm penetration depth) in multiparous ewes.

Trial 3: Comparisons of the penetration depth values between the ewes that lambed and the ewes that failed to lamb

Significant differences ($P<0.01$) were shown in the relation of the penetration depth values in the inseminated ewes that lambed and failed to lamb in each group (*Table* 2). Percentages indicated an increase in lambing for every 0.5 cm increase in depth of insemination. *Table 2* contains the total number of AM and PM inseminations

Table 1

Limits of penetration of modified Milovanov insemination catheter in ewe lambs, primiparous and multiparous ewes

Penetration depth (4)	Group A (1)		Group B (2)		Group C (3)	
	n	%	n	%	n	%
1 (<1cm)	82	20.1	14	3.3	14	3.1
2 (1-1.5 cm)	92	22.5	97	22.6	42	9.3
3 (1.5-2 cm)	110	27.0	122	28.4	99	21.8
4 (2-2.5 cm)	75	18.4	82	19.1	100	22.0
5 (2.5-3 cm)	36	8.8	55	12.8	94	20.7
6 (3-3.5 cm)	7	1.7	38	8.8	55	12.1
7 (3.5-4 cm)	6	1.5	19	4.4	30	6.6
8 (4 cm<)	0	0.0	3	0.7	20	4.4
Total (5)	408	100	430	100	454	100
Average (6)	2.84 ± 0.07^{a}		3.63 ± 0.07^{b}		4.32 ± 0.08^{c}	

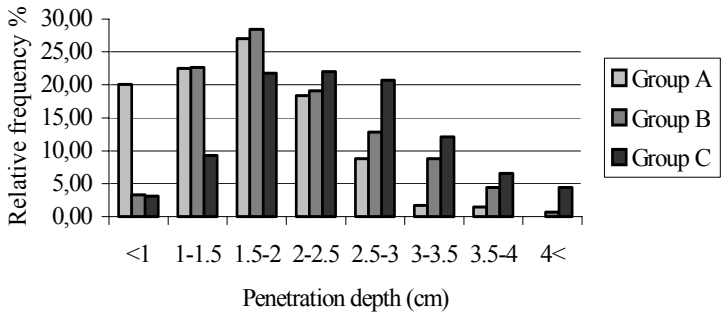
Group A: ewe lambs; Group B: primiparous ewes; Group C: multiparous ewes; n: number of penetrations. Values (means±SE) with different superscripts (a, b, c) differ significantly ($P<0.01$) among each group.

1. táblázat:A Milovanov katéter bevezethetőségének mértéke jerkékben, egyszer és többször ellett anyajuhokban

A csoport: jerkék(1), B csoport: egyszer ellett anyák(2), C csoport: többször ellett anyák(3), Behatolási mélység(4) Összesen(5), Átlag(6)

Figure 1

Changing of penetration depth of the catheter into the cervix in ewe lambs, primiparous and multiparous ewes



Group A: ewe lambs (*A csoport: jerkék*) Group B: primiparous ewes (*B csoport: egyszer ellett anyák*) Group C: multiparous ewes (*C csoport: többször ellett anyák*)

1. ábra: A katéter cervixbe vezethetőségének változása jerkékben, egyszer és többször ellett anyajuhokban

Függőleges tengelyen: relatív gyakoriság, vízszintes tengelyen: behatolási mélység

Table 2

Frequency table for the penetration depths of the catheter in ewes that lambed and failed to lamb

Penetration depth (6)	Lambing in Group A (1)				Lambing in Group B (2)				Lambing in Group C (3)			
	Yes (4)		No (5)		Yes (4)		No (5)		Yes (4)		No (5)	
	n	%	n	%	n	%	n	%	n	%	n	%
1 (<1 cm)	31	11.7	51	35.4	7	2.7	7	4.0	2	0.7	12	6.5
2 (1-1.5 cm)	51	19.3	41	28.5	42	16.4	55	31.6	21	7.8	21	11.3
3 (1.5-2 cm)	83	31.4	27	18.8	63	24.6	59	33.9	56	20.9	43	23.1
4 (2-2.5 cm)	59	22.3	16	11.1	55	21.5	27	15.5	60	22.4	40	21.5
5 (2.5-3 cm)	30	11.4	6	4.2	47	18.4	8	4.6	50	18.7	44	23.7
6 (3-3,5 cm)	5	1.9	2	1.3	29	11.3	9	5.2	41	15.3	14	7.5
7 (3.5-4 cm)	5	1.9	1	0.7	13	5.1	6	3.4	24	9.0	6	3.2
8 (4 cm<)	0	0	0	0	0	0	3	1.7	14	5.2	6	3.2
Total (7)	264	100	144	100	256	100	174	100	268	100	186	100
Average (8)	3.16±0.08		2.28±0.11		3.91±0.09		3.23±0.11		4.58±0.10		3.96±0.12	

Group A: ewe lambs, Group B: ewes after first lambing, Group C: ewes with multiple lambings. Values (mean±SE) with different superscripts are significant at P<0.01.

2. táblázat: Gyakoriségi táblázat a katéter behatolási mélységéről a termékenyítésre ellett és nem ellett állatok esetében

Ellési eredmények jerkéknél (A csoport)(1), Ellési eredmények egyszer ellett anyáknál (B csoport)(2), Ellési eredmények többször ellett anyáknál (C csoport)(3), Ellett(4); Nem ellett(5), Behatolási mélység(6), Összesen(7), Átlag(8)

Trial 4: Evaluation of the correlation between the depth of the penetration and the lambing rate

The correlation between the penetration depth of the catheter and the lambing rate are shown in *Table* 3. In case of cervico-uterinal insemination, 0.76 correlation was observed between the lambing rate and the insemination depth values in ewe lambs (P<0.05), 0.87 in primiparous ewes (P<0.02) and 0.79 in multiparous ewes (P<0.02).

DISCUSSION

The results of this study indicated significant differences in the penetration depth among the ewe lambs, primiparous and multiparous ewes. When the winding-ended catheter was used for the cervico-uterinal insemination. It was remarkable that the tip of the catheter could be introduced less than 1 cm into the cervix in 20% of the ewe lambs. The occurrence rate of this phenomenon was only 3% in primiparous and multiparous ewes The 2.5 cm line indicated the border between the mid-cervical and deep-cervical insemination (*Paulenz et al.,* 2002). Penetration depth of the catheter was deeper than 2.5 cm in 12% of the animals in ewe lambs 27% in ewes after first lambing, and 44% in ewes with multiple lambings. The reason for deeper penetrations in the respective ewes is probably because the cervix was dilated to a greater degree during the previous

Table 3

Correlation between the penetration depth values and the lambing rate in ewe lambs, primiparous and multiparous ewes

Penetration depth (5)	Lambing rate (1)		
	Group A (2)	Group B (3)	Group C (4)
1 (<1cm)	37.8	50.0	14.3
2 (1-1.5 cm)	55.4	43.3	50.0
3 (1.5-2 cm)	75.5	51.6	56.6
4 (2-2.5 cm)	78.7	67.0	60.0
5 (2.5-3 cm)	83.3	85.5	53.2
6 (3-3.5 cm)	71.4	76.3	74.5
7 (3.5-4 cm)	83.3	68.4	80.0
8 (4cm<)	-	-	70.0
Correlation (6)	0.76	0.87	0.79

Group A: ewe lambs; Group B: primiparous ewes; Group C: multiparous ewes. Level of significance in Group A is $P<0.05$, in Group B is $P<0.02$, in Group C is $P<0.02$.

3. táblázat: A katéter behatolási mélysége és az ellési arány közötti összefüggés vizsgálata jerkéknél, egyszer ellett és többször ellett anyáknál.

Ellési arány(1), Jerkék (A csoport)(2), Egyszer ellett anyák (B csoport)(3), Többször ellett anyák (C csoport)(4), Behatolási mélység(5), Korreláció(6)

lambings. The wider lumen made possible the deeper semen deposit site. We were not able to reach deeper than 4 cm insemination depth value in ewes that had not lambed before. On the basis of these results it would be more expedient to evaluate the correlation between the insemination depth, the pregnancy and lambing rates separately in ewe lambs, primiparous and multiparous ewes. Penetration depth values were not different between the morning and afternoon inseminations. The ewes were still in estrous at the 15.00 hour when the afternoon inseminations were completed. Perhaps that was the reason the penetration of the cervix did not differ. In contrast, insemination depth results were significantly deeper in ewes that lambed compared to those that failed to lamb to the AI.

The correlation coefficients demonstrate a close relationship between the insemination depth and the lambing rate. Taking into consideration the anatomy of the cervix, it is understandable. A great number of sperm practically come to a dead end in the rings of the cervix and cannot get into the body and horns of the uterus and the ampulla of the oviduct. Introducing the end of the inseminating catheter deeper into the cervix allows the sperm deposit through some rings but rarely through all the rings. In this situation semen enters directly into the body of the uterus. When semen cannot be deposited quite so deep into the cervix, a part of it or the whole quantity can flow back into the vagina. The acidic pH in the vagina is an unfavourable condition for the sperm. Deeper semen deposition saves the sperm from these unfavourable conditions so it would be expedient to do deep-cervical insemination. The authors of this study have done cervico-uterinal insemination as a routine practice for years and the lambing rate averages 60 to 73% after the first insemination with this simple insemination method

that can be easily adopted for daily practice. The lambing rates achieved with this technique are very comparable to lambing rates generally reported with laparoscopic AI and frozen semen (*Rodriquez et al.,* 1993).

With the use of 2 to 4ºC cooled, diluted, short-time preserved (2 to 3 days) ram semen, there is no need for laparoscopic technique so breeders can inseminate their ewes by themselves. It is advantageous to AI with short-time preserved ram semen that can be obtained from the AI centres easily. Short-time preserved ram semen can be transferred at least 500 km far from the AI centres and used safely in 2 to 3 days. In this case, breeders would get high quality semen from progeny-tested rams.

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