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ARTIFICIAL INSEMINATION PROGNOSIS IN CATTLE AT THE WORLD AND NATIONAL LEVEL

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Abstract

There are 20 years since the title "Artificial Insemination dynamics and prognosis in cattle" was the presented thesis by me as a graduate student. A 20 years period is long enough to find out if the dynamics and the prognosis were true. That time Artificial Insemination was appreciated as a strong mean to prevent venereal diseases, and to increase bulls' fertility. It was also seen as a needed biotechnical in ET technology. From the scientifically point of view spermatozoa sexing was then preview the term of fertilization ability of sperms being fully satisfied. In breeding practice AI was seen as a very efficient mean to induce genetic progress using progeny tested sires and ET as short term way to transfer breeds of interest to new areas. It was considered AI mostly will be applied in dairy breed where all active populations will be reproduced by artificial insemination. In extensive systems of beef cattle farming AI will be few extended because of too much labor needed. For Romania AI has to be extended since the action continued to be sustained by the public finance that paid the deep frozen semen used by cow owners. They had to pay inoculation of semen only. Excepting the last prognosis all other of them fulfilled. But AI association to the other biotechnologies development has shown richer than it was then specified. In the present form progresses in biotechnologies AI assisted will be more discussed. Sperm sexing, MOET, IVF, Embryo sexing, artificial identical twins genesis, Mammal animal clone production and engineering of transgenic organisms are mentioned in relation with new organism genesis and their security food or economic importance.

Key words: *biotechnologies of reproduction, IVF, mammal animal cloning.*

INTRODUCTION

About 20 years ago I have presented "Dynamics and Prognosis concerning Artificial Insemination in Dairy Cattle" as the graduate degree in animal husbandry (Paraschivescu M. Th., 1997). The subject was interesting that time because there was a political decision of changing Artificial Insemination (AI) organization from a state one to the private ownership, based on adopted German Project paid from UE founds.

My report had to preview the dynamics of AI spreading in dairy cattle production units that were privatized, as well, and to refer about the biotechnical progress using AI in bioengineering.

From biotechnological point of view, AI had got already that time, the top of possibilities in increasing the sires' fertility, since the deep frozen semen preserves its fertilizing power for very, very, long terms and the number of spermatozoa per doses could be decreased to 20 million or less. Great success was the high selection in precision of sires by progeny testing them for milk production. Prevention of genital diseases in cows was satisfactory solved as well. Better conception rate and lower cost per calving were wanted. Practical extension of AI in dairy cattle was to be expected everywhere, up to the degree of becoming exclusive even in the commercial farms. It was a large international offer of semen apart from many countries.

The spermatozoa sexing remained a question to be solved. The target of new approaches in AI biotechnical progress had become using AI in assisting embriotransfer biotechnology both *in situ* and *in vitro* fertilization in order to answer the specialization tendency of cattle breeds. New trends in cattle breeding required disposing of cows able to biologically producing *in vivo,* in their milk, organic medicines.

LATER EVOLUTION OF AI

From the time of the mentioned report presentation had past 20 years. Having in view that I have been interested afterwards in some AI assisted reproduction biotechnologies, I thought 20 years is enough time to appreciate if prognosis was made right or wrong. Further discussions will refer to the AI biotechnics targets concerning direct effect on the dairy farm economy and on the peculiar adaptation as an adjuvant biotechnics for new approaches to using biological production of cattle.

BASIS TARGETS OF AI BIOTECHNICS

The main targets of research in AI biotechnology during this period of time have been increased conception rate and reduced costs of artificial insemination along with controlling sex of the progeny and assisting bioengineering technologies for GMO.

In order to increase the conception rate and reduce the AT service costs 3 models were followed: a) "do it yourself" insemination model addressed to the cows' owners,(Hafez B., Hafez E.S.E., 2000, Paraschivescu M.Th., 2000, Robertson E., 1999) b) dispatching the frozen semen to small territorial depots of the private veterinarian net (Paraschivescu M., 1982) or to permanent AI points financed by the AI Centers and (Paraschivescu M., 1982) c) dispatching semen every day through the itinerants AI operators employed by the AI centers.(Oţel V. et al., 1967)

a) The "do it yourself" model was promoted by the American cooperative AI Centers in order to have better approach for the optimum insemination moment and a diminished insemination cost. The possibility of "Direct insemination", that uses the straw with deep frozen semen preserved in ethilenglicol crioprotector, without open it was well received especially by farmers for "do it yourself" model of artificial insemination (Robertson E., 1999).

b) Dispatching of frozen semen to small territorial depots of the private veterinarians reduced the transport cost per dose of semen. The model was successfully implemented by Neustadt am Eisch Kunstlike Besamung Verein, greatly reducing semen dispatching cost and creating a stronger relation between the AI Center, Veterinarians and farmers. The system of AI points is preserved in Romania, but their acting rules are still confused after SEMTEST enterprises were privatized.

c) Everyday dispatching of semen was secured by many AI Centers since the model ensures good instruction of inseminators under semen producer control.

The preferred model is a question of local decision of AI Centers.

Reducing semen preservation cost by lyophilization of sperms lost interest. (Oţel V. et al., 1967)

Sperm sexing, what means separating the X chromosomes caring spermatozoa from Y chromosome caring spermatozoa, has been tried in many experiences. The experiments' goal was to obtain doses of semen certainly containing one kind of chromosomes X or Y.

In this respect 2 hypotheses were thought. The first one intended to separate the spermatozoa based on the fact that the X chromosome is the largest one and the Y chromosome is the smallest one in the gamete genome. Par consequence the caring X chromosome spermatozoa should weigh more than the y caring ones and might be separated by centrifugation.

Even there were some claims of success, the hypothesis didn't confirm. Or the centrifuges weren't sensible enough or, more probably, there are other components of sperms varying in weight and covering the difference in weight between the two mentioned chromosomes.

The second hypothesis, which looks more hopefully, is based on the complementary of genes in their pair formation in diploid genomes.

In the diploid cells genes make pairs with their copies or their mutants, only. So a small segment of Y chromosome, treated to become fluorescent, is multiplied by PCR technique and mix with the diluted semen. Thus the caring Y chromosome spermatozoa attach the fluorescent segment in pairs of genes and became fluorescent them self.

The obtained material is passed in a continuous flow through a special item able to separate the "male" spermatozoa caring Y chromosome. This is the so called FISH biotechnics of sperm sexing. (FISH makes the acronym from

"Fluorescent *In Situ* Hybridization"). (Paraschivescu M., Tibără Dana, 1989).
If FISH biotechnics of sperm sexing will get commercial application more extension seems to have using X caring sperms in commercial dairy farms avoiding the birth of male calves which are not wanted.
That will reduce the quantity of necessary milk for producing the new heifers for the farm and a higher inside herd selection intensity. Using of Y chromosome sperms is convenient for dams (mother of bulls) the only ones which are in small number in breeds.
But, par consequence, no knowledge about the genetic merit of the female sibs of the bulls will be received for the animal model BLUP. Of course there will be an interest to have only male progeny out of dams that are progeny tested.

IN SITU AI ASSISTED BIOTECHNOLOGIES

The great success of AI in increasing fertility of special progeny tested sires induced idea of increasing fertility of dams, as well. But things look differently. Meanwhile spermatogenesis and spermiogenesis are continuous processes whose product is delivered out of the organisms, oogenesis is a stopped process, within which the order I oocytes preserved in meiotic arrest in ovarian follicles of female embryos. From there they restart to maturate, one by one, periodically in pubertal female and are fertilized inside her genital tract.
More than that, the cow or heifer uterus ordinarily accepts the development of only one embryo. It is obvious that in order to put in value more than one oocyte at one ovarian cycle the intended has to be assisted by *in situ* artificial insemination and completed using foster mothers (receptors) for outside development of embryo surplus. The issue act to obtaining higher fertility of one cow is to induce polyovulation instead of single ovulation.
That is possible by stimulating follicular maturation with the gonadotropic FSH pituitary hormone. The time of ovulation should be selected in such order that the polyovulation consequence should be longer and it is recommended that the artificial insemination to be done twice at about 10 hours interval.
The last target in the field is Multiple Ovulation Embryo Transfer (MOET). The last possibility to increase the number of ova that could be fertilized from a cow would be to repeat polyovulation at shortest possible terms. That means MOET. Hypothetically the shortest term in MOET is to apply the FSH treatment immediately after uterus washing for embryo collection and inseminate the donor 10 and 11 days later.
Next washing should have place 7 days later. But nobody organized such experiment. The shortest tried term by us was applying FSH at the end of the next heat after washing and planning the artificial insemination 10 and 11 days later (Paraschivescu, M.Th. et al., 2015). Unfortunately, because of poor research resources only two heifers could be used and so erratic answers were received. Doesn't matter what term from washing of the uterus to the new follicular stimulation is engaged it is strict to know how many times uterus might be washed staying healthy (Wrathbal A., Sietmoleler P., 1998). In the mentioned experiment (Paraschivescu M.Th. et al., 2015) this problem couldn't be included. Particularly for heifers it is important to know which is the earliest age (prepuberal heifers are not excluded) which is permitting follicular stimulation and how many times could be repeated without injuring their normal development and gynecology (Wrathbal A., Sietmoleler P., 1998). Full success of such experiments in this field will be when progeny tested dams might be selected in dairy breeds improvement. Nevertheless some special MOET farms were imagined (McGuirk B., 1993, Paraschivescu M. Et al, 1989, Paraschivescu M.Th., 2010).
The last two projects, communicated but not published, refer, the first one, to a **closed MOET Farm** (fig.1) receiving embryos from AI Centers interested in ET, selling male calves for progeny testing and using heifers as receptors (foster mothers) and the second one, to a **opened MOET Farm** (fig.2) receiving embryos from the same sources but using as surrogate mothers beef cattle heifers and selling progeny of both sexes. In the last case the interest for sperm sexing is obvious.

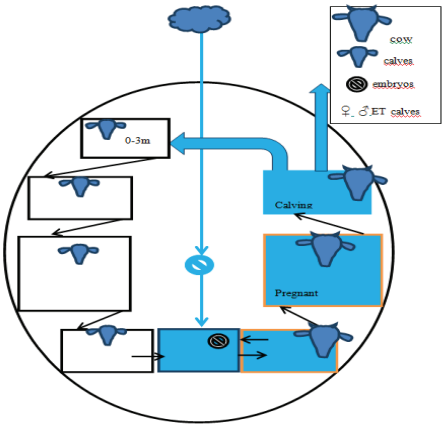

Fig.1 Closed dairy cattle MOET farm

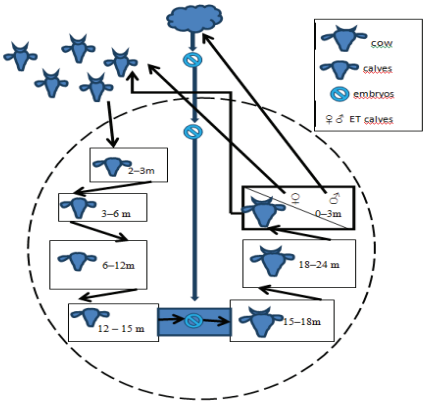

Fig. 2. Opened dairy cattle MOET farm

In the types of *in situ* AI attended biotechnologies embryo splitting and embryo cloning must be included.

IN VITRO AI ASSISTED BIOTECHNOLOGIES

Artificial insemination as the operation of fertilizing ova avoiding the mounting of females by males could be provided outside de female *in vitro* in aseptic laboratory conditions. The *In Vitro Fertilization* (IVF) gives the opportunity to access the zygote, which means that before the two genomes of the gametes are mixing (Vintilă I., 1997).

IVF difficulties are considerable. Ova have to be picked up from ovaries of living or slaughtered cows. Apart, it is necessary to have the ova as order II oocytes by inducing the maturation of ovarian follicles and keeping them. The spermatozoa have to be capacitated as the ones from the frozen semen are. IVF operation is done on clock glass under microscopic control. This way some progeny of very valuable dams could be obtained after their death.

A development of IVF is transgenesis used to obtain Genetically Modified Organisms (GMOs). Transgenesis is supposed to attach short segment of DNA to the zygote pronucleus. The foreign DNA segment attachment could be conceived by its mechanical inoculation into zygote pronucleus. Usually the male pronucleus, which is bigger, is preferred. Other means to attach the foreign DNA segment is to use compatible vectors. The vector carries the DNA segment and penetrates both pronuclei' membranes releasing the foreign matter in order to link to the respective genomes. Senday virus is the most used vector of DNA segments. Advantage of using vectors is that both pronuclei are modified and after amphimixis the chance of new genes to pair is increased. Transgenesis might be successful only when the foreign inoculated DNA segment is compatible to the host DNA. The chance to sexually reproduce an animal GMO is rare and is possible just in case both sexes genetically modified partners exist. But even in these cases the foreign genes could be eliminated during the reproduction process. Nature fights to preserve its order. The only convenient way to reproduce GMO animals is to clone them. The last procedure of artificial insemination is the mechanical monospermous fertilization of ova. It consists in preparing one sperm or head of one sperm in the wanted way, catching the piece with one inoculation needle and mechanically pushing it the cytoplasm of one mature ovocyte inside its original follicle.

In cloning superior animal organisms fertilization can be excluded if for cloning nucleus of somatic cells are used. In this case somatic diploid cells' nucleus extracted from the organism to be cloned have to be placed into the order II ovocyte cytoplasm. But the synchronization of the somatic cell nucleus formatting and of the ovocyte development has to be respected. The feasibility of such technic wonderfully was demonstrated by Wilmut

(Wilmut I and al. 1997, Wilmut I and al. 1998) when obtained Dolly, the famous creation of bioengineering.
Ignoring the great difficulties, the high costs, and the low rate of operating success, the IVF gives access to some fantastical opportunities in bioengineering. Such opportunities are using the genotypes of death female animals, practicing micro surgery on the haploid female or male genome before their amphimixis takes place, or passing over the breaks imposed by the genetic information as natural mechanisms of closed reproduction of genetic species as open ways to animal GMO bioengineering (Wiltbank M.C., 1997).
But bioengineering is costly and short time sustainable. Many times the trials to biologically reproduce animal GMO weren't successful. Species of genetic information controls the genotypes in the new generations of cloned organisms excluding the foreign genes. Cloning mammal organisms is the only hope of reproducing superior animal GMO.
The difficulties and costs of Transgenesis justify using it in pharmaceutical industry only. These biotechnics are beyond Artificial Insemination.

ARTIFICIAL INSEMINATION EXTENSION

The prognosis concerning the Artificial Insemination extension in dairy cattle breeding at the world level is confirmed as well, but not in this country. The stupid privatization of the former state SEMTEST enterprises and the clearing of the former elite dairy cattle farms with concentrated livestock, have generated a general disorder. Local AI managers weren't able to find solution and foreign specialists had no interest in repairing things.
The solution would be a simple one, namely to remake the former SEMTEST units as cooperatives of milking cows' owners. It was proposed to the EU team, but it was rejected. That was a mistake apart of Romanian leadership afraid to lose entering in Europe. Persistence of SEMTEST as local cooperatives justified maintaining elite dairy cattle farms, as the former so called "inercoop" cattle farms, for instance. The utility of the elite farms might provoke the interest of some investor in building large, concentrated, dairy cattle farms, may be at the beginning as elite and later as commercial dairy farms.
Therefore prognosis concerning AI extension in Romania didn't confirm, but for political reasons not for technical ones. Such mistakes are still going on. Promoting beef cattle husbandry, convenient for extensive agriculture, sustained by some Romanian officials, is not compatible with the Romanian agriculture specific based on hamlet location of labor neither for the European economy interested in intensive agriculture. That will decrease cattle breeders' interest for artificial insemination

CONCLUSION

Given the above information it is right to say that almost ideas of the technical prognosis thoughts 20 years ago were proven right. Additionally innovative ideas have been released globally as well.
As pure animal reproduction biotechnology, AI got the highest possible level. Much progress was wined in assisting other animal biotechnologies tending to GMO bioengineering.
Most progress concerning dairy cattle breeding refers to MOET. Unfortunately the two Romanian schemes of MOET farms were never published and no research unit lanced such projects because AI Centers acting in Romania are out of date.
Avant-garde research projects are directed to bioengineering as an AI in vitro modality to prepare medical bio products for human health care.
Concerning AI extension in dairy cattle breeding the trend was to become exclusive in most of the developed countries. In Romania things went wrong because of many political mistakes.
The future long term prognosis of AI seems to hit 3 targets: a) assisting the avant-garde biotechnics for transgenic organism enjoying pharmaceutical industry, b) assisting the MOET biotechnologies promoted to increase the female side fertility in dairy cattle breeding for breeds' improvement or higher yields of milk production in commercial dairy farms, c) extending it in current reproduction of dairy cattle to keep up the performance level of milk

production and to decrease the progeny cost up to the stage that the natural mating will be excluded.

On the contrary newly added ideas have completed the old ones worldwide.

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