# POSSIBILITIES TO INDUCE TWIN CALVING IN COWS BY EMBRYO-TRANSFER

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#### Abstract

The aim of our paper was to induce twin gestations in cows, by transferring frozen embryos. The embryos necessary for transfer were obtained from 12 donor cows from Romanian Black Spotted breed, super ovulated with FSH pituitary extract (Foltropin-V). Embryos were no surgically recovered at 7 days after the induced estrus, and those that were in 1 and 2 quality code (after IETS Manual) were frozen. At 11 recipient females we transferred embryos no surgically, one embryo for each uterine horn, 6 females were diagnose as pregnant (54,5%), from which 5 carried the gestation to term. From those, 3 cows had twin calving (60%) and 2 had simple caving (40%). 12 recipient cows received two embryos, by no surgical embryo -transfer, on luteal body ipsilateral uterine horn. 6 females have been diagnose pregnant (60%). From those, 2 cows had twinned calving (33,3%) and 3 cows (66,7%) had simple calving. The twin calving at the females that had one embryo transferred in each uterine horn was higher (60%) comparative to the females that had two embryos transferred into the luteal body ipsilateral uterine horn (33,3%).

Key words: cow, embryotransfer, twin calving

## INTRODUCTION

Twin calving inducing at monotocic species can be realized by administering low doses of folliclestimulating hormone (FSH or PMSG) or by embryo-transfer. In the case of embryo-transfer two embryos can be transferred at each recipient cow or an extra embryo can be transferred at the beginning of the natural gestation (days 6-7).

In dairy cows, to prevent the producing of sterile females (freemartinism), sexed embryos must be transferred, in meat cows not sexed embryos can be transferred [3].

The surgical transfer of an embryo in each uterine horn or two embryos in the ipsilateral luteal body can give gestation rates of 70%. The twin calving rate after the transfer of one embryo in each uterine horn is 70-72 %, compared with the gestation rate in the case of the transfer of two embryos in the same uterine horn (45%) [1].

Newcomb (1982) have reported gestation rates of 60% by nonsurgical transfer, form which 50% were twins.

The aim of our paper was to obtain twin calving from dairy cows by nonsurgical embryo-transfer.

## MATERIAL AND METHOD

The embryos necessary for embryotransfer were obtained from 12 donor cows of Romanian Black Spotted. Embryos donor cows were superovulated using the protocol described by Robertson Ed. (2003). As follicle-stimulating hormone we used pituitary extracts of FSH (Folltropin-V) and two subcutaneous implants with Norgestomet (Synchro Mate B).

Day	AM	PM			
0	Introduced the first SMB implant + 2	-			
	ml injectable SMB solution				
5	Introduced the second SMB implant	-			
	(without injectable solution)				
6	50 mg FSH	50 mg FSH			
7	50 mg FSH	50 mg FSH			
8	50 mg FSH	50 mg FSH			
9	50 mg FSH	50 mg FSH			
	+ 25 mg Lutalyse	+ 25 mg Lutalyse (redraw both SMB implants)			
10	-	-			
11	IA	IA			
12	IA	-			
18	Recovery, transfer or freezing	-			
21	25 mg Lutalyse	25 mg Lutalyse			

Figure 1. Donor cows superovulation inducing protocol with FSH and two Norgestomet implants (Robertson 2003)

The day in which the implants were introduced was considered the 0 day of the ovary stimulation protocol. The implant was applied to the internal face of the ear simultaneous with this we also administered 2 ml SMB (Syncro Mate-B) of injectable solution. In the 5<sup>th</sup> day we introduced the second SMB implant without administering the injectable solution. In the  $6^{th}$  day we started the superovulatory treatment with pituitary extracts of FSH (Folltropin-V), in a total dose of 400 mg, divided in 8 doses 50 mg each. The FSH doses were administered at 12 hours intervals. In the 9<sup>th</sup> day of the superovulatory treatment, in the same time with the last two FSH doses, we administered 25 mg Lutalyse (PgF2 $\alpha$ ). The two implants redrawn after the last were FSH administration (the 9<sup>th</sup> day). There were 3 A.I. performed, two in the morning and evening of the 11<sup>th</sup> day and the third in the morning of the 12 day. In the 18<sup>th</sup> day from the beginning of the hormonal stimulation (the 7<sup>th</sup> day of the induced estrus) embryos were non surgically recovered. At 3 days after embryo recovery 25 mg Lutalyse was administered to the donor cow.

Embryo quality was established after IETS Manual (1998). Embryos in 1 or 2 quality code were frozen by controlled freezing procedure, with the help of Biocool IV freezer.

In order to obtain twin calving, two embryos per female, one each uterine horn, were nonsurgical transferred at 11 recipient females, reproductively correlated with the embryo developmental stage. At 12 recipient females, there were transferred two frozen embryos both in the luteal body ipsilateral uterine horn.

## **RESULTS AND DISCUSSIONS**

The results obtained are presented in table 1 and 2.

In table 1, we presented the results obtained at the transfer of one embryo in each uterine horn.

At the 11 recipient females 22 embryos, appreciated as viable after thawing, were nonsurgically transferred. From the 11 females, 6 (54.5%) were diagnosed as pregnant, from which one aborted. 5 cows carried the gestation to term. From these 3 cows calved twins (60.0%), and 2 cows calved each one calf (40.0%).

In table 2 we presented the results obtained after the transfer of two embryos in the uterine horn ipsilateral to the ovary that presented the luteal body.

To the 12 recipient females a total of 24 embryos, appreciated as viable after thawing, were transferred. From the 12 females, 6 were diagnosed as pregnant (50.0 %). From the 6 pregnant cows, 2 had twin calving (33.3%), and 4 had simple calving (66.7%).

60.0

2

40.0

Results obla	ineu by transfer	ing one ei	noryo in eac						
Recipient	Transferred	Pregnant females		Calved females					
females	embryos	n	%	n	Twin o	calving	Simple	calvin	
(n)	(n)				n	%	n	0/2	

5

3

54.5

Table 1 Results obtained by transferring one embryo in each uterine horn

6

Table 2

11

Results obtained by embryo-transfer two embryos into the same uterine horn

Recipient	Transferred	Pregnant females		Calved females					
females	s embryos	n	%	n	Twin calving		Simple calving		
(n)	(n)				n	%	n	%	
12	24	6	50.0	6	2	33.3	4	66.7	

From these experiments it can be concluded that gestation rates after the transfer of two embryos, with the aim to obtain twin calving, are similar (54.5-60.0%).

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In respect to the twin calving, the proportion is higher (60.0%) to the recipient females at which there were transferred one embryo in each uterine horn compared to the recipient females at which there were transferred two embryos in the luteal body ipsilateral uterine horn (33.3%).

## CONCLUSIONS

- After the nonsurgical transfer of one embryo in each uterine horn, twin pregnancy rates are higher (60,0%), compared to the transfer of two embryos in the uterine horn ipsilateral to the ovary that the luteal body is present (33,3%).
- Because of the rising of the incidence of intersex females, resulted from the birth of twins of different sex, twin calving

with unsexed embryos can be used only in the case of meat calf's.

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