The 5th Winter Workshop of the Society for Biology of Reproduction

"Central and Local Regulations of Reproductive Processes"

Zakopane, Poland
February 13–15, 2019

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ORGANIZED by

Cracovian Section of the Society for Biology of Reproduction

Committee:

Prof. dr hab. Dorota Lechniak-Cieślak	– *Poznań University of Life Sciences*
Dr hab. Andrzej Herman, prof. nadzw.	– *Institute of Animal Physiology and Nutrition, Polish Academy of Sciences in Jabłonna*
Prof. dr hab. Wojciech Niżański	– *Wrocław University of Environmental and Life Sciences*
Prof. dr hab. Grażyna Ptak	– *Małopolska Centre of Biotechnology, Jagiellonian University in Kraków*
Prof. dr hab. Andrzej Sechman	– *University of Agriculture in Kraków*
Prof. dr hab. Jan Twardoń	– *Wrocław University of Environmental and Life Sciences*
Prof. dr hab. Edward Wierzchoś	– *University of Agriculture in Kraków*
Prof. dr hab. Adam Zięcik	– *Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn*

Local Organizing Committee:

Prof. dr hab. Barbara Gajda	– *National Research Institute of Animal Production, Kraków-Balice*
Dr hab. Katarzyna Knapczyk-Stwora	– *Jagiellonian University in Kraków*
Dr hab. Wiesława Młodawska	– *University of Agriculture in Kraków*
Dr hab. Edyta Molik	– *University of Agriculture in Kraków*
Dr hab. Anna Ptak	– *Jagiellonian University in Kraków*
Dr hab. Danuta Wrońska	– *University of Agriculture in Kraków*

Guest Editors:

Dr hab. Edyta Molik	– *University of Agriculture in Kraków*
Prof. dr hab. Edward Wierzchoś	– *University of Agriculture in Kraków*

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*Sicut dies juventutis sic et senectus tua.***

Pierwsza Międzynarodowa „Przed Szkoła" Biologii Rozrodu – Olsztyn 1992

„Advances and Perspectives in Reproductive Ebdocrinology of Domestic Animal"

Prelegenci zagraniczni: Australia – prof. G. Martin; Anglia – dr H. Dobson; Bułgaria – prof. L. Kanczew; Czechy – dr N. Tomanek; Finlandia – dr T.A. Sokka; Izrael – prof. M. Shemesh; Niemcy – E. Kanitz, dr K.P. Brusov, dr F. Elsasser, dr F. Schneider, prof. D. Schams; Szwecja – prof. S. Einersson; USA – prof. P. Dziuk, prof. G.L. Williams, dr J.D. Armstrong, prof. J. Tilton, prof. R.R. Kraeling.

*** Jakim kto był za młodu, takim jest na starość. (Horacy)*

Fot. nadesłał prof. Adam Zięcik

Słowo wstępne

Piąta Jubileuszowa Szkoła Zimowa Towarzystwa Biologii Rozrodu w Zakopanem organizowana przez środowisko Krakowskiego Oddziału TBR, zrzeszające pracowników naukowych Uniwersytetów Rolniczego i Jagiellońskiego oraz Instytutu Zootechniki PIB, to nie tylko fakt historyczny, lecz także wielki sukces działalności na rzecz wspólnego dobra – wielkiej bezinteresowności, współpracy, życzliwości, poświęcenia oraz zaangażowania w pokonywaniu wielu trudności formalnych oraz finansowych. A także – wynik wypracowania przez środowisko zasad bardzo skrupulatnej realizacji przyjętych przed laty założeń tych spotkań naukowych, odnoszących się do tematyki, miejsca, czasu, uczestnictwa, przygotowywania prezentacji i materiałów do druku. To obecnie już dostatecznie udokumentowany niepodważalny fakt, którym dziś możemy wszyscy się cieszyć. Jest on bowiem opisany po pierwsze historią uczestnictwa w tych spotkaniach naukowych ponad pięciuset osób, po drugie wydaniem w języku polskim i angielskim materiałów konferencyjnych w łącznym nakładzie około 900 sztuk egzemplarzy. Po trzecie, wygłoszeniem ponad 150 doniesień i przygotowaniem 600 posterów oraz odbyciem warsztatów szkoleniowych. I po czwarte – spełnieniem zamysłu, że Szkoły w Zakopanem będą miejscem, gdzie przede wszystkim młodzi pracownicy nauki, stypendyści, doktoranci będą mogli podzielić się swoimi osiągnięciami, przedyskutować niepowodzenia, nawiązać współpracę, czy też uzyskać informację na temat nowych rozwiązań metodycznych, aby móc zrealizować swe ambitne marzenia i „wypłynąć na głębię" – DUC IN ALTUM!

Szkoła Zimowa w Zakopanem, która wyrosła z potrzeby rozszerzenia aktywności naukowej i większej integracji członków Towarzystwa Biologii Rozrodu, jest stosunkowo nową tradycją, ale nawiązującą do spontanicznie organizowanych od około 30 lat: spotkań, sympozjów, zjazdów, poza przyjętymi statutowo – Kongresami Towarzystwa. Szkoła kultywuje więc i zachowuje to, co wcześniej pozwalało inspirować kolejne wyzwania w odpowiedzi na pojawiające się w światowej nauce nowe kierunki w badaniach podstawowych i utylitarnych w dziedzinie biologii rozrodu.

Niech więc już piąte nasze spotkanie, tutaj w Zakopanem w gościnnym „Rzemieślniku", a obecnie Hotelu PRL, potwierdzi Wasze i Nasze oczekiwania. Niech przyniesie „zdrowe owoce zaczynu intelektualnego". Niech pozwoli zająć się tym, co determinuje nasze życie. Niech pod Giewontem owionie nas świeże górskie powietrze, byśmy mogli też razem cieszyć się dwudziestoleciem powstania Towarzystwa Biologii Rozrodu (15 września 1998).

W imieniu Krakowskiego Oddziału
Towarzystwa Biologii Rozrodu
Przewodnicząca, dr hab. Edyta Molik
prof. dr hab. Edward Wierzchoś

Foreword

The 5th Jubilee Winter Workshop of the Society for Biology of Reproduction (SBR) in Zakopane, organized by the Cracovian Section of the SBR, which has as its members researchers from the University of Agriculture, the Jagiellonian University as well as the National Research Institute of Animal Production, is not only a historical fact, but also a remarkable success of the efforts towards the common good – selflessness, cooperation, friendliness, dedication, and commitment to overcome many formal and financial obstacles. It is also the result of meticulously following the principles of these scientific meetings, including the topics, venue, time, participation, preparation of presentations and materials of printing, which had been developed by the community in previous years. This is a well documented and undeniable fact, which all of us can enjoy now. Firstly, it is evidenced by the history of the participation of over five hundred people in these scientific meetings. Secondly, by the printing of around 900 copies of the conference proceedings in Polish and in English. Thirdly, by the presentation of more than 150 reports, preparation of 600 posters, and organization of training workshops. Fourthly, by realizing the idea that the Workshops in Zakopane will first of all provide a place for young research workers, scholarship holders and doctoral students to share their achievements, discuss failures, establish cooperation, and obtain information about new methods so as to accomplish ambitious dreams and to "go out into the deep" – DUC IN ALTUM!

The Winter Workshop in Zakopane, which grew out of the need to extend research activity and to better integrate members of the Society for Biology of Reproduction, is a relatively recent tradition, but it draws upon the meetings, symposia and conventions that have been spontaneously organized for around 30 years in addition to the statutory Congresses of the Society. In this way, the Workshop cultivates and preserves what previously inspired further challenges in response to new trends that emerge around the world in basic and practical research in the field of reproductive biology.

May our fifth meeting here in Zakopane, in the hospitable Hotel PRL (formerly "Rzemieślnik"), meet both your and our expectations. May it provide "an intellectual leaven that bears good fruit". May it allow us to address what determines our lives. May fresh mountain air at the foot of Giewont enfold us so that we can together celebrate the twentieth anniversary of the Society for Biology of Reproduction (15 September 1998).

On behalf of the Cracovian Section of the
Society for Biology of Reproduction
President Edyta Molik, PhD
Prof. Edward Wierzchoś, PhD

Conference program

The 5th Winter Workshop of the Society for Biology of Reproduction
„Central and Local Regulations of Reproductive Processes"

WEDNESDAY, FEBRUARY 13, 2019

14:00 Registration opens

THURSDAY, FEBRUARY 14, 2019

08:30-08:45 **Opening Ceremony – *Prof. Dr. hab. Andrzej Sechman***

08:45-09:30 **Plenary lecture – *Prof. Dr. hab. Wojciech Niżański***
HOW OLD IS TOO OLD – ANDROPAUSE IN ANIMALS AS IN HUMAN?

09:30–11:30 **Session 1**
FROM OOCYTE TO EMBRYO – BIOTECHNOLOGY IN ANIMAL REPRODUCTION
Prof. Dr. hab. Jan Twardoń, Dr. Magdalena Kowalik

09:30–09:45 Gajda B., Poniedziałek-Kempny K., Rajska I., Smorąg Z.
IN VITRO FERTILIZATION AND SUBSEQUENT DEVELOPMENT OF VITRIFIED PORCINE OOCYTES MATURED WITH THYMOSIN

09:45–10:00 Gąsior Ł., Ptak E.G., Polański Z.
THE INFLUENCE OF GENOTOXIC STRESS ON THE INCREASE OF mtDNA COPY NUMBER

10:00–10:15 Prochowska S., Ochota M., Niżański W., Partyka A., Kochan J., Młodawska W., Nowak A., Skotnicki J., Grega T., Pałys M.
THE EFFECT OF REDUCED OXYGEN TENSION ON FELINE OOCYTES MATURATION AND EMBRYO DEVELOPMENT *IN VITRO*

10:15–10:30 Rudnicka J., Gąsior Ł., Bisogno S., Fic K., Polański Z., Ptak G.E.
QUANTITATIVE IMAGING OF LIPIDS IN OOCYTES OBTAINED FROM DIAPAUSING MAMMALIAN SPECIES USING COHERENT ANTI-STOKES RAMAN SCATTERING (CARS) MICROSCOPY

10:30–10:45 Staszkiewicz-Chodor J., Sinderewicz E., Grycmacher K., Boruszewska D., Kowalczyk-Zięba I., Wocławek-Potocka I.
EXPRESSION OF PROSTAGLANDIN $F_{2\alpha}$ IN OOCYTES AND CUMULUS CELLS DERIVING FROM PUBERTAL AND PREPUBERTAL COWS, DEPENDING ON THE QUALITY OF THE OOCYTE

10:45–11:00 Szczepańska A., Kotlarczyk A., Dubełek R., Dziekońska A., Koziorowska-Gilun M., Kordan W., Wocławek-Potocka I., Korzekwa A.J.
IN VITRO FERTILIZATION OF RED DEER OOCYTES WITH FRESH AND FROZEN SEMEN AND BLASTOCYST MATURATION

11:00–11:15 presentation SanLab – sponsor

11:15–11:45 Coffee break

11:45–13:15 **Session 2**
CENTRAL AND METABOLIC REGULATIONS OF REPRODUCTION
Dr. hab. Andrzej Herman, Prof. nadzw., Dr. Małgorzata Szczęsna

11:45–12:00 Biernat W., Szczęsna M., Kirsz K., Zięba-Przybylska D.
THE EFFECT OF NUTRITIONAL STATUS ON RESISTIN'S-MEDIATED LEPTIN INSENSITIVITY IN SHEEP

12:00–12:15 Kieżun M, Dobrzyń K., Szeszko K., Rytelewska E., Kisielewska K., Gudelska M., Zaobidna E., Bors K., Wyrębek J., Mykytiuk A., Kamińska B., Smolińska N., Kaminski T.
EXPRESSION OF CHEMOKINE LIKE RECEPTOR 1 (CMKLR1/CHEMR23) IN THE PORCINE HYPOTHALAMUS DURING THE OESTROUS CYCLE

12:15–12:30 Kirsz K., Szczęsna M., Biernat W., Żebrowska M., Zięba-Przybylska D.
EFFECTS OF CENTRAL OREXIN A ON GONADOTROPINS AND PROGESTERONE SECRETION IN EWES IN THE LUTEAL PHASE OF THE ESTROUS CYCLE AND IN THE ANESTRUS

12:30–12:45 Kisielewska K., Rytelewska E., Gudelska M., Kieżun M., Zaobidna E., Dobrzyń K., Szeszko K., Wyrębek J., Bors K., Mykytiuk A., Kamińska B., Smolińska N., Kamiński T.
CHEMERIN EXPRESSION IN THE PORCINE PITUITARY DURING THE ESTROUS CYCLE AND EARLY PREGNANCY

12:45–13:00 Młotkowska P., Marciniak E., Misztal T.
EXPRESSION OF SELECTED GENES OF THE GONADOTROPIC SYSTEM IN SHEEP TREATED WITH STRESSFUL STIMULI AND ALLOPREGNANOLONE

13:00–13:15 Wartalski K., Gorczyca G., Wiater J., Duda M.
CHANGES IN PHENOTYPE OF OVARIAN MESENCHYMAL STEM CELLS INDUCED BY ANABOLIC STEROIDS

13:15–13:45 **Poster session no. I (sessions 1–4)**

13:45–14:45 Lunch

14:45-16:00 **Session 3**
ENVIRONMENTAL DETERMINANTS OF REPRODUCTION
Dr. hab. Anna Ptak, Dr. hab. Magdalena Socha

14:45–15:00 Gogola J., Hoffmann M., Ptak A.
PERSISTENT ORGANIC POLLUTANTS PRESENT IN HUMAN FOLLICULAR FLUID THROUGH MODULATING E2 AND IGF1 SECRETION BY ADULT GRANULOSA CELL TUMORS STIMULATE HUMAN GRANULOSA CELLS PROLIFERATION

15:00–15:15 Kowalik K., Kozubek A., Katarzyńska-Banasik D., Socha J., Zarabska A., Hrabia A, Sechman A.
NITROPHENOLS INHIBIT BASAL AND 8-Br-cAMP INDUCED STEROID HORMONE SECRETION BY OVARIAN FOLLICLES OF THE HEN (*GALLUS DOMESTICUS*)

15:15–15:30 Socha M., Drąg-Kozak E., Sokołowska-Mikołajczyk M., Chyb J.
EFFECT OF HERBICIDE ROUNDUP AND TAMOXIFEN ON PRUSSIAN CARP (*CARASSIUS GIBELIO* B.) OOCYTE MATURATION AND SECRETION OF 17α20β-P *IN VITRO*

15:30–15:45 Witek P., Matusiak J., Grzesiak M., Słomczyńska M., Koziorowski M., Duda M., Knapczyk-Stwora K.
NEONATAL EXPOSURE TO METHOXYCHLOR ALTERS PLASMA LEVEL OF FSH AND FSH RECEPTOR EXPRESSION IN OVARIAN FOLLICLES OF ADULT PIGS

15:45–16:00 Zajda K., Gregoraszczuk E.
AHR/ER CROSS TALK IN PAH MIXTURES ACTION ON CELL PROLIFERATION AND HORMONE SECRETION BY HUMAN GRANULOSA CELLS

16:00–16:15 Coffee Break

16:15–17:45 **Session 4**
MATERNAL-FETAL ADAPTATIONS DURING PREGNANCY
Prof. Dr. hab. Dorota Lechniak-Cieślak, Dr. Jacek Wawrzykowski

16:15–16:30 Gudelska M., Dobrzyn K., Kieżun M., Rytelewska E., Kisielewska K., Zaobidna E., Szeszko K., Wyrębek J., Bors K., Mykytyuk A., Kamińska B., Kamiński T., Smolińska N.
THE EXPRESSION OF CHEMOKINE-LIKE RECEPTOR 1 (*CMKLR1*) GENE AND PROTEIN IN THE PORCINE ENDOMETRIUM DURING THE OESTROUS CYCLE AND EARLY PREGNANCY

16:30–16:45 Guzewska M.M., Heifetz Y., Kaczmarek M.M.
SECRETION PATTERNS OF EXTRACELLULAR VESICLES DURING EARLY PREGNANCY IN THE PIG – *IN SITU* TRANSMISSION ELECTRON MICROSCOPY STUDY

16:45–17:00 Liu X., Schwarz T., Murawski M., Tayade Ch., Kridli R.T., Prieto Granados A.M., Sharma Ch., Bartlewski P.M.
MEASUREMENTS OF CIRCULATING PROGESTERONE AND ESTRONE SULFATE CONCENTRATIONS AS A DIAGNOSTIC AND PROGNOSTIC TOOL IN PORCINE PREGNANCY REVISITED

17:00–17:15 Machlowska J., Zacchini F., Arena R., Frolova A., Branicki W., Łabaj P., Bernhardt L., Haaf T., Ptak G.E.
UNIPARENTAL CONCEPTUS: TRANSCRIPTOME-WIDE INVESTIGATION OF GENOMIC IMPRINTING STATUS IN SHEEP PLACENTAE

17:15–17:30 Wawrzykowski J., Franczyk M., Kankofer M.
ANALYSIS OF GLYCOSYLATION PROFILE OF MEMBRANE PROTEINS IN PLACENTA OF COWS DURING PREGNANCY AND PARTURITION

17:30–17:45 Zacchini F., Stankiewicz A.M., Bernardt L., Haaf T., Ptak G.E.
GENOME-WIDE METHYLATION PROFILE OF PLACENTAE AND FETAL TISSUES DEVELOPED FOLLOWING ASSISTED REPRODUCTIVE TECHNOLOGIES

19:00 Dinner

FRIDAY, FEBRUARY 15, 2019

09:00–10:30 **Session 5**
MOLECULAR ANDROLOGY – THE COGNITIVE AND APPLICATIVE ASPECTS
Dr. hab. Katarzyna Knapczyk-Stwora, Dr. hab. Jolatna Opiela

09:00–9:15 Kamińska A., Marek S., Pardyak L., Pawlicki P., Bilińska B., Hejmej A.
THE EFFECT OF ANDROGEN SIGNALING DISRUPTION ON NOTCH PATHWAY IN PERIPUBERTAL RAT TESTIS – AN *IN VIVO* STUDY

09:15–09:30 Mietelska K., Orzołek A., Wysocki P., Kordan W.
DIFFERENCES IN SPERM PROTEINS PHOSPHORYLATION STATUS IN EVERY SEGMENT OF GOAT (*CAPRA HIRCUS*) EPIDYDIMIS

09:30–09:45 Miłoń A., Pawlicki P., Pardyak L., Kamińska A., Bilińska B., Kotula-Balak M.
ESTROGEN-RELATED RECEPTORS AND G PROTEIN-COUPLED ESTROGEN RECEPTOR IN RODENT LEYDIG CELLS

09:45–10:00 Pardyak L., Kamińska A., Brzoskwinia M., Marek S., Hejmej A., Kotula-Balak M., Jankowski J., Ciereszko A., Bilińska B.
ALTERED LEVELS OF JUNCTIONAL PROTEIN GENE EXPRESSION IN REPRODUCTIVE TISSUES ARE LIKELY RELATED TO THE APPEARANCE OF YELLOW SEMEN IN DOMESTIC TURKEYS

10:00–10:15 Partyka A., Ligocka Z., Rodak O., Dudek A., Niżański W., Grandhaye J., Jeanpierre E., Froment P.
THE EFFECT OF NATURAL AND PHARMACOLOGICAL AGENTS ADDITION ON THE QUALITY OF CRYOPRESERVED CANINE SEMEN

10:15–10:30 Koziorowska-Gilun M., Dziekońska A., Kotlarczyk A., Rafalska K., Purpurowicz P.S., Kordan W.
ANALYSIS OF MOTILITY AND SELECTED PARAMETERS OF THE ANTIOXIDANT STATUS OF EPIDIDYMAL SPERM OF RED DEER (*CERVUS ELAPHUS* L.) STORED IN ANDROMED® DILUENT

10:30–10:45 Coffee break

10:45–11:30 **Poster session no. II (sessions 5–6)**

11:30–13:30 **Session 6**
LOCAL REGULATION OF REPRODUCTIVE FUNCTIONS
Prof. Dr. hab. Grażyna Ptak, Dr. Marta Kieżun (UWM)

11:30–11:45 Dobrzyń K., Kowalik M.K., Kotwica J.
INFLUENCE OF STEROIDS ON THE EXPRESSION OF MEMBRANE PROGESTERONE RECEPTORS IN THE BOVINE PLACENTA

11:45–12:00 Grzesiak M., Słomczyńska M., Koziorowski M., Nowak S., Duda M., Knapczyk-Stwora K.
THE EFFECT OF NEONATALLY ADMINISTERED SEX STEROID AGONISTS AND ANTAGONISTS ON AMH-AMH RECEPTOR SYSTEM IN OVARIAN FOLLICLES AND AMH PLASMA LEVEL OF ADULT PIGS

12:00–12:15 Hoffmann M., Gogola J., Ptak A.
EFFECT OF APELIN, 17β-ESTRADIOL AND INSULIN-LIKE GROWTH FACTOR 1 TREATMENT ON OVARIAN CANCER CELL PROLIFERATION IN 2D AND 3D CELL CULTURE MODEL *IN VITRO*

12:15–12:30 Pawlicka B., Tomczyk I., Grzmil P.
DOES THE INTERACTION OF PXT1 AND BAG6 PROTEINS HAVE AN EFFECT ON THE SPERM QUALITY IN MOUSE?

12:30–12:45 Rytelewska E., Kisielewska K., Gudelska M., Dobrzyń K., Kieżun M., Zaobidna E., Szeszko K., Bors K., Wyrębek J., Mykytyuk A., Kamińska B., Kamiński T., Smolińska N.
CHEMERIN AS A HORMONE THAT MODULATES PROGESTERONE SECRETION BY THE PORCINE OVARY DURING THE OESTROUS CYCLE

12:45–13:00 Zygmuntowicz A., Markiewicz W., Smolińska N., Dobrzyń K., Jaroszewski J.J.
EFFECTS OF MONTELUCAST AND NIFEDIPINE ON THE UTERINE CONTRACTILITY IN IMMATURE AND PREGNANT PIGS

13:00–13:30 **Closing ceremony and presentation of awards for the best scientific presentations and posters**

14:00–15:00 Lunch

The team assessing poster presentations:
Dr. hab. Wiesława Młodawska
Dr. hab. Danuta Wrońska
Dr. hab Anna Korzekwa
Dr. hab Agnieszka Rak
Dr. Kamil Dobrzyń

Poster presentation

Poster session I

1. Bielas W., Rząsa A., Gil A., Niżański W. REPRODUCTIVE PERFORMANCE OF SOWS AFTER POST CERVICAL INSEMINATION WITH LIQUID SEMEN

2. Dzięgiel N., Jura J. EFFECTIVENESS OF TRANSFECTION WITH NANOPARTICLES OF RABBIT ZYGOTES-PRELIMINARY RESULTS

3. Fryc K., Kij B., Nowak A., Wierzbicka A., Murawski M., Kochan J. ANALYSIS OF MORPHOKINETIC OF OVINE EMBRYOS USING A TIME LAPSE SYSTEM – PRELIMINARY RESEARCH

4. Gogol P., Bryła M., Trzcińska M., Bochenek M. EFFECT OF SOYBEAN LECITHIN ON THE POST THAW QUALITY AND FERTILITY OF RAM SEMEN

5. Gorczyca G., Duda M. *IN VITRO* MATURATION OF PORCINE OOCYTES USING NOVEL TECHNIQUE OF LIQUID MARBLE BIOREACTORS.

6. Jurkiewicz J., Wierzchoś-Hilczer A., Stefan J., Opiela J. SUCCESSFUL DIFFERENTIATION OF EQUINE MESENCHYMAL STEM CELLS (MSCs) INTO OSTEOBLASTS, CHONDROCYTES AND ADIPOCYTES DERIVED FROM THE BONE MARROW COLLECTED POST SLAUGHTER

7. Poniedzialek-Kempny K., Rajska I., Gajda L., Gajda B. THE ABILITY OF EJACULATED, EPIDIDIMAL OR WITHOUT PLASMA BOAR SEMEN FOR *IN VITRO* FERTILIZATION

8. Rajska I., Poniedziałek-Kempny K., Soból K., Gajda B. THE EFFECT OF DIFFERENT ANTIOXIDANTS ON THE DEVELOPMENTAL COMPE-

TENCES OF PIG EMBRYOS OBTAINED AFTER *IN VITRO* FERTILIZATION

9. Samiec M., Skrzyszowska M., Opiela J. CAN EPIGENOMIC MODIFIER USED FOR *IN VITRO* MATURATION OF NUCLEAR RECIPIENT OOCYTES BE ABLE TO IMPROVE THE COMPETENCES OF SOMATIC CELL NUCLEI TO SUPPORT THE DEVELOPMENTAL POTENTIAL OF PORCINE CLONED EMBRYOS?

10. Drzewiecka K., Gromadzka-Hliwa K., Kłos J., Zięcik A.J. APPLYING OF INTRA- AND EXTRACELLULAR MEASUREMENTS OF cAMP FOR DETERMINATION THE POSSIBILITY OF LH RECEPTORS INTERNALISATION IN THE GRANULOSA CELLS OF OVARIAN PREOVULATORY FOLLICLES IN THE PIG

11. Małyszka N., Pawlak P., Lechniak-Cieślak D. FATTY ACID PROFILE IN FOLLICULAR FLUID AFFECTS THE QUALITY OF PORCINE CUMULUS-OOCYTE COMPLEXES *IN VITRO*

12. Molik E., Kosmatko J., Szczęsna M., Misztal T. THE ROLE OF TRH AND LENGHT DAY IN THE REGULATION OF GROWTH HORMONE SECRETION IN LACTATING SHEEP

13. Murawski M., Schwarz T., Paravinja V., Sohal J., Ahmadi B., Kridli R.T., Bartlewski P.M. EFFECTS OF SHORT-TERM LUPIN GRAIN FEEDING ON OVARIAN ACTIVITY IN NON-PROLIFIC POLISH MOUNTAIN EWES DURING THE BREEDING SEASON

14. Szczęsna M., Kirsz K., Wójcik K., Pisanko J., Wawrzyn A., Zięba D. EXPRESSION PROFILE OF LEPTIN RECEPTOR, PROLACTIN RECEPTOR AND SOCS-3 TRANSCRIPTS AT SELECTED STAGES OF FETAL DEVELOPMENT IN LAMBS

15. Tomczyk M., Wójcik M., Bochenek J., Pawlina B., Tomaszewska-Zaremba D., Antushevich A., Krawczyńska A., Herman A., Herman A.P. PERIPHERAL ADMINISTRATION OF CAFFEINE INFLUENCES THE SYNTHESIS OF GNRH AND LUTEINIZING HORMONE IN EWE DURING THE FOLLICULAR PHASE OF THE ESTROUS CYCLE

16. Zaobidna E., Kieżun M., Kisielewska K., Rytelewska E., Gudelska M., Dobrzyń K., Szeszko K., Wyrebek J., Bors K., Mykytiuk A., Kamińska B., Smolińska N., Kamiński T. CHEMOKINE LIKE RECEPTOR 1

(CMKLR1/CHEMR23) EXPRESSION IN THE PORCINE HYPOTHALAMUS DURING EARLY PREGNANCY

17. Likszo P., Jalali B.M., Skarżyński D.J. PROTEOMICS ANALYSIS OF THE PIG CORPUS LUTEUM DURING EARLY PREGNANCY

18. Dawid M., Mlyczyńska E., Kurowska P., Rak A. EFFECT OF APELIN ON THE ENDOCRINE FUNCTION OF THE HUMAN PLACENTA CELLS

19. Dobrzyń K., Kieżun M., Kisielewska K., Rytelewska E., Gudelska M., Szeszko K., Zaobidna E., Bors K., Wyrębek J., Mykytiuk A., Kamińska B., Kamiński T., Smolińska N. DETERMINATION OF G PROTEIN-COUPLED RECEPTOR 1 (GPR1) GENE AND PROTEIN EXPRESSION IN THE PORCINE ENDOMETRIUM DURING EARLY PREGNANCY AND THE OESTROUS CYCLE

20. Franczyk M., Wawrzykowski J., Kankofer M. EXTRACELLULAR MATRIX PROTEINS DURING PREGNANCY AND AT PARTURITION IN PLACENTA OF COWS

21. Mlyczyńska E., Kurowska P., Drwal E., Tworzydło W., Rak A. APELIN/APJ EXPRESSION IN THE HUMAN PLACENTA CELLS AND IT'S STIMULATORY ACTION ON CELL PROLIFERATION VIA APJ AND DIFFERENT KINASES ACTIVATION.

22. Najmuła J., Kaczmarek M.M. miR-23b-3p INHIBITS MIGRATION AND STIMULATE PROLIFERATION OF JEG-3 HUMAN TROPHOBLAST CELL LINE

Poster session no. II (sessions 5–6)

23. Brzoskwinia M., Pardyak L., Kamińska A., Marek S., Hejmej A., Bilińska B. TESTICULAR EXPRESSION OF NECTIN FOLLOWING SHORT-TERM POSTNATAL EXPOSURE TO FLUTAMIDE IN ADULT RAT

24. Duliban M., Dutka P., Kudrycka M., Gorowska-Wojtowicz E., Milon A., Pawlicki P., Kamińska A., Knapczyk-Stwora K., Hejmej A., Kotula-Balak M. IMPACT OF ESTROGEN-RELATED RECEPTOR (ERR) KNOCK DOWN ON EXPORTIN 5, DICER, DROSHA AND ARGONAUTE 2 EXPRESSION IN BANK VOLE (*MYODES GLAREOLUS*) LEYDIG CELLS *IN VITRO*

25. Fraser L., Mańkowska A., Brym P., Mogielnicka-Brzozowska M. ANALYSIS OF GENE TRANSCRIPT EXPRESSION IN BOAR SPERMATOZOA DIFFERED IN FREEZABILITY

26. Gajda L., Cegła M., Rajska I., Gajda B. EFFECT OF MEDIA ON THE DNA INTEGRITY OF FREEZE-DRIED BOAR SPERMATOZOA: PRELIMINARY STUDY

27. Kotlarczyk A.M., Szczepańska A., Górka P., Przybyło M., Kowalski Z., Korzekwa A. CHARACTERISTICS OF FRESH AND CRYOPRESERVED EPIDIDYMAL SPERMATOZOA OF MUNTJAC (*MUNTJACUS REEVESI*)

28. Kuzborska A., Mogielnicka-Brzozowska M., Zasiadczyk Ł., Fraser L., Kordan W. PROTEOMIC ANALYSIS OF STALLION SPERMATOZOA FOLLOWING LONG-TERM STORAGE IN LIQUID NITROGEN

29. Mańkowska A., Orzołek A., Kordan W. OPTIMIZATION OF THE SELECTED ISOLATION AND IDENTIFICATION PROCEDURES OF THE STALLION EPIDIDYMAL FLUID PHOSPHOPROTEINS

30. Marek S., Kamińska A., Pardyak L., Wróbel K., Kotula-Balak M., Bilinska B., Hejmej A. THE ROLE OF DELTA-LIKE 4 AND JAGGED 1 IN THE REGULATION OF ANDROGEN RECEPTORS EXPRESSION IN MOUSE SERTOLI CELLS

31. Pawlicki P., Miloń A., Duliban M., Kaczmarczyk M., Bilińska B., Rak A., Kotula-Balak M. ESTROGEN REGULATION OF THE INTERSTITIAL TISSUE IN BANK VOLE TESTIS

32. Czaja E., Knapczyk-Stwora K., Koziorowski M., Słomczyńska M. THE IMPACT OF EACs NEONATAL TREATMENT ON ERα AND GPR30 PROTEIN EXPRESSION IN ADULT PIG UTERINE

33. Gorowska-Wojtowicz E., Pawlicki P. Milon A., Kudrycka M., Dutka P., Bilińska B., Hejmej A., Kotula-Balak M. ROLE OF ESTROGEN-RELATED RECEPTORS (ERRs) IN THE MAINTENANCE OF STEROIDOGENIC FUNCTION IN MOUSE ADRENAL CORTEX CELLS

34. Knapczyk-Stwora K., Costa M.C., Grzesiak M., Witek P., Słomczyńska M., Koziorowski M.

35. EFFECT OF NEONATAL ANDROGEN AND ANTI-ANDROGEN EXPOSURE ON THE REGULATION OF PORCINE LUTEAL FUNCTION – INSIGHTS FROM A TRANSCRIPTOMIC APPROACH

36. Kunicka Z., Kurzyńska A., Szydłowska A., Kaczyńska B., Rosińska W., Golubska M., Bogacka I. PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR LIGANDS AFFECT THE EXPRESSION OF IL-1B AND IL-6 IN THE PORCINE ENDOMETRIUM ON DAYS 18-20 OF THE ESTROUS CYCLE

37. Kurowska P., Mlyczyńska E., Dupont J., Rak A. VASPIN AS A NEW ADIPOKINE IN THE PORCINE OVARIAN FOLLICLES: EXPRESSION, IT'S REGULATION AND IMPACT ON STEROID SYNTHESIS

38. Młodawska W., Konieczna A., Mrowiec P., Kochan J., Nowak A. ASSESSMENT OF MORPHOLOGY AND MITOCHONDRIAL MEMBRANE POTENTIAL OF SPERMATOZOA FROM THE DIFFERENT REGIONS OF DOMESTIC CAT EPIDIDYMAL DUCT – PRELIMINARY RESULTS

39. Murawski M., Schwarz T., Jamieson M., Bartlewski P.M. ECHOTEXTURAL CHARACTERISTICS OF THE MAMMARY GLAND DURING THE PERIOD ENCOMPASSING A PEAK OF LACTATION IN TWO BREEDS OF SHEEP VARYING IN MILK YIELDS

40. Socha J.K., Wolak D., Saito N., Sechman A., Hrabia A. AQUAPORIN 4 IN THE CHICKEN OVIDUCT DURING PAUSE IN EGG LAYING

41. Szydłowska A., Kurzyńska A., Kunicka Z., Mierzejewski K., Adamowicz J., Bogacka I. THE EFFECT OF PPAR LIGANDS ON THE EXPRESSION OF LIF AND IL-10 IN PORCINE ENDOMETRIUM DURING FOLLICULAR PHASE OF THE ESTROUS CYCLE

42. Tański D. EXPRESSION OF AQP1, 2, 5 AND 7 mRNA IN THEPIG UTERINE EPITHELIAL CELLS DURING FOLLICULAR PHASE

43. Wolak D., Socha J.K., Sechman A., Hrabia A. EXPRESSION AND ACTIVITY OF METALLOPROTEINASE-2 IN THE CHICKEN OVARY FOLLOWING TAMOXIFEN TREATMENT

Otwarcie Szkoły Zimowej TBR
Zakopane, 2019

Szanowny Prezesie Towarzystwa Biologii Rozrodu, Panie Profesorze Andrzeju Sechmanie,
Szanowna Przewodnicząco Oddziału Towarzystwa Biologii Rozrodu w Krakowie, Pani dr hab. Edyto Molik,
Czcigodni Członkowie Komitetu Naukowego i Organizacyjnego tegorocznej Szkoły TBR,
Drodzy i Dostojni Uczestnicy Piątej Zimowej Szkoły TBR w Zakopanem. Dostojni – bowiem „dostojeństwo Wasze wynika z dostojeństwa Waszych Uniwersytetów oraz Instytutów, w których trwa poszukiwanie prawdy" (JP II)

Bardzo serdecznie dziękuję Organizatorom kolejnej Piątej Szkoły TBR za uczynienie mi tego zaszczytu, iż mogę brać udział w otwarciu dzisiejszego naukowego spotkania pod nazwą „Szkoła Zimowa" w Zakopanem. Serce radują wszyscy Państwo – uczestnicy, którzy tak licznie przybyliście tutaj pod Tatry i Giewont z różnych stron Naszego Kraju. Przypisuje mi się udział sprawczy w dziele narodzin tych Konferencji. Nie jest to całkiem zasłużenie, gdyż w roku 2005 bez Pani Profesor Jolanty Polkowskiej i Pana Profesora Adama Zięcika, pierwszego Prezesa Towarzystwa, a później następnych Prezesów Zarządu Głównego, tj. Panów Profesorów: Dariusza Skarżyńskiego, Jana Kotwicy, a także obecnego Prezesa – profesora Andrzeja Sechmana te kolejne Szkoły nie zaistniałyby w działalności Krakowskiego Oddziału TBR. Nie jest to więc tylko mój udział, ale jak przy każdych narodzinach – o czym wszyscy zapewne tutaj są przekonani – musiała być płeć żeńska i Wspomożyciele. A poza tym, 15 lat temu byliśmy młodsi i w pełni wydolni.

Dziś wzruszony stoję przed Państwem i z zakamarków pamięci, zapominając o tym wszystkim co było niedobre, wydobywam budujące fakty i radość ze spełniającej się wizji organizowania co trzy lata „platformy", dostępnej szczególnie dla Młodych, którzy podejmują wysiłek twórczego zaangażowania się w dziedzinę nauki związanej z rozrodem – na poziomie badań podstawowych i utylitarnych. „Platformy" Młodych (cieszcie się Państwo, że tak pięknie możemy Was nazywać), ale zawsze z zaproszeniem ich liderów oraz wychowawców, troszczących się o rozwój i awanse naukowe tych pierwszych.

Czcigodni zebrani, doskonale wiecie, że stabilność, rozwój, szacunek i godność każdej naszej Rodziny zależą od tej osoby, która sprawuje, organizuje, „zarządza" oraz otacza opieką taką rodzinę. Wiemy też, jak trudne jest życie, gdy tej ważnej Osoby zabraknie bądź Jej nie ma. Obserwuję bowiem od wielu lat, iż w naszej tak licznej Szkolnej Rodzinie TBR też to wszystko się sprawdza. Od roku

2007, tj. od dwunastu lat taką osobą jest Pani dr hab. Edyta Molik. Najpierw jako magister, następnie doktor, doktor habilitowany i mam nadzieję, że tak będzie, gdy zostanie dopisane profesor. Brak mi dziś słów, abym mógł wypowiedzieć w tych podziękowaniach to wszystko co czuję, do czego jestem zobowiązany i co się Pani należy. Za te wprost niewiarygodne dokonania Pani Edyto! Jestem przekonany, że Ci co byli, Ci obecni i wszyscy przyszli to docenili, doceniają i będą doceniać – i będą wdzięczni. Dziękuję Pani Edyto najserdeczniej jeszcze raz. Zasługuje Pani na najwyższe uznanie i uszanowanie złożone tutaj publicznie w obecności Członków Towarzystwa Biologii Rozrodu, Zarządu Głównego i Oddziałów – uczestników tej kolejnej Ogólnopolskiej Konferencji. Niech Bóg wynagrodzi.

Nie do pomyślenia, aby nie zostały wymienione także pozostałe osoby, które włożyły wiele bezinteresownego wysiłku, zaangażowania i własnych inicjatyw w organizację wszystkich pięciu szkół. Rok 2007 – dr inż. Edyta Molik, dr hab. Dorota Zięba, dr hab. Andrzej Sechman. Rok 2010 – dr hab. Andrzej Sechman, prof. UR, dr hab. Danuta Wrońska-Fortuna, dr inż. Anna Hrabia, dr hab. Dorota Zięba, prof. UR, dr inż. Edyta Molik. Rok 2013 – prof. dr hab. Mirosława-Sokołowska Mikołajczyk, dr hab. Anna Hrabia. Rok 2016 – dr hab. Edyta Molik, dr hab. Anna Ptak, dr hab. Danuta Wrońska, dr Maria Mika. Rok 2019 – prof. dr hab. Barbara Gajda, dr hab. Katarzyna Knapczyk-Stwora, dr hab. Wiesława Młodawska, dr hab. Edyta Molik, dr hab. Anna Ptak, dr hab. Danuta Wrońska. Dla wszystkich tych osób Szkoły te były zaszczytną pracą na rzecz wspólnoty, wyzwaniem i misją! Dziękuję wymienionym i wierzę, że Dobry Stwórca też będzie im wszystkim to pamiętał.

Bardzo dziękuję Państwu, że zechcieliście wysłuchać tych moich emocjonalnych wynurzeń w tak ważnych dla mnie dniach jubileuszów. Dziękuję serdecznie organizatorom oraz uczestnikom V Zimowej Szkoły TBR w Zakopanem, że mogę spędzać razem z Państwem tych kilka dni. Radość młodości oraz przyjaźni, przeżywanie wspomnień, refleksje, chwile uniesień, zaduma – łagodzą świadomość przemijania. Wiek senioralny musi też mieć swoją godność bez względu na to, co narzuca trudna do okłamania biologia. Zaś zamknięta w czasie rzymska sentencja: *NIL EST BONAMENTE MELIUS (Nic nie jest lepsze w człowieku od jego rozumu)* – jest szczególna dla opisania tego wieku.

Życzę podobnych radości Wam Młodym. Narodzin dla nauki, dla przyszłych osiągnięć, dla prawdy. Starajcie się stawać Dostojnymi.

Niech Najwyższy darzy siłą i dobrymi myślami.
Nolite Timere! – Nie lękajcie się!

prof. dr hab. Edward Wierzchoś

Plenary lecture

HOW OLD IS TOO OLD – ANDROPAUSE IN ANIMALS AS IN HUMAN?

Wojciech Niżański

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There are scarce data in the literature on the reproductive performance in aged male animals. This topic was recently far better elaborated in human medicine. This is due to the obvious fact that men are becoming fathers later and later in life. Considering the fact that breeders of some species of animals register progeny born from old males with no age limits and bearing in mind demands of maximal reproductive use of potential of genetically valuable stud, the problem of aging in animal males is wortrhy of intensive clinical studies. It seems that especially males of companion animals, commonly used for breeding at advanced age may be considered as fairly suitable biomedical model in studies on human andropause. On the other hand all the relevant informations on the field of medicine are of value as the basis for considerations and inspiration for the studies performed on the field of veterinary. The question if results obtained in human medicine may be extrapolated on animal remains unsolved unless more detailed studies will be performed on this field. However from this point of view short review on data collected in human andrology may indicate the possible directions for further research in veterinary.

Recently a great debate is conducted on "Advanced Paternal Age" where men's fertility declines and chances for passing birth defects rise. In 1993, fathers aged 35–54 years accounted for 25% of live births within marriage, while ten years later this percentage was 40%. In the past the only concern of the research was whether or not sperm cells in older men was healthy enough to lead to conception. The research on Advanced Paternal Age was focused on "Time To Pregnant" (TTP), which is the time period it takes the sperm to result in pregnancy. Today, the focus has shifted from TTP to the relationship between paternal age and birth defects. The risk of birth defects related to Advanced Paternal Age is proven to be much greater than previously expected.

Aging in human and andropause. Endocrine function in human declines gradually with age and at age 75 years, mean plasma testosterone levels are only 65% of levels in young adults. Aging is accompanied by a series of signs and symptoms, many of which are rather similar to those observed in young hypogonadal males (14,15). Low circulating testosterone is correlated with low muscle strength, with high adiposity, with insulin resistance and with poor cognitive performance. It

was reported higher incidence of erectile dysfunction in aging men (1). Increased male age affects testicular function, reproductive hormone profiles, semen characteristics, sperm DNA integrity, de novo mutation rate, telomere length, chromosomal structure resulting in aneuploidies, genomic stability and epigenetic factors (15). These changes negatively affect fertility and reproductive outcomes, ART success, increase the risk of preterm parturition and spontaneous abortions in older couples (1,7). These are also factors contributing to higher incidences of congenital birth defects and fetal deaths. Increasing male age has been shown to be associated with numerous disorders like achondroplasia, autism, schizophrenia and bipolar disorders and other diseases in children of aged parents (14) At the age of 20, a sperm would have undergone 150 chromosomal replications, and at the age of 50, it would have gone through 840 replications. This increases the probability of replication errors in the germ line leading to the accumulation of mutations and hence increased de novo mutation rate in spermatozoa, which may increase the risk of embryonic and fetal malformations and spontaneous abortion. Epigenetic factors of aging are also warmly disussed. It has been reported that the age, father's nutrition and his exposure to toxicants is so strong that not only affects his offspring's epigenetic factors but also his grand-offspring epigenetics factors as well. It has been found that DNA methylation plays an important role in mammalian development and influences different processes like X-inactivation, genomic imprinting and embryo development as soon as the zygote is formed.

Dogs, which seems suitable biomedical animal model, are kept in human environment and it may be expected that environmental factors declining fertility of aged men may also negatively influence fertility of dogs. This was confirmed by study Lea et al. (8). It demonstrated rapid decline in male dog fertility, with potential link to environmental contaminants over the past three decades. Environmental contaminants found in commercially available pet foods were also detected in the sperm and testes of adult dogs causing a detrimental effects on sperm function. Over the 26 years of the study, authors found a decrease in the percentage of normal motile sperm. Between 1988 and 1998, sperm motility declined by 2.5 per cent per year. Then from 2002 to 2014 sperm motility continued to decline at a rate of 1.2% per year. In addition, that the male pups had an increased incidence of cryptorchidism.

General reproductive problems in aged dogs. Aged dogs may fail to achieve normal copulation due to lack of libido or a decrease in blood testosterone level. The quality of semen is often reduced in older males, which decreases the fertility rate. (6). Ssexual abstinence may cause a decrease in semen quality (5). Hypopituitarism, adrenal dysfunction, hypothalamic or pituitary tumors and prolactin adenomas may lead to infertility and azoospermia (4).

Changes of semen quality in normal aged dogs. It is generally assumed that age influences negatively the semen quality in dogs. Decrease in semen quality is different in normal healthy dogs which undergo senile changes and in dogs preseting differet forms of fertility disorders specific for older males (12). A correlation was confirmed between the age of the dog and the volume of the ejaculate

(3). **Motility.** Most studies performed on normal dogs did not prove aged-related changes in spermatozoal motility (12,13). Rota et al (13) did not found any differences of subjective motility between dogs 2–7 and >7 years old. Rijsselaere et al (12) used CASA system and similarly did not found any differences of motility characteristics between young and older dogs. However Hesser et al. (5) found that CASA progressive motility was lower in the senior male (>7 y) chilled samples compared to all other younger groups.

Morphology. The relationship between sperm morphology and fertilizing capacity of sperm cells is obvious (10,11). It was proved in dogs that less than 60% of morphologically normal sperm cells results in significantly poorer results of artificial insemination (11). There are some evidences that in older dogs the percentage of sperm cells presenting normal morphology gradually decreases (13). Rijseelaerre et al (12) revealed that the age was negatively correlated with the percentage of normal spermatozoa. Henrikse and Antonisse (cit. 12) revealed, that percentage of spermatozoa with normal morphology and Total Sperm Count were significantly affected by age. Regarding the type of predominant morphological abnormalities in older dogs it was revealed that more spermatozoa in aged dogs present defects of miedpiece and high percentage of sperm with cytoplasmatic droplets, which may express the degeneration of testicular and epididymal tissue. It was proved in *in vitro* functional tests, that presence of cytoplasmtic droplets in aged dogs negatively influences the affinity of sperm cells to bind zona pellucida (13). Taking into account, that quality of ejaculated spermatozoa is partly influenced by the effect of prostatic fluid on germ cells, Bhanmeechao et al. (2) checked the characteristics of epididymal sperm in four age groups of dogs: young (1–3 y), adult (3–6 y), old (6–9 y) and senile (>9 y). Similarly to ejaculated sperm, older dogs had higher percentages of spermatozoa having primary or secondary morphologically, defects compared to young and adult dogs. Positive correlations between age and sperm defects were observed for primary and secondary defects. **Total sperm count.** The data regarding number of sperm cells produced by aging dogs are not consistent. Rota et al (13) did not found decrease in sperm production in older dogs whereas Henrikse and Antonisse (cit. 12) found here negative relationship. According to some practical reviews and author's experience, older dogs tend to present symptoms resembling oligo-astheno-teratozospermia OAT (4).

DNA stability and mtDNA. Hesser and al (5) proved that for sperm chromatin structure of ejaculated sperm cells, the senior-aged group (>7 y) had a higher % COMPat than the middle-aged group. The SCSA data indicate that the older males had higher levels of cells outside of the normal population than the middle male group, with the young group being equal to both. SCSA results revealed a relatively narrow range of 0.5% to 4.5% COMPat. Sperm with abnormal DNA can fertilize initially but ultimately result in embryonic loss. This is in agreement with data confirmed by Nizanski et al. (10) who found that in subfertile dogs DFI is significantly increased beyond 5% DFI in comparison to normal, fertile dogs. Different results were obtained for epididymal sperm (2) and their DNA structure in

aged dogs is unchanged when compared to younger males. Mitochondrial DNA(mtDNA) was extracted in dogs and copy number was determined as an indicator of spermatogenic efficiency in ejaculated sperm. Its evaluation reported an average of 92 copies of mtDNA per sperm in both fertile and reproductively impaired males. Studs with impaired reproductive performance tended to have a higher copy number than the normal sample of studs, although not significantly. **Fertility of aged dogs.** It was proved that subfertile dogs produce sperm cells of poorer morphology, which may be associated with disruption of DNA structure, loos of intactness of membranes and decrease of mitochondrial protential (10,11). The poor semen quality in older dogs may influence negatively structural and functional properties of sperm cells and may negatively influence results of natural and artificial insemination. Rijselaere et al (12) reported that almost all of the sperm characteristics were significantly lower for dogs with poorer in vivo fertility results. Age-related changes in fertility were postulated for dogs, yielding lower conception rates and smaller litters after 7–8 years of age (6).

Freezability of semen in aged dogs and its usefulness for ART. The poorer quality of ejaculates of aged dogs may not only negatively influence fertility, but also may dimish their usefulness for ART. The ejaculate of poor motility, containing low number of sperm cells is difficult to preserve. The obvious fact, that cryopreservation is associated with loss of half population of viable/motile sperm cells, means that in many cases of older dogs only few frozen–thaw spermatozoa maintain normal fertilizg capacity post-thaw. The results of cryopreservation in such cases seems to be questionable. Also the presence of high number of sperm cells presenting morphological defects in fresh semen results in very poor freezability and very low number of motile spermatozoa possessing fertilizing ability after thawing. It was proved years ago that the presence of cytoplasmatic droplets, specific for older dogs (see above), results in very poor semen freezability, probably due to the fact of damagae of droplets at preservation and release of hydrolytic enzymes (9). On the basis on long term practical work of author on dog semen banking it was commonly seen that in older dogs >8 years of age freezability decreases, independently on the quality of fresh semen. In some cases, sperm cells in fresh semen of appartently perfect quality prefreeze are hard to successfully freeze and thaw. It may be due to latent changes of organelle, lack of stability of membranes and metabolic processes, which may result in susceptibility of cells to cold shock and cryodamage in aged dogs. In such cases it is not easy to recognize and predefine freezability of ejaculates in individuals. One of the fairy good indicator of freeezability may be prefreeze level of intracellular enzymes leakage from cells, morphology of sperm cells and percentage of cells of RAPID velocity detected on CASA (5).

To conclude the aging in human is detaily elaborated and many facts are known about the influence of the age on fertility and probability of the presence of abnormal pregnancy and congenital disorders in children of aged fathers. In model animals as dogs only scarce data were published on the relationship between the age of male and sperm quality and fertility. There are no data on the relationship between

the age of dogs and probability of pathology of pregnancy and the risk of congenital abnormalities in puppies of aged males. This is not explored field of veterinary medicine. The multicentric wide studies seems to be necessary on this topic. On the basis of such studies the procedures helpful to obtain healthy progeny may be elaborated like in human. On the basis of such studies more comprehensive methods of overcoming or retardation of age-dependent infertility/subfertility may be proposed in dogs.

References

(1) Belloc S., Hazout A., Zini A., Merviel P., Cabry R., Chahine H., Copin H., Benkhalifa M. How to overcome male infertility after 40: Influence of paternal age on fertility. Maturitas 2014; 78: 22–29.

(2) Bhanmeechao C., Srisuwatanasagul S., Prapaiwan N., Ponglowhapan S. Reproductive aging in male dogs: The epididymal sperm defects and expression of androgen receptor in reproductive tissues. Theriogenology 2018; 108: 74–80.

(3) Filipčik M., Vagenknechtova M., Hošek M., Jarinkovičova L. The effect of the age of dogs on their ejaculate. Acta Univ. Agric. Silvic. Mendel Brun. 2011; 59: 45–50.

(4) Fontbonne A. Infertility in male dogs: recent advances. Rev. Bras. Reprod. Anim., Belo Horizonte, 2011; 35: 266–273.

(5) Hesser A., Darr C., Gonzales K., Power H., Scanlan T., Thompson J., Love Ch., Christensen B., Meyers S. Semen evaluation and fertility assessment in a purebred dog breeding facility. Theriogenology 2017; 87: 115–123.

(6) Johnston S.D., Root Kustritz M., Olson P.N.S. Canine and Feline Theriogenology. WB Saunders Comp. Philadephia 2001.

(7) Kovac J.R., Addai J., Smith R.P., Coward R.M., Lamb D.J., Lipshultz L.I. The effects of advanced paternal age on fertility. Asian J. Androl. 2013; 15: 723–728.

(8) Lea R.G., Byers A.S., Sumner R.N., Rhind S.M., Zhang Z., Freeman S.L., Moxon R., Richardson H.M., Green M., Craigon J. & England G.C.M. Environmental chemicals impact dog semen quality *in vitro* and may be associated with a temporal decline in sperm motility and increased cryptorchidism. Sci. Rep. 2016; 6: 31281.

(9) Morton D.B., Bruce S.G. Semen evaluation, cryopreservation and factors relevant to the use of frozen semen in dogs. J. Reprod. Fertil. Suppl. 1989; 39: 311–316.

(10) Niżański W., Partyka A., Ochota M., Antończyk A., Mikołajewska N., Błasiak K., Mila H., Stańczyk E. Flow cytometric, computer assisted and traditional sperm analysis in fertile and subfertile dogs. Proc. 14th EVSSAR Congress “Advances in Feline Reproduction”, Milano, 11th March 2011, 52.

(11) Oettlé E.E. Sperm morphology and fertility in the dog. J. Reprod. Fertil. Suppl. 1993; 47: 257–260.
(12) Rijsselaere T., Maes D., Hoflack G., Kruif A. de,Van Soom A. Effect of body weight, age and breeding history on canine sperm quality parameters measured by the Hamilton-Thorne Analyser. Reprod. Dom. Anim. 2007; 42: 143–148.
(13) Rota A., Tesi M., Di Petta G., Sabatini C., Vannozzi I. A retrospective study on the relationships between semen quality, dogs' ageing and fertility. Proc. ISCFR VIII-EVSSAR XIX International Symposium on Canine and Feline Reproduction, Paris, June 22–25, 2016; 81.
(14) Sharma R., Agarwal A., Rohra V.K., Assidi M., Abu-Elmagd M., Turki R.F. Effects of increased paternal age on sperm quality, reproductive outcome and associated epigenetic risks to offspring Rep. Biol. Endocrin. 2015; 13: 35.
(15) Vermeulen A. Andropause. Maturitas 2000; 34: 5–15.

Session 1

FROM OOCYTE TO EMBRYO – BIOTECHNOLOGY IN ANIMAL REPRODUCTION

Prof. Dr. hab. Jan Twardoń
Dr. Magdalena Kowalik

Session 1

REPRODUCTIVE PERFORMANCE OF SOWS AFTER POST CERVICAL INSEMINATION WITH LIQUID SEMEN

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Introduction: The traditional method of artificial insemination of sows consists on depositing of boar semen directly into the uterine cervix of the female reproductive tract, using a special catheter for cervical insemination. The aim of the study was to assess the effect of intrauterine or post cervical insemination on the reproductive performance of sows.

Material and methods: The research was carried out on two large-scale farms, using 2122 multiparous crossbreeds PLW and PL sows. In the control groups, all females were inseminated with a routine intra cervical method (the volume and sperm concentration of the insemination dose, was: 80 ml and 3×10^9, respectively) In the experimental groups intrauterine insemination was performed using a special post cervical catheter (the volume and sperm concentration of insemination dose, was: 40 ml and 1×10^9, respectively). Inseminations were performed in the presence of the boar in individual pens. Pregnant females were moved to the pens where they remained until the prenatal period. A few days before the parturition, the females were moved to the farrowing pens. In the control and experimental groups of the sows the reproductive performance include farrowing rate, number of piglets born alive and stillborn. Data obtained in the breeding and farrowing pens, were analyzed for calculation of the sows' reproductive rates. The obtained data were subjected to statistical analysis using the Statistica PL package.

Results: There was significant difference between farrowing rate in the control and the experimental groups of sows. Significantly higher farrowing rate was found in the sows inseminated by the post-cervical method (93.9 vs. 88.8%). Others reproductive rates did not differ significantly.

Discussion: The obtained results of our research confirmed the hypothesis that artificial insemination with the help of special post cervical insemination catheters not only did not reduce the effectiveness of fertility rates of sows, but allowed to obtain a significantly higher percentage of farrowing rate. Summing up, the values of the obtained reproductive performance rates of sows inseminated with the catheters for intra uterine insemination are within the generally accepted norms for this species, which may result in higher economic income from pig farms. This study was supported by statutory research and development activity funds assigned to Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences.

Session 1

EFFECTIVENESS OF TRANSFECTION WITH NANOPARTICLES OF RABBIT ZYGOTES-PRELIMINARY RESULTS

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Transgenic animals have become the part of various biological research. From the literature it is known that nanoparticles have been successfully used for transfection of *in vitro* cell cultures. Nevertheless, there is little current information about using them in transgenesis.

To evaluate the possibility to transfect embryos with nanoparticles as gene carriers, two separate experiments were conducted with the use of spherical gold nanoparticles, sized 10 nm, with the surface modification of branched polyethylenimine (Au/bPEI) and gene construct pmaxFP-Red-n: an eukaryotic vector carrying the pmaxFP-Red gene coding expression of the red fluorescent protein. The aim of the first experiment was to assess the influence of Au/bPEI nanoparticles on rabbit embryos development. 392 zygotes obtained *in vivo* were subjected to microinjection into the perivitelline space. The concentrations of 5, 25, 50 and 100 ng/µl respectively were used for injection. Afterwards, the zygotes were cultured *in vitro* for 6 days. The toxic effect of the Au/bPEI nanoparticles was observed at the concentration of 100 ng/µl – only 23.3% of the embryos after microinjection reached blastocyst stage. The highest rate of hatching blastocysts was in the group injected with 5 ng/µl – 76.2%. The second experiment was to establish the most effective affinity ratio of DNA:nanoparticles complexes and to judge the possibility and the efficiency of transfecting embryos this way. To find the best affinity ratio, pmaxFP-Red-N vector and Au/bPEI nanoparticles were mixed in different proportions and examined by agarose gel electrophoresis. The ratio 1:2 (DNA 25: Au-PEI 50 ng/µl) was found to be the most effective in complex formation. Consequently, 298 rabbit zygotes were microinjected. Further, the embryos were *in vitro* cultured for 6 days. 53.8% of the transfected embryos were able to reach blastocyst stage, from which 19.4% showed expression of the FP-red.

Conclusions: the toxic effect of the Au/bPEI was at the concentration of 100 ng/µl. Complexes DNA: Au/bPEI mixed in the ratio 1:2 (25:50 ng/µl) effectively transfect rabbit embryos.

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Session 1

ANALYSIS OF MORPHOKINETIC OF OVINE EMBRYOS USING A TIME LAPSE SYSTEM – PRELIMINARY RESEARCH

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Objective: Using a non-invasive methods to select the most competent embryos is essential for *in vitro* fertilization procedure (IVF). Novel method of embryo assessment use the time-lapse imaging system to predicting *in vitro* fertilization outcome. Knowledge about timing of first cleaving of sheep embryos allows to create new criteria of embryos selection for embryo transfer in the small ruminant industry.

The aim of the study was to compare developmental potential of embryos, based on the timing of the first cleavage.

Materials and methods: Oocytes were collected from ovaries of slaughtered ewes. Oocytes were *in vitro* matured (IVM) using TCM 199 Earle's salts, HEPES-buffered supplemented with 10% FBS, 0.1 µl/ml FSH, 0.5 µl/ml LH by 24 h in condition of 38,5ºC and in air of 5% CO_2. Matured oocytes were fertilized by fresh ram semen capacitated in Sperm Air medium (Gynamed). Embryos after 24 h post inseminations were placed into special Primo Vision's dish and *in vitro* cultured in Cult medium (Gynamed). Embryo development was recorded every 10 min by using a time-lapse imaging system.

Results: From all collected and fertilized oocytes (596), 79 were selected to time-lapse observations, from which 69 (87.3%) were cleavage, 24 (34.78%) developed to morula stage, 8 (11.59%) developed to blastocyst stage and 4 (5.8%) were hatched. The earliest cleavages of embryos were observed at 19 h after IVF and the latest at 29 h 45'. The blastocyst cavity was formed between 115 h 44' and 146 h 25' after IVF.

Conclusions: Time-lapse system gives a possibility to follow up step by step view of embryo development for improving accuracy of morphology estimation of its development, while minimizing stress and disturbance to its developing *in vitro*. Morphokinetic evaluation of early sheep embryos development give better possibility for their objective selection in comparison to tradition methods. These preliminary results indicate the need to continue the research undertaken.

Session 1

IN VITRO FERTILIZATION AND SUBSEQUENT DEVELOPMENT OF VITRIFIED PORCINE OOCYTES MATURED WITH THYMOSIN

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Thymosin (TH) is a biologically active polypeptide released by thym. It protects cells from damage and blocks apoptosis. In our recent study we demonstrated the positive effect of supplementation of the maturation medium for oocytes with TH on the survival of vitrified porcine oocytes. The objective of the present study was to determine the ability for *in vitro* fertilization (IVF) of vitrified porcine oocytes matured with TH. Cumulus-oocyte complexes obtained from ovaries of slaughtered gilts were cultured in a modified TCM-199 medium supplemented with 0.5 mg/ml of synthetic TH (exp. group) or without TH (control group) for 42 h at 39ºC and 5% CO_2. After IVM, the oocytes from the exp. and control groups were vitrified using the OPS method described by Gajda et al. (*CryoLett*, 2015). Oocytes were equilibrated in 10% dimethyl sulfoxide (DMSO) and 10% ethylene glycol (EG) for 3 min. Oocytes with a minimum volume of vitrification solution (15% DMSO, 15% EG and 0.5 M sucrose) were loaded into the OPS straw. After 1 min, the straw was plunged into liquid nitrogen (LN_2). For warming, the OPS was quickly removed from LN_2, immersed in TCM-199 and sucrose for 5 min and washed in TCM-199. Oocytes of good quality were IVF. Zygotes were cultured in the NCSU-23 medium at 39ºC, in 5% CO_2 and 5% O_2, up to the blastocyst stage. The percentage of potential cleaved zygotes, morulae and blastocysts was evaluated. The blastocysts from the exp. (n=2) and control (n=7) groups were subjected to TUNEL assay. Statistical analysis was performed using the t-test and chi-square test. The survival rate of vitrified porcine oocytes matured in a medium supplemented with TH (exp. group) were higher (80%; $P<0.05$) than those of oocytes matured in a medium without TH (control group; 65%). After IVF in the exp. group the cleavage rate was 50.0% while in the control group it was 36.6%. However, no significant difference was observed in blastocyst rates between the groups (40.0 and 46.7%, respectively). It was observed that the mean number of cell nuclei in vitrified blastocysts did not differ between the exp. and control groups. On the other hand, in the exp. group the mean number of apoptotic nuclei (0.2) and

TUNEL index (0.4) was slightly lower than in the control group (0.7 and 1.6, respectively).

In conclusion, our experiments suggest that the presence of Thymosin during IVM improves cleavage rates and embryo quality of vitrified IVF embryos.

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Session 1

THE INFLUENCE OF GENOTOXIC STRESS ON THE INCREASE OF mtDNA COPY NUMBER

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Mitochondria are the most prominent organelles in oocytes. Mitochondrial function and dysfunction has been the subject of various studies in ovarian ageing and metabolic stress models. Mitochondria are inherited strictly from the mother and their poor quality may influence the women fertility. However, the overall mitochondrial impact on female fertility is yet to be elucidated. It was suggested that the oocytes with higher mtDNA copy number have higher meiotic and developmental competence. We observed that oocytes cultured *in vitro* and exposed to the oxidative/genotoxic stress, introduced with etoposide and tert-Butyl hydroperoxide (t-BHP) display elevated mtDNA copy numbers (278413 ± 47175 SD for control, 358028 ± 51455 SD for 500 nM t-BHP and 409265 ± 243905 SD for 100 µg/ml of etoposide), which was coincident with increased mitochondrial mass and elevated level of reactive oxygen species. Moreover, during the time when the mitochondrial copy number increased, we observed the mtDNA breaks repairs (until the 6 h after the stress removal). Nevertheless, the effectiveness of meiotic maturation of the oocytes upon etoposide/t-BHP treatment was severely compromised. We conclude that the oocytes may possess a mechanisms which compensates the loss of good quality mtDNA through both mtDNA replication and mtDNA repairs. This mechanism, however, may not fully restore the oocyte quality. Our results supports the recent suggestions that elevated number of mitochondria may serve as a predictor of the poor oocyte quality.

Session 1

EFFECT OF SOYBEAN LECITHIN ON THE POST THAW QUALITY AND FERTILITY OF RAM SEMEN

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The aim of the study was to investigate the effect of soybean lecithin as a substitute for egg yolk in milk and tris based extenders in ram semen cryopreservation. Twenty ejaculates were collected from four healthy, mature Wrzosówka rams (2–3 years of age). Each ejaculate was divided into four equal aliquots and diluted with four different extenders: 1) milk extender containing 5% egg yolk, 2) milk extender containing 1.5% soybean lecithin, 3) tris extender containing 20% egg yolk, 4) tris extender containing 1.5% soybean lecithin. Extended semen was loaded into 0.25 ml French straws, cooled and frozen in liquid nitrogen vapor. Total motility, curvilinear velocity, plasma membrane integrity and fertilizing ability of sperm were assessed after thawing. Total motility was lower ($P<0.05$) in tris-soybean lecithin extender when compared to other extenders. Curvilinear velocity was higher ($P<0.05$) for spermatozoa cryopreserved in milk-soybean lecithin extender compared to other extenders tested. For the percentage of live sperm no significant difference was observed between extenders. Twenty eight Wrzosówka ewes of 45–50 kg weight and 2–3 years old were used to determine the effect of extender on fertilizing ability of sperm. Semen was used frozen in two extenders ensuring the highest sperm quality after thawing: milk-soybean lecithin and tris-egg yolk. The lambing rate was higher (not statistically significant) in ewes inseminated with semen frozen in milk-soybean lecithin extender (42.9%) than in the tris-egg yolk extender (28.6%). In conclusion, milk-soybean lecithin extender can be an efficient alternative extender to cryopreservation of ram semen.

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Session 1

IN VITRO MATURATION OF PORCINE OOCYTES USING NOVEL TECHNIQUE OF LIQUID MARBLE BIOREACTORS

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The process of oocyte maturation takes place inside the ovarian follicles where it occupies central location and is surrounded by a few layers of granulosa cells named cumulus cells (CCs). This structure is called the cumulus-oocyte complex (COC). Cumulus cells play an important role during oocyte development, its competence acquisition, ovulation and fertilization. The communication within the COCs is bidirectional and coordinates functions of both cell types. CCs send information through the zona pellucida by gap junctions formed with the oocyte cell membrane. The maintenance of functional gap junctions is crucial for proper oocyte development during *in vitro* maturation. Traditionally COCs are cultured in cell culture medium's drop under a mineral oil (2D system). This method is used for short-time culture of COCs.

The aim of this work was to establish effective methods of COCs long-term culture in a 3D-system.

Two different types of 3D culture system were used: a novel technique in liquid marble bioreactor (LM) and alginate hydrogel capsule (AC). Porcine ovaries were excised from prepubertal gilts at a local slaughterhouse. Only healthy, medium-sized follicles (4–6 mm) were selected for COCs isolation under a stereoscopic microscope. COCs of grade I, possessing homogeneous ooplasm and uniform, compact CCs were considered for further 3D *in vitro* maturation procedure. In the 2D model of culture, after isolation and washing, COCs were transferred into the medium drop (TCM199, 50% follicular fluid) under mineral oil. In the 3D culture model COCs with a drop of medium were encapsulated with hydrophobic polytetrafluoroethylene powder particles, to form microbioreactors, defined as "Liquid Marbles". In the other 3D culture system tested, COCs were encapsulated in 5 μl alginate beads and then placed in 96-well plates. COCs matured for 96 hours. Every 24 hours ½ medium was changed. The degree of cumulus cell expansion and nucleus maturation rate were estimated under a light microscope. Only few oocytes maturing in the 2D model showed cumulus cell expansion, whereas in those maturing in both 3D culture models this process was much more advanced. The degree of maturation of cultured oocytes as measured based on the presence of the 1st polar body did not differ significantly between LM and AC. The polar body was not observed in the 2D-culture.

In conclusion, the 3D system can provide appropriate conditions for oocyte maturation in long-term in vitro culture.

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Session 1

SUCCESSFUL DIFFERENTIATION OF EQUINE MESENCHYMAL STEM CELLS (MSCs) INTO OSTEOBLASTS, CHONDROCYTES AND ADIPOCYTES DERIVED FROM THE BONE MARROW COLLECTED POST SLAUGHTER

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The aim of the study was to prove that the MSCs isolated from bone marrow collected post-slaughter from horses have the potential to differentiate *in vitro* into osteoblasts, chondrocytes and adipocytes. So far articles report derivation of MSC from the living animals. Methodology: The bone marrow (BM) was collected from slaughtered horses (1–3 years old) from sternal puncture. The volume of collected BM was 2–5 ml. The BM samples were collected into sterile flasks containing 1ml of heparin, stored at room temperature, and processed within 1 to 2 h after collection. MSCs were mixed with PBS (1:1) and isolated from BM by density gradient centrifugation at 400×g for 40 min at room temperature using Ficoll-Paque PREMIUM (GE Healthcare Bio-Sciences) medium. Primary cell lines were derived by *in vitro* culture in T75 flasks in low glucose-Dulbecco's modified Eagle Medium containing 10% FCS. After 1 passage horse MSCs were differentiated into adipocytes, chondrocytes and osteoblasts for 23–30 days of *in vitro* culture according to the protocols provided in Branly et al. (2017). Simultaneously the MSC were *in vitro* cultured in standard medium without differentiating factors, serving as control.

The cells subjected to differentiation and control cells were fixed in 1% PFA and stained to prove their differentiation: towards osteoblasts by von Kossa staining and/or Alizarin Red staining; towards chondrocytes by Alcian Blue staining and towards adipocytes by Oil Red O staining. Results: The positive staining was observed in all differentiated groups into adipocytes, chondrocytes and osteoblasts. The negative staining was observed in control cells.

Conclusion: The bone marrow from slaughtered horses can be used to isolate the MSCs which are morphologically and functionally correct. This way we proved that MSCs collected post mortem can be used in assisted reproductive technologies as well.

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Session 1

THE ABILITY OF EJACULATED, EPIDIDIMAL OR WITHOUT PLASMA BOAR SEMEN FOR *IN VITRO* FERTILIZATION

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Various aspects influence the ability of boar semen to fertilize. Among them is the type of semen (ejaculated, epididymal and without plasma). Seminal plasma contains various protein elements that stabilize spermatozoa and at the same time are a decapacitating factor (Kawano et al., 2010; Sancho and Vilagran, 2013). The aim of the study was to evaluate the effect of ejaculated and epididymal semen as well as semen without plasma on the ability to fertilize *in vitro* and the subsequent embryo development. Semen was obtained from 4 boars of different breeds at different ages (9 months to 3,5 years). Fresh semen after ejaculation, the epididymal semen obtained after slaughter and semen without plasma were diluted and assessed under a microscope. To remove the plasma from fresh semen ejaculates were centrifuged. Semen with 50% of motility underwent capacitation in a medium based on TCM-199 for 1 hour. Oocytes matured *in vitro* were used for *in vitro* fertilization. After IVF the zygotes were cultured in the NCSU-23 medium in an atmosphere of 5% CO_2 and 5% O_2 in humidified air up to the blastocyst stage. Statistical analysis was performed using the Chi^2 test. The results have been shown in Table 1.

Tabela 1. Comparison of fertilization effectiveness of ejaculated and epididymal boar semen and semen without plasma

Semen	Number of boars	Number of oocytes/ replication	Number of cleaved (%)	Number of morulas* (%)	Number of blastocysts* (%)
Ejaculated	2	503/16	270 (53.7)	162 (60.0)[a]	70 (25.9)[c]
Epididymal	2	107/4	59 (55.1)	33 (55.9)	18 (30.5)[e]
Without plasma	2	87/4	45 (51.7)	17 (37.8)[b]	2 (4.4)[d]

a,b; c,d; e,d – P<0,01 *****in relation to cleaved.

In conclusion, ejaculated and epididymal boar semen have a similar ability for *in vitro* fertilization. There were no differences in the percentage of cleaved, morula and blastocyst embryos obtained after *in vitro* fertilization with ejaculated and epididymal semen. Moreover the removal of seminal plasma from boar semen had a negative effect on its *in vitro* fertilization ability and embryo development.

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Session 1

THE EFFECT OF REDUCED OXYGEN TENSION ON FELINE OOCYTES MATURATION AND EMBRYO DEVELOPMENT *IN VITRO*

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The aim of this study was to evaluate the effect of reduced oxygen tension on the oocytes and embryo developmental competence in the domestic cat.

Material and methods: In the study we compared the results of oocytes maturation and embryo culture during two consecutive reproductive season (March to June). In the first season oocytes and embryos were cultured in the atmosphere of 5% CO_2 and 5% O_2 (reduced oxygen tension, study group I). In the second season oocytes and embryos were cultured in the atmosphere of 5% CO_2 and 20% O_2 (atmospheric oxygen tension, study group II). For both groups the ovaries were obtained from queens intended for routine castration procedures. The oocytes were collected by the scarification of ovarian cortex in a medium TCM 199 with Hanks salts, HEPES-buffered with 10% FBS and heparin. Oocytes (group I n=279, group II n=239) were transferred to maturation medium (TCM 199 with Earle's salts and HEPES-buffer, supplemented with 0.02 IU FSH/ml and 0.02 IU LH/ml) (1). After 24 hours, oocytes with the polar body, considered mature (group I: n=117, group II: n=147) were fertilized by ICSI with epididymal frozen-thawed semen. The presumptive embryos were cultured in groups in 50 µl droplets of commercial medium (Continuous Single Culture® supplemented with 10% Serum Substitute Supplement®, Irvine Scientific, Ireland) at 38.5°C in the atmosphere of reduced or atmospheric oxygen tension for 7 days. The number of cleaved oocytes, the number of embryos at morula stage and the number of embryos at blastocyst stage were noted 24 hours, 6 days and 7 days after ICSI, respectively. The experiment was performed in 17 replicates. The results were summed up within experimental groups and analyzed statistically with chi-square test. The results were considered significant at $P<0.05$.

Results: No statistically significant differences between experimental groups were observed for maturation rate (Group I mean = 48,7%, group II = 55,9%). In the group I significantly higher number of oocytes cleaved (61,5% vs 40,8%) but significantly less embryos reached blastocyst stage (6,9% vs 36,7%). No differences were noted for morula rate (56,9% vs 60%).

Conclusion: Due to the hampered development from morula to blastocyst stage, lowered oxygen tension is not advisable for in vitro embryo production in cats in commercial media used in this study.

Reference: 1) Waurich et al., Reproduction, 2010; 140: 531–540.

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Session 1

THE EFFECT OF DIFFERENT ANTIOXIDANTS ON THE DEVELOPMENTAL COMPETENCES OF PIG EMBRYOS OBTAINED AFTER *IN VITRO* FERTILIZATION

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The aim of the study was to determine the effect of adding 2 different antioxidants: resveratrol or vitamin C on the development of pig embryos obtained after *in vitro* fertilization (IVF) and *in vitro* culture (IVC).

The putative zygotes of experimental groups were cultured in the NCSU-23 medium with the addition of: a) 5.68 µM or 11.36 µM/mL of vitamin C or b) 0.5 µM/mL of resveratrol, while in the control group zygotes were cultured without antioxidants. Embryos were cultured at 39ºC and 5% CO_2, 5% O_2 and 90% N_2 in the air up to the blastocyst stage. The evaluation of embryo development was carried out every 24 hours under a stereoscopic microscope. Statistical analysis was performed using chi^2 test. It was observed that the cleavage rate was the highest in the group of embryos cultured with 11.36 µM/mL of vitamin C compared with embryos cultured with 5.68 µM/mL of vitamin C, 0.5 µM/mL of resveratrol and the control (25.2%; 19.5%, 17.0% and 23,7% respectively, no statistical differences). The percentage of blastocysts was significantly higher in the group of embryos cultured with 11.36 µM/mL of vitamin C than in the control group (46.0% and 27.5%, respectively $P<0.05$). There were no statistically significant differences in the percentage of blastocysts between the remaining experimental groups and the control.

The study showed that the presence of vitamin C has a positive impact on the development of pig embryos after IVF, while the addition of the investigated concentration of resveratrol does not improve the cleavage and blastocyst rates. However, the effect of resveratrol should be confirmed on a greater number of pig embryos.

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Session 1

QUANTITATIVE IMAGING OF LIPIDS IN OOCYTES OBTAINED FROM DIAPAUSING MAMMALIAN SPECIES USING COHERENT ANTI-STOKES RAMAN SCATTERING (CARS) MICROSCOPY

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Embryonic diapause (ED) is a period of temporary suspension of development at the blastocyst stage which occurs while waiting for the maternal implantation signal. ED characterized by minimal cell division is possible because of greatly reduced metabolism and likely due to the accumulation of energy reserves stored in lipid droplets. This fascinating phenomenon has been observed in over 130 species of mammals, ranging from bears and badgers to mice. The aim of this study was to find out if the quantity of energy reserves in the oocytes is correlated with the duration of embryonic diapause. To do this, we applied a novel microscopic technique, chemically specific, label-free - coherent anti-Stokes Raman scattering (CARS) to estimate the amount of lipid droplets in oocytes of several diapausing mammalian species with previously reported length of ED (including roe deer, mink, least weasel, tundra vole, bank vole, rat and mouse) and then to compare with previously reported length of ED. The CARS imaging enable to assess the number of droplets, their spatial distribution as well as to quantify the total amount of lipids in the cell. We observe a trend that the amount of energy stored in form of lipid droplets is related to the ED duration in given species. Data from our study suggest an important relationship between lipids' content in the oocytes and ED length. Further investigation, including analysis of higher number of diapausing species, will contribute to better understand the role of lipid during ED.

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Session 1

CAN EPIGENOMIC MODIFIER USED FOR *IN VITRO* MATURATION OF NUCLEAR RECIPIENT OOCYTES BE ABLE TO IMPROVE THE COMPETENCES OF SOMATIC CELL NUCLEI TO SUPPORT THE DEVELOPMENTAL POTENTIAL OF PORCINE CLONED EMBRYOS?

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The current study was undertaken to examine whether the epigenetic transformation of *ex vivo* maturing oocytes that was evoked by non-selective inhibitor of histone deacetylases (HDACs), termed as scriptaid, impacts their capacity to acquire the meiotic maturity and to direct the extracorporeal development of porcine nuclear-transferred (NT) embryos derived from foetal fibroblast cells (FFCs). Nuclear recipient oocytes had been matured for 20 to 21 h in TC 199 medium enriched with 10% porcine follicular fluid (pFF), 10% foetal bovine serum (FBS), 5 ng/mL recombinant human basic fibroblast growth factor (rh-bFGF), 10 ng/mL recombinant human epidermal growth factor (rhEGF), 0.6 mM *L*-cysteine, 1 mM dibutyryl cyclic adenosine monophosphate (db-cAMP), 0.1 IU/mL human menopausal gonadotropin (hMG) and 5 mIU/mL porcine follicle-stimulating hormone (pFSH). Afterwards, they were cultured for an additional 23 to 24 h in db-cAMP, hMG- and pFSH-deprived medium supplemented with 350 nM scriptaid. Reconstruction of enucleated metaphase II (MII)-stage oocytes was achieved *via* their electrofusion with nuclear donor fibroblast cells that was initiated by generating two successive DC pulses of 1.2 kV/cm for 60 μs. The same DC pulses that triggered the fusion of ooplast-FFC couplets were simultaneously applied to induce electrical activation of NT oocytes (clonal cybrids). Two-step *in vitro* maturation (IVM) in the scriptaid-depleted and scriptaid-enriched medium gave rise to reaching MII stage by 182/196 (92.9%)^{A} oocytes as compared to 151/187 (80.7%)^{B} oocytes in the scriptaid-untreated group [$^{A,B}P<0.01$]. The rates of cleaved embryos (143/168; 85.1%^{C}), morulae (107/168; 63.7%^{C}) and blastocysts (63/168; 37.5%^{C}) that developed from activated NT oocytes treated with scriptaid during IVM were found to be significantly higher than in the scriptaid-unexposed group (92/136; 67.6%^{D}, 59/136; 43.4%^{D} and 33/136; 24.3%^{D}, respectively) [$^{C,D}P<0.001$; χ^2 test]. In conclusion, improved competences of cloned pig embryos to complete their development to the morula/blastocyst stages seem to result from enhanced epigenetic reprogrammability and subsequently from elevated transcriptional activity of fibroblast cell-descended

nuclear genome in an epigenomically matured recipient ooplasm that has been subjected to non-specific scriptaid-mediated inhibition of HDACs.

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Session 1

EXPRESSION OF PROSTAGLANDIN $F_{2\alpha}$ IN OOCYTES AND CUMULUS CELLS DERIVING FROM PUBERTAL AND PREPUBERTAL COWS, DEPENDING ON THE QUALITY OF THE OOCYTE

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The role of cumulus cells is essential. They are necessary to prepare oocyte to maturation, fertilization and embryo development. Cumulus cells also participate in acquisition of the developmental competence and are the source of nutrients for oocyte. In the literature there is the model of good and poor oocyte quality. Due to it, oocytes obtained from mature cows are considered to be of good quality and oocytes obtained from immature calves are considered to be of poor quality.

Prostaglandins exert a strong influence on the physiology of the female reproductive system. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is synthesized in participation of synthase PGFS. $PGF_{2\alpha}$ acts via the interaction with the specific receptor PGFR. Literature data indicate the negative effect of $PGF_{2\alpha}$ on embryo quality and embryonic development in cow.

COCs from pubertal cows and prepubertal calves have been matured *in vitro* for 24 hours. After that, cumulus cells were mechanically separated from oocytes. From the oocytes and cumulus cells mRNA was isolated and the oocyte quality markers' expression in oocytes (*FST, GDF9, BMP15, OCT4*) and in cumulus cells (*CTSS, CTSZ, CTSB,CTSK*) as well as genes of $PGF_{2\alpha}$ synthesis pathway (*PGFS, PGFR*) in oocytes and cumulus cells were determined by Real-Time PCR.

Expression of mRNA of all examined factors was shown in oocytes and cumulus cells. Expression of oocyte quality markers in oocyte was significantly lower in the group of prepubertal calves, while expression of CTSs in cumulus cells of prepubertal cows was significantly higher ($P<0.05$). This data indicate good quality of oocytes obtained from pubertal cows and poor quality of oocytes obtained from prepubertal calves. What is more, expression of mRNA of *PGFS* was significantly higher in the oocytes obtained from prepubertal calves and the expression of mRNA of *PGFR* was significantly higher in cumulus cells of prepubertal cows ($P<0.05$). There were correlations between mRNA expression of

PGFS and oocyte quality markers in oocytes and between mRNA expression of *PGFR* and oocyte quality markers in cumulus cells.

Due to the appearance of correlations between mRNA expression of $PGF_{2\alpha}$ synthase and receptor with mRNA expression of oocyte quality markers, $PGF_{2\alpha}$ might be considered as the factor modulating mRNA expression of oocyte quality markers.

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Session 1

IN VITRO FERTILIZATION OF RED DEER OOCYTES WITH FRESH AND FROZEN SEMEN AND BLASTOCYST MATURATION

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Assisted reproduction techniques as superovulation, artificial insemination and embryo transfer are possible alternatives for the propagation of small populations. Many species of deer are rare, endangered and threatened in their natural habitat. The aim of our study was to define the conditions for IVM and IVF of oocytes in red deer as models for endengered related subspecies. Ovaries from red deer hinds were collected *post mortem*. Cumulus-oocyte complexes (COCs) were obtained by follicle fluid aspiration, tissue maceration and matured for 23 hours. For *in vitro* fertilization fresh epididymal semen was used for COCs collected from 14 hinds (group I) and frozen epididymal semen was used for COCs collected from 10 hinds (group II). Groups of COCs were co-incubeted with spermatozoa (Day 0) for 16 hours at 38.5°C in a 5% CO_2 humidified air atmosphere. At 16 h post insemination (Day 1) embryos were separated from cumulus cells by vortexing. Culture was carried out at 38.5°C in an atmosphere of 5% CO_2, 5% O_2, with high humidity for 9 days. The total number of COCs isolated from ovaries was: 347 from group I (164 aspirated from follicles and 183 from tissue maceration), and 305 from group II (128 COCs aspirated from follicles and 177 from tissue maceration). The cleavage rate was assessed 48 hours post insemination. In group I – 28% cleaved, in group II – 21%. The blastocyst rate was 60% in group I and 40% in group II. Conducted research shows successful methodology of *in vitro* maturation and fertilization of red deer oocytes and embryo culture with high blastocyst rate.

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Session 2

CENTRAL AND METABOLIC REGULATIONS OF REPRODUCTION

Dr. hab. Andrzej Herman
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Session 2

THE EFFECT OF NUTRITIONAL STATUS ON RESISTIN'S-MEDIATED LEPTIN INSENSITIVITY IN SHEEP

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If the major control pof seasonal changes in leptin sensitivity occurs at the hypothalamic level, questions regarding how this effect is mediated arise. Although several potential mechanisms to account for this process have been proposed the one that has received the most attention is the inhibition of intracellular leptin signaling by suppressor of cytokine signaling (SOCS-3). It was lately demonstrated that resistin is able to cause central leptin resistance in mice by increasing the circulating leptin concentration. Herein we examined the interaction of season and recombinant bovine resistin (rbresistin) on plasma concentrations of leptin, and mRNA expression of SOCS-3 in experiments conducted during both short (SD) and long days (LD). Thirty ewes of the Polish Longwool breed, a breed that exhibits strong seasonal reproduction, were ovariectomized with estrogen replacement. Intravenous treatments consisted of 1) Control (C; saline; n=10), 2) Low resistin (R1; 1.0 μg/kg body weight [BW]; n=10), and 3) High resistin (R2; 10.0 μg /kg BW; n=10). Blood samples were collected every 10 minutes during 4 h. After experiment ewes were euthanized and selected tissues were collected. The results indicated for the first time in sheep that intravenous infusion of resistin decreased ($P<0.001$) mean circulating concentrations of leptin in a dose-dependent manner during both seasons – LD and SD day. Furthermore, rbresistin increased ($P<0.001$) SOCS-3 mRNA expression in arcuate nucleus, proptic area and pituitary at both doses only during the LD when the leptin resistance/insensitivity phenomenon was observed. The data support the results of previous studies and findings on rodents model that one of the factors contributing to central leptin resistance is autosuppression, in which leptin /resistin stimulate the expression of SOCS-3 factor that inhibits leptin signaling. The increased expression of SOCS-3 in response to leptin and resistin may be a pivotal cause of the resistance/insensitivity, which is a pathological situation in obese individuals and a physiological fact in sheep during the long-day season. To the best of our knowledge, this is the first study to report a role for resistin in modulating the circulating leptin, and indicate that the ability of resistin to create this effect is somewhat seasonally-dependent. Further studies investigating the interaction of resistin and other adipokines are warranted.

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Session 2

APPLYING OF INTRA- AND EXTRACELLULAR MEASUREMENTS OF cAMP FOR DETERMINATION THE POSSIBILITY OF LH RECEPTORS INTERNALISATION IN THE GRANULOSA CELLS OF OVARIAN PREOVULATORY FOLLICLES IN THE PIG

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Polypeptide hormones, including luteinizing hormone (LH), act mainly trought metabotropic membrane receptors coupled to G proteins. G protein-coupled receptors are able to inhibit or stimulate the effector enzyme activity – adenyl cyclase. This enzyme catalyses the synthesis of cyclic adenosine monophosphate (cAMP) – the second messenger for LH and other hormones. LH receptors are expressed for example in granulosa cells in ovarian follicles. The G protein-coupled receptors may also undergo internalization as a result of repeated or prolonged hormonal stimulation. Recent literature data indicates that G protein-coupled receptors may continue cAMP signaling after their internalization in the granulosa cell (Lyga S. et al., Endocrinology, 157 (4): 1613–1621, 2016).

The aim of the study is to determine possibility of internalization LH receptors in granulosa cells in preovulatory ovarian follicles on the basis of intra- and extracellular cAMP levels evaluation. In the study ovaries containing ovarian follicles collected in the preovulatory period of estrous cycle from mature or immature pig were used. The first stage of the research was separation of the granulosa cells from theca by the method of Haney A.F. and Schomberg D.W. (Biol. Reprod., 19: 242–248, 1978). The next step was *in vitro* cell culture of non-adhered granulosa cells in incubator at 37°C with 5% CO_2 access. After overnight incubation, the cells were treated with IBMX (selective inhibitor of phosphodiesterase) and chellenged with exogenous recombinant LH at 10 and 1000 mIU/ml concentration. After 1 hour of incubation, the cells with the post-culture medium were collected to determine the intracellular and extracellular level of cAMP by immunoassay.

In preliminary pilot studies, significant changes in the amount of extracellular cAMP concentration in the medium after LH treatment were observed. Depending on the amount of exogenous LH hormone used, a “hormone dependent effect” was observed. The extracellular content of cAMP increased 1,4 times after stimulation (10 mIU/ml LH) granulosa cells collected from both immature and

mature gilts and 2,7 or 10,1 fold after stimulation (1000 mIU/ml) in granulosa cells from immature and mature gilts, respectively.

Since G protein-coupled receptors may continue cAMP signaling after internalization the examination of extra- and intracellular cAMP content will be performed during 24 hours of exogenous LH stimulation.

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Session 2

EXPRESSION OF CHEMOKINE LIKE RECEPTOR 1 (CMKLR1/CHEMR23) IN THE PORCINE HYPOTHALAMUS DURING THE OESTROUS CYCLE

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Reproduction in females is one of the most energy-intensive processes. Physiological mechanisms controlling energy homeostasis and reproductive functions are closely integrated. It is proposed that chemerin, a hormone produced mainly by the white adipose tissue, known for its influence on insulin sensitivity, energy homeostasis or obesity related parameters may also be engaged in the regulation of hypothalamo-pituitary-ovarian axis in gilts. The adipokine binds with three G-protein coupled receptors: chemokine like receptor 1 (CMKLR1/ChemR23), G protein-coupled receptor 1 (GPR1) and C-C chemokine receptor-like 2 (CCRL2). CMKLR1 has been investigated the most, and is known to act *via ERK1/2* and *Akt* kinases for signal transduction. We hypothesize that the hypothalamic structures responsible for GnRH production and secretion: medio basal hypothalamus (MBH), preoptic area (POA) and semi-median eminence (SME) are sensitive to chemerin. Therefore, the aim of this study was to determine the gene and protein expression of chemerin receptor CMKLR1 in the mentioned structures of the hypothalamus on days 2 to 3 (early luteal phase – formation of the corpus luteum), 10 to 12 (mid-luteal phase – fully active corpora lutea), 14 to 16 (late luteal phase – luteolysis) and 17 to 19 (follicular phase) of the oestrous cycle, using real-time PCR and Western Blot methods (n=5 in each experimental group). Data were analysed using one-way ANOVA. In MBH and POA, the highest *CMKLR1* mRNA content was observed on days 17 to 19 of the cycle, whereas the lowest on days 2 to 3 and 10 to 12, respectively. In SME, the gene expression was significantly lower on days 2 to 3 of the cycle when compared to other studied phases. In all the studied hypothalamic structures, higher CMKLR1 protein content was observed on days 10 to 12 of the cycle in relation to other phases of the cycle ($P<0.05$). The presented data indicated for the first time the presence of chemerin receptor CMKLR1 in the hypothamic structures responsible for GnRH production and secretion, which suggest its

sensitivity to chemerin. Moreover, the observed changes in the CMKLR1 gene and protein expression may be dependent on the phase of the oestrous cycle, namely the hormonal status of the animal.

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Session 2

EFFECTS OF CENTRAL OREXIN A ON GONADOTROPINS AND PROGESTERONE SECRETION IN EWES IN THE LUTEAL PHASE OF THE ESTROUS CYCLEA AND IN THE ANESTRUS

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The studies mainly on non-seasonal rodents indicate that orexin A (OXA) plays an important role in regulating the hypothalmo-pituitary gonadal (HPG) axis. However, the obtained results point out that the central impact of OXA on HPG axis is dual, depending on the conditions of the experiment. The aim of the present study was to further investigate the role of OXA in the regulation of reproduction in intact ewes (n=20) under natural physiological conditions, in the luteal phase of the estrous cycle (n=10) and in the anestrus (n=10). The treatment groups consisted of: Group 1 (control, n=5), Ringer-Locke buffer, pH 7.4; 2) Group 2 (n=5), bovine OXA; (0.3 μg/kg body weight). The Ringer-Locke buffer or OXA were injected centrally into the fourth ventricle at 1, and 2 hour after beginning of the 6-hours experiment (2 single injections). Blood samples (5 ml) were collected at 15-minutes intervals beginning one hour before the first injection. Four hours after the second injection, ewes were killed and the anterior pituitaries were isolated for measuring the concentration of GnRH receptor (GnRH-R) protein in the anterior pituitaries (AP) by ELISA. Radioimmuoassay revealed that OXA treatments inhibited ($P<0.001$) LH and FSH secretion in both examined periods and progesterone ($P<0.001$) during the luteal phase. The influence of OXA on LH and FSH release may be in part attributable to additional response of the pituitary to GnRH as under the influence of OXA the concentration of GnRH-R protein in the AP was down-regulated during the luteal phase ($P<0.01$) and in the estrus ($P<0.05$). We conclude that OXA may be a negative regulator of the gonadotropins secretion in ewes under natural physiological conditions, in periods when the HPG axis is suppressed by the sex steroid-negative feedback.

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Session 2

CHEMERIN EXPRESSION IN THE PORCINE PITUITARY DURING THE ESTROUS CYCLE AND EARLY PREGNANCY

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Adipose tissue is not only the site of energy storage, but also an endocrine organ. It secrets various biologically active compounds, including adipokines. Previous studies demonstrated that these substances affect the metabolic status of the organism and its reproductive functions. These hormones seems to be involved in the proper functioning of the female reproductive system in pigs by acting at all branches of the hypothalamic-pituitary-gonadal (HPG) axis. One of them may be chemerin. It is hypothesized that the expression of chemerin in the pituitary may depend on the phase of the oestrous cycle and early pregnancy. The aim of this study was to investigate chemerin mRNA and protein content in the porcine anterior (AP) and posterior (NP) pituitary lobes during different phases of the cycle: days 2 to 3, 10 to 12, 14 to 16 and 17 to 19, as well as during early pregnancy: days 10 to 11, 12 to 13, 15 to 16 and 27 to 28. Chemerin gene and protein expression were determined using the real-time PCR and Western blot methods, respectively. Data were analysed using one-way ANOVA. In AP, during the oestrous cycle, chemerin gene expression was higher on days 2 to 3 and 14 to 16 compared to days 10 to 12 and 17 to 19. The highest chemerin expression was noted on days 10 to 11, 15 to 16 and 27 to 28 compared to days 12 to 13 of gestation and 10 to 12 of the cycle. Chemerin protein concentration was higher on days 10 to 12 and 17 to 19 than on days 2 to 3 and 14 to 16 of the oestrous cycle. In the same tissue, protein content of chemerin was higher on days 10 to 11 of pregnancy compared to days 12 to 13, 15 to 16 and 27 to 28 of gestation and 10 to 12 of the cycle. In NP, chemerin mRNA content was lower on days 10 to 12 of the oestrous cycle compared to other days of the cycle. The highest mRNA level was reported on days 10 to 11 of gestation, lower on days 15 to 16, and the lowest on days 12 to 13 compared to days 27 to 28 of pregnancy and 10 to 12 of the cycle. In NP, chemerin protein concentration was lower on days 14 to 16 compared to other phases of the cycle. Chemerin protein levels were significantly higher on days 15 to 16 of pregnancy, than on the other days of gestation and days 10 to 12 of the oestrous cycle. Our results indicate that chemerin can be produced

locally in the pituitary. We demonstrated, for the first time, that chemerin expression levels in the porcine pituitary were dependent on the phase of the oestrous cycle and early pregnancy.

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Session 2

FATTY ACID PROFILE IN FOLLICULAR FLUID AFFECTS THE QUALITY OF PORCINE CUMULUS-OOCYTE COMPLEXES *IN VITRO*

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The ovarian follicle is a complex structure which includes the oocyte, follicular fluid (FF) and follicular cells. The quality of cumulus-oocytes complexes (COC) and preimplantation embryos may be positively or negatively affected by the particular fatty acids (FA). Recently, we described distinct FA profiles of FF from prepubertal and cyclic gilts and a beneficial role of FF from cyclic gilts in IVM of porcine oocytes. However, a huge heterogeneity in the total FA content have been noticed among the analyzed FF samples.

The aim of the study was to analyze the effects of the FA content (L – low versus H – high) in FF supplemented to IVM medium on the quality of porcine oocytes and surrounding cumulus cells (CC). Two FF batches were utilized that differed significantly in concentration of the total and the individual FAs.

COCs were aspirated from prepubertal gilts ovaries and matured in vitro (NCSU23, 10% FF, 10 U hCG, 10 U PMSG) for 44 h (24 h with hormones, 20 h without hormones). The two FF batches used were characterized by significantly different total FA content (H – 629.1 µg/ml; L – 423.0 µg/ml) as well as particular FAs content: AA (H – 62.6 µg/ml; L – 30.2 µg/ml), OA (H – 120.1 µg/ml; L – 81.8 µg/ml), SA (H – 106.1 µg/ml; L – 66.7 µg/ml), PA (H – 138.9 µg/ml; L – 102.6 µg/ml), LA (H – 107.6 µg/ml; L – 65.2 µg/ml). After IVM, oocytes and CCs were subjected to fluorescence staining (BODIPY 493/503) in order to visualize lipid droplets (LDs). Images were captured using z-stack confocal microscopy (Zeiss Airyscan) and processed by the ImageJ for calculations of LD number and area occupied by LD per cell, the level of fluorescence and oocyte diameter. Altogether 69 oocytes and 101 CC samples were analyzed.

The two oocyte groups and the corresponding CCs differed significantly in the LD fluorescence level (oocytes H – 531 354.41; L – 271 976.81; CC – H – 928 975.02; L – 786 490.71; $P<0.05$). Moreover, the CCs from the H group showed a significantly bigger area occupied by LD (H – 9.7283%; L – 6.7347%). The H oocytes displayed higher LD size (H – 8.36 μm^2; L – 8.08 μm^2) and diameter (internal: H – 120.3 µm; L – 123.8 µm; $P<0.05$; external H – 150.6 µm; L – 155.5 µm; $P<0.01$). The results suggest that the FA profile in FF supplemented to IVM medium affects the quality of COCs from prepubertal pigs. Besides, the biological importance of the FF used for IVM to oocyte (LD biogenesis) and embryo quality needs a careful investigation.

Session 2

EXPRESSION OF SELECTED GENES OF THE GONADOTROPIC SYSTEM IN SHEEP TREATED WITH STRESSFUL STIMULI AND ALLOPREGNANOLONE

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Stress-generating stimuli are integrated at the central nervous system (CNS) level and affect secretory activity of the hypothalamic-pituitary system. In the female's brain the synthesis of a neurosteroid – allopregnanolone (AL) is one of the many defense processes against stress. AL acts particularly through the GABAergic neurons, that have terminals located in the hypothalamus. The aim of this study was to investigate whether central administration of AL would affect the expressions of gonadotropin-releasing hormone (*GnRH*) and kisspeptin (*KISS-1*) genes in the mediobasal hypothalamus (MBH) and arcuate nucleus (ARC), respectively, as well as the expression of gonadotropin (*LHβ* and *FSHβ)* genes in the anterior pituitary (AP).

Adult, intact sheep (n=24) implanted with stainless steel guide cannula into the third brain ventricle (IIIv) were divided into 4 groups: i. infused for 3 days with Ringer-Locke (RL) solution (C group); ii. infused with RL and on the third day treated with stressful stimuli (isolation and partial immobilization; S group); iii. infused with AL (4 x 15 µg/60 µl/30 min for 3 days) and treated with stressful stimuli (AS group); and iv. infused with AL alone (A group). The animals were euthanized after the last infusion and the expression of selected genes was determined in the MBH, ARC and AP by Real Time-PCR.

A significant increase in the expression of GnRH mRNA in the MBH ($P \leq 0.01$) and a decrease in KISS-1 mRNA in the ARC ($P \leq 0.05$) were observed in the S group. In the AP, the expression of LHβ mRNA increased ($P \leq 0.05$) and that of FSHβ mRNA decreased ($P \leq 0.05$) in response to stressful stimuli. AL infused into the IIIv inhibited the expression of *GnRH* ($P \leq 0.05$), *KISS-1* ($P \leq 0.001$) and *FSHβ* ($P \leq 0.05$) genes in non-stressed sheep and had also an inhibitory effect on *KISS-1* ($P \leq 0.05$) and *FSHβ* ($P \leq 0.05$) genes under the influence of stress.

In conclusion, stress and AL differently affect the expression of genes in the gonadotropic system in sheep, indicating the inhibitory effect of AL.

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Session 2

THE ROLE OF TRH AND LENGHT DAY IN THE REGULATION OF GROWTH HORMONE SECRETION IN LACTATING SHEEP

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The correct course of lactopoiesis is crucial in the process of rearing offspring. Newborn animals require constant access to wholesome food that ensures their development and provides them with resistanceto adverse environmental conditions. The concentration of thyroid hormones in the blood is influenced by endogenous and environmental factors. The secretory function of the thyroid is determined by the hypothalamic-pituitary-thyroid axis (HPT) based on the principle of negative feedback. Until today, thyroid hormones were attributed the role of metabolic hormones. In recent years, attention was drawn to the role of thyroid hormones in the initiation and maintenance of lactation in small ruminants. The aim of the study was to determine the role of TRH factor in the regulation of growth hormone (GH) secretion. The study was performed on 20 sheep. Pituitary glands were collected on 40th day of lactation during the long (June, n=10) and short photoperiod (November, n=10). *In vitro* incubation was carried out for 3 hours at 37ºC. The control group (GK n=10) was incubated in medium alone, and experimental group in a medium with addition of exogenous TRH (36μg/ml of the medium). Studies have shown that the concentration of GH in June in the experimental group was significantly higher (39,4±6,4 μg/ml, $P \leq 0.01$) than in the control group (24.48±5.2 μg/ml). The administration of TRH in November has significantly ($P \leq 0.01$) raised the growth hormone secretion in experimental group (42.41±9.2 μg/ml) in comparison with the control group (37.63±7.4 μg/ml).

The results prove that TRH stimulates growth hormone secretion in ewes during lactation. The impact of TRH on somatotropic axis was depended on the length of the day.

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Session 2

EFFECTS OF SHORT-TERM LUPIN GRAIN FEEDING ON OVARIAN ACTIVITY IN NON-PROLIFIC POLISH MOUNTAIN EWES DURING THE BREEDING SEASON

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The physiological mechanism(s) by which supplementation with high-protein, high-energy lupin grain increases the ovulation rate in cyclic ewes remain(s) uncertain. The objective of this experiment was to employ videolaparoscopy (corpus luteum detection and enumeration) and transrectal ovarian ultrasonography (assessment of antral follicular numbers and kinetics) to determine ovarian effects of short-term lupin grain feeding in non-prolific Polish Mountain ewes. Estrus and ovulations were synchronized in 24 animals with progestogen-releasing sponges inserted for 12 days during the middle portion of the breeding season (September-October). Eight ewes received the maintenance (hay-only) diet (Ctrl), while the lupin-fed groups (n=8 each) received 1 x 500 g (Trt 1) or 2 x 250 g (Trt 2) of lupin grain per day, from Days 9 to 14 of the synchronized cycle (Day 0=first ovulation of the interovulatory period studied). There were no differences ($P>0.05$) in the mean ovulation rate between Ctrl animals (1.6±0.2; mean±SEM) and the flushed Polish Mountain ewes (Trt 1: 1.7±02 and Trt 2: 1.6±0.2). Ovarian antral follicles emerging in the penultimate wave of the estrous cycle in Trt 2 ewes had a longer ($P<0.05$) growth phase (Ctrl: 2.0±0.5 days; Trt 1: 1.8±0.4 days; and Trt 2: 3.8±0.5 days) and attained a greater ($P<0.05$) diameter before ovulation in comparison to the remaining two groups of animals (Ctrl: 5.5±0.2 mm; Trt 1: 5.2±0.2 mm; and Trt 2: 6.2±0.3 mm). A final wave of the interovulatory period emerged ~1 day earlier in Trt 2 than in Trt 1 ewes ($P<0.05$; 11.7±0.4 compared with 12.8±0.4 days post-ovulation). The nutritional supplementation increased ($P<0.05$) the number of 3-mm follicles in Trt 2 ewes (a difference between Trt 2 and Trt 1/Ctrl was most prominent on Day 10 or 1 day after the beginning of lupin feeding) but there were no effects of the flushing on daily numbers of medium-sized or large antral follicles throughout the entire observation period (Days 8 to 17). The present results indicate that short-term lupin

grain feeding from mid- to late luteal phase does not consistently enhance ovulatory responses in non-prolific genotypes of ewes. Two meals of lupin grain per day altered follicular lifespan and the timing of wave emergence, but they failed to increase the numbers and/or percentages of follicles ovulating in the penultimate and final waves of the estrous cycle studied.

Session 2

EXPRESSION PROFILE OF LEPTIN RECEPTOR, PROLACTIN RECEPTOR AND SOCS-3 TRANSCRIPTS AT SELECTED STAGES OF FETAL DEVELOPMENT IN LAMBS

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Leptin and prolactin play an important role in the regulation of energy homeostasis and thus are a potent regulators of metabolism, growth and development. They induce similar intracellular pathways of signal transduction. PRL-R (Prolactin Receptor) and LRb (long form of Leptin Receptor) lack intrinsic enzymatic activity and mediate signals by activation of JAK/STAT (Janus Kinases/Signal Transducer and Activator of Transcription) pathway. Suppressors of Cytokine Signaling-3 (SOCS-3) are important negative regulators of this cascade. The aim of this study was to assess LRb, PRL-R and SOCS-3 mRNA expression in selected tissues of lambs during fetal development.

Fetuses were obtained from 9 Polish Longwool ewes euthanized at 60, 90, and 120 d of pregnancy (3 animals per time-point). Ewes were mated after estrus synchronization and had twin pregnancies. Triplicates of tissues samples (from liver, lungs, kidneys, heart, brain and/or anterior pituitary) were collected from six fetuses at each stage of pregnancy. Real-time PCR was used to measure LRb, PRL-R and SOCS-3 mRNA expression. Expression levels were calculated using a relative quantification (RQ) analysis. Data were analyzed via one-way analysis of variance (ANOVA) using SigmaPlot statistical software followed by Tukey's multiple comparison test.

Results of this experiment proved that expression of LRb, PRL-R and SOCS-3 occur in almost every tissues that have been studied, however they exhibit various, tissue-specific pattern and their levels change during pregnancy. The highest level of LRb expression, relative to other tissues, was observed in the liver. The highest expression of PRL-R was observed in pituitary and the lowest in the lungs and kidneys. No expression of PRL-R was observed in the heart. When comparing the level of SOCS-3 transcripts, we found that the greatest expression occurred in the liver and lungs, and the lowest levels in tissues associated with the CNS. We also demonstrated that in majority of tissues (except the brain and pituitary) expression of target genes increased with the progression of pregnancy.

We shown that a LRb, PRL-R and SOCS-3 expression are tissue- and pregnancy-dependent, however a more complete analysis of the mechanisms of leptin and prolactin signaling at different stages of pregnancy could improve our knowledge about their roles in physiological and pathological processes during fetal development.

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Session 2

PERIPHERAL ADMINISTRATION OF CAFFEINE INFLUENCES THE SYNTHESIS OF GNRH AND LUTEINIZING HORMONE IN EWE DURING THE FOLLICULAR PHASE OF THE ESTROUS CYCLE

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Introduction: Caffeine is one of the most widely consumed pharmacologically active substances and it crosses the blood-brain barrier. It is postulated that caffeine may adversely affect fertility in female, although the exact mechanism by which caffeine disturbs the process of reproduction has not been known yet. A reproductive process in female is under the control of the neurohormonal system called the hypothalamic-pituitary-gonadal (HPG) axis. The hypothalamus plays a primary role in the regulation of reproduction, which is conditioned by tonic secretion of gonadotropin-releasing hormone (GnRH), which regulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone from the anterior pituitary (AP). It is worth noting that the presence of adenosine and ryanodine receptors by which caffeine exerts its biological effects was found both in the hypothalamus and pituitary gland.

Aim: The study was designed to determine the effect of peripheral administration of caffeine on the synthesis of GnRH and LH in the follicular phase ewes.

Material and methods: The study was performed on adult Blackhead ewes (n=24) during the follicular phase of the estrous cycle synchronized by Chronogest® CR method. The experimental procedures were conducted 24 h after intravenous injection of 500 i.u. PMSG. Ewes were divided into four groups: control (saline, iv., n=6), caffeine-treated: I (10 mg/animal, iv., n=6), II (20 mg/kg, iv., n=6) and III (40 mg/kg, iv., n=6). Animals were euthanized 3 hours after caffeine or saline injection. The preoptic area (POA) – hypothalamic structure playing key role in the GnRH-ergic activity and the AP were collected. The gene expression of GnRH and its receptor GnRH were assayed by Real-Time PCR. The content of GnRH in the

POA was assayed using ELISA. The protein expression of LH was determined by Western Blot.

Results: It was found that caffeine administrated at the highest dose stimulated ($P \leq 0.05$) the synthesis of GnRH as well as GnRHR mRNA expression in the POA. It was also determined that the same dose of caffeine increased ($P \leq 0.05$) both the expression of LH protein and GnRHR mRNA in the AP of follicular phase ewes.

Conclusion: The study showed that caffeine may influence the activity of the reproductive system in ewe by inducing the stimulation of GnRH and LH synthesis.

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Session 2

CHANGES IN PHENOTYPE OF OVARIAN MESENCHYMAL STEM CELLS INDUCED BY ANABOLIC STEROIDS

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Stem cells isolated from the adult porcine ovarian cortex seem to have the character of mesenchymal stem cells (MSCs). This has been confirmed in our earlier studies by the presence of MSC protein markers such as CD29, CD90 and CD105 at both protein (ICC / IF, WB) and mRNA (qRT-PCR) level. That is why the aim of this work was to investigate whether potentially carcinogenic anabolic steroids used in medicine and animal husbandry (boldenone and nandrolone) can induce differentiation of ovarian MSCs into cancer stem cells (CSCs) or endothelial cells what is the key event necessary for the initiation of neoangiogenesis and development of a potential tumor.

MSCs were isolated using the magnetic separation technology (MACS) based on the presence of SSEA-4 antigen from ovaries of 5 month old gilts. The MSCs were then cultured for 7 or 14 days in serum-free medium but with the addition of boldenone or nandrolone. After termination of culture, markers of CSCs (CD44, CD133) and endothelial cells (VE-cadherin and VEGFR-3 receptor) were identified (WB, qRT-PCR). After boldenone or nandrolone MSCs treatment, overexpression of both CD44 and CD133 markers was observed. Interestingly, administration of nandrolone led to an increased expression of CD44 protein after just 7 days of exposure, whereas nandrolone caused its overexpression after 14-days of culture. An increased expression of CD44 and CD133 at the mRNA level of CSCs markers was observed also after 14 days of culture. Differentiation of MSC into vascular endothelial cells was performed using various compounds including growth factors, e.g. VEGF, and bFGF. After 14 days of culture expression of endothelial markers such as VE-cadherin and VEGFR-3 receptor was examined. Expression of these markers tested by qRT-PCR was very high, similar to that in the aorta (positive control).

Based on these findings we can conclude that in the presence of anabolic steroids MSCs can change their phenotype as evidenced by the increased expression of CSCs markers. Furthermore, MSCs also very easily differentiate into endothelial cells, what can induce angiogenesis. Both, MSCs phenotype change and their ability

for differentiation into endothelium might be directly linked to ovarian carcinogenesis.

This research was supported by K/ZDS/008061 and project no. UMO-2016/21/N/NZ9/01205 from National Science Centre Poland.

Session 2

CHEMOKINE LIKE RECEPTOR 1 (CMKLR1/CHEMR23) EXPRESSION IN THE PORCINE HYPOTHALAMUS DURING EARLY PREGNANCY

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Previous studies demonstrated that adipokines, hormones produced by adipose tissue, affect the metabolic status of the body and its reproductive functions. We hypothesized that chemerin, a hormone from group of adipokines, is also engaged in the control of the reproductive system at the level of the hypothalamus. Chemerin binds with three G-protein coupled receptors: chemokine like receptor 1 (CMKLR1/ChemR23), G protein-coupled receptor 1 (GPR1) and C-C chemokine receptor-like 2 (CCRL2). The aim of this study was to determine *CMKLR1* gene and protein expression in the hypothalamic structures (medio basal hypothalamus – MBH, preoptic area – POA, median eminence – SME) during early pregnancy: on days 10 to 11 (transuterine migration of embryos), days 12 to 13 (maternal recognition of pregnancy), days 15 to 16 (implantation) and days 27 to 28 (end of implantation), as well as on days 10 to 12 of the oestrous cycle (mid-luteal phase). *CMKLR1* gene expression was examined using the real-time PCR method and corresponding protein was evaluated by Western blot analysis. Data were analysed using one-way ANOVA. In MBH, the highest *CMKLR1* gene expression was observed on days 15 to 16 of pregnancy, whereas the lowest on days 10 to 11 and 27 to 28 of gestation, and on days 10 to 12 of the cycle. The highest CMKLR1 protein content in MBH was found on days 27 to 28 of pregnancy compared to days 10 to 11 and 12 to 13 of gestation. In POA, the highest *CMKLR1* mRNA content was found on days 10 to 11 of pregnancy compared to other studied phases. Protein concentration of CMKLR1 observed in POA was the highest on days 27 to 28 of gestation compared to days 10 to 11 and 15 to 16 of pregnancy, and days 10 to 12 of the cycle. In SME, the highest *CMKLR1* gene expression was noted on days 27 to 28 of pregnancy, whereas the lowest on days 10 to 12 of the cycle. The highest protein level of the receptor was observed on days 10 to 12 of the cycle compared to all the studied periods of pregnancy ($P<0.05$). In this study we indicated, for the first

time, the presence of chemerin receptor CMKLR1 in the hypothamic structures responsible for GnRH production and secretion, which suggest its sensitivity to chemerin. The observed changes in the *CMKLR1* gene and protein expression across the periods of early pregnancy and on days 10 to 12 of the cycle suggest its dependence on the hormonal status of the animal.

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Session 3

ENVIRONMENTAL DETERMINANTS OF REPRODUCTION

Dr. hab. Anna Ptak
Dr. hab. Magdalena Socha

Session 3

PERSISTENT ORGANIC POLLUTANTS PRESENT IN HUMAN FOLLICULAR FLUID THROUGH MODULATING E2 AND IGF1 SECRETION BY ADULT GRANULOSA CELL TUMORS STIMULATE HUMAN GRANULOSA CELLS PROLIFERATION

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Granulosa cell tumors (GCT) arise from the granulosa cells that surround oocyte and belong to hormone-related cancers. Hyperoestrogenism observed in patients with GCT is connected with tumor production of oestrogens (Park at al., 2012). In the regulation of growth in ovarian cancer IGF1 also plays an important role. One of two subtypes of GCT is the adult form (AGCT) in most cases seen in adults. Recent evidences suggest that follicular fluid is not only rich in steroids, growth factors, but also in persistent organic pollutants such as perfluorooctane (PFOA), perfluorooctane sulfonate (PFOS), hexachlorobenzene (HCB), dichlorodiphenyldichlororthylene (p,p'– DDE) and polychlorinated biphenyl 153 (PCB153) (Petro et al., 2014, 2012). These compounds action as agonist or antagonist for hormone receptors thus they may potentially affect the hormones secretion by GCT.

The aim of this study was to identify if mixture and individual persistent organic pollutants present in follicular fluid effect on the basal E2 and IGF1 secretion in human AGCT. Moreover, we analyzed if secreted hormones by human granulosa cell tumors modulate the proliferation of non-cancer human granulosa cells.

In this study human GCT cell lines, KGN (RBRC-RCB1154, Riken Cell Bank, Ibaraki, Japan; after approval from Drs. Yoshiro Nishi and Toshihiko Yanase) representing adult granulosa cell tumors and human non-cancer granulosa cell lines HGrC have been used as in vitro model. KGN cells were cultured using a three-dimensional (3D) model to reflect tumor microenvironment. Secretion of hormones have been determined by ELISA kits and the proliferation of HGrC cell lines have been measured by AlamarBlue.

Firstly, we found that individual chemicals present in follicular fluid increase basal E2 secretion and IGF1 secretion by KGN cells. Secondly, results indicated that mixture also increases E2 and IGF1 secretion by KGN cell lines. However, the observed effect was lower than the predicted additive effect of each compound. Finally, this study has shown that conditioned medium harvested from KGN cell culture stimulate HGrC cells proliferation.

Taken together our results, we demonstrated that individual and mixture of persistent organic pollutants present in follicular fluid through modulating E2 and IGF1 secretion by adult granulosa cell tumors stimulate the proliferation of human non-cancer granulosa cells.

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Session 3

NITROPHENOLS INHIBIT BASAL AND 8-Br-cAMP INDUCED STEROID HORMONE SECRETION BY OVARIAN FOLLICLES OF THE HEN (*GALLUS DOMESTICUS*)

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4-nitrophenol (PNP) and 3-methyl-4-nitrophenol (PNMC) are environmental pollutants which are degradation products of widely used in agriculture insecticides (such as parathion and fenitrothion). The other source of PNP and PNMC are particles produced by diesel engines, so-called Diesel Exhaust Particles (DEPs). Mori et al. (2003) showed that 1 kg of DEPs contains 15 mg PNP and 28 mg of PNMC, and Mi et al. (2010) reported that the EU emits 240 000 tons of DEP into atmosphere per year. The influence of these pollutants on avian ovarian function is unknown, therefore, this study was designed to investigate *in vitro* effects of PNP and PNMC on basal and 8-Br-cAMP (cAMP analog and PKA activator) stimulated sex steroid secretion by preovulatory ovarian follicles of the laying hen. The fragments of granulosa and theca layers isolated 2 h after ovulation from the yellow hierarchical follicles (F3<F2<F1; 20–36 mm) of the Hy-Line brown laying hens (n=6) were used in the experiment. They were incubated for 24h at 39°C in medium supplemented with increasing doses of PNP or PNMC (10^{-8}, 10^{-6} and 10^{-4} M), 8-Br-cAMP (0.5 x 10^{-3} M), and 8-Br-cAMP with PNP or PNMC (10^{-6} M). Following the incubation medias were collected and progesterone (P4), testosterone (T) and estradiol (E2) concentrations were determined by RIA method. The data were statistically analyzed by two-way analysis of variance followed by Tukey's test at $P<0.05$. PNP and PNMC significantly reduced P4 secretion by the granulosa layer, and T and E2 secretion by the theca layer of the F3-F1 follicles; the highest effects were noticed in respect to the lowest dose of these nitrophenols (i.e. 10^{-8} M; $P<0.01$)). PNP and PNMC significantly ($P<0.01$) diminished 8-Br-cAMP stimulated T and P4 secretion by the granulosa and theca layers of these follicles, respectively. No effects of 8-Br-cAMP on E2 secretion by the theca layer of F3-F1 follicles were observed. Results of this experiment suggest that nitrophenols are potent inhibitors of sex steroid synthesis and/or secretion form chicken ovarian follicles. It can be suggested that nitrophenols may affect adenylyl cyclase/cAMP signaling pathway in the steroidogenic cells.

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Session 3

PROTEOMICS ANALYSIS OF THE PIG CORPUS LUTEUM DURING EARLY PREGNANCY

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The corpus luteum (CL) is a transitory endocrine gland necessary for the onset and maintenance of pregnancy in mammals. Most of the data on the mechanisms of CL function at the molecular level have come either from candidate gene or protein study and function approach or from genomic studies, but direct protein data are scarce.

The objective of this study was to generate a signature protein profile of CL during early pregnancy.

To address our aim, a proteomic approach was used to identify those proteins whose levels change significantly in the porcine CL as it progresses during early pregnancy, from day 12 to day 19. Corpus luteum from estrous synchronized pigs was collected on days 12 of cycle and days 12, 15 and 19 pregnancy. Proteins extracted from the tissue were resolved using iso electric focusing in pH range 4–7 followed by two-dimensional gel electrophoresis (2 DE). The analysis and comparison of gels from days 12, 15 and 19 of pregnancy to day 12 of cycle led to detection of significantly altered protein spots that were identified by MALDI TOF/TOF tandem mass spectrometry.

The use of 2 DE based analysis showed that 50, 85 and 100 proteins spots were significantly altered in days 12, 15 and 19 of pregnancy as compared to day 12 of cycle, respectively. We could identify a total of 225 proteins. There was a significant increase in the expression of proteins involved in process of translation such as elongation factor 1-beta, eukaryotic translation initiation factor, receptors for steroids such as membrane-associated progesterone receptor component 2 and oxidoreductases such as 2-oxoglutarate dehydrogenase in CL from pregnant animals on days 12, 15 and 19 of pregnancy as compared to CL from day 12 of cycle. On days 15 and 19 of pregnancy, an increase in the expression of proteins involved in chaperone activity such as heat shock protein beta-6, heat shock cognate 71 kDa protein, and in hormone such as prorelaxin precursor, enzymes such as succinyl-CoA ligase, was observed when compared to CL proteome obtained on day 12 of cycle. Furthermore, a decrease in actin-regulatory proteins such as macrophage-capping protein and proteins involved in protein degradation such as endophilin-B1 and galectin-1was observed in CL of pregnancy on days 12, 15 and 19 as compared to CL of cycle on day 12.

Our results indicate that protein profile of early pregnancy CL is associated with increased expression of proteins that take part in lipid metabolism, chaperone activity and process of translation.

Session 3

EFFECT OF HERBICIDE ROUNDUP AND TAMOXIFEN ON PRUSSIAN CARP (*CARASSIUS GIBELIO* B.) OOCYTE MATURATION AND SECRETION OF 17α20β-P *IN VITRO*

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The aim of the study was to evaluate the effect of Roundup and tamoxifen on the final stages of oocyte maturation, ovulation and secretion of 17α,20β-dihydroxy-4-pregnen-3-one (17α20β-P) in Prussian carp (*Carassius gibelio*, Bloch) females during the spawning season. Currently, Roundup, is the most popular pesticide in the world, which might be also used to control aquatic vegetation in ponds or lakes, so it was interesting to investigate its effect on fish reproduction, at the level of ovary. Taking into consideration the hormonal activity of Roundup probably through the estrogen receptor (ER), in this study we use simultaneously Roundup and tamoxifen, an estrogen receptor antagonist. The experiment was conducted on 6 sexually matured (GSI = 20,25%) Prussian carp females. Samples of oocytes (1 g) were incubated 24 hours in Cortland saline with the addition of: tamoxifen (10 ng ml^{-1} medium), Roundup (10 and 100 µg ml^{-1} medium) and/or carp pituitary homogenate (CHH-200 µg ml^{-1}). After 24 hours incubation, the samples of medium were taken for steroid level determination, and the oocytes were preserved in Serra liquid, then exposed in the turpentine oil. Next, oocytes were categorized according to a four-degree scale. Prior to the measurement of 17α20β-P with ELISA method, the medium samples were extracted with dichloromethane. After statistical analysis with Mann-Whitney test it was shown that simultaneous addition of tamoxifen and Roundup inhibited the final stages of Prussian carp oocyte maturation and ovulation. After 24 hours of incubation the secretion of 17α20β-P to the incubation medium was significantly decreased in all groups treated simultaneously with Roundup and tamoxifen. The highest level of tested steroid was noticed in groups treated with tamoxifen given alone as well as simultaneously with carp pituitary homogenate. But in groups were Roundup was given, the level of 17α20β-P was significantly lowered. Summing up obtained results, it was shown that Roundup and tamoxifen are able to affect the final process of oocytes maturation probably as a consequence of decreased level of 17α20β-P in Prussian carp females.

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Session 3

NEONATAL EXPOSURE TO METHOXYCHLOR ALTERS PLASMA LEVEL OF FSH AND FSH RECEPTOR EXPRESSION IN OVARIAN FOLLICLES OF ADULT PIGS

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Animals during fetal and neonatal life are susceptible to the environmental chemicals that may have long-term effects on reproductive function. Methoxychlor (MXC) is an organochloride pesticide with estrogenic, antiestrogenic and antiandrogenic properties. The use of MXC was banned in the USA in 2003 and in the EU in 2002, however MXC has been found to persist in the environment. Therefore, the objective of the conducted study was to determine the effect of neonatal exposure to MXC on the plasma FSH level and the expression of FSH receptor (FSHR) in the ovarian follicles of adult pigs. Animals were subcutaneously injected with MXC (20 µg/kg body weight) or corn oil (controls) between postnatal days 1 and 10 (n=4). Blood samples were drawn and ovaries were obtained from sexually mature gilts between days 8 and 11 of the estrous cycle. From each ovary, cortical fragments and small antral follicles (3–5 mm) were excised. Moreover preantral ovarian follicles population was enzymatically isolated from ovarian cortex and snap-frozen in liquid nitrogen for RNA and protein isolation. Plasma FSH was determined using Enzyme ImmunoAssay kit. FSHR mRNA expression was assessed by qPCR, while for FSHR localization immunohistochemistry was performed. Data were analyzed using Mann-Whitney U-test. MXC caused a decrease ($P<0.05$) in plasma FSH concentration and decrease in the expression of FSHR mRNA in small antral follicles ($P<0.001$) as compared with control. In both MXC-treated and control animals, FSHR has been localized in granulosa cells and in cytoplasm of oocytes in preantral follicles as well as in granulosa cells of small antral follicles. Concluding, neonatal exposure to MXC induces changes in FSH level and FSHR expression in small antral follicles in adult pigs, which may affect folliculogenesis.

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Session 3

AHR/ER CROSS TALK IN PAH MIXTURES ACTION ON CELL PROLIFERATION AND HORMONE SECRETION BY HUMAN GRANULOSA CELLS

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What is known already: Effects of PAHs in reproductive tissues could reflect their multiple modes of action, like activation of AhR-dependent metabolism of PAHs, ability of PAHs to activate ER, as well as endogenous hormones secretion. Strong evidence supports a role of the AHR in regulating the ovarian follicle growth of folliculogenesis. The research goal of the present study was to investigate mechanism of PAH mixture action on noluteinized granulosa cells, and to explore the possible involvement of AhR-dependent or AhR/ER/AR cross talk in its estrogenic/antiestrogenic effects.

Study design, size, duration: As a model we used HGrC1 cells possess the characteristics of granulosa cells in early stage follicles. We measured the concentrations of all 16 PAHs, which were identified as priority pollutants in maternal and cord blood. The analysis showed similar content of these compounds in both, suggesting that exposure to PAHs, during fetal life and in the postnatal period may contribute to abnormal ovarian function. The cells were exposed to the mixture 1 composed of all cancerogenic and non-cancerogenic compounds (M1) and mixture 2 (M2) composed of the five non-cancerogenic PAHs observed at the highest levels in maternal and cord blood. Activation of AhR, ARNT, AhRR, AR, ER, CYP1A1, COMT and CYP19 protein expression under the influence of both mixture has been evaluated. As an end point action on basal and FSH stimulated estradiol secretion and cell proliferation was determined.

MAIN results: M1 increased AhR and ARNT, parallel with a decreased in AhRR expression while, M2 had no effect on AhR and AhRR parallel with increased ARNT protein expression suggesting canonical in the case of M1 and non-canonical in the case of M2 mechanism of action. Additionally, both mixtures increased ERα and only M2 increased AR protein expression. Both mixture had no effect on FSHR expression however, had stimulatory effect on both basal and FSH stimulated cell proliferation parallel with inhibitory effect on E2 secretion and CYP19 protein expression. Gene silencing, confirm involvement of AhR, ER and AR in this action.

Conclusion: Naphthalene, phenanthrene, anthracene, fluoranthene, and pyrene present in the mixture as a new class of mixed AhR/ER agonists are

responsible for antiestrogenic action of mixture and can exert their effects at pituitary-gonadal axis to alter reproductive endocrine function.

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Session 4

MATERNAL-FETAL ADAPTATIONS DURING PREGNANCY

Prof. Dr. hab. Dorota Lechniak-Cieślak
Dr. Jacek Wawrzykowski

Session 4

EFFECT OF APELIN ON THE ENDOCRINE FUNCTION OF THE HUMAN PLACENTA CELLS

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Adipose tissue has many important functions in the human body, one of them is the ability to secrete hormones (adipokines); an example of them is apelin. It was first isolated in 1998 from extracts of bovine stomachs as an endogenous ligand of the previously identified APJ receptor. A number of studies have been conducted on the role of apelin in the regulation of cardiovascular function, water-electrolyte management, cell proliferation and apoptosis, and the course of angiogenesis. Our last study documented apelin/APJ expression in human placenta cells. However, effect of apelin on placental hormones secretion is still unknown.

The aim of this study was to investigate the effect of the apelin on progesterone (P4), estradiol (E2) and chorionic gonadotropin (hCG) secretion as well as steroidogenic enzymes 3βHSD and CYP19 protein expression in BeWo cell line. Apelin at doses: 0.02, 0.2, 2, 20 and 200 ng/ml were incubated for 24, 48 and 72 h. Then steroid hormones and hCG were tested using ELISA kits, while protein expression of steroidogenic enzymes by Western Blot. Statistical analysis were performed using GraphPad Prism 5 and a one-way ANOVA test.

We demonstrated that apelin significantly decreased both P4 and E2 secretion by inhibitory action on 3βHSD and CYP19 protein expression. Our preliminary data documented that apelin also have inhibitory effect on hCG. In conclusion, our findings indicate that apelin should be new considered a newly identified regulator of placental endocrinology; however molecular mechanism confirming presented results should be taken to consideration.

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Session 4

DETERMINATION OF G PROTEIN-COUPLED RECEPTOR 1 (GPR1) GENE AND PROTEIN EXPRESSION IN THE PORCINE ENDOMETRIUM DURING EARLY PREGNANCY AND THE OESTROUS CYCLE

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Chemerin belongs to the group of adipocyte-derived hormones. The hormone is known mainly from its role in the regulation of metabolism *via* food intake, energy homeostasis and insulin sensitivity regulation. Chemerin acts *via* three distinct receptors: chemokine like receptor 1 (CMKLR1/ChemR23), G protein-coupled receptor 1 (GPR1) and C-C chemokine receptor-like 2 (CCRL2). Growing body of evidence indicates that chemerin may be involved in the regulation of the female reproductive system, including the uterus. We hypothesize that the porcine endometrium may be sensitive to chemerin. Therefore, the present study analyses the expression of chemerin receptor, GPR1, at the gene and protein expression during the oestrous cycle: on days 2 to 3 (early luteal phase – formation of corpus luteum), 10 to 12 (mid-luteal phase – fully active corpora lutea), 14 to 16 (late luteal phase – luteolysis) and 17 to 19 (follicular phase), and early gestation: on days 10 to 11 (transuterine migration of embryos), 12 to 13 (maternal recognition of pregnancy), 15 to 16 (implantation), 27 to 28 (end of implantation) and 30 to 32 (placentation). The analysis of gene and protein expression were conducted using real-time PCR and Western Blot methods, respectively. Data were analysed using one-way ANOVA. During the oestrous cycle, the highest expression of *GPR1* gene was observed on days 14 to 16 of the cycle, whereas the lowest on days 2 to 3 and 17 to 19. The concentration of GPR1 protein during the oestrous cycle was the highest on days 14 to 16 of the cycle and did not differ between the other oestrous cycle periods. Comparing early gestation period and days 10 to 12 of the cycle, the highest expression of *GPR1* gene was observed on days 10 to 11 of pregnancy, lower on days 10 to 12 of the cycle, whereas the lowest, on days 27 to 28 and 30 to 32 of pregnancy. The highest content of GPR1 protein was observed on days 10 to 12 of the cycle, lower on days 10 to 11, 12 to 13 and 30 to 32 of gestation, and the lowest

on days 12 to 13 and 27 to 28 of pregnancy ($P<0.05$). This is the first study to indicate the expression of GPR1 in the porcine endometrium during the oestrous cycle and early pregnancy. The above data indicates that GPR1 is present in the porcine endometrium, which suggest its sensitivity to chemerin. Moreover, the observed changes in GPR1 expression varied across the examined periods of the cycle and pregnancy suggesting the dependence on the local hormonal milieu.

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Session 4

EXTRACELLULAR MATRIX PROTEINS DURING PREGNANCY AND AT PARTURITION IN PLACENTA OF COWS

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The objective of these preliminary studies was to detect the presence of selected non-collagenous extracellular matrix (ECM) proteins, such as decorin (DCN), glycodelin (PAEP) and dermatopontin (DPT) in bovine placental tissues during pregnancy and at parturition.

During the course pregnancy and periparturient period, characteristic changes in the composition and distribution of the ECM components are observed. We suspect, that DCN, PAEP and DPT may be associated with the maturation of the placenta and pregnancy development in cows due to their functions. DCN is involved the formation of collagen fibers during fibrillogenesis and has anti-adhesive properties. DPT interacts with DCN, accelerates collagen and fibronectin formations and contrary to DCN enhances cell adhesion. PAEP regulates fertilization, implantation, and placentation and exhibits negative influence on trophoblast invasion.

Placental tissues from healthy pregnant cows (2–4 months) were collected in slaughter house, while parturient tissues were obtained during caesarian section at term (n=9, 2 or more in each time point). Maternal and fetal parts of placenta were separated manually, subjected to homogenization and electrophoretic separation on polyacrylamide gels (16.5%) (Laemmli, 1970). The presence of examined proteins was verified by Western Blotting Towbin (1979) with antibodies for DCN sheep polyclonal anti-decorin antibody (ab35378, abcam), for DPT rabbit polyclonal anti-dermatopontin antibody (ab118710, abcam) and for PAEP rabbit polyclonal anti-human PAEP antibody (102-12554, RayBiotech).

Western blot analysis confirmed the presence of DCN with molecular mass of 40 kDa in all examined time points within fetal part of the placenta, whereas in maternal part DCN was merely at parturition. DPT was detected as a 24 kDa band in 2nd, 3rd and 4th month of pregnancy and at parturition both in fetal and maternal tissues. The expression of 17 kDa PAEP was noted in the 2nd month of pregnancy (fetal part) and in 4th month of pregnancy (maternal part). In addition, a highly glycosylated form of PAEP with molecular mass of 55 kDa was detected in whole period from 2nd month to delivery considering maternal part of the placenta. Within

fetal tissue there was no 55 kDa PAEP at parturition compared to pregnancy period, however in this time point 10 kDa fragments of the protein appeared.

Further studies confirming the role of examined proteins in bovine placenta are necessary.

Session 4

THE EXPRESSION OF CHEMOKINE-LIKE RECEPTOR 1 (*CMKLR1*) GENE AND PROTEIN IN THE PORCINE ENDOMETRIUM DURING THE OESTROUS CYCLE AND EARLY PREGNANCY

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Chemerin is secreted as a protein precursor – prochemerin. Active protein can be bound by three receptors: chemokine-like receptor 1 (CMKLR1), G protein-coupled receptor 1 (GPR1) and C-C chemokine receptor-like 2 (CCRL2). Chemerin can act pro-inflammatory and anti-inflammatory, and also participate in the regulation of metabolism and reproductive system. It seems that chemerin may be a link between the metabolic state of the body and reproductive ability. The research hypothesis assumes that *CMKLR1* gene and protein expression in the porcine endometrium depends on the phase of the cycle and/or pregnancy. The aim of this study was to determine the expression of *CMKLR1* gene and protein in the porcine endometrium (n=5) during the oestrus cycle: on days 2 to 3 (early luteal phase – corpus luteum formation), 10 to 12 (mid-luteal phase – fully functional corpus luteum), 14 to 16 (late luteal phase – phase of luteolysis) and 17 to 19 (follicular phase), and during early pregnancy on days: 10 to 11 (transuterine migration of embryos), 12 to 13 (maternal recognition of pregnancy), 15 to 16 (implantation), 27 to 28 (end of implantation) and 30 to 32 (placentation). To determine the expression of *CMKLR1* gene and protein were used real-time PCR and Western Blot analysis, respectively. Statistical analyses were performed using one-way ANOVA. During the oestrous cycle, in the endometrium, the highest *CMKLR1* gene expression was observed on days 17 to 19, while the lowest on days 14 to 16. The highest *CMKLR1* expression was noted on days 30 to 32 of pregnancy when compared to days 10 to 11, 15 to 16 and 27 to 28 of pregnancy, and days 10 to 12 of the cycle, whereas the lowest on days 27 to 28 of gestation in relation to other stages of pregnancy. The highest CMKLR1 protein content in the endometrium during the oestrous cycle was observed on days 14 to 16 compared to other phases of the cycle. The highest expression of CMKLR1 protein was observed on days 10 to 12 of the cycle and 30

to 32 of pregnancy when compared to days 10 to 11 and 12 to 13 of gestation. Presented data indicate, for the first time, the expression of CMKLR1 in the porcine endometrium which suggest that this tissue may be sensitive to chemerin. Changes in *CMKLR1* gene and protein expression during the oestrous cycle and early pregnancy suggest its dependence on the physiological status of the animals.

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Session 4

SECRETION PATTERNS OF EXTRACELLULAR VESICLES DURING EARLY PREGNANCY IN THE PIG – *IN SITU* TRANSMISSION ELECTRON MICROSCOPY STUDY

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Establishment of pregnancy in mammals involves activation of numerous cellular processes, mediated by outnumbered molecular pathways. In the pig, the process of embryo implantation starts after contact of conceptus with the uterine luminal (LE) or superficial glandular epithelium (GE), followed by apposition and adhesion of trophectoderm to the epithelia. Recent studies have highlighted the fact that maternal recognition of pregnancy and embryo implantation may rely on the processes not involving transcription and translation. The main role within this scenario is assigned to the extracellular vesicles (EVs). EVs represent double membrane covered vesicles released by cells, share surface receptors of the parental cells and transport several molecules (*e.g.*, mRNA and miRNA) to target cells. EVs can be generated *via* multiple biogenesis pathways and released from cells through different exocrine secretory mechanisms (*e.g.*, apocrine by membrane budding or merocrine by exocytosis). On the other hand, clathrin-maintained endocytosis is one of the routes of EVs uptake observed in target cells. In the present study, we used transmission electron microscopy (TEM), which is the most reliable method of characterization of EVs-mediated cell-to-cell communication, to show how EVs can be secreted from endometrial and trophoblast cells during early pregnancy in the pig.

Samples (uterus, trophoblast) were collected from crossbred gilts on day 12, 16, 17, and 20 of pregnancy [DP], fixed and resin embedded according to standard sample preparation protocol for TEM. Careful image analysis confirmed the presence of EVs in the lumen of uterine glands and multivesicular bodies located in the cytoplasm of LE, GE and trophoblast cells in all tested days of pregnancy. Large population of EVs of different size, attached to the plasma membrane of LE and trophoblast cells was identified. Closer look into apical cell surface revealed regions with apocrine and merocrine type of EVs' secretion in trophoblast cells and LE on 12 and 16 DP. Moreover, clathrin-coated pits were seen in trophoblast cells on 12 and 16 DP.

Our *in situ* TEM studies led us to conclude that in pigs EVs can be released through different mechanisms of secretion form both epithelial and trophoblast cells during early pregnancy. Moreover, accumulation of EVs on the surface of LE and trophoblast cells is a sign of ongoing cell-to-cell communication during the maternal recognition of pregnancy and embryo implantation.

Session 4

MEASUREMENTS OF CIRCULATING PROGESTERONE AND ESTRONE SULFATE CONCENTRATIONS AS A DIAGNOSTIC AND PROGNOSTIC TOOL IN PORCINE PREGNANCY REVISITED

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The main goal of this study was to examine the utility of measuring systemic concentrations of steroid hormones, namely progesterone (P_4) and estrone sulfate (E_1S), for monitoring the progression of porcine pregnancy and predicting sow fertility. There were three subsets of artificially inseminated (AI'd) sows used in the present experiments: i. animals sacrificed on gestational day 20 (gd20; n=16) or ii. gd50 (n=16; Experiment 1), and iii. animals maintained throughout pregnancy (n=24; Experiment 2). Blood samples (10 ml) were drawn from the orbital sinus and the endocrine data determined at different time points (around ovulation/artificial insemination (gd0=1st AI, gd1=2nd AI, and gd2) and maternal recognition of pregnancy (gd11) as well as on gd20 and gd50 (during two periods of increased embryonic/fetal mortality in swine) were examined for correlations with the numbers of healthy, arrested and reabsorbing embryos (Experiment 1) or with the number of live, stillborn and mummified piglets recorded at farrowing (Experiment 2). No correlations were recorded between circulating concentrations of both steroids and the numbers of healthy, arresting or reabsorbing conceptuses on gd20 or 50 (Experiment 1). The number of corpora lutea (CL) was directly related to the number of healthy embryos/conceptuses on gd20 and 50 (r=0.71, P=0.007 and r=0.76, P=0.0007, respectively) and the number of arresting embryos on gd20 (r=0.54, P=0.05), and negatively correlated with the number of reabsorbing embryos on gd20

(r=–0.53, P=0.05). In Experiment 2, circulating P_4 concentrations on gd11 related directly to the number of live-born piglets (r=0.46, P<0.04). Systemic E1S concentrations on gd0, gd1, gd2 and gd50 were correlated with the number of mummified conceptuses recorded at farrowing (r=0.50, P=0.03; r=0.59, P=0.01; r=0.48, P=0.04; and r=0.56, P=0.01, respectively) and plasma concentrations of E_1S on gd20 related directly to the number of stillborn piglets (r=0.60, P=0.02). In summary, the number of CL on gd20 and 50 is a reliable marker of embryonic/fetal pig status. Measurements of P_4 and E_1S on gd20 and 50 showed limited diagnostic value (i.e., were not indicative of the number of healthy and abnormally developing embryos/fetuses). However, measurements of circulating P_4 and E_1S concentrations during the periconceptional period and in the early/mid-pregnancy of sows have the makings of a practical method to predict gestational outcomes.

Session 4

UNIPARENTAL CONCEPTUS: TRANSCRIPTOME-WIDE INVESTIGATION OF GENOMIC IMPRINTING STATUS IN SHEEP PLACENTAE

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Genomic imprinting is an epigenetic phenomenon causing specific gene expression patterns in a manner characteristic to their parent of origin. Disturbances of genomic imprinting may lead to various placental disorders, like pre-eclampsia or intrauterine growth restriction. Imprint-free embryos are unable to create placental vascular network, which allows the transfer of nutrients and oxygen. The goal of this study was to reveal the effect of maternal and paternal genome on early placentation process in sheep (*Ovis aries*). This is a very adequate model for human pregnancy, in terms of similar physiology and placenta development. Transcriptome-wide sequencing (RNA-seq) was applied for identifying candidates of imprinted genes in ovine placentae, with paternal allele- and maternal allele- specific expression pattern. The investigation of imprinting concept was conducted on placentae of uniparental embryos, parthenogenotes (only maternal genome) and androgenotes (only paternal genome) collected at day 20 and 22 of sheep pregnancy, in comparison to biparental models, produced *in vivo*. RNA-seq study displayed dysregulation of imprinted genes: *PEG3* (P=1.06e-09), *PEG10* (P=3.98e-07), *SGCE* (P=2.71e-05), *MEST* (P=0.0002), *SNURF* (P=0.0002), *CDH18* (P=0.0004), *DLK1* (P=0.001), which were paternally expressed; whereas *SMOC1* (P=8.77e-05), *LDB1* (P=0.0001), *SALL1* (P=0.0003), were maternally expressed. According to available resources, genes *PEG3*, *MEST* and *DLK1* are already known to be paternally expressed in sheep, whereas seven of them are disclosed as new imprinted candidates, revealed for ovine placentae. The main functions of the imprinted genes included: mesoderm development; regulation of lipid storage; calcium ion binding; mediating cell

proliferation, differentiation and apoptosis; membrane organization; muscle organ development; calcium-dependent cell-cell adhesion; ureteric bud, kidney and ventricular septum development; ocular and limb development; hair follicle development; gastrulation with mouth forming second; negative regulation of transcription. The conducted whole-transcriptome investigation, represents a unique model for parent of origin genomic impact on early placentation of parthenogenetic and androgenetic models, which allow for better recognition of imprinting-related placental disorders.

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Session 4

APELIN/APJ EXPRESSION IN THE HUMAN PLACENTA CELLS AND IT'S STIMULATORY ACTION ON CELL PROLIFERATION VIA APJ AND DIFFERENT KINASES ACTIVATION

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Apelin is a bioactive peptide that was originally identified in bovine stomach extracts as the endogenous ligand of the orphan G protein-coupled receptor APJ. Apelin is one of the adipokines which has a pleiotropic effects; its role has been confirmed in the regulation of the cardiovascular system, fluid homeostasis or in carbohydrate metabolism. Recent studies show that apelin regulates steroid synthesis and proliferation in the ovarian cells. Moreover, higher serum apelin concentration was observed during first trimester of pregnancy. Nevertheless, expression and role of apelin in human placenta is not well understood.

The purpose of the study was firstly, demonstrate the expression of apelin/APJ in the human placental line BeWo, secondly investigate the dose-dependent effect of apelin on BeWo cells proliferation and then examine a molecular mechanism of apelin's effect on cell proliferation. We used BeWo cells, which reflecting villous cytotrophoblast. Apelin/APJ protein expression were measured using Western blot and immunocytochemistry. BeWo were incubated with apelin at different doses 0.02, 0.2, 2, 20 and 200 ng/ml for 24, 48 and 72 h, and then the proliferation was analysed by Alamar Blue test and Cell Counting Kit. Activations of extracellular signal-regulated kinases (ERK1/2), phosphatidylinositol 3 kinase/Akt (Akt), 5'-monophosphate-activated protein kinase (AMPK), signal transducer and activator of transcription 3 (Stat-3) kinases were tested by Western blot after 1–90 min of cells incubation with apelin (2 ng/ml). Using specific inhibitors of the mentioned above kinases and APJ we investigated cell proliferation. Statistical analysis were performed using GraphPad Prism 5 and a one-way ANOVA test.

We demonstrated significantly higher expression of APJ than apelin in placenta cells. Immunocytochemistry analysis shown strong localization of APJ in cell membrane, while apelin exhibited weak localization only in the cytoplasm. We

showed that apelin significantly stimulates cells proliferation via APJ and ERK1/2, AMPK and Stat-3 kinases activation. In conclusion, expression and stimulatory effect of apelin on placenta cell proliferation indicated important role of this adipokine in placenta physiology and should be considered a newly identified regulator of placenta development.

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Session 4

miR-23b-3p INHIBITS MIGRATION AND STIMULATE PROLIFERATION OF JEG-3 HUMAN TROPHOBLAST CELL LINE

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microRNAs (miRNAs), small molecules with a great potential for regulation of gene expression are crucial for many physiological and pathological processes. In humans, at least 30% of genes were shown to be regulated by miRNAs. Lately, aberrant miRNAs expression has been linked to various pregnancy complications. For example, miR-23b-3p was found among the miRNAs overexpressed in endometrium of patients with repeated implantation failure. It was suggested that increased level of miR-23b-3p in endometrium is associated with human embryo implantation defects, but mechanism still remains unknown. Thus, we decided to assess the effect of endometrial miR-23b-3p overexpression, acting in a paracrine manner, on human trophoblast cells. We delivered miR-23b-3p mimic by lipofection to human trophoblast tumour cell line JEG-3 and assessed its effect on the processes relevant to early pregnancy.

At first, we used *in silico* analysis using Ingenuity Pathway Analysis tools to show that miR-23b-3p targets can be involved, *i.a.* in apoptosis of embryonic cells (P=4.27E-05), proliferation of embryonic cell lines (P=0.001), migration of cells (P=0.0002) or growth of embryo (P=0.0003). To determinate the effect of miR-23b-3p on trophoblast cells migration, JEG-3 cells seeded on 24-well plates were transfected at about 80% confluency with 50 nM miR-23b-3p or control mimic for 12 hours. Once cells reached 100% confluency scratch was made and healing process was observed using a time-laps microscopy for 24 hours. Analysis of cell mobility ratio revealed decreased migration of miR-23b-3p-treated cells when compared to control mimic (P=0.0007; n=7). In the proliferation assay, cells were seeded on 96-well plate and transfected with 50 nM miR-23b-3p or control mimic. After 12 hours, CellTiter Proliferation Assay was performed. Positive effect of miR-23b-3p on JEG-3 cells proliferation was observed (*vs.* control mimic; P=0.0260; n=6).

Proper embryo implantation is dependent upon the proliferation, differentiation, migration and invasion of the trophoblast cells, strictly controlled by a variety of biomolecules. Any perturbation in expression of key factors controlling above mentioned processes can lead to pregnancy failure. Based on the results

presented herein, we suggest that miR-23b-3p, overexpressed in endometrium of patients with implantation failures, in a paracrine manner can affect expression of genes crucial for trophoblast cells function and survival, inhibiting their migration and stimulating their proliferation, what eventually could lead to pregnancy complications.

Session 4

ANALYSIS OF GLYCOSYLATION PROFILE OF MEMBRANE PROTEINS IN PLACENTA OF COWS DURING PREGNANCY AND PARTURITION

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The aim of this work was to assess the glycosylation profile of membrane proteins in bovine placenta during pregnancy and parturition.

Majority of membrane proteins of placental cells are N-glycosylated. N-glycosylation is a post-translational modification that can affect the spatial structure of proteins and thus their function and biological activity. Glycosylation processes can significantly affect the interaction of membrane proteins with other molecules and regulate adhesion, signal transduction or intercellular communication.

Protein profiles of placenta as well as plasma of cows in perinatal period have been partially described but this description does not include full profile of glycosylated proteins except from few individual glycoproteins.

Placental samples from healthy cows (3–5 months of pregnancy) obtained from slaughterhouse (n = 6, 3 on each time point) were separated into the maternal and fetal parts. At the time of parturition, tissues were collected during routine cesarean section (n=6).

Membrane proteins were separated using a membrane protein extract kit (Thermo Scientific ™ Subcellular Protein Fractionation Kit for Tissues). Separation of proteins in polyacrylamide gels was made using the technique of 1D and 2D-PAGE. After electrophoresis, the gels were stained for glycoproteins (Thermo Scientific ™ Pierce ™ Glycoprotein Staining Kit) and total protein (silver staining).

Staining specific for glycosylated proteins showed 5 fractions in placental samples from pregnancy and during parturition. Significant differences in intensity of staining were detected at 145, 120, 100 kDa during pregnancy as well as 52 and 41 kDa during pregnancy and parturition.

2D-PAGE analysis identified 456 membrane proteins in fetal part and 528 in maternal part of placenta. Statistical analysis (ANOVA $P<0.05$) showed significant differences between the following pairs: fetal placenta – 45 proteins between 3 and 4 months, 65 – between 3 and 5 months, and 52 – between 3 months and parturition. Maternal part: – 78 proteins between 3 and 4 months, 62 – between 3 and 5 months and 72 – between 3 months and delivery.

A proteomic map of membrane proteins was prepared with an indication of identified glycosylated proteins during pregnancy and delivery in cows. This map

shows dynamic changes during examined period of time. The results may serve for the identification of markers of pregnancy course and more complete description of the mechanism of placental cell adhesion.

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Session 4

GENOME-WIDE METHYLATION PROFILE OF PLACENTAE AND FETAL TISSUES DEVELOPED FOLLOWING ASSISTED REPRODUCTIVE TECHNOLOGIES

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Assisted Reproductive Technologies (ART) have contributed to the birth of over 5.4 milion babies worldwide. Evidence from human and animal studies showed that embryo adaptations, caused by sub-optimal *in vitro* conditions and/or manipulation, may cause epigenetic defects which may contribute to increased risk of diseases in adulthood. Aim of this study was to assess whether Blastomere Biopsy (BB), consisting in the removal of one cell from developing embryos to assess genetic normalcy before transfer, is a risk factor for the occurrence of epigenetic defects in placentae and fetal tissues. To this aim, mouse conceptuses at 18.5-day post coitum were obtained following BB, *in vitro* culture (IVC – embryos culture *in vitro* but not manipulated) and embryo transfer (ET- that is 3.5 dpc embryos transferred to recipient females). In vivo control group (CTR) consisted of naturally conceived pregnancy. At 18.5 dpc, placentae, fetal brain and liver were collected. At first, we found reduced fetal weight in BB fetuses vs IVC, ET and CTR ($P<0.05$) and placental overgrowth in BB vs CTR ($P<0.05$). Similarly, placental overgrowth has been observed in both IVC and ET vs CTR ($P<0.05$). Placentae, fetal livers and brains were analyzed by Reduced Representation Bisulfite Sequencing (RRBS). RRBS showed reduced content of methylated CpGs in BB placentae and fetal liver vs CTR ($P<0.05$), while comparable level of global methylation was observed in fetal brains ($P>0.05$). Reduced content of methylated CpGs was also observed in IVC and ET placentae versus CTR ($P<0.05$) and in IVC fetal liver versus CTR ($P<0.05$). In conclusion, our study showed that the occurrence of global epigenetic changes in placentae and fetal liver was mainly caused by IVC and/or ET, rather than BB.

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Session 5

MOLECULAR ANDROLOGY – THE COGNITIVE AND APPLICATIVE ASPECTS

Dr. hab. Katarzyna Knapczyk-Stwora
Dr. hab. Jolanta Opiela

Session 5

TESTICULAR EXPRESSION OF NECTIN FOLLOWING SHORT-TERM POSTNATAL EXPOSURE TO FLUTAMIDE IN ADULT RAT

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Spermatogenesis is regulated by testicular cell-cell interactions via specialized junctions. Cell-cell junctions between Sertoli cells and Sertoli cells and germ cells create a specialized microenvironment, through the formation of the blood-testis barrier (BTB). Nectin-2 is a calcium-independent cell-cell adhesion molecule that is one of the BTB components. The aim of this study was to investigate the expression of nectin in testis of adult rat after short-term postnatal treatment with anti-androgen flutamide compared to the respective control.

Flutamide (50 mg/kg bw) was injected into 82-days old Wistar rats every day in six doses. Testis samples from 90-day old control (n=8) and flutamide-treated rats (n=12) were used for all analyses. For routine histology and semi-thin sections, hematoxylin-eosin (H-E) and methylene blue stainings were performed. To measure relative transcript level for nectin qRT-PCR analyses were performed. Expression and localization of nectin protein were detected by western blot and immunohistochemistry, respectively, and characterized using qualitative and quantitative analyses. Each variable was tested by using the Shapiro-Wilk W-test for normality. Homogeneity of variance was assessed with Levene's test.

We did not find any morphological alterations in the seminiferous epithelium, however enlargement of the interstitial tissue was observed after flutamide exposure. Flutamide caused a statistically significant decrease in nectin expression at the mRNA ($P<0.05$) and protein level ($P<0.01$) as revealed by qRT-PCR and western blot analyses. Moreover, immunohistochemical analysis revealed decrease in the intensity of the staining for nectin protein ($P<0.05$) compared with control.

The results of this study showed that flutamide affects the expression of junction protein – nectin. The observed changes may have adverse effects on the homeostasis of the BTB and spermatogenesis.

Supported by a grant K/ZDS/008061.

Session 5

IMPACT OF ESTROGEN-RELATED RECEPTOR (ERR) KNOCK DOWN ON EXPORTIN 5, DICER, DROSHA AND ARGONAUTE 2 EXPRESSION IN BANK VOLE (*MYODES GLAREOLUS*) LEYDIG CELLS *IN VITRO*

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Our previous findings showed that expression pattern of the estrogen-related receptors (ERRs) in mouse Leydig cell depends on cell origin and cell physiological status. ERRα and ERRγ exhibited opposing regulatory properties in Leydig cells *in vitro* and *in vivo*. In addition, these receptors were regulated by xenoestrogens in mixture. Herein, we demonstrate ERR implication in epigenetic regulation of bank vole Leydig cell function. The bank vole is seasonally breeding rodent in which sex hormones level (both androgens and estrogens) is controlled by photoperiod.

ERRα and ERRγ were silenced *via* siRNA. Then the mRNA expression was examined, also protein expression and localization of molecules required for miRNA biogenesis and function (Exportin 5; Xpo5, Dicer, Drosha and Argonaute 2; Ago2) were analyzed. Additionally, global DNA methylation and histone deacetylation status together with estradiol secretion were determined.

In ERR-silenced vole Leydig cells, expressions of Xpo5, endonucleases and Ago2 were not changed. Independently of cell origin, immunolocalization of Dicer and Ago2 was either cytoplasmic and/or nuclear, while dependently on cell origin and ERR type knock-down localizations of Xpo5 and Drosha were always cytoplasmic. Absence of ERR affected cell methylation status (ERRα increased it; $P<0.01$ while ERRγ decreased it; $P<0.01$, $P<0.001$) but not affected histone deacetylases activity. ERRα and ERRγ silencing decreased ($P<0.01$, $P<0.001$) estradiol secretion by vole Leydig cells.

These novel observations provide a new insight into the role of ERRs that may be used as a molecular target for recognition and elaboration of epigenetic therapies in patients with estrogen level based testicular diseases.

This study was supported by a grant SONATA BIS5 2015/18/E/NZ4/00519 and partially by a grant OPUS12 K/PBO/000524 from National Science Centre, Poland.

Session 5

ANALYSIS OF GENE TRANSCRIPT EXPRESSION IN BOAR SPERMATOZOA DIFFERED IN FREEZABILITY

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In this study different software packages were used to analyze the differentially expressed (DE) gene transcripts in spermatozoa of the Polish large white (PLW) boar differed in semen freezability. Total RNA, isolated from spermatozoa of four boars with good (n=4) and poor (n=4) freezability each, was subjected to RNA-Seq. An average of 8 million reads per sample were obtained from paired-end sequencing (2 × 75 bp) on Illumina NextSeq 500 platform. RNA-Seq was mapped with the STAR software to the *Sus scrofa* 10.2 genome reference using AIR RNA-Seq data analysis software (Sequentia Biotech). Comparative analysis of expression of DE transcripts was performed with NOISeq, EdgeR and DESeq2 (Sequentia Biotech). KEGG pathway and Gene ontology (GO) terms were performed with the DAVID Tools. RNA-Seq analysis revealed a total of 10876 sperm transcripts, and most of the DE gene transcripts were significantly up-regulated (log2FC>1; FDR<0.05) in boars with poor semen freezability. The abundance of sperm transcripts varied among the software packages, being significantly higher with NOISeq. KEGG pathway showed that the DE gene transcripts were involved in Metabolic pathways and MAPK signaling pathway. DE gene transcripts related to catalytic activity were abundant in the GO molecular function category (*TRIT1* and *ATP6V0D2)*. Furthermore, GO biological process showed that the sperm transcripts were associated with the activation of transmembrane protein and cellular component assembly (*SMIM26* and *KIF3A*), whereas those related to plasma membrane were abundant in the GO Cellular component category (*BAMBI* and *PTPN2*). Among the DE gene transcripts detected in spermatozoa were those related to transcription factor activity (*NAFTC3*, *ING2* and *TFAP2C*), which are implicated in the regulation of gene expression. Transcriptome analysis provides valuable information about the expression profiles of gene transcripts of boar spermatozoa differed in freezability.

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Session 5

EFFECT OF MEDIA ON THE DNA INTEGRITY OF FREEZE-DRIED BOAR SPERMATOZOA: PRELIMINARY STUDY

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Lyophilization or freeze-dried (FD) sperm is a preservation method alternative to cryopreservation. However, when mammalian spermatozoa are freeze-dried, their motility is lost. The first successfully derived offspring from FD spermatozoa was reported in mouse (Wakayama and Yanagimachi, Nature Biotechnology, 1998). Recently, FD has been applied to several animal species. Many factors influence FD process, so it is necessary to find the strategies to control these factors, so as to maintain the sperm DNA integrity. The aim of this study was to examine the boar sperm DNA integrity which was freeze-dried in 2 media: TL-Hepes-PVA and TRIS. Both media were earlier used for FD in dog and cat spermatozoa. In addition, the impact of time storage was evaluated. Ejaculated sperm collected from 2 boars and diluted with Androhep extender were evaluated to assess sperm motility, centrifuged (17 min, 800 g, 17ºC) and equilibrated for 2 h in: TL-Hepes-PVA, TRIS and glucose/egg yolk (control) group. After equilibration, the semen (0.2 mL) was frozen in 2 mL vials and FD in Alpha 1-2 LD plus Lyophilizator (f. Martin Christ, Germany) for 18 h at 0.37 bar/-60ºC. The samples were stored at +4ºC for 7, 30 and 90 days. After rehydration, the motility of semen was evaluated microscopically and the DNA integrity was evaluated by flow cytometry using a sperm chromatin structure assay (SCSA). The data were analysed statistically by t-test. FD boar spermatozoa after rehydration were immotile. No significant differences were observed between experimental and control groups in the level of DNA damage of boar lyophilized semen stored for 7, 30 and 90 days. In conclusion, the results suggest that both media TL-Hepes-PVA and TRIS preserve DNA integrity of boar sperm during freeze-drying and storage for a short and long-term (90 days).

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Session 5

THE EFFECT OF ANDROGEN SIGNALING DISRUPTION ON NOTCH PATHWAY IN PERIPUBERTAL RAT TESTIS – AN *IN VIVO* STUDY

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In the testis, contact-dependent intercellular interactions are crucial for maintaining homeostasis and proper conditions for spermatogenesis. The canonical Notch pathway involved in contact-dependent signaling is an important regulator of proliferation, differentiation, and cell fate decisions in several tissues, including testis. Expression patterns of Notch pathway components show dynamic changes in rodent testis during the postnatal life and along the spermatogenic cycle of adult male. Androgens are hormones mediating a wide range of physiological responses and developmental processes in male gonad. Although numerous studies have been performed to characterize processes controlled by androgen action in rat testis, the role of androgen signaling in the regulation of Notch pathway activity in seminiferous epithelium has not been investigated yet. Therefore, the purpose of this study was to examine the interplay between Notch pathway and androgen signaling in rodent testis.

The present study was performed on testicular tissue isolated from peripubertal (5-week-old) Wistar rats (n=6 each group). Flutamide (50 mg⁄kg b.w. daily for 7 days) or ethylene-dimethane sulphonate (EDS; a single dose of 75 mg/kg b.w.) treatments were employed to reduce androgen signaling in rat testis. Control animals were given corn oil as a vehicle. Expression of Notch pathway proteins (Notch1, Jagged1, Hes1 and Hey1) was analyzed using Western blot followed by statistical analysis (U-Mann-Whitney test). Immunohistochemistry was performed to detect localization of activated Notch1 receptor in the testis.

Western blot analyses revealed downregulation of activated Notch1 receptor ($P<0.01$), Hey1 ($P<0.01$) and Hes1 ($P<0.01$) effector genes expression in rat testis after flutamide and EDS when compared to the control. In contrast, Jagged1 ligand was upregulated ($P<0.001$) in flutamide- and EDS-treated rats. Immunohistochemical analysis revealed the presence of activated Notch1 in the nuclei of Sertoli cells, spermatocytes, round and elongated spermatids, and in the Leydig cells. In experimental rats the localization of Notch1 did not change, but signal intensity decreased in spermatids when compared to the control.

The results showed that androgen signaling blockade in rodent testis induces changes in the expression of Notch signaling proteins, which may indicate a significant role of androgens in the control of Notch pathway activity during peripubertal period.

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Session 5

CHARACTERISTICS OF FRESH AND CRYOPRESERVED EPIDIDYMAL SPERMATOZOA OF MUNTJAC (*MUNTJACUS REEVESI*)

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The Chinese muntjac (*Muntiacus reevesi*) belongs to Cervidae family. This species is found in Taiwan and southeast China. Most cervides species living in temperate latitude are seasonally reproduced. Whereas some tropical deers are asesonal and they are fertile throughout the year. However, the sperm concentration value in autumn and winter is higher than in summer. The characteristics of the muntjac semen is useful to support breeding program of this species and to protect them from extinction. In this study were used the cauda epididymal semen collected by *post mortem* from 19 to 21 November from four male muntjacs (N=4). Analyses included the assessment of sperm motility and concentration in fresh semen using the Makler chamber. The semen were packaget in 0.25-ml straws and cryopreservation in Bioxcell (Bioxcell® IVM) extender. After thawing the semen, sperm motility was determined using CASA system. The sperm concentration in fresh semen was within the range from $5x10^8$/ml to $5x10^9$/ml and sperm motility 20–78%. In frozen-thawed semen, sperm motility was 5–29%. The muntjac cauda epididymal semen is characterized by high sperm concentration and sperm motility and can be used for cryopreservation using Bioxcell extender. There is no the presence of sperm in individuals slaughter in July and September on the contrary to semen presence in males collected in November. The research will be continued towards the enzymatic characteristics of the semen and the expression of factors influencing reproductive processes of the male muntjac, such as assessment of the activity of selected antioxidant enzymes, DNA integrity, structural and functional sperm membranes and apoptotic-like changes.

Session 5

ANALYSIS OF MOTILITY AND SELECTED PARAMETERS OF THE ANTIOXIDANT STATUS OF EPIDIDYMAL SPERM OF RED DEER (*CERVUS ELAPHUS* L.) STORED IN ANDROMED® DILUENT

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The objective of the conducted studies was to evaluate the motility and the activity of selected antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) of epididymal spermatozoa of red deer stored in AndroMed® extender. The cauda epididymal semen harvested by *post mortem* from 6 male red deer (culled during routine hunts in the Nowe Ramuki Forest District) were used in this study. Semen was storage in AndroMed® extender (Minitüb GmbH, Germany) at 5ºC for 6 days. Analysis included the assessment of sperm motility using CASA system (Hamilton-Thorne Biosciences, USA) and the SOD, CAT, GPx activity (U/10^9 spz) were carried out by using a spectrophotometer. Sperm motility analysis were performed on the first day of storage (D1) and on the following days (D3, D5 and D6), while the antioxidant activity of enzymes was determined on D1, D3 and D6.

In the studies, a significant decrease in sperm motility was observed after three days of storage. Significant ($P \leq 0.05$) differences in the analysis of the percentage of sperm showing movement (TMOT,%) were noticed between days D1 and D5 and these values were 88.2 ± 2.5 and 49.5 ± 8.1, respectively. Similar relationship was found in case of the PMOT analysis – these values were 50.2 ± 2.4 (D1) and 25.3 ± 5.5 (D5) respectively. The results concerning the antioxidant status revealed that the activity of SOD, CAT and GPx remained at a similar level to the D3 of storage and then decreased on the D6 of storage, however these changes were small and did not differ significantly ($P \geq 0.05$). The obtained results indicate that storage of the epididymal semen in the liquid state for 6 days significantly affects the motility and causes slightly changes in the antioxidant status of the red deer spermatozoa. Considering that motility is one of the main determinants of the quality

and suitability of semen for reproductive techniques, the AndroMed® diluent can be used most effectively to storage of red deer epididymal semen up to three days.

The research material was obtained under a cooperation agreement with the Nowe Ramuki Forest District.

The research was carried out as part of UWM 11.610.003-300 research.

Session 5

PROTEOMIC ANALYSIS OF STALLION SPERMATOZOA FOLLOWING LONG-TERM STORAGE IN LIQUID NITROGEN

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The study of sperm proteome provides valuable information about animal reproductive physiology, which is useful to improve fertility and semen preservation techniques. The aim of the study was to analyse changes in the proteome of spermatozoa varying in motility characteristics following long-term storage of stallion semen in liquid nitrogen (LN). Fresh ejaculates of 8 half-blood stallions were divided into two sperm motility groups: good and poor total motility (GTMOT and PTMOT, respectively). Ejaculates (n=9) were frozen and stored in LN for 9 to 16 years, prior to post-thaw sperm motility assessment with the CASA system. Frozen-thawed (FT) spermatozoa were classified as good motility, TMOT >39%, PMOT >18% (GMOT) and poor motility, TMOT <39%, PMOT <18% (PRMOT). Sperm extracts were prepared with the RIPA buffer, and were used for 2D-PAGE analysis. The PDQuest software was used to analyze the gels. Electrophoretic analysis revealed 6 unique polypeptides with molecular weight (MW) range 13.0–15.9 kDa and an isoelectric point (pI) of 5.3–9.3 range in fresh spermatozoa with GTMOT, whereas 9 unique polypeptides with MW range 20.0–59.6 kDa (2.6–9.2 pI range) were detected in spermatozoa with PRMOT. Furthermore, 2D-PAGE analysis identified 45 and 55 polypeptides in FT spermatozoa with GMOT and PRMOT, respectively. A total of 11 unique polypeptides with MW range 10.0–63.5 kDa (3.2–9.6 pI range) were detected in FT spermatozoa with GMOT, whereas 6 unique polypeptides with MW range 10.1–74.5 kDa (3.1–6.6 pI range) were identified in the PRMOT group. The expression of 2 polypeptides with MW 13.8 kDa and 17.4 kDa (3.1 pI range, respectively) was more prominent in FT spermatozoa with PRMOT. The high abundance of polypeptides, detected in FT spermatozoa with poor motility, might be due to the degradation of sperm proteins as a result of increased oxidation process during prolonged LN storage. Among the proteins showing marked changes in their expression are those that are involved in the response to oxidation-induced damage and anti-sperm immunity.

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Session 5

OPTIMIZATION OF THE SELECTED ISOLATION AND IDENTIFICATION PROCEDURES OF THE STALLION EPIDIDYMAL FLUID PHOSPHOPROTEINS

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During the epididymal transit the male gametes undergo, among others, changes in proteins phosphorylation degree. Surveys focused on phosphoproteome are limited by both low degree of protein phosphorylation and low abundance of phosphoproteins in the whole proteome.

The aim of the study was to optimize the isolation and identification procedures of the stallion epididymal fluid phosphoproteins conducted with use of one (1D-PAGE) and two-dimensional (2D-PAGE) electrophoreses and mass spectrometry (MALDI-TOF/TOF) method.

Phosphoproteins were isolated using PhosSelect Iron Affinity gelbed (Sigma-Aldrich, USA). Electrophoretic separations were performed on 10, 12 and 15% gels with use of Mini Protean II apparatus (BioRad, USA). Gels were stained with different dyes (Coomassie Blue R-250, Coomassie Blue Silver or quercetin). Interested phosphoproteins (10) were cut out from the gels and underwent trypsin digestion (Trypsin Profile IGD Kit, Promega, USA). Then they were subjected to mass spectrometry with use of different matrixes (HCCA, DHB or DHAP/DAHC) on Autoflex III Smartbeam MALDI-TOF/TOF mass spectrometer.

Phosphoproteins with high (250–100 kDa) and medium (100–50 kDa) molecular weights were separated on 12% gels the most precisely, while these with low molecular masses (<50 kDa) on gels with 15% density. It should be emphasized that a sizeable group of stallion epididymal fluid phosphoproteins was represented by low-molecular weight polypeptides. The best results of visualizing proteins gave the staining with use of Coomassie Brilliant Blue R-250 as a dye. No differences in sensitivity were observed in comparison to Coomassie Blue Silver staining ($P \geq 0.05$). By contrast, direct staining with use of quercetin had not brought expected results. We suppose that there might have occured some specific interactions between quercetin and SDS. Mentioned interactions might have either influenced the strength of quercetin and phosphoprotein fractions binding or weakened the fluorescence emission signal of stained phosphoproteins.

Results obtained by mass spectrometry were various depending on the type of the matrix. All chosen phosphoproteins, however not always with satisfactory

score, were identified with use of DHB. The DHAP/DAHC matrix solution was often subjected to induction and elimination from the plate. Results received after using DHAP/DAHC were similar to these obtained after usage of HCCA. Both matrixes should be not recommended in the course of identifying phosphoproteins.

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Session 5

THE ROLE OF DELTA-LIKE 4 AND JAGGED 1 IN THE REGULATION OF ANDROGEN RECEPTORS EXPRESSION IN MOUSE SERTOLI CELLS

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Spermatogenesis is a process controlled by male sex hormones, androgens. Numerous studies have considered Sertoli cells as major mediators of androgen action. Androgens act predominantly through the nuclear androgen receptor (AR) but they can also activate non-classical pathway by membrane receptor (ZIP9). Contact-dependent signaling, such as Notch pathway, also regulates seminiferous epithelium function. Notch pathway is a conservative way of intercellular communication wherein membranous ligand Delta-like (DLL) or Jagged (JAG) of one cell activates surface receptor Notch of the neighboring cell. The aim of this study was to explore the role of DLL4 and JAG1 in the regulation of AR and ZIP9 expression in TM4 mouse Sertoli cell line.

To perform ligand activation experiment TM4 cells were seeded into cell culture plates coated with recombinant ligands (rDLL4 or rJAG1) or bovine serum albumin as a control. After 24 hours cells were treated with Notch pathway inhibitor N-S-phenylglycine-t-butyl ester (DAPT; 25 and 50 μM) or a vehicle (dimethyl sulfoxide). Analysis of AR, ZIP9, Notch1 intracellular domain (NICD), HEY1, and HES5 mRNA and protein expression was carried out by real-time RT-PCR or Western blot, respectively. Statistical analyses were performed by one-way ANOVA, followed by Tukey's *post hoc* test.

Exposure of TM4 cells to either rDLL4 or rJAG1 increased NICD level ($P<0.001$) and upregulated Hey1 ($P<0.05$) and Hes5 ($P<0.001$, $P<0.05$) expression, which indicates the activation of Notch pathway by both ligands. AR mRNA expression was upregulated following rDLL4-induced activation of Notch pathway ($P<0.05$), but the AR protein level did not change in these cells. Similarly, exposure of Sertoli cells to rJAG1 did not affect AR expression. In contrast, ZIP9 mRNA and protein levels were clearly decreased after exposure to rJAG1 ($P<0.05$, $P<0.01$). DLL4 had no effect on ZIP9 expression. The addition of DAPT upregulated both AR and ZIP9 expressions ($P<0.01$; $P<0.001$), which confirms that Notch pathway is involved in the control of androgen receptors.

In summary, our results indicate that ZIP9 expression in TM4 cells is regulated by JAG1-mediated Notch signaling, whereas the regulation of AR expression by Notch pathway is DLL4- and JAG-independent. JAG1-Notch1 pathway may be therefore considered as a mechanism controlling membrane initiated androgen signaling in Sertoli cells.

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Session 5

DIFFERENCES IN SPERM PROTEINS PHOSPHORYLATION STATUS IN EVERY SEGMENT OF GOAT (*CAPRA HIRCUS*) EPIDYDIMIS

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It is widely known that phosphorylation/dephosphorylation processes are one of the most frequent posttranslational modifications regulating protein–protein activity in sperm. Many studies have demonstrated significant differences in sperm surface proteins and the mechanisms of protein modification during the epididymal maturation among various species so far.

The aim of this study was to analyze phosphoproteins profiles of spermatozoa derived from subsequent parts of buck epididymis.

Epididymides (n=6) were received after castration surgeries conducted during out of the breeding season. Obtained material was packed and transported to the laboratory in thermobox (5ºC). At first epididymides were centrifuged to separate epididymal fluid and then divided into three segments i.e. caput, corpus and cauda. Every part was washed with 0,85% NaCl and centrifuged at 2000 x g for 10 min threefold. Gained supernatants were transferred into new tubes and centrifuged at 10000 x g for 10 min in order to achieve sperm precipitation. Obtained pellets were diluted with 2 ml of RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 0,5% sodium deoxycholate, 0,1% SDS, pH=8.0). Phosphoproteins were isolated with use of PhosSelect Iron Affinity gelbed (Sigma-Aldrich, USA). Collected fractions were separated using Mini Protean II apparatus (BioRad, USA), stained with Coomassie Blue R-250 (BioRad, USA) and identified by mass spectrometry.

Separations performed by means of 1D-PAGE electrophoreses demonstrated approximately 20, 19 and 15 phosphoprotein bands in caput, corpus and cauda parts respectively. Phosphoproteins with molecular weight of 12,5, 11,5, 10,5 and 7,5 kDa underwent stronger phosphorylation in caput, whereas these with 33 and 28 kDa in corpus region. Phosphorylated polypeptides of about 74, 63 and 49 kDa were observed in both aforementioned parts of epididymis. In the cauda part there were a few polypeptides that were subjected to stronger phosphorylation i.e. 52, 27,5, 17, 16 and 14 kDa.

Separations conducted with use of 2D-PAGE method showed approximately 100, 80 and 50 spots in caput, corpus and cauda segments respectively. Some phosphoproteins appear to be constitutive as their presence was confirmed among all individuals. In caput that was 31, in corpus 19 and in cauda segment 5 phosphoproteins. The rest of the phosphoproteins profiles were diversified among individuals. The majority of proteins possessed molecular weights within the range of 80 to 20 kDa and pI from 4.0 to 8.0.

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Session 5

ESTROGEN-RELATED RECEPTORS AND G PROTEIN-COUPLED ESTROGEN RECEPTOR IN RODENT LEYDIG CELLS

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Estrogen-related receptors (ERRs) appeared to be important player in estrogen regulation. Three closely related members of ERRs (α, β and γ) were identified. ERRs share homology with estrogen receptors particularly in their DNA-binding and ligand-binding domains, DNA-response elements and target genes. In addition, G protein-coupled estrogen receptor (GPER), inducing rapid non-genomic estrogen responses, has been determined as a novel estrogen receptor. In physiology and pathology of reproduction the role and regulation of ERRs and GPER is still not fully discovered. The aim of the study was to examine the role of ERR and GPER in function of rodent Leydig cells.

In *in vitro* studies the primary Leydig cells and tumor mouse Leydig cells (MA-10) were used. In *in vivo* studies bank voles as well as mice were allotted into control and experimental groups (each group: n=5), receiving selective ERRα antagonist (XCT 790) (50 μg/kg bw) or selective GPER antagonist (G-15) (50 μg/kg bw). For routine morpho/histology, hematoxylin-eosin staining was performed. ERRs and GPER expression at mRNA level was examined by real-time qRT-PCR. Isolated tissues and cells were used for immunocyto-, immunohistochemical and Western blot analyses. Electron microscopic analysis of ultrathin testicular sections was performed with a JEOL 2100 HT TEM. Statistical analysis was performed on raw data using Statistica 10 software.

The expression of mRNAs and protein for ERRα, β, and γ was detected in primary and tumor Leydig cells. The expression of ERRs was always lower in primary Leydig cells. After ERRα blockage in bank vole testis enlarged interstitial tissue with Leydig cells was visible. The expression of mRNA for GPER was detected in immature, mature and aged mice. Depending on age, in G-15-treated mice, differences in structure and distribution of various Leydig cell organelles were observed.

In this study, we reported for the first time ERRs and GPER mRNA and protein expression in mouse and bank voles Leydig cells. Blockage of ERRα and GPER, respectively, resulted in morphological, ultrastructural and functional

changes of Leydig cells. The present data provides a new insight into the role of estrogen control in Leydig cell function.

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Session 5

ALTERED LEVELS OF JUNCTIONAL PROTEIN GENE EXPRESSION IN REPRODUCTIVE TISSUES ARE LIKELY RELATED TO THE APPEARANCE OF YELLOW SEMEN IN DOMESTIC TURKEYS

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It is well known that tight, adherens, and gap junctions are involved **in the regulation of** reproductive tissue function in male mammals. In birds, including domestic turkeys, intercellular interactions performed by junctional networks have not yet been studied. Furthermore, the cellular and molecular basis of yellow semen syndrome (YSS) in the turkey population remains poorly understood. Thus, the aim of the present study was twofold: first, to provide new information on the localization and expression of cell-cell junction proteins in the testis, epididymis, and ductus deferens of domestic turkeys, and second, to compare expression of junctional protein genes between two turkey population, one that produces white normal semen (WNS) and the other that produces yellow abnormal semen. Expression of *occludin*, *zonula occludens-1 (ZO-1), connexin 43 (Cx43), N-* and *E-cadherin*, and *β-catenin* genes were investigated using three complementary techniques: quantitative real-time polymerase chain reaction (qPCR), western blot, and immunohistochemistry. Alterations in localization and immunoexpression of junctional proteins were characterized using qualitative and quantitative analyses. All statistical analyses were performed using non-parametric Mann-Whitney U-test to determine which YSS values differed significantly from WNS values that served as controls.

Compared to WNS testis, epididymis, and ductus deferens, YSS tissues exhibited downregulation of occludin and β-catenin mRNA ($P<0.05$) and protein ($P<0.05$ and $P<0.01$, respectively) and upregulation of N- and E-cadherin mRNA ($P<0.001$, $P< 0.05$, $P <0.01$, respectively) and protein ($P<0.01$, $P<0.05$, and $P<0.05$, respectively). In contrast, ZO-1 and Cx43 mRNA and protein were upregulated in YSS testis ($P<0.05$ and $P<0.001$, respectively) but not in epididymis and ductus deferens; both mRNAs and proteins were downregulated ($P<0.05$) compared to the

respective WNS epididymis and ductus deferens. Altered staining intensity of immunoreactive proteins in YSS *versus* WNS reproductive tissue sections confirmed the gene expression results.

The present study is the first to demonstrate altered levels of junctional protein gene expression in reproductive tissues of male YSS turkeys. These findings may suggest that subtle changes in junctional protein expression affect the microenvironment in which spermatozoa develop and mature and thus may have an impact on the appearance of yellow semen in domestic turkeys.

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Session 5

THE EFFECT OF NATURAL AND PHARMACOLOGICAL AGENTS ADDITION ON THE QUALITY OF CRYOPRESERVED CANINE SEMEN

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Sperm cryopreservation is an assisted reproductive technique routinely used in canine species for genetic conservation. However, the DNA damages are still elevated during cryopreservation, limiting the fertilization rate. The present study was conducted to evaluate whether supplementation of semen extender with molecules modulating the metabolic activities can improve the quality of frozen-thawed canine spermatozoa. Two molecules: a synthetic, an antidiabetic drug – metformin and a natural polyphenol, an anthocyanidin in pigmented fruits – delphinidin, both known to modulate the mitochondrial metabolism and to limit the oxidative stress were evaluated. Semen samples were collected from 6 dogs Slovakian Hound, then pooled and cryopreserved with extender (Tris, citric acid, fructose and 20% egg yolk) and different combinations of metformin (50 μM and 500 μM) and delphidin (5 μM and 50 μM). After thawing, canine spermatozoa exposed to 50μM metformin increased the motility and rapid sperm analyzed by Computer-assisted sperm analysis in comparison to the control (57.0±5.3 vs. 40.6±4.5) and (34.4±3.3 vs. 21.4±3.9), respectively. In addition, delphinidin and metformin did not impact the membrane integrity, acrosome reaction or apoptosis rate. As expected, sperm metabolism was slightly modified by increasing the mitochondrial activity with metformin 50 μM and the NAD+ content, which plays a key role in mitochondrial function was higher with both ligands. Oxidative stress was decreased in both metformin or delphinidin conditions and was associated with a lower DNA damage. In conclusion, metformin and delphinidin are able to improve the quality of frozen-thawed semen when they are used during the cryopreservation procedure.

Session 5

ESTROGEN REGULATION OF THE INTERSTITIAL TISSUE IN BANK VOLE TESTIS

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Testicular interstitial tissue is composed of Leydig cells as well as macrophages, fibroblasts and telocytes (TC). The role of TC in the male gonad is scarcely know. Presumably, TC take part in maintaining tissue milieu and tissue contractile and secretory functions in various organs. Besides androgens, estrogens are important for proper function of testicular cells. Estrogen acts *via* nuclear estrogen receptors (ERs) and membrane G-coupled estrogen receptor (GPER). In addition, estrogen-related receptors (ERRs) are implicated in estrogen signaling interactions. These all receptors can exert genomic response and/or rapid signalling.

The aim of this study was to elucidate role of TC and their regulation by estrogens in the interstitial tissue of bank vole testis.

Bank voles were reared under one of two light cycles: long day (LD; 18 h light: 6 h darkness) or short day (SD; 18 h light: 6 h darkness). To gain a deeper insight into the ERR and GPER function in interstitial tissue, action of those receptors were blocked by GPER antagonist (G-15) and ERRα antagonist (XCT 790) (50 μg/kg bw).

XCT 790 and G-15 treatment increased interstitial tissue volume in both experimental groups. Additionally, ERRα blockage had a repercussion on localization and expression of steroidogenic proteins: lutropin receptor (LHR), translocator protein (TSPO), steroidogenic acute regulatory protein (StAR) and secretory proteins: insulin-like protein 3 (INSL3) and relaxin (RLN) in Leydig cells. Moreover, depending on the male age differences in localization of estrogen signaling molecules (ERs and cytochrome P450 aromatase) were observed after GPER-blockage. Based on ultrastructural, morphological and immunohistochemical [(IHC) for CD34 TC marker)] analyses, TC were detected in interstitial tissue. Telocytes number and distribution was changed after G15 treatment. Moreover, IHC and Western blot analysis of interstitial tissue confirmed expression and estrogen regulation of lipid balance molecules; leptin, adiponectin, and perilipin.

Our results demonstrated involvement of GPER, ERRs and their interactions with ERs in control of Leydig cell morphology, steroidogenic and secretory activities

as well as in control of TC number, distribution and regulatory functions in bank vole testis.

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Session 6

LOCAL REGULATION OF REPRODUCTIVE FUNCTIONS

Prof. Dr. hab. Grażyna Ptak
Dr. Marta Kieżun

Session 6

THE IMPACT OF EACs NEONATAL TREATMENT ON ERα AND GPR30 PROTEIN EXPRESSION IN ADULT PIG UTERINE

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During the early post-natal period in mammals development of uterine glands (adenogenesis) usually begins. In this process extensive cell proliferation of luminal epithelium causes budding of nascent glands. These critical events coincide with expression of estrogen receptor alpha (ERα) by nascent endometrial glands and stroma. Fully developed glands are responsible for sythesization and secretion complex array of enzymes, growth factors, cytokines, lymphokines, hormones and transport proteins. In pigs, first 10 days after birth are crucial for uterine glands development, so any abnormalities in this period can affect future reproductive abilities. Embryonic estrogens establish the signal for maternal recognition of pregnancy. Moreover, estrogens take part in regulation of epithelial proliferation which providing cells for secretory activity and morphological changes during early pregnancy. Due to that fact, uterine receptivity is crucial for embryo implantation and pregnancy development.

The aim of the study was to examine the effects of exposition to endocrine-active chemicals during neonatal period, on protein expression of ERα and GPR30 in adult pigs uterines. Neonatal pigs were injected with testosterone propionate (TP-20 mg/kg body weight (bw)), flutamide (Flu50 mg/kg bw), 4-tert-octylphenol (OP-100 mg/kg bw), ICI 182,780 (ICI-400 μg/kg bw) and methoxychlor (MXC-100 mg/kg bw,) between days 1 to 10 post partum (n=4 per each group) or corn oil (control). Uterine were collected from adult swines in their second estrous cycle. From each animal part of uterus was fixed for immunohistochemistry (IHC) and the other was frozen and used for Western blot analysis (WB). The FLU and OP treatments in neonatal period resulted in a significantly lower protein abundance of ERα compared to the control group, whereas in GPR30 protein no significant differences were observed. ERα was immunolocalized in the glandular epithelium (GE) of the uterus with differences in the intensity of staining.

Obtained data shows that expression of ERα in uterus of adult pigs is affected by EACs treatment in neonatal period, which is crucial for proper development of uterine glands. Changes in ERα expression may affect embryo implantation and the development of pregnancy.

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Session 6

INFLUENCE OF STEROIDS ON THE EXPRESSION OF MEMBRANE PROGESTERONE RECEPTORS IN THE BOVINE PLACENTA

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Progesterone (P4) affects on cells function by genomic mechanism through nuclear P4 receptors, and via nongenomic mechanism through the membrane P4 receptors. The expression of mRNA and protein for progesterone membrane receptor component (PGRMC) 1 and 2, PGRMC1 binding partner – SERBP1 (serpine mRNA binding protein) and membrane progestin receptor (mPR) α, β and γ was found in the bovine uterus and placenta. Since the regulatory mechanism of this expression is not explained we hypothesized that ovarian steroids can influence on gene expression for membrane P4 receptors in this organ.

In this purpose the placentome sections obtained from cows (n=5) in the II trimester of pregnancy were incubated for 6 and 24 h with: P4 (10^{-7}; 10^{-6}; 10^{-5} M), E2 (10^{-10}; 10^{-9}; 10^{-8} M) and P4 together with E2 ($10^{-7}/10^{-10}$; $10^{-6}/10^{-9}$; $10^{-5}/10^{-8}$ M) each in triplicate. The viability of tissue in the experimental conditions was determined by Alamar Blue test. After incubation, the concentration of P4, E2 and **prostaglandin E2** and F2α in medium and mRNA expression of studied genes were determined by an enzyme-immunoassay and Real Time PCR method, respectively.

Obtained data indicate that P4 in each of used doses and P4 (10^{-5} M) together with E2 (10^{-8} M) decreased ($P<0.05$) expression of mPRγ mRNA only after 6 h of incubation. There was no influence ($P>0.05$) of studied steroids on other genes expression after 6 and 24 h of incubation. These results suggest that P4 decreases expression of mPRγ, however E2 alone has no influence on expression of this receptor and it does not abolish the inhibitory effect of P4. Thus it is possible that P4 may down-regulate of mPRγ expression and this way influences on the placenta function.

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Session 6

ROLE OF ESTROGEN-RELATED RECEPTORS (ERRs) IN THE MAINTENANCE OF STEROIDOGENIC FUNCTION IN MOUSE ADRENAL CORTEX CELLS

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Estrogen-related receptors (ERRs) α, β and γ belong to the steroid hormone receptor family. These receptors play a key role in maintenance of lipid homeostasis and appear to be novel molecules implicated in interaction of estrogen signaling molecules. The aim of the study was to assess whether ERRs blockage or activation affects steroidogenesis through changes in the expression of steroidogenesis-regulating proteins in adrenal cortex cells.

Selective ERRα antagonist (XCT790) or ERRβ/ERRγ agonist (DY131) was injected (5μg/kg/every second day; five injections) into mature male mice. Additionally, human adrenocortical carcinoma cells (H295R) were treated with XCT790 or DY131 for 24h. For routine adrenal histology, hematoxylin-eosin staining was performed. To assess effects of XCT790 and DY131 on the expression of proteins: perilipin (PLIN), cholesterol side-chain cleavage enzyme (P450scc), steroidogenic-acute regulatory protein (StAR), translocator protein (TSPO), hormone sensitive lipase (HSL) and HMG-CoA reductase (HMGCR), western blot was carried out. Changes in protein localization were visualized by immunohistochemistry. Cholesterol content analysis were performed in both adrenals and H295R cells. For statistical analyses one-way ANOVA followed by the Tukey's *post-hoc* comparison test was used.

Histological analysis showed adrenocortical cell vacuolization after XCT790 exposition as well as inclusions of fibroblast-like cells after DY131. Western blot analysis revealed decreased expression of ERRα in adrenals and H295R cells after XCT790 treatment and increased expression of ERRβ and γ after DY131 treatment when compared to controls. In turn, in XCT790 treated adrenals increased expression of PLIN and decreased expression of StAR, TSPO, HSL was observed whereas in DY131 exposed ones increased expression of PLIN, P450scc, StAR and HSL and decreased of HMGCR expression was revealed. After ERRs blockage and activation, no significant changes in localization of steroidogenic proteins have been observed. Moreover, cholesterol content in adrenals and H295R

cells was decreased either after XCT790 or DY131 in comparison to respective controls.

To resume, ERRα, β and γ are emerging as important regulators of adrenal morpho-functional biology including their supervision of lipid homeostasis. Alterations of ERRs expression result in parallel perturbations in lipid homeostasis and steroidogenesis in cortical cells.

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Session 6

THE EFFECT OF NEONATALLY ADMINISTERED SEX STEROID AGONISTS AND ANTAGONISTS ON AMH-AMH RECEPTOR SYSTEM IN OVARIAN FOLLICLES AND AMH PLASMA LEVEL OF ADULT PIGS

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Recently we have shown that androgens and estrogens play crucial role in the assembly and further development of follicles in the neonatal porcine ovary. Anti-Müllerian hormone (AMH) is a marker of the population of small antral follicles and exerts inhibitory effect on primordial follicle recruitment as well as on the responsiveness of growing follicles to follicle-stimulating hormone. Therefore, the aim of the study was to determine the effect of neonatal exposure to sex steroid agonists and antagonists on plasma AMH level and expression of AMH and its receptor (AMHR) in preantral and small antral follicles of adult pigs. Animals were injected with testosterone propionate (TP, androgen activity, 20 mg/kg bw), flutamide (FLU, antiandrogen, 50 mg/kg bw), 4-tert-octylphenol (OP, estrogen activity, 100 mg/kg bw), ICI 182,780 (ICI, antiestrogen, 400 µg/kg bw) or corn oil (controls) between postnatal days 1 and 10 (n=4/each group). Blood samples were collected and ovaries were purchased from sexually mature gilts between days 8 and 11 of the estrous cycle. From each ovary, cortical fragments for enzymatic isolation of preantral follicles and small antral follicles (3–5 mm) were excised. Plasma AMH was determined using Enzyme ImmunoAssay kit. AMH and AMHR mRNA expression was assessed by qPCR, and AMHR was localized by immonohistochemistry. Data were analyzed using Mann-Whitney U-test. Only TP and FLU significantly decreased ($P<0.01$ and $P<0.05$, respectively) plasma AMH concentration. In preantral follicles, AMH mRNA was up-regulated by TP ($P<0.01$), while AMHR mRNA by TP ($P<0.001$) and FLU ($P<0.01$) when compared to control. In small antral follicles, TP ($P<0.05$) and FLU (0.05) increased, whereas OP ($P<0.01$) and ICI ($P<0.05$) decreased AMH transcript level. AMHR mRNA expression was up-regulated by FLU ($P<0.001$) and ICI ($P<0.05$) in comparison to

control. In all examined groups, AMHR was localized in the oocytes' cytoplasm of primordial follicles, oocytes' cytoplasm and granulosa cells of primary and secondary follicles, as well as granulosa and theca interna cells of small antral follicles. Overall, neonatal exposure to sex steroid agonists and antagonists may affect folliculogenesis in adult pigs *via* long-term effect on AMH level and AMH-AMHR system expression in preantral small antral follicles in adult pigs.

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Session 6

EFFECT OF APELIN, 17β-ESTRADIOL AND INSULIN-LIKE GROWTH FACTOR 1 TREATMENT ON OVARIAN CANCER CELL PROLIFERATION IN 2D AND 3D CELL CULTURE MODEL *IN VITRO*

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We previously demonstrated that apelin a bioactive adipocytokine with the serum concentration of 1.31 ± 0.12 ng/ml is expressed by epithelial ovarian cancer cell lines and acts as a mitogenic factor in these cells. Studies focused on adipokines and cancer etiopathogenesis have shown that alterations in adipokines expression affect cell proliferation, apoptosis, tumour invasion, and angiogenesis. Thus, we analyzed whether apelin can interact with two well know key regulators of ovarian cell functions: 17β-estradiol (E2) or insulin-like growth factor 1 (IGF-1) and regulate ovarian cancer progression.

Cell culture was performed in traditional two-dimensional (2D) monolayer model as well as 3D spheroid culture to better reflect cell-cell communication. The human ovarian serous carcinoma cell line OVCAR-3 were obtained from the ATCC. Cell proliferation was measured using the CellTiter-Glo Luminescent Cell Viability Assay (Promega). Basal expression of apelin was analyzed by real-time PCR (TaqMan Gene Expression, Applied Biosystems). Statistical analysis was performed using one-way ANOVA (Tukey's test, $P<0.05$).

Consistent with previous results, we found that in 2D cell culture apelin (2 ng/ml), E2 (1nM) and IGF-1 (100 ng/ml) stimulate OVCAR-3 cell proliferation (115%, 112% and 118%, respectively; $P<0.05$). Effect of tested compounds were similar in 3D model (apelin – 112%, E2 – 114%, IGF-1 – 112%; $P<0.05$). The combined treatment of apelin and IGF-1 does not change the proliferative effect of these compounds in monolayer and spheroid cell culture. However, treatment of E2 and apelin together decreased cell proliferation to the control cell level in both 2D and 3D model (99% and 102%, respectively; $P<0.05$). Moreover, we assessed that basal apelin transcript level was 1.0 RQ and 0.9 RQ in 2D and 3D model, respectively.

Taken together, our results show that apelin reverses E2 stimulatory action on OVCAR-3 cell proliferation, in the same way in 2D and 3D model of cell culture.

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Session 6

EFFECT OF NEONATAL ANDROGEN AND ANTI-ANDROGEN EXPOSURE ON THE REGULATION OF PORCINE LUTEAL FUNCTION – INSIGHTS FROM A TRANSCRIPTOMIC APPROACH

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Corpus luteum (CL) is a transient endocrine gland, which is comprised of steroidogenic cells, fibroblasts, endothelial cells, pericytes and immune cells. Its primary function is progesterone secretion. The life cycle of CL is strictly controlled by precise interaction of gonadotropins with intraluteal autocrine and paracrine signals. MicroRNAs (miRNAs) have emerged as new players in the regulation of developmental events for CL, and imbalance in their expression level can result in CL dysfunction. An altered androgen status (excess or deficiency) in neonates may affect ovarian function in adult life, including changes in epigenetic control of gene expression mediated by factors such as miRNAs. The current study was designed to gain insights into regulatory networks that mediate long-term impacts of androgen excess or androgen deficiency on CL function in pigs. Piglets were injected subcutaneously with testosterone propionate (TP, an androgen, at 20 mg/kg bw), flutamide (FLU, an anti-androgen, at 50 mg/kg bw) or corn oil (control) between postnatal Days 1 and 10 (n=3/group). CLs from sexually mature gilts were obtained between Days 8 and 11 of estrous cycle. Total RNA was extracted using miRCURY RNA Isolation Kit (Exiqon) and sequenced by using Illumina HiSeq 2000 system (EMBL Heidelberg Gene core facility) followed by bioinformatics to analyze levels of protein-coding transcripts and miRNAs. Potential miRNA-mRNA interactions were explored in silico. The luteal tissue showed 465 and 353 differentially expressed (DE) genes (padjusted<0.05; log2FC≥1.0) in response to neonatal TP and FLU treatment, respectively. Disruption of androgen signalling induced in neonates by androgen excess or deficiency affected genes linked to apoptosis, angiogenesis

and immune functions in adult pig CL. These results suggest an earlier timeline for the onset of luteolysis, although mechanisms for responses to TP or FLU are likely to differ. Furthermore, a set of miRNAs showed altered expression. Comparative analysis of known DE microRNAs in TP- and FLU-treated groups in comparison to control group revealed differences for 57 (9 up and 48 down) and 50 (7 up and 43 down) miRNAs, respectively. We propose that changes in selected miRNAs and mRNAs may in part account for long-term impacts of androgen excess or androgen deficiency on CL function.

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Session 6

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR LIGANDS AFFECT THE EXPRESSION OF IL-1B AND IL-6 IN THE PORCINE ENDOMETRIUM ON DAYS 18–20 OF THE ESTROUS CYCLE

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Cytokines are mediators of the immune response and they are crucial factors in the regulation of various physiological processes. Current knowledge indicates a close connection between the immune and reproductive functions. Changes in the synthesis of cytokines and hyperactivity of local pro-inflammatory factors play an important role in the pathogenesis of the endometrial tissue. The present study aimed to investigate the effect of PPAR selected ligands on the expression of pro-inflammatory cytokines, interleukin (IL)-1β and IL-6, in the porcine endometrium during LPS-induced inflammation on days 18–20 of the estrous cycle.

The endometrial tissue as collected from gilts (n=7) on days 18–20 of the estrous cycle. Endometrial slices were incubated *in vitro* for 24 h with lipopolysaccharide (LPS) or without LPS (control incubation) and then 6 h in the presence of PPAR ligands: PPARα agonist WY 14643 and antagonist MK-886; PPARβ/δ agonist L-165,041 and antagonist GW9662; PPARγ agonists: 15d-prostaglandin J_2 (PGJ2) or pioglitazone and antagonist T0070907. The expression of cytokines was determined by Real Time PCR. Statistical analysis was carried out by one-way analysis of variance for repeated measurements followed by the Duncan post-hoc test.

During physiological conditions (incubation without LPS), PPARα and PPARγ agonists stimulated IL-1β and IL-6 mRNA abundance in the porcine endometrium. During the LPS-induced inflammation, the ligands of all PPAR isoforms enhanced the expression of IL-1β mRNA, while the tested factors did not change IL-6 gene expression.

Our results demonstrate that PPARs are involved in the regulation of IL-1β and IL-6 expression in the porcine endometrium. The impact of the tested PPAR ligands is different and depends on the physiological status of animals.

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Session 6

VASPIN AS A NEW ADIPOKINE IN THE PORCINE OVARIAN FOLLICLES: EXPRESSION, IT'S REGULATION AND IMPACT ON STEROID SYNTHESIS

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VASP as a known as Visceral Adipose tissue- derived Serine Protease Inhibitor is a member of the serine protease inhibitor family. The existing literature indicate that its produced and secreted by adipose tissue and involvement in obesity, insulin resistance or pathogenesis of inflammatory reactions, however the lack described role in the female reproduction. It is now known that there is a close link between nutritional status and reproductive success of animals, including pigs. The aims of the study were to investigate VASP expression, it's regulation and impact on signaling kinases pathways and steroidogenesis in the ovary.

Ovarian follicles were obtained from mature sows at days 4–6, 10–12 and 16–18 of the estrous cycle. VASP mRNA, protein expression and cellular immunolocalization were determined by real time PCR, Western Blot and immunohistochemistry, respectively. Next we focused on effect of insulin, insulin-like growth factor type 1 (IGF-1) (10–100 ng/ml), gonadotropins (LH and FSH) (50–150 ng/ml) and steroid hormones: progesterone (P4), testosterone (T) and estradiol (E2) (10^{-8}–10^{-6} M) on VASP protein expression in *in vitro* cultures of granulosa and theca cells. Moreover, to detect VASP effect on kinases phosphorylation, VASP (1 ng/ml) was incubated for 1, 5, 15, 30, 45 and 60 min and then kinases (ERK1/2, Akt, Stat3, AMPK) and factor NFκB expression were analysed. Finally, to determine effect of VASP on steroidogenesis, VASP at doses 0,01, 0,1, 1, 10, 100 ng/ml was added and then steroids hormone (P4, T, and E2) and steroid enzymes (3βHSD, 17βHSD and CYP19A) expression were analysis. Statistical analysis were performed using Graph Pad Prism 5 and a one-way ANOVA test.

We demonstrated that both mRNA and protein level of VASP decreased in ovarian follicles during the estrus cycle. VASP was immunodetected in granulosa, theca cells and also in oocyte. We indicated that insulin, IGF-1, gonadotropins and steroids increased VASP expression in the ovarian follicles cells. Moreover, we showed that VASP stimulated phosphorylation of all studied kinases, while

decreased expression of NFκB (P>0.05). Our preliminary data indicated that VASP modulate steroid synthesis by ovarian cells.

To conclude, our study for the first time describe VASP as a new adipokine in the ovary; we showed VASP expression in porcine ovarian follicle and its regulatory factors, moreover VASP impact on kinases phosphorylation and steroid synthesis.

Supported by National Science Centre, Poland, project HARMONIA, no. 2016/22/M/NZ9/00316.

Session 6

ASSESSMENT OF MORPHOLOGY AND MITOCHONDRIAL MEMBRANE POTENTIAL OF SPERMATOZOA FROM THE DIFFERENT REGIONS OF DOMESTIC CAT EPIDIDYMAL DUCT – PRELIMINARY RESULTS

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During epididymal maturation, spermatozoa acquire progressive motility (PM) and fertilizing capacity. Mitochondria located in the midpiece produce the energy (ATP), which is needed to maintain crucial functions of sperm, including motility. The fluorescent dye (JC-1), has been used to identify sperm cells with high and low mitochondrial membrane potential ($\Delta\Psi_m$), which is an important indicator of mitochondrial function (Gravance et al., Theriogenology, 2000, 53: 1691–703). JC-1 forms aggregates in mitochondria with high membrane potential, emitting orange fluorescence. In case of low $\Delta\Psi_m$, JC-1 remains in monomeric form and exhibits green fluorescence. In the literature there is no information on sperm mitochondria functionality in domestic cat epididymal duct. The aim of the study was to characterize the sperm morphology, motility and mitochondrial membrane potential of sperm collected from different regions of domestic cat epididymis.

Testes-epididymides obtained from 6 cats after routine castration were used in the study. The caput, corpus and cauda of the epididymis were isolated, placed in ~1 ml of warm (37°C) Tris-extender and incised. Sperm suspension collected from each region of epididymis of each cat were processed separately. After estimation of morphology (eosin-nigrosin staining) and motility, the sperm were stained with JC-1 (Thermo Fisher Scientific, USA) for $\Delta\Psi_m$ evaluation, using fluorescence microscopy. Sperm were divided into three groups, according to fluorescence pattern of midpiece: I) – orange fluorescence; high $\Delta\Psi_m$; II) – heterogeneous orange/green fluorescence; moderate $\Delta\Psi_m$, and III) – green fluorescence; low $\Delta\Psi_m$. For statistical analysis Kruskal-Wallis test followed by Dunn's test were applied. It was found that the cytoplasmic droplet migration from a proximal to a distal position of midpiece occurs most intensively during sperm transit from the caput to the corpus of the epididymis. At the same time, the percentage of PM sperm as well as those exhibiting high $\Delta\Psi_m$ was low in the caput samples (3.3% and 10.6%, respectively), increased ($P<0.05$) in the corpus (38.3% and 42.1%) and remained almost unchanged in the cauda samples, reaching 52.5% and 55% respectively.

To sum up, the obtained results suggest that in domestic cat, cytoplasmic droplet migration and acquisition of motility take place during sperm cells transit from caput to corpus of the epididymis, and are closely related to mitochondria functionality and their membrane potential.

Financed from DS 3208.

Session 6

ECHOTEXTURAL CHARACTERISTICS OF THE MAMMARY GLAND DURING THE PERIOD ENCOMPASSING A PEAK OF LACTATION IN TWO BREEDS OF SHEEP VARYING IN MILK YIELDS

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Grey-scale ultrasonographic images are composed of numerous brightness elements called pixels. Computerized analysis increases precision of measurements and the range of detectable pixel variations. Echotextural variables determined with digital image analysis are objective measures of tissue echogenic properties and valuable indicators of corresponding histophysiological changes. The main goal of this study was to determine and compare ultrasonographic characteristics of the mammary gland in two genotypes of ewes varying in milk productivity at 2, 3 and 4 weeks after lambing (most sheep peak in their milk production ~4 weeks post-partum). We hypothesized that quantitative ultrasonographic characteristics of the mammary gland in the ewes of both genotypes would mainly reflect the varying rates of production and accumulation of its excretory product. Ultrasonographic images of the udder were obtained using the 5.0- and 7.5-MHz transducers, in coronal and sagittal planes, in four low milk-yielding Polish Mountain sheep and six high milk-yielding Olkuska ewes. All ultrasonograms were subjected to computer-assisted image analyses using commercially available image analytical software to determine pixel intensity (numerical pixel values-NPVs) and heterogeneity (pixel standard deviation-PSD) of the mammary gland parenchyma. During the 28-day period post-partum, the Olkuska sheep exceeded ($P<0.05$) Polish Mountain ewes in average milk yield (31.6±2.7 l and 25.0±4.2 l, respectively; mean±SEM) as estimated based on mean weight gains of the lambs. In animals examined with the 5.0-MHz transducer, mean NPVs of the mammary gland parenchyma in Olkuska ewes and mean PSD in both genotypes of ewes were lower ($P<0.05$) before than after milking. In addition, PSD recorded both before and after milking were lower ($P<0.05$) in the Polish Mountain compared with Olkuska sheep. Mean PSD values of the mammary gland

were less ($P<0.05$) before than after milking in Polish Mountain ewes and they were greater ($P<0.05$) in Olkuska compared with Polish Mountain ewes examined with the 7.5-MHz probe after milking. It can be deduced that both the milk quantity and chemical composition as well as histomorphology of the mammary gland tissue can impinge on echotextural attributes of the udder in different breeds of sheep. Our observations warrant future studies of the correlations between milk composition and ultrasonographic image attributes of the mammary parenchyma in ruminant species.

Session 6

DOES THE INTERACTION OF PXT1 AND BAG6 PROTEINS HAVE AN EFFECT ON THE SPERM QUALITY IN MOUSE?

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For the proper male gametes production two opposite process have to act in a balance manner. On the one hand germ cells proliferation is required to provide adequate number of sperm and on the other hand the elimination of damage germ cells, usually by apoptosis, assures high gametes quality. The *Pxt1* (peroxisomal, testis specific 1) gene encodes a protein consisting of 51 amino acids. The N-terminal part of PXT 1 contains functional BH3-like domain. The expression of the *Pxt1* gene is limited to male germ cells and starts from the 15th day of postnatal life. Analysis of transgenic mice revealed that overexpression of the *Pxt1* gene resulted in male germ cells apoptosis, finally leading to Sertoli cell-only (SCO) phenotype in older animals. It has been shown that mouse PXT1 protein interacts with BAG6 protein. The knockout of the *Bag6* gene leads to an increased apoptosis of male germ cells and lack of spermatozoa. The phenotype of the knockout mouse of the *Bag6* gene is very similar to the phenotype of mice overexpressing the *Pxt1* gene. It was additionally shown that the BAG6 protein protects the cells against PXT1 induced apoptosis.

The aim of this study was to check the hypothesis that the interaction of PXT1 and BAG6 proteins plays a key role in controlling male gametes quality.

To test this hypothesis a double knockout mouse model with disruption of both *Pxt1* and *Bag6* genes was created. 3.5-month-old $Pxt1^{-/-}Bag6^{-/-}$ male mice (from line called DKO1) were reared in order to identify male double gene knockouts ($Pxt1^{-/-}Bag6^{-/-}$) by standard genotype protocol using PCR analysis. Histological analysis of testis revealed normal spermatogenesis in two analysed mutant males. The number, morphology, membrane integrity and motility of sperm isolated from mutants was not significantly different from wild type control. These results clearly confirm our hypothesis and allow us to state that the main function of BAG6 in spermatogenesis is binding pro-apoptotic PXT1 and thus protecting germ cells from apoptosis. Furthermore, our preliminary study suggests, that in sperm with enhanced DNA double strand breaks PXT1 escapes from the BAG6 mediated regulation and induces apoptosis.

Experiments were performed in accordance with Polish Legal requirements of the use genetic modified organism (licence number 21/2018, 165/2012).

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Session 6

CHEMERIN AS A HORMONE THAT MODULATES PROGESTERONE SECRETION BY THE PORCINE OVARY DURING THE OESTROUS CYCLE

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The physiological mechanisms of the reproduction, energy homeostasis and immune response are closely related. It seems that chemerin may be involved in the regulation of all these processes. The presence of chemerin and its receptors were found in the ovaries of women, rats and cows. It has also been shown that chemerin affects the ovarian steroidogenesis in these species. Due to a lack of data in pigs, we hypothesized that chemerin may affect the hormone secretion by the porcine ovary, including progesterone (P_4), a key steroid hormone regulating female reproductive functions. Therefore, the aim of this study was to investigate the effect of chemerin on P_4 secretion by the porcine corpus luteum, theca interna and granulosa cells during the oestrous cycle. Corpora lutea were collected from gilts (n=5) on days 2 to 3 (early luteal phase – corpus luteum formation), 10 to 12 (mid-luteal phase – fully functional corpus luteum) and 14 to 16 (late luteal phase – phase of luteolysis) of the oestrous cycle. Theca interna and granulosa cells were obtained from pigs (n=5)
on days 17 to 19 of the cycle (follicular phase). Isolated cells were cultured *in vitro* in the presence of chemerin at the doses of 100 and 200 ng/ml. Progesterone concentration in the culture medium was examined by radioimmunoassay (RIA) method. Statistical analysis was performed by one-way analysis of variance (ANOVA). On days 2 to 3 of the cycle, chemerin stimulated P_4 secretion at the both studied doses. On days 10 to 12 and 14 to 16 of the oestrus cycle, secretion of P_4 was increased in the presence of chemerin at the higher dose (200 ng/ml). On days 17 to 19 of the cycle, chemerin at the both studied doses stimulated P_4 secretion by the theca interna and chemerin at the dose of 200 ng/ml caused an increase in the steroid secretion by the granulosa cells. In this study, we have demonstrated for the first time the relationship between chemerin and P_4 secretion by the porcine ovary. It may

suggest that chemerin modulates ovarian functions through the regulation of hormone secretion, thus affects the reproductive functions in gilts.

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Session 6

AQUAPORIN 4 IN THE CHICKEN OVIDUCT DURING PAUSE IN EGG LAYING

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Aquaporins (AQPs) are a family of membrane proteins that transport water and small molecules across the plasma membrane. AQPs have been found in many organs including the female reproductive system, where they play a crucial role in maintaining water flow between tissue components. So far, little is known about the expression and regulation of AQPs in the avian reproductive system. Therefore, the aim of the present study was to examine the expression of aquaporin 4 (AQP4) in the chicken oviduct during pause in egg laying induced by fasting or tamoxifen (TMX; estrogen receptor modulator) treatment.

In the first experiment (I), the laying hens were subjected to pause in laying by food deprivation for 5 days, and control ones were fed *ad libitum*. In the second experiment (II), the hens were injected daily with TMX until complete cessation of egg laying or vehicle (ethanol) for 7 days. Chickens (n=6 in each group) from the experiment I and II were sacrificed on day 6 or 8, respectively, and all sections of the oviduct, i.e. infundibulum, magnum, isthmus, shell gland and vagina were isolated. In tissues, the gene and protein expressions of AQP4 were examined by the qReal-Time PCR and Western blot, respectively. Furthermore, AQP4 was localized in the oviductal wall immunohistochemically.

In the hens of control groups, the relative mRNA expression (RQ) of AQP4 was very low in the magnum, isthmus and shell gland, and higher in the infundibulum and vagina. Fasting and TMX-treatment caused a decrease in the AQP4 transcript levels in the infundibulum by 86% ($P<0.001$) and the vagina by 56% ($P<0.05$), respectively. In both experiments, the band density of AQP4 protein was the highest in the infundibulum and shell gland. Starvation and TMX-treatment reduced ($P<0.01$, $P<0.05$) the AQP4 protein levels in the shell gland. The intensity of immunopositive reaction was as follows: the infundibulum $\geq$ vagina $>$ shell gland $\geq$ isthmus $>>$ magnum. In the control chickens the immunoreactivity for AQP4 in all oviductal segments was stronger compared with the fasted and TMX-treated hens.

The results obtained indicate that AQP4 takes part in the regulation of water transport required for the egg formation in the chicken oviduct. Since AQP4 might participate in water loss needed for downstream of apoptotic events, we propose that AQP4 may take part in the cell apoptosis, occurring in the regressing oviduct during pause in egg laying induced by different factors. Moreover, relationship between steroid action and AQP4 gene and protein expression is suggested.

Supported by DS-3243/KFiEZ.

Session 6

THE EFFECT OF PPAR LIGANDS ON THE EXPRESSION OF LIF AND IL-10 IN PORCINE ENDOMETRIUM DURING FOLLICULAR PHASE OF THE ESTROUS CYCLE

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Cytokines, mediators of the immune response, are important regulators of reproductive functions. Our previous results indicate an interaction between cytokines and peroxisome proliferator-activated receptors (PPARs) in the porcine endometrium during the luteal phase of the estrous cycle. The present study aimed to investigate the effect of PPAR ligands on the expression of anti-inflammatory cytokines, leukemia inhibitory factor (LIF) and interleukin (IL)-10, in the porcine endometrium during the follicular phase of the estrous cycle. In addition the effect of PPAR ligands on the LPS-treated endometrium has been also determined.

The endometrial tissue was collected from gilts (n=7) on days 18–20 of the estrous cycle. The tissue slices were incubated *in vitro* for 24 h in the presence of LPS or without LPS (control incubation). Then, the slices were incubated *in vitro* for 6 h in the presence of PPAR ligands: PPARα (agonist WY 14643 and antagonist GW 6471); PPARβ/δ (agonist L-165041 and antagonist GSK 3787) or PPARγ (agonists: 15d-prostaglandin J_2 (PGJ2) or pioglitazone and antagonist T0070907). The mRNA level in the tissue was determined by Real Time PCR. The effect of the treatment was established by one-way analysis of variance for repeated measurements followed by the Duncan post-hoc test.

During the physiological stage (incubation without LPS), the antagonists of all PPAR isoforms and agonists of PPARα and PPARγ (PGJ2) enhanced the expression of LIF mRNA in the porcine endometrium. Moreover, the ligands of PPARα and PPARγ increased IL-10 gene expression. During the inflammatory stage, the expression of LIF mRNA increased in the presence of PPARα agonist whereas the IL-10 transcript level enhanced after the treatment with the PPARγ agonist and PPARβ/δ antagonist.

Our results indicate that different isoforms of PPAR are engaged in the endometrial synthesis of LIF and IL-10 in the pig. The changes in the expression of LIF and IL-10 gene suggest diverse sensitivity of the tissue to the tested factors during the analyzed stages.

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Session 6

EXPRESSION OF AQP1, 2, 5 AND 7 mRNA IN THEPIG UTERINE EPITHELIAL CELLS DURING FOLLICULAR PHASE

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Aquaporins (AQPs) are proteins integrated with the cell membranes that form selective channels for water and other molecules. Therefore, it is suggested that AQPs may play a significant role in the regulation of water homeostasis in the reproductive system. Previous *in vitro* studies of uterine explants showed significant changes in the level of the protein expression and the transcript of AQP1 and 5. The aim of the study was to determine the effect of estradiol ($E_{2)}$,progesterone (P_4), arachidonic acid (AA), kinase A inhibitor (H-89) and selective inhibitor of MAP kinase kinases (PD98059) on the expression of AQP1, 2, 5 and 7 in the pig uterine epithelial cells.

Tissue samples were recovered from mature gilts at the follicular phase (days 18–20). The purpose of our study was to determinate changes in gene expression of AQPs in uterine epithelial cells. *In vitro* cell cultures were stimulated for 3 h with P_4, E_2, AA, H-89 and PD98059. H-89 and PD98059 were added in two doses – 1 and 10 mmol. Gene expression was determined by Real Time PCR. All data were analyzed by one-way ANOVA and least significant difference (LSD) post hoc test.

In the present study we observed an increased expression of *AQP1* in endometrial cells stimulated by H89 (1mM) for 3 h in compare to the control and estradiol stimulation. The increased expression of*AQP2* in endometrial cells was observed only under the influence of H89 (1 mM) in compare to control, estradiol and arachidonic acid stimulation. No significant differences were observed in expression of *AQP5.* In case of *AQP7* gene expression increase was observed in cells stimulated by progesterone and H89 in two doses, 1 mM and 10 mM in compare to control and estradiol stimulation.

Our results indicate that particular isoforms of AQPs are stimulated by different factors. In turn, changes in the expression of *AQP1* and *AQP2* genes, regulated by H89 (1 mM) shown similar pattern. It can be caused by close affinity of these isoforms. The expression of AQP7 change different, probably because it acts as aquaglyceroporin. Also signaling pathway may be different for classical aquaporins. In the future study we plan to incubate cells with tested factors longer and check how the expression of the studied genes change.

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Session 6

EXPRESSION AND ACTIVITY OF METALLOPROTEINASE-2 IN THE CHICKEN OVARY FOLLOWING TAMOXIFEN TREATMENT

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Metalloproteinases (MMPs) are a group of proteolytic enzymes which take part in the extracellular matrix turnover, essential for the ovary development, function and regression. There is limited information concerning the involvement of MMPs and their tissue inhibitors (TIMPs) in the chicken ovary regression as well as hormonal regulation of MMPs. The aim of the present study was to examine the expression of MMP-2 and its tissue inhibitor TIMP-2, and MMP-2 activity in the chicken ovary after tamoxifen (TMX; estrogen receptor modulator) treatment.

The laying hens were injected daily with TMX at a dose of 6 mg/kg of b.w. until complete cessation of egg laying. Control chickens received vehicle (etanol). Birds were sacrificed on day 8 of the experiment, and ovarian stroma with primordial follicles and white (1–4 mm), yellowish (4–8 mm), small yellow (8–12 mm), and yellow preovulatory follicles (F3-F1; 20–36 mm) were isolated. From the largest follicles the theca and granulosa layers were separated. In tissues, the expression of MMP-2 and TIMP-2 mRNAs and proteins were determined by qReal-time PCR and Western blot, respectively. The activity of MMP-2 was investigated with Biotrak activity assay system.

The relative expression (RQ) of MMP-2 and TIMP-2 mRNA was found in all examined ovarian compartments. Within the ovary the expression of MMP-2 mRNA was the lowest in the granulosa layer of F3-F1 follicles. The lowest expression of TIMP-2 mRNA was observed in the granulosa layer of F3 and F2. Treatment of hens with TMX elevated the level of MMP-2 transcript in the theca layer of F1 follicle, and reduced the expression of TIMP-2 mRNA in the theca layer of F3 and in the granulosa layer of F3-F1. Both latent and active forms of MMP-2 protein were detected by Western blot analysis in all examined tissues. The relatively lowest abundance was observed in the granulosa layer of F3-F1 in comparison with other ovarian tissues. TMX caused increase in abundance of active form of MMP-2 protein in all tissues. The total activity of MMP-2 (ng/mg protein) was the lowest in the granulosa layer, and the highest in the theca layer of the largest follicles. It was higher in the theca of F3-F1 and in the granulosa of F2, and lower in the white, yellowish and small yellow follicles of TMX-treated chickens.

The results obtained indicate that MMP-2 and TIMP-2 participate in the chicken ovary remodeling. Moreover, involvement of estrogen in the regulation of MMP-2 and TIMP-2 gene expression and/or activity is strongly suggested.

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Session 6

EFFECTS OF MONTELUCAST AND NIFEDIPINE ON THE UTERINE CONTRACTILITY IN IMMATURE AND PREGNANT PIGS

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A proper uterine contractility depends on many factors and determines many reproductive functions including embryo transport, implantation, gestation and parturition. It has been found that leukotriene receptors are present in the endometrium and myometrium in cattle, horses, humans and pigs. Moreover, it is suggested that calcium channel blockers attenuate the constrictor effects of cysteinyl leukotrienes (Cys-LTs) in the uteri of sheep. Therefore, the aim of the study was to investigate the effect of montelukast [(MON), a CysLT1 receptor (CysLTR1) antagonist] administered alone or in combination with nifedipine [a calcium (Ca^{2+}) channel blocker] on the contractile activity of uterine smooth muscles in immature and pregnant pigs.

The study was conducted on sexually immature (n=8) and pregnant (on days 27–28 of pregnancy; n=8) pigs. The uteri were placed on ice and strips of smooth muscles were collected from the middle part of the horns. Strips of the myometrium (MYO) were suspended in Krebs-Ringer solution. After 60–90 min of preincubation, the strips were stimulated as follow: a) MON and nifedipine at a concentrations of 10^{-8}-10^{-4} M were administered alone at 15-minute intervals; b) MON at concentration of 10^{-4} M, was administered 15 min before the treatment with increasing concentrations of nifedipine. The smooth muscle contractility was determined with a Hugo Sachs Elektronik equipment for measuring isometric contractions.

MON administered alone caused a significant decrease in amplitude ($P<0.001$) and frequency ($P<0.01$), but did not exert a significant impact on tension of MYO in pregnant pigs as well as did not change the examined parameters in immature pigs as compared to the period before the treatment. Nifedipine significantly decreased amplitude ($P<0.001$), frequency ($P<0.001$) and tension ($P<0.05$) in pregnant pigs as well as amplitude and frequency ($P<0.001$) in immature pigs.

In pregnant pigs nifedipine administered after MON at all concentrations causeds significantly higher decrease in amplitude and frequncy as compared to the action of nifedipine administered alone whereas in immature pigs significantly higher decrease was observed only in amplitude and frequency after administration of nifedipine in the highest concentration. The obtained results indicate that the influence of MON and nifedipine on the contractile activity of the porcine uterus is dependent on the hormonal status of animal. Moreover, the blockade of CysLTR1 enhances the relaxing effect of nifedipine.

Indeks nazwisk
Index of names

Spis treści – *Contents*

NOTES

NOTES

NOTES

NOTES

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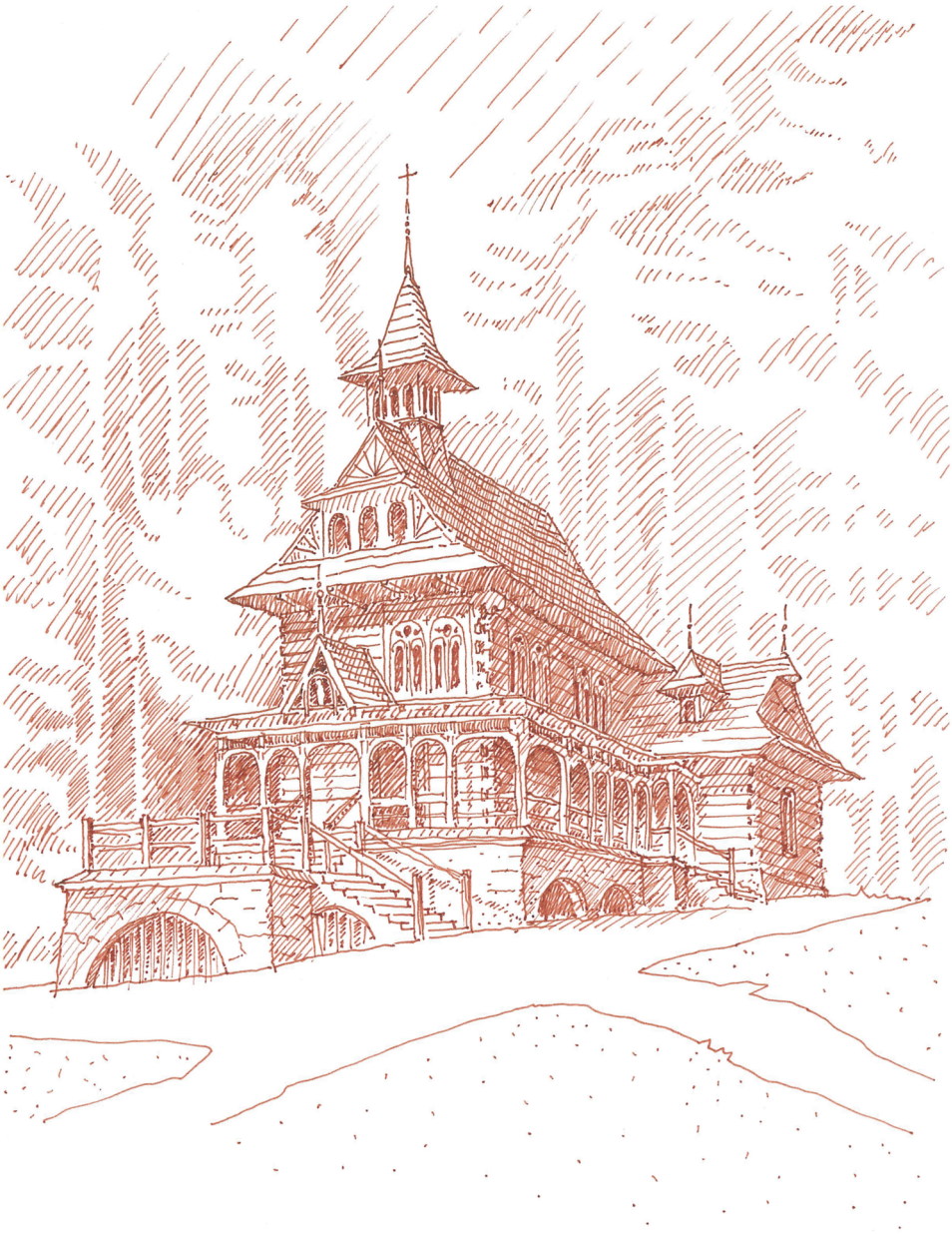

Kaplica NSJ w Zakopanem – Jaszczurówce wg projektu S. Witkacego (1907)

Rys. mgr inż. arch. Andrzej Mikulski, autor projektu Centrum Świętego Jana Pawła II „Nie lękajcie się" w Krakowie