4. VH13 mulberry variety was approved as official national variety by Scientific Council of Ministry of Agriculture and Rural Development in April 2006 and disseminated to the provinces in northern and central provinces. VH13 hybrid mulberry variety had been awarded the Gold Cup in 2007 and agriculture VIFOTEX award in 2010.

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23. APPLICATION OF BIOTECHNOLOGY IN ANIMAL HUSBANDRY IN SOUTHERN PROVINCES

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SUMMARY

Application of biotechnology in animal husbandry has been encouraged by governmental policies. Our initial results in this aspect showed that reproductive biotechnologies including sexed semen, ovum pick-up can be used into in vitro embryo production in order to produce high quality animal breed; molecular biology have been applied successful to detect pathogen of serious disease (PRRS) or congenital genetic disease (BLAD). Our research trends focus on (i) combining reproductive biotechnology with gene assisted selection for producing high quality animal breed (ii) combining genotype selection (MAS, GAS, SNPs) with phenotype selection (BLUP) in animal selection and breeding programmes, especially on improving quality of animal products (iii) combining molecular biology with conventional methods in diagnosis of animal diseases, especially on genetic diseases.

Keywords: Porcine Reproductive and Respiratory Syndrome, Bovine Leukocyte Adhesion Deficiency, Gene Assisted Selection, Best Linear Unbiased Prediction, Ovum Pick-Up, In Vitro Embryo Production

I. INTRODUCTION

Application of Biotechnology in animal husbandry (2008-2020) has been confirmed by

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Governmental Decision (Decision 14/2008/QĐ-TTg) '...Improving reproductive biotechnologies, especially in cell biotechnologies including semen, embryo freezing; embryo transfer, *in vitro* fertilization. Application of molecular marker, transgenic technology for selection and production of high yield, quality domestic animal and embryo sexing. Researching and improving capacity in vaccine production, especially in some dangerous diseases in domestic animal …'

Biotechnology Department, which belongs to IAS, has conducted some researches in above contents. Some our initial results of biotechnology application in animal husbandry have been summarized in the report.

II. REPRODUCTIVE BIOTECHNOLOGIES IN DOMESTIC ANIMAL

The use of Multi Ovulation and Embryo Transfer (MOET) technology in cattle breeding has been continued to increase (especially in the dairy cattle) over the past 30 years with the movement toward genetic improvement as opposed to the production of desirable phenotypes. This technology is influencing the direction of cattle breeding industries; the numbers are small but the impact is high. Like ET, technology for *in vitro* embryo production (IVEP) in cattle has also encountered many challenges on the path toward widespread commercial application. However, IVEP technology will be more useful when combined with sperm-sorting technology. This combination will produce the foreseen sexual embryos which create suitable calves to different objectives of production (beef or dairy industry). IVEP in cattle by using sorted semen within conditions of new lab is also a primary objective of this study.

Two necessary materials of IVEP are oocyte and semen, stable and good oocyte supply is being main constraint of IVEP. In Vietnam where bad oocytes of old/ill cow at slaughter house was not good for IVEP and that's why the technique like OPU (Ovum Pick-up) is one of the alternatives to get developmentally competent oocytes. IVEP in dairy cattle get more difficult, most of dairy cow were only eliminated by too old or problems of reproduction after many treatments with hormone, it means that the ovary was influenced seriously. So, oocytes from slaughter ovary of dairy cow were not high developmental competence. Evidence suggests that the intrinsic quality of the oocyte is the key factor determining the proportion of oocytes developing to the blastocyst stage. The oocyte maturation is a complicated process and the selection of oocytes for culture *in vitro* only by morphology is not enough to obtain better IVM and IVF. There is considerable evidence that the medium and conditions of oocyte culture also impact on the development of bovine embryos *in vitro*. These processes involve numerous levels of checks, balances and are sensitive to regulation of endogenous and exogenous factors.

Our experiments aim to improve efficiency of IVEP in dairy cattle. These techniques can be used to produce high quality dairy cattle.

2.1. Material and Methodologies

Experiment 1. Using sexed semen in invitro bovine embryo production

- Oocytes were collected from ovary after slaughtering.

- Frozen sorted-semen was imported from O'Connor Land & Cattle company (Canada)

- Procedure of IVM (*In vitro* Maturation), IVF (*In vitro* Fertilization) and IVC (*In vitro* Culture) were implemented by NLBC's guide book (Japan).

- Sex of embryo was re-examined by PCR (Kameyama Yuichi, 2000)

- Comparing the efficiency of IVEP by using two different semen types, normal semen and sorted semen (only sperm with X chromosome).

Experiment 2. Using Ovum Pick-Up to supply stable, good oocytes to IVEP

- Specific ultrasound system (HS-2000V, Japan) with transvaginal probe has been used to collect oocyte on ovary of living cows.

- Frozen normal semen was product of Moncada center

- Procedure of IVM, IVF and IVC were implemented by NLBC's guide book (Japan).

- Number of aspirated oocytes in every OPU is major evaluation criteria

Experiment 3. Supplementing Follicular Fluid and IGF-1 into IVM and IVC medium

- Follicular Fluid was collected from dominant follicle on slaughter ovary of dairy cows and sterilized by 20μm filter.

- Hormone IGF-1 was imported from Sigma-Aldrich

- Procedure of IVM, IVF and IVC were implemented by NLBC's guide book (Japan).

- Comparing the rate of mature oocyte in basic medium (TCM-199 + 5% Calf Serum, control) with basic medium adding 5% Follicular Fluid.

- Comparing rate of blastocyst embryo in basic medium (CR1aa + 5% FCS, control) with basic medium adding 50ng/ml or 100ng/ml IGF-1.

2.2. Results and discussion

Experiment 1. Using sexed semen in invitro bovine embryo production

Result showed that sorted-semen can be used in *in vitro* bovine embryo production with 29.9% fertilization rate and 35% morula and blastocyst rate. There was no significant difference in efficiency of normal semen and sorted semen in *in vitro* bovine embryo production (P>0,05).

Table 1. Using sorted semen in *in vitro* bovine embryo production

***In vitro* Fertilization**	Type of Semen	
	Normal semen	Reducing sugar
Number of matured oocytes	67	904
Number of zygotes	20	204
Fertilization rate	29.9%	22.6%

Experiment 2. Using Ovum Pick-Up to supply stable, good oocytes to IVEP

Our initial result showed that OPU technique is rather difficult and complex, but we can conduct OPU technique in both slaughter ovary and live cow. After six trials, average of oocytes per OPU was 1.8 oocytes/OPU, it was lower than previous result of Nguyen Van Ly et al (2006) with 3.98 oocytes/OPU. Successful rate of OPU (aspirated oocytes per detected follicles) was 21.2% lower than result of Cech et al (2011) with 50.9%, but the successful rate varied from 20% on small follicle ≤12mm to 76.7% on large follicle >12mm.

Experiment 3. Supplementing Follicular Fluid and IGF-1 into IVM and IVC medium

Our initial results showed that: Rate of mature oocyte have been improved significantly in IVM basic medium adding 5% follicle fluid. It reached 77.8% compared with 61.8% in control. Bovine morula and blastocyst rate of IVC medium, which was added 50ng/ml or 100ng/ml IGF-1, were 48.28% or 45.95% comparing with 28.79% in control (without IGF-1). Initial results of adding IGF-1 into IVC medium proven that our researchs is right when focusing on improving culture medium.

Table 2. Supplementing follicle fluid (5%) into medium of *in vitro* maturation

	IVM medium	
	Control (TCM199+5%CS)	Basic Medium+5% FF
Initial Oocytes	223	122
Matured Oocytes	138	95
Rate of matured oocytes	61.8%	77.8%

Table 3. Supplementing IGF-1 into medium of *in vitro* culture

	IVC medium (CR1aa) adding IGF-1		
	0 ng/ml	50 ng/ml	100 ng/ml
Matured oocytes	129	124	140
Number of zygotes	66	58	74
Rate of zygote (%)	51.16	46.77	52.86
Number of morula & blastocyst	19	28	34
Rate of morula & blastocyst (%)	28.79	48.28	45.95

III. MOLECULAR BIOLOGY IN DOMESTIC ANIMAL

DNA marker is an identifiable DNA fragment or sequence that can be used to detect DNA polymorphism. Different types of markers are available, including RFLP, RAPD and AFLP (involving the use of restriction enzymes and the polymerase chain reaction), minisatellites, microsatellites, single nucleotide polymorphism (SNP). Markers that have a statistical association with a phenotypic trait can be used for selection of animals for the desired phenotype (marker-assisted selection-MAS). Molecular markers may also be used to increase the efficiency of the introduction (introgression) of genes from one breed into another through repeated backcrossing of a recipient breed. Now, genetic selection can be conducted directly on the QTL (gene assisted selection-GAS) or SNPs (based on micro-array chip) and combine with phenotype selection (MA-BLUP).

PCR is being a useful method for diagnosing pathogenous factors. This technique has been extremely useful in epidemiology, enabling comparison of isolates of a particular pathogen. Real time PCR offers a much greater sensitivity for the identification of pathogens and is especially useful when the pathogen is available only in small numbers or is difficult to culture. For more precise phenotype and genotype analysis, sequence analysis can also be conducted.

Our research trends focus on using MAS, GAS combining with BLUP (conventional method) in breeding programmes of domestic animal. In addition, application of molecular biology for determining popular pathogenous factors, especially congenital genetic diseases, in animal husbandry is major consideration of our research.

3.1. Material and Methodologies

Experiment 1. Application of PCR-RFLP for detecting BLAD in dairy cattle population in Ho Chi Minh city

- 268 blood samples, 684 milk samples and 18 frozen samples were obtained from dairy cattle with different cross-bred rate of Holstein Friesian (HF)

- The protocol of Laura-Lee Boodram (2004) for extracting DNA from blood samples, the protocol of F. d'Angelo (2007) with some modifications for extracting DNA from milk samples and the protocol of Luciana A. Ribeiro (2006) with some modifications for extracting DNA from frozen semen samples have been chosen

- Using PCR-RFLP protocol developed by Kriegesmann et al (1997). In order to determine the area of mutation in PCR products, the PCR products were digested with TaqI endonuclease enzyme.

- The gene frequencies of BLAD-free cattle, BLAD carriers and BLAD-affected cattle were calculated based on the Hardy-Weinberg law.

Experiment 2. Using real time RT-PCR for detecting PRRS virus in swine population in Dong Nai province

- 220 blood samples and 25 fresh semen samples were collected

- ELISA method by using HerdCheck* PRRS Virus Antibody Test Kit 2XR (IDEXX Laboratories Inc. USA)

- Real-time One-step RT PCR with Taqman® Probe by using iScriptTM One-Step RT-PCR kit for Probe (Bio-Rad). Primer and probe of EU strain developed by Kleiboeker et al (2005) and NA strain developed by Lurchachaiwong et al (2007)

- Comparing precise diagnosis level between ELISA and real time RT-PCR.

3.2. Results and Discussion

Experiment 1. Application of PCR-RFLP for detecting BLAD in dairy cattle population in Ho Chi Minh city

There was no homozygote BLAD animal. The mutant BLAD allele frequency in the Holstein cross-bred cattle in Ho Chi Minh city was 0.001. Results of the age distribution of BLAD-carrier showed that one calf and one heifer was detected, ratio of BLAD-carrier was 1.06% (1/94) in surveyed calves and 0.28% (1/357) in surveyed heifers, the gene frequency of BLAD was 0.0053 in surveyed calves and 0.0014 in surveyed heifers. Survey on the HF crossbred groups showed that both of BLAD-carriers were cattle with 87.5% HF blood, the ratio of BLAD-carrier was 0.29% and the gene frequency of BLAD was 0.001 in dairy herd with 87.5% HF blood. No BLAD-carrier was detected in dairy herd with 50% HF blood and 75% HF blood. However, most of dairy cattle in Ho Chi Minh city were HF crossbred cattle with more 75% HF blood.

Table 4. BLAD genotype on surveyed dairy cattle with different HF cross-bred rate

Type of breed	Number of samples	Type of BLAD genotype			
		Normal	BLAD carrier	Rate of BLAD carrier	Gene frequency of BLAD
50% HF	27	27	0	0	0
75% HF	241	241	0	0	0
≥ 87,5% HF	684	682	2	0.29	0.0015
Pure HF (Frozen Semen *)	18	18	0	0	0
	970	968	2	0.2061	0.0010

*Note: * Frozen semen of pure HF bulls have been used in Ho Chi Minh city*

Experiment 2. Using real time RT-PCR for detecting PRRS virus in swine population in Dong Nai province

Among blood samples, there were 56% (28/50) positive samples with EU strain, 30.76% (8/26) positive samples with NA strain. Among fresh semen samples, there were 84% (21/25) positive samples with NA strain. Among negative samples with ELISA, 25% samples were positive with real

time RT-PCR. It proven that real time RT-PCR was more precise than ELISA by detecting directly on antigen. It was confirmed by Dee et al (2003). Their results demonstrated that pigs with negative or low positive ELISA S/P ratios may harbor infectious PRRSV and that ante mortem diagnostic samples may not be capable of accurately predicting the PRRSV status of individual animals that originate from PRRS-positive populations.

IV. RESEARCH TRENDS IN ANIMAL BIOTECHNOLOGY

Based on lead of Government and MARD, combining our research conditions and real demand of animal husbandry in southern provinces, some trends of animal biotechnology should be done as follows:

- Researching continuously reproductive biotechnologies in combination with gene assisted selection to produce high quality, yield animal, especially in dairy cattle which is priority domestic animal in southern provinces

- Increasing application of molecular biology in breeding programmes of domestic animal, combining genotype selection (MAS, GAS, SNPs) with phenotype selection (BLUP) in order to produce high quality animal and improve quality and quantity of animal products.

- Applying quickly molecular biology on diagnosis of popular diseases of domestic animal such as FMD, PRRS, PED…, especially on congenital genetic disease such as BLAD, CVM, DUMPS in cattle or Scrotal hernia, Large Umbilical hernia, Atresia Ani in female, Cryptorchid in male, Hermaphrodite pig with large clitoris, Splayleg or spraddle leg in pig.

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