USE MOET PROGRAMME FOR DEVELOPMENT AND CONSERVATION OF SOME RACES OF BOVINE IN ROMANIA

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Abstract

In Romania there are populations of endangered cattle as Grey Steppe breed and Pinzgau of Transylvania and Romanian Buffaloes number is a marked decline. An important role in preserving the "ex situ" is biotechnology breeding populations vulnerable to conserve animal genetic information but not breeding material: gametes usually stored sperm as semen coming from a single parent (haploid) and embryos with 2 parents (diploid) population structure that allows preservation by freezing.
Material and method
MOET program implementation (Multiple Ovulation Embryo Transfer) to 4 gray cattle breed cow, repeated ovarian stimulation with FSH and PMSG intervals: 55, 60, and 115 and 157 days, and at 2 Holstein Friesian cattle every: 52 and 87 days.
Results
Following these repeated treatments Grey Steppe breed cows resulted in 99 of the corpus luteum (11CL/DR) from or taken Embryonic Formations 69 (7.66 FE / DR), of which 40 were embryos transferred and of these, 27 were frozen and stored in liquid nitrogen at -196 ° C. Following repeated application poliovulations FSH treatment every 52 to 87 days, 2 heifers HF, ovaries responded with a total of 31 CL 6.2-CL / DR to who prelevat12 FE and 2.4 FE / DR and 12 embryos were frozen blastocyst. FSH were poliovulations with a 4 cows and 6 Pinzgau of Transylvania Romanian buffaloes, a total of 10 female donors who have repeatedly poliovulations treatment, and the average number was the 8.4 CL / D, 1 CL lower than the average female with repeated poliovulations, the average number was 3.3 EFF EFF / D 3 also less than 6.7 EFF in the group of females with repeat poliovulations treatment.
Conclusion
We conclude that after several repeated hormone treatments poliovulations 6 embryo donor females found that health in general and particularly genital tract not suffer in comparison with the group of 10 females poliovulations once and morphology embryos was very good, allowing freezing and storage in gene banks

Key words: biotechnology, embryon, reproduction, bovine

INTRODUCTION

MOET raises fertility poliovulator only females treated several times to IA. Assuming that a cow M. T. 5 descendants are obtained by IA and are obtained by Moet 15, fertility has increased 3 times.
Scientific research in biotechnology breeding and their applications in practice zoo veterinary aimed mainly at increasing the number of livestock, livestock production, under increasing fertility, fertility and prolificacy. Preservation "ex situ" breeding animal biotechnology is to conserve animal genetic information but not breeding material: usually gametes, sperm stored as semen coming from a single parent (haploid) and embryos with 2 parents (diploid), which allows preservation of population structure by freezing (1.3). This method of protecting biodiversity is particularly useful for populations of rare or vulnerable state, not the obvious and immediate value in terms of trade and production. Of bovine monotocic species that an estrous cycle develops and open one mature follicle, ovulation latest development applied in the practice of multiple embryo donors became Moet. Moet relies on increasing donor embryo female fertility and hence the possibility of a higher selection intensity on the female sex bulls used in AI The multiple ovulation does not increase prolificacy donors because they do not girl born not many products, but only their fertility, their participation in designing a larger number of individuals in subsequent generations (5). Moet is a concept that can be used success, using embryo transfer in animals (6,8).

MATERIALS AND METHODS

All stages and phases of MOET program took place in SCDCB Dancu Grey Steppe breed Iasi (7), in Hunedoara County of Transylvania Pinzgau breed, SCDC Şercaia for buffalo and I.C.D.C.B. Baloteşti for Holstein-Friesian.
In our research conducted over several years at different locations bovine and we tried to give an answer to: poliovulations number of repetitions to be performed without damaging the overall health potential donor female genitalia and especially to preserve biological quality embryos and from what age can induce poliovulations the vines.
MOET concept (Multiple Ovulation Embryo Transfer) after M.PARASCHIVESCU (5) was initiated by Nicholas and Smith (1983) who considered embryo transfer (ET) as applied biotechnology to optimize genetic improvement of dairy cattle. Is multiple ovulation embryo transfer

stage biotechnological which makes a repeated poliovulations donor female (9). Poliovulation is the phenomenon of ovarian stimulation with FSH hormones or P.M.S.G. luteal administered during the oestrus following which are initiated and developed more follicles than normal is usually one species monotocic (6.8).

Steps in application MOET

Step - 1. Taken and preparation of donor females (D) embryos, comprising:

- Phase field: identifying females and gynecological clinical exameul, have more than 60 days after calving, the estrous cycle at regular intervals and without uterine infections, can take blood samples to determine the metabolic and hormonal profile in order to correct ration (9)

-phase Laboratory to determine progesterone (P4) and estrogen (E) ELISA kit DRG using EIA156.) (8.9)

Step- 2. Inducer poliovulation the donor and IA:

Includes phase field (application protocols poliovulaţie hormones FSH).

Protocol No. ET. .. / 2012

FARM:

Race ..... no. registration: D1 = ... D2 = ... ; Last calving: D1 .....D2 ..... + minimum 60 days post partum

V1. E.T.R. time-PGF 2 α .. I - date estrus after PGF 2 α I: D1and D2 and after 24 hours of estrus D1 and D2 is PGF 2 α I . R1; R2; R3: R4: R5 .

V2.+11days of PGF if I fall in heat will be the second PGF 2 α in D1 andD2 Day of estrus after PGF 2 α II and after 24 hours of estrus D1 and D2 is PGF 2 α II R1;R2; R3; R4; R5 . + 10 days after oestrus in D1 and D2 be Varianta1 (V1) and Version 2 (V2)

Poliovulation treatment of donors:

Hormone: FSH Pluset - company Serono, Italy, Laboratory of Spain

Day-1:….2012-AM-h7,00 -4 ml., im FSH/D1+D2

- PM-h.19 - 4 ml., im. FSH / D1+ D2

Day-2:….2012-AM-h7.00 -3ml., im. FSH/D1+D2

- PM-h.19 -3 ml., im, FSH / D1+ D2

Day-3: .2012 - AM-h.7,00 -3 ml., im. FSH / D1 + D2 of PGF 2 α II / R1-R5

- PM-h.19 -3 ml., Im, FSH + PGF 2 α / D1+D2

Day-4: ..... 2012 comments:

Day-5: ..... 2012-hour appearance will mark the donors who come into oestrus oestrus will ad. a 5 ml of receiver before performing PM

18-I h has I.A. x Code…….Taurus……..
Day-6: ..... 2012 AM-h II IA
+ 7 days after IA
Day of collection: 2012
Step - 3. Prelevation embryonic formation of embryos from donor female uteru. Phase Field: embryonic formation was taking the 7-day after IA, non-surgical techniques via the cervix, repeated lavage each uterine horn part, using type Folley catheters and Madi Dulbeco washing with PBS-BSA 0, 4%, and embryo collection has a special filter 22μ diameter.
Step - 4. Examination room equipped with a TV stereolupe and software acquisition and storage of images. Phase Laboratory formations were identified embryonic samples were evaluation and rated by international standards IETS Manual (2.4).

RESULTS AND DISCUSSION

Results concretized by the number of good embryos and blastocyst morula obtained from each donor handmade and embryos frozen embryo transfers, are presented in Table 1.
From Table no. 1, it follows that: 4 Grey Steppe cattle breed, repeated ovarian stimulation with FSH and PMSG intervals: 55, 60, 115 and 157 days, and at 2 Holstein Friesian cattle every: 52 and 87 days. Following these repeated treatments Grey Steppe breed cows resulted in 99 of the corpus luteum (11CL/DR) from or taken Embryonic Formations 69 (7.66 FE / DR), of which 40 were embryos transferred and of these, 27 were frozen and stored in liquid nitrogen at - 196 ° C. Following repeated application poliovulations FSH treatment every 52 to 87 days, 2 heifers HF, ovaries responded with a total of 31 CL 6.2-CL / DR to who prelevat12 FE and 2.4 FE / DR and 12 embryos were frozen blastocyst. FSH were poliovulate with a 4 Transylvanian Pinzgauer breed cows and laughter consisted of 34 ovarian CL, respectively 8,5 / D, which were taken 10 FE 2.5-EF / D and were frozen and stored 7 embryos. Were also poliovulate with FSH of 6 Romanian buffaloes and ovarian laughter was the CL 50 or 8.4 / D, which were taken 23 FE or 3.8 FE / D and have, were frozen and stored 16 embryos.

Table no. 1

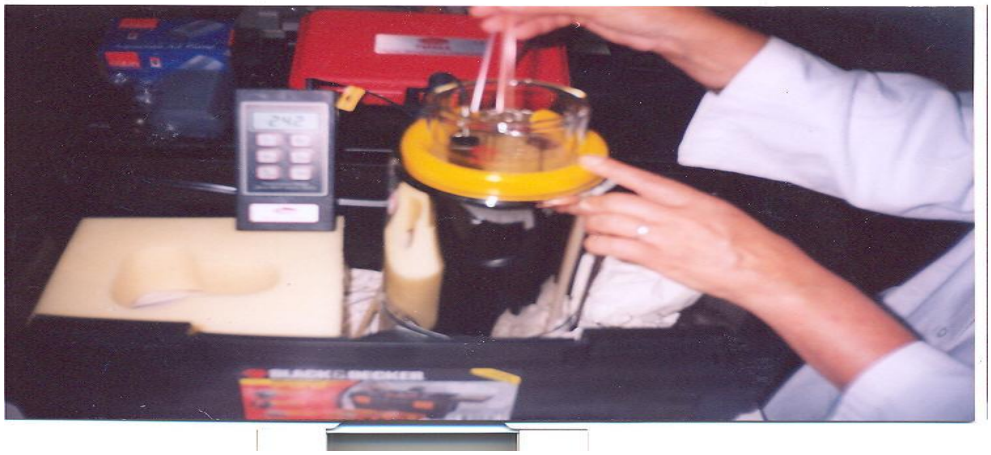
RESULTS OF COLLECTION EMBRYOS FROM DONOR

No Crt.	Matricola of the donor	Date of collection day 7th	Interval in days from removals and FSH / PMSG	No. C.L. and Embryonic Formations Collected		Quality embryos M= morula Bl= blastocyst E.D.= embryos degenerate			Embryos transfer	Embryos frozen in EG or GL
				Nr. C.L.	Nr. F.E.C.	M	Bl	E.D. Ov.		
1	9999 Rec.1	05.04.06	FSH1000	9	6	2	1	3	3	-
	9999 Rec.2	05.06.06	I-II=60	17	7	0	0	7	0	0
2	0005 Rec.1	05.06.06	FSH	6	5	-	4	1	4	
	0005 Rec.2	08.10.06	I-II=115	9	0	0	0	0	0	0
3	0007 Rec.1	08.10.06	FSH	11	9	-	7	2	-	7 - EG
	0007 Rec.2	15.03.07	I-II=157	16	16	3	9	4	-	12 -GLY
	0007 Rec.3	10.05.07	II-III=55	13	12	5	7	-	6	6 - EG
4	0006 Rec.1	15.03.07	PMSG	13+6chi	9	-	1	8	-	1 - GLY
	0006 Rec.2	10.05.07	I-II=55	5	5	1	-	4	-	1 - EG
	4 Donoros Grey Step		m=55 M=157	99 (11 /R)	69 (7,66/R)	11	29 = 40 (5,44 Bl / D)	29	13	27
1	3618HFViţea	11.06.12	FSH500ui	7	4		4	0	0	4- EG
	3618 HF-14 l	03.08.12	I-II=52	7	7		7	0	0	7- GLY
2	1092HF heifer	11.06.12	FSH500ui	4+11chi	0		0	0	0	0
	1092HF-15 l	03.08.12	I-II=52	9	0		0	0	0	0
	1092HF	30.10.12	II.III=87	4+10chi	1		1	0	0	1- EG
	TOTAL 2-D Heifers HF		m=52 M=87	31 (6,2/R)	12 (6/R)	0	Bl-12			12
	6 donors Repet poliovulation		m=52 M=157	CL.130 (9,28/R)	FER.81 (6,7/R)	11	Bl-41 Total 52	29	13	39
1	39721-Pz	04.04.11	FSH500ui	11	0		0	0	0	0
2	91641-Pz	04.04.11	FSH	12	6		3	3	0	3-E.G.
3	0749-PZ	15.04.11	FSH	0	0		0	0	0	0
4	9506-Pz	15.04.11	FSH	11	4		0	0	0	4-E.G.
	TOTAL 4 -D. Pinzgau de Transilvania			34 (8,5 /D)	10 (2,5/D)	0	Bl-3	3		7
1	2761- B.	28.07.10	FSH1000	8	5		0	5	0	5- GLY
2	0014- B	28.-7.10	FSH1000	5	3		0	3	0	3-GLY
3	0133-B	23.08.10	FSH1000	8	0		0	0	0	0
4	26888-B	23.08.10	FSH1000	12	7		0	7	0	0
5	93037-B	29.03.11	FSH1000	10	5		5	0	0	5-E.G.
6	26810-B	29.03.11	FSH1000	7	3		3	0	0	3- E.G.
	TOTAL 6 donors Buffalo			50 (8,3 /D)	23 (3,8/D)		Bl-8	15	0	16
	10- D. poliovulation			84 (8,4/D)	33 (3,3/D)		Bl-11	18	0	23 Total 62

Step - 5. Freezing embryos
Laboratory-phase: good and very good embryos in morula stage (M) or blastocyst (Bl) were processed by passing at least 5 successive baths BSA0 PBS + 4%, each time changing the pipette tip and the last bath was used trypsin were then împaietaţi in the freezing glycerol or ethylene glycol, the Mid sparkles between two bubbles and two columns environment.
What 27 Grey Steppe breed embryos were frozen in glycerol (Gly) 10% - 13 and Ethylene Glycol (EG) of 1.4 M -14. The straw will write the code number of staff authorized to transfer, date of collection, number of embryos and stage of development (M or BI), registration number and name of donor-breed, number and name mareicol bull-breed. Freezing these embryos was gray cattle breed with a portable freezer E.Robertson type that does not use electricity. (Foto.nr.1)

Foto.nr.1

Portable freezer type E.Robertson



Portable freezer equipment is:
- exterior that Dewar vessel serves only as a container for liquid nitrogen (LN2).
- Inner Dewar vessel having double walls and a controlled vacuum from walls, like a thermos, is the only piece absolutely necessary to freeze embryos.
To start freezing, inner Dewar vessel filled with 350 to 400 ml of ethyl alcohol or methyl and outer Dewar vessel immersed in liquid nitrogen LN2.
Straws embryos are placed in the bowl with alcohol and freezing diagram starts at room temperature 18 -20 ° C with a rate decrease of 0.5 to 0.7 of

temperature ° C / min up to - 6 ° C, when alcohol and sparkles vessel is removed from the vessel with nitrogen and induce crystallization (seeding) with forceps previously cooled in liquid nitrogen. When all the sequins were plucked and alcohol temperature reached - 7 ° C, the vessel with alcohol and sparkles again to reintroduce nitrogen vessel where cooling is continued until the temperature of -32 degrees. Then sequins to remove and plunge into liquid nitrogen at -196 ° C, then pass the goblet and stored in the container.

Others, 35 embryos, collected from other races donor HF, Pz and Romanian Ox were frozen in 1.4 M EG was used freezer type Freeze Control (Photo No. 2.) With a rate of decrease of 1 C / min to -7 C, when the seeding induced for 10 minutes, then the temperature of 0.5 C / min.până at - 35 C, then plunges directly into liquid nitrogen

Foto nr.2

Freezing of gametes and embryos automatic with freezer

FREEZE CONTROL®

CryoPreservation Systems

CONCLUSION

It was found that the health in general and genital apparatus especially the 6 female donor (4-gray cattle and 2-HF) embryos, had suffered from the 14 treatments with FSH hormone repeated at intervals minimum of 52 days and a maximum of 157 days and ovaries response was 9.28 on average CL / DR to the average of 8.4 CL / D of the 10 donor females once poliovulate (4-Pz., and 6 buffalo).

The 2 HF heifers that were repeated hormone treatments with FSH at a minimum intrval 52 days and maximum 87 days, were aged 14 months and 15 months-3618-1092 and post-treatment ovarian response was an

average of 6.2 CL / DR without further consequences of cyclic ovarian activity.

From 6 donors were sampled 52 high-quality embryos, 39 embryos were frozen: 20 to Gly and 19 in EG and stored in liquid nitrogen containers at -196 ° C, the genetic resource banks: SCDCB-Dancu, General Berthelot and CSCBA "Acad.David Davidescu" - Bucharest. Were immediately transferred to 13 recipient embryos Grey Steppe breed.

MOET (Multiple Ovulation Embryo Transfer) is a biotechnology applicable in optimizing embryo transfer genetic improvement for multiplication and preservation of breeds of cattle.

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