

## ARTIFICIALLY FORCED FERTILITY IN DAIRY CATTLE EMBRYO TRANSFER

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### **Abstract**

*Forced increased fertility in cattle can be obtained by multiple ovulation embryo transfer (MOET). This paper presents results of a trial to shorten interval between two pollyovulation treatments to the term determined by the natural estrus shown by donors after the previous embryos' collection. Pollyovulation was provoked using FSH. Experiment started with 4 Romanian Black and White heifers, 17 month aged, but only two of them answered to the treatment for estrus synchronization. Both these heifers have shown heat in the same day, at 11 days after uterine washing. A total number of 27 yellow bodies were counted (14 units in one heifer and 13 in the other), but only 12 embryos were collected (11 embryos from the first heifer and 1 embryo from the second). There is no explanation of the fact. The resulted interval between pollyovulation treatments was 53 days, 7 days less than the 60 days interval recommended by literature. More interesting was the presence of a total 26 atretic follicles in the 4 days (diestrus state) of embryo collection. They must pertain to the first wave of follicles starting maturation after prostaglandin injection given at embryo collection to prevent pregnancy. The fact suggests the possibility to stimulate these waves of follicles to have shorter interval between pollyovulation treatments. It is counted that the interval could be shortened up to 25 days. If that is possible MOET could be applied in heifers to increase selection precision of mother of sire dams by progeny testing them. New idea is to use MOET in dams of known genetic merit to discover better compatibility of parental pairs to obtaining the wanted type of the breed. Experiments are needed also to establish if more frequent uterine washings cause or don't cause alteration of uterine mucosa functions.*

**Key words:** dairy cattle, embryo transfer, poliovolutions, cow fertility.

## INTRODUCTION

Berge S. says "fertility is the property of mammals to give birth to viable progeny" (Berge S., 1965). That means fertility (F) is a trait of organisms like anyone else which can be measured for individual animals or for groups of animals. Fertility quantification is done by the number of progenies given in determined time, usually per year or per life. Paraschivescu M proposes to measure the fertility of cows also by the calving interval (CI) (Oțel V., Paraschivescu M., Mihăilescu C., 1967).

Artificial Insemination in dairy cattle has given the possibility to tremendously increase the fertility of bulls. Par consequence that has permeated selecting sires for artificial insemination with great intensity and precision (vertical genetic improvement) and also a large dissemination of their traits within the breeds (horizontal genetic improvement). Results are already wonderful. Theoretically nothing could be added in testing bulls.

But people wants more and try to get it by a higher precision in selecting sires dams. They might get it if would induce sufficiently high fertility in heifers tested to become mother of sires and in cows with known genetic merit already selected as breed dams. Such hopes are related to increased performances of Multiple Ovulation Embryo Transfer (MOET) especially by shortening the interval between embryo collections.

As it is known cow fertility is low because ovogenesis is stopped in the embryonic stage of ontogenesis and the number of emitted gametes is limited at the number of the primary follicles in the ovary. In addition, naturally, only one ovary follicle is dehiscent in one estrus cycle, even more of them entered maturation. More than that after the ovum fertilization a long gestation has to be run in order to allow the development and growth of one new born calf. Pregnancies with twins are not well sustained by dairy cows and the trait is not wanted in the commercial dairy farms. Then it is necessary to call for receptor foster (or surrogate) mothers after pollyovulation by hormonal treatments has been induced in donor mothers.

The present experiment is limiting its goal to start investigations concerning the shortest

interval between ovulations keeping acceptable pollyovulation rate and normally formatted embryos. Questions as having good conception rate in receptors after embryo transfer or of normal fertility in donors after embryos' collections are not covered by the present paper. Nevertheless the experiment was put on donor heifers, having in view future research on this theme.

## MATERIALS AND METHODS

For the initial estrus synchronization of donors we disposed of 4 heifers aged 17 months. They were clinically inspected for the normal development of the ovaries and of the genital tract. Nevertheless only 2 of them had well formatted yellow body and responded to the normal standard of diestrus after prostaglandin treatment for estrus synchronization. Treatment has been done at 8 hour in the afternoon and the two of them answering to the treatment showed heat after in the third day after 72 hours from the prostaglandin injection.

That was the 0 day of the pollyovulation treatment. Maturation of ovary follicles started in the 11<sup>th</sup> day. (E. Robertson 1999).

Both heifers received the same treatment by intramuscularly injection following the next schedule (Tobă G. et al., 2011):

-11<sup>th</sup> day at 7 hour a.m. 4 ml FSH,  
-11<sup>th</sup> day at 7 hour p. m. 4 ml FSH;  
-12<sup>th</sup> day at 7 hour a.m. 3 ml FSH,  
-12<sup>th</sup> day at 7 hour p.m. 3ml FSH;  
-13<sup>th</sup> day at 7 hour a.m. 3ml FSH, 13<sup>th</sup> day 7 hour p.m. 3ml FSH + 2ml PGF<sub>2α</sub>.

In the 14<sup>th</sup> day from the treatment supervision for proestrus signs was provided. No sign was noticed.

In the 15<sup>th</sup> day at 17 o'clock 3 ml of GnRH (Receptal) were inoculated and after 2 hours (at 19 o'clock) heifers have been inseminated. Insemination was repeated after 12 hours at 7 o'clock in the next morning.

Embryo collection was provided by uterus washing with buffered solution in the 7<sup>th</sup> day after insemination. It started with the counting of number of yellow bodies and of atretic follicles on the ovaries. Inoculation of 2 ml of PGF<sub>2α</sub> and 3ml of GnRH was done after washing in order to avoid occasional pregnancy.

The next follicular stimulation was decided to take place after the first not induced estrus by repeating the same procedure for pollyovulation as before.

The entire experiment protocol with the two accepted heifers is presented in table 1. There is a perfect synchronization of the ovarian activity in both heifers and in both cycles

Table 1 Treatment timing in the two heifers

Donor heifer	Order of treatment	The 0 Day	Follicular stimulation	Insemination	Washing
3618	I	19.05	30.05-01.06	04.06	11.06
	II	11.07	22.07-25.07	28.07	03.08
1092	I	19.05	30.05-01.06	04.06	11.06
	II	11.07	22.07-25.07	28.07	03.08

Collected embryos were classified for the biological quality and were frozen in yellow straws when ethylene glycol crioprotector was used (first collected embryos) and in white straws when glycerol as cryoprotective agent was used (second embryo collection).

## RESULTS AND DISCUSSIONS

Two heifers have out of four shown well developed ovaries after the first estrus synchronization. The 50% of not answering to the treatment heifers at 17 month of age show unfair life conditions of the replacement heifers of the farm.

The 0 day interval between the two embryo collections was 53 days, 1 week less than the minimum interval recommended by literature. It includes 11 days after the naturally expressed estrus by the heifers, what happened to take place in the same day. The term of 11 days is the middle of diestrus when the prostaglandin injection for new synchronization was done.

Trying to understand how the 53 days interval is formatted we will find: the above mentioned 11 days, other 11 days + 3 days for the next pollyovulation treatment, 3 days up to heifers' insemination and 7 days passed up to the second embryos' collection. That means a total

of 35 days. There is a difference of 18 days that remains for the natural estrus cycle proposed by the experiment protocol to have place between the two induced heats. This assertion has to be accepted since it respects the variability limits of normal ovary cycle (21±3 days) in cattle.

We can conclude that the applied hormonal treatment did not impaired normal function of the ovary. We can consider, also, that the second estrus synchronization wasn't necessary since the natural estrus was synchronized in the two heifers. If that's true the inter pollyovulation treatments' interval might be reduced to 39 days (the actual 53 days interval - (11 + 3 days for the second induced estrus synchronization).

The ovaries' answers to poliovolutions stimulation are presented in table 2.

Table 2 Ovary answer to the poliovolutions stimulations

Donor heifer	Order of treatment	Ovary answer		
		Yellow bodies	Antral follicles	Collected embryo
3618	I	3 L + 4 R	3 L + 3 R	4
	II	4 L + 3 R	1 L + 1 R	7
1092	I	2 L + 2 R	6 L + 5 R	0
	II	5 L + 4 R	3 L + 4 R	1

Looking figures in Table 2 first thing to be noticed is the big difference between heifers concerning the number of collected embryos. After the first induced poliovolutions 4 embryos have been collected from the 3618 heifer and 0 embryos were collected from the heifer 1092. At the second attempt the difference was even greater (7 to 1). Since the embryo washing was done by the same operator using the same procedure the difference must have a biological explanation. Which one it might be we can't say.

No unfertilized ova were found 7 days after follicular dehiscence.

Remarkable thing is the number of antral follicles in the 7<sup>th</sup> day after insemination what

means in the 8<sup>th</sup> day after the previous estrus. Totally in 4 diestrus states 26 antral follicles were detected. They must pertain to the first wave of follicles entering maturation of the cycle induced by the prostaglandin injection that has commanded estrus. Then why not try to stimulate maturation of this wave of follicles, too?

There is a hypothesis that has to be verified. Using the same presented schedule this stimulation can start 13 days after the prostaglandin injection. Then 5 days later the donor heifer can be inseminated. If maturation had success embryos should be collected after 7 days. Thus the interval between two successive washings is reduced to 25 days (the above 13 + 5 + 7 days). That makes possible to have 5 embryo collections within 101 days. If 5 embryos were collected by one washing we would dispose of 25 embryos to be transferred. With the 50% sex rate in embryos and 60% pregnancy success obtained using E. Robertson's procedures of freezing embryos under ethylene glycol protection, about 7 – 8 daughters might be got from one donor heifer (Paraschivescu, M. Th., 2010). If the number of successive polyoovulation stimulations is increased the donors' fertility is larger.

Now, what benefit could be obtained increasing females' fertility in cattle breeding?

The question has particular response in case of heifers and in case of adult cows.

Since in dairy cattle the genetic merit of heifers for milk production can be estimated only by ancestors' performances and the heritability coefficient is low ( $h^2=0, 2$ ) there is no interest to increase their fertility. Nevertheless it could be of interest if heifer would receive such a high fertility as to be progeny tested for milk production (Paraschivescu M. et al. 1989). In this case dams' selection to produce sires for artificial insemination will become much precise. But the breeder will be confronted with some difficulties. The first one is the future dam must have enough number of daughters to be tested on progeny (in bull progeny test at least 30 daughters are required for a 70% repeatability of the result) (Paraschivescu M. et al. 1989). The second one is the desire to have the result of the test as early as possible so that

she would get in calf at least 4 -5 times after the result of her progeny test is known. Other way the procedure is too costly to be practically applied (Paraschivescu, M. Th., 2010). To reduce costs sexing embryos for female become of interest.

If the donor is a dam of known genetic merit increasing her fertility is always desired. Progeny of any sex are wanted.

The forced fertility could bring a new idea in using the breeding stock. That is to find out the genetic combinability of partners. The idea is used in poultry breeding when industrial hybrids are obtained. In cattle when a registered cow receives the merit of dam after her first parturition she can be included in an individual test program for compatibility with bulls of different families. The program can start with the first follicular stimulation for polioovulations in the 13<sup>th</sup> day from day of heat. Then the treatment schedule mentioned in the present paper could be applied.

## CONCLUSION

Efficient polioovulations and embryo collection can be applied using the treatment schedule and uterus washing terms presented in this paper. Individual differences in the in the heifers answer to the treatment can't be excluded. Further ovary function is not affected by the treatment.

The presence of many atretic follicles on ovaries in the day of embryo collection suggests that the 53 days interval between treatments for polyoovulation could be shortened up to 25 days if a new stimulation of ovary follicles stimulation is promoted 15 days after embryo collection and prostaglandin injection has to avoid pregnancy.

Experiments to estimate the effects of more frequent uterine mucosa must include longer chains MOET components. Success of these procedures opens new ways to use MOET in genetic improvement of breeds.

With heifers increased precision in selecting dams as mother of bulls for AI could be obtained testing them by progeny.

With cows of known genetic merit the compatibility of dam and sire pair could be discovered if insemination of donors cows at

repeated embryo collections is done using semen of different bulls.

The program gives the possibility to elect among the best made combination of parental pairs.

In order to reduce cost of such a program cheap receptor heifers of beef breeds. Using receptor heifers there is chance to have better embryo transfer pregnancy rates than using receptor cows (Paraschivescu M.Th. et al.,2003).

The main risk of forced increased fertility results from infiltration of uterine tissues during washing embryos. Less danger is apart Prostaglandin or FSH hormones since they have a great division speed.

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