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Embryo transfer results in endangered cow breeds in Latvia

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Abstract. Since 2017 multiple ovulation (MO) and embryo transfer (ET) were used to save endangered cow breeds in Latvia. The aim of this work was to analyse results to establish factors influenced recipients' pregnancy obtained up to now. Recipients age, induced (IRC) or native reproductive cycle (NRC), level of estradiol (E2) and progesterone (P4) in recipients' blood on ET day, stage and quality of embryo, fresh or thawed embryo usage and person provided ET were taken into account. Repeated artificial insemination (AI) had been provided in 19 (22.1%) recipients before ET. Pregnancy was accepted in 23 out of 76 recipients (30.3%) until now. Higher pregnancy results were obtained using fresh embryos (19 out of 53 (35.8%)) vs thawed embryos (4 out of 23 (17.4%)), ($P < 0.05$). The IRC was more productive than NRC ($P < 0.05$). The development stage, quality of embryos, E2 level were significant factors to reach pregnancy ($P < 0.05$) but recipients age, P4 level and person provided ET did not influence the pregnancy rate in the present study ($P > 0.05$). More successful pregnancies were reached using embryos obtained from donors which had a higher glucose and cholesterol level in blood ($P = 0.05$). In conclusion, our newly educated MOET team should gain their experience and results could be improved using IRC in recipients, fresh embryos, 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.

Key words: cow embryo, embryo transfer, recipients.

INTRODUCTION

Thanks to ERAF project No.1.1.1.1/16/A/025, 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. Mainly our endangered breed cows are kept on small farms without well-established management but it is important factor for cows' health and productivity despite the modesty of the native breeds (Cielava et al., 2017). Latvian Blue cows are suffering never from *Bovine leukaemia*, and mastitis is not common for them (Grīslis, 2006). Latvian Brown cows inherit durable leg, foot and hoof health from their ancestors (Šematoviča et al., 2017). Feed's composition and quality besides amount of milk, influences milk fat and protein content (Frorip et al., 2012; Šematoviča et al.,

2017a). It is reported that Latvian native breed cows have high milk fat content, milk fat and protein ratio (Paura et al., 2012; Smiltiņa et al., 2015). 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.

Successful ET depends on the embryo quality and subsequently on the donor-cow factors such as: genetic qualities, nutrition, health and housing conditions (Hasler et al., 1987; Son et al., 2012; Vieira et al., 2014; Diskin et al., 2016; Mikkola, 2017; Abdelatty et al., 2018). The housing system directly affects dairy cows' longevity, health of the udder and calving performance (Leso et al., 2019).

Recipient depending factors are very important and also widely investigated. Some of them are ovarian functionality and hormonal status (Hasler et al., 1980; Rao & Yelisetti, 2013). Within the framework of the present work because of Latvian society was not informed enough about MO, embryo flushing (EF) and ET procedures influence on animal's health, the animals' owners quite often allowed us to use heifers for ET which were after unsuccessful artificial insemination (AI) or quite old ones. Although heifers-recipients used were without clinical signs of illness.

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

MATERIAL AND METHODS

More than 150 heifers (different breeds and crossbreed) were accepted for a recipient role. These animals usually were kept on other farms than donor-cows. Different housing system (tied - 39 (51.3%) and free - 37 (48.7%)) and different feeding were provided (totally mixed ration and conventional feeding). Water was free available by automatic waterers. Animals were active and interacted with the personnel and each other appropriately depending on their individual character. Only 86 heifers (13–37 months of age, 330–400 kg bodyweight) became the recipients. It was too early to detect pregnancy in 10 recipients (11.6%). Cloprostenol (*Oestrophan*, Bioveta) was used to synchronize the oestrus cycle in recipients using two injections with 11 days of interval in 60 (69.8%) recipients, but in 26 (30.2%) recipients the 7th day of native reproductive cycle was used. It was a random day of the reproductive cycle when heifers received the first injection of Cloprostenol. An epidural anaesthesia in recipients was done using 2.0 mL Procamidor (*Procaine hydrochloride* 20.0 mg mL^{-1}, *Richter Pharma*) before ET.

Blood samples were taken to evaluate the ovaries' functional activity in heifers on the 7th day of the reproductive cycle. Concentration of E2 and P4 were analysed in the blood sera using Enzyme-Linked Fluorescent Assay method in the accredited laboratory (LVS EN ISO/IEC 17025:2005) at the Institute of Food Safety, Animal Health and Environment 'BIOR'. Some parameters of embryo donors were taken into account (blood glucose, cholesterol, P4 and somatic cell count in milk).

Heifers' ovaries were investigated by rectal palpation on the 7th day of the reproductive cycle (common ovarian status, presence, location and consistency of *corpus luteum* (CL)) before the potential process of ET. Poor quality of CL was the reason to decline a heifer to become a recipient because of inappropriate reaction to hormonal treatment through synchronization process or NRC. If the CL was well expressed, at least 10–12 mm large and compact, ET was carried out. Embryos were transferred to the same uterus horn to the current CL. If fresh embryos were used, it was done 6–8 h after EF from the donor cow. If frozen embryos were used, thawing was provided shortly before ET.

Embryos were evaluated and classified according to the International Embryo Technology Society standards and related guidelines (Bo & Mapletoft, 2013). The quality of totally 86 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%).

Donors mainly were located on other farms than recipients. Before transferring, embryos were flushed out from the donors using BoviFlush Recovery medium (Minitube) and stored at room temperature in BoviHold medium (Minitube). Embryos for cryopreservation were put in straws with BoviFreeze medium (Minitube) and embryo thawing was provided using Portable Incubator (Minitube). Embryos were carried to recipients in the ET devices heater V2.0 with a carrying strap, +25 °C (Minitube), and ET was done immediately.

Fresh embryos transfer was done in 57 (66.3%) recipients and thawed embryos were transferred to 29 (33.7%) recipients. Transferring was provided by three 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 AND DISCUSSION

Pregnancy was accepted in 23 out of 76 recipients (30.3%) by manual palpating 60-75 days after ET. Usually pregnancy should be approved earlier but our native breed cows are located in very different regions of Latvia. Significantly higher pregnancy results were obtained using fresh embryos (19 pregnancies out of 53 (35.8%) in comparison with of using thawed embryos (4 out of 23 (17.4%)), ($P < 0.05$). The cryopreservation is a physico-chemical process which has a negative influence on fertility and quality of embryos. A tremendous improvement was carried out to ensure a quality and fertility of embryos within the last decades (Saragusty & Arav, 2011; Huang et al., 2019). Nowadays cryopreservation allows to reach good pregnancy results in recipients using thawed embryos, and methodology is still improving (López-Damián et al., 2020).

Recipient factors could be very important for a successful ET outcome (Ferraz et al., 2016). 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. It remains unknown exactly how many of these recipients did not save transferred embryo at all, how many of them saved pregnancy, and how many micro-abortions occurred. It could be suspected that embryo survive failure is recognisable by too late rebreeding (Diskin et al., 2016). In our work it could be 38.2%, but it should be taken into account how accurately the owners of recipients had evaluated heifers' oestrus signs.

The IRC (54 recipients, 71.1%) was more productive than that of the NRC (22 recipients, 28.9%), ($P < 0.05$). A higher pregnancy rate using IRC was also noticed by other scientists (Hasler et al., 1987). 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%). It could be explained with the more comfortable obstacles for expression of estrus signs to establish the first reproductive cycle day and better welfare conditions to save pregnancy after ET on the 7th day of the reproductive cycle.

In the present work, the development stage of embryo might have been a quite significant factor to reach recipients' pregnancy ($P = 0.07$) because most of pregnancies obtained were using compact morula (18 pregnancies out of 23 (78.3%). However, it should be mentioned that compact morulas totally were used in 45 recipients and 27 of them did not became pregnant ($P > 0.05$). An early blastocysts were used in 16 recipients, and a successful outcome was in three recipients (18.8%). Nine blastocysts were transferred and one pregnancy was detected (11.1%). Six expanded blastocysts were transferred and only one pregnancy was approved (16.7%). All pregnancies obtained by thawed embryos were fulfilled using embryos of the stage of the compact morula.

Table 1. Progesterone and estradiol 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

* ($P < 0.05$).

Recipients which become pregnant were younger than unsuccessful pregnancy recipients (16.6 ± 2.33 and 18.0 ± 4.11 months respectively, ($P > 0.05$)).

Steroid hormone level in recipients' blood on ET day are presented in Table 1. In the present study, on the ET day the E2 level in blood was lower in recipients which

became pregnant in comparison to the recipients which did not became pregnant (9.7 ± 1.81 and 13.3 ± 10.83 pg mL^{-1} respectively, $P < 0.05$). The level of steroid hormone P4 is very important to save the pregnancy and it depends on CL functional quality (Lonergan et al., 2007; Stevenson & Lamb, 2016). It was established that in the recipients which had become pregnant after ET, the level of P4 was higher on the 7th day of the reproductive cycle than recipients which failed to be pregnant (Rao & Yeliseti, 2013). In our study, P4 level had no statistically significant differences for a successful pregnancy and unsuccessful pregnancy in the recipients' blood (19.8 ± 7.87 and 22.7 ± 12.08 nmol L^{-1}, respectively, ($P > 0.05$)). The similar observation was revealed by other scientists who established no significant differences regarding P4 level in the heifers' blood which became pregnant and heifers which did not save a pregnancy later (Hasler et al., 1980). A more important factor established is CL quality, diameter and size (Nogueira et al., 2012). We noticed that a quite strong functionality of CL was necessary to save pregnancy using thawed embryos. Level of P4 to obtain only 4 pregnancies using thawed embryos were 27.4 ± 9.15 nmol L^{-1}.

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 ($P < 0.05$), (Table 2). Training and experience are very important conditions besides the factors related with donors, recipients, embryos, equipment, and methods used (Seidel, 1984).

Table 2. Pregnancies obtained by different persons A, B and C

	ET/ pregnancies totally	Pregnancies totally %	Fresh ET/ pregnancies	Fresh ET pregnancies %	Thawed ET/ pregnancies	Thawed ET pregnancies %
A	41 / 10	24.4	28 / 8	19.5	13 / 2	15.4
B	33 / 13	39.4	25 / 11	33.3	8 / 2	25.0
C	2 / 0	0.0*	0 / 0	0	2 / 0 *	0.0
TOTAL	76 / 23	30.3	53 / 19	35.8	23 / 4	17.4

* ($P < 0.05$).

We have found out that the background of embryos is important because 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 $486.4 \pm 1{,}073.88$ thousand mL^{-1}). Pregnancy rate between those donors' embryos was statistically significant ($P < 0.05$). Interrelation between the udder health and reproductive performance regarding pregnancy loss is actual still now (Dahl et al., 2020). The positive influence of the embryo donor blood glucose and cholesterol level on embryo harvest was reported earlier (Choe et al., 2012).

Season is mentioned as important factor for a successful MO, EF and ET (Hasler et al., 1987; Vieira et al., 2014). In the present work, 44 (58.7%) ET were provided in the winter-spring season, but 31 (41.3%) ET were done in summer-autumn period. Sixteen pregnancies were obtained in the winter-spring season but only seven pregnancies in summer-autumn period. This difference is not statistically significant because of number of unsuccessful ET at these periods.

Because of MOET was restarted after more than 35 years' interruption in Latvia the new staff had been trained. The experienced professionals have reached 70% pregnancy rate using fresh and 60% using thawed embryos (Gadisa et al., 2019). We did not find the individual factors that were decisive for the recipients' pregnancy. Using *IBM SPSS Statistics 21 software General Linear Model*, we have found the significance of interactions of individual components such as: recipient reproduction history, IRC and person provided ET. So we must to improve some areas of our work regarding technique, donors, recipients and embryos.

CONCLUSIONS

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.

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