ARBC2018 at Taipei Zoo, Taiwan

13th Asian Reproductive Biotechnology Congress, 3~6 May 2018

Reproductive biotechnology for defining, delineating and displaying the world of human and animal

A. Reproductive medicine
B. Fertility preservation
C. Assisted reproduction
D. Cell and Tissue bio-banking
E. Animal cloning
F. Embryo development
G. Cell reprogramming
H. Biology and Economics

3-6 May
2018

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Reproductive Biotechnology

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CONTENTS

B. Fertility preservation

C. Assisted reproduction

D. Cell and Tissue bio-banking

E. Animal cloning

F. Embryo development

G. Cell reprogramming

H. Biology and Economics

Previous Meetings of ARBC

Introduction of Sponsors

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Venue: International Conference Hall
Third Floor, Panda House, Taipei Zoo
No. 30, Section 2, XinGuang Road, WenShan District, Taipei City 11656 Taiwan

Reproductive Biotechnology	Abstracts	Species
A. Reproductive medicine	6	Farm Animal; Zoo Animal; Endangered Species
B. Fertility preservation	15	Human; Farm Animal; Zoo Animal; Lab Animal; Amphibia; Endangered Species
C. Assisted reproduction	11	Human; Farm Animal; Zoo Animal; Lab Animal; Endangered Species
D. Cell and Tissue bio-banking	9	Farm Animal; Zoo Animal; Endangered Species
E. Animal cloning	4	Farm Animal; Endangered Species
F. Embryo development	11	Farm Animal; Lab Animal
G. Cell reprogramming	7	Farm Animal; Lab Animal
H. Biology and Economics	4	Farm Animal

Oral Presentation Schedule (Panda House)

Time	3 May (Thu)	4 May (Fri)	5 May (Sat)	6 May (Sun)
08:30	Registration/ *Third Floor of Panda House*	Registration	Registration	Registration
09:00		Plenary Speaker Thomas B. Hildebrandt (Germany)	Plenary Speaker Pascal Mermillod (France)	Plenary Speaker Pierre Comizzoli (USA)
09:40		Cell and Tissue bio-banking (I)	Reproductive medicine (II)	Cell reprogramming (II)
10:00	Opening Ceremony Group Photo		Assisted reproduction (II)	
10:30	Tree Planting			
11:00	Plenary Speaker Janine L. Brown (USA)	Health break	Health break	Health break
11:40	Reproductive medicine (I)	Animal cloning	Embryo development (I)	Assisted reproduction (III)
12:00				
12:30	Lunch break	Cell reprogramming (I)	Lunch break	Lunch break
13:00		Lunch break		
13:30	Fertility preservation (I)		Embryo development (II)	Fertility preservation (III)
14:00		Zoo Tour: ● Tour African Animal ● Tour Forest Animal ● Tour Formosa Animal ● Tour Animal Medical Center		
14:30			Biology and Economics (I)	Biology and Economics (II)
15:00	Health break		Health break	Health break
15:30	Assisted reproduction (I)		Fertility preservation (II)	Cell and Tissue bio-banking (II)
16:00				
16:30				Closing Ceremony
17:00	Reception/ *Insect House Lobby*			
17:30				Gala Dinner/ *Gate Square* The Outstanding Paper Award of Young Scientists
18:00				
19:00	600 meter night trip walking with Firefly			
20:00				
21:00				

Hand-On Training Schedule (maximum 7 people per class)

Hand-On Training	Location	3 May (Thu)	4 May (Fri)	5 May (Sat)	6 May (Sun)	Instructor
Flow Cytometric Sperm Assay	Room 301	15:00~17:00		10:00~12:00	10:00~12:00	Hsiu-Lien Lin, Ting-Yung Kuo, Chiao-Chien Chu
Oocyte Freezing	Room 302		10:00~12:00	15:00~17:00		Yu-Shin Chen, Ting-Chieh Kang, Fung-Hsiang Chu, Jenn-Rong Yang

Poster Presentation Preference Session & Exhibition

09:00AM~16:30PM 3~6 May	Third floor at International Conference Hall of Panda House

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ARBC2018TW

Oral Presentation Schedule (Panda House)

Time	3 May (Thu)	Presenter/ Paper Title
08:30 09:00 09:30	Registration/ *Third Floor of Panda House*	All Participants
10:00	Opening Ceremony Group Photo	Invited Guests All Participants
10:30	Tree Planting	All Participants
11:00	Plenary Speaker	Janine L. Brown (USA) *HORMONE MONITORING: AN IMPORTANT TOOL FOR BREEDING MANAGEMENT OF WILDLIFE SPECIES*
11:40	Reproductive medicine (I)	Yu-Chia Chang (Taiwan) *CHARACTERIZATION OF NONINVASIVE MONITORING REPRODUCTIVE ENDOCRINE PROFILES FROM THE CAPTIVE AND FIELD EURASIAN OTTER (LUTRA LUTRA) IN TAIWAN* Bo Xiong (China) *THE PROTECTIVE ROLE OF MELATONIN IN PORCINE OOCYTE MEIOTIC FAILURE CAUSED BY THE EXPOSURE TO BENZO(A)PYRENE*
12:10		Noboru MANABE (Japan) *PRODUCTION OF PRION GENE KO COW TO PREVENT SPONTANEOUS BSE AND THEIR CHARACTERISTICS*
12:30~	Lunch break	Education Center
13:30	Fertility preservation (I)	Na Li (France) *APPLICATION OF FLOW CYTOMETRY IN SEMEN ANALYSIS*
14:00		Koshiro Watanuki (Japan) *GAIN: GREAT APE INFORMATION NETWORK FOR BEST CARE AND MANAGEMENT OF CAPTIVE APES IN JAPAN*
14:30		Jiehuan Xu (China) *IMPROVING PRACTICAL USE OF BOAR SEMEN CRYOPRESERVATION: CURRENT UNDERSTANDING* Mark Wen Han Hiew (Malaysia) *EFFECT OF STORAGE TEMPERATURE OF EXTENDERS ON THE QUALITY OF BOAR SEMEN*
15:00	Health break	*Third Floor of Panda House*
15:30	Assisted reproduction (I)	Ampika Thongphakdee (Thailand) *REPRODUCTIVE SCIENCE AND TECHNOLOGIES FOR ENHANCING WILDLIFE CONSERVATION: THAI EXPERIENCES* Eufrocina P. Atabay (Philippines) *IMPROVED PREGNANCY THROUGH SYNCHRONIZED OVULATION AND FIXED TIME AI IN WATER BUFFALOES*
16:00		Jonathan T. Aaltonen (USA) *APPLYING ASSISTED REPRODUCTIVE TECHNOLOGIES TO WILDLIFE CONSERVATION MANAGEMENT*
16:30		Muhammad Usman Mehmood (Pakistan) *EFFECT OF INSEMINATION TIMING FOLLOWING 5 OR 7 DAYS CIDR CO-SYNCH IN NILI RAVI BUFFALO HEIFERS* Nam-Hyung Kim (Korea) *SPINDLE FORMATION AND ASYMMETRIC CELL IN MAMMALIAN OOCYTES*
17:00~ 19:00	Reception/ *Insect House Lobby*	All Participants
19:00~ 20:00	600 meter night trip walking with Firefly	All Participants

Hand-On Training Schedule (maximum 7 people per class)

Hand-On Training	Location	3 May (Thu)	4 May (Fri)	5 May (Sat)	6 May (Sun)	Instructor
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Oocyte Freezing	Room 302		10:00~12:00	15:00~17:00		Yu-Shin Chen, Ting-Chieh Kang, Fung-Hsiang Chu, Jenn-Rong Yang

Poster Presentation Preference Session & Exhibition

09:00AM~16:30PM 3~6 May	Third floor at International Conference Hall of Panda House
Embryo development	Jin-Hoi Kim (Korea) *SINGLE CELL RNA-SEQ ANALYSIS PROVIDE DEEPER MOLECULAR INSIGHTS INTO ABNORMAL MATURATION OF TUBASTATIN A-TREATED MOUSE OOCYTES* Xue-Fang Wang (China) *TREATMENT WITH RESVERATROL DURING IN VITRO MATURATION IMPROVES PORCINE OOCYTE QUALITY AND EMBRYONIC DEVELOPMENT*

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Oral Presentation Schedule (Panda House)

Time	4 May (Fri)	Presenter/ Paper Title
08:30	Registration	*Third Floor of Panda House*
09:00	Plenary Speaker	Thomas B. Hildebrandt (Germany) *THE ART OF ART IN WILD PATIENTS*
09:40	Cell and Tissue bio-banking (I)	Bertrand Pain (France) *BAT STEM CELL LINES FOR BIODIVERSITY CONSERVATION AND VIRAL STUDIES*
10:10		Chia-Xin Lee (Taiwan) *SEMEN CRYOPRESERVATION AND QUALITY OF FROZEN-THAWED SEMEN IN TAIWAN HOLSTEIN BULL* Hsiu-Lien Lin (Taiwan) *SEPARATION OF ROOSTER SPERMATOZOA IN SILICA-BASED COLLOIDAL MEDIUM BY CENTRIFUGATION* Lilian P. Villamor (Philippines) *ANIMAL GENETIC RESOURCES THROUGH CRYOBANKING IN THE PHILIPPINES: MANAGEMENT INITIATIVES AND FUTURE DIRECTION*
10:40		Boripat Siriaroonrat (Thailand) *RESEARCH AND CONSERVATION PROGRAMS AT ZPO*
11:00	Health break	*Third Floor of Panda House*
11:30	Animal cloning	Rangsun Parnpai (Thailand) *HOW TO IMPROVE SUCCESS OF INTER-SPECIES SOMATIC CELL NUCLEAR TRANSFER IN ENDANGERED SPECIES*
12:00		Xiang-Xing Zhu (China) *GENERATION OF TRANSGENIC-CLONED GUANGXI HUANJIANG XIANG PIGS SYSTEMICALLY EXPRESSING GREEN FLUORESCENT PROTEIN* Xi-jun Yin (China) *DOUBLE-MUSCLED PIGS CLONED USING MSTN-DISRUPTED FIBROBLASTS* Zifei Liu (China) *IGF2-H19 LOCUS METHYLATION STATUS IN CLONED GOAT FIBROBLAST CELLS*
12:30	Cell reprogramming (I)	Yuka Miki (Japan) *KNOCKOUT ANALYSIS OF OOCYTE-SPECIFIC MULTI-COPY GENE, OOG1*
13:00 13:30	Lunch break	Lunch Box for tour activity
14:00 14:30 15:00 15:30 16:00 16:30	Zoo Tour: ● Tour African Animal ● Tour Forest Animal ● Tour Formosa Animal ● Tour Animal Medical Center & Conservation and Research Center	● Tour African Animal *Group A:* *14:00~14:30* *Group B:* *14:40~15:10* ● Tour Forest Animal *Group C:* *15:00~15:30* *Group D:* *15:40~16:10* ● Tour Formosa Animal *Group E:* *16:00~16:30* *Group F:* *16:40~17:10* ● Tour Animal Medical Center & Conservation and Research Center (maximum 50 people per group) *Group G:* *14:00~14:50* *Group H:* *15:00~15:50*
17:00~		Free Activity

Hand-On Training Schedule (maximum 7 people per class)

Hand-On Training	Location	3 May (Thu)	4 May (Fri)	5 May (Sat)	6 May (Sun)	Instructor
Flow Cytometric Sperm Assay	Room 301	15:00~17:00		10:00~12:00	10:00~12:00	Hsiu-Lien Lin, Ting-Yung Kuo, Chiao-Chien Chu
Oocyte Freezing	Room 302		10:00~12:00	15:00~17:00		Yu-Shin Chen, Ting-Chieh Kang, Fung-Hsiang Chu, Jenn-Rong Yang

Poster Presentation Preference Session & Exhibition

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Oral Presentation Schedule (Panda House)

Time	5 May (Sat)	Presenter/ Paper Title
08:30	Registration	*Third Floor of Panda House*
09:00	Plenary Speaker	Pascal Mermillod (France) *MIMICKING THE MATERNAL ENVIRONMENT TO OPTIMIZE THE PRODUCTION OF EMBRYOS IN VITRO IN WILD AND DOMESTIC MAMMALS*
09:40	Reproductive medicine (II)	Rong Chen (China) *THE RESEARCH OF THE BREEDING OF NOMASCUS GABRIELLAE IN NANJING HONGSHAN FOREST ZOO IN CHINA*
10:00	Assisted reproduction (II)	Wanlaya Tipkantha (Thailand) *UPDATE ON REPRODUCTIVE TECHNOLOGIES FOR ENDANGERED CLOUDED LEOPARD* Nguyen Thi Hong (Vietnam) *INVESTIGATION OF CYCLE SYNCHRONIZATION AND ARTIFICIAL INSEMINATION FOR BREEDING INDUSTRY IN RABBITS*
10:30		Tamas Somfai (Japan) *COMPARISON OF TWO OOCYTE MATURATION MEDIA FOR IN VITRO PRODUCTION OF BLASTOCYSTS IN AN INDIGENOUS VIETNAMESE MINIATURE PIG*
11:00	Health break	*Third Floor of Panda House*
11:30	Embryo development (I)	Huiyan Xu (China) *BUFFALO OOCYTES MATURED IN VITRO WITH ACETYL-L-CARNITINE IMPROVES CRYOTOLERANCE DUE TO CHANGE IN MITOCHONDRIAL FUNCTION AND THE MEMBRANE LIPID PROFILE* Jeongwoo Kwon (Korea) *INHIBITION OF MEK1/2 AND GSK3 (2I SYSTEM) AFFECTS EPIGENETIC MODIFICATION AND EARLY DIFFERENTIATION OF PORCINE PARTHENOTES*
12:00		Kyung-Tae Shin (Korea) *THE TOXIC EFFECT OF AFLATOXIN B1 ON EARLY PORCINE EMBRYONIC DEVELOPMENT* Min-Jung Seong (Korea) *CPEB2 IS REQUIRED FOR TIGHT JUNCTION ASSEMBLY FOR ESTABLISHMENT OF PORCINE TROPHECTODERM EPITHELIUM*
12:30 13:00	Lunch break	Education Center
13:30	Embryo development (II)	Nguyen Thi Nhung (Vietnam) *EFFECT OF DONER CELL TYPES ON THE DEVELOPMENT OF PIG EMBRYOS PRODUCED BY SOMATIC CELL NUCLEAR TRANSFER* Qing Guo (China) *JNJ-7706621 IMPROVES THE IN VITRO DEVELOPMENT COMPETENCE OF PORCINE PARTHENOGENETIC ACTIVATION AND SOMATIC CELL NUCLEAR TRANSFER EMBRYOS*
14:00		Yu-Jin Jo (Korea) *WHAMM PLAY ESSENTIAL ROLES IN SPINDLE FORMATION AND CAGE ACTIN MESH OF MATURING MOUSE OOCYTES* Zhao-Bo Luo (China) *CO-TREATMENT WITH REPSOX AND LBH589 IMPROVES THE IN VITRO DEVELOPMENTAL COMPETENCE OF PORCINE SOMATIC CELL NUCLEAR TRANSFER EMBRYOS* Zheng-Wen Nie (Korea) *THIAMETHOXAM INHIBITS BLASTOCYSTS EXPANDING AND HATCHING VIA ACTIVATION OF ROS-INDUCED DNA DAMAGE CHECKPOINT IN PIGS*
14:30	Biology and Economics (I)	Kim Dung Pham Thi (Vietnam) ENHANCEMENT UTILIZATION OF REPRODUCTIVE TECHNOLOGY IN PIG HERDS OF VIETNAM TO MEET THE INDUSTRY DEVELOPMENT POLICY Ruth Miclat-Sonaco (Philippines) *TECHNICAL TRAINING INPUT OF PIG REPRODUCTION IN LOCAL AND INTERNATIONAL FARMERS AT PHILIPPINE ITCPH*
15:00	Health break	*Third Floor of Panda House*
15:30	Fertility preservation (II)	Jing Pang (China) *INFLUENCES OF DIFFERENT DIETARY ENERGY LEVEL ON SHEEP TESTICULAR DEVELOPMENT ASSOCIATED WITH AMPK-ULK1 AUTOPHAGY PATHWAY*

		Shao-Chen Sun (China) *MELATONIN PROTECTS OOCYTES FROM MEHP EXPOSURE INDUCED MEIOSIS DEFECTS IN PORCINE*
16:00		Shuangshuang Geng (China) *THE PRIMARY RESEARCH OF OVER EXPRESSION OF GDNF GENE IN BUFFALO TESTIS SERTOLI CELLS* Wenjun Zhou (Korea) *FIPRONIL CAUSES APOPTOSIS DURING MEIOTIC MATURATION IN PORCINE OOCYTES*
16:30		Yong-Han Kim (Korea) *ACENTRIOLAR MICROTUBULE ORGANIZATION CENTERS AND RAN-MEDIATED MICROTUBULE FORMATION PATHWAYS ARE BOTH REQUIRED IN PORCINE OOCYTES* Tingting Li (China) *THE ESTABLISHMENT OF IN VITRO CULTURE SYSTEM OF BUFFALO SPERMATOGONIAL STEM-LIKE CELLS*
17:00~		Free Activity

Hand-On Training Schedule (maximum 7 people per class)

Hand-On Training	Location	3 May (Thu)	4 May (Fri)	5 May (Sat)	6 May (Sun)	Instructor
Flow Cytometric Sperm Assay	Room 301	15:00~17:00		10:00~12:00	10:00~12:00	Hsiu-Lien Lin, Ting-Yung Kuo, Chiao-Chien Chu
Oocyte Freezing	Room 302		10:00~12:00	15:00~17:00		Yu-Shin Chen, Ting-Chieh Kang, Fung-Hsiang Chu, Jenn-Rong Yang

Poster Presentation Preference Session & Exhibition

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Venue: International Conference Hall

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Oral Presentation Schedule (Panda House)

Time	6 May (Sun)	Presenter/ Paper Title
08:30	Registration	*Third Floor of Panda House*
09:00	Plenary Speaker	Pierre Comizzoli (USA) *GONADAL TISSUE PRESERVATION IN RARE AND ENDANGERED SPECIES*
09:40	Cell reprogramming (II)	Ying-Jie Niu (Korea) *PINK1 IS ESSENTIAL FOR MAINTENANCE OF MITOCHONDRIAL MORPHOLOGY IN PORCINE PREIMPLANTATION EMBRYOS* Xiaoxiao Gao (China) *ROLES OF FIBROBLAST GROWTH FACTOR 9 AND LOC105611671 IN HU SHEEP TESTOSTERONE BIOSYNTHESIS*
10:10		Mei-Fu Xuan (China) *MYOSTATIN DEFICIENCY INCREASES TYPE II FIBER FORMATION IN SKELETAL MUSCLE BY REGULATING THE EXPRESSION OF MYOD AND MEF2 IN NEWBORN MYOSTATIN-KNOCKOUT PIGS* Jenn-Rong Yang (Taiwan) *CURRENT DEVELOPMENT OF PORCINE EMBRYONIC STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS IN TAIWAN*
10:40		Shuting Wang (China) *EXPRESSION AND LOCALIZATION OF MECP2 GENE IN THE REPRODUCTIVE ORGANS OF HU SHEEP* Kaiping Deng (China) *CARCASS TRAITS, MEAT QUALITY, ANTIOXIDANT STATUS AND ANTIOXIDANT GENE EXPRESSION IN MUSCLE AND LIVER OF HU LAMBS FED PERILLA SEED*
11:00	Health break	*Third Floor of Panda House*
11:30	Assisted reproduction (III)	Pei-Shiue Tsai (Taiwan) *THE EFFECTS OF TYPE I COLLAGENASE ON THE DEGELIFICATION OF CHIMPANZEE (PAN TROGLODYTES) SEMEN PLUG AND SPERM QUALITY: IMPROVING GENETIC DIVERSITY OF ZOO CAPTIVE CHIMPANZEE*
12:00		Thanh Quang Dang-Nguyen (Japan) *GERMINAL VESICLE TRANSFER AS A RESCUE TOOL*
12:30 13:00	Lunch break	Education Center
13:30	Fertility preservation (III)	Ting-Yung Kuo (Taiwan) *SCREENING ON SPERM CHROMOSOMAL BREAKAGE AND OXIDATION LEVEL OF YOUNG BREEDING BOARS* Jian Zheng (China) *COMPARATIVE TRANSRIPTOME RESEARCH IN HU SHEEP HYPOTHALAMUS ASSOCIATED WITH PROLIFICACY*
14:00		Chiao-Chien Chu (Taiwan) *SPERM MATURITY OF GROWTH PERFORMANCE TESTED BOARS* Yu-Che Chiu (Taiwan) *ESTABLISHMENT OF CULTURE CONDITIONS FOR PRIMORDIAL GERM CELLS FROM TAIWAN COUNTRY CHICKEN*
14:30	Biology and Economics (II)	Thanee Pak-Uthai (Thailand) *ECONOMIC INPUT OF LIVESTOCK DEVELOPMENT IN FARMER BASED THAILAND* Xiaolei Yao (China) *AGE-ASSOCIATED EXPRESSION OF VDR AND VIT D METABOLIZING ENZYMES IN MALE REPRODUCTIVE TRACT AND SPERM OF SHEEP*
15:00	Health break	*Third Floor of Panda House*
15:30	Cell and Tissue bio-banking (II)	Kazuhiro Kikuchi (Japan) *INTERNATIONAL COLLABORATION FOR CONSERVATION AND UTILIZATION OF PIG RESOURCES - SATREPS ACTIVITY IN VIETNAM*
16:00		Long Xie (China) *EFFICIENT DERIVATION OF PRIMORDIAL GERM CELLS FOR GENETIC PRESERVATION OF INDIGENOUS CHICKEN* Yi-Chen Chen (Taiwan) *IN VITRO CULTURE AND CHARACTERISATION OF DUCK PRIMORDIAL GERM CELLS* Jun-Xia Wang (China) *CRYOPRESERVATION OF PIG PANCREATIC ISLET WITH TREHALOSE CRYOPROTECTANT*
16:30	Closing Ceremony	All Participants

17:00		
17:30	Gala Dinner/	All Participants
18:00	*Gate Square*	The Outstanding Paper Award of Young Scientists
19:00		
20:00		
21:00		

Hand-On Training Schedule (maximum 7 people per class)

Hand-On Training	Location	3 May (Thu)	4 May (Fri)	5 May (Sat)	6 May (Sun)	Instructor
Flow Cytometric Sperm Assay	Room 301	15:00~17:00		10:00~12:00	10:00~12:00	Hsiu-Lien Lin, Ting-Yung Kuo, Chiao-Chien Chu
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ARBC2018TW

Email:

Name:

Hand-On Training

Sperm Assay (maximum 7 people per class)

Choice of Class	Class DATE Selection		
1ST	☐3 May (Thu)	☐5 May (Sat)	☐6 May (Sun)
2ND	☐3 May (Thu)	☐5 May (Sat)	☐6 May (Sun)
3RD	☐3 May (Thu)	☐5 May (Sat)	☐6 May (Sun)

Oocyte Freezing (maximum 7 people per class)

Choice of Class	Class DATE Selection	
1ST	☐4 May (Fri)	☐5 May (Sat)
2ND	☐4 May (Fri)	☐5 May (Sat)

Zoo Tour Group Booking (Friday Afternoon of May 4th)

Choice of Tour	☐A ☐B	☐C ☐D	☐E ☐F	☐G ☐H
Zoo Tour: ● Tour African Animal ● Tour Forest Animal ● Tour Formosa Animal ● Tour Animal Medical Center & Conservation and Research Center	● Tour African Animal *Group A:* *14:00~14:30* *Group B:* *14:40~15:10*	● Tour Forest Animal *Group C:* *15:00~15:30* *Group D:* *15:40~16:10*	● Tour Formosa Animal *Group E:* *16:00~16:30* *Group F:* *16:40~17:10*	● Tour Animal Medical Center & Conservation and Research Center (maximum 50 people per group) *Group G:* *14:00~14:50* *Group H:* *15:00~15:50*

Hand-On Training Schedule (maximum 7 people per class) (Panda House)

Hand-On Training	Location	3 May (Thu)	4 May (Fri)	5 May (Sat)	6 May (Sun)	Instructor
Flow Cytometric Sperm Assay	Room 301	15:00~17:00		10:00~12:00	10:00~12:00	Hsiu-Lien Lin, Ting-Yung Kuo, Chiao-Chien Chu
Oocyte Freezing	Room 302		10:00~12:00	15:00~17:00		Yu-Shin Chen, Ting-Chieh Kang, Fung-Hsiang Chu, Jenn-Rong Yang

Zoo Tour on the Afternoon of May 4th (Fri)

Zoo Tour: ● Tour African Animal ● Tour Forest Animal ● Tour Formosa Animal ● Tour Animal Medical Center & Conservation and Research Center	● Tour African Animal *Group A:* *14:00~14:30* *Group B:* *14:40~15:10*	● Tour Forest Animal *Group C:* *15:00~15:30* *Group D:* *15:40~16:10*	● Tour Formosa Animal *Group E:* *16:00~16:30* *Group F:* *16:40~17:10*	● Tour Animal Medical Center & Conservation and Research Center (maximum 50 people per group) *Group G:* *14:00~14:50* *Group H:* *15:00~15:50*

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Reproductive Biotechnology	Abstracts	Species
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C. Assisted reproduction	11	Human; Farm Animal; Zoo Animal; Lab Animal; Endangered Species
D. Cell and Tissue bio-banking	9	Farm Animal; Zoo Animal; Endangered Species
E. Animal cloning	4	Farm Animal; Endangered Species
F. Embryo development	11	Farm Animal; Lab Animal
G. Cell reprogramming	7	Farm Animal; Lab Animal
H. Biology and Economics	4	Farm Animal

Theme	Presenter (alphabetical order)	Paper Title	From
A	**Bo Xiong**	THE PROTECTIVE ROLE OF MELATONIN IN PORCINE OOCYTE MEIOTIC FAILURE CAUSED BY THE EXPOSURE TO BENZO(A)PYRENE	China
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G	**Yuka Miki**	KNOCKOUT ANALYSIS OF OOCYTE-SPECIFIC MULTI-COPY GENE, OOG1	Japan
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THE PROTECTIVE ROLE OF MELATONIN IN PORCINE OOCYTE MEIOTIC FAILURE CAUSED BY THE EXPOSURE TO BENZO(A)PYRENE

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Introduction

Benzo(a)pyrene (BaP), a widespread environmental carcinogen found in particulate matter, 2.5 μm or less (PM2.5), has been shown to have toxicity at the level of the reproductive systems. Acute exposure to BaP has transient adverse effects on the follicle growth, ovulation and formation of corpora lutea, which results in transient infertility. However, the molecular mechanisms regarding how BaP influences the oocyte maturation and whether melatonin restores the BaP-induced meiotic failure has not yet been determined.

Materials and Methods

Porcine oocytes were randomly assigned to control, BaP-exposed and melatonin-supplemented groups. Acquisition of oocyte meiotic competence was assessed using immunostaining, fluorescent intensity quantification and/or immunoblotting to analyse the cytoskeleton assembly, mitochondrial integrity, cortical granule dynamics, ovastacin distribution, ROS level and apoptotic rate. Fertilization ability of oocytes was examined by sperm binding assay and IVF.

Results and Discussion

BaP exposure resulted in the porcine oocyte meiotic failure via impairing the meiotic apparatus, showing a prominently defective spindle assembly, actin dynamics and mitochondrion integrity. In addition, BaP exposure caused the abnormal distribution of cortical granules and ovastacin, which were consistent with the observation that fewer sperm bound to the zona pellucida surrounding the unfertilized BaP-exposed eggs, contributing to the fertilization failure. Conversely, melatonin supplementation recovered, at least partially, all the meiotic defects caused by BaP exposure through inhibiting the rise in ROS level and apoptotic rate.

HORMONE MONITORING: AN IMPORTANT TOOL FOR BREEDING MANAGEMENT OF WILDLIFE SPECIES

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As more species reach vulnerable or endangered status, captive breeding is increasingly viewed as a means to sustain important insurance populations in case of catastrophic extinctions. Unfortunately, many captive populations are not self-sustaining. As reproduction is key to species survival, understanding how to control and monitor ovarian function is vital. It is now clear that the ability to track reproductive activity via hormones is vital to developing successful *ex situ* breeding programs, through both natural and assisted reproductive means. What we have learned is that a diversity of reproductive mechanisms exists across taxa. The development of noninvasive monitoring techniques to analyze hormones or their metabolites excreted in urine or feces, greatly expanded our ability to study rare species, including in the wild. Perhaps it should not be surprising then, that major differences in metabolism and routes of excretion exist, not only between species, but also between hormone types within a species. This means that a species by species, and sometimes hormone by hormone, approach is needed for developing effective reproductive monitoring and control strategies. Over the past 30 years, our laboratory has developed and validated a number of reproductive assay techniques, which has led to our amassing a database of ovarian cycle dynamics on over 100 species. Even among related species, there are marked differences in seasonal, environmental and social influences on ovarian cycle dynamics, ovulatory mechanisms, and responses to assisted reproductive/ovulation induction protocols. This presentation summarizes comparative ovarian function research on some of the most well-studied species in our laboratory: felids, elephants, rhinos, tapirs and the giant panda, and how that information has been used to aid *ex situ* management. Each of these species represents a range of reproductive strategies, from the highly seasonal, monestrus giant panda to the aseasonal, polyestrus elephant. Some species exhibit spontaneous ovulations, while others are induced ovulators or both, with variations in ovarian cycle lengths that range from a few days to several months. These differences reinforce the need for studies of species basic biology to optimize breeding strategies.

PRODUCTION OF PRION GENE KO COW TO PREVENT SPONTANEOUS BSE AND THEIR CHARACTERISTICS

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Prion (Pr) protein plays a pivotal role in the infection of bovine spongiform encephalopathy (BSE). The aim of our research was to make Pr gene homo-knockout (KO) cows and to reveal their characteristics. Firstly, hetero Pr gene was knockouted in fibroblastic cells prepared from female Japanese black embryo. Somatic cell nucleus with Pr gene hetero KO was transferred into oocyte of female Japanese black. After activation and in vitro culture, somatic cell nucleus of blastocyst cell was transferred into oocyte to make Pr homo gene KO embryo. The Pr gene homo KO embryos were transferred in to the uterus of female Japanese black. Nine cows were born. Detailed data of Pr distribution in organs of wild-type and somatic cell nuclear cloning Japanese black cows were demonstrated as follows: Pr protein were shown in peripheral organs: 7 skeletal muscles, 5 areas of intestinal tracts and 5 areas of tongue (5- to 31- fold less Pr protein than the brain where high levels of that, 9.0-11.0 μg/g, were noted). Histochemistry showed that Pr protein was expressed in nerve cells not only in brain but also in the tongue, skeletal muscles and intestines, indicating that these organs may serve as potential sources of BSE infection. No positive reaction for Pr mRNA or for Pr protein was noted in Pr gene homo KO cows, indicating that the KO cows have no possibility of BSE infection. Molecular biochemical characteristics on protein expression, epigenesist etc. were also revealed in the KO cows. In conclusion, to produce the KO cattle is useful and the only way to make safe bovine derived foods, drugs and medical materials.

MIMICKING THE MATERNAL ENVIRONMENT TO OPTIMIZE THE IN VITRO PRODUCTION OF EMBRYOS IN WILD AND DOMESTIC MAMMALS

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The preservation of endangered wild species and domestic breeds represents a challenging perspective regarding quick decline of biodiversity in the world. A wide range of reproductive biotechnologies are now available to facilitate the conservation and genetic management of mammals threatened with extinction (1). The maternal environment offers optimized conditions for gametes maturation, fertilization and embryo development, based on a constant dialogue between somatic and germinal tissues, guarantee for the success of reproduction. Bypassing this dialogue through in vitro technologies, such as in vitro production of embryos (IVP), leads suboptimal regulation of early events with negative impact of reproductive success and offspring health. Since IVP techniques offer a wide range of applications for the spreading and amplification of valuable genotypes, it seems important to understand the mechanisms of this dialogue and find some ways to reproduce it in vitro in view to optimize the output of the technique. In deer species, it has been clearly shown that the presence of oviduct epithelial cells (OEC) can improve the rate and quality of embryo development of embryos produced from oocytes collected from slaughtered females in red deer (2,3) or from living females by laparoscopic ovum pick up (LOPU) in sika deer (4). In bovine, we showed that this beneficial effect of OEC relies on a molecular dialogue with early developing embryos, modulating the gene expression profiles of both partners (5). In pigs, we showed that oviduct fluid can regulate the polyspermy, which is a major problem for IVP in this species (6,7). Recently, extracellular vesicles such as exosomes emerged as a new paradigm in cell communication, beyond the traditional ligand – receptor pathways, triggering intracellular signaling cascades. These membrane vesicles of 50-150 nm can transport proteins, mRNA and ncRNA from secreting cell to target cells and modify their properties. We found exosomes in OF, containing proteins, and different RNAs, including microRNA (8) and we showed that these exosomes are able to reproduce the effects of OEC on embryo development in cattle (8) as well as the effect of OF on pig IVF (Unpublished data). In conclusion, mimicking the maternal environment in vitro by using cells or fluid from the oviduct can improve the IVP success, at least a part of this effect may be mediated by extracellular vesicles. Further studies of these vesicles will allow to set up optimized IVP conditions allowing the efficient production of high viability embryos

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THE RESEARCH OF THE BREEDING OF NOMASCUS GABRIELLAE IN NANJING HONGSHAN FOREST ZOO IN CHINA

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Nomascus gabriellae and *Nomascus leucogenys* are belong to Hylobatidae family. They are classified as Endangerd on the IUCN Red List and list on Appendix I of CITES. We have one of largest captive *Nomascus gabriellae* populations in China. To measure the level of urinary progesterone (P4) and level of urinary Estradiol-17beta (E2), urinary data were then combined with their knowledge of endocrine, physiology and reproduction to infer their length of ovarian cycle.
We wanted to know the breeding situation of captive *Nomascus gabriellae*. Mitochondrial cytochrome C oxidase subunit I (COI) gene were used as the DNA barcoding to identify *Nomascus gabriellae* from *Nomascus leucogenys*. These results can help us to achieve the purpose of protecting the species.

CHARACTERIZATION OF NONINVASIVE MONITORING REPRODUCTIVE ENDOCRINE PROFILES FROM THE CAPTIVE AND FIELD EURASIAN OTTER (*LUTRA LUTRA*) IN TAIWAN

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Eurasian otter *(Lutra lutra)* has been categorized as endangered animal in "Wildlife conservation Act" of Taiwan. Among Taiwan area Kinmen island is the only left area with stable population of this species. However Eurasian otter do not reproduce well in captivity. The Reproductive profile in Eurasian otter is difficult to quantify because little is known about the complex endocrine interactions controlling in the reproductive cycle. Our main objectives were to characterize endocrine parameters by longitudinal monitoring of fecal hormone metabolites. In this study, we attempted to identify seasonality breeding, estrus and pregnancy states, sexual maturity, and predict the date of birth in Eurasian otters by examining fecal hormones and behavior. Fecal samples were collected from 3 captive Eurasian otters (1 female and 2 male) housed at Taipei zoo (from Sub-adults to adulthood) and field samples were collected from Kinmen Qionglin Reservoir (2017-2018) . Noninvasive methods were validated for monitoring fecal metabolites of reproductive hormone (Estrogen, Progesterone, Testosterone) by EIA.

In captivity, fecal estrogens and progesterone in females did not vary by season or month, nor did fecal testosterone in males. During this study, the female one became pseudopregnany one time and pregnant two times. Her sexual maturity age is at 3 years old, total gestation length was 56 and 58 days, and without delayed implantation. Pseudopregnany exhibited a similar progesterone peak of 55 days which could not be differentiated from pregnancy. Both pregnancy were characterized by a moderate rise in fecal progesterone for 5days post ovulation followed by a marked increase. Due to insufficient study size in the field sample, we could not see significant changes in the reproductive cycle during different seasons. These findings represent the first comprehensive information on normative endocrine of the Eurasian otter in Taiwan. However due to difficulty fecal collection in the wild field, more survey is necessary in the future study.

SPERM MATURITY OF GROWTH PERFORMANCE TESTED BOARS

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Introduction

Mitochondria is a key factor in providing energy for motility and fertilization of sperm. The energy required for sperm metabolism is provided by mitochondria. Mitochondrial integrity measurement is an important basis for sperm quality determination. Sperm maturity evaluation of young boars could be done by mitochondrial integrity measurement via sperm flow cytometer. The objective of this study is conducted to measure the sperm concentration and mitochondrial integrity by using flow cytometer to evaluate the semen productive ability and maturity of high feed efficiency young boar, and try to apply the elite young boar for the reproduction of breeding stock and the production of meat pig early.

Materials and Methods

A total of 1,079 finished test boars from 3 breeds (Duroc, Landrance and Yorkshire) in class 201507, 201509, 201510, 201511, 201601, 201603, 201604, 201605, 201607, 201609, 201610, 201611, 201701, 201703, 201704 and 201705 of the Pig Performance Testing Station of National Animal Industry Foundation were used at this project. We collected the semen 20 days before the auction and stored at 17℃. The collected semen were immediately analyzed the sperm concentration and mitochondrial integrity at least 5,000 sperm each semen to assess the semen productive ability of young boar.

Results and Discussion

The results showed that the sperm concentration and mitochondrial integrity of the young boars from Duroc (n=701), Landrance (n=270) and Yorkshire (n=108) were 340 ± 106 (10^6/ml), 376 ± 119 (10^6/ml), 289 ± 126 (10^6/ml) and 57 ± 21.4 (%), 58.9 ± 24.1 (%), 54 ± 23.3 (%), respectively. These data indicated that young boars with better feed efficiency have decreased sperm concentration and mitochondrial integrity.

COMPARATIVE TRANSRIPTOME RESEARCH IN HU SHEEP HYPOTHALAMUS ASSOCIATED WITH PROLIFICACY

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Hu sheep is unique in China, the world famous prolific sheep breeds, with early sexual maturity, a seasonal breeding and prolificacy. This study aims to investigate the key functional genes associated with prolificacy by a hypothalamus transcriptome analysis. We performed RNA-seq and bioinformatics analysis between Hu sheep with high prolificacy (H, n=3, litter size=3 for three consecutive lambing) and low prolificacy (L, n=3, litter size=1 for three consecutive lambing), then the expression levels of differentially expressed (DE) genes in the hypothalamus tissue were verified by real-time reverse transcription quantitative PCR (qRT-PCR). By comparing and analyzing transcriptome data, 56 DE genes were obtained, 24 of them were up-regulated and 32 of them were down regulated between the H and L group. GO classification and annotation analysis showed that these DE genes are closely related to biological processes such as organ development, signal transduction and transcriptional regulation. Meanwhile, molecular events such as DNA binding, protein binding and ATP binding are also involved. KEGG pathway analysis showed that the neural signal pathway, GABA signaling pathway might play an important role in hypothalamus regulation of ovulation and follicular development. The qPCR analysis of 5 randomly selected DE genes were consistent with sequencing results. This study provides a basis for further explanation of the basic molecular mechanism of hypothalamus, but also provides a new idea for a comprehensive understanding of prolificacy characteristics in Hu sheep.

Key words: Hu sheep; hypothalamus; transcriptome; qRT-PCR

IMPROVING PRACTICAL USE OF BOAR SEMEN CRYOPRESERVATION: CURRENT UNDERSTANDING

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Artificial insemination (AI) using fresh semen has been more and more widely used in commercialized pig production. The storage time of fresh semen is quite limited, normally less than 3 days. Cryopreservation of boar semen is of importance and significance for pig production. The worldwide use of boar frozen semen only accounts for around 1% currently. Cryopreservation of boar sperm has still remained at the laboratory stage. In recently years, with the quickly improvement of diluents and cryoprotective agents, cryopreservation of boar semen has been made much progress. In our laboratory, we optimized a protocol to cryopreserve boar semen. The 0.25 ml plastic straws were used as the sperm carrier. First, the boar semen was pre-diluted with ZORLESCO (ZO) solution and pre-equilibrated at room temperature for 1 h. After adding extender I, spermatozoa were equilibrated at 5 °C for 1.5 h; then an equal volume of extender II was added and the spermatozoa equilibrated for 2 h. The resulting spermatozoa were loaded into 0.25 ml straws, equilibrated for 10 min at 3 cm above the surface of liquid nitrogen (LN), then promptly submerged into LN. When thawing, straws were incubated in a water bath at 37 °C for 30 s. This procedure yielded the highest post-thaw motility of 0.58 ± 0.03 and plasma integrity of 63.2 ± 1.2%, together with a normal acrosome in 51.4 ± 2.6% of spermatozoa. Abnormal spermatozoa after freezing represented only 14.0 ± 3.0%, which is the lowest figure ever published to date. We also used the high density of spermatozoa (1×10^9 spermatozoa/ml) for the cryopreservation and each straw of boar semen could be used for one insemination after thawing. Twice inseminations of a sow at heat using those frozen boar semen could result in 33.3% gestation in sow (6/18). Therefore, this technology enables it possible to reproduce pigs using frozen semen insemination.

INFLUENCES OF DIFFERENT DIETARY ENERGY LEVEL ON SHEEP TESTICULAR DEVELOPMENT ASSOCIATED WITH AMPK-ULK1 AUTOPHAGY PATHWAY

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Introduction

Energy balance is an important feature for spermatozoa production in the testis. The 5'-AMP-activated protein kinase (AMPK) is a sensor of cell energy, has been implicated as a mediator between gonadal function and energy balance. Herein, we intended to determine the physiological effects of AMPK on testicular development in feed energy restricted and compensated pre-pubertal ram.

Materials and Methods

Lambs had restricted feeding for 2 months (M) and then provided compensatory feeding for another 3 M. Feed levels were 100% (control), 15% and 30% of energy restriction (ER) diets, respectively. The testes were collected for qRT-PCR, WB, IHC and HE analysis.

Results and Discussion

The results showed that lambs fed the 30% ER group had significantly lower testicular weight ($P < 0.05$) and spermatids number in the seminiferous tubules, but there was no difference between control and 15% ER groups ($P > 0.05$). Meanwhile, 15% ER and 30% ER group induced testis autophagy and apoptosis through activating AMPK-ULK1 (ULK1, Unc-51 like autophagy activating kinase) signal pathway. And with characterization of increased Beclin-1 and Light chain 3-II/Light chain 3-I (LC3-II/LC3-I) ratio, up-regulated the ratio of pro-apoptotic Bcl-2-associated X protein (BAX) and anti-apoptotic B-cell lymphoma 2 (Bcl-2), as well as activated AMPK, phosphorylated AMPK(p-AMPK) and ULK1. Furthermore, a compensation of these parameters occurred when the lambs were re-fed with normal energy requirement after restriction. Taken together, dietary energy levels influence testicular development through autophagy and apoptosis interplay mediated by AMPK-ULK1 signal pathway, which also indicates the important role of the actions of AMPK in the testis homeostasis.

GAIN: GREAT APE INFORMATION NETWORK FOR BEST CARE AND MANAGEMENT OF CAPTIVE APES IN JAPAN

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Introduction

The Great Ape Information Network (GAIN) is part of the National Bio Resource Project (NBRP) administered by the Ministry of Education, Culture, Sports, Science and Technology, Japan. It started in 2002 and had been run by the Primate Research Institute, Kyoto University. GAIN is collecting individual data on all captive apes in Japan for the purpose of promoting non-invasive scientific research, animal welfare and population management. (GAIN; http://www.shigen.nig.ac.jp/gain)

Materials and Methods

GAIN has collected historical and current individual data on all apes; chimpanzees, bonobos, gorillas, orangutans and gibbons housed in Japan and made this information available on an open-access database. For collecting such information, we have visited zoos and research institutions keeping apes to make personal networks. When an individual dies and the dead body is available for research, GAIN can inform researchers about the samples for use in non-invasive research.

Results and Discussion

According to current data by GAIN, there are 317 chimpanzees, 6 bonobos, 21 gorillas, 46 orangutans and 180 gibbons in Japan, as of April 23rd, 2018. Including historical individuals, data for around 2000 individuals is stored in the GAIN database. Owing to our real-time database and zoos-researchers network, GAIN can provide a variety of information on best care and breeding of apes. Morphological, genetic, reproductive and physiological specialists are included among our collaborative researchers. In this presentation we will also introduce a rare case of a captive chimpanzee's genital swelling during her lactation period.

EFFECT OF STORAGE TEMPERATURE OF EXTENDERS ON THE QUALITY OF BOAR SEMEN

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Introduction

In artificial insemination, semen extenders are important to maintain the quality and survivability of sperm. This study aimed to determine the effects of different storage temperatures of KRUUSE Beltsville Thawing Solution (BTS) powder on motion characteristics, live percentage and morphology of extended boar semen.

Materials and Methods

Semen samples were collected from seven different boars. Each sample was mixed with 3 different BTS extenders that were stored at 4°C, 16°C and room temperature prior to preparation of the extender. All the extended semen samples were kept at 16°C and evaluated at 24 hour intervals for 3 days. Data was analyzed with one-way ANOVA (SPSS 22) with $P < 0.05$ considered statistically significant.

Results and Discussion

General motility and live percentage of spermatozoa decreased gradually over time for all temperatures. However, there was no significant difference in the general motility, live percentage and abnormal morphology between all temperatures. There was a positive correlation ($P < 0.05$) between general motility and live percentage of the sperm. Negative correlation ($P < 0.05$) was observed between both general motility and live percentage with the abnormal morphology percentage. In conclusion, storing the extender in room temperature prior to preparation can maintain the highest quality of extended boar semen. However, statistically there was no difference in the survivability of sperm in extended boar semen for all storage temperatures.

APPLICATION OF FLOW CYTOMETRY IN SEMEN ANALYSIS: SEMEN ANALYSIS BY FLOW CYTOMETRY APPROACH TO PREDICT AND TO IMPROVE THE FERTILITY

Na Li, Agnès Camus and Guy Delhomme

Semen analysis is widely used to evaluate semen quality. The assessment by classical microscopic observation remains subjective, cannot suit the need of semen processing centers and final customers with higher requirements of semen quality control. Technologies developed from the medical-food processing have been applied to semen evaluation. Routine tests can be evaluated in short time by CASA (Computer Assisted Semen Analysis) system: concentration, morphology, motility, kinematics etc. Flow cytometry, a more advanced technology, has also been applied to study semen functional parameters in a timely manner. The review will focus on the flow cytometry approach, and aims to establish the relationship between semen analysis *in vitro* and field fertility *in vivo*. In this study, the semen functional parameters measured by flow cytometer are primarily assessed, i.e., sperm membrane integrity, mitochondrial activity, potential oxidation, acrosome status and DNA compaction. The fertility prediction model is also discussed. In equine, the correlation values between the predicted and the real fertility values was up to 0.942. Reliable results obtained with CASA (IVOS II®, Hamilton Thorne, USA) and flow cytometry (EasyCyte®, Millipore, USA). These results allow the opportunity to standardize semen analysis. Combination of IVOS2 and EasyCyte® give a full characterization of the semen quality. Semen analysis becomes a new perspective to predict the male fertility potential, to improve semen process and field fertility of domestic animals.

GONADAL TISSUE PRESERVATION IN RARE AND ENDANGERED SPECIES

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There is enormous potential in applying human-related fertility preservation strategies to wild animals, especially approaches that could assist managing or ‘rescuing’ gametes and gonadal tissues of genetically valuable individuals. Indeed, one of the highest priorities in wildlife ex situ management is sustaining all existing genetic diversity to (1) preserve heterozygosity to avoid inbreeding depression and (2) ensure species integrity and the persistence of genomic adaptability to environmental changes. There are components of the rapidly growing field of onco-fertility in men and women that are highly compatible with preserving valuable germplasms of individuals or populations of threatened wildlife. Strategies associated with ovarian or testicular tissue cryopreservation followed by in vitro culture are especially attractive for protecting and extending fertility. Given adequate attention and more basic studies, we predict that these approaches could assist in the intensive and practical management of gene diversity in endangered species.

MELATONIN PROTECTS OOCYTES FROM MEHP EXPOSURE INDUCED MEIOSIS DEFECTS IN PORCINE

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Introduction

In 2011, DEHP (plasticizer) was reported to illegally be added in food and beverage products in Taiwan, which caused great concerns about food safety worldwide. DHEP has multiple toxic effects to human and animals like endocrine disruption and cardiotoxicity. However, the toxic effects of DEHP on mammalian oocyte quality are still unclear.

Materials and Methods

We used porcine oocyte as model to explore the effects of active metabolite of DEHP - MEHP on oocyte maturation and we also studied the effects of melatonin administration on MEHP exposure-induced meiosis defects.

Results and Discussion

Our results showed that exposure to MEHP significantly decreased the oocyte polar body extrusion. Cell cycle progression, meiotic spindle organization and actin assembly were all disturbed after MEHP exposure. Moreover, the DNA and histone methylation levels were also affected, showing with altered 5mC and H3K4me2 levels. While MEHP exposure induced meiotic defects were all remarkably ameliorated by the administration of melatonin in porcine oocytes. We further tried to explore the causes of MEHP toxicity on oocytes, and we found that MEHP exposure resulted in significant elevations of oxidative stress and induced early apoptosis as well as autophagy, while melatonin administration could reduce these. Taken together, our results indicated that MEHP exposure induced deterioration of oocyte quality, whereas melatonin supplement showed amelioration on oocyte maturation through its rescue effects on oxidative stress mediated apoptosis and autophagy.

THE PRIMARY RESEARCH OF OVER EXPRESSION OF GDNF GENE IN BUFFALO TESTIS SERTOLI CELLS

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Introduction

Glialcellline-Derived Neurotrophic Factor (GDNF) is a neurotrophic factor firstly cloned from mouse brain tissue by Lin *et al.* in 1993, which can also be secreted by Sertoli cells. It has a variety of physiological functions and plays an important role in the nervous system and maintenance of spermatogonial stem cells. Up to date, the study of in vitro culture of buffalo spermatogonial stem cells (SSCs) usually supplement with mouse-derived GDNF, but the proteins for its variety of the structure and modification in different animal species may affect the culture efficiency of SSCs in vitro. In the present research, the gene of buffalo GDNF was integrated into PiggyBac expression vector to make it stably expressed in Sertoli cells, which provided the basis for the related research of buffalo SSCs.

Materials and Methods

The buffalo brain tissue was used as material to extract total RNA which was reversed by transcription. Then the GDNF fragment was cloned, and the product was taken from the agarose gel and connected with T vector to construct the Piggybac recombinant plasmid. The prepubertal buffalo testes (3 to 7 months) were used as materials. The Sertoli cells were purified by two-step enzymatic digestion combined with differential planting method, which were identified by WT1 and GDNF antibodies. The PB-GDNF plasmid was stably transfected into Sertoli cells by lipofectamine 2000. The SSCs were co-cultured with PB-GDNF-Sertoli cells and Sertoli cells as feeder layers, respectively. Afterwards, we compared the expression of PLZF, NANOS2 and DDX4 in these two kinds of SSCs, which we obtained.

Results and Discussion

We constructed the GDNF-Piggybac eukaryotic expression vector which was transfected into Sertoli cells. The quantitative real-time PCR results showed that the expression of GDNF gene in PB-GDNF-Sertoli cells was significantly higher than that of Sertoli cells. Meanwhile, the expression of the specific markers PLZF, NANOS2 and DDX4 in the PB-GDNF-Sertoli-SSC group was significantly higher than in the Sertoli-SSC group. In conclusion, we successfully constructed buffalo-derived GDNF eukaryotic over expression vectors and a buffalo Sertoli cell line which could over express the GDNF gene in vitro. This research could provide a new method for the maintenance of the pluripotency of buffalo SSCs in vitro.

THE ESTABLISHMENT OF IN VITRO CULTURE SYSTEM OF BUFFALO SPERMATOGONIAL STEM-LIKE CELLS

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Introduction

Spermatogonial stem cells (SSCs) are precursor cells of sperm, providing continual motility for spermatogenesis, while also ensuring the transfer of genetic material between parent and offspring. The research on SSCs is of great significance for regenerative medicine, species preservation, and production of transgenic animals. In the previous studies, we used DMEM/F12 as basal medium, got the compact and round cell clusters, however, they were different from the morphology of SSCs in model organisms. The objective of this paper was to isolate, characterize and establish a new culture system of buffalo spermatogonial stem-like cells (SSC-like cells) in vitro.

Materials and Methods

We used the specific markers OCT4, DDX4, THY-1, PGP9.5 and PLZF to perform in situ staining of prepubertal buffalo testis (3 to 7 months) by paraffin section immunofluorescence staining. The single SSC-like cells were purified by two-step enzymatic digestion combined with differential planting method. The $DDX4^{+}$ and $PGP9.5^{+}$ cells were counted before and after the purification, meanwhile the cells also were detected by the quantitative real-time PCR. After the SSC-like cells were cultured for three passages, they were identified at the protein level and transcription level, respectively. Afterwards, we conducted the in vitro induction experiment with RA in this culture system.

Results and Discussion

The immunofluorescence staining showed that all of the specific markers were expressed in the SSC-like cells, which means that the materials we used really contain SSC-like cells, which could provide a source of cells for our follow-up study. Meanwhile, about 60% of SSC-like cells could be enriched through differential planting method. During our in vitro culture processes, a remarkable result was found that, serum-free culture system appeared as more suitable for the proliferation of buffalo SSC-like cells. At the initial stage of culture, SSC-like cells were mainly in the state of A_{single} or A_{paired}, which were typical proliferation forms of stem cells, with the prolongation of the culture time, showed a typical string-like proliferation mode, notably. The result of in vitro induction experiment illustrated that the differentiation genes such as Stra8 and Rec8 were highly expressed in the induced SSC-like cells in vitro, while the expression of the pluripotency gene NANOS2, PLZF and germ line gene DDX4 were decreased. In conclusion, we established a method to obtain a large number of buffalo SSC-like cells, and optimized the in vitro culture system. These results could provide the basic platform for buffalo SSC-like cells studies, such as its long-term culture, gene editing, the mechanism of self-renewal and differentiation and so on.

SCREENING ON SPERM CHROMOSOMAL BREAKAGE AND OXIDATION LEVEL OF YOUNG BREEDING BOARS

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Introduction

High standards of semen quality and non-reduction in sperm fertility in livestock are of economic relevance due to its association with fertility and offspring results for animal industry. The objective of this study was to screen and estimate genetic parameters and genetic trend for new sperm parameters such as sperm chromosomal breakage and oxidative damage of on-farm young breeding boar.

Materials and Methods

124 ejaculates collected from 40 Duroc, 24 Landrace and 16 Yorkshire boars. The data was collected over 2 years (2014-2015) from a breeding farm located in the north part of Taiwan. New sperm parameters of semen quality traits considered per ejaculate were % of intact membrane spermatozoa (VA), viable and intact acrosomal status (AS), mitochondrial activity (MA), DNA compaction (DC) and viable nonoxidized (NO) were assessed with Flow cytometry (Guava easyCyte microcapillary flow cytometer with CytoSoft software; Guava Technologies Inc., Hayward, CA, USA; distributed by IMV Technologies). Genetic heritability parameters and new sperm parameters were estimated by VCE6.0 with the univariate model of Restricted Maximum Likelihood.

Results and Discussion

Heritability estimates of VA, AS, MA, DC and NO were 0.33, 0.42, 0.22, 0.08 and 0.41 respectively. Genetic correlations among those of new sperm parameters of flow cytometric measurement varied from -0.451 to 1.0 among breeds of pigs. In summary, the deterioration of genetic ability indicated that new sperm parameters should be taken into account for boar selection at young age and may further to improve and enhance the overall fertility of breeding boars.

FIPRONIL CAUSES APOPTOSIS DURING MEIOTIC MATURATION IN PORCINE OOCYTES

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Introduction

Fipronil is a widely used and highly effective phenylpyrazole pesticide, which can kill pests through blocking GABA (γ-aminobutyric acid) gated chloride channels. However, it is found that fipronil and its metabolites have detrimental effects on mammalian blood cells, nerve cells and spermatogonia causing increased level of ROS and DNA damage. On the contrary, studies on the effect of fipronil on the female mammalian reproductive system have hardly reported, especially on oocytes.

Materials and Methods

In this study porcine oocytes were chosen as a target. And Immunofluorescence, qPCR, Time-lapse and etc. were used to observe the changes in mitochondria function and cell cycle.

Results and Discussion

The results showed that the first polar body extrusion rate significantly decreased and oocytes arrested at GV stage when treated with 100 μM fipronil ($p < 0.05$). Besides, the apoptosis and autophagy activities were enhanced in 100 μM fipronil treatment group oocytes ($p < 0.05$). Meanwhile, the fipronil caused a significant increased ROS level and more severe DNA damage inside the oocytes ($p < 0.05$). In the 100 μM fipronil group, the mitochondrial membrane potential and expression of Bcl-xL dropped and the cytochrome c was released from the mitochondria ($p < 0.05$). Furthermore, the result showed a delayed increase of MPF activity during GVBD in the treatment group, which was also consistent with the lag in cyclin b1 degradation. In conclusion, the fipronil causes apoptosis and cell cycle arrest in the porcine oocyte during maturation because of the increased ROS level and DNA damage.

ACENTRIOLAR MICROTUBULE ORGANIZATION CENTERS AND RAN-MEDIATED MICROTUBULE FORMATION PATHWAYS ARE BOTH REQUIRED IN PORCINE OOCYTES

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Introduction

Mammalian oocytes lack centrioles, but can generate bipolar spindles using several different mechanisms. Full knowledge of the different mechanisms of spindle assembly in various mammalian oocytes is lacking. In this study, we show that both MTOC-mediated and Ran-mediated microtubule formation are required for the functional meiotic metaphase I spindle generation in porcine oocytes. The results demonstrate that the stepwise involvement of Ran- and MTOC-mediated microtubule assembly is crucial for the formation of meiotic spindles in porcine oocytes, indicating the diversity of spindle formation mechanisms among mammalian oocytes.

Materials and Methods

Porcine oocytes were obtained from 30~40 ovaries using 18-gauge micro needles and were transferred into *in vitro* maturation medium. Localization of MTOC and spindle were tested by immunofluorescent staining of Centrosomal protein of kDa 192 (Cep192) and α-tubulin. Function of MTOC was tested by using microinjection of Cep192 dsRNA and treatment of Polo-like kinase 1 inhibitor. Relative gene expression related with Cep192 was quantified by RT-PCR. The Cep192 and spindle protein levels were quantified using ImageJ software. The average fluorescence intensity per unit area within a region of interest was determined. Function of Ran-mediated microtubule assembly was tested by the expression of dominant negative or constitutively active Ran mutants using microinjection of cRNA.

Results and Discussion

Cep192 is localized at the spindle at Metaphase I and Metaphase II stage microtubules, but is absent at the initial microtubule polymerization at GVBD stages. Injection of dsRNA specific for porcine Cep192 depleted the Cep192 mRNA levels by as much as 20% of the levels in the negative control GFP dsRNA–injected oocytes. Treatment of the PLK1-specific inhibitor, BI-2536, decreased Cep192 and alpha-tubulin levels, similar with phenotype Cep192 knockdown. Microtubule and Cep192 fluorescence levels were severely diminished following the injection of the dominant negative or constitutively active Ran mutants. Collectively, the data demonstrate significant mechanistic differences in the spindle formation mechanism between mammalian oocytes, including human, mouse, and porcine oocytes.

ESTABLISHMENT OF CULTURE CONDITIONS FOR PRIMORDIAL GERM CELLS FROM TAIWAN COUNTRY CHICKEN

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Introduction

Primordial germ cells (PGCs), the progenitor cells of gametes, carry the genetic information throughout generations. Hence, PGCs hold the potential to produce transgenic animals and serve a tool for the conservation of endangered animals due to their germ line-transmissible ability. The aim of this study was to isolate, characterize and establish an optimal culture condition for Taiwan country chicken primordial germ cells (cPGCs).

Materials and Methods

Chicken embryos from the country chicken of National Chung Hsing University strain L2 were collected at stage 18-20 (3-3.5 days after incubation). The blood was drawn from the dorsal aorta of embryos using a fine glass micropipette, and then washed with DMEM containing 10% fetal bovine serum (FBS). The cPGCs were isolated by gradient centrifugation with 6.3% and 16% of Ficoll, and allocated into three culture media, i.e. DMEM-10%FBS-5% chicken serum (CS) supplemented with bFGF-LIF, bFGF-LIF-SCF, and bFGF-LIF-SCF-BMP4. Cell proliferation and expression of pluripotency markers were analyzed.

Results and Discussion

The cPGCs were cultured for 7 days and then subcultured every 5 days. Results showed that the total cell number decreased in three groups after culture, but the numbers in bFGF-LIF-SCF group are higher than those in the other groups after 2 passages. The immunocytochemical staining results demonstrated that some cultured cells expressed SSEA-1, indicating that the current three culture systems are able to maintain the cell pluripotency in short term.

REPRODUCTIVE SCIENCE AND TECHNOLOGIES FOR ENHANCING WILDLIFE CONSERVATION: THAI EXPERIENCES

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Degradation and fragmentation of habitats, illegal hunting and climate change directly affect natural wildlife populations. Many attempts including captive breeding and reintroduction have been done actively for their long-term survival. Zoological Park Organization (ZPO) contributes modern zoo strategies to wildlife conservation by maximizing opportunities to engage in conservation-relevant research, especially reproductive science to enhance captive breeding programs. The prioritized research species are Thai native and globally important species i.e. Eld's deer (*Rucervus eldii*), Asian elephant (*Elephas maximus*), Eastern Sarus crane (*Grus antigone sharpii*), Malayan tapir (Tapirus indicus), goral (*Naemorhedus griseus*) and wild felids. In the forum, the presentation will give the information of fundamental reproductive science knowledge and some efforts of applying basic to advanced assisted reproductive technologies (ARTs) i.e. artificial insemination (AI), in vitro fertilization (IVF), and embryo transfer (ET), somatic cell nuclear transfer (cloning) and induced pluripotent stem cell (iPSC) for saving endangered species from extinction.

Keywords: wildlife, reproduction, assisted reproductive technology

IMPROVED PREGNANCY THROUGH SYNCHRONIZED OVULATION AND FIXED TIME AI IN WATER BUFFALOES

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Introduction

Water buffaloes are important contributors to milk and meat production thus playing a critical role in achieving agricultural productivity and food sufficiency. In the Philippines, Genetic Improvement Program for buffaloes has been implemented mainly through AI following estrus synchronization over the last three decades. However, the poor estrus expression and detection in buffaloes limits the full potential of AI technology in buffalo propagation. More recently, an Intensified Reproductive Management System has been developed which involved the application of Fixed Time Artificial Insemination (FTAI) technology in buffaloes in the country.

Materials and Methods

Two studies were conducted to assess the efficiency of FTAI technology in water buffaloes. Study 1 validated the efficacy of the widely used ovulation synchronization protocols for FTAI. Non-pregnant animals were randomly allotted into: Protocol A: OVSYNCH, animals received the 1st Gonadotropin–releasing hormone (GnRH) injection (day 0), Prostaglandin (day 7), 2nd GnRH (day 9), and timed AI (day 10). In protocol B: progesterone-supplemented Ovsynch (CIDR-SYNCH), the procedure is similar with A except that the Controlled Internal Drug Release (CIDR) device insertion was done simultaneous with the 1st GnRH injection. Study 2 was conducted to compare the effects of ovulatory hormones: GnRH and human chorionic gonadotropin (hCG) in optimizing CIDR-SYNCH protocol. Animals were randomly assigned into protocol A: CIDR-SYNCH-GnRH or protocol B: CIDR-SYNCH-hCG. Follicular dynamics were monitored by ultrasonography at days 0, 7, 8, 9 and 10. Pregnancy diagnosis was performed at day 35 and 60 post-AI.

Results and Discussion

Study 1 revealed a pregnancy rate of 31.63% for OVSYNCH and 43.06% for CIRD-SYNCH which are significantly different ($P<0.05$). For Study 2, the mean follicle diameters of >9mm (day 0), >10mm (day 7), >11mm (day 8), and >12mm (day 9) were not significantly different between the two protocols. However, the size of preovulatory follicles at day 10 for hCG (14.0mm) against 12.89mm for GnRH, and subsequent ovulation rate of 90.09% (vs. 70% for GnRH) were significantly different ($P<0.05$) between the two treatments. The pregnancy rate of 58.04% achieved with CIDR-SYNCH-hCG is significantly higher ($P<0.05$) than that of CIDR-SYNCH-GnRH (43.06%). Essentially, the efficiency of OVSYNCH in terms of pregnancy (31.63%) can be improved with CIDR-SYNCH protocol (43.06%), and the substitution of GnRH with hCG resulted in the largest diameter of preovulatory follicle, highest ovulation, and a pregnancy rate of 58.04%. The size of the follicle at the end of the treatment can serve as a key indicator of an effective ovulation synchronization protocol in water buffaloes.

APPLYING ASSISTED REPRODUCTIVE TECHNOLOGIES TO WILDLIFE CONSERVATION MANAGEMENT

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Non-intervention with endangered wildlife species reproduction is no longer an option in this era of accelerated species extinction, as we enter a possible 6th mass extinction event Assisted Reproductive Technologies (ART) will prove invaluable to save some species. Many species reproductive traits and physiology are ill defined due to a lack of basic research and access to meaningful study numbers. While basic research is fundamental, the Omaha's Henry Doorly Zoo and Aquarium's (OHDZA) desire for applied sciences with clear results coupled with efforts being made in the zoo world to use non-invasive techniques with respect to animal welfare issues has led to some mixed, yet interesting, results. This presentation will candidly cover some of the successes and failures of the Reproductive Sciences Lab at OHDZA in the past decade. Detailed topics will include attempts at creating, isolating, and large scale production of recombinant tiger hormones with the use of Chinese Hamster Ovarian (CHO) cell and other cell cultures transfected with recombinant tiger LH and FSH sequences. A novel delivery method modified for ART hormones via an inert, biodegradable polylactide co-glycolide polymer-based gel that allows for a controlled release rate for super ovulation in tigers. The preliminary success of a cryopreservation study that resulted in freezing whole live Aurelia aurita jelly polyps. Other topics briefly touched on will address the development of a density gradient insert that has become a World Health Organization (WHO) recommendation for all human fertility clinics worldwide. Development of voluntary semen collection methods for gorilla and Philippine crocodile through positive reinforced behavioral training, successful snake artificial insemination and hormone induced spermiation or egg laying in Malagasy powder blue reed frog and Mississippi gopher frog resulting in healthy offspring.

EFFECT OF INSEMINATION TIMING FOLLOWING 5 OR 7 DAYS CIDR CO-SYNCH IN NILI RAVI BUFFALO HEIFERS

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The aims of the present study were to compare the pregnancy rates in relation to optimal time of AI 5-day or 7-day CIDR Co-synch in Nili Ravi buffalo heifers. A sum of eighty Nili Ravi buffalo heifers were randomly separated into two treatments. First treatment (n=40) was subjected to controlled internal drug release (CIDR) containing 1.38 grams progesterone for 5 days while the second treatment (n=40) received CIDRs for 7 days. On CIDRs removal both treatments received 150 μg of prostaglandin intramuscularly. Furthermore, after CIDRs removal each treatment was divided into two subgroups regarding timing of AI. In 5-day CIDR Co-synch (n = 20) animals were injected 100 μg of GnRH intramuscularly and inseminated co-currently at 72 hrs after CIDRs removal and the remaining half (n=20) were injected and inseminated co-currently at 84 hrs of CIDRs removal. Similar treatments were given to the 7 days CIDR Co-synch group after 72 hrs and 84 hrs of CIDRs removal. Artificial insemination was done by using frozen, thawed semen of a buffalo bull. An ultrasound was performed for follicular dynamics, ovulation and pregnancy rates. The follicular growth rate was tended to be high in 7-day as compared to 5-day CIDR Co-synch (1.5 ± 0.3 vs. 1.3 ± 0.4 mm/day; $P = 0.060$). Ovulatory follicle size was significantly higher in 7-day than 5-day CIDR Co-synch (11.8 ± 1.1 vs. 11.1 ± 1.1 $P = 0.01$). Ovulation rate was significantly higher in 7-day CIDR Co-synch as compared to 5-day CIDR Co-synch treatments (95% vs. 80%, $P = 0.043$). There was a non-significant difference in pregnancy rates buffalo heifers inseminated either 72 hrs (30%) or 84 hrs (50%) in 5-day CIDR Co-synch treatment; whereas in 7-day CIDR Co-synch pregnancy rates were higher ($P < 0.05$), at 84 (65% odd ratio = 5.5) than at 72 hrs (25%). In conclusion the buffalo heifers treated with 7-day CIDR Co-synch at 84 hrs improves fertility.

Key Word: Buffalo Heifers, CIDR-Co-synch, GnRH, Pregnancy rate, Fixed time artificial insemination.

SPINDLE FORMATION AND ASYMMETRIC CELL IN MAMMALIAN OOCYTES

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Before fertilization mammalian oocytes generate a meiotic spindle and undergo chromosome segregation to yield an egg that is ready for fertilization. Herein, I review the recent advances in understanding the mechanisms controlling formation of the meiotic spindle in metaphase I (MI) and metaphase II (MII) in mammalian oocytes, and focus on the differences between mouse and human oocytes. Unlike mitotic cells, mammalian oocytes lack typical centrosomes that consist of two centrioles and the surrounding pericentriolar matrix proteins, which serve as microtubule-organizing centers (MTOCs) in most somatic cells. Instead, oocytes rely on different mechanisms for the formation of microtubules in MI spindles. Two different mechanisms have been described for MI spindle formation in mammalian oocytes. Chromosome-mediated microtubule formation, including RAN-mediated spindle formation and chromosomal passenger complex-mediated spindle elongation, controls the growth of microtubules from chromatin, while acentriolar MTOC-mediated microtubule formation contributes to spindle formation. Understanding the mechanisms of MI/MII spindle formation, spindle assembly checkpoint, and chromosome segregation, in mammalian oocytes, will provide valuable insights into the molecular mechanisms of human infertility

INVESTIGATION OF CYCLE SYNCHRONIZATION AND ARTIFICIAL INSEMINATION FOR BREEDING INDUSTRY IN RABBITS

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Introduction

A program of developing rabbit breeding at large industrial scale has been developing in Vietnam in recent years. The purpose of this study is to establish a method of cycle synchronization using eSG and HCG produced by Southeast Asia Biotechnology Center (SABC) for developing rabbit artificial insemination (A.I) under conditions of intensive farming.

Materials and Methods

Experiments were carried out on New Zealand white rabbits with average initial age of five months, including 45 does and 15 bucks. Estrus synchronization and ovulation induction were conducted using ECG and HCG produced by SABC. The experimental rabbit does were divided into 3 groups (5 does/ each, 3 replicates): First group (G1) considered as a control group including does with natural estrus and mating was carried out with fertile bucks. The second group (G2) included does with natural estrus receiving A.I and administration of a dose of 50 IU/animal of HCG (i.m) at the time of insemination to induce ovulation. The third group (G3) included does treated of estrus synchronization by administration (i.m) of a dose of 60 IU eSG/doe 48 h before A.I and 50 IU/animal of HCG (i.m) at the time of insemination. For A.I, two ejaculates with an interval of 30 min per male were collected using an artificial vagina 1 h before the insemination. Sperm evaluation including volume, mass motility and percentage of motile sperm were estimated according to the methods described by Beatty (1957) and Björndahl et al, (2003). Ejaculates possessing more than 70% of motility rate were used for dilutions in TCG (Tris-Citric-glucose buffer) at concentration 100×10^6 sperm/ml. Does were inseminated with 0.5 mL of semen dilution containing 50×10^6 million total sperm/female. Hand palpation at twelve days post mating was carried out to detect the pregnancy. Pregnancy rate at D12, pregnancy rate at birth (number of does giving birth), number of total and live-born, number of kits/does were noted after kindling. A $\chi 2$-test was performed to study differences between treatments.

Results and Discussion

The pregnancy rate estimated at D12 was 77, 80 and 80 % and pregnancy rate at birth was 66, 80 and 80 %, respectively for the groups 1, 2, and 3. The total number of kits born were 65, 88 and 103, indicating the mean number of kits born/recipient 6.5 ± 0.5; 7.3 ± 0.5; and 8.6 ± 0.4, respectively for the groups 1, 2, and 3. In conclusion, the obtained results showed significant positive influence of treatment method on the does kindling. The produced eCG and hCG can enhance the application of artificial insemination to improve reproductive efficiency in rabbit intensive industrial production.

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THE EFFECTS OF TYPE I COLLAGENASE ON THE DEGELIFICATION OF CHIMPANZEE (*Pan troglodytes*) SEMEN PLUG AND SPERM QUALITY: IMPROVING GENETIC DIVERSITY OF ZOO CAPTIVE CHIMPANZEE

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Introduction

Semen from the chimpanzee species becomes a colloidal solid after ejaculation. The formation of this copulatory plug is believed to prevent additional spermatozoa of subsequent mating events from accessing the ova. However, this naturally preserved strategy hampers the processes for sperm preparation.

Materials and Methods

Sixteen semen samples were collected from 4 individuals using artificial vagina. Type I collagenase was used for co-incubation with semen ejaculates. Sperm concentration, motility, morphology, viability were assessed manually or with computer assisted sperm analysis system (CASA). Immunofluorescent and immunoblotting against specific antibody were used to evaluation collagenase effects on sperm capacitation and acrosome integrity.

Results and Discussion

We showed that incubation of chimpanzee ejaculates with 0.1% type I collagenase efficiently and significantly ($p<0.05$) releases 2.7-fold more spermatozoa from the coagulated ejaculates, and this degelification process did not alter sperm morphology or viability; nor did it stimulate spontaneous capacitation or an acrosome reaction as assessed by tyrosine phosphorylation and peanut agglutinin stains; moreover, based on computer assisted sperm analysis assay, motility-related parameters remained similar to those of untreated spermatozoa. Moreover, we observed that post collagenase treatment, 2.5% glycerol as a cryoprotectant, resulted in better acrosome integrity and 7.8% glycerol maintained sperm motility better than that of 2.5% glycerol. Our results demonstrated for the first time that type I collagenase can be used to obtain a significantly higher number of spermatozoa from colloid chimpanzee semen ejaculate without affecting the physiological properties of spermatozoa, and these results are critical for the subsequent gamete development. Our results would benefit sperm preparation processes and cryopreservation efficiency per ejaculate. These results would also benefit the genetic diversity of the chimpanzee species, using sperm cells from less dominant individuals, and for achieving better pregnancy success in primates with significantly higher amounts of sperm for artificial insemination.

COMPARISON OF TWO OOCYTE MATURATION MEDIA FOR IN VITRO PRODUCTION OF BLASTOCYSTS IN AN INDIGENOUS VIETNAMESE MINIATURE PIG

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Our aim was to establish an efficient *in vitro* embryo production system for Ban miniature pigs, native and endemic to the north part of Vietnam. We compared two maturation media, a non-defined (ND) one and a defined one (D) in terms of blastocyst development and quality after *in vitro* maturation (IVM), fertilization (IVF) and subsequent embryo culture. Immature cumulus-oocyte complexes (COCs) were obtained from all antral follicles (irrespective of size) of the ovaries of 7-8-month old Ban gilts (20-25 kg each) slaughtered on arbitrary days of estrus. The COCs were subjected to IVM either in TCM-199 medium supplemented with 10% (v/v) porcine follicular fluid (ND group) or in POM medium supplemented with 10 ng/ml epidermal growth factor (D group). In both groups the media was supplemented with 10 IU/ml eCG and 10 IU/ml hCG and oocyte maturation was synchronized by 1 mM dibutyryl cAMP during the first 22 h of IVM. IVF was conducted by frozen-thawed epidiymal Ban sperm. Zygotes were cultured in PZM-3 medium for 7 days. Polar body extrusion, cleavage, blastocyst rates and total cell numbers in blastocysts were compared in 5 replications. Results were analyzed by t-test. There was no significant difference ($P>0.05$) between the ND and D groups in terms of oocyte maturation (67.9% and 74.1%, respectively), cleavage (56.0% and 52.3%, respectively), and blastocyst development rates (17.3% and 22.4%, respectively). The mean total cell numbers were significantly ($P<0.05$) higher in blastocysts of the D group than in those of the ND group (77.5 vs 51.9, respectively). In conclusion, the efficacy of the two media was similar in terms of blastocyst production; however, better quality embryos were obtained by the defined one. This study was supported by SATREPS from Japan Science and Technology Agency (JST) and Japan International Cooperation Agency (JICA).

GERMINAL VESICLE TRANSFER AS A RESCUE TOOL

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We examined whether germinal vesicle transfer (GVT) can help to produce matured oocytes derived from freeze-dried oocytes, and to improve maturation rate and developmental competence of growing oocytes. In order to explore the possibility of using GVT to support freeze-drying of pig oocytes, nuclear materials of freeze-dried oocytes were injected into enucleated fresh oocytes. In an attempt to reduce the size of GV to increase survival rate following GVT, oocytes were treated with resveratrol briefly after *in vitro* maturation (IVM). Although resveratrol did not reduce the size of GV ($p = 0.094$), it did significantly enhance the survival rates following GV transfer ($p < 0.001$). Following transfer of freeze-dried GVs into enucleated fresh oocytes, there were three out of 126 reconstructed oocytes reached the metaphase-II stage (2.4 ± 1.4%). Although this proportion was significantly lower ($P < 0.05$) than that of the IVM control group (83.2 ± 2.5%), it was comparable with the GVT control group (7.4 ± 2.7%). Oocytes in IVM control group were matured *in vitro* without any treatment or manipulation, whereas oocytes in GVT control were not introduced to freeze-drying but were exposed to GVT. In order to improve the maturation rate and developmental competence of growing oocytes collected from early antral follicles in pigs, we used two methods 1) fusion of a growing oocyte with the cytoplast from a fully-grown oocyte (CFR group), and GVT (GVTR group). The maturation rate of GVTR oocytes was significantly improved (18.8 ± 3.5%) compared with that of growing oocytes (0.0%). The proportion of oocytes that reached the metaphase-II (M-II) stage in the CFR group (37.8 ± 2.0%) was significantly higher ($P < 0.05$) than that in the GVTR group, although still lower than that in the control group (75.2 ± 4.4%). No blastocyst was derived from growing oocytes. Among in vitro fertilized GVTR oocytes, 3.0 ± 1.9% developed into blastocysts; however, this percentage showed an insignificant increase compared with the GR group. On the other hand, the percentage of CFR embryos that developed into blastocysts (12.0 ± 4.3%) was significantly higher than that of GR embryos (0.0%), although still lower than that of control embryos (27.0 ± 5.5%). Total cell number in blastocysts in the GVTR group (23.3 ± 6.9) was significantly lower ($P < 0.05$) than that in the control group (50.4 ± 5.0). Meanwhile, the total cell number in blastocysts derived from CFR oocytes (36.3 ± 4.8) was comparable to that of the control group. In summary, the use of GVT helped to produce matured oocytes derived from freeze-dried GVs although with rather low rate, and significantly improve maturation rate and developmental competence of growing oocytes.

THE ART OF ART IN WILD PATIENTS

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Synopsis

Progressive habitat destruction and fragmentation is causing an accelerated loss of planetary biodiversity. A key mission of the international zoo community is to counteract this by using successful captive **breeding programs of endangered species** and their reintroduction into the depopulated natural habitat.

This presentation will discuss the incorporation of **novel assisted reproduction technologies** (ART) into classical captive breeding programs. The principal remit of captive breeding programs is to preserve the maximum gene pool of the target species. Traditionally, this required the exchange of potential breeding partners between zoological institutions. However, animal transportation poses several risks including (i) high risk of disease transmission, (ii) stress-induced infertility or partner incompatibility and (iii) high financial and logistic efforts. Thus the utilization of ART could serve as a real alternative to eliminate these problems.

An additional challenge exists when dealing with critically endangered species or populations (e.g. the northern white rhino or the Bornean subspecies of the Sumatran rhino). In this situation, the effective founder population is already too small for successful species recovery programs. Therefore, the incorporation of new genetic technologies and the future **production of artificial gametes** using stem cell technologies will be required for saving such species. This will open up a totally new dimension in animal conservation and will strengthen the application of ART in wildlife.

The final part of the lecture will present some of the more unusual **reproductive strategies and curiosities in the animal kingdom**. The basic processes behind many of these surprising adaptations are not fully understood. These traits demonstrate the amazing information we have yet to learn from many creatures written in the "Library of Evolution" and we may never do so prior to their extinction.

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UPDATE ON REPRODUCTIVE TECHNOLOGIES FOR ENDANGERED CLOUDED LEOPARD

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Clouded leopards are endangered felids of Southeast Asia that the wild populations are declining due to habitat loss and hunting in range countries. The male aggression, high incidence of abnormal sperm and uncertain ovarian activity of females have been stated that limit the captive breeding success. Therefore, the development of optimal assisted reproductive technologies for this species is recommended. Over two decades, several assisted reproductive techniques have been applying in clouded leopard to overcome the limitation that effected on breeding success in both genders in term of reproductive biology, physiology and behavior. In male, semen collection and sperm preservation protocol have been optimized for genome resource banking and further assisted techniques applying. According to a high incidence of abnormal sperm in most captive males, sperm selection was attempted. In our recent study two sperm preparation methods included 1) simple washing or 2) the single layer centrifugation (SLC) method was applied prior to cryopreservation (n=12). The sperm motility, intact acrosome and sperm with normal tail in chilled and frozen-thawed semen samples were increased after processed by SLC. The heterologous IVF in the SLC-processed group had significantly higher fertilization rate than the simple washing group ($P <0.05$). In female clouded leopard, the fecal steroid hormone metabolites (i.e. estradiol, progesterone, glucocorticoid) have been studied for their reproductive physiology (estrous cycle, ovulation pattern and pregnancy). Exogenous gonadotropins administration were also attempted to stimulate ovarian function for assisted reproductive techniques such as artificial insemination (AI), in vitro fertilization (IVF) or others. Our current study, we have been attempting to optimize stimulating protocol for AI using eCG and pLH with promising results and less adverse effects for animals. To date, three reports of artificial insemination success has reported from fresh semen (Howard et al., 1996), chilled semen (Tipkantha et al., 2016) and frozen semen (Comizzoli et al., 2017). In conclusion, to select a good quality spermatozoa for further apply with optimal assisted techniques would be promising for propagation and sustainable the genetic variation of this endangered species in the future.

BAT STEM CELL LINES FOR BIODIVERSITY CONSERVATION AND VIRAL STUDIES

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Bats represent, after rodents, the largest group of mammals with more than 1,000 species, representing nearly 20% of mammalian biodiversity. Present in almost all latitudes and climates, they also occupy a very large number of ecological niches. But paradoxically, aside from studies on the ecology and adaptation to the environment of these animals, very few studies have been and are being carried out on the original physiology of these species. Their role as a reservoir of many viruses is de facto one of the first reasons to study the reproduction, metabolism, immunology and genomics of these species, disciplines closely associated with the study and understanding of the host-virus interaction. But despite their key roles in numerous ecological niches, numerous bat species are over the world also endangeered species. Understanding and deciphering their physiology is is also imperative to better protect them. Beside, bats are an important reservoir for some of the deadliest viruses, including Filoviruses, Hénipaviruses, Arenaviruses and Coronaviruses (Wang and Cowled, 2015). Most of these viruses cause haemorrhagic fevers, respiratory and neurotropic disorders in humans, livestock and wildlife, but no clinical symptoms have ever been observed in bats. Despite their key role in the outbreak and spread of these zoonotic epidemics, the physiology of the bat is poorly studied, particularly the immunology of bats and their immune response to a viral infection. Regarding the difficulty of having large colonies of bats and infected animals, an alternative is to develop in vitro models to study the cellular response and more particularly the innate immunity response, essential in the antiviral response. Here, we will present an innovative approach to generate bat stem cell lines by somatic reprogramming. By using those new non invasive procedures, those reprogrammed cells could contribute both to maintain the genotypes and biodiversity of numerous bat species and to help deciphering their role as host reservoir of numerous pathogens.

RESEARCH AND CONSERVATION PROGRAMS AT ZPO

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Zoological Park Organization is responsible for education, preservation and reproduction, and management of zoo to be the place for relaxation of general public. Apart from that, it is the organization that is responsible for coordination and operation for the zoos. Currently, there are 8 zoos under its responsibility. The target of the operation are wild animal preservation for study, research and sending back to the nature, promote good quality of life for world animal, provide integrated education about wild animal, improve management, administration and entertainment standard to genuinely be the preservative tourist attraction.

SEMEN CRYOPRESERVATION AND QUALITY OF FROZEN-THAWED SEMEN IN TAIWAN HOLSTEIN BULL

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Introduction

Semen cryopreservation has been one of the important reproductive techniques and widely used in the farm animal industry. Additionally, it could be served as transmission of genetic materials and preservation of gene banks. However, cryopreservation may result in impairing spermatozoa, including reduction of sperm viability, motility, fertilization ability, acrosome reaction, cytoplasmic membrane integrity, and increasing DNA damage. Due to a high content of unsaturated fatty acids in the cell membranes, and lack of significant antioxidants contained in the cytoplasmic components, sperm cells are susceptible to lipid peroxidation by O_2^- and H_2O_2.

Materials and Methods

The youth bulls were chosen in line with Taiwan DHI data ranking and also be the Ten Tons Cow's offspring. Comparison of the quality of frozen-thawed semen among five different extenders was conducted, which are two commercial cryopreservation extenders, i.e. Bioxcell and OptiXcell, and Tris-citric acid-fructose with 8% low density lipoprotein (TCF-8% LDL) extender supplemented with various concentrations of Glutathione (GSH), i.e. TCF-8% LDL, TCF-8%LDL+0.5 mM GSH and TCF-8% LDL+1 mM GSH. Semen were collected from three Holstein bulls by using an artificial vagina, then were diluted with different extenders accordingly to bring to 2~3 x 10^8 cell/ mL in the final concentration. After frozen- thawed semen, the percentage of live sperm, motility and the acrosome integrity of spermatozoa were recorded by CASA and flow cytometry.

Results and Discussion

The results showed that the OptiXcell and TCF-8% LDL+0.5 mM GSH extenders performed well on sperm motility (68.63% vs. 62.05%), viability (60.92% vs. 58.76%) and integrity of mitochondria (72.42% vs. 63.95%) after thawing. In addition, the acrosome integrity (39.05%) is higher in the group of TCF-8% LDL+0.5 mM GSH than other groups.

SEPARATION OF ROOSTER SPERMATOZOA IN SILICA-BASED COLLOIDAL MEDIUM BY CENTRIFUGATION

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Introduction

Moderate sperm separation can eliminate debris, immature and abnormal spermatozoa from semen ejaculation to collect functional spermatozoa for improving the efficiency of fertilization. Density gradient media have been used extensively in separating cells. However, only a few studies have attempted this technology in rooster spermatozoa. Therefore, the purpose of this study was to evaluate the effects of two silica-based colloidal media on rooster spermatozoa separation. The data obtained from these experiments will benefit the development of a feasible sperm purification protocol for the rooster.

Materials and Methods

Forty adult Taiwan Native chicken L7, L9, L11 and L12 roosters in Taiwan were used in this study. Semen was routinely collected once a week, and pooled semen was diluted 1:3 with PBS immediately after. The isotonic Percoll® and Percoll® PLUS (GE Healthcare, Uppsala, Sweden) were mixed with 1x PBS to prepare 60%, 70%, 80% and 90% Percoll and Percoll PLUS solutions, respectively, and then transferred 2 mL of these solutions each to a 15 mL conical plastic centrifuge tube. Diluted semen was gently placed on top of the solutions and centrifuged at 800 g for 20 minutes. After centrifugation, the sperm pellet was washed with PBS and resuspended in Poultry media (IMV, L'Aigle, France). Sperm viability and motility were analyzed by microcapillary flow cytometry (Guava Technologies Inc., Hayward, CA, USA; distributed by IMV Technologies) and CASA (Ceros II, IMV, L'Aigle, France).

Results and Discussion

The percentage of viable/motile/progressive sperm was significantly enhanced after Percoll 70%, 80% and 90% centrifugation by 13.1%/14.3%/26.0%, 17.3%/16.1%/27.3%, and 19.8%/16.4%/28.0%, respectively ($P < 0.01$). The similar results were also found in Percoll PLUS 70%, 80% and 90% centrifugation with 11.1%/13.3%/23.6%, 17.7%/15.7%/21.4%, and 17.4%/16.8%/19.9% increments, respectively ($P < 0.01$). Our results initially showed that either Percoll or Percoll PLUS centrifugation could be a practicable procedure for rooster spermatozoa separation. Further studies on the effects of these procedures on the fertilizing capacity of rooster spermatozoa will be determined.

CRYOPRESERVATION OF PIG PANCREATIC ISLET WITH TREHALOSE CRYOPROTECTANT

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Introduction:

Pig pancreatic islets are being considered as an alternative source for clinical islet transplantation, but the technique for pig islet cryopreservation is not well established. Trehalose is a sugar that can withstand prolonged periods of desiccation of plants and animals, which is also a non-permeating cryoprotective agent, has been documented as less toxic and highly efficient during cryopreservation of various cells or organisms. However, it has never been applied to the cryopreservation of the pig pancreatic islets.

Materials and Methods:

In this study, the selective osmotic shock method was used to isolate a number of pancreatic islets of Large White pigs, and the isolated islets were in-vitro cultured for four days. We used the dithizone (DTZ) staining, the hoechst/propidium iodide (PI) double staining and the glucose for stimulating insulin secretion to detect the viability and function of islets. After thawing, the recovery rate and insulin release of islets and mRNA level of INS, GCG, Bcl-2 and Bax genes in islets were analysed.

Results and Discussion:

The results of DTZ staining and hoechst/PI double staining showed that the isolated pig islets using the selective osmotic shock method had intact morphology and preferable viability. And then the insulin release level showed that the islet function was sustained and it's favorable cryopreservation time was 1 day after in-vitro culture ($p<0.01$). Furthermore, we conformed that the optimal concentration of trehalose in the islet cryopreservation was 0.1 mM/L, resulting from the recovery rate, insulin release, and mRNA level ($P<0.05$). Finally, it was reported that the effect of trehalose and glycerol dealing with cryopreservation of pig pancreatic islets were not different. As a result, trehalose was the one potential and less toxic cryoprotectant for pig pancreatic islet cryopreservation.

INTERNATIONAL COLLABORATION FOR CONSERVATION AND UTILIZATION OF PIG RESOURCES -SATREPS ACTIVITY IN VIETNAM-

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Pig genetic resources in local regions have been in dangerous situation because their numbers of breeds and individuals are reducing dramatically after the introduction of commercial breeds for expecting high productivity. For overcoming these situations, intense activities such as cryobanking of gametes, gonadal tissues and early-stage embryos of rare (endangered) breeds are necessary. For example in Japan, our organization, of which name was National Institute of Agrobiological Sciences, had established a Genebank Project with the financial support from the Ministry of Agriculture, Forestry and Fisheries, Japan. On the other hand, sustainable utilization of pig genetic resources will help the advanced conservation of pig genetic resources at low cost. However, in some cases in countries having rare pig breeds, system of the conservation and utilization of genetic resources have not fixed yet; in other words, international collaboration between these types of countries is now necessary. We are now carrying out one project called Science and Technology Research Partnership for Sustainable Development (SATREPS)* project entitled “Establishments of cryobank system for native pig resources and of sustainable production system” between Japan and Vietnam. In the present symposium, we would like to introduce general outline of this project.

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Keywords: Pig, Bio-diversity, Cryobank, Sustainable Preservation

ANIMAL GENETIC RESOURCES CONSERVATION THROUGH CRYOBANKING IN THE PHILIPPINES: MANAGEMENT INITIATIVES AND FUTURE DIRECTIONS

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Introduction

Buffaloes, cattles, and caprines play a significant role in maintaining the livestock genetic diversity of an ecosystem. Cryobanking or commonly known as gene banking has been utilized worldwide and serves as a mechanism for conserving animal genetic resources (AnGR).

Materials and Methods

In the Philippines cryobanking settings, management initiatives consist of various components such as information dissemination, collection of AnGR for processing of samples (e.g. species identification, cryopreservation, test of infectious diseases), databank, and utilization. The collection of AnGR was of two types, reproductive cells and non-reproductive cells.

Results and Discussion

Samples collected and stored covering the years 2012-2017 provides the following outlook: total collection and cryopreservation of 304,934 units of semen was particularly from 88.4% buffaloes, 11.2% cattle, and 0.4% caprine. These were reserved genetic materials from improved breeds for meat, milk, and draft. Also, non-reproductive cells from blood and DNA were mainly from swamp buffaloes for genetic diversity study for conservation management. Future directions of the Cryobank features its role in management where it involves up to the gene banking of genetic materials from diverse indigenous species and introduced breeds in the country. This unit will also continue to pursue advancement in structured databank, duplication of stored genetic resources from within the country and even those coming from overseas, establishment of screening protocols for infectious and genetic defects, and aassist in the establishment of legal policies, rules, and regulations related to cryobanking activities. The effects of changing environment that threaten the lives and genetic diversity of livestock are also being given utmost considerations.

EFFICIENT DERIVATION OF PRIMORDIAL GERM CELLS FOR GENETIC PRESERVATION OF INDIGENOUS CHICKEN

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Introduction

Primordial germ cells (PGCs) are promising tools for producing transgenic chickens or preserving genetic resources in endangered birds or domestic avian species. Procuring PGCs from embryonic tissues, as described in the literature, is inefficient, unstable, and time-consuming. Moreover, germline transmission of PGCs tends to be decreasing with extended culture in vitro. Therefore, we set up to develop a robust method for isolation and rapid propagation of PGCs derived from embryonic gonads.

Materials and Methods

Chicken PGCs used in culture system optimization were labeled with EGFP. A total of 2,000 PGCs were seeded in different culture systems mKO (modified knockout DMEM), mKO-Feeder, FACs and cKO-F. Cell proliferation were examined in day 1, 3, 6, using a BioTek Cytation5 system.

In primary culture, PGCs were isolated from the Donglan chickens, a black chicken breed native to Guangxi Province, China. PGCs were examined every two days in the first 20days. Immunocytochemistry and RT-PCR were performed to confirm the expression of germ cell specific biomarker in the established cell lines. PGCs labeled with GFP were injected into recipient White Leghorns chicken embryos to evaluate the migration potential. Germline chimera were bred to wide-type chicken and donor-derived chicken is examined by the presence of black feather or skin in F1 chicken.

Results and Discussions

PGCs were more proliferative in mKO and mKO-Feeder than in the other two culture system. But while mKO-F system was used in the primary PGCs culture, cells ceased to grow 6 days. Then we used a hanging-insert system to obviate the direct contact of PGC and feeder cells in primary culture. Surprisingly, PGCs derived in mKO-FI (mKO-feeder-inserts) presented a robust proliferation and grew to 10^6 in 14 days. Proliferative cell lines could be established in over 90% of the isolations, much higher than those in FACs and cKO-F ($P < 0.01$). Germ cell specific biomarkers, such as Nanog, pouV, Cvh, Dazl, Cdh, were detected by RT-PCR in the derived PGCs. And immunocytochemistry also showed that SSEA1 and DAZL were positive in these cells. In addition, these cells maintained gonad migration ability and up to 64% of germline-transmission efficiency was seed in F1 chicks. The mKO-FI culture system optimized in our research would not only allow efficient genetic banking of chicken species, but also facilitate production of chicken bearing a desired phenotype via genomic editing.

IN VITRO CULTURE AND CHARACTERISATION OF DUCK PRIMORDIAL GERM CELLS

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Introduction

The aim of the study is to isolate, cultivate *in vitro* and characterise duck primordial germ cells (PGCs).

Materials and Methods

Firstly, we derived both Muscovy duck circulated PGCs (MDcPGCs) and gonadal PGCs (MDgPGCs) using a modified condition for chicken PGC culture. Using this method, gonadal PGCs (gPGCs) were also isolated from other duck breeds, Pekin duck and a hybrid mule duck. For all three duck breeds, cultured gPGCs were characterised by periodic acid-Schiff (PAS), by immunostaining and by analysing the expression of germline- and pluripotent-associated genes. In addition, the functional assay with transplantation was also conducted.

Results and Discussion

MDgPGCs were found to exhibit proliferative performances in FAot, an aseric chemically-defined medium. A significant increase in cell proliferation was obtained by the third day of primary culture. Moreover, these cultured gPGCs were characterised by typical markers and able to colonise gonads after transgenic labelling and injection into recipient embryos. Altogether, the results demonstrate that duck PGCs can be isolated, expanded and still retain their developmental potential. However, additional factors appear to be required and identified in order to establish long-term culture.

HOW TO IMPROVE SUCCESS OF INTER-SPECIES SOMATIC CELL NUCLEAR TRANSFER IN ENDANGERED SPECIES

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It has been shown that advanced reproductive biotechnologies such as artificial insemination (AI), in vitro fertilization (IVF) and embryo transfer (ET) are the important tools for conservation of endangered species. Since the birth of first cloned mammalian animal using somatic cell nuclear transfer (SCNT) was announced in 1997, several live cloned domestic, laboratory and companion species have been produced using homologous oocytes. In endangered species, there are extremely lack of homologous oocytes and recipients as perform in traditional SCNT. Interspecies SCNT (iSCNT) is an alternative technique for producing embryos and offspring of endangered species. The iSCNT procedure need the oocytes of animals and also the female recipients which are the same genus of individual that we want to propagate. The advanced of iSCNT already demonstrated in ovine, bovine and feline families. Even though live offspring iSCNT of these three species have been produced, but still have very low success. There are numerous technical and biological factors affecting the success of iSCNT. The low frequency of successful has been associated with abnormal nuclear and epigenetic reprogramming, mitochondrial heteroplasmy as well as incompatibilities between the nucleus and cytoplasm that could possibility impair embryo development, high abortion rate, anatomical and physiological abnormalities of fetus, offspring died immediately before and after birth. Numerous factors which involve in low efficiency of embryo production and survival of live birth need to be examined. In this review we will discussed how to improve success rate of iSCNT in endangered species. This work was supported by Suranaree University of Technology.

GENERATION OF TRANSGENIC-CLONED GUANGXI HUANJIANG XIANG PIGS SYSTEMICALLY EXPRESSING GREEN FLUORESCENT PROTEIN*

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Introduction

Guangxi Huanjiang Xiang pig is a unique miniature pig strain that is originally from Huanjiang Maonan Autonomous County of Guangxi, China, and it has great potential in agricultural and biomedical research. Although cloning and genetic modification of pigs would enhance their application value,cloning of Guangxi Huanjiang Xiang pig has not yet been reported. The present study aimed to generate transgenic-cloned Huanjiang Xiang pigs, laying the foundation for the conservation and utilization of this valuable swine resource.

Materials and Methods

Fibroblasts were isolated from tails of Huanjiang Xiang pig, and genetically modified using Xfect transfection. Positive transgenic fibroblasts were selected by G418 resistance screening, and positive transgenic fibroblasts expressing green fluorescent protein (GFP) were used as donor cells for reconstructing transgenic embryos by somatic cell nuclear transfer (SCNT). Embryos transfer was performed at the day of SCNT. Pregnancy was monitored, and the surrogates were delivered by natural parturition on Day 114 to 120 of gestation (SCNT was performed on Day 0). Genomic DNA was extracted from tail biopsies of newborn cloned piglets and PCR reactions were used for the detection of GFP gene integration. One transgenic-cloned offspring was autopsied to collect organs and tissues for histological analysis of GFP expression.

Results

Positive transgenic fibroblasts expressing GFP were selected by G418 resistance screening and then were used as donor cells for reconstructing transgenic embryos by SCNT. After the transfer of 504 transgenic SCNT embryos to two surrogate recipients, one of which was pregnant and gave birth to three live piglets. Overall cloning efficiency was 0.6%. One piglet died at Day 7 after birth because of the feeding problem. Another one was autopsied for histological analysis, and the 3rd transgenic-cloned Huanjiang Xiang pig is still alive with clinically healthy. All of three piglets were positive transgenic identified by PCR reaction, and the histological analysis showed that GFP transgene systemically expressed in the transgenic cloned Huanjiang Xiang pig.

Conclusion

The successful generation of transgenic-cloned Huanjiang Xiang pigs lays the foundation for the conservation and utilization of this valuable swine resource.

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DOUBLE-MUSCLED PIGS CLONED USING MSTN-DISRUPTED FIBROBLASTS

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Introduction

Myostatin (MSTN) plays a negative role in regulating the formation and differentiation of pig skeletal muscle, which might prevents the growth of muscle cells and muscle fibers. The deletion or reduction of this gene could cause excessive muscle development of animal body. Also, it has important relation with the growth, development and the fatty deposits of pigs, and it could be used to improve and adjust the development of pigs and the proportion of lean meat and fat.

Materials and Methods

A transcription activator-like effector nuclease (TALEN) pair targeting exon 1 of the swine MSTN gene was constructed and used to transfect porcine fetal fibroblasts (PFFs), along with a surrogate reporter plasmid. MSTN-mutated PFFs were enriched by magnetic separation and used to produce homozygous MSTN-KO boars in a two-step cloning process via somatic cell nuclear transfer (SCNT).

Results and Discussion

In the first step, 428 of SCNT embryos transferred to two recipient sows, five and nine fetuses were obtained on gestation days 27 and 36, respectively. All fetuses were used to establish PFF cell lines, which were analyzed for MSTN gene mutations by T7E1 and Sanger sequencing. Eight of the fourteen fetuses (57%) showed mutations at the MSTN loci, with three having biallelic and five having monoallelic mutations. In the second step, a PFF cell line with biallelic MSTN mutations ($MSTN^{-/-}$), consisting of a 2-bp deletion in one allele and a 4-bp deletion in the other allele, was used as a donor to generate cloned pigs via SCNT. Of 646 cloned embryos transferred into three surrogates, 14 live and two stillborn piglets were delivered. At birth, these piglets were hardly distinguishable phenotypically from wild-type piglets. However, starting at 2 month of age, they showed visually-clear hypermuscular characteristics. Comparisons of the magnitude of increase in dressing percent and loin eye size and the magnitude of decrease in backfat thickness in 6-month-old cloned and age-matched wild-type pigs support the $MSTN^{-/-}$ genotype of the former. $MSTN^{-/-}$ boars developed and grew normally to sexual maturity, after artificial insemination gave birth to normal offspring.

IGF2-H19 LOCUS METHYLATION STATUS IN CLONED GOAT FIBROBLAST CELLS

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Introduction

Previous study showed a decrease in efficiency or even a failure over repeated iterations in somatic cell nuclear transfer (SCNT). DNA methylation in cloned offspring should be responsible for this inefficiency or failure. Hypothetically, SCNT itself may influence the methylation of *IGF2-H19* locus in cloned goat fibroblast cells that could be donor cells in SCNT, which resulted in inefficient serial recloning.

Materials and Methods

Goat ear fibroblast cells (GFC, control group) were obtained from the ear of a 2-month-old healthy female Saanen dairy goat (Yangling, China).Cloned goat ear fibroblast cells (CFC, experimental group) were obtained from the ear of a 2-month-old healthy female cloned goat that was produced by SCNT (using GFC as donor cells). When two groups were cultured to passage 5, using cytometry for cell growth curves, flow cytometer for cell apoptosis, Real-Time PCR for gene relative expression level and bisulfite sequencing PCR for the methylation status of differentially methylated region (DMR).

Results and Discussion

In this study, we found that there is no obvious morphological difference between CFC and GFC, while cell proliferation and apoptosis in CFC had significantly changes. Furthermore, we also observed dysregulation on *Dnmts* and *Tets* genes expression, and aberrant DNA methylation on *IGF2-H19* locus. Compared with amphigory, SCNT embryo had a low survival rate and cloned offspring had a high morbidity and mortality rates. Ear fibroblast cells played an important role in SCNT, and its DNA methylation had a great impact on recloning efficiency. Therefore, we concluded that SCNT resulted in aberrant methylation of *IGF2-H19* locus by influencing *Dnmts* and *Tets* genes expression, which could affect genomic imprinting, and then influenced the efficiency on serial recloning.

BUFFALO OOCYTES MATURED IN VITRO WITH ACETYL-L-CARNITINE IMPROVES CRYOTOLERANCE DUE TO CHANGE IN MITOCHONDRIAL FUNCTION AND THE MEMBRANE LIPID PROFILE

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Introduction

The cryopreservation of oocytes is an essential strategy used to accelerate genetic improvements in livestock and lipid content dictates crucial physical and chemical properties, particularly membrane fluidity. The addition of ALC to the IVM medium influenced the distribution of lipid acid in lamb fresh oocytes and then improved oocytes quality and embryo development. However, whether ALC supplementation in oocytes mature medium impact the phospholipid profile of the oocyte membrane after vitrification still need to be discovered.

Materials and Methods

Cumulus-oocyte complexes (COCs) were matured and vitrified as three sets: Fresh, matured in unsupplemented maturation medium, not vitrified; 0 mM ALC vitrification, matured in unsupplemented maturation medium, then vitrified; 2.5 mM ALC vitrification, matured in maturation medium supplemented with 2.5 mM ALC, then vitrified. We then evaluated embryonic development, mitochondrial DNA copy number (mtDNA), mitochondrial membrane potential (MMP), and the lipid profile of oocyte membrane as markers for oocyte quality after vitrification.

Results and Discussion

Supplementation with ALC during IVM significantly improved the rates of oocyte cleavage, morulae and blastocysts and increased MMP after vitrification compared to unsupplemented vitrified oocytes ($P < 0.05$). Used multivariate data analysis based on positive ion matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) data, identified five significant phospholipid ions (m/z 728.7[PC (32:3)], 746.9[PC (32:5)], 760.6[PC (34:1)], 768.8[PC(P-36:3)], and 782.6[PC (36:4)]; $P < 0.05$) were significantly more abundant in fresh oocytes than in unsupplemented vitrified oocytes. Meanwhile, three phospholipid ions (m/z 734.6[PC (32:0)], 760.6[PC (34:1)], and 782.6[PC (36:4)]; $P < 0.05$) were higher abundant in the ALC-supplemented vitrified oocytes compared to the unsupplemented vitrified oocytes. In Conclusion, supplementation of ALC during IVM might improve buffalo oocyte quality after vitrification by enhancing mitochondrial function and altering the phospholipid composition of the vitrified oocyte membrane.

INHIBITION OF MEK1/2 AND GSK3 (2I SYSTEM) AFFECTS EPIGENETIC MODIFICATION AND EARLY DIFFERENTIATION OF PORCINE PARTHENOTES

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Introduction

Preimplantation development of mammalian embryos features several embryonic differentiations that yield the fetus and placenta. In the morula stage of embryos, first cell lineage determinations occur, and cells in an embryo differentiate as the inner cell mass (ICM) or trophectoderm (TE) cells. Embryonic stem cells (ESCs) can be isolated from the ICM in blastocysts prior to the differentiation to the three embryonic germ cell layers (ectoderm, endoderm, and mesoderm). Inhibition of both MEK1/2 and GSK3 (2i system) facilitates the maintenance of naïve stemness for embryonic stem cells in various mammalian species. However, the effect of the inhibition of the 2i system on porcine early embryogenesis and epigenetic modifications is unknown. We investigated the effect of the 2i system on early embryo development, pluripotent gene expressions, and epigenetic modification.

Materials and Methods

Aspiration of follicle fluid from porcine ovaries were done using an 18-gauge needle with a 10 mL syringe. COCs were cultured in a 4-well cell culture dish with in vitro maturation medium. Denuded oocytes were washed three times in PBS-BSA and activated using an Electro Cell Manipulator 2001 (BTX, Inc., San Diego, CA, USA) on 280 mM mannitol medium supplemented with 0.01 mM CaCl2 and 0.05 mM MgCl2. Activated oocytes were placed on PZM-5 medium containing 7.5 μg/mL cytochalasin B for 3 h. Embryos were washed three times and cultured in PZM-5 medium containing dimethylsulfoxide (DMSO, 0.2%; Control group), MEK1/2 inhibitor (PD0325901, 4 μM), GSK3 inhibitor (CHIR99021, 0.3 μM), or 2i inhibitor (PD0325901 4 μM + CHIR99021 0.3 μM), for 144 h at 38.5°C in an atmosphere containing 5% CO2.

Results and Discussion

Inhibition of MEK1/2 (by PD0325901) and/or GSK3 (by CHIR99021) did not change the developmental potential of porcine parthenogenetic embryo blastocysts, but improved blastocyst quality, judged by the blastocyst cell number, size, and decreased number of apoptotic cells. Expression levels of OCT4 and SOX2, the primary transcription factors that maintain embryonic pluripotency, were significantly increased by 2i treatments. Epigenetic changes, including decreased Histone 3 Lysine 9 trimethylation (H3k9me3), increased Histone 3 Lysine 9 acetylation (H3k9ac), and increased demethylation at the satellite I regions were observed upon 2i treatment. The collective results indicate that 2i system in porcine embryos improved blastocyst quality by regulating of epigenetic modification and pluripotent related gene expression.

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SINGLE CELL RNA-SEQ ANALYSIS PROVIDE DEEPER MOLECULAR INSIGHTS INTO ABNORMAL MATURATION OF TUBASTATIN A-TREATED MOUSE OOCYTES

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Introduction: In our previous study, we observed that Tub-A treatment induced an increased level of the acetylation of α-tubulin, and a failure of spindle migration and actin cap formation. Most Tub-A treated oocytes were arrested in an MI-like or a GVBD-like stage and decreased mTOR (spindle formation factor) and mDia1 (inhibitor of actin assembly) in an HDAC6 expression-dependent manner. We wonder if TubA only afftects HDAC6 expression. Therfore the purpose of this study is to clarify the cause of the phenomenon through RNA-seq anaysis.

Materials and methods:

Tubastatin A treatment: Tubastatin A HCl (Catalogue no. 27108) was obtained from BPS Bioscience (San Diego, USA). The compound was dissolved in DMSO and further diluted in saline to the 10 and 20 μM concentration. Oocytes in the control group were incubated with the same amount of solvent alone (DMSO) for 12 h.

RNA-sequence analysis: The library was prepared using an Illumina NextSeq (Illumina) according to the manufacturer's instructions. The gene annotation of the mouse reference mm10 from UCSC genome (https://genome.ucsc.edu) in GTF format was used as gene models, and the expression values were calculated in Fragments Per Kilobase of transcript per million fragments mapped (FPKM) unit.

Results and discussion: In the Tub-A treated group, 3665 genes were differently expressed, compared to untreated group (GV or MII stage oocytes). Of them, 1,547 and 2,118 genes in TubA treated group are up- or down-regulated. Also, 718 (4.2%), 723 (4.3%), and 294 (1.7%) genes were specifically expressed at GV, MII, and Tub-A-treated oocytes, respectively. Of note, p53 signaling pathway, MAPK signaling pathway, Wnt signaling pathway, and Notch signaling pathway related gene expressions in TubA treated group are significantly increased, whereas metabolism, DNA replication, and oxidative phosphorylation related gene expression are significantly decreased. Furthermore, TubA treated group showed significant alternations in histone methyltransferase-related gene expression, indicating that abnormal meiotic maturation of mouse oocytes by TubA treatment is caused by combined inhibitory effects such as HDACs and Sirtuins, but not HDAC6 inhibitory effect alone. RNA-seq and in silico pathway analysis demonstrate the potential for using mouse oocytes as an in vitro platform for the systematic validation of chemotherapeutic targets.

THE TOXIC EFFECT OF AFLATOXIN B1 ON EARLY PORCINE EMBRYONIC DEVELOPMENT

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Introduction

Aflatoxin B1 (AFB1) is a type of mycotoxin produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. AFB1 is considered as the most toxic mycotoxin owing to its toxic effect on health. Many mycotoxins display cytotoxicity due to the excessive production of reactive oxygen species (ROS) that result in oxidative stress. Excessive ROS leads to the induction of DNA damage, apoptosis, decreased ATP production, and further detrimental effects. Our study indicates that exposure to AFB1 impairs porcine preimplantation embryonic development due to ROS-induced DNA damage repair failure, apoptosis, and autophagy.

Materials and Methods

In this study, we are used the porcine embryos and token to ROS staining, TUNEL assay, Immunofluorescence, BrdU analysis, commet assy, RT-PCR to observe the changes in ROS generation, apoptosis, mitochondria function and cell cycle.

Results and Discussion

The results showed that the presence of AFB1 induced the generation of reactive oxygen species (ROS). And excessive ROS caused DNA damage Additionally, AFB1 also disrupted the DNA damage repair through the regulation of 53BP1. TUNEL assay confirmed the generation of apoptosis, further resulting in the occurrence of autophagy. These results showed that the presence of AFB1 impaired porcine early embryonic development through oxidative stress, as well as DNA damage and repair, apoptosis, autophagy.

CPEB2 IS REQUIRED FOR TIGHT JUNCTION ASSEMBLY FOR ESTABLISHMENT OF PORCINE TROPHECTODERM EPITHELIUM

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Introduction

After fertilization, the embryo undergoes continuous mitotic cell division and loses distinct cell boundaries during the process of compaction. After compaction, the first epithelial cells are established in the outer portion of this structure, which contains a cavity formed by water influx brought about by an ion gradient, water channels, and tight junction (TJ) assembly. Recent study reported that apical localization of TJP1 (ZO-1) transcripts mediated by post-translational mechanisms for TJ assembly and mammary epithelial cell polarity. Cytoplasmic polyadenylation element binding protein (CPEB) is an RNA-binding protein that promotes elongation of poly(A) tails and regulates wild post-translational mechanisms. CPEB depletion in mammary epithelial cells is known to disrupt tight junction (TJ) assembly via mislocalization of TJP1, but the role of CPEB in the biological functions associated with TJs in trophectoderm epithelium has not yet been studied.

Materials and Methods

Cumulus-oocyte complexes were collected from porcine ovaries obtained from a local slaughterhouse and incubated in in vitro maturation medium for 44 h at 39 °C. Parthenogenetic activation was used an Electro Cell Manipulator 2001 (BTX, Inc., San Diego, CA, USA) on 280 mM mannitol medium supplemented with 0.01 mM CaCl2 and 0.05 mM MgCl2. Using a FemtoJet microinjector (Eppendorf, Hamburg, Germany) and a TE2000-U inverted microscope (Nikon Corporation, Tokyo, Japan), CPEB2 dsRNA (1 μg/μL) was then injected into the cytoplasm after 6 h of electrical stimulation. After injection, the embryos were placed in PZM-5 solution and cultured in an incubator for 144 h.

Results and Discussion

CPEB2 was detected in both the nuclei and apical cytoplasm at the 4- and 8-cell stages, localized to cell-cell contact after the initiation of the morula stage. Its depletion led to retarded blastocyst formation caused by impaired TJ assembly. Moreover, transcription of TJ-associated genes, including TJP1, CXADR, and OCLN, was not affected, but the corresponding proteins were not properly localized at the apical cell membrane in morulae, suggesting that CPEB2 confers mRNA stability and/or determines subcellular localization for translation. Remarkably reduced relative levels of TJP1 transcripts bearing the 3′-untranslated region were noted, indicating that CPEB2 mediates TJP1 mRNA stability. In conclusion, our findings demonstrate that because of its regulation of TJP1, CPEB2 is required for TJ assembly during porcine blastocyst development.

EFFECT OF DONER CELL TYPES ON THE DEVELOPMENT OF PIG EMBRYOS PRODUCED BY SOMATIC CELL NUCLEAR TRANSFER

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Donor cell type and number of cloned embryos transferred to an individual surrogate are two major factors that affect to the successful rate of somatic cell nuclear transfer (SCNT) in pigs. The aim of this study is to compare the influence of different donor cell types on the quality of pig embryos produced by SCNT. Piezo method was used to enuclear Landrace oocytes collected after in vitro maturation and transfer donor cell. Three types of donor cell (cumulus cells of Landrace, fibroblasts of Landrace, fibroblasts of Ban) corresponding three groups 1, 2, 3 were used as donor cells. The nucleus-transferred oocyte was then electrofused by a pulse of direct current 1,5kV/cm, 100μs in 0.28mM Manitol. Reconstructed embryos were cultured in IVC Pyruvate-Lactate medium for the first 2-day and IVC Glucose for the next 5 days. The results showed that there were differences between the three groups in the rate of cleavage, blastocyst and the number of cells per blastocyst. The rate of blastocyst and the number of cell per blastocyst in groups 2 were higher than those in group 1 and group 3 (14.5 vs 9.9; 11.0, $p < 0.05$, and 44.9 vs 38.6; 36.5, $p < 0.05$, respectively). The rate of blastocysts in group 3 was higher than in group 1 (11.0 vs 9.9, $p < 0.05$, respectively) but the number of cell per blastocyst was lower than that in group 1 (36.5 vs 38.6). In conclusion, our findings suggests that both breed and cell type influences the quality of cloned embryos and Landrace's fibroblasts using as donor cell resulted in a higher cloning efficiency by comparison with Landrace's cumulus cells and Ban minipig fibroblasts.

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Keyword: *somatic cell nuclear transfer, pig, donor cell types.*

JNJ-7706621 IMPROVES THE IN VITRO DEVELOPMENT COMPETENCE OF PORCINE PARTHENOGENETIC ACTIVATION AND SOMATIC CELL NUCLEAR TRANSFER EMBRYOS

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Introduction

Oocyte activation by fertilization or parthenogenetic activation (PA) is mainly regulated by M-phase-promoting factor (MPF), a composite of cyclin and p34cdc2 (CDK1). Hunter (1995) reported that CDK1 activity is stimulated by the phosphorylation of Thr161, but is suppressed by phosphorylation of Tyr15. JNJ-7706621, a novel 3,5-diamino-1,2,4-triazole, can inhibit many protein kinases at different concentrations, such as CDK1, CDK2, and CDK4. Therefore, we speculate that the CDK1 inhibitor JNJ-7706621 can modulate the growth of pig PA or somatic cell nuclear transfer (SCNT) embryos in vitro by regulating the activity of MPF.

Materials and Methods

Cumulus-oocyte complexes were obtained from porcine ovaries derived from a local abattoir. This study focused on the effect of different concentrations of JNJ-7706621 on PA or SCNT embryo development. Comparison analysis of porcine embryo in vitro development competence, the phosphorylation of Tyr15 and Thr161, the level of MPF activity, the expression of apoptosis-related and pluripotency-related genes.

Results and Discussion

A significantly higher percentage of PA and SCNT embryos reached the blastocyst stage after exposure to 10 μM JNJ-7706621 for 4 h compared to embryos exposed to 5 μg/mL cytochalasin B for 4 h. The rate of Tyr15 phosphorylation of the CDK1 was significantly elevated and Thr161 phosphorylation of CDK1 was significantly lower in the JNJ-7706621-treated group compared to cytochalasin B or non-treated group. Similarly, the level of MPF in embryos was significantly lower in the JNJ-7706621-treated group compared to the cytochalasin B-treated and non-treated groups. The expression of Oct4 was significantly elevated in the JNJ-7706621-treated group compared to the cytochalasin B-treated group. Our results support the hypothesis that JNJ-7706621 improves the early development of PA and SCNT porcine embryos by suppressing the activity of CDK1 and a concomitant reduction in the level of MPF.

TREATMENT WITH RESVERATROL DURING *IN VITRO* MATURATION IMPROVES PORCINE OOCYTE QUALITY AND EMBRYONIC DEVELOPMENT*

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Introduction

The developmental competence of somatic cell nuclear transfer (SCNT) embryos from porcine *in vitro* matured (IVM) oocytes is lower than that of *in vivo* counterparts. Insufficient cytoplasmic maturation of porcine IVM oocytes is thought to be responsible for this low efficiency. The aim of the present study was to determine whether the supplementation of resveratrol during IVM can improve porcine oocyte quality and SCNT embryo development of these oocytes.

Materials and Methods

Porcine Cumulus-Oocyte-Complexes were cultured in medium supplemented with different concentration of resveratrol (0, 0.1, 1.0, 5.0 and 10.0 μM). At 44h post IVM, the polar body extrusion was examined to evaluate the nuclear maturation rates. H2DCFDA and 2-NBDG staining were used to assay intracellular reactive oxygen species (ROS) levels and glucose uptake capability of oocytes, respectively. Actin and tubulin staining were used to assay cytoskeleton structure. In addition, qRT-PCR was used to examine the expression of apoptosis and oxidative stress-related genes including *Bax*, *Bcl2*, *CAT* and *SOD1*. Finally, the developmental competency of parthenogenetic activation (PA) and SCNT embryos derived from IVM oocytes were used to determine whether the treatment of resveratrol during IVM could improve the subsequent embryonic development.

Results and Conclusion

The results showed that: (1) after 44h of IVM, no significant difference was observed in nuclear maturation rates of the 0.1, 1.0, and 10.0μM resveratrol groups (75.1%, 78.2% and 79.0% respectively) compared with that of the control group (72.6%, $P > 0.05$), but the nuclear maturation rate of 5.0μM resveratrol group (84.5%, $P < 0.05$) was significantly increased; (2) resveratrol treatment at the optimum concentration of 5.0 μM significantly reduced intracellular ROS, increased the ability of glucose uptake, and improved cytoskeletal dynamics, indicating that treatment of 5.0 μM resveratrol can improve the quality of porcine IVM oocytes; (3) there was significantly lower expression of the proapoptotic gene (*Bax*) and higher expression of the antiapoptotic gene (*Bcl2*) in oocytes with matured nucleus ($P < 0.05$), and the catalase (*CAT*), superoxide dismutase 1 (*SOD1*) mRNA gene expression were significantly higher than that in the control group ($P < 0.05$); (4) blastocyst formation rate (15.2% vs 12.5%, $P < 0.05$) and total cell numbers (37.7 vs 30.5, $P < 0.05$) of 5 μM resveratrol treated IVM oocytes group after SCNT were significantly higher than the control group. Collectively, our results showed that treatment with 5.0 μM resveratrol during IVM could improve porcine oocyte quality by decreasing intracellular ROS level, increasing glucose uptake capability and regulating gene expression, and then enhancing subsequent embryo development.

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WHAMM PLAY ESSENTIAL ROLES IN SPINDLE FORMATION AND CAGE ACTIN MESH OF MATURING MOUSE OOCYTES

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Introduction

Dynamic actin filament polymerization and depolymerization is essential for various cellular processes including cell migration, rotation, cytokinesis and mammalian oocyte maturation. Various actin-binding proteins (ABPs) regulate actin cytoskeletors reorganization. WHAMM (WAS Protein Homolog Associated with Actin, Golgi Membranes and Microtubules) plays a role in actin nucleation, Golgi membrane association and microtubule binding. The WHAMM protein is a nucleation-promoting factor that activates the Actin-related protein 2/3 complex (Arp2/3) and initiates growth of new actin filaments by binding to existing actin filaments. WHAMM also functions in regulation of transport from the endoplasmic reticulum to the Golgi complex and in maintenance of the Golgi complex near the centrosome. However, the roles of WHAMM in mammalian oocyte maturation are poorly understood.

Materials and Methods

Germinal vesicle (GV)-intact oocytes were collected from the ovaries of 6–8-week-old imprinting control region (ICR) mice and cultured in M16 medium (Sigma) under paraffin oil at 37°C in 5% CO2.Oocytes were collected in M2 medium for immuno-staining and microinjection after culturing for various amounts of time. Function of WHAMM was tested by using microinjection of WHAMM dsRNA. Relative gene expression related with WHAMM was quantified by RT-PCR. The WHAMM and F-actin levels were quantified using ImageJ software. The average fluorescence intensity per unit area within a region of interest was determined.

Results and Discussion

Presence of WHAMM in mRNA and protein level have been confirm during all stage of mouse oocyte maturation. It mainly localized in spindle pole and appears enriched in near the chromosome during maturation. By knock down, we confirmed that WHAMM is regulator for formation of spindle. And also WHAMM affect the localization of MTOC during the spindle formation. Taken together, these findings show that WHAMM is one of essential component of the actin cytoskeleton machinery that crucial roles in oocyte maturation, presumably by controlling formation of normal length spindle by activating the cage actin mesh by Arp2/3 complex.

CO-TREATMENT WITH REPSOX AND LBH589 IMPROVES THE IN VITRO DEVELOPMENTAL COMPETENCE OF PORCINE SOMATIC CELL NUCLEAR TRANSFER EMBRYOS

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Introduction

Accumulating evidence suggests that aberrant epigenetic reprogramming and low pluripotency of donor nuclei lead to abnormal development of cloned embryos and underlies the inefficiency of mammalian somatic cell nuclear transfer (SCNT). This study comparison analysis different histone deacetylase inhibitors (HDACis) on porcine SCNT embryo in vitro development. Demonstrated that porcine SCNT embryo treated with HDACi and combined with a small molecule RepSox on in vitro development.

Materials and Methods

The protocols of IVM and SCNT were adapted from a previous study (Yin et al. Biology of Reproduction, 2002). Porcine embryos were treated with VPA, MGCD0103, M344 and LBH589 after SCNT respectively. The best HDACi was combined with RepSox on porcine SCNT embryos. The porcine embryo in vitro development competence, histone modification level and pluripotency-related genes expression were analyzed.

Results and Discussion

LBH589 significantly increased the blastocyst formation rate compared with MGCD0103, M344 and control. In addition, co-treatment RepSox and LBH589 significantly increased the expression of pluripotency-related genes at both 4-cell and blastocyst stage, including NANOG, POU5F1, and SOX2. Moreover, co-treatment of LBH589 and RepSox improved epigenetic reprogramming by histone acetylation and methylation. In summary, combination of LBH589 and RepSox increases expression of developmentally important genes, optimizes epigenetic reprogramming, and improves the in vitro development of porcine SCNT embryos.

THIAMETHOXAM INHIBITS BLASTOCYSTS EXPANDING AND HATCHING VIA ACTIVATION OF ROS-INDUCED DNA DAMAGE CHECKPOINT IN PIGS

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Introduction

Thiamethoxam (TMX) is a neonicotinoid insecticide. It has specific high toxicity to insects. So far, residues of TMX have been detected in rice hull, bran, and polished rice grains. The quantified values are greater in hull and in rice bran. Early embryo quality is vital for fertility. Overmuch production of ROS can override an embryo's antioxidant defenses producing oxidative stress and triggering apoptosis, necrosis and/or permanent DNA-damage response (DDR) in the developing early embryo. Relative studies point that TMX hepatotoxicity is significant to mammals in acute test, but it is unknown on accumulated chronic toxicity to early embryo development of mammals. Therefore, it is necessary to make it clear.

Materials and Methods

The embryos were obtained by *in vitro* maturation of oocytes, parthenogenetic activation and *in vitro* culture early embryo during which embryos were exposed to TMX. Localization or relative content of TUNEL, BrdU and DNA damage checkpoint components were tested by immunofluorescent staining. Genes expression related with ROS and hatch were quantified by RT-PCR. The ROS content was quantified using the DCHFDA method. Activity of $p34^{cdc2}$ was tested with MESACUP CDC2 kinase assay kit. Data were analyzed using SPSS software.

Results and Discussion

Expanding and hatch of blastocysts treated with TMX decreased by 21.73% and 16.71% compared with control, respectively. The rate of cell proliferation decreased by 44.33% compared with expanding blastocyst of control. Increased ROS, γH2AX and mRNA of *sod1*, and decreased transcription of *Mnsod* and *Gpx1* appeared in TMX group. In addition, activity of MPF decreased by 31.41% in expanding blastocysts. These data indicate that TMX has a bad impact on blastocyst quality. In conclusion, TMX inhibits blastocysts expanding and hatching by ROS-induced DNA damage checkpoint activation, which is consistent with relevant studies in zebrafish embryos.

CURRENT DEVELOPMENT OF PORCINE EMBRYONIC STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS IN TAIWAN

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Porcine pluripotent stem cells such as embryonic stem cells (pES) and induced pluripotent stem cells (piPS) have been successfully established in Taiwan. These cells survived repeated subcultures, maintained typical colony morphology, normal karyotype, embryoid body (EB) formation, pluripotent cellular markers and continued expressing green fluorescent protein (GFP). They could differentiate into cell lineages representative to three germ layers after *in vitro* induction. The pigs, on the basis of their similarity in anatomy, immunology, physiology, and biochemical properties, have been wide used as model animals in the study of various human diseases. The therapeutic implications of pES and piPS have been demonstrated in modeling of Parkinson's disease, spinal cord injury and osteoporosis in rats, as well as autotransplantation to porcine model for Parkinson's disease and osteoporosis. Therefore, further in-depth study of of pES and piPS would immediately benefit the biomedical research and regenerative medicine of cell-based therapeutic strategy in humans.

CARCASS TRAITS, MEAT QUALITY, ANTIOXIDANT STATUS AND ANTIOXIDANT GENE EXPRESSION IN MUSCLE AND LIVER OF HU LAMBS FED PERILLA SEED

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Introduction

The increase of n-3 polyunsaturated fatty acid in livestock meat products can effectively improve meat fatty acid composition. However, unsaturated fatty acids are more easily oxidized, which can lead to deterioration of meat products. Perilla seed (PFS) rich in α-linolenic acid and phenolic antioxidants is a promising option to modify fatty acid composition of animal-derived food product through use as a feed supplement. Therefore, this study evaluated these potential effects of PFS supplementation on Hu lamb products.

Materials and Methods

Sixty Hu lambs (3 month old) were randomly divided into four experimental groups receiving diets containing 0%, 5%, 10% or 15% perilla seed (CD, 5%PFSD, 10%PFSD and 15%PFSD, respectively). During the 84 days experimental period, these groups were fed the assigned diets *ad libitum*. At the end of experiment, animals were transported to slaughter house.

Results and Discussion

In the present study, the lambs grew at similar rates and thus had similar carcass weights at the end of the feeding period, probably because of the similarity in dry matter intake in isocaloric and isonitrogenous diets. In addition, supplementation of lamb diets with PFS had no effect on drip loss, cooking loss or shear force. However, the intramuscular lipids gradually increased with increasing levels of PFS supplementation. Although ingestion of food enriched with PUFA can increase oxidative stress in tissues, it can also increase antioxidant defences and reduce free radical oxidation damage. Therefore, the inhibition of lipid oxidation in tissues with higher n-3 PUFA content probably be related to the antioxidant activity of phenolic compounds in PFS and an increase of expression of the antioxidant enzymes.

MYOSTATIN DEFICIENCY INCREASES TYPE II FIBER FORMATION IN SKELETAL MUSCLE BY REGULATING THE EXPRESSION OF MYOD AND MEF2 IN NEWBORN MYOSTATIN-KNOCKOUT PIGS

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Introduction

Myostatin (MSTN) is primarily expressed in muscle and plays an important role in muscle and fat development in pigs. The lack of myostatin leads to a muscle hypertrophy and increases fast fiber (type II) formation. Several reports suggest that myogenic differentiation factor (MyoD) is required for fast fiber formation and myocyte enhancer factor 2 (MEF2) is responsible for slow fiber (type I) formation. In order to elucidate whether MSTN gene could induce muscle fiber type change by these factors or not, myostatin-knockout pigs were analyzed.

Materials and Methods

Three skeletal muscles, namely the biceps femoris (BF), diaphragm (DP) and semitendinosus (ST) were excised from newborn wild-type (WT), $Mstn^{+/-}$ and $Mstn^{-/-}$ pigs. Samples were examined by hematoxylin-eosin staining and ATPase staining for histomorphology analysis. Furthermore, expression patterns of MyoD and MEF2 were detected by quantitative RT-PCR.

Results and Discussion

Hematoxylin-eosin staining revealed the muscle fiber cross-sectional area (CSA) in myostatin-knockout pigs is significantly larger than in WT pigs, but muscle fiber CSA of $MSTN^{-/-}$ is smaller than $MSTN^{+/-}$; this result may be related to the birth weight. ATPase staining revealed increased type II fibers with a concomitant decrease in type I fibers in muscles of myostatin-knockout pigs. The composition rate of type II fiber in $MSTN^{+/-}$ pig is higher than in $MSTN^{-/-}$ pig. Quantitative RT-PCR results revealed that MyoD expression of muscle tissue was 1.3~2 fold greater ($p<0.01$) in $MSTN^{+/-}$ pig and 1.8~3.5 fold greater ($p<0.01$) in $MSTN^{-/-}$ pig compared with wild type. However, MEF2 mRNA levels were significantly lower in $MSTN^{+/-}$ pig compared with wild type pig ($p<0.05$).

EXPRESSION AND LOCALIZATION OF MECP2 GENE IN THE REPRODUCTIVE ORGANS OF HU SHEEP

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Introduction

The human X-chromosomal gene methyl-CpG-binding protein 2(MECP2) was identified as an epigenetic factor capable of binding to methylated DNA. MECP2 is involved in fertility and early development; however, its involvement in the reproduction and fertility of females remains unclear. Therefore, the present study aimed to examine the presence of MECP2 in the reproductive organs of ewes during the estrus period.

Materials and Methods

Female reproductive organs (ovaries, uteri, and oviducts) were collected from eight adult ewes at the Jiangyan Experimental Station (Taizhou, Jiangsu Province, China). Phenotypic traits are characterized by high fecundity and low fecundity (n=4).

Results and Discussion

Results showed that MECP2 mRNA and protein were present in some reproductive organs of ewes during the estrus period. The highest levels of MECP2 mRNA and protein occurred in the uterine horn and oviductal ampulla and the lowest in the uterine cervix and oviductal infundibulum. Higher levels of MECP2 messenger RNA and proteins occur in the ovaries and oviductal ampulla of high-fertility ewes. Immunohistochemical analysis revealed that MECP2 protein was mainly located in luminal and glandular epithelial cells of the uterus and oviduct, and in the theca and granulosa cells of the ovary. Further studies are required to evaluate the function of MECP2 in reproduction.

ROLES OF FIBROBLAST GROWTH FACTOR 9 AND LOC105611671 IN HU SHEEP TESTOSTERONE BIOSYNTHESIS

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Introduction

Fibroblast growth factor 9 (FGF9) is a member of the FGF family. In mice and human embryos, FGF9 has a crucial role in early gonadal development and germ cell maturation. Previous reports have focused mainly on the roles of FGF9 in males and females of humans and rodents, and there is less information about FGF9 and its upstream lncRNA *Loc105611671* in sheep. Therefore, we explored the roles of FGF9 and *Loc105611671* in ovine testis development and steroidogenesis.

Materials and Methods

Fifteen healthy Hu male sheep in various developmental stages, including 3 M (months; n=5), 9 M (n=5), and 24 M (n=5) were used in research. Serum FGF9 and testosterone concentrations were detected by ELISA; qRT-PCR and Western blot analysis were performed to measure expressions of genes and proteins in testis. siRNA transfection was performed in Leydig cells.

Results and Discussion

In this study, we discovered the level of serum FGF9 had a significant increase ($P <$ 0.05) in 9 M compared to 3M of male sheep, as well as the level of *FGF9* mRNA in ovine testis. Furthermore, the levels of the *Loc105611671* mRNA and FGF9 protein increased in ovine testis from 3 M to 24 M. This suggested that FGF9 and *Loc105611671* may be associated with ovine testis development and steroidogenesis. To investigate the role of *Loc105611671* and FGF9 in testosterone biosynthesis, the levels of testosterone and testosterone synthesis-related enzymes were measured. We observed significant decreases in secreting testosterone levels and the levels of testosterone synthesis-related enzymes in Leydig cells after downregulating *Loc105611671* or *FGF9* expression using siRNA ($P <$ 0.05). The results indicated *Loc105611671* act as a positive regulator of FGF9, which can regulate the secretion of testosterone.

PINK1 IS ESSENTIAL FOR MAINTENANCE OF MITOCHONDRIAL MORPHOLOGY IN PORCINE PREIMPLANTATION EMBRYOS

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Introduction

PINK1 selectively locates to the outer membrane of impaired mitochondria and promotes their autophagy. PINK1 regulates mitochondrial dynamics through promoting mitochondrial fission in Drosophila and has a role Parkinson's disease. Mitochondrion is an important organelle in mammalian preimplantation embryos, but few studies focus on the mitochondrial dynamics during preimplantation embryo development.

Materials and Methods

To investigate whether PINK1 are required for mitochondrial dynamics in porcine preimplantation embryos, gene knockdown were engaged in the present study. After injection of PINK1 dsRNA, the parthenotes was cultured in vitro to blastocyst stage. Immunofluorescence and qPCR were used to observe the changes in mitochondrial quality and oxidative stress.

Results and Discussion

The results showed that the blastocyst formation was compromised significantly (42.9±1.9% vs. 36.2±1.4%, $p<0.01$) after PINK1 knockdown. Furthermore, knockdown of PINK1 induced mitochondrial elongation (1.93±0.06μm vs. 3.03±0.09μm, $p<0.001$) and mitochondrial copy number reduce (1.01±0.07 vs. 0.80±0.04, $p<0.05$). The total ROS (10.79±0.97 vs. 20.46±1.86, $p<0.001$) and mitochondrial derived ROS (6.69±0.71 vs. 13.06±3.07, $p<0.05$) was observably increase, but the mitochondria were seriously depolarized. In addition, both of the autophagy (9.17±4.01 vs. 32.57±7.35, $p<0.05$) and apoptosis (6.80±1.18% vs. 18.00±3.21%, $p<0.01$) were significantly increase after mitochondrial elongation. In conclusion, these data suggest that PINK1 promotes mitochondrial fission in porcine preimplantation embryos.

KNOCKOUT ANALYSIS OF OOCYTE-SPECIFIC MULTI-COPY GENE, *OOG1*

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Sex-specific genes in germ cells are necessary to keep the sex of germ cells. The cascade of sex-specific gene control over the down-stream genes supports proper germ cell differentiation. *Oog1* is an oocyte-specific gene and seems to function as a transcription factor during meiosis. In knock down (KD) analysis, it has been suggested that *Oog1* suppresses sperm-specific genes and activates oocyte-specific genes in oocytes to maintain the female germ cell sex. In *Oog1*-KD oocytes, sperm-specific genes such as Tektin2 (*Tekt2),* Tudor Domain Containing 6 (*Tdrd6),* Kelch Like Family Member 5 (*Klhl5)* and Transition Protein 2 (*Tnp2)* were upregulated and oocyte-specific linker histone *H1foo* was downregulated. Significant phenotype, however, is not observed in *Oog1*-knockdown mice. We thought that knock out (KO) analysis is more effective because any mRNA is not produced. We tried to produce *Oog1*-KO mice using CRISPR/Cas9 system. It is challenging because *Oog1* has 5 copies. The two copies locate on chromosome 4 and the others locate on chromosome 12. We used gRNAs shared by all 5 copies, which of two are on the exon 3 and the other is on the exon 4. Genetically modified (GM) mice produced using three gRNAs are 8 males and 10 females. GM mice produced using one gRNA are 5 males and 2 females. To perform mutation analysis, five pairs of PCR primers specific to each copy were designed to amplify only targeted copies. In one GM mouse line, one copy in chromosome 4 is defected largely enough to be recognized by the size of DNA amplified by PCR.

TA cloning shows that another copy in chromosome 4 is also defected in both alleles although the mutation can not be recognized by the size of DNA amplified by PCR. In another GM mouse line, 3 copies of *Oog1* in chromosome 12 are all mutated. DNA size amplified by PCR indicates that one copy is defected largely and another copy has extra DNA insertion. TA cloning showed that the remaining one copy has a few bases deletions in both alleles. We next intend to generate complete KO mice by mating these two CRISPR/Cas9 GM mouse lines and examine the detail function of *Oog1.*

ENHANCEMENT UTILIZATION OF REPRODUCTIVE TECHNOLOGY IN PIG HERDS OF VIETNAM TO MEET THE INDUSTRY DEVELOPMENT POLICY

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Pig production accounts for 60% of the value of the Vietnamese livestock industry. Pig population are 29 million heads, ranked first in ASEAN, ranked second in Asia, in the top 15 countries with the largest pig population in the world. Growth rate of pig population by the period from 2007 to 2017 is 0.91% per year. It is estimated that there are 3 million pig farms in the country and about 500,000 breeding households. Pig Farm with big scale is account for 75% of pig production. The percentage of livestock using industrial bran is about 70%. The productivity of Vietnamese pigs is low, ranging from 17 to 24 weans/sow per year. The output of animal feed Industrial changed from 400,000 tons in 1993 to 23.15 million tons in 2016 (of which 20.15 million tons of animal feed and 3 million tons of aquatic feed).
Many supplying companies with the most famous pig breeds in the world have developed in Vietnam. The number of pigs imported in 2016 is 9,521 pigs (Landrace, Duroc, Yorkshire and Pietrain Breed) from the United States, Canada, Denmark, Taiwan, France, England and Belgium. Modern technologies have been transferred and Vietnamese farmers have accessed technology of breeding, gene selection, and artificial insemination in order to improve reproductive performance of sows. Selected research to improve meat yield and reproductive performance has been carried out with proper housing design to minimize environmental pollution and to adapt climate change. However, 8 main difficulties of pork production are identified as 1)Production of markets is lack of connection and overall management, many intermediaries, production costs are high; 2)infectious diseases are difficult to control, especially blue ear disease and FMD; 3)Restructuring livestock production is still low, slow to change, low investment accumulation; 4)Slaughter processing is not well managed and well planned; 5)Production organization is weak, reducing the competitiveness of products; 6)Support policy is not synchronous; 7)Institutional deficiencies are related to land, credit, insurance ...; 8)The chain linkage is unspecified, fragmented, unconnected ...etc. In the next 10 years, 1) the number of large farms increased, pork production in farms will account for more than 70% by 2027; 2) small-scale production fell sharply (5-7% per year), equivalent to less than 30% of small household; 3) pig farming is primarily a "game" of business and "professional" ranchers; 4) a strong chain linkage between famers, slaughterhouses and distributors. By 2027, livestock registration nationwide and strongly developing traceable food chain will be required. Developing many types of organic pig production and industrial slaughter density will increase with branded processed pork products to export. In general, the pig industry in Vietnam has many advantages, but will face great challenges due to increasing competition. Vietnamese pork is not only competitive with American pork, Danube, Brazil, Holland, but also China and Russia; high cost of livestock production and difficulties in controlling the environment. To overcome these challenges, core solutions need to be developed as the state develops the overall management strategy for the livestock sector. In conclusion, Application of pig reproduction science and biotechnology on breed development, quality control of feeds, and breeding technology to reduce product costs, to improve the meat quality, and to increase the competitiveness of pork products.

ECONOMIC INPUT OF LIVESTOCK DEVELOPMENT IN FARMER BASED THAILAND

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Livestock in Thailand plays a critical role in economy by contributing to securing rural livelihoods, raising national income, reducing poverty, improving human nutrition, earning or saving as well as trading. Livestock are capital assets since replacement can be bred and kept within the herd. However we need to add more fund to new investment. Livestock production provides employment through the livestock system which we can classify the land use into three categories: 1) ‘grassland-based’ pastoralism and ranching 2) ‘mixed-farming’, either rainfall or irrigation and 3) ‘landless livestock production’. Livestock development could be motivated through extension with technology transfer which has four main parts. They are developing livestock breeds which are suitable to Thailand’s environment to improve production, developing livestock feeds which are produced from local material and/or agriculture by-products, e.g. maize tree, cassava and cassava leaves, mulberry leaves and pine apple peels, encouraging farmers to use agriculture machinery and implementing GAP farm management to create consumer’s confidence through food safety, traceability and friendly environment. The output is to increase productivity and decrease production cost while the outcomes are to encourage farmers to extend competitive capacity and have occupation sustainability which leads to increase income whereas to offer safety food to both domestic and foreign consumers.

AGE-ASSOCIATED EXPRESSION OF VDR AND VIT D METABOLIZING ENZYMES IN MALE REPRODUCTIVE TRACT AND SPERM OF SHEEP

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Introduction

The cellular response to Vitamin D (Vit D) is complex and depends not only on Vitamin D receptor (VDR) but also on activity of Vit D-metabolizing enzyme (CYP2R1, CYP27A1, CYP27B1, and CYP24A1). To enhance our knowledge of Vit D function in male reproduction, this study evaluated the expression of VDR and Vit D-metabolizing enzymes in the ram reproductive tract at different ages and in spermatozoa.

Materials and Methods

15 Hu sheep were divided into three groups (3 M, 9 M and 24 M). The epididymis and testes were collected for qRT-PCR, WB and IHC analysis. The cauda epididymis and fresh ejaculate spermatozoa were collected from 24 Hu sheep for IF, qRT-PCR and WB analysis. Sperm motility was detected by CASA and divided into high-(≥80%) and low-motility (≤50%) group.

Results and Discussion

VDR and Vit D-metabolizing enzymes were found to be expressed in the ram testes, epididymis, and mature spermatozoa suggests that Vit D might play key roles in spermatogenesis and sperm maturation. VDR and CYP24A1 were mainly concentrated in the mid-piece of ejaculated or cauda epididymis spermatozoa or both. Moreover, VDR and CYP24A1 expression was higher in high- than in low-motility sperm, which suggests that VDR and CYP24A1 can serve as biomarkers of semen quality. Lastly, the marked higher VDR and CYP27B1 expression levels in ejaculated than in cauda epididymis spermatozoa, which suggests that Vit D may be necessary for the motility of spermatozoa. These findings provide further compelling evidences of the pivotal role of Vit D in male reproductive functions, which could suggest the influence of Vit D on the efficiency of sheep fertility. However, the underlying mechanisms have not been completely clarified; therefore, further investigation is required to elucidate the functions of Vit D_3 in reproduction.

TECHNICAL TRAINING INPUT OF PIG REPRODUCTION IN LOCAL AND INTERNATIONAL FARMERS AT PHILIPPINE ITCPH

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ITCPH (International Training Center on Pig Husbandry) is the only training center in Asia specializing on pig husbandry. ITCPH was established in 1985 as a foreign-assisted project of the government of Netherlands. ITCPH is now one of the 17 training centers of the Agricultural Training Institute (ATI) whose mother agency is the Department of Agriculture. ITCPH is a government-training center dedicated to the empowerment of our clientele to enable them to achieve their goals of productivity and profitability. Our clientele includes (non) government extension workers, farmers, entrepreneurs, private extension service providers, agricultural teachers and professors, members of rural-based organizations and cooperatives, and interested private individuals. National Trainors' Course on Pig Husbandry is one of the regular courses of ATI-ITCPH offered once a year. It is a course for extension workers / technicians of government and non-government organizations; and teachers / instructors of agricultural colleges and universities, handling pig husbandry subjects and / or in-charge of pig farm project in the campus. ITCPH provides classroom instructions coupled with practical classes, following the principle of "learning by doing" thru hands-on training; has intensive guidance by highly competent technical staff whose local and international trainings include both large and small-scale pig farming practices; and is well-equipped with facilities modest accommodations at the ATI-ITCPH Dormitory and Guest House.

Previous Meetings of ARBC

Name	From	To	Location
1st	2004-04-12	2004-04-16	Ho Chi Minh, Vietnam
2nd	2005-11-02	2005-11-07	Bangkok, Thailand
3rd	2006-11-29	2006-12-03	Hanoi, Vietnam
4th	2007-11-24	2007-11-28	Singapore
5th	2008-11-26	2008-11-30	Kunming, China
6th	2009-11-16	2009-11-20	Siem Reap, Cambodia
7th	2010-11-08	2010-11-12	Kuala Lumpur, Malaysia
8th	2011-10-25	2010-10-30	Guilin, China
9th	2012-10-23	2012-10-28	Manila, Philippines
10th	2013-08-19	2013-08-25	Ho Chi Minh, Vietnam
11th	2014-11-03	2014-11-08	Bangkok, Thailand
12th	2015-11-26	2015-11-29	Hanoi, Vietnam

FOSS

CombiFoss ™ 7

- Testing an unparalleled 19 parameters including new differential somatic cell count(DSCC) in six seconds from same milk sample.
- Differential SCC for better mastitis management.
- Superior performance at 600 samples per hour.

BacSomatic™

- Small and handy instrument for Bacteria Count and Somatic Cell Count.
- Offers a rapid alternative to manual assay or semi-automated methods requiring reagent handling.

IDEXX

Laboratory-Based Milk Pregnancy Testing

- Cow and goat milk samples.
- Pregnancy associated glycoproteins (PAGs).

Rapid Visual Pregnancy Test

- Detecting without ELISA instrumentation and read visually.
- Early identification of open (nonpregnant) cows and heifers.

Dairy Test

- Detects antibiotic residues in milk.
- Cow, Goat, Sheep, Mare, Buffalo, Camel.
- Read results after 6 minutes.

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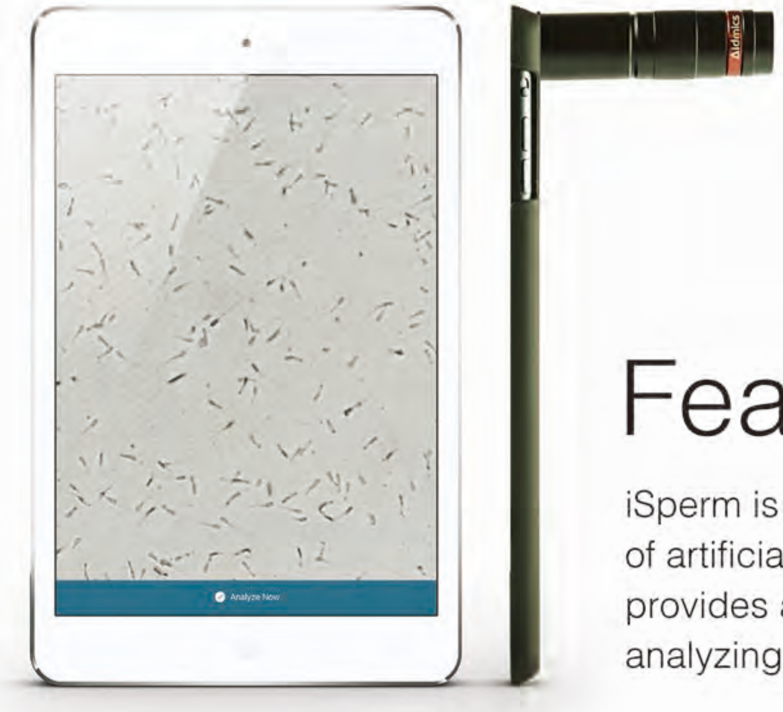
iSperm™

https://isperm.aidmics.com/



Features

iSperm is designed for vet, simplifying the process of artificial insemination and production control. It provides a user-friendly app to accomplish sperm analyzing precisely and efficiently.

iSperm Animal Sperm Analyzer

Function

Motility: Total Motility

Analysis Time: 10 sec video analysis

Progressive motility: LIN/STR/VSL/VAP/VCL

Concentration: Total Concentration、Dilution Doses

Sample: Fresh semen、Diluted semen、Frozen semen

Supported Species: Swine / Poultry / Canine / Caprine / Bovine / Equine

Web Site QR Code

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