



A085E TAI/FTET/AI

What could be the impact of cervical mucus removal after oestrus synchronization on artificial insemination outcome in INRA180 prolific sheep?

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Keywords: Oestrus synchronization, mucus, artificial insemination.

Artificial insemination (AI) is an important tool that improves the chance of using superior rams (Arranz et al., *Renc. Rech. Rum*, 15, 359-362, 2008) and helps to control genitally diseases. During the AI process, the administration of exogenous progestagen to synchronize oestrus in ewes is one of the most important steps. However, the administration of such hormones has resulted in contradictory reports of both increased and decreased mucus production, which could affect the outcome of AI. The present work aimed to study the effect of mucus removal after oestrus sychronisation of INRA180 prolific adult ewes. A total of 84 ewes (2.5 to 3 years old) that have been managed under natural reproduction system were used to make 3 groups: group 1 (control) reproduce naturally, group 2 inseminated without removing the mucus and group 3 inseminated after mucus removal. For each group, two different doses of eCG (250 IU vs 300IU) have been used. The ewes were treated with intravaginal progesterone sponges (20 mg Flurogestone acetate, Pharmavet) for 14 days, and then injected with equine chorionic gonadotropin (eCG) at 250 IU or 300IU (Folligon®, Pharmavet) during Jun 2016. They were naturally mated (n=20 with 250 IU, and n=20 with 300 IU) or inseminated after removing the mucus (n=12 with 250 IU, and n=12 with 300 IU) or not (n=12 with 250 IU, and n=12 with 300 IU). The mucus was removed from animals in standing position using vaginal speculum. A split-plot design was adopted and all analyses were performed using JMP SAS v11. Fertility and prolificacy data were assessed by χ^2 analysis of contingency tables. The results showed that the natural mating revealed the highest fertility rates (80 to 90%). In both 250 IU and 300 IU groups, the treatment (removing the mucus or not) was highly significant ($P<0.05$). The conception rates were 32% and 40% respectively for the ewes receiving 250 IU and 300 IU of eCG. The mucus removal has significantly improves the fertility as this parameter increased to 59% and 67% respectively in 250IU and 300 IU. Recently, it has been reported that the cervicovaginal mucus proteome of the ewe undergoes natural variation across the oestrous cycle, and is significantly altered by progesterone synchronisation (Maddison et al., *Journal of Proteomics*, 155, 1-10, 2016.). This could explain a part of the result obtained in this work. The prolificacy was not improved ($P>0.05$) and it varies from 1.44 to 1.89. In conclusion, the present study revealed that it is possible to improve the fertility rate after artificial insemination by means of mucus removal after oestrus induction. Further protocols are planned on a large group of animals and will focus mainly on the eCG doses (300 IU) showing the greatest fertility rate. A part of this work was supported by CNRST of Morocco (PPR15/47).



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Comparative study of ewe's cervix anatomy of two Moroccan breed: A necessary step before the artificial insemination

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Keywords: Boujaâd ewes, D'man ewes, cervix anatomy.

The anatomy of the cervix represents a major constraint for developing the trans-cervical artificial insemination (TCAI) in sheep. Thus, the aim of this study was to compare this parameter in two Moroccan indigenous breeds. A total of 250 uteri Boujaâd (non-prolific, n = 187) and D'man (prolific, n = 64), with different ages (2,4,6 and 8 teeth) (Hemming, J.Wildl. Manag. 33, 552–558,1969) were collected from slaughterhouses at Bejaad and Errachidia respectively. The uteruses were transported at 5°C to the laboratory, and were cleaned in order to perform various measurements. For each sample, the weight of the uterus, length of the cervix, depth of penetration (using the artificial insemination gun), cervical grade (Kershaw *et al.*, Theriogenology 64, 1225-1235, 2005), and the number of cervical rings were recorded. All data were analyzed using the Statistical Analysis System software JMP (SAS version 11), by means of a factorial design ANOVA. The statistical model included the breed and age as fixed effects. When statistically significant differences were detected, the Tukey's post hoc, was used to compare the means and standard errors, considering the significance level of $P < 0.05$. Data are expressed as the mean \pm SD. The cervical grade data were assessed by X2 analysis of contingency tables. Independently on the ewes age, this study showed significant differences in the cervical grades between the two breeds. According Kershaw *et al.*, (Theriogenology 64, 1225-1235, 2005) the grades were as follow: grade 1 (11.90%) vs (12.82%), grade 2 (52.38%) vs. (69.23%) and grade 3 (35.71%) vs. (17.95%) respectively for Boujaâd and D'man. Furthermore, the average length of the cervix was 54.47 ± 13.60 mm for Boujaâd ewes instead of 41.53 ± 9.54 mm for D'man ($P < 0.05$). In addition, there were significant differences between breeds in the number of cervical rings (4.56 ± 1.32 for Boujaâd vs. 3.91 ± 1.09 for D'man), weight of the uterus (46.04 ± 18.21 g for Boujaâd vs. 37.39 ± 13.15 g for D'man) and the depth of penetration percentage (34.18% for Boujaâd vs. 41.70% for D'man). While the penetration depth of the insemination gun was not significantly different between the two breeds (18.06 ± 7.95 mm). Age had a significant effect on all studied parameters within each breed. Generally, in ageing ewes, the cervix tended to become longer with loose folds. As a conclusion, there was an apparent difference in the complexity of the cervix between Boujaâd and D'man breeds and between age within each breed, with a marked complexity in the Boujaâd ewe, which may make trans-cervical artificial insemination more difficult in this breed.



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Analysis of sperm-induced neutrophil extracellular traps (NETs) formation in the bovine system

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Keywords: sperm, PMN, neutrophil extracellular traps (NETs).

The natural site of semen deposition is the vagina in cattle and sperm migrate into the uterus leaving the bulk of the seminalplasma (SP) behind. However, current artificial insemination introduces variable amounts of SP into the uterus, which naturally remains in the vagina. While neutrophils combat microbial contamination and eliminate excess/dead sperm, their presence at the time of semen deposition reduces fertility. Beside phagozytosis and secretion of immune modulators, polymorphonuclear neutrophils (PMNs) are able to form "Neutrophil Extracellular Traps" (NETs) extruding their DNA into the extracellular environment. These are web-like structures, mainly composed of chromatin. Bovine PMN were isolated via Ficoll gradient centrifugation from peripheral blood. Frozen/thawed sperm cell suspensions (SCS) of bulls with proven fertility were used. The visualization and identification of NETs was achieved by scanning electron microscopy (SEM) or via fluorescence microscopy analysis, respectively. For NET induction, PMN and SCS were co-cultured for different time points (0, 15, 30, 45, 60, 120, 180 min). NET induction of sperm and supernatant alone was also measured. Zymosan was used to induce the formation of NETs in bovine PMN as positive control. Quantification of NETs formation was performed by spectrofluorometric analyses using an automated plate monochrome reader (Varioscan Flash; Thermo Scientific). Scanning electron microscopy as well as fluorescence microscopy analyses revealed that the exposure of bovine PMN to frozen/thawed bovine SCS trigger the formation of NETs. After quantification, fluorescence intensities (FI in arbitrary units, AU) indicate that sperm alone led to significantly reduced fluorescence intensities suggesting that the extender and the remaining seminal plasma are affecting NET formation to a higher extent. A significant increase in FI was seen until 60 min of incubation indicating that NET formation might be finished at that time point. Furthermore, no differences in FI were assessed with motile or immotile sperm indicating that the release of NETs is independent on sperm motility. These data show that bovine sperm are able to induce NETs formation. The financial support of the Förderverein Bioökonomieforschung e.V. (FBF) is gratefully acknowledged.



A088E TAI/FTET/AI

Automated activity monitoring of estrus in recipient heifers: a retrospective study in an embryo transfer center

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Keywords: automated activity monitor, recipient, estrus.

For several years, EVOLUTION has set-up a new strategy to optimize its selective breeding programs by genotyping embryos after biopsy, keeping them frozen until their transfer once selected based on their estimated breeding value. Since only the best embryos are transferred, the management of recipients become essential and to succeed in this task, a recipients center has been opened to transfer embryos from our dairy breeding programs. The aim of this retrospective study is to highlight some zootechnical results obtained with an automated activity monitoring of estrus in recipient heifers. 209 Holstein heifers weighting more than 400 kg were used to receive frozen biopsied embryos. Most of them wear activity monitors (HEATIME®) and their heats were synchronized by groups of 5 to 15 using the following protocol: D0, Norgestomet implant insertion, Norgetsomet and Buserelin injection (CRESTAR Pack®) – D10 Cloprostenol (ESTRUMATE®) injection – D11 implant removal – D12 to D14 heat observation. The objective of this protocol is to transfer embryo between 6 and 7 days after heat detection, at D19. 383 heats were detected and followed by a transfer for 295 of them while the full activity profile was recorded for 219 of them. Each heifer was allowed up to 3 transfers before leaving the breeding program. The HEATIME® profile allows the record of the beginning of the peak activity, the increase of activity at the peak and the duration of the peak activity. The pregnancies were checked by ultrasonography at day 30 and confirmed between day 55 and day 65. 419 synchronization protocols were performed : 87 heifers were synchronized once, 62 twice, 40 three times and 20 more than three. Regarding the beginning of activity peak, 2% occurred in less than 12 hours after implant removal, 12% between 12 and 24 h, 41 % between 24 and 36 h, 17% between 36 and 48 h, 17% between 48 and 72 h, 9% between 72 and 96 h and 2% after more than 96 h. The mean activity increase at the peak during heat is 85 % \pm 16 and the mean of peak activity duration is 14.5 h \pm 6.1. For the 295 transfers, the pregnancy rate is 56%. This rate differs according to intensity of peak activity ($P < 0.05$) with 42.5% when the increase was strictly less than 90 % and 57.5% for increase of 90% or more. No effect of protocol or transfer rank can be observed. No significant effect either of the recipient's stage could be shown as the pregnancy rates are 54 % (7/13), 61 % (27/44), 56% (128/227) and 45 % (5/11) for transfers done between [4-5[, [5-6[, [6-7[and [7-8] days after the heat, respectively. This kind of automated activity monitoring allows us to have detailed description of recipient's heats which is not the case when recipients are in partners' farms. Recorded information will allow us to discriminate more closely the recipients based on estrus characteristics or stage.



A089E TAI/FTET/AI

Progesterone concentrations on the day of re-insemination on farms using artificial insemination services and on herd-owner insemination farms

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Keywords: cow, oestrus, pregnancy.

The objective was to examine whether training background of artificial inseminator (herd-owner inseminators=OWNER, AI technicians=AI-T and fertility consultants=FC) has an effect on pregnancy rate and if additional training helps AI professionals to detect cows not in oestrus. A total of 1584 re-AI occasions on 754 farms were included. Whole milk samples were collected for progesterone (P4) analysis (RIA) from all cows submitted to re-AI and data including farm type, previous breeding attempts, oestrus signs and evaluation of uterine tone, slipperiness of the cervix and co-operation of the cow was collected. Further breeding attempts and next calving or culling date were sought from registers. AI occasions were divided into three categories based on P4 concentrations; <6 nmol/l: no luteal activity and cow could be in oestrus, 6 to 10 nmol/l: some luteal activity and >10 nmol/l: high luteal activity and cow was not in oestrus. A proportion of 7.7% of cows offered for re-AI had P4 concentration >10 nmol/l. There was no statistical difference between OWNERS and farms using AI service. OWNER farms chose for AI more cows with intermediate P4 values than farms using AI service (OWNERS: 82.7% <6 nmol/l, 9.8% from 6 to 10 nmol/l and 7.5% >10 nmol/l and farms using AI-service: 86.2%, 5.9% and 7.8%, respectively). AI-Ts recommended no AI significantly less than FCs: 1.6% versus 4.9%. Both groups were equally right: 71% and 68% of cows recommended no AI had high P4 concentration. Finally, in the three P4 categories, AI-Ts and FCs inseminated 86.3%, 6.5%, 7.2% and 89.2%, 5.5%, 5.3% of cows, respectively. As of more courageous rejection of cows with high P4, FCs inseminated statistically significantly more cows at <6 nmol/l and less cows at 6 to 10 nmol/l than OWNERS. 36.7% of cows finally inseminated got pregnant and there was no significant difference between OWNERS and farms using AI service (37.1% versus 36.4%). FCs had significantly higher pregnancy rates than AI-Ts (39.6% versus 32.6%). The proportion of cows inseminated during the luteal phase has increased in Finland from 4.4% (Laitinen 1983, Oestrus confirmation, pregnancy diagnosis and postpartum ovarian follow-up of the Finnish dairy cows by milk progesterone assay: Effects of breed, season, feed and sampling on milk progesterone levels, PhD thesis) to 7.7% in the past 30 years. This should be taken into account in the education of AI professionals and OWNERS. The ability of the inseminators to detect the cows not in oestrus and to reject them can be strengthened through training. More accurate rejections yield a higher pregnancy rate. The best indicative and predictive oestrous signs detected by the inseminator at the time of AI are uterine tone and consistency of the vaginal mucus. Behaviour of the cow at AI did not predict the P4 concentration. Acknowledgements The study was supported by the MAILI project of Savonia University of Applied Sciences financed by the European Union.