Scientific Works. Series C. Veterinary Medicine. Vol. LX (2)
ISSN 2065-1295, ISSN Online 2067-3663, ISSN CD-ROM 2343-9394, ISSN-L 2065-1295

HARVESTED EMBRYOS ASSESSMENT FOLLOWING POLIOVULATION USING FSH IN COWS WITH A VIEW TO THE BIODIVERSITY OF THE SPECIES

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Abstract

The paper aimed to present the possibility of obtaining a larger number of calves from valuable cows, increasing their contribution to genetic progress, completing the amelioration programs by artificial insemination, using poliovulation and embryo transfer. We used in this study 15 donor cows from the Montbeliarde breed belonging to three different farms from southern Romania. The current paper presents poliovulation results with a view to the number and quality of embryos harvested following the FSH poliovulation in decreasing doses. In the 16-th day of the protocol, the embryos were harvested and morphologically assessed before transferring or freezing them. After harvesting the embryos through the lavage of the uterine horns, they were transferred in special plates and they were identified employing a Nikon magnifier loupe. They were also evaluated employing an inverted Nikon Eclipse TS 100 microscope. Following the morphological assessment of the 116 harvested formations, they were classified as follows: 2 compact morulas (1.72%), 94 early blastocysts (81.03%), 13 blastocysts (11.21%) and 7 unfertilized ovules (6.03%). By employing this protocol, there were no instances of cystisation of ovarian follicles. This fact proves that the FSH administered in decreasing doses leads to a very uniform ovulation and, implicitly, to a smaller number of late ovulated follicles as compared to other poliovulation protocols.

Key words: *cow, embryos, poliovulation, protocol, FSH*

INTRODUCTION

Embryo transfer provides the possibility of obtaining a larger number of calves from valuable cows, increasing their contribution to genetic progress, completing the amelioration programs by artificial insemination (Betteridge, 1986, 2003; Evans, 2005).

Embryo transfer is very effective in the selection programs, in countries with advanced animal husbandry, approximately 95% of tested bulls from milk breeds come from embryos transfer. This percentage reflects the impact of biotechnology in the selection programs (Hasler, 2003).

The importance of this study lies in the possibility of determining a poliovulation in cows subjected to embryo transfer in order to improve its results (Bîrţoiu et all, 2006, 2007).

It also seeks, through this paper, standardization of protocols to be applied in the Laboratory of Biotechnologies of the Clinic of Obstetrics and Gynaecology of the Faculty of Veterinary Medicine Bucharest and externalization of the services to individuals and businesses, farms and breeders alike (Viţălaru, et all, 2008, Viţălaru and Bârţoiu, 2008).

MATERIALS AND METHODS

15 donor cows belonging to the Montbeliarde breed, from three different farms in southern Romania, were included in a poliovulation survey.

The current paper presents the poliovulation results with a view to the number and quality of harvested embryos following a FSH poliovulation in decreasing doses.

Day 0 of the poliovulation protocol in the donor cow was actually the 9th day of its sexual cycle. On day 0 of the protocol, the Periodestrol was introduced. On day 4 of the protocol, the cow received FSH (Folltropin) 4

ml (140 U.I.) i.m., in the morning at 08:00, and 4 ml (140 U.I.), in the evening at 20:00. On days 5, 6 şi 7 of the poliovulation protocol, the treatment was repeated in decreasing doses. On day 5 of the protocol, the cow received FSH (Folltropin) 3 ml (105 U.I.) i.m., in the morning at 08:00, and 3 ml (105 U.I.), in the evening at 20:00. On day 6 of the protocol, the cow received FSH (Folltropin) 2 ml (70 U.I.) i.m., in the morning at 08:00, and 2 ml (70 U.I.), in the evening at 20:00. On day 7 of the protocol, the cow received FSH (Folltropin) 1 ml (35 U.I.) i.m., in the morning at 08:00, and 1 ml (35 U.I.), in the evening at 20:00. On day 7 of the protocol, $PgF2_{\alpha}$ was added, 500 mcg at every 12 hours, usually at 08:00 and 20:00, and the pridoestrol was retrieved.

The oestrus appeared within an average of 48 hours from the last $PgF2_{\alpha}$ dose. The female donor was artificially inseminated when the oestrus was detected, and two other additional times 12 hours apart.

2,5 ml Buserelin acetate were administered per cow before performing the first artificial insemination, immediately after detecting the oestrus. The employed product was Receptal, from Intervet.

On day 16 of the protocol, the embryos were harvested and they were morphologically assessed before transferring or freezing them.

After harvesting the embryos through the lavage of the uterine horns, they were transferred in special plates and identified with a Nikon magnifier loupe and later on evaluated using an inverted Nikon Eclipse TS 100 microscope. The harvested embryos were rinsed after the identification process in order to be morphologically assessed. The embryonic formations were examined using a Nikon magnifier loupe within the farm and, when possible, they were frozen and brought to the Biotechnology and Reproduction Laboratory within the Faculty of Veterinary Medicine, where they were examined using an inverted Nikon Eclipse TS 100 microscope.

RESULTS AND DISCUSSIONS

Using the decreasing FSH protocol, we have harvested several formations inserted in Table 1.

Table 1. Evolution of Dairy Cows during the period 1990-2010 (thousand heads)

Total	EMBRYOS						Unfertilized oocytes	
	Compact morulas		Ealry Blastocysts		Blastocysts			
	No.	%	No.	%	No.	%	No.	%
116	2	1.72	94	81.03	13	11.21	7	6.03

Following the morphological assessment of the 116 harvested formations, they were classified as follows: 2 compact morulas (1.72%), 94 early blastocysts (81.03%), 13 blastocysts (11.21%) and 7 unfertilized ovules (6.03%).

By employing this poliovulation protocol, various results will be obtained. The 15 donors undergoing this protocol produced 158 corpora lutea. The distribution of these corpora lutea on the two ovaries was unequal (85 on the left ovary, so 53.8%, and 73 on the right ovary, so 46.2%).

116 formations were harvested in total from both ovaries. Only 7 oocytes were identified as being unfertilized (6.03%), representing a smaller percentage than the one offered by the literature in the field (Viţălaru, 2008).

With regard to the embryonic formations, the majority was represented by early blastocysts, namely 94 (81.03%), as compared to the 13 blastocysts (11.21%) and the 2 morulas (1.72%).

One needs to underline the fact that by using this protocol there was no follicle cystisation present.

This fact demonstrates that the FSH administered in decreasing doses leads to a very uniform ovulation and, implicitly, to a smaller number of late ovulated follicles as compared to other existing poliovulation protocols.

When closely examined with an inverted Nikon Eclipse TS 100 microscope, the embryonic formations had various aspects as presented in Figures 1-6.

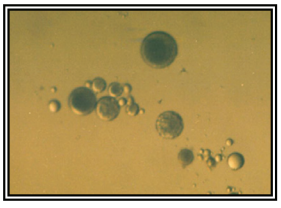

Figure 1. Embryos examined in Nikon Stereo Magnifier Loupe (compacting morulas and blastocysts) (orig.)

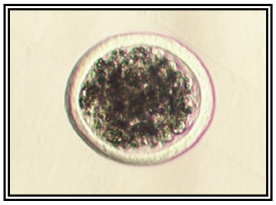

Figure 2. Compacting morula examined with Nikon Inverted Microscope (magnification 40x) (orig.)

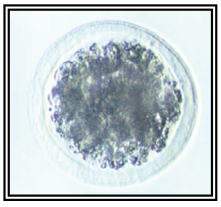

Figure 3. Compacting morula examined with Nikon Inverted Microscope (magnification 40x) (orig.)

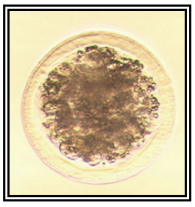

Figure 4. Compacting morula examined with Nikon Inverted Microscope (magnification 40x) (orig.)

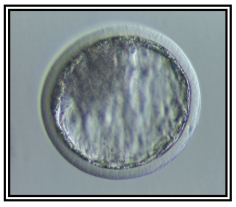

Figure 5. Blastocyst examined with Nikon Inverted Microscope (magnification 40x) (orig.)

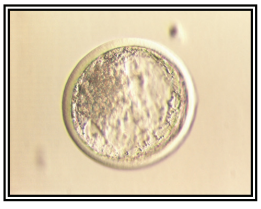

Figure 6. Blastocyst examined with Nikon Inverted Microscope (magnification 40x) (orig.)

CONCLUSIONS

A batch of 15 cows was submitted to an induced poliovulation by using a FSH protocol in decreasing doses (Folltropin + Pridoestrol + $PGF2_{\alpha}$ + Gn-RH) for all animals in the survey.
Following the FSH doses, embryos in all stages were harvested, from compact morulas, to blastocysts, and to a small number of unfertilized oocytes resulting from a late ovulation.
The 15 donors submitted to this protocol produced 158 corpora lutea which were unequally distributed on the two ovaries (85 on the left ovary, so 53.8%, and 73 on the right ovary, so 46.2%).
The majority of the harvested formations was represented by early balstocysts (81.03%).
This protocol did not result in any follicle cystisation.
The number of harvested embryos did not correspond to the number of corpora lutea diagnosed on the ovaries (the number is smaller). It is possible that a number of embryos still remained in the uterus, thus making necessary the administration of $PGF2_{\alpha}$ to all donors after harvesting in order to avoid any unwanted gestation.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Şcoala Postdoctorală pentru Biodiversitate Zootehnică şi Biotehnologii Alimentare pe Baza Ecoeconomiei şi Bioeconomiei Necesare Ecosanogenezei, Contract no. POSDRU/89/1.5/S/63258, beneficiary: INSTITUTUL NAŢIONAL DE CERCETĂRI ECONOMICE „COSTIN C. KIRIŢESCU" AL ACADEMIEI ROMÂNE (INCE).

REFERENCES

Betteridge, K.J., 1986 - Increasing productivity in farm animals. In Austin, C.R. & Short, R.V., eds. Reproduction in mammals: 5. Manipulating reproduction, p 1–47. Cambridge, Cambridge University Press.

Betteridge, K.J., 2003- A history of farm animal embryo transfer and some associated techniques. Anim Reprod Sci; 79:203-244.

Bîrţoiu, I.A., Viţălaru, B.Al., Ochea, M., Ruxandra Badea, 2007 - Comparative studies regarding the

synchronization between the receivers and donors estrous in Montbeliarde cows, Revista Română de Medicină Veterinară, vol.17,2, 339-348; ISSN:1220-3173.

Bîrţoiu, I.A., Viţălaru, B.Al., Ochea, M., Tobă, G., Badea, Ruxandra, Crivineanu, Maria, Rotaru, Elena, Tobă, G.L., Badea, I., 2006 - Researches regarding the efficiency of different protocols of inducing the poliovulation in Montbeliarde cows, Scientific Works C series, Veterinary Medicine, XL, ISSN:1222-5304.

Bîrţoiu, I.A., Ochea, M., Viţălaru, B.Al., Şonea, Al., Badea, I., 2006 - Researches regarding the embryo recovery by different non-surgical procedures in Montbeliarde cows, Scientific Works C series, Veterinary Medicine, XL, ISSN:1222-5304.

Evans, G., Faul, A., Bielanski, A., Renwick, S., Van Derlinden, I., 2005 - Risk analysis and international trade principles applied to the import, INRA Productions Animales.

Hasler, J.F., 2003 - The current status and future of commercial embryo transfer in cattle. Anim Reprod Sci; 79:245-264.

Viţălaru, B. Al., Ochea, M., Ruxandra Badea, Ţogoe, D., Rotaru, Elena, Diniţă, D., Diaconescu, Al., Bîrţoiu, A., 2008 - Rezultate obţinute în urma transferului de embrioni la vaci din rasa Montbeliarde, Lucrări ştiinţifice FMV Iaşi.

Viţălaru, B. Al., Birtoiu, I.A., 2008 - Cercetări privind performanţele la fătare, în urma embriotransferului, la vaci din rasa Montbeliarde, Buletin USAMV-CN, 65(1-2)/2008 (-), ISSN 1454-2382

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