ABSTRACT

Keywords: cow, embryo transfer, embryos, hormones, FSH,

The main objectives of this thesis were updating information on embriotransfer in cows as a component of reproductive biotechnologies.

Studies have focused more on the improvement of techniques for increasing the reproductive potential of females with high genetic value.

The thesis that is called ,, ***Contribution to improuved embryo transfer biotechnology in cattle*** ” includes a number of 221 pages and is divided into two main parts. The work includes 61 figures and 28 tables.

The first part of the work is carried out on 58 pages and contains four chapters, representing a critical literature review of current knowledge about organogenesis, morphological and reproductive function in cow, physiology and reproductive biotechnology in this species.

The first chapter deals with the organogenesis of the reproductive system in cow and the mechanisms through which different stages of female genital segments are born.

The second chapter provides information about the cow genital morphology and the role of each segment in the formation and development of a new life.

The third chapter provides information on reproductive physiology in cows showing the neuroendocrine mechanisms in detail that control the sequence of events in the reproductive function of cows. The central nervous system through its control mechanisms sends the command to the responsible organs for the secretion of reproductive hormones insuring the reproductive function.

Chapter IV provides data about the current state of biotechnology embryo tranfer in the world. During its ten chapters it describes in detail each step of this technique.

It is also shown how these techniques of embryo transfer can increase ten times or more in a year the rate of reproduction valuable cows and five times or more their productive life.

The second part of the paper has 98 pages and 7 chapters which present personal research and partial conclusions.

At the end of the paper the general conclusions and bibliographical sources are presented followed by annexes.

The interest in studying embryo biotechnology stems from the fact that after the year 2000, the massive investments made in Romania in large dairy farms required a rapid finding of genetic improvement methods to increase the production and the productivity.

Chapter V refers to the purpose of the work, the objectives and importance of the many factors that influence the success of embryo transfer.

This paper followed several important objectives that have been systematized on several stages, to comply with a chronological succession: donor selection on morpho-productive criteria; the selection of the recipients; finding new treatment protocols for superovulation; choosing an efficient method of embryo collection; evaluation and implanting the collected embryos; choosing an optimal cryopreservation protocol; using embryos in treating infecundity conditions of cows.

For all these major objectives were sought procedures, which eliminates stress for livestock and facilitate the work of practitioners so that embryo transfer can become a routine labor.

Chapter VI refers to research on induction superovulatoin in donor cows. This chapter is divided into three subchapters.

The first chapter refers to necessary work materials (producing animals used meaning 25 cows of Holstein, Montbeliarde and Normand breeds that were applied superovulation treatments) and methods used (the superovulation protocols).

Commercial products used were: Pridoestrol®, Folligon®, PRID®, Folltropin®, Pluset®, Prosolvin®, Prostol®.

The second subchapter describes the results obtained from the four groups of donors that have applied different superovulation protocols .

A poor response to superovulation treatment (an average of 4.8 embryos collected per donor, a rate of average recovery of 39.13%) was observed in group P1, which was superovulated with Folligon® product.

A poor response to treatment was observed in superovulation group P1 (an average of 4.8 embryos collected per donor rate of average recovery of 39.13%), to which was used the product Folligon.

Equine chorionic gonadotropin (PMSG), the active substance of the product Folligon®, due to the half time too long, causes several waves of follicular development without the synchronization of ovulation.

The P3 group, where the product used was Pluset® (without the implant with slow release intravaginal progesterone ‘’PRID®’’) had a better answer, with an average of 8 embryos collected per donor and a recovery average rate of 66.66%. The lack intravaginal implant PRID®, resulted in secretion of inhibin by the dominant follicle which blocked the development of several follicles in the next follicular wave.

The other two groups P2 and P4 which used Folltropin® product administered via classical (intramuscular) and mixed way (epidural and intramuscular) the superovulation response to treatment was significantly superior to lots P1 and P3 and relatively close together. Significantly, the better response was influenced by the products used and the way of administration.

Thus, the group P2 yielded an average of 9.1 embryos per donor collected and an average recovery of 77%, and in group P4 after analyzing the results, an average of 8.8 embryos per donor cow and an average recovery rate of 78.57%.

Similar results show that the administration of FSH superovulation protocol - by mixt administration (epidural and intramuscular) is an alternative which can be implemented in embryo transfer programs.

Chapter VII of this thesis deals with the synchronization of oestrus stage between donor and recipient.

It is also structured in three subchapters: the first subchapter describes the materials and methods, the second issue concerns the results obtained from the four synchronization protocols, and the third subchapter describes the partial conclusions.

225 recipients cows were selected, they were divided into four groups, which were applied to three different treatment protocols. So groups S1 and S2 applied the same synchronization protocol but with different commercial products: S1 (Pridoestrol® and Prosolvin®) and S2 (PRID® and Prosolvin®) The S3 group had a protocol of syncronization with the random administration of prostaglandin F2α, and in group S4 synchronization protocol was applied with synthetic analogue of prostaglandin administrated after ultrasound morphometric of the yellow body.

After analyzing the results obtained we observed a procent of synchronization of 90-91% in the S1 and S2 groups with an interval between estrus donors-recipients + 0.19 hours in S1group and + 0.20 hours in the S2 group.

In the S3 group treated randomly with synthetic prostaglandin analog F2α achieved a 62% estrus synchronization with a range between donors-recipients + 0.32 hours.

The best result was observed in S4 group who achieved an 95% synchronization, and estrus interval donors-recipients + 0.17 hours. This is due to the benefit offered by ultrasound examining and monitoring .

Chapter VIII refers to the collection of the embryos as biotechnology milestone in embryo transfer. It is divided into three subchapters as: materials and methods, results and some partial conclusions. In the first section are presented the list of materials and equipment

needed as: flushing uterine solutions, Luer or Foley dual path catheters, type "Y" duct, glasses filters collectors pore size of 80 microns, plates, stereomicroscope for quality evaluation of embryos, syringes, etc.

Uterine flushing was done by two methods: first LFC (each horn flushing method) with catheter placement in deeper level in uterine horn and second LBS method (flushing bicornual simultaneously) with catheter placement before the cervix.

After the execution of both working methods we have different results, namely: by means of LFC method were harvested in a total of 69 embryo compared to the LBS method with 117, a percentage difference of 41.03%.

With regard to the average number of collected embryos were observed per donor comparative differrences per donor between the two methods, the mean number of 6.9 embryo per donor collected by the LFC method to 9 embryos collected per donor by LBS method, a percentage difference between the two methods of 23.34%.

Regarding the average of transferred embryos per donor it was a significant percentage 30,49%, meaning 5.7 embryos transferred by LFC method were classified compared to 8.2 tranferred embryos per donor by LFB method.

In conclusion the LBS method (bicornual simultaneously flushing) is more effective, easier to perform, more convenient and faster than LFC method (lavage each separate horn) and can be applied by practitioners.

Chapter IX refers to the stage of embryo freezing, a way for long-term storage of embryos collected when there are not enough receivers synchronized or when they want an international trade.

It is divided into two parts: the first part concerns the materials needed for freezing methods, sequins and suction pipette for embryo Criocell freezing plant, liquid nitrogen and methods of work and preparation for freezing embryos. We worked after two freezing protocols for two batches of embryos.

The protocol used for the batch of embryos C1 was the one with the onset of freezing at a temperature of 0 ° C and the batch of embryos C2 protocol was the onset of freezing at room temperature.

The results obtained in this chapter are in close correlation with the results of **Chapter X**, with pregnancy rates obtained from embryos implant, as these chapters are interlinked across two stages of embryo biotechnology. Pregnancy rate remains the only method of assessing the effectiveness of freezing.

Chapter X refers to implant fresh or frozen embryos. This chapter is divided into three subchapters: materials required and the methods used: Three groups of recipients synchronized special implant gun (with rounded metal insert to facilitate crossing of the cervix), procaine 2%, syringes, needles, ultrasound Tringa Linear device for the exam of the ovaries and the determination of uterine horn that will make the implant.

Subchapter two makes the analysis of results from implantation, and some partial conclusions from section three.

Analysis of the results obtained after controlling pregnancy through ultrasound shows that: in T1 group (where fresh embryos were implanted) achieved pregnancy rate was 67% representing a total of 57 confirmed gestation of 85 embryos implanted.

The T2 recipients group, which were implanted frozen embryos from C1 group (starting from the temperature of 0°C), the rate of pregnancy obtained was 53%, being confirmed 19 gestation at the control of gestation from 36 embryos implanted.

In T3 recipients group which were implanted with frozen embryos from C2 group (with room temperature for freezing departure) the rate of pregnancy was 48%, being confirmed at the control of gestation 20 gestations of 42 embryos implanted.

Comparative analysis of batches shows that the best rate of gestation (67%) was observed in T1 group which was used to implant fresh embryos, the rate of pregnancy is within the range 60-70% reported by IETS (International embryo Society). **[www. iets.com]**

Regarding the rate of pregnancy among T2 and T3 group (which were implanted frozen embryos) there is a 5% difference between the two groups (53% in Q2) compared to (48% in Q3) implying that freezing embryos method in C1 group (departing frozen at 0 ° C) was more effective than the method of embryo freezing in C2 group, due to the shorter exposure time of embryos outside the uterus.

Chapter XI covers acquired infecundity disorders using embryo transfer therapy. In this chapter, all three subchapters are structured on materials and methods, results and discussion and partial conclusions describeing the way for producing some very good female that can continue even if the reproductive productive activity ended.

For this chapter were treated by embryonic implant a group of six cows with previous genital tract obstruction, diagnosed by testing the permeability with fenolsulftalein.

After the safeguards were diagnosed gestation 3 gestation, which means that were recovered 50% of cows that were reformed.

Embryo transfer therapy for infecundity states may be an alternative for females saving and extending their productive activities.

Chapter XII describes the general conclusions of this study.

Chapter XIII refers to some practical recommendations for field technicians.

The bibliography contains a 128 number of references that have been cited along the thesis of which 21 romanian and 107 foreigners.