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Embryo transfer results in endangered cow breeds in Latvia

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Introduction cow embryo collection and transfer is a developing field since 2017 in Latvia. Our main aim is to save endangered cow breeds in Latvia. A new staff was educated to provide multiple ovulation (MO), embryo flushing and embryo transfer (ET) in the cow. In Latvia endangered cow breeds (Latvian Brown, Latvian Blue and Danish Red) are on extinction line. Small number of animals exist and this was the main reason why almost all intended cows were used to obtain embryos despite the fact that many of them did not meet conditions necessary for a good donor cow status.

The aim of this work was to analyze recipients' pregnancy results in relation to different factors: age and reproduction history, natural (NRC) or induced reproductive cycle (IRC) using, estradiol (E2) and progesterone (P4) concentration in blood. Factors of embryos were: fresh or thawed; development stage and quality. There were taken into account person who provided embryo transfer procedure and season. The background of embryos was analyzed: glucose and cholesterol level in donor-cow blood on embryo obtaining day and somatic cell count in milk on the nearest milk recording day.

Materials and methods. Recipients were 76 heifers (13-37 months of age, 330 - 400 kg bodyweight). Cloprostenol (Oestrophan, Bioveta) was used to induce IRC in 60 (69.8%) recipients, but in 26 (30.2%) recipients the 7th day of NRC was used. An epidural anaesthesia was done using 2.0 mL Procamidor (Procaine hydrochloride 20.0 mg mL-1, Richter Pharma) before ET. Blood samples were taken to evaluate concentration of E2 and P4 using Enzyme-Linked Fluorescent Assay. Some parameters of embryo donors were taken into account (blood glucose, cholesterol, P4 and somatic cell count in milk). The quality of transferred embryos was: good - 38 (44.2%), fair - 44 (51.2%) and poor - 4 (4.7%), but their development stages were: stage III - morula (3 embryos, 3.5%), stage IV - compact morula (49 embryos, 57%), stage V - early blastocysts (19 embryos, 22.1%), stage VI - blastocysts (9 embryos, 10.5%), and stage VII - expanded blastocysts (6 embryos, 7%). Fresh ET was done in 57 (66.3%) recipients and thawed ET in 29 (33.7%). ET was provided by 3 persons (A - 48 recipients, 55.8%), B - 2 recipients, 2.3%) and C - 36 recipients, 41.9%). Data are expressed as the mean ± SD, percentage and independent samples t-tests were performed for statistical analysis considering the significance level of $P < 0.05$ using IBM SPSS Statistics 21 software.

Results. Pregnancy was accepted in 23 out of 76 recipients (30.3%); ET (19 pregnancies out of 53 (35.8%) vs thawed ET (4 out of 23 (17.4%)), ($P < 0.05$). Despite the unsuccessful AI was provided in 19 recipients (25%), before they were accepted for the recipients' role, it was not a statistically significant factor for the successful ET outcome ($P > 0.05$). Thirteen recipients (17.1%) expressed oestrus in the following reproductive cycle, 11 recipients (14.5%) were in heat in the second reproductive cycle, but 29 recipients (38.2%) were in the heat just after 3 and more reproductive cycles after ET. The IRC (54 recipients, 71.1%) was more productive than that of the NRC (22 recipients, 28.9%), ($P < 0.05$). Weak statistically significant correlation was established between pregnancy rate and recipients housing system ($r = 0.32$, $P < 0.001$). In tethered heifers group pregnancy saved 9 out of 39 (23.1%), but in free heifers group 14 out of 37 (37.8%). The most of pregnancies obtained were using compact morula (18 pregnancies out of 23 (78.3%). Pregnancy rate was not different significantly between the person A and B providing ET ($P > 0.05$), but ET procedures provided by person C were unsuccessful ($P < 0.05$). A season was is not statistically significant factor ($P>0.05$) in our study.

All pregnancies obtained by thawed embryos were fulfilled using embryos of the stage of the compact morula. more successful ET results were reached if embryos were obtained from donors which had a higher level of glucose in the blood (3.1 ± 0.39 and 2.8 ± 0.42 mmol L–1 respectively), higher level of cholesterol in the blood (5.6 ± 0.91 and 4.9 ± 1.11 mmol L^{-1}) and lower somatic cell count in milk (214.5 ± 121.94 and 486.4 ± 1,073.88 thousand ml^{-1}). We have found the statistical significance of interactions of individual components such as: recipient reproduction history, IRC and person provided ET.

In conclusion, our newly educated and trained MOET team should improve experience to rise the rate of recipients' pregnancy. Very specific reasons were not revealed for quite poor results. Our success could be improved using induced reproductive cycle in recipients, fresh embryos, by improving technique of cryopreservation, and more attention could be paid to the donor-cows management in order to reach more qualitative embryos. Somatic cell count in the donors' milk could be one of the indicator to avoid unsuccessful embryo obtaining. Future monitoring and investigation must be provided to improve pregnancy results in recipients in our country.

Table 1. P4 and E2 level in recipients' blood on ET day

	Progesterone (nmol L^{-1})	Estradiol (pg mL^{-1})
Pregnant altogether (n = 23)	19.8 ± 7.87	9.7 ± 1.81
Un-pregnant altogether (n = 53)	22.7 ± 12.08	13.3 ± 10.83*
Pregnant using fresh embryo (n = 19)	18.2 ± 6.80	9.7 ± 1.93
Pregnant using thawed embryo (n = 4)	27.4 ± 9.15*	9.7 ± 1.35
Un-pregnant using fresh embryo (n = 34)	21.1 ± 9.79	12.2 ± 8.41
Un-pregnant using thawed embryo (n = 19)	25.4 ± 15.18	15.2 ± 14.15
Induced reproductive cycle (n = 54)	20.5 ± 10.81	11.5 ± 6.85
Natural reproductive cycle (n = 22)	25.2 ± 10.78	13.9 ± 13.56
Pregnant using natural reproductive cycle (n = 4)	29.7 ± 7.9	9.7 ± 1.35
Pregnant using induced reproductive cycle (n = 19)	17.7 ± 6.2	9.7 ± 1.93
Un-pregnant using natural reproductive cycle (n = 18)	24.1 ± 11.5	14.9 ± 14.96
Un-pregnant using induced reproductive cycle (n = 35)	22.0 ± 12.45	12.5 ± 8.28

* ($P < 0.05$)

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