10th Meeting of the Society for Biology of Reproduction

Warsaw, September 12th - 14th, 2024

CONFERENCE BOOK

Towarzystwo Biologii Rozrodu
Society for Biology of Reproduction

Institute of Animal Reproduction and Food Research Polish Academy of Sciences in Olsztyn

Faculty of Biology · University of Warsaw

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Ministry of Science and Higher Education
Republic of Poland

Doskonała Nauka

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WELCOME MESSAGE

Welcome to Warsaw!

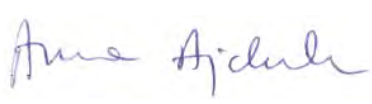
We are honored to welcome you to the **10th Meeting of the Society for Biology of Reproduction (Towarzystwo Biologii Rozrodu, TBR)** held in Warsaw, Poland, from September 12th to 14th, 2024. This event is particularly special as TBR celebrates its 25th anniversary. Like all our meetings over the past 25 years, our conference aims to provide a friendly and inspiring environment for researchers from Poland and beyond to exchange knowledge and ideas across various aspects of reproductive biology.

The scientific programme includes a range of themed sessions dedicated to:

- Gamete development and function
- Mechanisms of early embryonic development
- Assisted reproduction and preservation of fertility
- Embryo-maternal interactions and pregnancy
- Hormonal, neuronal, and immune regulation of reproduction
- Environmental influence on reproductive function
- Gonadal development and function.

During the conference, the laureates of the TBR Medals and the Prof. Władysław Bielański Award will be announced. To celebrate 25 years of TBR, we have also scheduled a special session on the history of our Society. Additionally, the conference features workshops on scientific career development for young scientists and an outreach event on reproductive biology.

On behalf of the Organizers, we wish you a great time in Warsaw, filled with scientific inspiration and fruitful networking. TBR meetings are not only about science but also about reconnecting with old friends and making new ones. We hope this meeting will be no different.

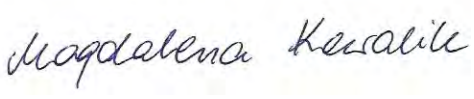


Anna Ajduk

Chair of the Scientific Committee

Anna Piliszek

Chair of the Organizing Committee



Magdalena Kowalik

Vice-chair of the Organizing Committee

A brief history of the Society for Biology of Reproduction

Prof. Adam J. Zięcik

How did it begin?

In the late 20th century, Poland lacked a scientific society dedicated to researchers working on human and animal reproduction, similar to the British Society for Reproduction and Fertility or the American Society for the Study of Reproduction. The inspiration to create such a society arose among members of the Committee of Animal Reproduction Biology of the Polish Academy of Sciences, chaired by Prof. Jerzy Strzeżek, and the Commission on Reproductive Biology of the Medical Sciences Division of the Polish Academy of Sciences, chaired by Prof. Maciej Kurpisz.

The first meeting of the founding members of the Society took place on **January 6, 1998**, at the Polish Academy of Sciences in Warsaw. Attendees included Professors Bernard Barcikowski, Szczepan Biliński, Czesław Błaszak, Zdzisław Boryczko, Jerzy Jakowicki, Tadeusz Krzymowski, Maciej Kurpisz, Zbigniew Kwias, Andrzej Łukaszyk, Longin Marianowski, Alina Midro, Marek Niemiałtowski, Bożena Olszańska, Jadwiga Przała, Janusz Rząsa, Zbigniew Samborski, Marian Semczuk, Zdzisław Somrąg, Stanisława Stokłosowa, Jerzy Strzeżek, Marek Świtoński, Andrzej Tarkowski, Lidia Wenda-Różewicka, Witold Woźniak, Adam J. Zięcik, Lech Zwierzchowski, as well as dr. hab. Leszek Bablok and dr. Dorota Fiszer.

During this meeting, the state of reproductive research in Polish scientific institutions was discussed, and the prospects for the activities of the new Society were outlined, including the possibility of establishing a new scientific journal that would cover topics related to human and animal reproduction. The Temporary Board of the Society was elected, consisting of: chairman – Prof. Adam J. Zięcik, vice-Chairman – Prof. Jerzy Jakowicki, secretary – Dr. Władysław Kordan, treasurer – Dr. Bożena Szafrańska. A formal motion to establish the new Society was voted on, and after discussion, the name Society for Biology of Reproduction (Towarzystwo Biologii Rozrodu) was adopted, marking the official beginning of its activities.

The first meeting of the Temporary Board of the Society for Biology of Reproduction took place on **April 16, 1998**. During this and subsequent meetings, the Board members drafted the statute of the Society, decided on the name of the journal "Reproductive Biology," and elected Prof. Stanisława Stokłosowa as Editor-in-Chief.

The Society was officially registered on **September 15, 1998**, at the First Civil Division of the District Court in Olsztyn.

The Society meetings

The 1st Meeting of the Society took place on June 4-5, 1999, in Mierki near Olsztyn. The chairman of the Organizing Committee was Prof. Adam J. Zięcik. The inaugural lecture, with the Polish title “Co tam Panie w embriologii (ssaków)? Zarodki trzymają się mocno” ("What's Up with Embryology (of Mammals)? The Embryos Are Holding Strong"), was delivered by Prof. Andrzej Tarkowski. The conference materials were published in the journal "Postępy Biologii Komórki" (Supp. 12, 1999). During the 1999-2001 term, the Main Board of the Society consisted of: chairman – Prof. Adam J. Zięcik, vice-chairwoman – Prof. Lidia Wenda-Różewicka, secretary – Dr. Barbara Gawrońska (Dr. Wiesław Demianowicz, Dr. Aneta Andronowska), with the Audit Committee led by Prof. Barbara Bilińska. The Society branches were chaired by: Maria Bruska –

Poznań, Bronisława Chełmońska – Wrocław, Mariusz Majewski – Olsztyn, Bożena Olszańska – Warsaw, Janusz Rząsa – Kraków, Marian Semczuk – Lublin, and Jan Udała – Szczecin.

The 2nd Meeting took place on June 5-8, 2001, in Warsaw. The chairwoman of the Organizing Committee was Prof. Bożena Olszańska. The conference began with a symposium entitled "Future Prospects of Nongonadal Actions of LH/hCG in Reproductive Biology and Medicine", which included participation from Prof. William Hansel from the USA. The inaugural lecture "Ethical Aspects of Reproductive Research in Humans" was delivered by Prof. Jacek Zaremba. During the Main Board meeting on November 23, 2001, the Władysław Bielański Award was established, and the Award Committee was selected: chairman – Prof. Jerzy Strzeżek, vice-chairman – Prof. Adam J. Zięcik, and members – Prof. Bronisława Chełmońska, Prof. Maciek Kurpisz, and Prof. Janusz Rząsa.

The 3rd Meeting was held on September 4-7, 2002, in Międzyzdroje, and it was organized in collaboration with the Society of Histochemists and Cytologists. The chairpersons of the Organizing Committee were Prof. Lidia Wenda-Różewicka and Prof. Jan Udała. During the General Assembly of the Society members on September 5, 2002, changes to the statute were adopted, shortening the term of office of the Society's authorities to 3 years, and a new Main Board was elected for the 2002-2005 term: chairman – Prof. Adam J. Zięcik, vice-chairman – Prof. Dariusz Skarżyński, treasurer – Dr. hab. Bożena Szafrańska, secretary – Dr. Aneta Andronowska.

Subsequent meetings of the Society took place in various regions of Poland: 4th Meetig in Kraków (September 22-24, 2005, Prof. Edward Wierzchoś as the chairman of the Organizing Committee), 5th Jubilee Meeting in Wrocław (September 10-13, 2008, Prof. Jan Twardoń), 6th Meeting in Polańczyk (September 7-10, 2011, Prof. Marek Koziorowski), 7th Meeting in Toruń, held jointly with the Polish Society of Reproductive Medicine, the Section of Fertility and Infertility of the Polish Gynecological Society, and the Center for Human Fertility Studies (September 11-13, 2014, Prof. Jan Kotwica), and the 8th Meeting in Olsztyn (September 7-9, 2017, Dr. hab. Monika M. Kaczmarek). Due to the COVID-19 pandemic, the 9th Meeting, which was to be held in Poznań, was organized online on September 2-4, 2021, with Prof. Dorota Cieślak and Dr. hab. Ewelina Warzych-Player as the chairpersons of the Organizing Committee. On September 12-14, 2024, we will gather for the 10th Jubilee Meeting in Warsaw, with Dr. hab. Anna Piliszek and Dr. hab. Magdalena Kowalik as the chairpersons of the Organizing Committee.

Winter Conferences of the Society

When discussing important initiatives of the Society, it is worth mentioning five Winter Conferences organized by the Kraków Branch of the Society. These meetings took place in Zakopane in the years 2007, 2010, 2013, 2016, and 2018. The Organizing Committee of the first conference included Prof. Edward Wierzchoś, Dr. hab. Andrzej Sechman, Dr. Edyta Molik, and Dr. hab. Dorota Zięba. The aim of these conferences was to provide **a platform for doctoral students and young researchers to present their latest research** in the field of human and animal reproductive biology. These conferences also served to integrate the scientific community within the Society for Biology of Reproduction and the Committee on Reproductive Biology of the Polish Academy of Sciences.

Reproductive Biology

The journal Reproductive Biology was established through the initiative of the Society and the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn. The first issue was published **in June 2001** with a print run of 400 copies. Key roles in the creation of the journal were played by Prof. Stanisława Stokłosowa, who served as the first Editor-in-Chief, and Dr. Renata Ciereszko, the Editorial Secretary, who succeeded Prof. Stokłosowa in 2012. Prof. Nafis Rahman took over as Editor-in-Chief in 2015, leading the journal for the next eight years in collaboration with the publisher Elsevier BV. The journal quickly gained international recognition and a high scientific standard. At the beginning of 2024, Prof. Dorota Zięba-Przybylska and Prof. Mariusz Kowalewski became the new Editors-in-Chief, with the mission of promoting the translational character of the journal. Their goal is to apply research conducted on animals, including domestic species, to human medicine, representing a significant step forward in the development of interdisciplinary research.

The Prof. Władysław Bielański Award

The Prof. Władysław Bielański Award, **established in 2001**, is granted to young researchers for outstanding work in the field of reproductive biology, conducted in Poland. To date, seven individuals have received the award: Dr. Karolina Piotrowska (2002), Dr. Marta Wańkowska (2008), Dr. Michał Wróbel (2011), Dr. Agnieszka Rak-Mardyła (2014), Dr. Joanna Nynca (2017), and Dr. Maciej J. Śmiałek and Dr. Justyna Gogola-Mruk (2020). In 2024, this distinction was awarded to Dr. Maria M. Guzewska, who will deliver a lecture on her achievement during the 10th Jubilee Meeting of the Society.

The Society for Biology of Reproduction Medal

The Medal of the Society is **the highest honor** awarded by the Society for groundbreaking discoveries in reproductive biology and for organizational contributions to the developments of the Society. Over the 25 years of the Society's existence, 14 individuals have received this medal: Prof. Stanisława Stokłosowa (2012), Prof. Tadeusz Krzymowski (2012), Prof. Edward Wierzchoś (2013), Prof. Adam J. Zięcik (2013), Prof. Leszek Bablok (2013), Prof. Lidia Wenda-Różewicka (2014), Prof. Nafis Rahman (2015), Prof. Renata Ciereszko (2017), Prof. Barbara Bilińska (2020), Prof. Ewa Gregoraszczuk (2020), Prof. Jan Kotwica (2020), Prof. Dariusz J. Skarżyński (2020), and Prof. Sławomir Wołczyński (2020). During the 10th Jubilee Meeting, the medal will be posthumously awarded to Prof. Bronisława Chełmońska, and it will also be given to Dr. hab. Aneta Andronowska, Dr. hab. Edyta Molik, Prof. Zdzisław Gajewski, and Prof. Janusz Rząsa.

Krótka historia Towarzystwa Biologii Rozrodu

Prof. dr hab. Adam J. Zięcik

Jak to się zaczęło?

Pod koniec XX wieku w Polsce nie istniało towarzystwo naukowe, które by skupiało naukowców zajmujących się rozrodem człowieka i zwierząt, podobne do brytyjskiego Society for *Reproduction and Fertility* czy amerykańskiego *Society for the Study of Reproduction*. Inspiracja do stworzenia takiego towarzystwa zrodziła się wśród członków Komitetu Biologii Rozrodu Zwierząt Użytkowych Polskiej Akademii Nauk, które przewodniczącym był prof. Jerzy Strzeżek, oraz Komisji Biologii Rozrodu Wydziału Nauk Medycznych Polskiej Akademii Nauk, której przewodniczącym był prof. Maciej Kurpisz.

Pierwsze zebranie członków założycieli Towarzystwa odbyło się **6 stycznia 1998 roku** w siedzibie Polskiej Akademii Nauk w Warszawie. W spotkaniu wzięli udział profesorowie: Bernard Barcikowski, Szczepan Biliński, Czesław Błaszak, Zdzisław Boryczko, Jerzy Jakowicki, Tadeusz Krzymowski, Maciej Kurpisz, Zbigniew Kwias, Andrzej Łukaszyk, Longin Marianowski, Alina Midro, Marek Niemiałtowski, Bożena Olszańska, Jadwiga Przała, Janusz Rząsa, Zbigniew Samborski, Marian Semczuk, Zdzisław Somrąg, Stanisława Stokłosowa, Jerzy Strzeżek, Marek Świtoński, Andrzej Tarkowski, Lidia Wenda-Różewicka, Witold Woźniak, Adam J. Zięcik, Lech Zwierzchowski, a także dr hab. Leszek Bablok i dr Dorota Fiszer.

Podczas tego zebrania omówiono stan badań nad rozrodem w ośrodkach naukowych w Polsce i zarysowano perspektywy działalności nowego Towarzystwa oraz możliwości powołania nowego czasopisma naukowego, które obejmowałoby tematykę rozrodu człowieka i zwierząt. Wybrano Tymczasowy Zarząd Towarzystwa, który tworzyli: Przewodniczący – prof. Adam J. Zięcik, Zastępca Przewodniczącego – prof. Jerzy Jakowicki, Sekretarz – dr Władysław Kordan, Skarbnik – dr Bożena Szafrańska. Przegłosowano formalny wniosek o powołanie nowego Towarzystwa i po dyskusji przyjęto nazwę Towarzystwo Biologii Rozrodu, co formalizowało początek jego działalności.

Pierwsze zebranie Tymczasowego Zarządu Towarzystwa Biologii Rozrodu (TBR) odbyło się **16 kwietnia 1998 roku**. Podczas tego oraz kolejnych spotkań członkowie Zarządu opracowali statut Towarzystwa, ustalili nazwę czasopisma „Reproductive Biology" i wybrali prof. Stanisławę Stokłosową na stanowisko Redaktora Naczelnego.

Towarzystwo zostało zarejestrowane **15 września 1998 roku** w I Wydziale Cywilnym Sądu Okręgowego w Olsztynie.

Zjazdy Towarzystwa

Pierwszy Zjazd TBR miał miejsce 4-5 czerwca 1999 roku w Mierkach koło Olsztyna. Przewodniczącym Komitetu Organizacyjnego był prof. Adam J. Zięcik. Inauguracyjny wykład, pod jakże polskim tytułem – „Co tam Panie w embriologii (ssaków)? Zarodki trzymają się mocno", wygłosił prof. Andrzej Tarkowski. Materiały zjazdowe zostały opublikowane w czasopiśmie „Postępy Biologii Komórki" (Supp. 12, 1999). W kadencji 1999-2001 Zarząd Główny Towarzystwa tworzyli: przewodniczący – prof. Adam J. Zięcik, wiceprzewodnicząca – prof. Lidia Wenda-Różewicka, sekretarz – dr Barbara Gawrońska (dr Wiesław Demianowicz, dr Anetę Andronowska). Komisję Rewizyjną prowadziła prof. Barbara Bilińska. Przewodniczącymi oddziałów TBR byli: Maria Bruska – Poznań, Bronisława Chełmońska – Wrocław,

Mariusz Majewski – Olsztyn, Bożena Olszańska – Warszawa, Janusz Rząsa – Kraków, Marian Semczuk – Lublin, Jan Udała – Szczecin.

Drugi Zjazd TBR odbył się w dniach 5-8 czerwca 2001 roku w Warszawie. Przewodniczącą Komitetu Organizacyjnego była prof. Bożena Olszańska. Zjazd rozpoczęło sympozjum pt. „Future Prospects of Nongonadal Actions of LH/hCG in Reproductive Biology and Medicine", w którym udział wziął między innymi prof. William Hansel z USA. Wykład inauguracyjny pt. „Aspekty etyczne badań nad rozrodem u ludzi" wygłosił prof. Jacek Zaremba. Na posiedzeniu Zarządu Głównego TBR, które odbyło się 23 listopada 2001 roku, ustanowiono Nagrodę im. Władysława Bielańskiego oraz wybrano skład Kapituły Nagrody: przewodniczącym został prof. Jerzy Strzeżek, zastępcą przewodniczącego – prof. Adam J. Zięcik, a członkami – prof. Bronisława Chełmońska, prof. Maciek Kurpisz i prof. Janusz Rząsa.

Trzeci Zjazd TBR miał miejsce w dniach 4-7 września 2002 roku w Międzyzdrojach i został zorganizowany we współpracy z Towarzystwem Histochemików i Cytologów. Przewodniczącymi Komitetu Organizacyjnego byli prof. Lidia Wenda-Różewicka i prof. Jan Udała. Podczas Walnego Zgromadzenia członków Towarzystwa, które odbyło się 5 września 2002 roku, uchwalono zmiany w statucie dotyczące skrócenia kadencji władz Towarzystwa do 3 lat oraz wybrano nowy skład Zarządu Głównego na kadencję 2002-2005: przewodniczącym został prof. Adam J. Zięcik, wiceprzewodniczącym – prof. Dariusz Skarżyński, skarbnikiem – dr hab. Bożena Szafrańska, sekretarzem – dr Aneta Andronowska.

Kolejne Zjazdy TBR miały miejsce w różnych regionach Polski: IV Zjazd w Krakowie (22-24.09.2005, prof. Edward Wierzchoś jako przewodniczący Komitetu Organizacyjnego), V Jubileuszowy Zjazd we Wrocławiu (10-13.09.2008, prof. Jan Twardoń), VI Zjazd w Polańczyku (7-10.09.2011, prof. Marek Koziorowski), VII Zjazd w Toruniu połączony ze zjazdami Polskiego Towarzystwa Medycyny Rozrodu, Sekcją Płodności i Niepłodności Polskiego Towarzystwa Ginekologicznego, Ośrodkiem Studiów na Płodnością Człowieka (11-13.09.2014, prof. Jan Kotwica), VIII Zjazd w Olsztynie (7-9.09.2017, dr hab. Monika M. Kaczmarek). Ze względu na pandemię COVID-19, IX Zjazd, który miał odbyć się w Poznaniu, został zorganizowany w formie on-line 2-4 września 2021 roku, z prof. Dorotą Cieślak i dr hab. Eweliną Warzych-Player jako przewodniczącymi Komitetu Organizacyjnego. W dniach 12-14 września 2024 roku spotykamy się podczas X Jubileuszowego Zjazdu w Warszawie, z dr hab. Anną Piliszek i dr hab. Magdaleną Kowalik jako przewodniczącymi Komitetu Organizacyjnego.

Zimowe konferencje Towarzystwa

Pisząc o ważnych inicjatywach Towarzystwa, warto wspomnieć o pięciu Zimowych Konferencjach zorganizowanych przez Krakowski Oddział TBR. Spotkania odbyły się w Zakopanem w latach 2007, 2010, 2013, 2016 i 2018. W skład Komitetu Organizacyjnego pierwszej konferencji wchodzili prof. Edward Wierzchoś, dr hab. Andrzej Sechman oraz dr Edyta Molik i dr hab. Dorota Zięba. Celem tych konferencji było **umożliwienie prezentacji przez doktorantów i młodych pracowników nauki** najnowszych badań z zakresu biologii rozrodu człowieka i zwierząt. Konferencje te służyły także integracji środowiska naukowego w ramach TBR oraz Komitetu Biologii Rozrodu Polskiej Akademii Nauk.

Reproductive Biology

Czasopismo "Reproductive Biology" zostało stworzone z inicjatywy TBR oraz Instytutu Rozrodu Zwierząt i Badań Żywności Polskiej Akademii Nauk w Olsztynie. Pierwszy numer ukazał się w **czerwcu 2001 roku** w nakładzie 400 egzemplarzy. Kluczowe role w tworzeniu czasopisma

odegrali prof. Stanisława Stokłosowa, która pełniła funkcję pierwszego Redaktora Naczelnego, oraz dr Renata Ciereszko, sekretarz Redakcji, która w 2012 roku zastąpiła prof. Stokłosową. W 2015 roku obowiązki Redaktora Naczelnego przejął prof. Nafis Rahman, który kierował czasopismem przez kolejne 8 lat, już we współpracy z wydawnictwem Elsevier BV. Czasopismo szybko zyskało międzynarodowe uznanie i wysoki poziom naukowy. Na początku 2024 roku nowymi Redaktorami Naczelnymi zostali prof. Dorota Zięba-Przybylska i prof. Mariusz Kowalewski, którzy przyjęli misję promowania translacyjnego charakteru "Reproductive Biology". Celem jest wykorzystanie badań prowadzonych na zwierzętach, w tym gatunkach zwierząt domowych, w medycynie człowieka, co stanowi istotny krok w rozwoju badań interdyscyplinarnych.

Nagroda im. prof. Władysława Bielańskiego

Nagroda im. prof. Władysława Bielańskiego, **ustanowiona w 2001 roku**, jest przyznawana młodym badaczkom i badaczom za wybitne prace z zakresu biologii rozrodu, które zostały wykonane w Polsce. Do tej pory, nagrodę otrzymało siedem osób: dr Karolina Piotrowska (2002), dr Marta Wańkowska (2008), dr Michał Wróbel (2011), dr Agnieszka Rak- Mardyła (2014), dr Joanna Nynca (2017), a także dr Maciej J. Śmiałek i dr Justyna Gogola-Mruk (2020). W roku 2024 wyróżnienie to zostało przyznane dr Marii M. Guzewskiej, która podczas X Jubileuszowego Zjazdu TBR wygłosi wykład na temat swojego osiągnięcia.

Medal Towarzystwa Biologii Rozrodu

Medal TBR jest **najwyższym wyróżnieniem** przyznawanym przez Towarzystwo za przełomowe odkrycia w dziedzinie biologii rozrodu oraz za działalność organizacyjną na rzecz rozwoju TBR. Przez 25 lat istnienia Towarzystwa, medal ten otrzymało 14 osób: prof. Stanisława Stokłosowa (2012), prof. Tadeusz Krzymowski (2012), prof. Edward Wierzchoś (2013), prof. Adam J. Zięcik (2013). prof. Leszek Babloka (2013), prof. Lidia Wenda-Różewicka (2014), prof. Nafis Rahman (2015), prof. Renata Ciereszko (2017), prof. Barbara Bilińska (2020), prof. Ewa Gregoraszczuk (2020), prof. Jan Kotwica (2020), prof. Dariusz J. Skarżyński (2020) i prof. Sławomir Wołczyński (2020). A podczas X Jubileuszowego Zjazdu TBR uhonorowani zostaną: śp. prof. Bronisława Chełmońska, dr hab. Aneta Andronowska, dr hab. Edyta Molik, prof. Zdzisław Gajewski oraz prof. Janusz Rząsa.

In memoriam - prof. dr hab. Bożenna Olszańska (1934-2023)

Dr hab. Anna Piliszek

Prof. Bożenna Olszańska (1934-2023) graduated from the Faculty of Biology and Earth Sciences at the University of Warsaw in 1956. In 1966, she obtained a Ph.D. in natural sciences from the Department of Radiochemistry and Applied Physics at the Timiryazev Academy of Agricultural Sciences in Moscow. In 1985, she earned her habilitation degree at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences (PAS), and in 1999, she was awarded the title of full professor of agricultural sciences.

Prof. Olszańska's scientific activity was primarily associated with the Institute of Genetics and Animal Breeding, PAS (now the Institute of Genetics and Animal Biotechnology, PAS) in Jastrzębiec, where she had been employed since 1965. During this time, she completed numerous internships at foreign institutions, including Universite Libre de Bruxelles (Belgium), Institut d'Embryologie in Nogent-sur-Marne (France), Institut Jacques Monod in Paris (France), and the Roslin Institute (UK).

Prof. Olszańska's research primarily focused on the biotechnology of bird embryos, using the Japanese quail (*Coturnix japonica*) as a model. Among her most significant scientific achievements were the analysis of gene expression during oogenesis and early bird embryo development, including the quantitative determination of the accumulation and distribution of maternal RNA in bird oocytes, the detection of nucleolytic enzymes (RNases, DNases) in bird oocytes, and the identification of melatonin receptor transcripts in oocytes and cleavage-stage bird embryos. Prof. Olszańska was the author of numerous research papers and a co-author of two books, including "Organization of the Early Vertebrate Embryo" (1994). Her work received several awards, including those from the Scientific Secretary of the PAS (1974, 1983), and a distinction from the Fifth Division of the PAS (1997).

Prof. Olszańska's indisputable contribution to the advancement of avian reproductive biology was the establishment of original methods for ovulation and fertilization of bird oocytes *in vitro.* The significance of these innovative methods for the field is highlighted by the fact that researchers from many international scientific institutions, including the Roslin Institute (Scotland, UK), INRA (France), the University of London (UK), as well as from India, Ukraine, and Japan, often undertook months-long research internships in Prof. Olszańska's laboratory in Jastrzębiec to learn the techniques she developed and to conduct joint research.

Prof. Olszańska was a member of the Polish Biochemical Society and the Warsaw Scientific Society, as well as a member of the Commission of Bird Reproduction under the Committee on Animal Reproduction Biology, PAS.

Prof. Olszańska was one of the founding members of the Society for Biology of Reproduction (TBR), and the first chairperson of its Warsaw Branch (from 1999 to 2002). She co-organized the 1st TBR Meeting in 1999 in Mierki near Olsztyn, served as the Chair of the Organizing Committee for the 2nd TBR Meeting held in 2001 in Warsaw, and was a member of the Scientific Committee for the 3rd TBR Meeting in Międzyzdroje in 2002.

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Prof. Bożenna Olszańska (1934-2023) ukończyła studia na Wydziale Biologii i Nauk o Ziemi Uniwersytetu Warszawskiego w 1956 r. W roku 1966 uzyskała stopień doktora nauk przyrodniczych w Katedrze Radiochemii i Fizyki Stosowanej Akademii Rolniczej im. Timiriaziewa w Moskwie, w roku 1985 – stopień doktora habilitowanego w Instytucie Biochemii i Biofizyki PAN, a w roku 1999 tytuł profesora nauk rolniczych.

Działalność naukowa prof. Olszańskiej związana była przede wszystkim z Instytutem Genetyki i Hodowli Zwierząt PAN (obecnie Instytut Genetyki i Biotechnologii Zwierząt PAN) w Jastrzębcu, którego pracownikiem była od roku 1965. W tym czasie odbyła wiele staży w placówkach zagranicznych, m.in. w Universite Libre de Bruxelles (Belgia), Institut d'Embryologie w Nogent-sur-Marne (Francja), Institut Jaques Monod w Paryżu (Francja) oraz w Roslin Institute (Wielka Brytania).

Badania prof. Olszańskiej dotyczyły przede wszystkim biotechnologii zarodków ptaków, a korzystała w nich z modelu przepiórki japońskiej (*Coturnix japonica*). Do jej najważniejszych osiągnięć badawczych należy zaliczyć analizę ekspresji genów w oogenezie i wczesnym rozwoju zarodka ptaka, w tym ilościowe określenie akumulacji i rozmieszczenia macierzystego RNA w oocytach ptasich, wykrycie obecności enzymów nukleolitycznych (RNaz, DNaz) w oocytach ptaka czy stwierdzenie występowania transkryptów receptorów melatoniny w oocytach i bruzdkujących zarodkach ptasich. Prof. Olszańska była autorką wielu prac badawczych oraz współautorką 2 książek, m.in. „Organization of the Early Vertebrate Embryo" (1994). Prace jej autorstwa zostały wyróżnione nagrodami Sekretarza Naukowego PAN (1974, 1983), oraz wyróżnieniem Wydziału V PAN (1997).

Bezspornym wkładem prof. Olszańskiej w rozwój biologii rozrodu ptaków było opracowanie oryginalnych metod owulacji i zapłodnienia oocytów ptaków *in vitro*. Znaczenie tych nowatorskich metod dla rozwoju dziedziny podkreśla fakt, że przedstawiciele wielu zagranicznych jednostek naukowych, m.in. z Roslin Intitute (Szkocja, Wielka Brytania), INRA (Francja), University of London (Wielka Brytania) oraz Indii, Ukrainy czy Japonii niejednokrotnie odbywali wielomiesięczne staże naukowe w laboratorium prof. Olszańskiej w Jastrzębcu, aby nauczyć się opracowanych przez nią technik i prowadzić wspólne badania naukowe.

Prof. Olszańska była członkiem Polskiego Towarzystwa Biochemicznego oraz Towarzystwa Naukowego Warszawskiego, a także członkiem Komisji Rozrodu Ptaków przy Komitecie Biologii Rozrodu Zwierząt Użytkowych PAN.

Prof. Bożenna Olszańska była jednym z członków-założycieli Towarzystwa Biologii Rozrodu), a także pierwszą przewodniczącą Oddziału Warszawskiego TBR w latach 1999-2002. Była współorganizatorką I Zjazdu TBR w roku 1999 w Mierkach k/Olsztyna, przewodniczącą Komitetu Organizacyjnego II zjazdu TBR, który odbył się w 2001 w Warszawie oraz członkiem Komitetu Naukowego III Zjazdu TBR w Międzyzdrojach w 2002 roku.

REPRODUCTIVE BIOLOGY

ELSEVIER

Reproductive Biology is an international, peer-reviewed journal published quarterly. It is dedicated to disseminating high-quality research in all aspects of reproductive biology. The journal welcomes original research articles, short communications, technical notes, reviews, and mini-reviews, offering a comprehensive platform for exchanging knowledge.

Reproductive Biology covers a wide range of topics within reproductive biology and medicine, with a special focus on translational research. The journal serves as a bridge between studies in human medicine and animal research, emphasizing the concept of One Health. The journal publishes cutting-edge research on reproductive physiology, endocrinology, reproductive immunology (including endocrine-related cancers), obstetrics and gynecology, andrology, infertility, embryology, assisted reproduction, contraception, and animal breeding and reproduction, with particular attention to domestic, large, and small animals.

Reproductive Biology key mission is to promote the translational nature of research, fostering connections between human and animal studies to enhance our understanding of reproduction across species.

We encourage all Members and Friends of the Society for Biology of Reproduction to publish their research results in Reproductive Biology. **This is our journal!**

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Towarzystwo Biologii Rozrodu
Society for Biology of Reproduction

Institute of Animal Reproduction and Food Research PAS

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CONFERENCE PROGRAMME

Programme overview

Conference venue:
Faculty of Biology, University of Warsaw
Miecznikowa 1, 02-096 Warsaw

12.09.2024 – Day 1

9:00-12:00	Preconference workshops for young researchers: **WORKSHOP 1** (for young researchers with a PhD degree) – '*Kamienie milowe na drodze do owocnej kariery naukowej*' [workshop in Polish] hosted by **Piotr Jaworski** **WORKSHOP 2** (for PhD students) – *'How to live with research failures and not be discouraged'* [workshop in Polish or English] hosted by **Paweł Frelik** and **Monika M. Kaczmarek**
From 11:00	Registration
12:00-13:00	Lunch
13:00-13:15	Official opening of the conference
13:15-14:00	**Sesja I. Historia, teraźniejszość i przyszłość Towarzystwa Biologii Rozrodu** [session in Polish] **Adam Zięcik** (Instytut Rozrodu Zwierząt i Badań Żywności PAN, Olsztyn, Poland) **Monika M. Kaczmarek** (Instytut Rozrodu Zwierząt i Badań Żywności PAN, Olsztyn, Poland)
14:00-14:30	**Ceremonia wręczenia Medali TBR** [award ceremony in Polish] Laureaci: • śp. prof. dr hab. Bronisława Chełmońska • dr hab. Aneta Andronowska • dr hab. Edyta Molik • prof. dr hab. Zdzisław Gajewski • prof. dr hab. Janusz Rząsa
14:30-15:00	**Prof. Władysław Bielański Award Ceremony** **Laureate's lecture:** **L.1** '*What if everything wraps around extracellular vesicles? Novel role of embryonic signals in shaping EV-mediated cell-to-cell communication during pregnancy*' **Maria Guzewska** (Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland)
15:00-15:30	Coffee break

15:30-17:30	**Session II. Gamete development and function** Chairs: **Marta Olszewska** (Institute of Human Genetics PAS, Poznań, Poland) and **Julia Gabryś** (University of Agriculture in Krakow, Kraków, Poland) **15:30-16:15 - Invited lecture:** **L.2** *'MAIA, the human-specific oocyte ligand, facilitates gamete membrane fusion'* **Katerina Komrskova** (Institute of Biotechnology CAS, Vestec, Czech Republic) **16:15-17:30 - Short talks selected from abstracts:** **O.2.1** *'Impact of NANOS1 variant on early embryonic human germ cell development: altered ribonucleoprotein interactome'* **Matisa Alla** (Institute of Human Genetics PAS, Poznań, Poland) **O.2.2** *'The influence of age and the timing of the reproductive season on sperm quality in African penguins (Spheniscus demersus)'* **Paweł Borecki** (Zoo Wrocław and Wrocław University of Environmental and Life Sciences, Wrocław, Poland) **O.2.3** *'Impact of Sperm Fractioning on Chromosome Positioning and Chromatin Integrity'* **Zuzanna Graczyk** (Institute of Human Genetics PAS, Poznań, Poland) **O.2.4** *'Spatiotemporal distribution of mitochondria during meiotic progression in bovine oocytes'* **Zofia Madeja** (Poznań University of Life Sciences, Poznań, Poland) **O.2.5** *'Diet induced hyperhomocysteinemia impairs mitochondria content and lipid metabolism in mice oocytes, as revealed by single cell RNA-seq and fluorescent labelling of intracellular organelles'* **Zuzanna Gonera** (Poznań University of Life Sciences, Poznań, Poland)
17:30-18:00	Coffee break
18:00-19:00	**Biologia rozrodu dla każdego – wydarzenie popularnonaukowe online** [an outreach event in Polish] *'Komórki macierzyste pomiędzy nauką a medycyną'* **Zofia Madeja** (Uniwersytet Przyrodniczy w Poznaniu, Poznań, Polska)

	'Czy dieta ketogeniczna może wpływać na płodność?' **Piotr Kaczyński** (Instytut Rozrodu Zwierząt i Badań Żywności PAN, Olsztyn, Polska)
18:00-19:00	Poster session (odd numbers)
19:00-22:00	Welcome party Faculty of Biology, University of Warsaw

13.09.2024 – Day 2

From 9:00	Registration
9:00-11:00	**Session III. Mechanism of early embryonic development** Chairs: **Maciej Murawski** (University of Agriculture in Krakow, Kraków, Poland) and **Patrycja Konowrocka** (Institute of Genetics and Animal Biotechnology PAS, Jastrzębiec, Poland) **9:00-9:45 - Invited lecture:** **L.3** *'Post-transcriptional regulation cell fate acquisition during preimplantation mouse embryo development; the emerging role of mTOR and p38-MAPK signalling'* **Alexander W. Bruce** (University of South Bohemia, České Budějovice, Czech Republic) **9:45-11:00 - Short talks selected from abstracts:** **O.3.1** *'Effects of early-life malnutrition on reproductive performance and early embryonic development'* **Dominika Kawka** (Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland) **O.3.2** *'Role of visfatin (Nampt) on early embryogenesis. Studies on siRNA induced gene knockdown mouse model'* **Patrycja Kurowska** (Jagiellonian University in Kraków, Kraków, Poland) **O.3.3** *'XIAP role in executing apoptosis in parthenogenetic porcine and bovine embryos'* **Piotr Pawlak** (Poznań University of Life Sciences, Poznań, Poland) **O.3.4** *'Characterization of the dynamics of the RUNT-related transcription factor RUNX1 and its localization and expression in extra-embryonic lineages in the mouse blastocyst'* **Roberto de la Fuente** (The University of Manchester, Manchester, UK and Institute of Genetics and Animal Biotechnology PAS, Jastrzębiec, Poland)

	O.3.5 *'The velocity of cytoplasmic movement in trophectoderm cells as a noninvasive marker of mouse embryo quality'* **Agnieszka Walewska** (University of Warsaw, Warsaw, Poland)
11:00-11:30	Coffee break + group photo
11:30-13:30	**Session IV. Assisted reproduction and preservation of fertility** Chairs: **Barbara Grzechocińska** (Warsaw South Hospital, Warsaw, Poland) and **Bartłomiej Wysoczański** (The Kielanowski Institute of Animal Physiology and Nutrition PAS, Jabłonna, Poland) **11:30-12:15 - Invited lecture:** ***L.4*** *'DNA fragmentation, oxidative stress and what should men do about it?'* **Darren Griffin** (University of Kent, Canterbury, UK) **12:15-13:30 - Short talks selected from abstracts:** **O.4.1** *'Evaluation of the effectiveness of gamete preservation in roe deer'* **Anna Korzekwa** (Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland) **O.4.2** *'Sucrose and trehalose: cryoprotectants for chicken semen cryopreservation'* **Azindokht Babapour** (Wrocław University of Environmental and Life Sciences, Wrocław, Poland) **O.4.3** *'Parthenogenetic development of human and bovine oocytes activated after slow-freezing or vitrification, may serve as a potent tool of cryopreservation efficacy evaluation instead of fertilization'* **Krzysztof Papis** (Fertility Clinic nOvum and Warsaw University of Life Sciences, Warsaw, Poland) **O.4.4** *'Testicular biopsy in azoospermic patients after cryptorchidism surgery'* **Jan Karol Wolski** (Fertility Clinic nOvum, Warsaw, Poland) **O.4.5** *'Results of Ovum Pick Up performed in Poland and combined with ICSI results'* **Magdalena Profaska** (University of Agriculture in Krakow, Kraków, Poland)
13:30-14:30	Lunch
14:00-15:00	Poster session (even numbers)

15:00-16.45	**Session V. Embryo-maternal interactions and pregnancy** Chairs: **Zofia Madeja** (Poznań University of Life Sciences, Poznań, Poland) and **Maria Pia Viscomi** (Institute of Genetics and Animal Biotechnology PAS, Jastrzębiec, Poland) **15:00-15:45 – Invited lecture:** **L.5** 'Different and together: cells at the border between mother and fetus' **Francesco Colucci** (University of Cambridge, Cambridge, UK) **15:45-16:45 - Short talks selected from abstracts:** **O.5.1** *'Estradiol and progesterone membrane receptors as potential novel regulators of endometrial receptivity'* **Maria Sztachelska** (Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland) **O.5.2** *'Using single cell transcriptomic and 3D models to study immune-endometrial-fetal interactions in physiological and pathological contexts'* **Ludivine Doridot** (Institute Cochin, Paris, France) **O.5.3** *'Preconceptional innate immunomodulation leads to a partial correction of gestational complications induced by endometriosis in a murine model'* **Kheira Bouzid** (Institute Cochin, Paris, France) **O.5.4** *'Regulatory B cells and IL-33 in normal pregnancy and after miscarriage in women'* **Anna Chełmońska-Soyta** (Hirszfeld Insitute of Immunology and Experimental Therapy PAS and Wrocław University of Environmental and Life Sciences, Wrocław, Poland)
16.45-17:15	Coffee break
17:15-19:00	TBR General Assembly
20:00-24:00	Concert and Gala dinner Novotel Warszawa Airport Hotel, 1 Sierpnia 1, 02-143 Warsaw

14.09.2024 – Day 3

From 9:00	Registration
10:00-12:00	**Session VI. Hormonal, neuronal, and immune regulation of reproduction** Chairs: **Tomasz Misztal** (The Kielanowski Institute of Animal Physiology and Nutrition PAS, Jabłonna, Poland) and **Oana Lupu** (Medical University of Białystok, Białystok, Poland) **10:00-10:45 - Invited lecture:** **L.6** *'Mechanisms underlying sex-specific prenatal programming of the offspring's brain and behaviour by maternal psychosocial stress'* **Paula Brunton** (University of Edinburgh, Edinburgh, UK) **10:45-12:00 - Short talks selected from abstracts:** **O.6.1** *'GW0742, by reduction of energy metabolism, decreases viability of adult ovarian granulosa cell tumor'* **Justyna Gogola-Mruk** (Jagiellonian University in Kraków, Kraków, Poland) **O.6.2** *'Expression of asprosin/furin/Olfr734 in mouse hypothalamus and pituitary fluctuates along oestrus cycle and differs in diet-induced obese mice'* **Dominika Wachowska** (Jagiellonian University in Kraków, Kraków, Poland) **O.6.3** *'The role of ghrelin and serotonin receptors in the activation of IP3/DAG signaling pathway in the melatonin biosynthesis during the breeding season in ewes'* **Katarzyna Kirsz** (University of Agriculture in Krakow, Kraków, Poland) **O.6.4** *'Th2 specific interleukins: IL-4 and IL-13 – profibrotic response and beyond – transcriptomic and functional in vitro study on mare endometrial fibroblasts'* **Anna Wójtowicz** (Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland) **O.6.5** *'Yellow semen syndrome in reproductive turkeys (Meleagris gallopavo) might be connected with altered content and polarization of macrophages in testis, epididymis, ductus deferens, and Bursa of Fabricius'* **Ewa Drzewiecka** (Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland)
12:00-12:30	Coffee break

12:30-14:30	**Session VII. Environmental influence on reproductive function** Chairs: **Anna Ptak** (Jagiellonian University in Kraków, Kraków, Poland) and **Magdalena Skowrońska** (Medical University of Białystok, Białystok, Poland) **12:30-13:15 - Invited lecture:** **L.7** *'Glyphosate based herbicides in bird and human fertility'* **Joelle Dupont** (French National Institute for Agriculture, Food, and Environment (INRAE), Nouzilly and Tours University, Tours, France) **13:15-14:30 - Short talks selected from abstracts:** **O.7.1** *'Support of the reproductive potential by immune system in males of roe deer is dependent on the habitat'* **Ummu Gulsum Boztepe** (University of Agriculture in Krakow, Kraków, Poland) **O.7.2** *'Factors stimulating movement of fish spermatozoa'* **Katarzyna Dziewulska** (University of Szczecin, Szczecin, Poland) **O.7.3** *'Can Staphylococcus bacteria support male fertility?'* **Monika Frączek** (Institute of Human Genetics PAS, Poznań, Poland) **O.7.4** *'The impact of selected pesticides on rooster fertility and reproductive hormones levels'* **Agnieszka Partyka** (Wrocław University of Environmental and Life Sciences, Wrocław, Poland) **O.7.5** *'Novel Plasticizers, Bisphenol S and F negatively affect Meiotic Maturation and Spindle Structure of Mouse Oocytes'* **Oliwia Jędruch-Smulska** (University of Warsaw, Warsaw, Poland)
14:30-15:30	Lunch
15:30-17:30	**Session VIII. Gonadal development and function** Chairs: **Kamila Kusz-Zamelczyk** (Institute of Human Genetics PAS, Poznań, Poland), **Wojciech Niżański** (Wrocław University of Environmental and Life Sciences, Wrocław, Poland) and **Kinga Kamińska** (Jagiellonian University in Kraków, Kraków, Poland) **15:30-16:15 - Invited lecture:** **L.8** *'Studying neglected cell populations of the developing testis and their functions'* **Serge Nef** (University of Geneva, Geneva, Switzerland)

	16:15-17:30 - Short talks selected from abstracts: **O.8.1** *'HAND2, a GATA4 interacting protein is a new potential genetic player in human gonadal development as its variant is associated with 46,XY gonadal dysgenesis'* **Kamila Kusz-Zamelczyk** (Institute of Human Genetics PAS, Poznań, Poland) **O.8.2** *'Impaired steroidogenesis in obese female mice is driven by Nodal suppression in theca cells'* **Karolina Wołodko** (Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland) **O.8.3** *'Visfatin Impact on Angiogenesis, Proliferation, and Apoptosis in the Porcine Corpus Luteum'* **Ewa Mlyczyńska** (Jagiellonian University in Kraków, Kraków, Poland) **O.8.4** *'Patients with premature ovarian failure have reduced glucose bioavailability in the ovarian follicle'* **Weronika Marynowicz** (Jagiellonian University in Kraków, Kraków, Poland) **O.8.5** *'Variability of boar testicles echotextural image during the immunocastration procedure'* **Tomasz Schwarz** (University of Agriculture in Krakow, Kraków, Poland)
17:30-18:00	Closing remarks

Preconference workshops

Our meeting is accompanied by **preconference workshops** for young scientists that will be held in **the Faculty of Biology, University of Warsaw, on September 12th, 2024, at 9:00 am.**

WORKSHOP 1 (for young researchers with a PhD degree)
Milestones on the road to a fruitful scientific career

TEAM. Amundsen and Scott expeditions - Can the experiences of polar explorers from over 100 years ago help in team management? **Hosted by Piotr Jaworski.**

The aim of the workshop is to improve motivation and increase the level of knowledge about project management and the team functioning. Based on the history of two famous expeditions to the South Pole, the greatest challenges facing project teams will be presented.

Can the experiences of Pole conquerors from over 100 years ago help in managing a project team? Definitely yes. The extreme conditions in which they had to achieve their goals allowed them to gather extraordinary knowledge about teamwork. It is this knowledge supported by the most interesting findings of contemporary researchers that will be the subject of the training.

WORKSHOP 2 (for PhD students)
How to live with research failures and not be discouraged

FAILURES: STIGMA or OPPORTUNITY. Can we survive in the scientific environment driven by metrics and success, where shortcomings and failures abound? **Hosted by Monika M. Kaczmarek and Paweł Frelik**

In an environment driven by metrics and success, shortcomings and failures are often perceived as stigmas that make one unfit for the profession. This perception of the scientific career is then internalized by both young and experienced researchers, often leading to emotions ranging from a sense of inadequacy to the impostor syndrome to depression. Our workshop aims to disenchant failure and frame it as a normal and even necessary element of academic life and research. In that, we will look at selected case studies and discuss possible ways of handling crises, arguing that failure is an inescapable and necessary part of being a researcher.

Piotr Jaworski – Certified auditor, mentor, and business trainer. Team leader with over 20 years of experience. Graduate of the Warsaw School of Economics (SGH), Kozminski University and Maria Curie-Skłodowska University. Head of audit teams in many organizations (ARiMR, PGZ S.A., PKP Intercity, PKP PLK). For over 20 years, he has been assessing organizations and projects as an auditor and evaluator. Expert in management and control systems. He began to gain extensive experience in this field in the late 1990s as coordinator of the European Program at the Institute of Public Affairs. After the end of the conflict in the Balkans, as an OSCE employee, he implemented the peace plan in Serbia and Macedonia, learning in practice what a multicultural team is all about. As the owner of a consulting company, he has developed and implemented internal control and risk

management systems in several hundred units of the public finance sector, including the largest Polish metropolises - Warsaw, Wrocław, Gdańsk. Former head of the Collegium Wratislaviense Mentor School. Lecturer in mentoring and at postgraduate studies at the Warsaw School of Economics. (SGH), Poznań University of Economics and Business, and Catholic University of Lublin. Author of articles on evaluation, control, audit and project management. Co-author of the book "Academic mentoring program in practice". Author of an online course on remote team management and coaching management style.

Paweł Frelik – Associate Professor in the American Studies Center, University of Warsaw. His research interests include audiovisual media, popular genres, and politics of/in culture. He is the author of over 150 international publications and sits on the editorial boards of Science Fiction Studies (USA), Extrapolation (USA/UK), and Journal of Gaming and Virtual Worlds (UK).
He also co-edits the New Dimensions in Science Fiction book series at the University of Wales Press. In 2012-14, he was President of Science Fiction Research Association (USA) and now serves as President of International Association for the Fantastic in the Arts (USA). In both positions, he has been the first researcher from outside the United States and Great Britain.

Monika M. Kaczmarek – Associate Professor in the Institute of Animal Reproduction and Food Research PAS in Olsztyn. Her research interests are broad, spanning multiple disciplines, but are primary focused on unrevealing the molecular mechanisms of progeny-maternal interactions during pregnancy and the postnatal period. She has received numerous scientific awards and fellowships, including recognitions from the Foundation for Polish Science, the Polish-American Fulbright Commission, and the Alexander von Humboldt and Hertie Foundations. With over 70 scientific articles and several book chapters published, she adeptly combines her passion for science with activities in popularization and organization. Since 2020, she has been a member of the Council of the National Science Center.

Outreach event

Our conference is accompanied by an **online outreach event in Polish** entitled **'Biologia rozrodu dla każdego'** ('Biology of reproduction for everyone') consisting of two lectures dedicated to different aspects of reproductive biology. It will be held **on September 12th, 2024, at 18:00.**

18:00-18:30 - *'Komórki macierzyste pomiędzy nauką a medycyną' (Stem cells between science and medicine)* **Zofia Madeja** (Uniwersytet Przyrodniczy w Poznaniu, Poznań, Polska)

18:30-19:00 - *'Czy dieta ketogeniczna może wpływać na płodność?' (Can ketogenic diet affect fertility?)* **Piotr Kaczyński** (Instytut Rozrodu Zwierząt i Badań Żywności PAN, Olsztyn, Polska)

The outreach online event is dedicated primarily to secondary and high school students, but everyone is welcome. The lectures will be transmitted live on the TBR FaceBook profile:

https://www.facebook.com/TowBiolRozrodu

Zofia E. Madeja is a biotechnologist working in the Department of Genetics and Animal Breeding (Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences), where she conducts scientific research and teaches students. She is an expert in embryology, genetics, molecular biology, and biotechnology applied to animal reproduction. She gained professional experience through research internships in renown scientific institutions, including MTT Agrifood-Research Finland and the University of Cambridge. She has been the recipient of numerous scholarships and grants, including the Homing/Powroty grant of the Foundation for Polish Science, the Minister of Science and Higher Education Scholarship for Outstanding Young Scientists, and grants from the National Science Centre. Currently, she is working on a new project aimed at understanding how mitochondria regulate and affect the quality of embryos (their potential for proper development).

Piotr Kaczyński works at the Institute of Animal Reproduction and Food Research PAS in Olsztyn. He developed his scientific expertise during research internships at scientific institutions such as the Functional Genomics Laboratory in Munich and the Swiss Federal Institute of Technology in Zurich. For his scientific achievements, he was awarded the scholarship for young scientists conducting innovative research (DrINNO) and the Minister of Education and Science Scholarship for Outstanding Young Scientists. His research focuses on the molecular interactions between embryos and the maternal organism during early pregnancy. The goal of his latest project is to investigate whether a ketogenic diet affects the quality of oocytes and whether it can influence the health of offspring.

LAUREATES

Prof. Władysław Bielański Award:

Dr Maria Guzewska has been working at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn since 2016. She defended her doctoral thesis, titled "*Biogenesis and release of extracellular vesicles during communication between the embryo and the maternal endometrium during early pregnancy in pigs*" in 2023 with distinction. Her scientific work focuses on the biogenesis of extracellular vesicles (EVs) and their crucial involvement in establishing embryo-maternal communication at the early stages of pregnancy. **Dr Guzewska received the Władysław Bielański Award for her collection of interrelated scientific publications describing the role of embryonic signals in the EV-mediated cell-to-cell communication during pregnancy.**

Medals of the Society for Biology of Reproduction (TBR Medals):

Prof. dr hab. inż. Bronisława Chełmońska (1934-2020) dedicated her professional life to the innovative exploration of the connections between the physiology of reproductive processes and their impact on the breeding performance of domestic birds. She was a distinguished scientist and the founder of the Polish school of poultry insemination. Her research interests were primarily centered on the biology of the reproductive organs of male domestic birds. She not only studied the physiological basis of spermatogenesis but also examined the internal and external factors influencing semen quality, especially during storage in liquid nitrogen and its fertilization capacity. She highlighted the distinctiveness of geese reproductive physiology compared to other domestic bird species and successfully obtained hybrids of wild and domestic geese. She also initiated studies on the creation of genetic reserves for birds. As a long-standing head of the Department of Poultry Breeding at the Wrocław University of Life Sciences, Prof. Chełmońska was an exceptional academic teacher and mentor to many generations of students and researchers in reproductive biology. As a recognized scientist, she was a honorary member of the World Poultry Science Association, the Scientific and Technical Council on Insemination of the Minister of Agriculture, the Commission on Biology of Bird Reproduction of the Committee on Biology of Reproduction of Farm Animals PAS, and the Board of Directors of the Polish Zootechnical Society. She was a founding member of the Zootechnical Society,the Society for Biology of Reproduction (TBR) and the founder and the first president of the Wrocław Branch of TBR. Prof. Chełmońska received numerous honors, including the Knight's Cross of Polonia Restituta, the Medal of the National Education Commission, the Golden Cross of Merit, and the badge “Meritorious for the Wrocław Province and the City of Wrocław.” She was known for her kindness and cheerful demeanor, and she was passionate about reliable and open science. **Prof. Chełmońska was posthumously awarded the TBR Medal for creating a scientific school of reproductive biology of domestic birds and her involvement in the foundation of TBR and its Wrocław Branch.**

Dr hab. Aneta Andronowska is a professor at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences. She conducts research on the reproductive biology of farm animals, focusing on immuno-endocrine and molecular mechanisms involved in embryo-uterine interactions during early pregnancy and the role of chemokines in the peri-implantation period in the porcine uterus. Currently, she is involved in studying the impact of extracellular vesicles isolated from follicular fluid on spermatozoa function. She has been actively involved in the creation and development of the Society for Biology of Reproduction (TBR) since its beginning. Since 1999, she has played a significant role in the activities of the Olsztyn Branch of the Society, and since 2002, in the activities of the Society's Main Board. Within the Olsztyn Branch, she served as Secretary for two terms (1999-2002 and 2002-2005). On the Society's Main Board, she held various positions across multiple terms: Secretary of the Board (2002-2005; 2005-2008) and Treasurer of the Board (2011-2014 and 2014-2017). Prof. Andronowska has also been instrumental in organizing and co-organizing numerous conferences for the Society for Biology of Reproduction. Notable contributions include serving as a member of the organizing committee for the 6th Congress of the Society for Biology of Reproduction (September 7-10, 2011, Polańczyk, Poland); Main Secretary of the "Animal Models for Human Health" conference (June 7-8, 2013, Łańsk, Poland); Main Secretary of the workshop "Pigs Reproduction for Practitioners" (June 8, 2013, Olsztyn); Main Secretary of the 9th International Conference on Pig Reproduction (ICPR, June 9-12, 2013, Olsztyn); and Co-organizer of the 8th Congress of the Society for Biology of Reproduction (September 7-9, 2017, Olsztyn). **Prof. Andronowska was awarded the TBR Medal for her long-term and multifaceted organizational activities aimed at the development of TBR.**

Dr hab. Edyta Molik, prof. URK, has been conducting research on the influence of day length and melatonin signals on the secretion of gonadotropic, lactogenic, and metabolic hormones in both seasonal and non-seasonal sheep. Apart from her scientific work, she has been for many years actively involved in the activities of the Society for Biology of Reproduction (TBR). In 2005, she was a member of the organizing committee for the 4th Symposium of the Polish Society for Biology of Reproduction and Joint Polish-Japanese Seminar. Since 2007, she has co-organized five conferences on "Central and Local Regulations of Reproductive Processes," which are held in Zakopane (2007 - 1st Winter Conference, 2010 - 2nd Winter Conference, 2013 - 3rd Winter Workshop, 2016 - 4th Winter Workshop, 2019 - 5th Winter Workshop). From 2010 to 2013, she served as the Treasurer of the TBR Branch in Kraków, from 2013 to 2021 - she was presided the Kraków Branch. Between 2015 and 2018, she presided the Prof. Władysław Bielański Award Committee. As part of her work in the TBR branch in Kraków, she organized scientific meetings in collaboration with national scientific institutions and members of the Ukrainian Academy of Sciences. **Prof. Molik was awarded the TBR Medal for her longstanding organizational activity for TBR, in particular for organizing the TBR Winter School series in Zakopane.**

Prof. dr hab. Zdzisław Gajewski has been associated with the Faculty of Veterinary Medicine at the Warsaw University of Life Sciences (SGGW) throughout his entire professional career. He currently heads the SGGW Center for Translational Medicine. He was the initiator and project leader for the establishment of the Diagnostic and Experimental Center and the Center for Regenerative Medicine. For his professional activities, he has been awarded the Gold Medal for Long Service (2013), the Individual Award of the Minister of Education and Science (2015 and 2023), the Medal of the Commission for National Education (2016), and numerous individual and team awards from the Rector of SGGW. **Prof. Gajewski was awarded the TBR Medal for his organizational work for TBR and 'Reproductive Biology' journal, as well as for developing telemetry-based EMG methods for studying reproductive system of females.**

Prof. dr hab. Janusz Rząsa worked until his retirement at the University of Agriculture in Kraków. His research focused on bird physiology, with particular emphasis on the endocrine regulation of reproductive functions in female domestic birds. Among his greatest scientific achievements are characterizing the biological properties of hormones in the neural part of the pituitary gland in domestic birds, describing the interaction of vasotocin and prostaglandins in egg transport through the avian oviduct, determining ovarian steroidogenic activity in various physiological states, and defining the physiological role of biogenic amines in the functioning of the avian ovary and oviduct. Throughout his career, he has published 426 works, including 160 original scientific papers and 240 conference communications. He also co-authored two textbooks. Prof. Rząsa was committed to organizational work and served as Dean of the Faculty of Animal Husbandry from 1990 to 1996 and as Head of the Department of Animal Physiology from 1994 to 2009. He was actively engaged in organizing doctoral studies at his university. As a founding member of the Society for Biology of Reproduction (TBR), Prof. Rząsa served on the TBR Board and chaired the Kraków Branch of TBR from 1999 to 2002. He is currently an honorary member of TBR. Additionally, he was a member of the Committee on the Biology of Reproduction of Farm Animals of the Polish Academy of Sciences, Chairman of the Committee on Bird Reproductive Biology, and Vice-Chairman of the Committee on Biology of the Kraków Branch of the Polish Academy of Sciences (2004-2010). Prof. Rząsa's contributions have been recognized with the Knight's and Officer's Crosses of the Order of Polonia Restituta and the Medal of the Commission for National Education. He has received awards from the Minister of Science, Higher Education and Technology five times, as well as numerous awards from the Rector of the University of Agriculture in Krakow. **Prof. Rząsa was awarded the TBR Medal for his outstanding scientific achievements in avian reproduction, his involvement in the foundation of TBR, and his efforts in organizing the TBR Branch in Kraków.**

INVITED SPEAKERS

Invited speakers:

Alexander W. Bruce (University of South Bohemia, České Budějovice, Czech Republic) graduated from University of Leeds (UK) and obtained his post-doctoral training in Sanger Institute (Hinxton, UK) and in University of Cambridge (UK). From 2010 he has been a head of the Laboratory of Early Mammalian Development in University of South Bohemia (České Budějovice, Czech Republic). He investigates molecular mechanisms underlying the formation of the three mammalian blastocyst lineages (extraembryonic trophectoderm and placenta and the pluripotent epiblast). His lab has particular focus on dynamic regulation of the cytoskeleton and metabolic influences that impinge on protein translation of specific mRNA transcripts under the regulation of the p38-MAPKi stress kinase and mTOR signalling pathways.

Paula J Brunton (University of Edinburgh, Edinburgh, UK) is a Senior Lecturer in the Centre for Discovery Brain Sciences, University of Edinburgh, UK and an Associate Professor at the Zhejiang University-University of Edinburgh Joint Institute in Haining, China. She received her PhD in Neuroendocrinology from the University of Edinburgh in 2002. Her expertise lies in the area of stress neurobiology, neuroendocrinology and behaviour, with key research themes focused on understanding the impact of maternal stress exposure during pregnancy on the mother, the pregnancy, her offspring and on subsequent generations. Paula is a member of the Board of Trustees of the British Society for Neuroendocrinology and a Senior Editor for the Journal of Neuroendocrinology.

Francesco Colucci (University of Cambridge, Cambridge, UK) trained as a doctor at the University of Bari (M.D. 1991) and learnt immunology at the University of Umeå (Ph.D. 1997). He studied natural killer (NK) cells during his post-doc at the Necker Hospital in Paris and became member of the Pasteur Institute in Paris (2000), group leader at the Babraham Institute in Cambridge (2004) and professor of immunology at the University of Cambridge (2010). His team continues to study how NK cells work in immunity and reproduction. He is fellow, director of studies in medicine and graduate admissions tutor at King's College, Cambridge, manager of the Cambridge Centre for Trophoblast Research, advisor of the Ceppellini School of Immunology in Naples, and visiting professor at the University of Turin.

Joëlle Dupont (French Research for Agriculture, Food and Environment Institute (INRAE), Nouzilly and Tours University, Tours, France) is a director of research at the INRAE in France at Nouzilly and a deputy director of the Reproductive Physiology and Behavior Unit. Her current research interests include: the role of adipokines in the reproductive tract in domestic animals and human, and the impact of endocrine disruptors and plant extracts on fertility. Joëlle authored over 230 papers and her work has been cited more than 13000 times.

Darren Griffin (University of Kent, Canterbury, UK) received his BSc and DSc degrees from the University of Manchester and his PhD from University College London. After postdoctoral stints at Case Western Reserve University and the University of Cambridge, he landed his first academic post at Brunel University, before settling at the University of Kent, nearly 20 years ago. He is a Fellow of the Royal College of Pathologists, the Royal Society of Biology and the Royal Society of Arts and is President of the International Chromosome and Genome Society. A world leader in cytogenetics, he performed the first successful cytogenetic PGT and played a significant role in the development of Karyomapping, an approach he now applies to cattle and pigs. He has co-authored ~400 scientific publications, mainly on the cytogenetics of reproduction and evolution, recently providing insight into the karyotypes of dinosaurs. He is a prolific science communicator, a part time TV presenter, and an enthusiastic proponent of interdisciplinary research endeavour. He has supervised 40 PhD students to completion and his work appears consistently in the media. He runs a vibrant research lab of ~25 people (including a programme of externally supervised students) and maintains commercial interests relating to the outcomes of research findings.

Katerina Komrskova (Institute of Biotechnology CAS, Vestec, Czech Republic) studied biology and chemistry at the Faculty of Science, Charles University, Czech Republic, where she also obtained a doctoral degree in Developmental Biology. She is currently a head of the Group of Reproductive Biology, Institute of Biotechnology of the Czech Academy of Sciences, and an associated professor in the Department of Zoology, Faculty of Science, Charles University. Her research is focused on studying the molecular mechanisms of fertilization and the nature of specific sperm proteins that play a key role in sperm maturation, sperm-egg interaction and fusion, and early embryo development. Her expertise covers monitoring sperm quality in men with infertility issues and patients with testicular cancer and other pathological challenges. She designs novel diagnostic tools for quality assessment and gamete selection to be utilised in human Assisted Reproduction and livestock breeding strategies.

Serge Nef (University of Geneva, Geneva, Switzerland) is a professor at the Faculty of Medicine, University of Geneva. He investigates the molecular mechanisms regulating gonadal differentiation and testicular function in mammals. His laboratory uses molecular and cellular techniques, mouse functional genomics, and human genetics to investigate the complex gene networks that regulate primary sex determination, testis development, and function. In recent years, his laboratory has developed expertise in analyzing single-cell RNA sequencing data. This allows better characterization of cell lineages in vivo and the study of the complex mechanisms of gonadal differentiation in combination with transgenic mouse models. In particular, his laboratory is investigating how cell fate decisions are made during testicular and ovarian differentiation.

ABSTRACTS
INVITED LECTURES

Invited lectures:

L.1

What if everything wraps around extracellular vesicles? Novel role of embryonic signals in shaping EV-mediated cell-to-cell communication during pregnancy

Maria M. Guzewska

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In mammals, blastocyst implantation is restricted to a specific period known as the "window of implantation", a limited timeframe when blastocyst competency aligns with the receptive state of the uterus. To achieve this receptivity, the endometrium undergoes significant molecular and cellular changes, essential for successful implantation of embryo(s) at the exact stage of synchronized development. Current research underscores the importance of a coordinated, timed, and bilateral dialogue between the developing embryo and the uterine surface epithelium. This dialogue is mediated by various embryonic signals, such as estradiol (E2) and prostaglandin E2 (PGE2) in domestic pigs, or chorionic gonadotropin in humans. Over the past decade, the role of extracellular vesicles (EVs) has been increasingly recognized as critical in this embryo-maternal communication. EVs, particularly their molecular cargo, are now seen as crucial modulators and triggers of intercellular signaling both locally and across distant cells, playing a pivotal role in the successful establishment of pregnancy.

Results published in the award-winning publications have unequivocally shown that during the peri-implantation period – specifically during the highly conserved apposition and attachment phases among mammalian species – the porcine trophoblast and uterine endometrium secrete EVs as key mediators of cell-to-cell communication. Notably, during gestation days 10-12, the EV biogenesis machinery and the patterns of EV cargo sorting are influenced by pregnancy-related factors, including E2, PGE2, and microRNAs (miRNA). This interaction results in both the mother and the developing embryo releasing a unique blend of EVs. Additionally, miR-125b-5p, which was found to be encapsulated in EVs present in the uterine lumen, has been shown to affect EV biogenesis machinery and cargo sorting patterns, leading to the formation and release of a distinct population of EVs by trophoblast cells.

These findings unveil a previously undescribed interconnection between the crucial phases of early pregnancy, the factors secreted during these phases, and the molecular machinery governing the biogenesis and release of EVs. For the first time, embryonic signals such as E2, PGE2, and miRNAs have been identified as key modulators of EV biogenesis and release during early pregnancy events in mammals. With recent scientific advancements, EV-mediated cell-to-cell communication has emerged as a central focus, highlighting the potential to use EVs as biomarkers for physiological processes or pathological conditions during pregnancy. Moreover, utilizing EVs as vehicles for delivering personalized molecular offers a promising novel approach to addressing implantation failures and reducing embryo mortality in mammals.

Awarded studies were financed by National Science Centre Poland (018/29/N/NZ9/02331) and Ministry of Science and Higher Education within KNOW Leading National Research Centre Scientific Consortium "Healthy Animal – Safe food" (KNOW2016/IRZBŻ/PRO1/01/5).

L.2

MAIA, the human-specific oocyte ligand, facilitates gamete membrane fusion

Katerina Komrskova[1,2], Jana Vondrakova[1], Michaela Frolikova[1], Lukas Ded[1], Jiri Cerny[3], Pavla Postlerova[1,4], Veronika Palenikova[1], Ondrej Simonik[1], Krystof Basus[1], Eliska Valaskova[1], Radek Machan[5], Allan Pacey[6], Zuzana Holubcova[7,8], Pavel Koubek[9], Zuzana Ezrova[10], Zuzana Nahacka[10], Soojin Park[11], Ruiwu Liu[12], Raghavendran Partha[13], Nathan Clark[14], Jiri Neuzil[10,15], Masahito Ikawa[11], Kent Erickson[16], Kit S. Lam[12], Harry Moore[17]

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Sperm-oocyte membrane interaction and fusion during fertilization is mediated by various molecules that are located on both the oocyte and sperm membranes. Previously, Izumo1, Juno and CD9 were identified as the critical proteins involved in sperm-oocyte fusion and fertilization. It was shown in mouse, that at the point of sperm adhesion to the oocyte, Juno associates with monomeric Izumo1 on sperm membrane, resulting in Izumo1 dimerization and followed by a Juno removal from the oocyte surface. It implies that other additional receptor on the oocyte membrane is required to play a role in sperm-oocyte fusion. We used a random one-bead one-compound (OBOC) combinatorial peptide library, which represented synthetic human egg mimics and identified a novel ligand as Fc receptor-like 3 (FcRL3), named MAIA after the mythological goddess intertwined with JUNO. Interaction between MAIA and JUNO on the oolemma was indicated by co-localization and proximity ligation assays and was confirmed by transmission electron microscopy (TEM) of the close association of both proteins on *microvilli*. Transfected human embryonal kidney cells co-

expressing MAIA and JUNO permitted human sperm to bind and fuse *in vitro*, which was further supported by newly designed sperm - cell GFP fusion assay. Structural modelling suggests that MAIA forms a highly stable interaction with the IZUMO1/JUNO dimer complex, enhancing binding to IZUMO1 on the shedding of JUNO to elicit tight apposition of membranes and to permit specific gamete fusion. MAIA forms a highly stable interaction with the known IZUMO1/JUNO sperm-egg complex, permitting specific gamete fusion. The wide diversification of the MAIA/FcRL3 receptor family during evolution is likely to confer species-specificity during sperm-egg recognition and gamete fusion. The complexity of the MAIA isotype may offer a cryptic sexual selection mechanism to avoid genetic incompatibility and achieve favourable fitness outcomes.

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L.3

Post-transcriptional regulation cell fate acquisition during preimplantation mouse embryo development; the emerging role of mTOR & p38-MAPK signalling.

Alexander W. Bruce

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Preimplantation mouse embryo development involves the temporal-spatial specification and segregation of three blastocyst cell lineages: trophectoderm (TE), primitive endoderm (PrE) and epiblast (EPI). Whilst the power of mouse genetics has enabled great strides in identifying key genetic regulators of cell fate, with particular respect to the composition of the transcriptome and the establishment of important gene regulatory networks, the study and contribution of related post-transcriptional mechanisms is comparatively impoverished. Here I will summarise some of our work focussing on the key central metabolic regulatory kinase mammalian target of rapamycin (mTOR) and the classically recognised stress kinase p38-MAPK (of the mitogen-activated-kinase superfamily). Specifically, how during highly defined developmental time windows both mTOR and p38-MAPK activity, functioning via regulatory interfaces controlling protein synthesis, are required to facilitate the separation of the TE and inner-cell-mass cell populations, as well as facilitating the specification and differentiation of the PrE lineage from the pluripotent EPI, during blastocyst ICM maturation. Hence, highlighting specific control of protein synthesis as important regulatory nodes for the successful emergence of appropriately specified per-implantation stage blastocyst embryos.

L.4

DNA fragmentation, oxidative stress and what should men do about it?

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In making a sperm, premeiotic divisions, meiosis and spermiogenenesis are all essential prerequisites. These, plus fertilization and all subsequent mitotic divisions, require a properly functioning DNA structure. A good human sperm has 23 Chromosomes including an X or a Y, undamaged/non-fragmented DNA, appropriate balance of protamines and histones, good mitochondrial function, a functional acrosome for fertilization, normal morphology and a defined chromatin packaging/organization. Here, the telomeres should be near the periphery and the centromeres and sex chromosomes centrally located. By contrast, a bad sperm may have damaged/fragmented DNA, poorly defined chromosomal organization/packaging, poor protamines/histone balance/mitochondrial function/acrosome/morphology and/or sperm aneuploidy (e.g. both an X and a Y chromosome). Sperm DNA damage can be defined as any chemical change in the normal structure of the DNA, and sperm DNA fragmentation (DNAFrag) is one of the most common. Multiple meta-analyses indicate normal DNA structure may influence embryo development, implantation and pregnancy in both natural and assisted reproduction. Altered DNAfrag is thus associated with reduced fertilization rates, lower blastulation rates, reduced implantation, lower pregnancy and live birth rates, altered obstetric outcomes and aneuploidy (paternal and maternally derived) in embryos. Indeed, DNAfrag is a much improved predictor of fertilization rates and live birth outcomes than semen analysis alone. Oxidative stress is associated with DNA fragmentation and an oxidative-reductive BALANCE is essential for normal sperm function. Both oxidative distress and sustress can lead to DNA damage and fragmentation. There are many ways to determine oxidative stress including measurement of reactive oxygen species (ROS), measurement of oxidation-reduction potential (ORP) or determining DNAfrag. The latter typically involves flow cytometry and can be manifested in the TUNEL assay or the AOFT (Acridine Orange Flow Cytometry Test). DNAfrag testing should be considered when there is unexplained infertility, sub-optimal semen analysis, recurrent IVF/ICSI failure, for embryo development, recurrent miscarriage or part of routine work-up. Here, I will report the development of a screening service for this purpose, outlining ongoing projects. Post-screening typical interventions include microfluidic sperm selection, antioxidant treatment, lifestyle changes, treatment for varicocoel or PICSI. In this talk I will consider the evidence base for DNAfrag screening in the context of assessing the evidence base for all areas of reproductive, compared to other forms of, medicine. I will argue that all roads lead to DNAfrag and ORP testing for a large proportion of patients.

L.5

Different and together: cells at the border between mother and fetus

Francesco Colucci

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Cells from different people don't mix easily, except for a good reason: reproduction. In haemochorial placentation, two genetically different individuals share tissues so intimately and for months. On the other hand, transplantation works only if powerful drugs suppress the forces of immune rejection. Histocompatibility genes are the most variable in all known mammalian genomes and, when not matched, cause rejection. And yet, nearly ninety years and five Nobel laureates after the discovery of histocompatibility genes, we have not cracked the code of how maternal immune cells and fetal trophoblast interact despite the half-mismatch. The placenta has a peculiar pattern of histocompatibility gene expression that resembles that of cells infected by viruses specialising to evade specific immunity. This suggests that hundreds of million years of co-evolution between immunity and placentation has resolved the potential conflict at the border between mother and fetus in mammals. The immunology challenge has evolved into a peaceful physiological process where tissue immune cells in the uterus have become integral to the physiology of placentation and fetal growth. I'll review our work determining the landscape of diverse immune cells at the maternal-fetal interface, and their gene expression. I'll discuss how both maternal and fetal histocompatibility genes influence fetal growth. Finally, I will share new, unpublished data that shed new light on the biology of natural killer cells.

L.6

Mechanisms underlying sex-specific prenatal programming of the offspring's brain and behaviour by maternal psychosocial stress.

Paula J. Brunton

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Prenatal stress (PNS) can program neuroendocrine and behavioural changes in the offspring. Often this programming is maladaptive and sex-specific. For example, adult male, but not female PNS offspring, display heightened anxiety-like behaviour; whereas both sexes show hyperactive hypothalamo-pituitary-adrenal (HPA) axis stress responses. We aimed to investigate the mechanisms underlying these programmed phenotypes in PNS offspring and whether they can be reversed, focussing on a role for neurosteroids. Moreover, it is unclear how the effects of maternal stress are conveyed to the fetus. Direct transfer of maternal glucocorticoids is often presumed; however, protective mechanisms including maternal HPA axis hyporesponsiveness and placental 11β-hydroxysteroid dehydrogenase-2 (11βHSD2), should limit mother-to-fetus glucocorticoid transfer in pregnancy. Therefore, we also investigated the mechanisms involved in stress transmission from mother to fetus.

Using a social stress model in pregnant rats, we found evidence for reduced central neurosteroid production in both male and female PNS offspring. Moreover, administration of

systemic neurosteroids or up-regulation of central gene expression for neurosteroid-synthesising enzymes normalised aberrant HPA axis stress responses in PNS offspring. Corticosterone secretion was significantly greater in stressed dams compared with controls, but there was little impact on fetal circulating corticosterone concentrations and no change in the fetal brain. The 11βHSD2 placental barrier also appeared intact, minimising glucocorticoid transfer across the maternal-fetal interface. However, oxidative stress evidently does play a role in fetal programming. Social stress increased oxidative stress markers in the mother, placenta and fetus; and maternal antioxidant treatment during pregnancy prevented some of the adverse phenotypes in the offspring.

In conclusion, down-regulation of central neurosteroid production may underlie enhanced HPA axis stress responses in PNS offspring. Maternal glucocorticoids do not appear to be directly involved in mediating the programming effects of maternal stress on the fetus, but may act in an indirect, and sex-dependent manner to induce oxidative stress.

L.7

Glyphosate based herbicides in bird and human fertility

Joëlle Dupont

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Glyphosate (Gly) is the active molecule of non-selective herbicides used in conventional agriculture. Some evidence shows that exposure to Glyphosate-Based Herbicides (GBH) can affect fertility in animal models. However, few data exist on humans but also birds that can be easily exposed through their cereal-based diet. In Europe, GLY authorization in agriculture has been extended until 2034. Meanwhile the toxicity of GBH in humans and birds is still in debate. In birds, we investigated the effects of chronic dietary exposure to GBH and the potential reversibility on the fertility and embryo development in chickens. In our protocol, adult hens were exposed to GBH (47 mg kg-1/day-1 Gly equivalent corresponding to half of the No-Observed-Adverse-Effect-Level as defined by European Food Safety Authority in birds, GBH group (GBH), n = 75) or not (Control group (CT), n = 75) for 6 weeks. Then, both CT and GBH groups were fed for 5 more weeks without GBH exposure. We showed that the dietary chronic exposure of broiler hens to GBH induces an accumulation of Gly in the egg yolk resulting in severe early embryonic mortality and a delayed embryonic development in survivors that were abolished two weeks after the end of GBH exposure. In human, we analysed the Gly concentration by LC/MS-MS in the seminal and blood plasma in an infertile French men population (n=128). We detected for the first time Gly in the human seminal plasma in significant proportions and we showed that its concentration was four times higher than those observed in blood plasma. We also observed a strong positive correlation between plasma blood Gly concentrations and plasma seminal Gly and 8-OHdG concentrations, the latter reflecting DNA impact. Taken together, our results suggest a negative impact of Gly on the human and bird reproduction and possibly on their progeny.

L.8

Studying neglected cell populations of the developing testis and their functions

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Gonadal sex determination represents a unique model for studying cell fate decisions. However, a complete understanding of the different cell lineages forming the developing testis and ovary remains elusive. The widespread adoption of advanced sequencing technologies, such as scRNA-seq, has provided the field of developmental biology with an opportunity to discover previously unrecognized cell types, such as short-lived progenitors or rare cell lineages. By combining single cell transcriptomic analyses during the critical period of sex determination with *in vivo* lineage tracing, we will describe the specification and differentiation of several previously neglected gonadal cell lineages that give rise to multiple cell types such as rete testis cells, peritubular myoid cells, as well as fetal and adult Leydig cells.

ABSTRACTS
ORAL PRESENTATIONS

Oral presentations:

Session II. Gamete development and function

O.2.1

Impact of NANOS1 variant on early embryonic human germ cell development: altered ribonucleoprotein interactome

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Approximately 15% of couples worldwide experience infertility, often attributable to genetic factors. NANOS1, an RNA-binding protein crucial for germ cell development in Drosophila, regulates this process through post-transcriptional gene regulation via the 3'UTR of target mRNAs. The importance of NANOS1 in human fertility is underscored by the discovery of the v-NANOS1 p. [Pro34Thr; Ser78del] variant, associated with the absence of germ cells in seminiferous tubules of infertile patients. Preliminary data on transient overexpression of affected v-NANOS1 protein in a seminoma-derived cell line revealed a significant reduction in cell viability, contrasting with the anti-apoptotic effect observed in the wild-type counterpart.

Thus, the primary goal is to gain a comprehensive insight into the mechanistic dynamics of the NANOS1 ribonucleoprotein interactome during the early development of human primordial germ cells, particularly when the protein is affected. Utilising a human embryonic stem cell model tagged with a td-Tomato marker allows for cell sorting into germ cells and soma during in-vitro differentiation. Enhanced cross-linking immunoprecipitation and RNA sequencing of sorted cells were applied to inducibly-overexpressed NANOS clonal cell lines, v-NANOS1 and wt-NANOS1, to identify bound RNA targets and their differential expression. Significantly, at the germ cell stage expressing v-NANOS1, upregulation of bound canonical WNT signalling targets is accompanied by an increase of late mesoderm markers and downregulation of early germ cell markers, suggesting a transition to a more somatic cell profile. Components of the canonical WNT pathway, particularly β-catenin bound to v-NANOS1, seem to activate extracellular matrix adhesion targets, promoting germ cells' transition towards mesodermal lineages. This study underscores the substantial impact of the p. [Pro24Thr; Ser78del] mutation on the NANOS1 interactome and early germ cell development, potentially elucidating observed infertility phenotypes.

This research was supported by a grant from the National Science Centre Poland, OPUS 2019/35/B/NZ1/01665 to JJ.

O.2.2

The influence of age and the timing of the reproductive season on sperm quality in African penguins (*Spheniscus demersus*)

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The African penguin *Spheniscus demersus* is one of the most common penguin species kept in zoos. Its stable captive population holds potential for future reintroduction initiatives as its population in the wild is rapidly decreasing. To help secure genetic diversity for future generations, assisted reproduction techniques such as semen cryopreservation could be applied. However, it is crucial to assess species-specific semen characteristics first.

Our study explores the assessment of African penguin semen quality in two age categories (young and mature) and in samples collected during and outside the reproductive season. We applied the traditional method of eosin-nigrosin staining to describe sperm morphology. Additionally, we used advanced methods including a computer-assisted sperm analyzer (CASA) to assess sperm motility parameters, and flow cytometry with fluorescent markers to evaluate plasma membrane integrity, acrosome integrity, mitochondrial potential, apoptosis, and chromatin status.

With the exception of some sperm motility parameters, better sperm quality, assessed through various spermatozoa characteristics, was determined in mature males compared to young individuals. Semen from young males had more motile and progressive spermatozoa (both $P < 0.05$) than semen from mature penguins. However, mature males' spermatozoa revealed greater viability ($P < 0.05$) and higher levels of acrosome integrity ($P < 0.001$) compared to the spermatozoa of young males. Additionally, acrosome integrity was higher ($P < 0.05$) in samples collected during the reproductive season compared to those collected outside the reproductive season. No other spermatozoa characteristics were affected by the timing of the season. We found high variability in sperm viability between samples assessed by eosin-nigrosin smears and by flow cytometry.

This study serves as preliminary research on the application of flow cytometry for assessing African penguin semen characteristics, which is crucial for the future implementation of assisted reproductive technologies (ARTs), such as artificial insemination (AI) and semen cryopreservation, in this species.

This research is funded by the 6th edition of the Implementation Doctorate Programme by the Ministry of Science and Higher Education.

O.2.3

Impact of sperm fractioning on chromosome positioning and chromatin integrity

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Sperm chromosomes are non-randomly organized in nuclear space. Incorrect chromosome organization might affect good sperm morphology, reducing male fertility. Nuclear architecture may can regulate genome functioning, influencing early embryonic development.

The study aimed to determine if selecting spermatozoa with good motility and/or morphology, defines specific chromosome positioning.

Semen samples from 5 control normozoospermic males were fractionated via swim up (selecting motile spermatozoa) or Percoll gradient (selecting spermatozoa with good motility and morphology). Chromatin integrity was evaluated using aniline blue (AB) for sperm chromatin protamination status, and acridine orange (AO) for sperm DNA fragmentation. Fluorescence in situ hybridization (FISH) was applied to analyse the positioning of chromosomes 4, 7, 8, 9, 18, X and Y.

AB staining showed that the average level of sperm DNA protamination is higher in selected high-quality spermatozoa compared to the non-fractionated ejaculated samples ($p<0.05$). AO staining showed no differences between fractions.

In spermatozoa with good motility and/or morphology, chromosome 4 moved towards the acrosome, and the distance between chromosomes 4 and 8 increased, when compared to non-fractionated sperm. In motile sperm, chromosomes 18, X, and Y shifted to the nuclear periphery, and the distance between chromosomes 18 and Y increased, while in sperm with good motility and morphology chromosomes 8, 9, and Y relocate to the nuclear periphery, with increased distances observed between chromosomes 7 and 9 increased compared to non-fractionated sperm ($p<0.05$).

High-quality sperm selectioning promotes repositioning of sex chromosomes towards nuclear periphery, the first region known as interacting with the ooplasm during fertilization. XY positioning is crucial for chromatin remodeling and paternal genome organization during early embryogenesis. High-quality sperm has properly protaminated chromatin, indicating effective both fractionation methods used. These findings highlight the potential importance of selecting high-quality sperm for assisted reproductive technologies.

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O.2.4

Spatiotemporal distribution of mitochondria during meiotic progression in bovine oocytes

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Oocyte's cytoplasm provides maternal factors and organelles necessary to support embryonic development. Mitochondria-(mt) supply energy for oocyte maturation. They associate with microtubules, for quick movement towards certain compartments of the cell. Presumably mt stabilise the karyokinetic spindle and facilitate proper chromosome segregation. Aneuploidies are the leading cause of embryo-developmental arrest. This study investigated mt-kariokinetic-spindle associations during meiosis. Bovine oocytes (n=95) were in-vitro matured (IVM) and analysed at 6-time-points post-IVM (17-23h). To visualise the spindle, oocytes were immunostained for α-Tubulin, mt labelled with Mitotracker, chromatin marked with DAPI and imaged in super-resolution confocal-microscopy. The results prove previous findings showing that at Metaphase-I, mt migrate towards the ooplasm periphery. α-Tubulin labelling revealed that mt co-localise with the kariokinetic spindle, and that a certain pool of mt is retained within the polar body (PB). Interestingly, our study reveals a novel observation suggesting that mt may associate with the midbody (MB). In all cases, in Telophase-I a strong Mitotracker signal was detected at the MB. After Telophase-I completion and PB extrusion, the mt signal was still visible as a remnant associated with the PB. Analogous observations were made for zygotes and early embryos, where Mitotracker was detected in a region separating the two PBs. This observation should be treated carefully, as no other published evidence exists that indicate MB-mitochondria association. Studies on yeast and HeLa cells show that mt migrate towards the cleavage furrow of the dividing cell. The enrichment of mt at cell division site occurs simultaneously with the appearance of the contractile ring (a structural element of the MB), which recruits microtubules and membrane abscission machinery. The role of MB in gametes remains unclear. Recent study on mice shows that MBs are translationally active, thus in asymmetric cell division (meiosis), MBs may help to retain these transcripts within the oocyte.

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O.2.5

Diet induced hyperhomocysteinemia impairs mitochondria content and lipid metabolism in mice oocytes, as revealed by single cell RNA-seq and fluorescent labelling of intracellular organelles

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Elevated serum homocysteine (Hcy), termed hyperhomocysteinemia (HHcy), disrupts oocyte quality and embryonic/fetal development. HHcy also deregulates methylation and acts as a pro-oxidant. We aimed to analyze cytoplasmic factors important for energy provision and regulation of oxidative stress in single oocytes, along with ovarian single-cell transcriptomes to assess how dietary-induced HHcy affects oocyte quality.

HHcy was induced in 7-week-old C57BL/6 female mice by providing 1% Methionine (Met) or 0.1% Hcy in drinking water for 5 weeks, while the control group remained on plain water. Ovaries and oocytes were collected post-mortem. Methods included Chromium Single Cell Multiome ATAC+Gene Expression (10xGenomics) of whole ovaries, single oocyte qPCR to estimate mtDNA Cox1 and Cytb gene copy numbers, and fluorescent staining combined with confocal imaging and ImageJ analysis for mitochondrial content, lipid droplets (LD), ROS, and glutathione (GSH) levels in GV oocytes.

We found upregulation of 50 and 48 genes in the Met and Hcy groups, respectively, compared to the control. Top pathways enriched in the Met group included lipid and steroid metabolism, while in the Hcy group, translation and apoptosis regulation were prominent. LD fluorescence intensity was significantly higher in the Met (37%) and Hcy (27%) groups compared to the control. Both Met and Hcy oocytes had significantly lower (24%-28%) mtDNA copy numbers compared to the control, which corresponds with mitochondrial content assessed by MitoTracker staining. Despite similar ROS levels, lower mtDNA copy numbers suggest elevated ROS levels in both HHcy groups, alongside significantly decreased GSH levels.

HHcy may disrupt oocyte energy production and promote a pro-oxidant environment with increased ROS and decreased GSH. Additionally, gene expression analysis revealed impaired lipid metabolism in the Met group and hyperactive ribosome biogenesis in the Hcy group. These combined effects suggest that dietary hyperhomocysteinemia compromises oocyte quality, potentially leading to reduced embryonic competence in a mouse model.

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Session III. Mechanism of early embryonic development

O.3.1

Effects of early-life malnutrition on reproductive performance and early embryonic development

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Environmental factors modulate developmental trajectory of organisms and overall physiological well-being by inducing changes in gene expression and systemic functions. Maternal diet deficits can have profound effects on progeny health, including reproductive fitness. Our goal was to explore the impact of transient undernutrition during the early postnatal period on the reproductive efficacy, gamete competence, and the subsequent embryonic development.

Newborn C57BL/6 mice (F1) were assigned to: 1) a control group (CON) with mothers fed ad libitum, and 2) a lactation undernutrition group (LUN) with mothers receiving 50% of the daily chow consumed by control dams. After weaning, F1 offspring had unlimited access to food. Adult mice were bred accordingly (female x male): CONxCON, LUNxCON, CONxLUN, and LUNxLUN. To evaluate reproductive performance over time, breeding pairs were tracked for

the number of pups and litters produced over a 20-week period. The identical pairing scheme was employed for the *in vitro* fertilization trial. Post-fertilization, embryonic development was monitored and gene expression (Cdx2, Nanog, Nr1h3, Cidec, Acsl1, Dnmts) was assessed upon reaching the blastocyst stage.

The CONxLUN breeding pair exhibited decreased reproductive capacity, evidenced by the lowest number of pups in liter and the overall lowest production of offspring. Additionally, impaired reproductive fitness was noted in the LUNxLUN group. Interestingly, these observations were not reflected in the percentage of fertilized oocytes and the trajectory of *in vitro* embryo development. However, expression of genes related to lipid homeostasis and DNA methylation was affected in blastocysts conceived by undernourished parents.

In summary, nutritional deficiencies experienced early in life hinder the reproductive capacity of both males and females. This study emphasizes the role of nutritional history of parents in programming the developmental trajectories of next generations, which are evident even at early developmental stages.

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O.3.2

Role of visfatin (Nampt) on early embryogenesis. Studies on siRNA induced gene knockdown mouse model

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Visfatin/NAMPT, the hormone/adipokine exerting a pleiotropic effect, is also perceived as an important factor in the regulation of reproductive processes. Previous studies confirmed its involvement in the control of porcine pituitary and ovary function including oocytes maturation and improves this process in obese and old individuals. Besides, other adipokine such as leptin is necessary for the proper embryonic development and its lack leads to reproductive pathologies and infertility. Therefore, the aim of this study was to determine the role of visfatin in the early embryogenesis in mice.

Firstly, Nampt mRNA expression was examined in mice embryos from the 1-cell to the blastocyst stage by real-time PCR. Next, we silenced the mRNA expression of Nampt at the 1 cell stage using siRNA and evaluated: i) embryo development rate (microscopy), ii) expression of markers for early embryogenesis (Mater, Nobox, HSF1), proliferation (PCNA), apoptosis (Caspase 3, Bax, Bcl2) and blastocyst differentiation (Oct4, Nanog, Cdx2, Gata6, Sox2, Sox17, Eomes) (real-time PCR), iii) the percentage of OCT4, NANOG, CDX2 and GATA6 positive cells in the blastocysts (immunofluorescence). Statistical analysis was performed in GraphPad Prism software.

We showed that Nampt transcript level was decreased with embryo development stage and its knockdown had no effect on the percentage of embryos developed to the blastocyst stage.

However, lack of visfatin was linked with decreased antiapoptotic Bcl-2 mRNA expression in the 4-cell embryos, while increased Nanog mRNA level and Gata6 positive cells in the blastocyst (n=8, p<0.05).

To summarize, we showed that visfatin is an essential factor in early mouse embryogenesis, however future studies are necessary to understand the mechanism of visfatin action and the link between visfatin and implantation rate.

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O.3.3

XIAP role in executing apoptosis in parthenogenetic porcine and bovine embryos

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Apoptosis is a gene-directed biological process that leads to self-controlled cell death without inflammation. Here, we aimed to examine apoptosis occurrence in porcine and bovine blastocysts of parthenogenetic (PA) origin as well as molecular regulation of this process. Additionally, the role of XIAP, a known caspase blocker was evaluated by supplementing culture media with its inhibitor, embelin.

Porcine and bovine cumulus oocyte-complexes (COCs) were matured *in vitro* and activated with ionomycin (5uM) followed by 6DMAP (2mM) treatment and cultured up to the blastocyst stage. The apoptotic blastomeres have been detected with TUNEL and confocal microscopy. The transcriptomes (RNA-seq) of the individual embryos were analyzed according to our published protocol. We selected differentially expressed genes (DEGs) from Gene ontology term (GO) „apoptosis" using following assumptions (P<0.05; -11). Embryos were cultured with embelin at concentrations of 5 and 10µM from day 5 to 7.

Only 21% of porcine PA embryos showed at least one apoptotic nucleus, contrary to 100% of bovine embryos (P<0.01). Moreover, apoptotic index (apoptotic/all blastomeres) accounted for 10.9% in bovine and only 0.6% for porcine embryos (P<0.01). Single embryo RNA-seq revealed 50 DEGs out of 83 apoptotic genes between embryos of both species. Among these, 4 nucleases (DNASE1L3, DNASE2, PLSCR1, EXOG) and 5 of the caspase family genes were upregulated in bovine embryos. In porcine embryos, we observed significant upregulation of XIAP which inhibit the Caspase 3, 7 and 9, known apoptosis executors. Culturing of porcine embryos with XIAP inhibitor, embelin, revealed apoptotic blastomeres in all embryos at morula and blastocyst stage with apoptotic index ranging between 2-25%. In conclusion, species-specific differences in expression profile of apoptotic genes exist. In porcine PA embryos, XIAP seems to play pivotal role in preventing apoptosis that may be useful in establishing of parthenogenetic embryonic stem cell lines.

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O.3.4

Characterization of the dynamics of the RUNT-related transcription factor RUNX1 and its localization and expression in extra-embryonic lineages in the mouse blastocyst

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In mammals, embryo development entails the expression of different members of the RUNX (Runt-related transcription factors) family in a variety of stages and tissues. Previous reports have indicated transcriptional activity of the hematopoiesis regulator RUNX1 in the pre-implantation mouse blastocyst, despite the lack of hematopoietic activity within this period. Our analyses revealed that RUNX1 is present during the pre-implantation stages in both extra-embryonic lineages, the trophectoderm (TE) and the primitive endoderm (PrE), but not in the pluripotent epiblast (Epi). We have characterized the localization dynamics of RUNX1 along with GATA4, a typical PrE marker, and have found interdependence between the expression levels of both proteins. Furthermore, by using a Cre-lox system-generated Runx1bRFP mouse strain, we have shown that Runx1b expression is dramatically upregulated in the PrE concomitant with the increased expression of GATA4 and the physical segregation from the Epi. We have also found a different response in RUNX1 expression to effectors of the FGF signalling pathway between the PrE and the TE. Our characterization of Runx1b-deficient embryos has demonstrated the involvement of RUNX1b protein specifically, and no other members of the RUNX family, in regulating the expression of the PrE marker GATA4. Altogether, our results point to a novel role for RUNX1b in defining the extra-embryonic lineages and in guaranteeing the proper architecture of the mouse blastocyst.

O.3.5

The velocity of cytoplasmic movement in trophectoderm cells as a noninvasive marker of mouse embryo quality

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Implantation abnormalities are a major source of mammalian reproductive loss. It is known that the proper functioning of the trophectoderm is crucial for the appropriate implantation process. Some of the most important determinants of trophectoderm functionality are its biomechanical properties, which are reflected, among others, as the velocity of cytoplasmic movement. In our studies, we measured the velocity of cytoplasmic movement in trophectoderm cells of mouse blastocysts using time-lapse imaging and the Particle Image Velocimetry method, to find whether this property could reflect the embryo's ability to implant. We showed that the cytoplasmic velocity differs in embryos at different stages of late preimplantation development and between polar and mural trophectodermal cells. Additionally, we discovered a relationship between the size of the outgrowth formed in the *in vitro* implantation test and the cytoplasmic velocity in trophectodermal cells. Next, we checked whether the velocity of cytoplasmic movements may be affected by (i) the quality of the keratin cytoskeleton - because keratins are key regulators of trophectoderm function; and (ii) maternal and *in vitro* postovulatory aging - because these processes highly affect embryo quality. We found that the depletion of keratin 8 or keratin 18 increased the velocity of cytoplasmic movement e.g., depletion of Krt8 increased the velocity of cytoplasm in mural trophectodermal cells in E4.5 embryos in comparison to the control. However, we did not observe the impact of aging on the velocity of cytoplasmic movement and the mRNA levels for Krt8 and Krt18. The above data suggest that the cytoplasmic velocity depends on the keratin cytoskeleton and may be a valuable and non-invasive biomarker of embryo quality and its ability to implant. However adverse effects of maternal and *in vitro* postovulatory aging on the embryos' development most likely do not result from changes in the biomechanical properties of the trophectodermal cells.

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Session IV. Assisted reproduction and preservation of fertility

O.4.1

Evaluation of the effectiveness of gamete preservation in roe deer

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European roe deer is a widely distributed deer species, therefore we used it as a model to develop artificial reproductive technology (ART), which can be used for endangered deer species. The aim was to evaluate effectiveness of the methods of semen cryopreservation and oocyte vitrification in roe deer. The testes and ovaries were collected post mortem immediately after they were hunted (N=24). The motility parameters: CASA: total motility, progressive motility and morphology between the fresh semen (FS) and after cryopreservation (AC) were compared. Hyaluronic binding assays (HBAs) were carried out for FS, and the mitochondrial membrane potential of the sperm in the frozen–thawed semen suspension (flow cytometry) was determined for AC. Half of the oocytes were fertilized and the other half underwent viability measurement (MTT) and vitrification. After ten days, the oocytes were thawed and assessed for viability. The fresh oocytes were fertilized with thawed semen, and the embryos were cultured until reaching the blastocyst stage. The numbers of isolated oocytes, cumulus–oocyte complexes (COCs), cleaved embryos, expanded blastocysts, and embryos collected (day 6 - 9 of the culture) were evaluated. For FS, HBA showed viability rate of 61.9%. Higher percentages of the morphology parameters were observed in FS compared to AC, whereas the motility and progressive movement were greater in AC semen ($P \leq 0.001$). The viability of AC semen was 50.5%, and the mitochondrial membrane potential of the thawed semen was 40.6%. In total, 311 oocytes from 8 does were collected. From 150 COCs, 125 blastocysts developed. The viability rate of the fresh oocytes was 98%, whereas after vitrification 81% ($P \leq 0.001$). We showed that oocyte vitrification and cryopreservation of roe deer semen are effective and can be implemented into ART for other deer species.

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O.4.2

Sucrose and trehalose: cryoprotectants for chicken semen cryopreservation

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This study investigates the impact of sucrose and trehalose on chicken semen quality after cryopreservation, that is crucial for genetic resource conservation. Despite advancements, cellular injuries often compromise post-thaw sperm viability and fertility.

The semen was collected from ten Green-legged Partridge roosters by dorso-abdominal massage twice weekly, and then was pooled. Pooled sample was divided into control group subjected to cryopreservation with 6% dimethylformamide (DMF), trehalose with 3% DMF (T400 and T500 mM), sucrose with 3% DMF (S300 and S500 mM) and then were frozen in straws. Sperm motility (MOT, PMOT, VAP, VSL, VCL) was analyzed using the CASA system. Flow cytometry assessed plasma membrane integrity, mitochondrial membrane potential, acrosome integrity, early apoptosis, lipid peroxidation, intracellular calcium levels, and DNA fragmentation.

T500 mM showed the highest motility ($P<0.01$), while the control group had the lowest motility and the fewest slow spermatozoa ($P<0.01$). S300 mM had the highest curvilinear (VCL) ($P<0.05$) and average path velocities (VAP) ($P<0.01$) but the highest DNA fragmentation ($P<0.05$).

The highest and lowest plasma membrane integrity were exhibited in the control and S500 respectively ($P<0.05$). S300 mM demonstrated the highest mitochondrial potential ($P<0.05$) but also had the most live cells with damaged acrosome ($P<0.01$). Lipid peroxidation was highest in the control group and lowest in T500 mM ($P< 0.05$).

The control group had the fewest apoptotic cells, while T500 mM had the lowest among the test groups. The control group had the highest number of live spermatozoa with low calcium levels, ($P<0.05$). T400 mM and T500 mM exhibited the lowest DNA fragmentation ($P<0.05$). The control group exhibited the highest number of intact acrosome spermatozoa ($P<0.05$).

Since T500 mM improved sperm motility, reduced oxidative stress, and minimized DNA fragmentation while also decreasing apoptotic cell death, it could be considered as a good cryoprotectant for chicken semen cryopreservation.

O.4.3

Parthenogenetic development of human and bovine oocytes activated after slow-freezing or vitrification, may serve as a potent tool of cryopreservation efficacy evaluation instead of fertilization.

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An efficient, modified approach to slow-frozen human oocyte thawing and dilution methods have already been demonstrated (Papis et al., 2015). It was based on a retrospective comparison with traditional (commercial) treatment of slow-frozen oocytes. An indirect experimental confirmation of such retrospective analyses, by means of the parthenogenetic development of thawed or warmed, chemically activated oocytes, is shown here.

Human MII oocytes were slow-frozen using a commercial solution consisting of 1.5 M propanediol + 0.2 or 0.3 M sucrose or vitrified using commercial solution based on DMSO/ethylene glycol mixture. Bovine MII oocytes were vitrified using VS14 vitrification solution and an original microdroplet vitrification method (Papis et al, 2000). After thawing, slow-frozen oocytes were subjected to the modified protocol of propanediol dilution, according to the method shown already (Papis et al., 2015). After thawing/warming oocytes were subjected to the chemical activation. Activation was performed using 15 min exposition to a standard calcium ionophore solution (CultActive, Gynemed) followed with treatment in 2mM 6-DMAP diluted in MHM medium (Fujifilm) and incubated 3h in 37ºC. *In vitro* culture was performed in Continuous Single Culture medium (Fujifilm) under time-laps control (EmbryoScope). Survival of oocytes and post-activation development ratios were calculated and analysed using non-parametric one-way ANOVA tests (Statistica 12.0).

High survival rate was obtained in both studied groups (slow freezing 91.9%, vitrification 98.9%, NS). *In vitro* culture tended to be more efficient in group of embryos obtained from slow-frozen oocytes in comparison with vitrified ones (20% vs 14.5% of blastocysts out of activated oocytes, respectively, NS). Out of 50 warmed and activated bovine oocytes 2 (4%) parthenogenetic blastocysts were developed.

The results presented here, demonstrate the relatively high ratio of human parthenogenetic embryo development, regardless of cryopreservation method used. It confirms our previous retrospective clinical observations and the utility of chemical activation in a such application.

O.4.4

Testicular biopsy in azoospermic patients after cryptorchidism surgery

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Cryptorchidism - common malformation of the male genitourinary system; correlates with gestational age, birth weight of newborns: 1.0–9% of premature births,>2500 g.; 1.1–45.3% preterm infants, low birth weight infants. Reduces individual fertility: unilateral 10-30%, bilateral 38%. Causes azoospermia: unilateral 13%, bilateral up to 90%. It correlates with an increased risk of germinal testicular cancer (most often seminoma). Relative risk of testicular cancer with orchidopexy prior to 13 yo 2. 2/ > 13 yo 5. 4 - the need for early orchidopexy. In azoospermia, the procedure for obtaining sperm is a gonadal biopsy. The presence of sperm qualifies for IVF-ICSI (in-vitro fertilization-intracytoplasmic sperm injection).

During 2018-2024, 822 testicular biopsies were performed: 432 TESA (testicular sperm aspiration), 390 m-TESE (microdissection testicular sperm extraction). Patients after cryptorchidism surgery: TESA 28/432 (6.5%); m-TESE 50/390 (12.8%). Each azoospermic patient was diagnosed by European Association of Urology Guidelines. TESA - outpatient procedure, short intravenous anesthesia (procedure time 15-30 minutes); m-TESE one-day surgical procedure, general anesthesia, with laryngeal mask (60-90 minutes). Prevention of infection: cephalosporin single dose (TESA), 5 days treatment (m-TESE). Techniques: TESA – transdermal gonadal puncture with a Menghini biopsy needle; m-TESE – surgical extraction of promising areas of tubules, Leica M8602x2 microscope, 25x magnification. The fragments of the collected samples were fixed in Bouin fluid for histopathological examination; the remaining tissue were preserved in liquid nitrogen (-196C).

Sperm found: 14/28 TESA (50%), 21/50 m-TESE (42%). Complications: I st. Clavien-Dindo. Genetic tests (1 balanced translocation, 6 CFTR gene mutations), age of surgery (3-37 years) did not correlate with biopsy results.

Patients after cryptorchidism surgery with azoospermia have a chance to obtain sperm by TESA testicular biopsy (50%)/m-TESE (42%) and be included in IVF-ICSI procedure.

According to the EAU Guidelines, the orchidopexy procedure should be performed no later than 18 months of age, which has a protective effect on spermatogenesis.

O.4.5

Results of Ovum Pick Up performed in Poland and combined with ICSI results

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The *in vitro* production of equine embryos, using ovum pick-up (OPU) and intracytoplasmic sperm injection (ICSI), is becoming more popular for breeding sport horses. The results of this complicated procedure are variable and hard to predict. The purpose of the research is to show the results of OPU performed in Poland compared with ICSI performed in Italy, at the Avantea laboratory.

OPU was performed from all ovarian follicles with min. 4mm of diameter presented on the mare's ovaries. In total, procedure was carried out 51 times on 42 mares. A total of 538 oocytes were obtained and transported to Avantea by air transport, of which 299 oocytes were injected, until finally received 69 embryos.

Session V. Embryo-maternal interactions and pregnancy

O.5.1

Estradiol and progesterone membrane receptors as a potential novel regulators of endometrial receptivity

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Infertility is becoming an increasingly widespread problem in the society that needs increased attention. In addition to the ovarian or fallopian tube factors, we can observe growing evidence of the importance of the endometrial factor as a cause of lack of embryo implantation and female infertility. Endometrium is a unique tissue that undergoes repeated changes in each menstrual cycle in preparation for embryo implantation as a result of a fine balance between actions of estradiol and progesterone. The role of their nuclear receptors in endometrial biology has been extensively studied. Yet, evidence has been mounting, supporting a significant role of non-classical rapid steroid signaling mechanisms in uterine biology.

Our preliminary results showed that the lack of decidualization is a common problem among infertile patients. Around 50% of endometrial tissues collected from IVF patients could not undergo decidualization *in vitro* and did not secrete prolactin after 9 days of E2 and progesterone stimulation. We showed that not only nuclear receptors, but also membrane

estrogen (GPER) and progesterone receptors (mPRs), may be one of the crucial factors regulating changes occurring during decidualization.

We determined that progesterone alone is not able to induce prolactin secretion, and we suggest that there might be additional pathway involved. Inhibition of GPER lead to prolactin secretion downregulation by decreasing cAMP level in endometrial cells. Stromal cells stimulated with progesterone bound to bovine albumin, unable to penetrate inside the cell, did not secrete prolactin. Thus, mPRs may have inhibitory role in prolactin secretion regulation.

These findings may indicate novel factors activated during decidualization, that enables the embryo invasion and implantation. Understanding the molecular mechanisms of estrogen and progesterone membrane receptors action could serve as a foundation for future successful diagnostic techniques thus might be promising for patients suffering from idiopathic infertility.

O.5.2

Using single cell transcriptomic and 3D models to study immune-endometrial-fetal interactions in physiological and pathological contexts

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In mammals, for a successful reproduction, there is a need for a successful implantation of a blastocyst on the endometrial mucosa followed by the establishment of an appropriate maternal-fetal interface. Hormonal response and immune cells are recognized to induce an implantation-prone endometrium. The endometrium is essential to allow a suitable placentation. Both endometrium and the maternal fetal interface are highly dynamic tissue that undergo massive changes across the cycle or the gestation respectively. Furthermore, the immune cells within the endometrium and at the maternal fetal interface are important for both implantation and the maintenance of pregnancy. Indeed, there is a necessity for an immune tolerance of the semi-allograft that is the fetus, the immune cells are also thought to be involved in the timely initiation of parturition. However, the intricate interaction between the different cell types within the endometrium and the maternal fetal interface are still not clearly understood. Pathological context, such as endometriosis, can alter the endometrium and the maternofetal interface establishement. Endometriosis is a complex inflammatory gynecological disorder characterized by the presence of endometrium-like tissue in ectopic regions, and it is associated with a molecularly altered eutopic endometrium and an immune dysfunction, both aspects being important for implantation and placental development. In addition to the fertility issues associated with endometriosis, there is also an increased risk of miscarriage. New technological advancement such as single cell transcriptomic allows to explore the endometrium and maternal fetal interface with unprecedented details, at different time points. 3D culture models allow to explore in detail the response to external factors (hormones, microbial derivatives). Here, we will show how we used both these approaches to explore the interactions between the different cell types at different stages of the cycle/gestation in women and mice with or without endometriosis.

O.5.3

Preconceptional innate immunomodulation leads to a partial correction of gestational complications induced by endometriosis in a murine model

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Endometriosis is a common chronic gynecological disease in women of childbearing age. It can cause pain, infertility and pregnancy complications such as miscarriages, low-birth-weight babies and even premature deliveries. Today, we do not fully understand the mechanisms associated with pregnancy complications in endometriosis. We suspect the inflammatory environment and the altered eutopic endometrium to be deleterious to the normal progression of pregnancies.

Our team has previously shown in a mouse model of endometriosis that immunomodulatory treatment with low-dose lipopolysaccharides (LPS) limits the inflammation associated with the disease and reduced lesion size. In this study, we aim to determine the immunomodulatory effect of low-dose LPS on gestation complications induced by endometriosis.

We will present here transcriptomic analysis of early placentas (E9.5) at a single nuclei level in the context of endometriosis and endometriosis with preconceptional LPS treatment. We will also focus on the immune aspect of the placental development by presenting the results of transcriptome analysis of sorted immune cells from these early placentas and physiological late gestation placentas. We will show that the LPS treatment leads to a partial correction of the pregnancy complications caused by endometriosis, associated with a correction of the inflammatory transcriptomic profile induced by the disease.

O.5.4

Regulatory B cells and IL-33 in normal pregnancy and after miscarriage in women

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The key to maintaining pregnancy is the balance between pro-inflammatory and anti-inflammatory responses, in which immune cells such as lymphocytes T and B play a pivotal role. In particular, although the role and regulation of the population of B regulatory cells (Breg) in pregnancy remain unresolved, their engagement in tolerance maintenance seemed crucial.

Interleukin 33 (IL-33) is an alarmin, a member of IL-1 family produced and released by damaged or necrotic cells. It has a broad pleiotropic action that influences differentiation, maintenance and function of immune cell types via the ST2 receptor. It has also been shown to be chromatin-associated in the nuclei and can regulate transcription.

To investigate the interrelation between IL-33 and its receptor in the context of Breg cells-maintained tolerance in women after miscarriage we determined the frequencies of Breg

subpopulations: B1a, B10, immature B cells, plasmablasts, and memory B cells, and the expression of ST2 receptor on these cells in blood from pregnant women and from women after miscarriage. We also analyzed the concentration of IL-33 and its soluble receptor sST2 in serum from these patients.

In women after miscarriage an increased percentage of B cells (CD19+), immature B cells, plasmablasts, and memory B cells with expression of ST2 receptor within total lymphocytes, but a decreased percentage of B10 ST2+ cells within total CD19+ cells, compared to the control group were observed. The median of B10 ST2+ and B1a ST2+ B cells was significantly increased in patients after miscarriage. There were no significant differences between serum level of IL-33 and sST2.

Our findings indicate that ST2 receptor is present on regulatory B cells of pregnant and post-abortive women in different proportions depending on the subpopulation of these cells. B1a and B10 subpopulations seem to be involved in immune regulation by IL-33/ST2.

Session VI. Hormonal, neuronal, and immune regulation of reproduction

O.6.1

GW0742 by reduction of energy metabolism decreases viability of adult ovarian granulosa cell tumor

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Epidemiological study show that thyroid dysfunction is connected with an increased risk of ovarian cancer and reduced survival time for patients with ovarian cancer (Minlikeeva et al., 2017). Moreover, data indicated that ovarian granulosa cell tumor (GCT) express thyroid hormone receptor α and β (TRα and TRβ), which may point to an important role for T3 in ovarian cancer cells (Heublein et al. 2022).

Ovarian GCT is rare and poorly characterized type of ovarian cancer, among which we distinguish adult granulosa cell tumor (AGCT) without well-developed treatment. Chemical analyses revealed the antagonistic effect of synthetic compound GW0742 on nuclear thyroid hormone receptors TRα and TRβ (Perez Diaz et al., 2016).

Therefore, the aim of the study was to examine influence on viability and energy metabolism of AGCT cell line.

The KGN cells (human AGCT-derived cell line) (Riken Cell Bank) were exposed to GW0742 (1 – 100 µM) and viability was analyzed by PrestoBlue™ Cell Viability Reagent (Thermo Fisher). Level mitoATP and glycoATP was measured using Seahorse XFp Analyzer by Seahorse ATP Rate Assay (Agilent).

Firstly, we demonstrate that GW0742 in dose dependent manner decrease viability of KGN cells after treatment by 24 and 48h. Additionally, we observed reduction ATP Production Rate by KGN cells after 24h. Our studies show that reduction of ATP Production generated by both glycolysis and mitochondrial respiration. Result of oxygen consumption rate (OCR) and

extracellular acidification rate (ECAR) confirm this data. Interestingly, we indicated that basal level of glycoATP production rate is higher than mitoATP production rate in KGN cells.

These studies stated that blocking TRα and TRβ by GW0742 contributes to reduction ATP production during glycolysis and mitochondrial respiration and decrease viability of KGN cells.

The work was supported by the programme “Excellence Initiative-Research University” at the Jagiellonian University in Kraków, Poland.

O.6.2

Expression of asprosin/furin/Olfr734 in mouse hypothalamus and pituitary fluctuates along oestrus cycle and differs in diet-induced obese mice

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Asprosin, a novel adipokine, is a circulating hormone primarily secreted by white adipose tissue and encoded by exon 65 and 66 of the fibrillin 1 (Fbn1) gene. The protein product of Fbn1, profibrillin-1, undergoes proteolytic cleavage by the furin enzyme to produce fibrillin-1 and asprosin. Asprosin has been implicated in the development of diabetes, obesity, insulin resistance, cancer, and polycystic ovarian syndrome. Additionally, it affects various cellular and physiological processes, including appetite stimulation via orexigenic neurons in the hypothalamus, insulin secretion, glucose release, enhancing male fertility in rodents and bovine ovary functions through activation of Olfr734, an olfactory G-protein-coupled receptor. However, its potential impact on the upper branches of female reproductive system has not been investigated.

This study aimed to investigate the expression of asprosin, furin, and Olfr734 in the mouse hypothalamus and pituitary across the oestrous cycle and in obese individuals. We used a C57BL/6J female mouse diet-induced obesity model. Hypothalamus, pituitary, adipose tissue samples were collected from control mice at proestrus, estrus, and diestrus, and from diet-induced obese mice in the estrus phase. The mRNA and protein expressions of asprosin/furin/Olfr734 were assessed using RT-qPCR, Western blot, and immunohistochemistry respectively. Statistical analysis was conducted using Graph Pad Prism 10 software with one-way ANOVA and Tukey's test ($n = 5$, $p < 0.05$).

Our results indicate that asprosin, furin, and Olfr734 mRNA and protein levels fluctuate throughout the oestrous cycle in the mouse hypothalamus and adipose tissue. In contrast, transcript levels in the pituitary remain stable across all phases. Furthermore, in obese mice, asprosin, furin, and Olfr734 are differentially expressed in the examined tissues compared to control mice.

These preliminary findings suggest asprosin may play a modulatory role in the upper branches of the female reproduction, influenced by the body's metabolic status.

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O.6.3

The role of ghrelin and serotonin receptors in the activation of IP3/DAG signaling pathway in the melatonin biosynthesis during the breeding season in ewes

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The *in vivo* and *in vitro* studies confirmed that melatonin (MEL) secretion is regulated by serotonin type 2 (5HT2) receptor and ghrelin (GHRL) in seasonally reproductive sheep. Herein, we investigated the role of inositol trisphosphate (IP3)/diacylglycerol (DAG) signaling pathway in MEL biosynthesis after treatment of the pineal gland (PG) with GHRL, mCPP (a mixed agonist of the 5-HT2B/2C), and RS102221 (a highly selective antagonist of the 5-HT2C). The PG were collected after sunset from 8 ewes during the breeding season (November). The PG were transected sagittally into strips (~30 mg) with each equilibrated in 1.0 ml of DMEM for 60 min, followed by a 4-h incubation in medium alone (control) or containing: GHRL (100 ng/ml); GHRL (100 ng/ml)+m-CPP (10 µM); RS102221 (10 µM)+m-CPP (10 µM). The PG explants were harvested every 60-min, frozen in liquid nitrogen, and stored at –80°C until ELISA for quantitative determination of IP3, DAG, protein kinase C (PKC), and western blotting for detection of phosphorylated serotonin N-acetyltransferase (pT31-AANAT) expression. Samples of medium were stored at -20°C until RIA for MEL. Only mCPP treatment led to an accumulation of IP3 ($P < 0.05$). Administration of GHRL decreased ($P < 0.05$) DAG concentrations as compared with the control and GHRL+mCPP group. The RS102221+mCPP reduced ($P < 0.05$) DAG and PKC concentrations compared to all experimental groups. GHRL and RS102221+mCPP inhibited ($P < 0.05$) the secretion of MEL as compared to control, whereas treatment with GHRL+mCPP increased ($P < 0.01$) the concentration of MEL in relation to all experimental groups. Similar changes were observed in pT31-AA-NAT expression. These results indicate that GHRL and 5-HT2 receptors are involved in the regulation of MEL biosynthesis and secretion through activation of DAG/PKC intracellular signal cascade.

This work was supported by a grant from the National Science Center (NCN 2012/05/B/NZ4/02408).

O.6.4

Th2 specific interleukins: IL-4 and IL-13 – profibrotic response and beyond – transcriptomic and functional *in vitro* study on mare endometrial fibroblasts

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T helper 2 cells (Th2) secreted interleukin (IL)-4 and IL-13 are described as proinflammatory and profibrotic factors in multiple organs and tissues. The precise role of IL-4 and IL-13 in tissue fibrosis remains unclear. In this study, the mare with endometrosis, characterized by endometrial fibrosis, was employed as a non-traditional large animal model to investigate the effect of IL-4 and IL-13 on fibroblasts isolated from healthy (category I) and moderately fibrotic (category IIB) endometrium. We aimed to determine the impact of IL-4 and IL-13 on the proliferation, migration, and transcriptomic changes of fibroblasts isolated from category I and category IIB endometria.

IL-4 and IL-13 increased the proliferation of fibroblasts from both endometrial categories ($p<0.05$). Following 96 hours of IL-4 treatment, the migration of fibroblasts derived from category I endometrium was reduced ($p<0.05$). IL-4 treatment resulted in the differential expression of 1228 and 1187 genes (DEGs) in fibroblasts derived from category I and IIB endometria, respectively, with 897 DEGs common to both categories. Category I-specific DEGs were involved in the sphingolipid signalling pathway, efferocytosis, and ferroptosis, while category IIB-specific DEGs were found to be involved in i.a. vascular smooth muscle contraction and MAPK signalling pathway according to the KEGG database. The treatment of fibroblasts with IL-13 resulted in 471 and 465 DEGs in categories I and IIB, respectively, with 305 DEGs common to both categories. The DEGs identified exclusively in category I were found to be enriched for glycolysis and gluconeogenesis KEGG pathways, whilst category IIB-specific DEGs were assigned to nucleotide sugars, amino acids, and steroids biosynthesis.

The results suggest that Th2-specific cytokines influence endometrial function and gene expression, potentially contributing to endometrosis. Fibroblasts respond differently based on fibrosis severity, highlighting the need to distinguish cellular responses in varying fibrosis degrees for future studies.

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O.6.5

Yellow semen syndrome in reproductive turkeys (*Meleagris gallopavo*) might be connected with altered content and polarization of macrophages in testis, epididymis, ductus deferens, and Bursa of Fabricius

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Yellow semen syndrome (YSS) in turkeys decreases the reproductive potential of tomes. We hypothesize that YSS development may be connected with altered macrophage content and polarization in the testis, epididymis, ductus deferens, and Bursa of Fabricius in YSS turkeys.

Testes, epididymis, ductus deferens, and Bursa of Fabricius were isolated from toms producing yellow (YSS, n = 4) and white semen (WS, n = 4), defined based on protein concentration in seminal plasma (WS <20 mg/mL; YSS >55 mg/mL). Cells were isolated from tissues using a fragment seeding approach in high-glucose DMEM with 20% FBS and 1% antibiotic-antimycotic solution until reaching 80% confluence, collected with trypsin, and cryopreserved. Next, cells were subjected to flow cytometry after staining with primary anti-CD14, anti-iNOS, and anti-IL10 antibodies. Only CD14+ cells were considered in analyses. The percentage of CD14+ iNOS+ (M1, pro-inflammatory type) and CD14+IL10+ (M2, anti-inflammatory type) macrophages were compared with a t-test (statistically significant differences at $P < 0.05$).

In testis, epididymis, and ductus deferens the total percentage of M1 or M2 macrophages did not change between WS and YSS. A higher percentage of M1 and M2 macrophages was observed in the Bursa of Fabricius in YSS birds. In testis and ductus deferens M1 macrophages dominated in both WS and YSS tissues. In the epididymis and the Bursa of Fabricius, the percentage of M1 compared to M2 in WS and YSS did not differ.

In conclusion, an increased percentage of M1 and M2 macrophages in the Bursa of Fabricius in YSS indicates activation of the immune system, possibly contributing to the YSS pathological condition. Regardless of the YSS disease, M1 macrophages represent the majority in the testis and ductus deferens and may contribute to the efficient removal of abnormal spermatids during spermatogenesis in the testis and abnormal spermatozoa stored in ductus deferens.

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Session VII. Environmental influence on reproductive function

O.7.1

Support of the reproductive potential by immune system in males of roe deer is dependent on the habitat

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The reproductive period of roe deer is characterized by the rutting season from mid-July to mid-August. Following the rutting season, testosterone (T4) concentration decreases. By regulating inflammation and eradicating pathogens, the immune system ensures the reproductive tract's integrity, thereby promoting optimal conditions for gametogenesis and fertility. Interleukins (IL)s play a vital role in male reproduction by affecting sperm functionality and fertility outcomes. High level of IL-1, a proinflammatory cytokines have been associated with reduced sperm quality and motility. Additionally, Toll-Like Receptors (TLR)s are part of defense system as well by identification specific molecules associated with pathogens and initiate inflammatory responses to eliminate them.

The aim is to determine protein expression of IL-1, TLR1, TLR3 and TLR6 in kidney and their correlation with fecal testosterone levels in male roe deer from three habitats.

Experimental material was kidney and feces from males (3-5 years old, post mortem) collected between 20th July and 10th August 2023 from three habitats: forest (Strzałowo Forestry, N=9), field (Ciechanów Forestry, N=9) and mosaic (Velenje hunting region, Slovenia, N=7). Protein expression (western blotting) in kidney and T4 concentration (ELISA) in feces after extraction was examined.

The lowest protein expression for all examined factors was observed in males from forest habitat with simultaneously the highest concentration of T4 ($P<0.05$). Whereas the lowest concentration of T4 was evaluated in males from mosaic habitat together with higher protein expression of immune parameters ($P<0.05$).

Condition of males from forest habitat based on immune parameters seem to be the most anticipated during the reproductive season. The studies are continued in direction of immune status examination in other tissues of roe deer.

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O.7.2

Factors stimulating movement of fish spermatozoa

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Fish are a diverse group of vertebrates. In male reproductive tract spermatozoa are in a resting state maintained by the composition of seminal plasma (osmolality of approximately 300 mOsm kg-1). Gametes thrown into the water are activated to move. The aim of the research was comprare influence of selected ions and non-ionic compounds dissolved in water on spermatozoa activation in representatives of four taxonomic groups. The study included chub (*Squalius cephalus, Leuciscidae, Cypriniformes*), pikeperch (*Sander lucioperca, Percidae, Perciformes*), sea trout (*Salmo trutta m. trutta, Salmonidae, Salmoniformes*) and burbot (*Lota lota, Lotidae, Gadiformes*). The percentage of moving spermatozoa and movement parameters were recorded using computer-aided sperm analysis (CASA).

Chub and pikeperch spermatozoa were activated/blocked by the osmotic pressure of the external environment. Chub spermatozoa were immobilized in a solution with an osmolality of 300 mOsm kg−1. This pressure corresponds to a 150 mM concentration of sodium and potassium ions or a non-ionic solution of 240 mM sugar (osmolality 280-290 mOsm kg−1). The best sperm movement parameters were achieved in a 60-90 mM sodium or potassium ion (osmolality 150-200 mOsm kg-1). In pikeperch very small batches of spermatozoa were motile in a solution isotonic to seminal plasma. The environment with an osmotic pressure of 400 mOsm kg-1 has a completely immobilizing effect. The best movement parameters were achieved by spermatozoa of this species in water with a pH of 8. Sea trout and burbot spermatozoa are sensitive to potassium ions. Motility of the gametes was inhibited by potassium ions at a concentration of 8 mM (48 mOsm kg−1) or an osmolality above (450 mOsm kg−1). The optimal environment for activaton of the male gametes was a solution of 80-130 mOsm kg−1 (30-60 mM NaCl).

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O.7.3

Can *Staphylococcus* bacteria support male fertility?

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High-throughput sequencing studies have changed the current approach to the role of male genital tract infections in male fertility. In this context, little is known about commensal seminal microbiota. The study aimed to determine the associations between the seminal microflora composition and standard/extended semen parameters. The study design included three research groups: fertile men (n=14), infertile men with varicocele (n=51), and infertile men with cryptorchidism in childhood (n=26). Standard semen analysis was performed according to the 5th WHO laboratory manual. The qualitative and semi-quantitative composition of the semen microbiome was examined using the Next Generation Sequencing (NGS) technique of the V3-V4 variable region in the 16S rRNA gene. In turn, an immunosorbent ELISA test was used to assess the level of new anti-inflammatory IL-37 in seminal plasma. As for microbiota diversity, no significant differences among the studied groups were noted. However, the most operational taxonomic units were detected in patients with varicocele. A total of six bacterial taxa were identified to be differentially abundant among the studied groups. Of these, the *Alphaproteobacteria* class, the *Staphylococcaceae* family, the *Staphylococcus* genera, and the *Staphylococcus sp.* Icri14 species showed a significantly higher abundance in fertile men compared to the infertile groups (padj.<0.05). In turn, the *Flavonifractor* genus and the *Bifidobacterium bifidum* species were principally represented in cryptorchid men. In the same group of patients both progressive and total sperm motility increased with the presence of the *Staphylococcaceae* family and the *Staphylococcus* genus. Moreover, a significant negative correlation between the *Staphylococcus sp*. Icri14 and the IL-37 concentration in the fertile group was noted (r=-0.8243, padj.=0.0143). The findings, for the first time, showed *Staphylococcus* as a fertility-associated genus due to the interaction between host and microbiota with an immune response.

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O.7.4

The impact of selected pesticides on rooster fertility and reproductive hormones levels

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Agricultural pesticides are a major category of endocrine-disrupting chemicals affecting non-target organisms globally. Birds, especially chickens, are exposed to pesticides through their cereal-based diet. This study evaluated the effects of three commonly detected pesticides—tebuconazole (TEB), imidacloprid (IMI), and glyphosate (GLP)—and their mixtures at maximum residue limits in chicken feed on rooster fertility.

Eighty 24-week-old roosters were divided into eight groups and exposed to the following treatments for six weeks: TEB, IMI, GLP, TEB+IMI, TEB+GLP, IMI+GLP, TEB+IMI+GLP, and a control group (CTR). After six weeks, all groups were fed a pesticide-free diet for an additional four weeks. During both periods, we assessed fertilization and hatchability rates, and measured reproductive hormone levels in blood plasma.

Results indicated that all pesticides, except GLP and TEB+IMI, significantly reduced fertility and hatchability rates from set eggs compared to the control (CTR) during the exposure period. The IMI group exhibited significantly lower hatchability rates from fertilized eggs and higher embryo mortality after six weeks of exposure. After the break, fertilization rates remained lower in all experimental groups except TEB. Hatchability rates from set eggs were higher in the control compared to the GLP, TEB+IMI, TEB+GLP, and TEB+IMI+GLP groups. There were no significant differences between groups in hatchability rates from fertilized eggs or mortality rates after the break.

Additionally, a significant decrease in progesterone levels was observed in all groups except IMI after six weeks of pesticide exposure. There was no significant effect on estradiol levels, but testosterone levels decreased in the TEB+IMI and IMI+GLP groups. After the break, there were no differences between the control and tested groups in progesterone and estradiol levels, but a reduction in testosterone concentration persisted in the TEB+GLP, IMI+GLP, and TEB+IMI+GLP groups.

Overall, dietary exposure to pesticides in roosters reduces fertility and hatchability rates and decreases progesterone and testosterone levels.

O.7.5

Novel plasticizers, bisphenol S and F negatively affect meiotic maturation and spindle structure of mouse oocytes

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Of all the pollutants released into the environment every year by human activity, endocrine-disrupting chemicals (EDCs) are among the most dangerous. Nowadays, they are considered as one of the potential reasons for a growing health problem – infertility. Plasticizers, such as bisphenol A and its analogues, bisphenol S (BPS) and bisphenol F (BPF), are among the most frequently used EDCs. In the current study, we focused on BPS and BPF that have become increasingly popular substitutes for BPA. Until now, they were detected in human body fluids, including (for BPS) follicular fluid. Nonetheless, BPS/BPF impact on the reproductive system, fertility and the quality of mammalian oocytes remains still largely unknown. In our preliminary study, we checked whether BPS and BPF act on *in vitro* meiotic maturation of mouse oocytes and structure of their metaphase II spindles. Briefly, we cultured prophase I oocytes in a medium supplemented with increasing concentrations of BPS (2, 20, 200 ng/ml) or BPF (0.2, 2, 20 ng/ml), corresponding to their reported or expected concentration in human follicular fluid. Each experimental variant was conducted in at 3 repetitions. After achieving metaphase II stage, oocytes were fixed, stained (for β-tubulin and DNA) and then their spindles were classified as normal or abnormal (lack of bipolar shape and/or misaligned chromosomes). Our results demonstrated that 20ng/ml BPF negatively affected oocyte meiotic progression, increasing the number of oocytes that underwent lysis. We also showed that 20ng/ml BPS exposure, although did not alter meiotic maturation rates, significantly elevated the percentage of abnormal meiotic spindles. Concurrently, 20ng/ml BPS added during *in vitro* maturation modified spindle morphology (for spindles classified as normal): spindles were elongated and the ratio of spindle length to width was elevated compared to control groups.

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Session VIII. Gonadal development and function

O.8.1

HAND2, a GATA4 interacting protein is a new potential genetic player in human gonadal development as its variant is associated with 46,XY gonadal dysgenesis

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Our knowledge of the genetic mechanisms of gonadal development remains incomplete, as the pathogenic variant is unknown in more than 50% of patients with 46,XY gonadal dysgenesis (GD).This study aimed to identify the genetic causes of 46,XY GD using whole exome sequencing, seeking new genes and pathways involved in early gonadal development.

In a female 46,XY GD patient of Ukrainian origin, we identified a heterozygous mutation in the GATA4 (GATA Binding Protein 4) gene, combined with a heterozygous mutation in HAND2 (Heart And Neural Crest Derivatives Expressed 2). Both were inherited from the patient's mother and maternal grandmother. Although GATA4 mutations have already been described as causative for 46,XY GD, the specific mutation found in our patient was also present in a boy without sexual development abnormalities, suggesting it alone does not explain the GD phenotype. In contrast, mutations in the HAND2 gene have not been reported so far in any disorder of gonadal development. Interestingly, HAND2 encodes a basic helix-loop-helix transcription factor that is essential for cardiac morphogenesis, where it interacts with the GATA4 transcription factor. Remarkably, the *Hand2* gene has recently been reported to be robustly expressed in the early androgenital primordium of mice, but its importance in human gonadal development has not been previously suggested. Here we propose for the first time that the interaction between HAND2 and GATA4 proteins is critical not only for heart but also for gonadal development and that the co-occurrence of HAND2 and GATA4 gene mutations present in our patient may underlie 46,XY GD. We are currently investigating how these mutations affect the HAND2/GATA4 interaction, which may reveal a new oligogenic mechanism in gonadal development.

O.8.2

Impaired steroidogenesis in obese female mice is driven by Nodal suppression in theca cells

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Induced obesity (DIO) in mice leads to ovarian leptin resistance. Presently, we investigated whether susceptibility to obesity impacts ovarian performance in mice. We hypothesise that impaired Nodal signalling in theca cells (TC) dysregulates ovarian steroidogenesis following DIO.

C57BL/6J (B6) mice were submitted to chow diet (CD) or high fat diet (HFD) for 16 weeks. The animals were culled, and TC and granulosa cell (GC) fractions collected from ovaries followed by immunofluorescence (IF) or mRNA isolation and real-time PCR (RT PCR) analysis. Next, ovarian explants derived from DIO protocol were cultured *in vitro* for 12h and treated with Nodal and SB431542, a Nodal receptor inhibitor.

The B6 HFD mice presented significant increase in body weight ($p<0.0001$) and fat mass (FM) ($p<0.0001$). Intensity of perilipin1 staining following IF of ovarian sections was decreased in B6 HFD ($p<0.01$), particularly in TC from antral follicles. RT PCR analysis in TC revealed decreased mRNA level of *Lhr* ($p<0.05$), *StAR* ($p<0.001$), *Nr5a1* ($p<0.05$) and *Era* ($p<0.05$) in HFD mice. Moreover, Nodal signalling suppression in TC from HFD mice was demonstrated, as mRNA levels of Nodal and its receptors Acvr2b, and Alk7 ($p<0.01$, $p<0.05$ respectively) were decreased. Treatment of ovarian explants from CD with SB431542 downregulated mRNA levels of *Lhr* ($p<0.05$), *Star* ($p<0.05$), *Nr5a1* ($p=0.06$). In the rescue experiment, ovaries from HFD mice were treated with Nodal, resulting in the upregulation of *Lhr* ($p=0.05$), *Star* ($p<0.05$) and *Era* ($p<0.05$) in TC.

Overall, impaired steroidogenesis in obese mice is associated with dysregulation of the Nodal signalling in TC. Imbalanced steroidogenesis may impact oocyte development and quality in obese mothers, so further studies are needed to explore the underlying interactions.

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O.8.3

Visfatin Impact on Angiogenesis, Proliferation, and Apoptosis in the Porcine Corpus Luteum

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Visfatin, a hormone belonging to the adipokines' group plays an important role in regulating the hypothalamic-pituitary-ovarian axis. Our previous studies confirmed visfatin's presence in the porcine corpus luteum (CL) and its modulatory effect on endocrine functions indicating that it is an important luteotropic factor. This study aims to elucidate visfatin's impact on angiogenesis, proliferation, and apoptosis in the porcine CL.

Luteal cells collected in the mid-luteal phase were cultured and treated with visfatin (1-100 ng/mL) and FK866, an enzymatic activity blocker of visfatin. After 24 hours, the secretion of angiogenic factors (VEGFA, bFGF2, ANG-1, ANG-2) was measured via ELISA, while the expression of their receptors (VEGFR1, VEGFR2, FGFR1, FGFR2, TIE2) was assessed by Western blot. Luteal cell proliferation was analyzed using the alamarBlue assay. DNA fragmentation and caspase-3 and -7 activity were examined using Cell Death Detection Kit and CaspaseGlo 3/7 assays, respectively. qRT-PCR was employed to measure mRNA concentration of key apoptotic factors. Statistical analysis was performed in GraphPad Prism8.

Results showed that visfatin inhibited the secretion of most angiogenic factors, except for bFGF-2 and ANG-2, which were stimulated by visfatin at the dose of 1 and 10 ng/mL, respectively. Protein expression of angiogenic receptors decreased with visfatin treatment. Visfatin increased the percentage of viable cells and reduced caspase-3/7 activity, though it did not affect DNA fragmentation. mRNA concentration of caspase-3, -8, -9, and Bax decreased depending on the visfatin dose while Bcl-2 expression increased in response to 100 ng/mL of visfatin. The presence of FK866 mostly abolished observed effects, highlighting the involvement of enzymatic form of visfatin in the action on the investigated processes in the porcine CL.

These findings confirm that visfatin modulates angiogenesis in the pig CL and is a luteotropic factor through its pro-proliferative and anti-apoptotic action.

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O.8.4

Patients with premature ovarian failure have reduced glucose bioavailability in the ovarian follicle

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The incidence of premature ovarian insufficiency (POI) is a leading cause of infertility in women and requires infertility treatment. Idiopathic POI affects women under the age of 40 and is often referred to as premature menopause. Literature shows that higher serum glucose concentrations are associated with high FSH levels in POI patients.

The aim of this study was to determine whether POI is associated with glucose bioavailability to granulosa (GCs) and cumulus (CCs) cells, measured here by glucose concentration in follicular fluid (FF) and expression of glucose transporters.

The study, approved by the Ethical Committee of the Jagiellonian University (1072.6120.30.2023), included human granulosa cells isolated from the follicular fluid of 47 women undergoing IVF. POI was defined according to the criteria of the European Society of Human Reproduction and Embryology (age<40, AMH<1.1 ng/ml). Human GCs isolated from FF of POI and control women were cultured for 24h as an *in vitro* model. Expression of *GLUT1*, *GLUT4*, *SLC5A1* and *SLC5A2* genes was determined by RT-qPCR. Glucose levels in FF were established using a Glucose Colorimetric Detection Kit (EIAGLUC, Invitrogen).

W showed that there were no differences in BMI and glucose concentrations between FF from POI and control patients. The expression of the classic ovarian glucose transporters, *GLUT1* and *GLUT4*, was significantly lower in GCs and CCs from the POI group. Moreover, the expression of alternative glucose transporters, *SLC5A1* and *SLC5A2*, was not detected.

These results show that GCs and CCs of POI have lower expression of classic glucose transporters. Thus, despite the same amount of glucose in FF, the bioavailability of glucose is limited. These results suggest that disruption of glucose bioavailability in POI patients may lead to impaired energy metabolism in ovarian cells and affect female fertility.

This work was supported by the National Science Centre (NCN), Poland [2022/45/B/NZ7/00254].

O.8.5

Variability of boar testicles echotextural image during the immunocastration procedure

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Surgical castration of young boars become more and more controversial procedure in pork production. Thus, alternative methods seem to be more ethical and have the same goal - prevent boar taint of pig meat. The aim of the study was to analyze changes in testicular development using USG scanning during two-phase procedure of immunocastration. The research has been carried out using 30 polish landrace boars in Experimental Station in Chorzelów (NRIAP). Animals were purchased in the age of 9 week and divided into 2 groups. 15 boars were subjected to immunocastration procedure using Improvac® (EG), and another 15 were served as controls (CG). In 11th week of live boars' testicles were diagnosed using USG scanning (Aloka PS2, 6 MHz linear array probe). A week later the 1st injection of Improvac was made. In 14th week the 2nd scanning of testicles was performed, followed by the 2nd injection 2 weeks later. In the age of 18 weeks the last (3rd) USG scanning was performed for final diagnosis of testicles status. Mean parameters of testicles did not differ significantly between groups in the 1st scanning (diameter 19.2±2.8 and 18.5±2.9; mean echotexture 16.8±8.3 and 14.9±5.1 in EG and CG respectively). Differences between groups did not occur after the 1st Improvac application (diameter 21.5±7.3 and 23.8±4.7; mean echotexture 22.3±10.3 and 24.6±9.0 in EG and CG respectively), however, significant difference ($P<0.001$) was seen between the 1st and 2nd scanning in CG. Significant differences between groups ($P<0.001$) in every analyzed testicle parameter were found during the 3rd scanning (diameter 28.4±5.2 and 49.5±6.4; mean echotexture 37.2±9.9 and 57.3±6.0 in EG and CG respectively). Differences were also visible inside groups between the 2nd and 3rd scanning, however, in CG they were greater than in EG. To conclude, Improvac® is effective in inhibiting testicular development only in two-phase procedure.

ABSTRACTS
POSTERS

Posters:

P.1

Does lipid peroxidation and enzymatic antioxidant activity differ in fresh semen of mature and aged dogs?

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The study aimed to assess the fresh semen of mature and senile dogs to determine whether sperm quality declines with advanced aging and whether membrane lipid peroxidation, seminal plasma and spermatozoa antioxidant activity, and DNA damage are involved in this process. For this purpose, the sperm-rich fractions of the ejaculate were collected from 20 mature (2 to 4 years) and 20 senile (≥ 9 years) dogs, with 2 or 3 ejaculates per dog, totaling 107 ejaculates. The samples were evaluated for various parameters, including sperm concentration, total sperm output, acrosome-membrane integrity and permeability, mitochondrial potential, DNA fragmentation (TUNEL), lipid peroxidation, and seminal plasma and spermatozoa superoxide dismutase, catalase, and glutathione peroxidase activities. The semen of mature dogs exhibited superior values for motility, concentration, total sperm output, plasma membrane integrity and permeability, and high mitochondrial potential compared to that of senile dogs ($p < 0.05$). Conversely, no correlation was found between age and lipid peroxidation, DNA damage, or enzymatic antioxidant activity. In conclusion, the sperm quality of dog semen declines with advanced aging. However, this decline is not associated with oxidative damage to the plasma membrane and DNA or decreased antioxidant activity, as reported in bulls and men.

P.2

A search for NANOS1 interacting proteins to elucidate structural and functional impairments caused by a variant associated with infertility

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The NANOS family of proteins specifically expressed in germ cells are essential for maintaining germ cell fate and survival across species by inhibiting somatic fate. NANOS is a highly conserved Zn-finger (CCHC)2 RNA-binding protein (RBP) interacting with RNAs and proteins.

Its post-transcriptional repressor activity is mediated by recruiting the CCR4-NOT deadenylation complex to RNA targets. In humans, a NANOS1 variant carrying a double mutation p.[Pro34Thr; Ser78del] (further referred to as mut-NANOS1) is associated with the complete absence of germ cells in the seminiferous tubules of the patients (Sertoli Cell Only Syndrome) and causes NANOS1 functional switch from anti- to pro-apoptotic.

Our molecular dynamics simulations revealed conformational structural changes in the mut-NANOS1. Specifically, the NOT1 interacting motif was affected. Additionally, the distance between Zn atoms of (CCHC)2 domains was shortened making them inaccessible for interactions. We performed a global screening for the wt- and mut-NANOS1 binding proteins in the W15 human embryonic stem cell lines, engineered for wt- and mut-NANOS1 overexpression. These can efficiently differentiate into human primordial germ cells (PGCs). To begin with, we selected the pre-mesendodermal stage (preME, competent stage for PGC). We used co-immunoprecipitation and an eight-plex tandem mass tag (TMT) labelling to identify and quantify the proteins. Among the 20 proteins identified in wt-NANOS1, we found wt-specific VIGILIN, another RBP as a potential putative interacting partner. It influences mRNA stability and selective translational repression. Interestingly, out of 22 interactors of mut-NANOS1, we found mut-specific AT-rich interactive domain-containing protein 1A (ARID1A), critical for the commitment of cell lineages to mesodermal origin. With these preliminary results we hypothesise that through binding to ARID1A, mut-NANOS1 asserts preME cells towards differentiation of mesodermal derivatives rather than germ cells. Validation of these results is ongoing.

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P.3

Seasonal and nutritional changes in the short form of leptin receptor expression and the VEGF system in the choroid plexus, arcuate nucleus and anterior pituitary in leptin/MTS-leptin- and resistin-treated sheep

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Seasonal reproduction is strongly influenced by metabolic status. Many factors that affect reproduction, appetite, and energy expenditure have been described. Many of these factors are adipokines, hormones produced mainly by white adipose tissue. New roles for adipokines in fertility, reproduction, and metabolism have recently emerged, particularly since the description of leptin, and resistin. The blood-brain barrier (BBB) is a key player in adipokine signaling from the periphery to the central nervous system (CNS). The short form of the leptin receptor (LeptRa) plays a key role in the transport of leptin to the central nervous system. Here, MTS-leptin and recombinant ovine (ro) leptin-mediated expression of LeptRa and VEGFA and VEGFR2 in selected hypothalamic nuclei, the choroid plexus (ChP) and anterior pituitary (AP) were analyzed considering the photoperiod and acute fasting (experiment 1) and nutritional status (experiment 2) of ewes. In experiment 1, sixty sheep were fed normally or fasted for 72 h and received one injection of saline, MTS-leptin or roleptin 1 h prior to

euthanasia. *LeptRa* mRNA transcript levels and VEGF system protein concentrations were detected in the arcuate nucleus (ARC) and ChP predominantly in the short day (SD) period and in the AP in the long day (LD) period. In experiment 2, an altered diet for 5 months created lean or fat sheep. Twenty sheep were divided into four groups: the lean and fat groups were given saline, while the lean-R and fat-R groups received resistin 1 h prior to euthanasia. The results of the experiments showed for the first time the effect of MTS-leptin/roleptin on the expression of LeptRa, protein concentrations of VEGFA, and VEGFR2 in selected brain regions, namely, the AP, ChP, and ARC, in the context of nutritional status, resistin, and photoperiod.

P.4

Fatty acid transporter expression in peri-implantation conceptuses and developing placenta of the pig

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The proper development of early embryos and further growth of fetuses rely on maternal-derived nutrients. Among them, polyunsaturated fatty acids (PUFA) determine the metabolic status of the cell, modulate the expression of genes involved in cellular homeostasis and are important for cell membrane composition. Thus, an efficient uptake of PUFA must exist in the placenta. The aim of the study was to examine the expression of solute carrier 27A (SLC27A) proteins, which function as transmembrane FA transporters, in porcine conceptuses/ trophoblasts during the period of early placenta formation. Gilts were slaughtered on various days of early pregnancy to collect conceptuses (days 10-11, 12-13, and 15-16; n = 6-8/group), trophoblast tissue (days 18-20, 25, and 30; n=5-6/group), and embryos (days 18-20). The abundance of *SLC27A1*, *SLC27A4*, and *SCL27A6* transcripts was examined using Real-time PCR, while protein expression was determined with Western blot and immunohistochemistry. Data were analyzed using one-way ANOVA or Student's t-test. All examined SLC27A proteins were detected in conceptuses and in the trophoblast-epithelial unit of the placenta. *SLC27A1* mRNA and protein expression was greater in day 30 trophoblasts as compared with days 12-13 conceptuses ($P<0.05$). The abundance of *SLC27A4* decreased in filamentous conceptuses collected on days 12-16 as compared with days 10-11 spherical conceptuses ($P<0.01$). *SLC27A4* mRNA and protein levels were greater on days 18-20 as compared with days 12-13 ($P<0.00$). The average abundance of *SLC27A6* transcripts was about 50-fold lower in days 18-30 trophoblasts compared with days 10-13 conceptuses. The expression of SLC27A1 and SLC27A4 proteins was greater in trophoblast tissue than embryos on days 18-20 of pregnancy ($P<0.01$). In conclusion, changes in *SLC27A* expression point to the existence of an active transport of PUFA into porcine trophoblast cells to support embryo and early placenta development.

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P.5

The expression and function of sirtuin 2 and sirtuin 3 at the maternal-conceptus interface during early pregnancy in pigs

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Sirtuins (SIRT1-7), NAD^+-dependent deacetylases, affect diverse cellular processes. Yet the reproductive functions of sirtuins remain unclear. The present study aimed to examine 1) the expression of SIRT2/3 in conceptus trophoblast (Tr) and endometrial tissue of early pregnant gilts and 2) the effect of SIRT2/3 on proliferation of Tr and endometrial luminal epithelial (LE) cells. Endometrial and/or Tr tissue from days 15, 20, 25, and 30 of pregnancy (n=24 in total) was used to analyze SIRT2/3 expression by immunohistochemistry and/or qPCR. For proliferation assay, Tr cells from day 15 conceptuses and LE cells from uteri of day 12 cyclic gilts (n=4-5) were used. Cellular expression of both sirtuins was confirmed by immunofluorescence. Tr and LE cells were exposed to enzymatically active SIRT2 and SIRT3 proteins or pharmacological SIRT2 and SIRT3 inhibitors (AGK2 and 3-TYP, respectively). Data were analyzed using ANOVA or Student's t-test. Positive staining for SIRT2/3 was observed in endometrial epithelium and stroma on days 15-30 of gestation. Trophoblasts of day 20 and day 30 embryos also showed strong immunoreaction for SIRT2/3. The expression of *SIRT2* mRNA increased in Tr tissue between days 15 and 30 of pregnancy ($p<0.001$). The profile of *SIRT3* transcript abundance in Tr tissue did not change during the studied period. Cultured Tr and LE cells positively stained for SIRT2/3 protein presence. SIRT2 stimulated proliferation of Tr ($p<0.01$) and LE cells ($p<0.05$), whereas AGK2 reduced the number of viable LE cells ($p<0.05$). Similarly, SIRT3 stimulated proliferation of Tr cells ($p<0.01$). The SIRT3 inhibitor, in turn, decreased the number of viable LE cells ($p<0.001$). In summary, SIRT2/3 are present at the maternal-conceptus interface during implantation and early placentation in the pig and seem to be important for proliferating activity of Tr and LE cells.

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P.6

Ultrasound imaging of the brain and head development in sheep during the embryo-fetal period

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Prenatal development in sheep, as in other mammals, involves a series of continuous, repeating processes. They concern, among others, organogenesis - an important period for the organs that are developing at this time. In this aspect, the development of individual

structures of the brain, as well as the head, may become particularly important in relation to the further proper development and functioning of the embryo/fetus. Therefore, the aim of this study was to determine the echogenicity and morphometric features of developing brain and head structures in sheep in the prenatal period using ultrasound examination.

The study also determined the time at which cerebral vessels could be imaged for the first time. The research was carried out on 12 pregnant Suffolk sheep, which underwent cyclic ultrasound examination (EDAN U50 scanner) throughout the entire pregnancy.

Imaging and biometric measurements for choroid plexus width, biparietal diameter (BPD), occipital-nasal length (ONL), eye socket width and eye socket height were performed in B mode, while the Color-Doppler technique was used to image the cerebral vessels. The conducted research indicates the possibility of using ultrasound examination to monitor the development of the brain and head in sheep in the prenatal period.

The results obtained in this regard showed significant differences in the examined biometric parameters of the brain and head between individual periods of embryo-fetal development ($P<0.01$). It has also been shown that it is possible to image cerebral vessels in sheep already in the first trimester of pregnancy.

P.7

Resveratol supplemented extender for short-term storage of honey bee (*Apis melifera*) drone semen - preliminary studies

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The study aimed to evaluate the effects of resveratrol on honey bee dron spermatozoon at liquid storage for 6 weeks at 15-16°C. Semen was collected from Apis m. carnica sexually mature individuals aged 14-21 days using a calibrated glass pipette. All semen samples were pooled together for elimination individual variations. The pooled semen was diluted in four extenders: Ext1 (0.3 g glucose; 0.21 g $NaHCO_3$; 2.43 g sodium citrate; 0.41 g KCl in 100 ml redistilled water), Ext 2 (Ext1 with 5µM resveratrol), Ext 3 (Ext1 with 10µM resveratrol) and Ext 4 (Ext1 with 15µM resveratrol). Semen was stored in glass capillaries according to the methodology of Collins (J Econ Entomol, 93: 568-571, 2000). Semen quality was evaluated on the day of collection (fresh semen) and after 3 and 6 weeks of storage. Sperm viability was assessed on an epifluorescence microscope (Nikon Eclipse E600, Japan) using propidium iodide staining, determining % of viable sperm with intact membrane integrity. The data were analysed by ANOVA, and the significance of the difference between the means was determined using Duncan's test at $P<0.05$. Our study results revealed that, the lowest percentage of viable spermatozoa was identified after 3 weeks of storage the drone semen in Ext3 and Ext4. Evaluation of semen motility after 6 weeks of storage, revealed that a significantly higher percentage of motile sperm was observed in extender Ext2 (73.5±3.1) compared to Ext1 (61.7±2.9), Ext3, (60.8±1.8) and Ext4 (43.2±2.0). Also, after 6 weeks of storage, the highest percentage of viable sperm was observed in extender Ext2 (71.5±2.5).

The study showed that extender supplemented with 10µM resveratrol have beneficial effects on honey bee drone sperm parameters after storage for 6 weeks at 15-16°C.

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P.8

VASPIN in human placental cells: expression, hormonal regulation and its role on trophoblast endocrinology and signaling pathways

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VASPIN is an adipokine described in 2005 in the adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Vaspin action is linked with the regulation of energy balance food intake, preadipocyte differentiation, insulin sensitivity, glucose tolerance and reproduction. Both vaspin and its receptor GRP78 level dynamic changes across trimesters; altered levels are implicated in pregnancy pathologies, suggesting their potential roles as biomarkers or contributors to complications.

Our studies were conducted on human placental cell lines, JEG-3/BeWo (n=5), and terminal physiological placentas obtained after delivery. We examined mRNA expression and immunolocalization of vaspin/GRP78 in JEG-3/BeWo, and maternal/fetal placental parts. Effect of progesterone (P4), estradiol (E2), chorionic gonadotropin (hCG), and insulin (INS) with involvement of extracellular signal-regulated kinase (ERK 1/2) on vaspin/GRP78 level, was evaluated in JEG-3 cells. Vaspin's impact on P4, E2, hCG, and placental lactogen (hPL), alongside ERK1/2 and protein kinase A (PKA) phosphorylation was studied in BeWo/placental villous cells.

We confirmed the mRNA expression of vaspin/GRP78 in JEG-3/BeWo and compartments of human placentas. In JEG-3 and BeWo, vaspin was immunolocalized in the perinuclear and cytoplasmic regions, while GRP78 was in the cytoplasm, perinuclear region, and nucleus. In the maternal placental part, vaspin was present in capillary epithelium and decidual cells, whereas GRP78 was in decidual cells. In the fetal part, vaspin was detected in cytotrophoblasts and syncytiotrophoblasts, while GRP78 was exclusive to syncytiotrophoblasts. We noted that selected steroids and protein hormones modulated vaspin and GRP78 levels; the molecular

mechanism of P4, hCG, and insulin action involved the ERK1/2 pathway. Also, vaspin time- and dose-dependent regulated secretion of P4, E2, hCG, and hPL via ERK1/2 or PKA.

In conclusion, we underscored the significance of the vaspin/GRP78 system in the endocrine regulation of the human placenta, highlighting its potential to impact hormonal fluctuations throughout pregnancy.

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P.9

Cryopreservation-induced changes in the phosphoproteome of Siberian sturgeon spermatozoa in relation to cryoprotectant MeOH and DMSO

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Sperm cryopreservation plays a crucial role in the conservation of aquatic species but induces significant biochemical alterations. Our recent study demonstrated that despite similar quality of cryopreserved Siberian sturgeon semen, dimethyl sulfoxide (DMSO) cryopreserved semen had reduced fertilization success compared to methanol, revealing cryoprotectant-dependent alterations in the sperm proteome. However, information on changes in the semen phosphoproteome is lacking. This study examined the phosphoproteome changes in Siberian sturgeon spermatozoa cryopreserved with methanol (CryoMeOH) and DMSO (CryoDMSO) using LC-MS/MS analysis. We analyzed 1,590 phosphopeptides from 809 proteins, identifying 299 phosphopeptides with differential expression between fresh and cryopreserved spermatozoa (q-value ≤ 0.05, fold change > 1.2). In CryoDMSO, 156 phosphopeptides (231 phosphosites) from 114 proteins were significantly altered, with 63 upregulated and 56 downregulated proteins. In CryoMeOH, 195 phosphopeptides (260 phosphosites) from 127 proteins were altered, with 81 upregulated and 53 downregulated phosphoproteins. Functional enrichment analysis revealed that upregulated phosphoproteins in both groups were linked to cilium movement and protein binding. CryoMeOH specifically affected phosphoproteins related to cilium beat frequency and the elongation factor complex, while CryoDMSO was associated with calmodulin binding and the phosphorylase kinase complex. Downregulated phosphoproteins were connected to catabolic processes and phosphatidylinositol phosphate 5'-phosphatase activity, localized in the cytoplasm. CryoDMSO-specific downregulation involved nuclear pore assembly and microtubule processes, whereas CryoMeOH impacted intracellular transport. Ingenuity Pathway Analysis indicated significant changes in cilium assembly and glucose metabolism in both cryopreservation groups. DMSO uniquely affected RHO signaling, SUMOylation, and microRNA biogenesis, while MeOH influenced mitotic processes and phosphatidylinositol metabolism. This pioneering study offers a comprehensive characterization of the phosphoproteome changes in Siberian sturgeon spermatozoa due to cryopreservation, highlighting the distinct effects of DMSO and methanol. Most importantly, our results suggest that DMSO can influence signaling pathways involved in fertilization and early embryonic development.

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P.10

Preliminary results of vitrification of *in vitro* matured wisent oocytes

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Our team success in developing a method for oocyte maturation, fertilisation of mature oocytes and *in vitro* embryo culture, as well as cryopreservation of wisent (European bison) embryos opens new perspectives for this near-threatened species (Duszewska et al., 2018, Reprod Domest Anim. 2018,53,818-821; Duszewska et al., Animals 2022,11,12:1239-1241). Cryopreservation of gametes (spermatozoa and oocytes) is a crucial tool for conserving the germplasm of this species. While sperm cryopreservation has already been developed, the cryopreservation of oocytes still poses a formidable substantive and methodological challenge. Therefore, the aim of the present study was the vitrification of *in vitro* matured wisent oocytes. In this study, two female wisents were culled out of the reproductive season (October–March) for reasons unrelated to fertility. Immature COCs were aspirated from ovarian follicles and matured for 30 h. After maturation, COCs were denuded, and their morphology was analysed. Only matured oocytes were then vitrified in a two-step manner (VS1, VS2). They were subsequently placed in straws and stored in LN2 for one week. After, matured oocytes were thawed, and their morphology was determined. The preliminary results indicate that 78,57% (11/14) of oocytes matured, and 71,42% (10/14) were classified for vitrification. Only 10% (1/10) of vitrified oocytes survived and showed normal morphology after thawing. In conclusion, the low survival rate of thawed oocytes indicates the need for further research to increase the efficiency of vitrification of wisent mature oocytes. This is a crucial step towards practical germplasm wisent preservation, that is, preserving the reproductive potential of this near-threatened species.

P.11

Effect of liquid storage time at 5°C on motility, viability and morphology of epididymal spermatozoa of European red deer (*Cervus elaphus elaphus*) subjected to cryopreservation

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The aim of the study was to evaluate effect of storage time at 5°C on the motility, viability and morphology of epididymal spermatozoa of European red deer subjected to cryopreservation. We used spermatozoa obtained from the cauda epididymis of six males shot during the rut of

the hunting season. Before the freezing process, the samples of sperm were stored in a liquid state at 5°C for six days (D1-D6). Salomon's extender was used for liquid storage and cryopreservation, without and with the addition of glycerol (6%), respectively. The analysis of sperm quality included the assessment of sperm motility (using the CASA system), assessment of viability (cytometric method) and assessment of morphology (Giemsa staining). Analyses were performed before and after thawing during D1, D2, D4 and D6. Data were processed through statistical analysis. Prior to the freezing process, the quality (motility and viability) of liquid-stored spermatozoa differed significantly ($p \leq 0.05$) between D1 and D6. The cryopreservation process was shown to significantly affect spermatozoa motility, viability and percentage with morphological defects regardless of the day of storage. With the duration of storage of spermatozoa in the liquid state, quality was shown to decrease after thawing. The analysis showed significant differences in the quality of cryopreserved spermatozoa between storage days D1 and D4, and these values (%) for the percentage of spermatozoa showing total motility were 60.7±3.8 and 47.5±10.5, respectively and for viability were 60.4±2.9 and 53.9±8.01, respectively. Conclusions: Sperm from the cauda epididymis of European deer stored in a liquid state for up to six days can be used for cryopreservation, and the cryopreservation of sperm stored in a liquid state for up to four days can be considered the most optimal.

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P.12

Effect of IVM media supplementation with n6/n3 fatty acids on the quality of bovine oocytes and blastocysts

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In vitro-produced embryos have reduced developmental competence compared to their *in vivo* counterparts thus the culture conditions are crucial. Besides, the quality of blastocysts is strictly related to the quality of oocytes which is affected by the composition of IVM media. According to the literature, unsaturated fatty acids (FAs) influence oocyte maturation when added to IVM medium. Alpha-linolenic FA (ALA/n3) improved the developmental competence of bovine oocytes by preventing apoptosis in cumulus cells, reducing ROS accumulation in oocytes, and supporting development to the blastocyst stage. The linoleic FA (LA/n6) had diverse effect. Although effects of the two FAs differ, the most important is the n6/n3 ratio. In this study, we evaluated the impact of the two FAs supplemented to IVM media at 3:1 ratio (n6/n3) on the quality of oocytes, cumulus cells (CC) and resulting blastocysts.

Cumulus-oocyte complexes (COCs) were matured in medium supplemented with n6/n3 FAs at a 3:1 ratio (75µM/ 25µM). Selected COCs were fertilized to obtain blastocysts whereas some of them were analyzed (oocytes for ROS and GSH; CC for apoptosis). Day 7 expanded

blastocysts were subjected to TUNEL to determine cell count and apoptotic index (AI). Altogether, 358 oocytes, 9 CC samples (1000 cells each), and 92 blastocysts were analyzed. In comparison to the control group, the n6/n3 oocytes were characterized by reduced contents of intracellular ROS and GSH. No differences were noticed in the AI index of cumulus cells.

Regarding blastocysts, the total cell count was significantly higher in the n6/n3 group than in the controls and no effect was observed for apoptotic index.

In conclusion, supplementing IVM medium with n6/n3 FAs has a beneficial effect on oocytes (reduced ROS) and resulting blastocysts (higher cell count).

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P.13

Stage-dependent potential of rabbit ICM to differentiate into extraembryonic lineages

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Early stages of embryonic development of placental mammals entail of differentiation of the first cell lineages – the pluripotent epiblast (Epi), and the extra-embryonic primitive endoderm (PrE, also called hypoblast) and trophectoderm (TE). The proper differentiation of these lineages is a prerequisite for further development, and abnormalities in this process may result in stunted development already at implantation in all mammalian species, including humans. Here we investigated how the cellular plasticity of early mammalian embryos relates to the developmental time scale and changes in gene expression using rabbit isolated ICMs.

While determine the timing of lineage commitment of the inner cell mass (ICM) vs TE, we first identified GATA3 as an early marker of rabbit TE, and CDX2 as a marker of mature TE, and then studied the dynamics of rabbit blastocyst formation using time-lapse imaging.

We then analysed the developmental potential of rabbit ICMs isolated by immunosurgery and subsequently cultured in vitro, showing that they retain the ability to regenerate TE up to mid-blastocyst stage (stage VII), in contrast to mouse blastocyst, which lose this ability soon after cavitation. We further observed that rabbit ICMs isolated from later blastocyst stages lose the ability for TE specification, instead forming a halo-like cavity with an outer layer of endodermal cells.

Our data further indicate that in mammalian embryos the potential for TE differentiation gives way to the formation of a different type of extraembryonic epithelial layer, suggesting a potential common mechanism of pluripotency restriction between eutherian mammals.

Recent reports suggest that plasticity of the extraembryonic lineages in mammalian embryos might be extended in species other than the mouse. Moreover, there is a potential common mechanism that might give way the potential to form trophectoderm into another epithelial lineage.

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P.14

Alterations in connexin 43 gene and protein expression in the hen ovary following tamoxifen treatment

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Connexin 43 (Cx43) is a major Cx within the mammalian ovary, where takes part in proper follicle development. As a component of the gap junctions, it facilitates transfer of small molecules between neighboring cells. Information concerning the expression and regulation of Cx43 in the chicken ovary is largely unknown. The present study aimed to investigate the expression of the Cx43 gene (GJA1) and protein as well as the immunolocalization of Cx43 in the hen ovary in relation to follicle development. Moreover, the effect of tamoxifen (TMX; an estrogen receptor blocker) treatment on the Cx43 expression in the ovary was examined at the mRNA (by qRT-PCR) and protein (by Western blotting) levels. The results demonstrated differences in Cx43 mRNA transcript and protein abundances among ovarian white follicles, yellowish follicles, small yellow follicles, and the largest yellow preovulatory follicles (F3–F1). In general, Cx43 was more abundant in large (preovulatory) than small (prehierarchical) follicles and in granulosa cells compared with theca cells. Furthermore, the effect of TMX treatment depended on the stage of follicle development and the layer of the follicular wall. Ovarian regression after TMX treatment was accompanied by an increase in Cx43 abundance in most ovarian follicles, which may impact the formation and function of Cx43 hemichannels. Our results showed, for the first time, the differences in Cx43 mRNA transcript and protein abundances between ovarian follicles, suggesting the potential involvement of this gap junction protein in the regulation of ovarian folliculogenesis. In addition, the results point to a potential role for estradiol in regulation of Cx43 transcription and/or translation in the hen ovary. Understanding the impact of Cx43 in mechanisms underlying ovarian follicle development and function may be of considerable importance for poultry egg production.

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P.15

Exploring the influence of extracellular vesicles from small and large ovarian follicles on cumulus cells expansion and transcriptome alterations in equines: a preliminary study

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Cumulus expansion process is vital for the oocyte maturation, fertilization, and subsequent embryo development. Granulosa cells release factors into the follicular fluid (FF), inducing paracrine signaling essential for proper folliculogenesis. The FF is rich in proteins, nucleic acids, and extracellular vesicles (EVs). These particles facilitate intercellular communication in the ovarian environment by transporting bioactive components, potentially enhancing oocyte development during *in vitro* maturation (IVM). The research hypothesized that EVs from small (< 20 mm) and large (> 35 mm) ovarian follicles could influence cumulus cells (CC) expansion and transcriptome alterations.

FF was aspirated post-mortem from ovaries without a corpus luteum, and EVs were isolated through ultracentrifugation and characterized. The IVM process was conducted in commercial EQ-IVM medium (IVF-BioScience) with or without EVs (200 µg of EV protein/ml). EV internalization during IVM was assessed via fluorescent particle labeling and confocal microscopy. Cumulus expansion was assessed by measuring COCs before and after IVM, followed by isolating and storing CC at -80°C. Subsequently, RNA extraction will be conducted, cDNA libraries generated, and changes in the CC transcriptome investigated using next-generation sequencing.

Confocal microscopy confirmed the internalization of EVs by COCs. Nanoparticle tracking analysis revealed an average EV size of 108.4 nm, with 90% of particles being 150.7 nm or smaller. Flow cytometry showed that 52.6% of EVs expressed the surface marker CD63 and 15.6% expressed CD81. Transmission electron microscopy verified the distinctive disk-shaped morphology of nanovesicles. EVs derived from both small and large follicles have significantly contributed to cumulus expansion in mare compacted COCs, however, this effect was not observed in expanded COCs. Follicular EVs appear to effectively support the physiological needs of gametes. Nonetheless, further research is required to evaluate the impact of EVs from different estrous cycle phases on maturing COCs.

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P.16

The effect of antioxidants (resveratrol and ascorbic acid) on the developmental competence of feline oocytes after vitrification

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Recent studies have indicated that an increase in reactive oxygen species (ROS) levels in thawed oocytes causes a temporary, dynamic loss of mitochondrial membrane potential, resulting in decreased adenosine triphosphate (ATP) production and potentially impairing embryo development. This research aimed to evaluate the developmental potential of feline oocytes following *in vitro* maturation (IVM), vitrification, and post-warming incubation with two antioxidants: resveratrol and ascorbic acid.

Following vitrification and thawing, oocytes were incubated with either 0.2 µM, 2 µM, or 20 µM resveratrol or ascorbic acid for 2 hours. After the 2-hour incubation, the warmed oocytes underwent *in vitro* fertilization (IVF), and the resulting presumptive zygotes were cultured and their development assessed. In the resveratrol-treated groups, the highest cleavage rate was observed with 0.2 µM resveratrol (88.34%), significantly higher than the control group (75%). Conversely, in the ascorbic acid-treated groups, the highest cleavage rate was achieved with 0.2 µM ascorbic acid (37.5%), which was still lower compared to the resveratrol-treated groups.

These findings suggest that resveratrol exerts a beneficial effect on embryonic development, contingent on the concentration used. While vitrification commonly leads to reduced developmental potential in embryos due to cryopreservation-induced damage, the application of low concentrations of resveratrol improves the success of the procedure.

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P.17

The brief coexistence of the transferred somatic nucleus and the parthenogenetic pronucleus in the cytoplasm of an activated oocyte does not preclude the development of the reconstructed embryo to the blastocyst stage

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Somatic cell nuclear transfer (SCNT) is a laboratory strategy for creating a group of genetically identical animals that constitute a clone. In a classical approach, the nucleus of a somatic cell is deposited in the cytoplasm of the egg cell just after its enucleation. In our approach, the nucleus of a follicular cell was transferred to the cytoplasm of an intact MII oocyte, which contains its own genetic material. After artificial activation, both parthenogenetic and transferred nuclei coexisted in a common cytoplasm for several hours. Afterwards the parthenogenetic pronucleus was removed from this cell by a selective enucleation. When the experimental embryos were cultured *in vitro*, 13.5% of them reached the blastocyst stage. Chromosome preparation using an air-dried method confirmed that blastocysts were the result of SCNT.

P.18

Catalase and superoxide dismutase expression in the chicken oviduct following tamoxifen treatment

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The imbalance between free reactive oxygen species (ROS) production and elimination leads to the damage of important biomolecules and cells. Previous studies in hens showed that treatment with tamoxifen (TMX; estrogen receptor blocker) modulates oxidative stress and causes the reproductive system regression. The present study aimed to examine the mRNA and protein abundances, as well as immunolocalize the key enzymatic antioxidants, i.e. catalase (CAT) and superoxide dismutase (SOD), in the laying hens' oviduct after TMX treatment. Birds were treated daily with TMX until a pause in egg-laying occurred in all hens and then euthanized (day 8 of the experiment). Quantitative real-time PCR and western blot analyses showed the presence of CAT and SOD transcripts and proteins, respectively, in all oviductal segments, i.e., the infundibulum, magnum, isthmus, shell gland and vagina. In laying hens (control), the mRNA expression of CAT was the highest in the shell gland, lower in the isthmus and the lowest in other oviductal parts, whereas protein abundance was the highest in the magnum, lower in the isthmus and the lowest in other segments. The SOD transcript and protein abundances only were lower in the magnum than in other segments.

Immunoreactivity for CAT and SOD proteins was present in all layers of the oviductal wall, but the intensity of staining depended on the cell type. TMX treatment changed CAT and SOD expression and the effect of TMX depended on gene, protein, cell type, and oviductal part. Generally, CAT abundance was elevated, while SOD abundance was reduced under TMX treatment. These results point to the significance of CAT and SOD in the maintenance of proper oviduct health and function. Changes in ROS scavenging enzymes after estrogen receptor blockage indicate the importance of estrogen in the regulation of oxidative status in the hen oviduct.

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P.19

Dietary supplementation with raspberry or strawberry seed oils impacts folliculogenesis, hormonal parameters and fatty acid profile in the juvenile rabbit ovary

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This study aimed at evaluating the effect of raspberry and strawberry seed oils, which are rich in polyunsaturated fatty acids, on the course of folliculogenesis, hormonal parameters and fatty acid profile in the rabbit ovary. Female Termond White rabbits at 35 days of age were randomly assigned into three groups (n=6/group): a control group (C), a group receiving a diet enriched with a 1% addition of raspberry seed oil (RO), and a group receiving a diet enriched with a 1% addition of strawberry seed oil (SO). All animals were fed ad libitum until 12 weeks of age and then were slaughtered. Ovaries were collected for histological evaluation and fatty acid profile analysis, whereas blood samples were centrifuged, and plasma was used for steroids (progesterone, testosterone, estradiol), follicle-stimulating hormone (FSH) and anti-Müllerian hormone (AMH) concentration analyses. The addition of both RO and SO resulted in decreased number of primary follicles ($p<0.05$, respectively) and increased number of antral follicles ($p<0.05$, respectively) in comparison to the C group. Following RO supplementation, the plasma progesterone and estradiol concentration was elevated ($p<0.001$, respectively), while the addition of SO increased only estradiol level ($p<0.001$). The plasma FSH level was higher ($p<0.05$, respectively) in both experimental groups, while the AMH concentration significantly decreased ($p<0.01$) following SO addition. Regarding fatty acid profile in the rabbit ovary, significant effect was observed mainly after SO supplementation, which led to increased content of lauric acid ($p=0.0097$), myristic acid ($p=0.005$), myristoleic acid ($p=0.005$), palmitoleic acid ($p=0.005$), and linoleic acid ($p=0.018$), and decreased content of stearic acid ($p=0.005$), arachidic acid ($p=0.051$), eicosapentaenoic acid ($p=0.051$), docosatetraenoic acid ($p=0.007$), and docosapentaenoic acid ($p=0.009$). In conclusion, both oils affected the follicle development in the rabbit ovary via regulation of related hormones level. Additionally, SO changed ovarian fatty acid profile that might affect folliculogenesis as well.

P.20

Characteristics of liquid-preserved semen collected from native Polish Crested Chickens

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Effective procedures for short-term storage of semen in a liquid state is of practical interest in the management of native chickens kept under biodiversity conservation programs. The aim of the study was to assess: 1) sperm motility using CASA; 2) sperm viability using Vybrant Apoptosis Assay Kit and LIVE/DEAD Sperm Viability Kit; and 3) sperm chromatin damage (DFI) using sperm chromatin structure assay (SCSA) of semen collected from native Polish Crested Chickens (PCr) in comparison to Hy-Line Brown (HLB) rooster. Semen samples were obtained using the abdominal massage technique (5 times, total of 30 samples/ genotype) and stored for 48 h at 5 ºC. The evaluation of sperm quality was performed at 0, 24 and 48 h of storage. At 0 h, PCr semen was characterized by proportion of total motility (MOT) and LiveSYBR-14+/PI- sperm greater than 93.0 %, while sperm with apoptotic-like changes YO-PRO-1+/PI- was 3.1 % and DFI-% was 4.8 %. It was found that proportion of sperm YO-PRO-1+/PI- , sperm with DNA fragmentation, deadSYBR-14-/PI+, and dyingSYBR-14+/PI+ were lower after storage of semen from PCr compared to HLB. Semen storage resulted in a gradual reduction in MOT and moderate changes in kinetic motility parameters in both genotypes. Compared to 0 h, after 24 h and 48 h, MOT decreased by 10.7 % and 24.9 %, respectively. Also, a gradual increase in DFI-% with storage time was noted. Compared to 0 h, after 24 h and 48 h, DFI-% increased by 5.5 % and by 17.4 % for PCr and by 10.5 % and 25.9 % for HLB. In summary, MOT, motility parameters, and DNA integrity were more affected than other sperm functions during storage of PCr semen. It can be suggested that PCr semen retains high fertility potential when stored in liquid for no longer than 24 hours.

P.21

Changes in cell proliferation and apoptosis and expression of connexin 43 in gander testes in relation to the annual period

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The mechanisms of seasonal alterations in the morphology, histoarchitecture and testicular function in birds are not fully elucidated. The aim of the present study was to determine the rate of cell proliferation and apoptosis and these processes-related gene expression (PCNA,

caspase-3), as well as abundance and localization of connexin 43 (Cx43) in gander testes during the annual cycle.

Gander testes (n = 28) were collected at 5 stages, i.e., prebreeding (PrB), peak of activity (PR), postbreeding (PoB), nonbreeding (NB), and onset of reactivation (OR). After evaluation of morphometry and testicular histology the following parameters were investigated: the number of proliferating (IHC) and apoptotic (TUNEL method) cells; mRNA (qRT-PCR) and protein (Western blot) expression of PCNA, caspase-3 and Cx43 (GJA1 gene); and localization (IF) of Cx43.

At the NB stage, there was a decrease in testicular weight and morphometry of the seminiferous tubules (STs) compared to the PR and OR stages. The number of proliferating cells was higher at PrB and PR than at the PoB stage. The number of apoptotic cells was higher at PoB and NB than at PrB and OR stages. Thus, cell proliferation-to-apoptosis ratios were lower at PoB and NB than in other stages. Moreover, at the last stages, the levels of PCNA mRNA transcript abundances were lower that at PrB and PR. The annual stage-dependent expression of the Cx43 gene as well as the localization pattern and distribution of Cx43 protein in STs were found.

During gander testis development, regression, and recrudescence, the interaction between processes of cell proliferation, apoptosis, and alterations in the localization of Cx43 protein in the germinal epithelium occurred. The balance between these processes may determine the occurrence of seasonal changes in the fertility of domestic ganders.

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P.22

Activity and immunolocalization of DNA-methyltransferases in porcine corpus luteum during early pregnancy and the estrous cycle

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Corpus luteum (CL) is a temporary endocrine gland secreting progesterone which is a key hormone for implantation of embryos and early pregnancy maintenance. Expression of genes involved in CL formation is regulated by different molecular mechanisms associated with RNA and protein molecules, epigenetic mechanisms, and signal transduction pathways. All of them can act at different times and sites during transcriptional or translational processes. However, the role of DNA methylation in controlling luteal gene expression in pigs has not been studied yet. We recently evidenced that expression of DNA methyltransferases (DNMTs) differs in porcine CL during the estrous cycle and pregnancy. The aim of the present study was to evaluate the activity of DNMTs and to localize DNMT1 protein in CL during the estrous cycle and pregnancy.

Porcine CLs were collected form gilts on day 12 of pregnancy and the estrous cycle (n=7 per group). The activity of DNMTs was determined in nuclear extracts from collected CLs by using commercially available kit. Immunolocalization of DNMT1 was performed by

immunohistochemistry in paraformaldehyde-fixed paraffin-embedded tissues of porcine corpora lutea collected on day 12 of the estrous cycle and pregnancy.

We found that DNMTs activity was significantly elevated in CLs collected from gilts on day 12 of pregnancy compared to CLs collected from gilts on day 12 of the estrous cycle ($p<0.05$). The expression of DNMT1 protein was localized in large and small luteal cells and in blood vessels of porcine corpora lutea.

Summarizing, increased activity of luteal DNMTs during early pregnancy suggest DNA methylation as a novel potential mechanisms of gene regulation in porcine corpus luteum during early pregnancy.

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P.23

Characteristic of porcine follicular fluid-derived extracellular vesicles following vitamin D3 and insulin *in vitro* treatment

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Extracellular vesicles (EVs) represent a heterogeneous population of membrane-bound nanoparticles, which are released by various cell types. Recent evidence highlights the crucial role of EVs in the intrafollicular communication via specific cargo (DNA, RNA, miRNA, proteins, lipids), determining follicle growth and functions. Given that vitamin D3 (VD) and insulin (I) are well-known regulators of ovarian processes, the question arises whether they are able (alone or in combination) to influence EVs release within porcine ovarian follicle. This study aimed to investigate the effect of VD and I on the follicular fluid-derived EVs morphology (transmission electron microscopy), concentration, size distribution, zeta potential (nanoparticle tracking analysis) and proteome cargo (liquid chromatography-tandem mass spectrometry coupled with the TMT-isobaric mass tag labeling). Whole porcine antral follicles (n=12/each group) were incubated *in vitro* for 12 hours with compounds (VD, I, VD+I) or without any treatment (C; control). Then, follicular fluid was collected for EVs isolation using size-exclusion chromatography. In all groups, EVs represent a typical cup-shaped vesicular morphology. In the I and VD+I groups, the average particle size decreased in comparison to the VD group ($p<0.05$), while EVs concentration was lower in the I group than in the VD and VD+I groups ($p<0.05$). Global proteomic analysis showed 3977 proteins in the EV samples. A comparative proteomic analysis (the cutoff value of 1.5-fold change and $q<0.05$; R package v4.2.3) revealed 19, 16, 8, 8 and 10 differentially abundant proteins (DAPs) between comparisons: C vs VD, C vs I, C vs VD+I, VD vs VD+I and I vs VD+I, respectively. Functional analysis of DAPs using KOBAS-i and STRING database (B-H FDR $p<0.05$) revealed that most of them are assessed to protein metabolism and ribosomes, indicating potential involvement of EVs in the basal cellular

processes, such as protein synthesis. VD and I could impact ovarian functions via EVs-mediated pathway.

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P.24

The role of LATS1/2 kinases in the first cell fate decision in preimplantation rabbit embryo development

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The initial cell fate determination in mammalian embryogenesis is a pivotal process that delineates the segregation of cells into the trophectoderm (TE) and the inner cell mass (ICM). This decision is regulated by specific signalling pathways and transcription factors, with the Hippo pathway playing a central role. LATS1/2 kinases, as essential components of the Hippo pathway, regulate the phosphorylation and localization of YAP/TAZ transcriptional co-activators. In outer cells destined to form the TE, reduced Hippo pathway activity results in diminished LATS1/2 kinase activity, allowing YAP/TAZ to translocate into the nucleus, where they interact with TEAD4 to induce expression of TE-specific genes such as CDX2 and GATA3. Conversely, in inner cells, active Hippo signalling activates LATS1/2 kinases, leading to the phosphorylation of YAP/TAZ, retaining them in the cytoplasm, and preventing TEAD4 activation, thus supporting ICM-cell fate. Such mechanism has been shown to be active in several mammalian species.

To further analyse the role of LATS1/2 in early mammalian development, we conducted experiments on preimplantation rabbit embryos. Two-cell stage embryos (E1.0) were collected and cultured for 96 hours *in vitro*. The control group was cultured in RDH +BSA medium, while the experimental groups were cultured in RDH + BSA medium with LATS and TEAD inhibitors (10 µM: LATS-IN-1, MGH-CP1, TED-347). Immunostaining was conducted using markers for trophectoderm (GATA3), ICM (SOX2) and active YAP. Quantitative analysis revealed a significant decrease in the number of SOX2-positive cells (epiblast) and an increase in GATA3-positive cells (TE) in the experimental groups compared to the control.

Our results suggest that in the rabbit embryo, inhibition of LATS1/2 kinases skews cell fate towards the TE lineage at the expense of the ICM, highlighting the crucial role of LATS1/2 in early embryonic development and cell fate decisions.

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P.25

Can carbon monoxide (CO) as a humoral light signal affect HIF-1α levels in the preoptic area of the hypothalamus of the domestic pig?

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Carbon monoxide infused into the angular vein of the eye in the ocular sinus influences the regulation of biological clock genes by modulating the activity of the clock transcription factors BMAL1:CLOCK/NPAS2 in the hypothalamus of the domestic pig. HIF-1α is a transcription factor activated under hypoxia and its expression follows circadian rhythms. The presence of E-box sequences in the 5'-UTR of the HIF-1α gene, which are recognised by BMAL1:CLOCK/NPAS2, led us to speculate that CO may modulate HIF-1α expression in the hypothalamus via a humoral pathway. We used tissues from the hypothalamic preoptic area (pa) of three groups of pigs: N-animals kept in 12h light/12h dark; K-animals kept in the dark plus autologous plasma infusion with physiological CO concentration; CO-animals kept in the dark plus infusion of autologous plasma with increased concentration of CO. The animals were slaughtered: at 12 o'clock (middle of the subjective day) and 24 o'clock (middle of the subjective night). mRNA expression levels were determined by qPCR while protein levels by Western Blotting. Significantly lower ($p \leq 0.01$) mRNA levels were shown in hypothalamic tissue at night in the N and CO groups. There were no differences in mRNA levels in the K group (darkness plus infusion of autologous plasma with physiological CO concentration). Protein levels showed inverse correlations in groups N and CO with higher protein levels in hypothalamic tissue obtained at night and in group K statistically higher ($p \leq 0.001$) protein levels in hypothalamic tissue obtained at night compared to tissue obtained during the day. Conclusions: 1.HIF-1α can be regulated by both neuronal (light) and humoral (CO) pathways. 2.CO modulates HIF-1α expression in the hypothalamus through mechanisms related to the biological clock. 3.Differences in mRNA and protein levels in the same groups may indicate an effect of CO on translation efficiency.

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P.26

The regression of trophectodermal protrusions from the surface of the inner cell mass of the mouse blastocyst correlates with the formation of primitive endoderm

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This study aimed to ascertain the presence of trophectoderm protrusions covering the surface of the inner cell mass at various stages of blastocyst development. Experiments were conducted on E3.5 and E4.5 mouse embryos to investigate whether trophectoderm

protrusions could be detected using latex beads labeled with fluorescein. Subsequently, it was assessed whether the disappearance of trophectoderm protrusions covering the inner cell mass is correlated with the formation of the primitive endoderm layer in the blastocyst.

The utilization of latex beads labeled with fluorescein proved effective in detecting trophectoderm cells. In this study, latex beads were endocytosed by the outer embryo cells, enabling the observation of their protrusions through confocal microscopy.

Throughout the study, a correlation was observed between the number of blastocyst cells and the extent of coverage of the inner cell mass by trophectoderm protrusions. As the blastocyst developed, the trophectoderm protrusions covering the inner cell mass diminished. No protrusions were observed in E4.5, where the cells in the inner cell mass were sorted, and a layer of primitive endoderm cells formed on the inner cell mass surface.

P.27

The phenotype, sex ratio and gonadal development in triploid rainbow trout (*Oncorhynchus mykiss*) x brook trout (*Salvelinus fontinalis*) hybrids

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Viral haemorrhagic septicaemia (VHS) and infectious hematopoietic necrosis (IHN) are currently widespread around the world, posing significant challenge to rainbow trout farms, as there are no effective vaccines or medications available. Extensive research on the salmonids have revealed that certain charr species (*Salvelinus sp*.) exhibit resistance to mentioned infections. Unfortunately, the aquaculture production of charrs is less profitable than that of rainbow trout (*Oncorhynchus mykiss*). Recently, crosses between rainbow trout and brook trout (*Salvelinus fontinalis*) have garnered attention as a promising avenue for transferring charr's resistance to VHS and INH. However, each newly induced interspecific fish crosses should be carefully evaluated to determine the best phenotypes and genotypes before commencing commercial production. In the present study, the phenotype, genetic sex ratio and gonadal development characteristics were carried out in juvenile (15 months old), sub-adult (22 months old) and adult (30 months old) triploid crosses between mentioned species. The obtained results revealed the presence of three distinctive phenotypes with the following frequencies: f=50.8% for Phenotype 1, f=31.7% for Phenotype 2 and f=17.5% for Phenotype 1/2. A significant skew in genetic sex ratio was also recorded, with 33.3% females and 66.7% males among the examined hybrids. Genetic males were exclusively detected among individuals bearing Phenotype 1, which developed macroscopically visible and histologically functional testes in the second year of life. In turn, a comparable proportion of male and female individuals were observed within individuals carrying Phenotypes 2 and 1/2, among which males developed macroscopically distinguishable and histologically functional testes in the third year of life. In the case of females underdeveloped intersex or completely reduced gonads were recorded. Our study revealed that individuals bearing Phenotype 2 represents the greatest value for the

commercial production because of their unique salmon-like appearance and late maturation after reaching market size (males) or complete sterility (females).

P.28

Importance of miRNA (miR-21, miR-34a, miR-132 and miR-503) in process of progesterone synthesis in granulosa cells of the porcine preovulatory follicle

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In the pig and other species progesterone is a key steroid hormone in the regulation of many reproductive processes. The process of steroidogenesis, where the synthesis of progesterone takes place, is very complicated. Several studies and our earlier results suggested that short, non-coding miRNAs, involved in the negative regulation of gene expression, participate in regulation of steroidogenesis. The aim of the study was to determine the effect of selected miRNAs in expression of genes involved in progesterone production in granulosa cells (GC) on *in vitro* model. After transfected with miRNA, GC were treated with luteinizing hormone (LH; 24, 48, 72 hours) to stimulate expression of enzymes and factors regulating steroidogenesis. The experiment was performed on GC obtained from preovulatory follicles (<6 mm), collected from mature gilts (n=8).

The *in vitro* experiment performed on GC revealed that concentration of progesterone was higher in medium with GC transfected with miR-21, miR-34, miR-132, miR-503 ($p<0.05$) after 48- and 72-hours incubation with LH than in medium with GC without transfection. We observed an increasing expression of CYP11A1 and STAR mRNAs in GC transfected with miR-21, miR-34, miR-132, miR-503, as well as increasing protein expression of HSD3B1, STAR and CYP11A1 in GC transfected with miR-21, miR-34, miR-132 after 48 hours. Also, increased mRNA/protein expression of CYP11A1, HSD3B1, STAR in GC transfected with miR-21, miR-34, miR-132 after 72 hours were documented. Furthermore, mRNA/protein level of transcription factors of ovarian steroidogenesis (CREB1 and ATF4) were increased in GC transfected with miR-21, miR-34, miR-132 and miR-503 after 72 hours. There were no changes in the expression of analyzed transcript or protein in 24 hours incubation.

In conclusion, presented data suggest that miR-21, miR-34, miR-132 and miR-503 may play an important role in mechanisms of progesterone production during steroidogenesis.

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P.29

The interplay between anti-Müllerian hormone and mir-181a in uterine leiomyoma

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The pathogenesis of uterine leiomyoma (UL) involves epigenetic processes which modulate gene expression by micro-ribonucleic acid (miRNA) variation, such as miR-181. miRNA-targeted pathways are likely to regulate genes by involving multiple growth factors. The anti-Müllerian hormone (AMH) is a potential suppressor in benign Müllerian duct-derived conditions such as endometriosis, adenomyosis and UL. Very few studies have explored the link between AMH and miR-181, none of which were conducted on UL. Hence, we investigated the effects of AMH on miR-181 and proliferation rates in UL. Both AMH and its receptor, AMHRII, were overexpressed in UL compared to normal myometrium. miR-181a was also overexpressed and increased even more in the presence of AMH. Their interaction led to a decrease in UL proliferation; this, combined with mRNA levels of SMAD1, as well as PI3K suggests the involvement of two other signaling pathways. Decoding these molecular mechanisms will be of tremendous value for creating targeted therapeutic strategies for UL, as no safe, effective medication has been developed yet.

P.30

Effects of *Lepidium meyenii* supplementation in diets on lipid content and mRNA expression in oocytes of prepubertal gilts

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Peruvian maca (*Lepidium meyenii*) is a plant used in human dietary as a supplement to enhance fertility and support the treatment of respiratory disorders, rheumatism and anemia. Few studies also shown that supplementation of maca positively impact mouse and human sperm semen parameters, enhancing the acrosomal reaction and motility primarily due to the content of macamides - bioactive plant metabolites found in it. Limited research explores the impact of maca supplementation on female reproductive parameters and oocyte developmental potential. Therefore, this study aimed to analyze porcine oocyte cytoplasmic and molecular maturation parameters in response to maca added to a feed of growing pigs. .

Cumulus-oocyte complexes (COCs) and follicular fluid (FF) were obtained from prepubertal gilts divided into control (fed standard diet, n=10) and experimental (fed standard diet with addition of 1% powdered maca roots, n=10) groups after 108 days of feeding. The relative transcript

levels of genes involved in folliculogenesis (GDF9), fatty acid metabolism (ACACA, SCD, PNPLA2, SREBF1, GSTA2), free radical metabolism and antioxidant stress (SOD1 gene) were analyzed in oocytes using qPCR along with lipid droplet (LD) content measurements (confocal microscopy). Fatty acid profile was analysed by gas chromatography in follicular fluid.

The analysis of total 544 oocytes revealed significant increase in lipid droplet content in experimental oocytes compared to control (568 vs 284 respectively; $p<0.01$) alongside with significantly smaller LD size indicating biogenesis. Similarly, we found increased fluorescence level of LD in cumulus cells in maca group ($p<0.01$) which may indicate lipid storage. No significant differences between studied groups were found in fatty acid profiles in FF nor in the oocyte gene expression levels of the studied genes. This research provides insights into diet induced lipid storage in oocytes however the potential role of maca action needs to be elucidated.

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P.31

Application of FISH technique with centromeric probes for rapid karyotype assessment in Bovidae embryos and stem cell studies - a practical approach

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Chromosome stability is fundamental for proper embryonic development. Aneuploidies are the main cause of developmental arrest and concomitant decline in *in vitro* embryo production (IVP), embryo implantation, early miscarriages and congenital anomalies. Except for human, cattle is the only organism for which IVP is done on an a large scale. Due to numerous similarities in preimplantation development between cattle and human, bovine embryos are considered to be a relevant model to study early mammalian development. Despite many years of research, the IVP conditions still remain suboptimal. Having a quick, and efficient method to verify chromosomal stability may prove beneficial both to embryologists and researchers. Pluripotent stem cells derived from embryos (ESC) and reprogrammed somatic cells (iPSC) of large animals are a valuable tool for biomedical research. Prolonged exposure to various chemical compounds that alter the activity of signalling pathways may have a negative effect on maintaining genomic stability in cells. Chromosomal aberrations have been systematically described in ESC and iPS cells. Therefore, we have designed a rapid fluorescence in-situ hybridisation (FISH) method based on a sub-centromeric probe (Cot1-DNA) specific to bovine autosomes. This probe may be used on both interphase and metaphase chromosomes, allowing to omit metaphase-arrest step in FISH-specimen preparation. The designed probes were tested on bovine (Bos taurus) metaphase oocytes, preimplantation embryos (blastocysts) and primary wisent (Bison bonasus bonasus)

fibroblasts and iPS lines derived from adult fibroblasts (both species share 60XY karyotype). As a control, to verify the origin of the iPS lines derived in dual-species culture systems (on mouse embryonic fibroblasts, MEFs), mouse Cot1-FISH probe was also developed. Our results showed that that the designed probes are applicable for determining the karyotype stability and iPS origin during co-culture of wisent iPS with MEFs. Moreover, these probes may be successfully applied to monitor embryonic chromosomal abnormalities.

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P.32

Orotic acid: a potential modulator of mitochondrial function in HGrC1 cells

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Introduction: Orotic acid (OA) is a natural product found in sheep's milk that acts as a precursor in the pyrimidine nucleotide biosynthesis pathway. The majority of articles on the administration of OA and its salts focus on its therapeutic benefits, but its effects on the female reproductive system are not clearly defined. Our previous study suggested that mitochondria are a target of OA action.

Methods: In our study, we determined the effect of orotic acid on the human non-luteinised HGrC1 cell line. Cells were grown in two-dimensional monolayer culture and exposed to OA at a dose of 100 nM for 24 hours. The energetic phenotype and ATP production from glycolysis and mitochondria simultaneously was performed using a Seahorse XF analyser (Agilent). Mitochondrial activity was measured using JC-1 stain and visualised by fluorescence microscopy.

Results: Our study found that OA increased mitochondrial activity in a human ovarian granulosa cell line. Interestingly, 24 hours of treatment with OA resulted in increased basal oxygen consumption and mitoATP production, leading to the dominance of oxidative phosphorylation as the main process of ATP production.

Conclusions: In conclusion, orotic acid treatment induced aerobic cell metabolism and mitochondrial activity. The acceleration of mitochondrial activity, resulting in better cell condition, may be beneficial and may be used in the design of future therapeutics.

P.33

Central effect of kynurenic acid on the expression of GnRH-LH/FSH axis genes in sheep

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Kynurenic acid (KYNA) is one of the highly neuroactive products formed in the central nervous system (CNS) and various peripheral tissues during the enzymatic transformation of tryptophan in the kynurenine metabolic pathway. The aim of this study was to investigate whether KYNA, acting centrally, could affect the secretory activity of the hypothalamic-pituitary GnRH-LH/FSH axis in sheep. Initially, the effect of KYNA on the expression of GnRH gene in the mediobasal hypothalamus (MBH) and preoptic area (POA), as well as LHβ, FSHβ and GnRHR genes in the anterior pituitary (AP) was examined. The anestrous sheep (n=18), implanted with stainless steel guide cannula into the third brain ventricle (IIIv) were divided into 3 groups and treated with: i. intracerebroventricular (icv.) infusion of Ringer-Locke solution for 3 days; ii. icv. infusion of the lower dose of KYNA (4×5 µg/60 µL/30 min) for 3 days; and iii. icv. infusion of the higher dose of KYNA (4×25 µg/60 µL/30 min) for 3 days. After the last infusion, the animals were euthanized and the selected structures were dissected from the brain to determine the gene expression. A significant increase ($P<0.05$-$P<0.001$) in the relative expression of GnRH mRNA was observed in the MBH after the infusion of both doses of KYNA, but in the POA the most effective was only the higher dose ($P<0.001$), as compared to the control. In the AP, both doses of KYNA increased ($P<0.05$-$P<0.001$) the expression of LHβ mRNA, but only the higher dose was effective for FSHβ mRNA ($P<0.001$). In the case of GnRHR mRNA, the lower dose of KYNA reduced the expression ($P<0.01$), while the higher one caused a significant increase ($P<0.05$). In conclusion, KYNA, a kynurenine pathway metabolite, has the stimulatory effect on the GnRH-LH/FSH axis in sheep, at least at the gene expression level.

P.34

Are phototextural characteristics of ovine presumptive zygotes indicative of their subsequent developmental potential *in vitro*?

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In vitro embryo production (IVP) has widespread implications for animal husbandry and conservation programs. The goal of achieving pregnancy with IVP systems calls for non-invasive and accurate methods of embryo selection. The present study set out to examine whether phototextural attributes of ovine presumptive zygotes were reliable markers of embryo viability and developmental potential *in vitro*. Sheep oocytes were obtained after slaughter from nine cycling Polish Longwool ewes by ovarian scarification. Following *in vitro* maturation and

fertilization of oocytes with frozen-thawed ram semen, the development of embryos (n=37) to the blastocyst stage was monitored with time-lapse imaging technology. Ovine embryos were retrospectively classified as non-arresting (developing to the blastocyst stage; n=6) and arresting (embryos that did not divide or arrested before the 8th mitotic division; n=31). Commercially available image analytical software (ImageProPlus®) was used to generate bitmaps of the regions of interest encapsulating zygotic cytoplasm. Subsequently, a proprietary in-house developed computer algorithm (r-Algo) was employed to determine if there were clusters of pixels for which first-order phototextural characteristics of presumptive zygotes (i.e., mean pixel intensity (MPI), heterogeneity (MPH), and concentration (MPC)) differed significantly between the two subsets of embryos. Algorithmic analysis of presumptive zygote microphotographs has revealed the existence of specific pixel ranges for which statistically distinctive (P<0.004) phototextural properties existed between future arresting and non-arresting ovine embryos (MPI: pixel range of 73-75; MPH: 36-42; and MPC: 105-106). Computerized sequestration of ovine presumptive zygote images is a promising non-invasive method of predicting the developmental potential of *in vitro*-derived sheep embryos.

P.35

Assessment of Tebuconazole's *in vitro* impact on chicken sperm functions

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Introduction: Tebuconazole (TEB) is a widely used triazole fungicide frequently detected as pesticide residues in grains. This experiment aimed to examine how different concentrations of TEB affect rooster sperm motility parameters and their characteristics *in vitro*.

Materials and Methods: The experiment involved 10 Greenlegged Partridge roosters. Semen was collected twice a week via dorso-abdominal massage, then pooled. Different TEB concentrations (0, 0.1, 1, 10, 100 µM) were added and incubated for 1 and 3 hours at 37°C. Sperm motility parameters were assessed using the CASA system, measuring percentage of motile spermatozoa (MOT), percentage of progressively motile spermatozoa (PMOT), velocity along the path (VAP), progressive velocity (VSL), and velocity in a curvilinear line (VCL). Flow cytometry analysis measured sperm plasma membrane integrity using SYBR-14 and propidium iodide (PI), mitochondrial membrane potential with JC-1, acrosome integrity using lectin PNA Alexa Fluor 488 conjugate, and early apoptosis with YO-PRO1. Active caspases in apoptotic cells were detected using Caspase 3/7 Green Ready, intracellular calcium levels with Fluo-3 AM, and lipid peroxidation with C11-BODIPY581/591.

Results: TEB exposure led to a significant decrease (P<0.05) in cells with high Ca2+ levels and a decrease (P<0.01) in lipid peroxidation. Lectin staining showed the control samples had the highest (P<0.01) number of cells with damaged acrosomes. JC-1 staining indicated reduced (P<0.01) mitochondrial potential in TEB-treated samples. Regarding early apoptosis, the lowest and highest TEB doses maintained the same level of apoptotic cells as the control, while the intermediate dose reduced the number of apoptotic cells. TEB did not significantly (P>0.01) affect sperm viability, motility parameters, and caspase activity.

Conclusions: Tebuconazole may impact sperm function-related processes such as calcium homeostasis, lipid peroxidation, acrosome integrity, and mitochondrial function without significantly affecting viability and motility.

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P.36

Searching for genomic variants and methylation level alterations in sperm DNA of infertile brothers with oligoasthenoteratozoospermia

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Infertility affects approximately 10-18% of couples at reproductive age, with about 50% of all the cases originated from the male side. An aetiology of infertility includes hormonal disruptions, environmental hazard factors, and genetic factors. Genetic background determines ~10-15% of reproductive fertility cases. But diagnosis for ~20-25% of infertile males still remains idiopathic. Recent years have shown an increasing role of epigenetic background in proper gametogenesis or fertilization process (incl. imprinting or developmental genes). Thus, it seems to be reasonable to investigate both: genomic and epigenomic context of infertility.

An aim of our study was to find the genetic variants and altered methylation level regions causative for observed decreased sperm quality in two familial cases of infertile brothers with decreased sperm concentration and similar morphology abnormalities of the sperm head and acrosome.

Blood DNA samples from members of two families (F-I: 4 brothers, F-II: 3 brothers, father) were sequenced (whole genome sequencing WGS, IlluminaNovaSeq6000). Sperm DNA samples were evaluated by whole genome methylome sequencing (WGMS, Illumina Infinium_MethylationEPICBeadChip_850k_array). Immunofluorescence for candidate genes was applied (Leica DM5500, LASX software). Characteristics of semen quality, incl. chromatin integrity tests were performed: TUNEL, aniline blue, acridine orange stainings.

We have found genomic variants in a.o: ADCY10, DDX34, DDX3X, TCTN1, PABPC3, LENG9, USP8, while changed methylation pattern was documented for: SLC35F2, ATF3, GYS2, TBCD and VOPP1 genes. Revealed genes are known as involved in gametogenesis, proper cilia functioning or related to processes linked with reproduction and can suggest the observed sperm phenotypes.

This study emphasizes the strong need for genomic and epigenomic surveys in patients with severely decreased sperm quality. Particular sperm parameters (microscopically visible as identical) can reveal various genomic background. Thus, collating of WGS and WGMS should be reasonable to get more detailed diagnostics of observed abnormalities.

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P.37

The possible effect of phoenixin on the pituitary gonadotrophic cells' secretory activity in sheep. Preliminary results

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Phoenixin (PNX) is a highly conserved neuropeptide throughout vertebrate species, existing in two active molecular forms, PNX-14 and PNX-20, and exhibits the same biological activities in various structures, including the pituitary gland. PNX interacts with the GPR173 receptor, expressed in, among others, pituitary and ovary, suggesting a link between PNX and the gonadotrophic axis.

The aim of this study was to determine whether PNX modulates the secretory activity of pituitary gonadotrophic cells in sexually mature sheep.

The experiment was conducted on sexually mature Old-type Polish Merino sheep (n=16). All of experimental animals had stainless steel cannulas implanted directly into the IIIv of the brain. After convalescence and synchronisation of oestrus, the sheep were divided into 3 groups. The following intracerebroventricular infusions were conducted: control group received Ringer-Locke solution in a dose of 480µl/day, group I and group II received PNX in a dose of 10 and 80µg/480µl/day, respectively. On the day 3 of the infusion blood samples has been collected from the animals (from 08:00 a.m. to 02.00 p.m.). Immediately after the experiment all sheep were euthanized, pituitaries and plasma samples were stored for Real-Time RT qPCR, histological and radioimmunoassay analysis.

Quantitative analysis of the images demonstrating immunoreactive LH material shows that in group I the percentage of area exhibiting positive LH staining in the pituitary decreased in comparison with control group, however, in group II the results were the opposite, resulting in increased staining.

Based on the results obtained, it can be concluded that PNX can modulate the secretory activity of pituitary gonadotrophic cells in sheep.

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P.38

Characterizing healthy and pathological microbiomes in the canine reproductive tract using next-generation sequencing

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Inflammatory conditions of the female reproductive tract of bacterial etiology are common in veterinary practice. The microbiome composition in the reproductive tract of bitches depends on many factors, yet differences between physiological and pathological flora remain unclear. Conventional culture protocols reveal only 1-10% of the actual microbiota. Next-Generation Sequencing (NGS) based on 16S rRNA gene sequencing offers precise bacterial identification, aiding in classification and phylogenetic analysis. This study aims to reveal the composition of vaginal and uterine microbiomes in healthy bitches and those with pathological changes using NGS and to define bacterial taxa associated with pathologies.

Swabs were collected from the vagina and uterus of 16 bitches (7 pathological, 9 healthy), aged 1.5 to 15 years. Vaginal swabs were obtained before ovariohysterectomy (OVH), while uterine swabs were taken immediately after OVH. Microbiota DNA was isolated, and the V3-V4 regions of the 16S gene were amplified and sequenced using MiSeq. Bioinformatic and phylogenetic analyses were performed using the kraken2 package. Statistical analyses were conducted using R.

Between 263 to 511 and 316 to 524 bacterial species were identified in the vagina and uterus, respectively. These bacteria were categorized into four phyla in the vagina (*Proteobacteria, Bacteroidetes, Firmicutes, Tenericutes)* and three in the uterus (*Proteobacteria, Bacteroidetes, Firmicutes*). Healthy bitches showed higher bacterial abundance in the uterus and greater species diversity in the vagina. Pathological vaginal microbiomes exhibited significant quantitative diversity. Uterine microbiomes consistently differed from vaginal microbiomes with significant inter-individual variability. Three marker families for the pathological status were identified (*Pasteurellaceae, Campylobacteraceae, Bacteroidaceae*).

NGS-based microbiome studies present novel avenues for diagnosing reproductive tract infections, facilitating early detection and treatment monitoring. Further research is essential to pinpoint bacterial strains indicative of pathological conditions and those with potential probiotic properties.

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P.39

Unsymmetrical segregation of lipid stores and mRNAs during first cleavage of porcine parthenogenetic embryos revealed by single cell RNA-seq and fluorescence staining

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The very first stages of mammalian embryonic development are governed by transcripts and proteins of maternal origin. At strictly species-specific time, following a certain number of mitotic divisions, embryonic genome activation occurs to take control over further development. Two models are proposed for segregation of transcripts, proteins and organelles during first cell divisions in early embryos, the “partitioning errors” and “transcriptional noise”. Based on our previous observations on lipid content in preimplantation embryos we aimed to analyse the differences in the number of lipid droplets and transcriptional profile of daughter blastomeres during first cleavage.

Porcine cumulus oocyte-complexes (COCs) were matured *in vitro* and activated with ionomycin (5uM) followed by 6DMAP (2mM) treatment and cultured using Primovision time laps system to monitor timing of first cleavage. The 2-cell stage embryos were fixed and stained with Bodipy 493/503 to visualize lipid droplets (confocal microscopy). In parallel embryos were disaggregated and single blastomeres used for transcriptomic scRNA-seq analyses. RNA was isolated using Smarter Ultra Low Input RNA Sequencing kit for Illumina (Clontech) followed by cDNA synthesis, enrichment and library prep using Nextera XT (Illumina). RNA-Seq was performed using Novaseq platform (Illumina).

No difference was found in LD number between cleaved and uncleaved embryos. However, only 25% of embryos showed similar distribution of lipid droplets in 2-cell stage. Blastomeres differed in LD content between in range from 16,7% to 561,7%. scRNA-seq revealed on average 14126 aligned transcripts in single cells. All embryos showed differentially expressed genes (from 96 to 489) between daughter blastomeres. Top enriched pathways include meiosis, proteolysis, phosphorylation, mitochondrial respiratory chain, embryo development, mTOR signaling and cell-cell junction development but remain distinct between the embryos. These data show uneven distribution of oocyte storage materials during first cleavage after activation which may influence cell fates during subsequent mitotic divisions.

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P.40

Expression of omentin-1 in porcine corpus luteum and its *in vitro* effect on luteal cells proliferation, apoptosis and progesterone secretion

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The corpus luteum (CL) is a transient endocrine gland that plays a central role in regulating the reproductive cycle. Excess adipose tissue can affect the proliferation/apoptosis ratio and progesterone (P4) synthesis in the body through the secretion of adipokines, including omentin-1 (ITLN1), whose function is maintaining the body's energy homeostasis. Moreover, our previous study noted expression of ITLN1 in porcine ovarian follicles and its action on the granulosa cell's function. Therefore, this study aimed to determine the expression of ITLN1 in porcine CL and its effect on luteal cells functions: proliferation, apoptosis and P4 secretion.

To assess the mRNA and protein expression of ITLN1 as well as immunolocalization, (PCR, Western blot, immunohistochemistry) the CL was collected from Large White pigs on days 2-3, 10-12, and 14-16 of the estrous cycle. To determine the *in vitro* effect of ITLN1 on insulin receptor (INSR) levels (ELISA); viability/proliferation/apoptosis (AlamarBlue, BrdU, caspase-3/7 activity assays, the levels of proliferating cell nuclear antigen- PCNA and caspase-3 via PCR, ELISA); as well as P4 secretion to culture medium (ELISA), the luteal cells from 10-12 days of the estrous cycle were cultured with ITLN1 (10-100 ng/mL) by 24 h. Statistical analysis was performed using one-way analysis of variance ($p < 0.05$, $n = 5$).

We showed that ITLN1 is expressed in CL, and its level (gene and protein) increased with the progression of the estrous cycle in pigs. What is more, ITLN1 modulates (enhanced/had no impact/reduced) the INSR levels as well as the viability, proliferation, caspases activity, the levels of PCNA, caspase-3 and P4 secretion in the porcine CL cells.

In conclusion, we found that INTL1 expression depends on the luteal phase stage and affects luteal cells functions, indicating that ITLN1 is a new regulator of porcine reproduction.

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P.41

The neuroendocrine regulation of reproduction, the role of QRFP43

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43RF-amide (QRFP43) is one of the RF-peptides regulatory peptides family and its expression has been demonstrated in all vertebrate groups. C-terminal sequence of this peptide, which is responsible for its biological activity, remained unchanged in the course of evolution. Such kind of phenomenon is characteristic for the compounds performing the key vital functions in the organism. The aim of this study was to verify the research hypothesis, which assume that QRFP43 can modulate gonadotrophic axis activity in sheep.

The experiment was performed on sexually mature Polish Merino sheep (n=48). In all experimental animals, oestrus synchronisation was performed 21 days before intracerebroventricular infusion. Animals were divided into 3 groups and the following types of infusion into the third ventricle of the brain was performed:

1. Ringer-Locke solution 480µl/day (control group)
2. QRFP43 in dose 10µg/480µl/day (RFa10 group)
3. QRFP43in dose 50µg/480µl/day (RFa50 group)

Blood samples has been collected from animals on Day 3 of infusion (from 08:00 a.m. to 02.00 p.m.). Immediately after the experiment all sheep were slaughtered, and selected structures of the hypothalamus, pituitaries and plasma samples were stored for Real Time RT qPCR, histological and radioimmunoassay analysis.

QRFP43 was found to downregulate *Kiss* mRNA expression in the MBH and reduce the level of IR material in ME. This resulted in a reduction of GnRH IR material in the ME. QRFP43 increased plasma FSH levels while decreasing LH levels.

On the basis of obtained results, it can be concluded that QRFP43 may modulate the activity of the gonadotrophic axis activity at the level of the hypothalamus and may represent another neuromodulator of reproductive processes in animals.

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P.42

The impact of omentin-1 on the levels of gonadotropin and selected adipokines in anterior pituitary cells. Studies on normal-weight Large White and fat Meishan pigs

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Background: Omentin-1 is an adipokine, a protein produced by adipose tissue, which plays a crucial role in regulating numerous metabolic and inflammatory processes. Literature data indicate its significant role in reproduction, however, its role in neurohormonal processes remains unclear. Our previous study identified omentin-1 gene and protein expression in porcine anterior pituitary (AP) cells. This study aims to evaluate omentin-1's effect on the expression and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as on selected adipokines (adiponectin, leptin, chemerin, resistin, vaspin, visfatin, apelin) and their receptors in Large White pigs with normal body weight and Meishan pigs, a model for obesity research.

Methods: In the experiments, a real-time PCR technique was utilized to assess the gene expression levels of LH and FSH, as well as the examined adipokines, in porcine AP cells. Additionally, ELISA assays were used to measure their secretion in the culture medium. The statistical analysis employed two-way ANOVA, followed by Tukey's test ($n=5$; $p < 0.05$).

Results: Results showed that omentin-1 significantly modulated LH and FSH secretion at a dose of 50 ng/mL in Large White pigs. Omentin-1 at doses 10 and 50 ng/mL reduced adiponectin secretion in Meishan pigs but stimulated AdipoR1 expression in Large White pigs. A breed-dependent effect on leptin secretion was noted at a dose of 100 ng/mL. Omentin-1 also influenced the expression of the chemerin receptor (CMKLR1), apelin, and visfatin, depending on the breed.

Conclusion: Differences in hormonal and metabolic responses between breeds suggest that omentin-1 may significantly regulate AP cells and metabolic processes, potentially improving health and reproductive efficiency in pig farming. Further research will explore the regulatory molecular mechanisms of omentin-1 in AP cells.

P.43

The DDX53 (CAGE) as potential regulator of RNA metabolism in human spermatogenesis

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Male infertility has become a global health concern, but the genetic factors that contribute to spermatozoa differentiation still remain to be discovered. RNA metabolism plays a crucial role in the precise regulation of sophisticated gene expression patterns, required for the successful development of male gametes during spermatogenesis.

Recent studies using WGS and WES screenings performed on samples collected from nonobstructive azoospermia (NOA) patients revealed a novel possibly associated with spermatogenesis failure gene DDX53 [1,2]. The DDX53 (DEAD-box helicase 53, also known as CAGE) is an intronless gene located on the X chromosome and is predominantly expressed in the subset of germ cells in human testis. Although other studies investigated DDX53 protein function in the context of various cancer types where its expression is also detectable, our project focuses on the role of DDX53 in human spermatogenesis. We examined DDX53 function and targets using the human seminoma cell line (TCam-2) as an *in vitro* male germline model. Our eCLIP and RNA-seq data show that DDX53 protein binds directly a wide pool of RNA targets, drives transcriptome changes in human cells, and also is potentially involved in alternative splicing of RNA transcripts. Further, we investigated the localization of DDX53 protein using Western Blot, immunofluorescence staining, and confocal microscopy, revealing that it is mainly located in the cytoplasm, but is also present in the nucleus of TCam-2 cells.

Herein we present potential DDX53 RNA targets and protein interactors (detected by using Co-IP-MS) and show that this protein acts as an important regulator of RNA metabolism in male germ cells.

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P.44

Effect of melatonin on early neurulation by using human embryonic stem cells

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Neurulation is a crucial process in early human development, where the neural plate forms the neural tube, which later becomes the brain and spinal cord. Melatonin, a hormone primarily produced by the pineal gland, is known for regulating sleep-wake cycles. Emerging research suggests melatonin also plays a significant role in early neural development, particularly through maternal contributions. This study aimed to examine the effect of melatonin supplementation on early human neurulation, specifically the neural tube formation process, using human embryonic stem cells (hESCs) as a model.

Melatonin receptor (MT) expression in hESCs was examined by immunocytochemistry before induced differentiation. Neural tube differentiation was initiated by cultivating hESC clumps in a medium with or without melatonin (10 µM) supplementation for seven days. Subsequently, cells were plated on dishes for neural tube evaluation under phase-contrast microscopy. Gene expression was analyzed via RT-PCR, and protein expression was assessed through immunocytochemistry. Statistical analysis, utilizing analysis of variance (ANOVA), examined the neural tube formation rate and gene expression.

The results indicated that hESCs expressed MT1 and MT2. Neural tube characteristics, including a radiated and elongated arrangement of cells, were observed in all groups. The number of neural tubes in the melatonin-treated group (85±6.5) was significantly higher than in the non-treated groups (35±4.8) ($P < 0.05$). Gene expression analysis revealed a decreasing trend in OCT4 and NANOG in melatonin-treated cells ($P < 0.05$), while the expression of SOX1, SOX2, PAX6, and Nestin in melatonin-treated cells was significantly higher than in the non-treated groups ($P < 0.05$). Additionally, immunocytochemistry results showed that OCT4, PAX6, SOX1, SOX2, Nestin, N-cadherin, and E-cadherin were detected in both melatonin-treated and non-treated groups.

The presence of melatonin in the neural tube differentiation medium affected the morphology of the neural tube and early neural-specific gene expression of hESCs.

P.45

Visfatin as a regulator of ovarian follicles functioning in pigs

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Visfatin (VIS) is classified as a coenzyme involved in cellular metabolism and an adipokine. Research also suggests that VIS may be involved in the regulation of female reproductive processes. Our previous studies have shown that VIS is produced locally in porcine corpora lutea and affects their functioning in a manner dependent on the dose of VIS and the phase of the oestrous cycle. We therefore hypothesised that VIS may regulate the functioning of ovarian follicles in pigs as well. To verify this hypothesis, we examined the effect of VIS on the proliferation and apoptosis of granulosa and theca interna cells – two key cell layers essential for the proper functioning of the ovarian follicles. Additionally, we investigated the impact of VIS on the secretion of progesterone and oestradiol by these cells.

With this aim, large preovulatory follicles were harvested from gilts (n=5) during the follicular phase. After isolation via enzymatic digestion, the cells were preincubated for 24 hours, followed by incubation for an additional 24 hours with medium (controls), VIS at sub-physiological, physiological, and supra-physiological doses, and/or FK866 – a specific and selective VIS inhibitor. Cell proliferation was assessed using an Alamar Blue® assay, and apoptosis was determined by flow cytometry. Hormone concentrations in the culture media were determined by radioimmunoassay.

For both cell types, it was shown that VIS at a sub-physiological dose had an anti-apoptotic effect, whereas VIS at a supra-physiological dose increased cell proliferation. Moreover, VIS at all studied doses was found to stimulate the secretion of progesterone and oestradiol by granulosa and theca interna cells. Further, co-treatment of the cells with VIS and the FK866 blocker abolished the observed VIS impact. Thus, the obtained results confirm that VIS may be a regulator of ovarian follicles functioning in pigs.

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P.46

Progesterone can regulate the DNA methylation in the cow's corpus luteum during the estrous cycle

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DNA methylation is a process that causes gene expression inhibition. The enzymes catalyzing this reaction are DNMT methyltransferases, of which the key ones are DNMT1, DNMT3a, and DNMT3b. The demethylation restores the inhibited gene's expression using the action of dioxygenases which include TET1, TET2, and TET3. Changes in the expression and activity of DNMTs and TETs can affect the function of the corpus luteum (CL). the aim of this study was to determine if alterations in DNMT and TET activities, as well as the mRNA expression of DNMT1, DNMT3a, DNMT3b, and TET1, TET2, and TET3, could be observed in the cyclic CL of cows. Whether the listed changes may show a correlation with progesterone (P4) levels throughout the cycle. The material consisted of CLs from days 2–5, 6–10, 11–16, and 17–20. Gene expression was measured by real-time PCR, while DNMT and TET activities respectively using luminescent and fluorescent ELISA kits. Expression of all DNMTs was the highest on days 2-10 and then decreased while the highest expression of TET1 and TET2 on days 6-16, and TET3 at the end of the cycle. DNMT activity was low on days 2-16 and increased towards the end of the cycle. In contrast, TET activity was high on days 2–16 and decreased in the last phase of the cycle. The expression levels of all enzymes, excluding DNMT1 and TET3, showed a positive correlation with P4 levels. Furthermore, we observed a correlation only between the activity of TET and the level of P4 that CL secretes during the cycle. Thus, the changes in DNMT and TET activities, gene expression levels for individual enzymes involved in both DNA methylation and demethylation and their correlation with P4 may suggest that this hormone regulates DNA methylation.

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P.47

Can high levels of simple sugars in diet affect the occurrence of ovulatory infertility in women by increasing oxidative stress?

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Environmental factors can significantly affect a woman's fertility by disrupting hormone function and reducing egg quality. Vitamins and minerals with antioxidant properties can play an important role in female fertility. Lifestyle factors such as an unhealthy diet, chronic stress, smoking, and excessive alcohol consumption can also negatively affect fertility. In the case of an unhealthy diet, a factor that may contribute to reproductive dysfunction is the high content

of simple sugars in the diet. Their excessive amounts in the diet can lead to the formation of reactive oxygen species (ROS). An imbalance between ROS production and antioxidant defenses in turn leads to the appearance of oxidative stress.

Some studies show that increased fructose exposure increases ROS levels in several types of tissues, such as white adipose tissue (WAT) cells. In addition, a diet high in fructose has been shown to affect intestinal permeability, abdominal obesity, insulin signaling, and reproductive function. Although glucose also increases ROS levels, fructose has been shown to induce ROS production to a greater degree than glucose.

The results of a preliminary study conducted on women with ovulatory infertility (study group) and women without ovulatory infertility (control group) have shown a statistically significant (p=0.03) difference in daily glucose intake (g/d) among women with ovulatory infertility (Me = 9.25; Q1 = 8.33; Q3=12.01) compared to women in the control group (Me = 6.70; Q1 = 5.30; Q3=7.20). In addition, statistically significant differences (p=0.01) have been found in daily fructose intake (g/d). Women with ovulatory infertility have a statistically higher daily fructose intake (Me = 14.08; Q1 = 11.60; Q3=14.72) than women in the control group (Me = 7.53, Q1 = 6.08, Q3=8.97).

Most studies focused on the effects of glucose and fructose on oxidative stress are limited and often involve the animal model. For this reason, more well-designed studies are needed to confirm the existence of a causal relationship.

P.48

Isolation and characterization of soluble scavenger receptor cysteine-rich domain-containing protein from turkey (*Meleagris galopavo*) seminal plasma

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Soluble scavenger receptor cysteine-rich domain-containing protein (SSc5D) from turkey seminal plasma has been recently identified in our laboratory. SSc5D is a member of scavenger receptor cysteine-rich (SRCR) superfamily belonging to the pathogen recognition receptors playing a role in the regulation of innate immune responses. The purpose of the present study was to isolate and characterize the SSc5D from turkey seminal plasma. The SSc5D of 16 kDa was firstly isolated by ion exchange, gel filtration and reversed phase chromatography, identified using mass spectrometry and used as antigen for immunization. Next, a large quantity of pure SSc5D protein, designated for protein characterization, has been isolated through immunoaffinity. The molecular weight and isoelectric point were determined using electrophoretic methods. The identification of phosphorylation sites was performed before and after TiO2 enrichment by LC-MS/MS. Glycopeptide and releasing N- and O-glycans analysis were performed by LC-MS/MS. An efficient isolation procedure has been developed for turkey SSc5D of 16 kDa. Using immunoaffinity, we isolated at least seven protein bands, which were identified as SSc5D (accession number XP_010705458) with molecular weight ranging from 11 to 63 kDa

and pI in the range 4.6 to 5.0. Three phosphopeptides phosphorylated at six sites (Ser122, Ser137, Ser271, Thr284, Ser416, Ser419) were found in SSc5D protein. N-glycosylation of SSc5D was confirmed for two sites (Asn36 and Asn329). N-glycans included high mannose, complex-type, and hybrid structures and features like high mannose and LacdiNac epitopes were fucosylated and sialylated. Fifteen sites of O-glycosylation were confirmed. O-glycans included mucin-like core structures were mostly fucosylated. Analysis of turkey SSc5D sequence revealed presence of four tandem SRCR domains and shows similarities to other avian group B SRCR proteins. Further studies, including antimicrobial function, should prioritize investigating the role of SSc5D in avian reproduction.

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P.49

Post-thaw incubation temperature influences canine sperm quality

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The viability of canine sperm after thawing can be preserved for a maximum of six hours. However, researchers are divided in their opinions as to the optimal temperature for post-thaw incubation (1).

The aim of this study was to determine the effect of post-thaw incubation temperature on the quality of cryopreserved canine sperm. Sperm-rich fractions of ejaculates from four dogs were collected and frozen following a 0.25 mL-straw protocol. The frozen-thawed semen samples were aliquoted into three parts and stored at 17°C, 25°C, and 37°C, respectively, for up to six hours. During incubation, sperm motility parameters (total motility - TMOT and progressive motility - PMOT) were measured every hour using the CASA HTM-IVOS. Post-thaw sperm assessment (conducted 0, 1, 2, and 3 h after thawing) included the determination of sperm plasma membrane integrity (SPMI, SYBR-14/PI fluorescent staining) and a simultaneous evaluation of plasma and acrosomal membrane integrity, and mitochondrial membrane potential (IPIAH, FITC-PNA/JC-1/PI fluorescent staining).

In each hour of incubation, sperm pellets were characterized by the highest values of TMOT and PMOT at a temperature of 17°C. With the progress of incubation, the values of SPMI and IPIAH decreased in all semen samples (incubated at 17°C, 25°C, and 37°C). This trend was observed primarily in semen samples incubated at 37°C, and it was less drastic in the samples incubated at lower temperatures (17°C and 25°C).

The results of this study indicate that the quality of thawed canine sperm is determined by incubation temperature. It can be concluded that post-thaw incubation of frozen-thawed canine semen at a temperature of 17°C allows high sperm quality to be maintained for up to six hours, which can facilitate artificial insemination.

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P.50

Leptin signaling in ovine placenta

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Pregnancy regulates leptin physiology by influencing its synthesis, availability, and activity. Leptin acts on target organs through the long form of the leptin receptor (LRb), which induces the JAK/STAT3 signaling pathway. This pathway may be negatively regulated by cytokine signaling-3 (SOCS-3) suppressor, which not only inhibits leptin signaling but is also an important factor responsible for maintaining the normal course of pregnancy, placental and fetal development. The short isoform of leptin receptor (LRa) serves as a specific leptin transport system, affecting its availability for target tissue.

To investigate the factors involved in leptin signaling during pregnancy, we determined the expression levels of LRa, LRb, and SOCS-3 in the placenta and the concentration of leptin in the blood plasma of 12 Polish Longwool ewes euthanized on days 30, 60, 90, and 120 of pregnancy. Real-time PCR was used to measure transcript expression. Leptin concentration was determined by radioimmunoassay. Data were analyzed by ANOVA using SigmaPlot software.

It was shown that both forms of leptin receptor were expressed from the first period of pregnancy and their expression level increased ($P<0,001$) with the subsequent stages of pregnancy, reaching the highest level on day 120 of gestation. The relative increase in expression between early and late pregnancy was greater for LRa compared to LRb (1600-fold vs. 40-fold, respectively). Placental SOCS-3 expression also increased ($P<0,01$) during pregnancy. Between 30 and 120 days of pregnancy, a 13-fold increase in SOCS-3 expression was observed.

The results suggest that the placenta may be a target organ for leptin. Changes in the abundance of LRa in the ovine placenta may be necessary to ensure the proper distribution of leptin, and variability in the expression of key elements of the leptin signaling pathway (LRb and SOSC-3) may be important for the proper functioning of the placenta, pregnancy, and fetal development.

P.51

QRFP43 modulate the hypothalamic-pituitary-thyroid axis activity

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The hypothalamic-pituitary-thyroid (HPT) axis is one of the important regulators of energy status and can directly and indirectly affects the regulation of metabolism in all body cells. On the other hand, QRFP43 which belongs to the RF-amide peptides family, is involved in the control of feeding behavior and may be another neuropeptide that participatie in the regulation of HPT axis.

The aim of this study was to investigate the role of QRFP43 in modulations of HPT axis activity in sheep.

The experiment was performed on sexually mature Polish Merino sheep (n=48). Stainless steel cannulas were implanted directly into IIIv of the brain in all experimental animals, which were divided into 3 groups. The following types of infusion into the third ventricle of the brain was performed: Control group (Ringer-Locke solution 480µl/day), RFa10 group (QRFP43 in dose 10µg/480µl/day) and RFa50 group (QRFP43 in dose 50µg/480µl/day). Blood samples has been collected from animals on Day 3 of infusion (from 08:00 a.m. to 02.00 p.m.). Immediately after the last infusion, animals were weighed, anaesthetised, and selected structures of the hypothalamus, pituitaries and plasma samples were stored for further analysis.

It was found that QRFP43 inhibit the expression of TRH mRNA expression in MBH as well as TRHR mRNA expression at the level of the pituitary. Exogenous QRFP43 administration led to decrease in the TSHβ mRNA expression in the pituitary, while increased amounts of IR TSH materials in pituitary cells were also observed. These changes led to increase in the mean plasma TSH concentrations. Furthermore, radioimmunology analyses showed that icv administration of QRFP43 decreased FT4 concentration and simultaneously increased FT3 plasma concentration.

On the basis of obtained results, it can be concluded that QRFP43 can modulate the HPT axis in sheep.

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P.52

PFASs dysregulate mitochondrial function in mouse ovarian granulosa cells

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Background: Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are known as endocrine disrupting chemicals due to their ability to disrupt reproductive function and hormone signalling. PFAS are known to be found in drinking water, personal care products, food packaging, furniture, etc. Some studies have shown that exposure to PFAS is associated with increased concentrations of these compounds in blood and ovarian follicular fluid (FF), and as a result can disrupt ovarian function.

Aim of the study: We aimed to determine whether a mixture of PFASs, reflecting the profile found in FF, affects mitochondrial function in mouse granulosa cells (GCs).

Methods: Granulosa cells were isolated from mature ovaries of female outbred OF1 mice. GC cells were treated with PFAS mixtures of test compounds named M6 (PFOS, 22.4 ng/ml; PFOA,

14.5 ng/ml; PFHxS, 21.3 ng/ml; PFDA, 0.9 ng/ml; PFHpA, 0.6 ng/ml; PFUnDA, 0.4 ng/ml; PFNA, 2 ng/ml) at 3 concentrations (0.1-, 1- and 10-fold dilutions) for 5h and 24h respectively. Mitochondrial activity (MitoTracker Deep Red dye), mitochondrial membrane potential (ΔΨm, JC-1 dye) and reactive oxygen species (ROS) levels (H2DCFDA dye) and ATP levels (Seahorse ATP Rate Assay) were then measured.

Results: Following short-term exposure to PFAS mixtures, we observed induction of reactive oxygen species and mitochondrial activity. In addition, log-term exposure caused decreased APT levels in parallel with disruption of ΔΨm in granulosa cells.

Conclusions: PFAS exposure decreased ATP production and increased oxidative stress, potentially leading to granulosa cell metabolic dysfunction or senescence.

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P.53

Assessment of relative mitochondrial DNA copy number in embryo spent medium to predict embryonic quality and chromosomal abnormalities

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In assisted reproductive technology, cell-free DNA (cfDNA) extracted from the embryo spent medium is one of the non-invasive methods used as starting material for predicting embryonic quality. This cfDNA includes both genomic DNA (gDNA) and mitochondrial DNA (mtDNA). cfDNA arises from the rupture of embryonic cells during division or from cell-secreted exosomes. This process may result in the loss of important embryonic organelles, such as mitochondria, which are energy-related structures in the cell, potentially affecting embryonic development. Therefore, the objective of this study is to examine the relationship between the relative mtDNA copy number and the quality of the embryo, as well as abnormalities in the number of chromosomes in the embryo.

Thirty-four zygotes obtained from 8 women undergoing intracytoplasmic sperm injection (ICSI) treatments were cultured in a time-lapse incubator until reaching the blastocyst stage. Afterward, trophectoderm (TE) cells from each embryo were removed via a biopsy procedure to perform pre-implantation genetic testing for aneuploidy (PGT-A), and the embryos were frozen. Subsequently, the embryo spent medium was collected to quantify relative mtDNA copy number by quantitative PCR. The mtDNA copy numbers of normal- and abnormal embryos were statistically compared using the Mann-Whitney test and the Kruskal-Wallis test.

Regarding the results, no statistically significant differences were found in embryo quality when comparing the relative mtDNA copy number with various parameters: percentage of fragmentation (<10%, 10-25%, and ≥25%) or the grades of the inner cell mass (ICM) and trophectoderm (TE) (grades A, B, C). Additionally, regarding chromosomal abnormalities based on PGT-A results, although the aneuploid group tended to have a higher relative mtDNA copy number than the euploid group, no statistically significant differences were observed.

Therefore, based on the present study, it can be concluded that the relative mtDNA copy number is not associated with embryo quality and chromosomal abnormalities.

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The impact of bisphenol A on mitochondrial function in *in vitro* matured mouse oocyte

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Mitochondria play a pivotal role during oocyte maturation, as they provide ATP required for this stage of development. Multiple studies have demonstrated that BPA, an endocrine-disrupting compound commonly used in the production of epoxy resins and polycarbonate plastics, impedes the structure and function of mitochondria in oocytes, resulting in increased oxidative stress and a reduction in mitochondrial membrane potential (MMP). However, in the majority of these studies, BPA was used in very high concentrations, limiting the physiological significance of the results. Therefore, the objective of this project was to investigate whether BPA in concentrations corresponding to those in human follicular fluid affects mitochondrial function and the redox state during mouse oocyte maturation. Mouse oocytes at the germinal vesicle (GV) stage, derived from 1–2-month-old females, were matured *in vitro* with BPA at concentrations of 2, 20, and 200 ng/ml, or with corresponding concentrations of BPA dilutant. We observed no significant differences in meiotic maturation between these groups. First, the redox state of metaphase II (MII) oocytes was assessed. To this end, monochlorobimane was employed as an indicator of glutathione levels. No significant differences were observed between oocytes treated with the abovementioned BPA concentrations and their respective controls. Subsequently, the MMP was determined using the mitochondrial membrane potential indicator JC-1. Our data indicates that there are no significant differences between MMP in oocytes matured with the abovementioned BPA concentrations and the corresponding controls. To ascertain whether maternal age affects mitochondrial susceptibility to BPA, we analysed mitochondrial membrane potential in oocytes obtained from 14- to 16-month-old females. Once more, none of the BPA concentrations affected MMP in oocytes. In summary, our data indicates that BPA at physiological concentrations of 2, 20, and 200 ng/ml does not affect mitochondrial function in mouse oocytes.

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P.55

Comparatively assessing the adipogenic derivatives of mesenchymal stem cells between wild boars and domestic pigs – profiling the transcripts coding for adipokines and their receptors

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The aim of the study was to comparatively examine the quantitative profiles of mRNA transcripts coding for selected adipokines (adiponectin and visfatin) and adiponectin receptors in undifferentiated mesenchymal stem cells (MSCs) and their adipogenically differentiated derivatives. MSCs were isolated post mortem from wild boar adipose tissue (wbAT) and porcine adipose tissue (pAT) explants. By using real-time PCR, the relative abundances (RAs) pinpointed for adiponectin (AdipoQ), adiponectin receptor 1 (AdipoR1), adiponectin receptor 2 (AdipoR2) and visfatin (NAMPT) mRNAs were inter-specifically analyzed for wbAT- and pAT-derived MSCs as well as their cell counterparts undergoing *in vitro* differentiation into adipocytes (Tukey's post hoc test followed by one-way ANOVA). All the experiments were independently performed in biological and technical triplicates. There were no significant differences in the inter-species variability between RAs identified for mRNA transcripts encoding the selected adipokines and their receptors ($p \geq 0.05$). But it is worth noting that RA for AdipoQ mRNA tended to be higher in wbAT-MSCs as compared to their pAT-derived counterparts. Moreover, after differentiation of wbAT-MSCs into adipocytes, an insignificant decrease in the expression profile of AdipoQ transcripts has been shown as compared to that recognized for undifferentiated MSCs. Conclusively, the present study provides the empirical justification and research highlights targeted at developing the *in vitro* comparative models of tracking the extent of molecular inter-specific consanguinity between transcriptomic profiles related to adipogenic differentiation of wbAT- and pAT-derived MSCs. Elaborating such ex vivo models might be helpful for exploring inter-transcriptomic pathways of recruiting MSC differentiability arising from up- and/or downregulation of expression profiles noticed for mRNAs encoding adipokines and their receptors, depending on metabolic disturbances resulting from obesity and superobesity in closely related mammalian species, including primates and humans.

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P.56

Devising the effective strategies for ex situ establishing somatic cell lineages in roe deer and wild boars as a promising frontier in biotechnological protection of free-living animals

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The objective of current research was to establish somatic cell lines from tissues of fauna representatives inhabiting the Małopolska region. The material was obtained from animals hunted between June 23 and July 17, 2023, in accordance with Polish regulations. To isolate Leydig cells, muscle cells, chondrocytes, and keratinocytes, testes, pubococcygeus muscles, skin fragments, and ear cartilage were collected from three specimens of roe deer and three specimens of wild boar. The enzymatic isolation of Leydig cells from testicular tissues of roe deer and wild boars was performed with the triple 10-min use of 0,25% trypsin solution. The isolated cells exhibited viability ranging from 86% to 89%. They were seeded in FBS-enriched DMEM on collagen-coated culture plates. The medium was replaced every two days, and the first passage was conducted after 10 days of culture. The cells were subsequently passaged twice more, and the resulting Leydig cell lines, which exhibited classical morphology and numerous lipid droplets, were cryopreserved. Additionally, pubococcygeus muscle cells, keratinocytes, and chondrocytes were isolated by 2-h liberase-mediated incubation. The obtained purified cell suspensions were analysed for cell viability. Trypan blue staining confirmed that the suspensions contained viable muscle cells, keratinocytes, and chondrocytes in extents of 84%, 83%, and 86%, respectively. Similar to the Leydig cells, these cells were seeded on collagen-coated culture dishes using FBS-supplemented DMEM, which was replenished every two days. The first passage was achieved after 7 days of initial culture, and after two consecutive passages, the cells were cryopreserved. Collectively, development of efficient conditions of ex situ establishing a variety of somatic cell lines stemming from roe deer and wild boars opens up the new possibilities for restoration of endangered or extinct wild mammalian species in Poland. This potential might be harnessed for the purpose of genetic rescue of wildlife by applying assisted reproductive technologies.

P.57

The BTBR mouse model of autism displays elevated placental efficiency and metabolic imbalance

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Growing evidence indicates that autism spectrum disorders have their origins during fetal stages of development, however, little is known about the role of the placenta in the occurrence of neurodevelopmental disorders. This study examined fetal and placental growth, histological

landmarks of placental efficiency and metabolism in the BTBR T+ Itpr3tf/J (BTBR) mouse model of autism, and the C57BL/6J (B6) control strain. Placentas were collected from BTBR and B6 pregnant females at 12.5 and 15.5 days post-coitum (dpc) (n=15/group, 3 litters/group) and investigated through PAS carbohydrate-positive staining, lipid-positive Bodipy and Oil Red O stainings, and qPCR. BTBR fetuses and placentas were lighter and smaller than B6 controls at both time points analyzed, however, their fetal/placental weight ratio was significantly higher than controls, accompanied by a larger labyrinth area, indicative of higher placental efficiency. BTBR placentas were also characterized by altered lipid metabolism, and strain-specific gene expression metabolic landmarks. Overall, this study sheds light on the possible involvement of placental metabolic functions in shaping the BTBR mouse's metabolic and behavioral phenotype.

This research was financed by the National Science Center, Poland, grant number 2020/39/B/NZ4/02105.

P.58

Extracellular vesicles supplementation during IVM supports energy metabolism of bovine cumulus-oocyte complexes and affects blastocyst quality

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Metabolism disturbances during cumulus-oocyte complexes (COCs) maturation result in reduced competence of oocytes and embryos. Crucial aspects of metabolism involve lipids and glucose, which are widely known energy sources for COCs. Extracellular vesicles (EVs) present in the follicular environment may be important for the energy production processes.

The aim of this study was to evaluate the role of EVs in energy metabolism during bovine COCs *in vitro* maturation. COCs were collected from bovine ovaries, matured in basic medium supplemented with lipid or glucose metabolism inhibitors (etomoxir or iodoacetate+DHEA, respectively) and supplemented or not-supplemented with EVs (+ vs -), resulting in the following groups: control (CON+ vs CON-), glucose metabolism inhibition (IODH+ vs IODH-) and lipids metabolism inhibition (ETO+ vs ETO-). Hatched blastocysts have been collected for the lipidomic analysis (MRM-profiling) on day 8 of culture.

EVs supplementation compensated for the negative effect of inhibitors, increasing the MII rate in oocytes of both experimental groups IODH+ vs IODH- ($p<0.01$) and ETO+ vs ETO-($p<0.05$). IODH+ group also showed increased blastocyst formation rate when compared to IODH- ($p<0.01$). Interestingly, EVs supplementation led to a higher hatched blastocysts rate in all groups (CON+ vs CON- $p<0.01$; IODH+ vs IODH- $p<0.01$; ETO+ vs ETO- $p<0.05$). Lipidomic analysis of blastocysts identified 207 lipid species, mostly revealing increased content in groups supplemented with EVs. The enrichment analysis showed significant changes in IODH+ vs IODH- and ETO+ vs ETO- groups with regard to the following pathways activity: Sphingolipid Metabolism (involved in cell polarization and differentiation), Phospholipid Biosynthesis (involved in membranes function) and Arachidonic Acid Metabolism (involved in prostaglandin synthase production). Our observations indicate a positive effect of EVs supplementation during IVM on the quantity and quality of resulting blastocyst.

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P.59

An ex vivo study of the effects of endocrine-active compounds on uterus of naturally and artificially fed piglets: impact on antioxidant enzymes activity, cell proliferation and apoptosis

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This study assessed the effects of the anti-androgen 2-hydroxyflutamide (2Hf), the environmental estrogenic compound 4-tert-octylphenol (OP), and the metabolite of the organochlorine insecticide methoxychlor (HPTE) on reactive oxygen/nitrogen species (ROS/RNS) production, antioxidant enzymes activity, proliferation, and apoptosis in uterine explants of sow-fed and formula-fed piglets. Uterine explants from 10-day-old sow-fed (n=5) or formula-fed (n=5) piglets were incubated for 20 hours in the M199 medium supplemented with 2Hf (1.7 x 10-4M), OP (10-6M), and HPTE (5 x 10-7M). Controls were incubated without endocrine-active compounds (EACs). Total ROS/RNS and antioxidant enzymes activity were assayed using the OxiSelect™ *In Vitro* ROS/RNS Assay Kit (Cell Biolabs) and colorimetric assays kits (Cayman Chemical), respectively. In uterine slices of sow-fed piglets, compared to controls, higher levels of ROS/RNS ($p<0.001$) as well as catalase and glutathione peroxidase activities ($p<0.001$, $p<0.05$, respectively) were observed upon incubation with OP. Additionally, catalase activity was higher ($p<0.001$) in 2Hf-incubated uterine slices. In formula-fed piglets, compared to controls, glutathione S-transferase activity was higher ($p<0.05$) upon incubation with 2Hf. Analysis of apoptosis by TUNEL method revealed a higher number of apoptotic cells in 2Hf- and OP-incubated uterine slices ($p<0.001$, $p<0.05$, respectively) of sow-fed piglets, compared to controls. Analysis of PCNA-positive cells in sow-fed piglets showed diminished uterine cell proliferation ($p<0.05$) upon incubation with the examined compounds, although the effect depends on the type of uterine cells. Conversely, in formula-fed piglets, compared to controls, there was elevated uterine cell proliferation ($p<0.001$), especially within the myometrium. Overall, our results suggest that EACs, particularly those with antiandrogenic and estrogenic properties, may impact neonatal uterine development by altering ROS/RNS levels, antioxidant enzymes activity, as well as cell proliferation and apoptosis. It also appears that sow-based natural feeding does not protect against the EACs-associated adverse effect on the uterus.

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The influence of photoperiod on inflammation-induced changes in the expression of biological clock genes in the ovine anterior pituitary

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The pituitary has a functional, internal circadian clock that is involved in the regulation of many cellular functions, including hormonal and transcriptional processes, as well as cell growth and death. Disturbances in the expression of clock genes in the anterior pituitary (AP) may therefore lead to changes in its secretory activity.

The study aims to determine the effect of endotoxin-induced inflammation on the expression of biological clock genes in the AP of ewes under two different photoperiodic conditions.

Two analogous experiments were carried out: in short-day photoperiod (SD) and long-day photoperiod (LD). The estrous cycles of ewes in SD were synchronized using the Chronogest® CR method. In each experiment, sheep were divided into two groups: day (n = 12) and night (n = 12), in which two subgroups were created: control (n=6; iv. NaCl) and LPS-treated (n=6; iv. 400 ng/kg). Experimental agents were administered 3 h before the middle of the light or dark phase of the day, and the animals were euthanized after another 3 h. The gene expression of CLOCK, BMAL, CRY1, and CRY2 in the AP was assayed by qRT-PCR.

Endotoxin administration reduced ($p<0.05$) the expression of CRY 1 and CRY2 regardless of photoperiodic conditions. Immune stress decreased ($p<0.05$) nocturnal CLOCK gene expression in SD conditions and diurnal expression in LD conditions. Inflammation reduced both diurnal ($p<0.05$) and nocturnal BMAL gene expression in SD conditions and nocturnal expression in LD.

Studies have shown that inflammation affects the expression of biological clock genes in the AP, in a manner that is partially dependent on photoperiodic conditions. Disruption of the internal circadian clock in the AP cells may be another mechanism by which bacterial infection causes hormonal disruptions.

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P.61

Regulation of aquaporin 4 gene and protein expression in the domestic hen ovary

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Aquaporin 4 (AQP4) belongs to a family of proteins responsible for transporting of water across the membrane under a osmotic gradient. The expression and localization of AQP4 have been demonstrated in the hen ovarian follicles at different stages of development. These data suggest participation of AQP4 in water movement required for follicle growth and maturation; however, the regulation of this channel protein expression in the avian ovary is largely unknown. This study aimed to examine whether mRNA and protein expression of AQP4 is regulated by gonadotropins and estrogen. For this purpose, hens were treated with equine chorionic gonadotropin (eCG) or tamoxifen (TMX, estrogen receptor modulator). Ovarian white, yellowish, and granulosa and theca layers of the largest yellow preovulatory follicles (F3-F1) were harvested from control, eCG- or TMX-treated (daily until cessation in egg-laying) hens. AQP4 transcript was detected in all examined ovarian tissues of control and treated groups. Real-time polymerase chain reaction and western blot analyses revealed changes in the expression of AQP4 following eCG and TMX. The injections of eCG decreased mRNA transcript abundance of AQP4 in the theca layer of the F3 follicle. TMX treatment lowered AQP4 transcript abundance in the white follicles and increased it in the theca layer of F3-F1 follicles. Moreover, TMX caused a decrease in protein abundance in the theca layer of the F2 follicle. We propose a relationship between gonadotropins (mainly FSH) and estradiol action and AQP4 gene and protein expression in the chicken ovarian follicles.

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P.62

The development of ex vivo hypothalamic-pituitary model. An alternative approach to *in vivo* neuroendocrinological studies

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Conventional cell and tissue cultures are inadequate for accurately reflecting the complexity of human organs, thereby limiting their effectiveness in disease modelling, drug development, and the study of organ and tissue physiology. A promising alternative lies in microfluidic cell cultures conducted on chips, commonly referred to as ""organ-on-a-chip"" (OOC) systems. These systems are engineered to induce microflow of culture medium through cells or tissues

embedded within specially designed chips or matrices, simulating the physiological environment of an organ.

The objective of our study was to replicate the hypothalamic-pituitary axis using OOC technology in order to investigate the modulatory effect of phoenixin (PNX) on the secretion of key hormones within the gonadotrophic axis.

The model design involved culturing slices of hypothalamic and pituitary tissues obtained from the sexually mature Old-type Polish Merino Sheep (n = 16) on a semi-permeable membrane positioned between glass slides placed in a dedicated apparatus. These explants were interconnected via capillaries, allowing for perfusion with a total of seven different media combinations containing PNX, an antagonist for NPY1R and NPY5R, using a peristaltic pump. The perfusates were collected at specific 'checkpoints' following parallel passages through the hypothalamus and through both the hypothalamus and pituitary slices, thus allowing for the measurement of changes in key reproductive hormones such as LH and FSH within the same slices. This aimed to elucidate the impact of PNX on tissue interactions and hormone secretion.

This model offers invaluable insights into neuroendocrinology, replicating interactions between the hypothalamus and pituitary. It provides a useful tool for understanding hormone secretion mechanisms within the gonadotropic axis. The OOC technology offers information that traditional cell models may not fully capture, due to their limitations in replicating actual tissue architecture and physiological conditions.

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Progestin and adipoQ receptor (PAQR) 7 and PAQR8 knockdown affects the function of bovine endometrial endothelial cells

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Our previous research showed changes in mRNA and protein levels of membrane P4 receptors: PAQR7 and PAQR8 in the endometrium during the estrous cycle and the first trimester of pregnancy in cows. Moreover, higher protein expression of the receptors was observed in the luminal and glandular epithelial cells and the endothelial cells of blood vessels. This may suggest that PAQR7 and PAQR8 can take part in the regulation of uterus function via modulation of endothelial cells of blood vessels. Therefore, the aim of this study was to investigate the involvement of PAQR7 and PAQR8 in secretion, proliferation, and migration processes in bovine endometrial endothelial cells. The research concerned the mRNA expression of selected genes including those related to prostaglandins synthesis (COX1, COX2, PGES, PGFS), growth factors (FGF2, IGF1, TNFα, VEGF), proliferation (TEK) and apoptosis (BCL, BAX, CASP3, CASP8) in cultured endometrial endothelial cells before and after PAQR7 and PAQR8 silencing, respectively. The results showed that the PAQR7 and PAQR8 silencing down-regulated COX1, IGF1, BAX, CASP8 and TEK mRNA expressions and up-regulated FGF2 mRNA expression. Moreover, we found that the silencing of PAQR8 down-regulated TNFα and up-regulated COX2 mRNA expression. Additionally, the silencing of both receptors increased PGE2 and PGI2 secretion but inhibited the proliferation and migration processes in endothelial cells. Therefore, it can be assumed that PAQR7 and PAQR8 can regulate the synthesis of

prostaglandins, cell proliferation, migration and apoptosis in the bovine endothelial cells and this way they may control endometrium function.

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Investigating the functional interactions and compensatory mechanisms of TEAD family transcription factors in the mouse embryos

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TEAD1 and TEAD4 proteins are part of the TEAD family of transcription factors, crucial for regulating developmental processes, from blastocyst formation to organ ontogenesis. Limited studies suggest potential compensatory functions between TEAD factors in knockout mice myocytes. However, it remains unknown whether TEAD1 and TEAD4 can substitute for each other's functions in the preimplantation embryo. The aim of this study was to determine whether TEAD1 and TEAD4 can compensate each other functions in driving lineage specific cell population in preimplantation mouse embryos. The temporary suppression of *Tead* genes was achieved by introducing silencing RNA targeting either the *Tead1*, the *Tead4* transcripts, or both, into embryos via electroporation, or in ES cells by lipofection. Embryos/ES cells transfected with non-silencing siRNA served as controls. Embryos were cultured *in vitro* until the blastocyst stage, and the presence of TEAD1, TEAD4 proteins, and blastocyst lineage-specific markers was analysed using immunostaining. Furthermore, the expression levels of all *Tead* genes were assessed using qPCR in both embryos and ES cells. Our preliminary findings suggest that temporary suppression of Tead1 and/or Tead4 gene expression induces compensatory mechanisms, promoting embryonic survival and development despite limited expression of either factor.

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P.65

Immunolocalization of vitamin D receptors and metabolic enzymes in embryos and larvae of common carp (*Cyprinus carpio* L.)

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Vitamin D, known for its pleiotropic effect in organisms, plays a crucial role in various physiological processes. While its positive impact of reproduction in mammals has been established, its potential role in the reproductive processes of fish remains unexplored. The aim of this study was to immunolocalize vitamin D receptor (VDR), protein disulphide isomerase family A member 3 (PDIA3) and metabolic enzymes (CYP27B1 and CYP24A1) in embryos and larvae of common carp (*Cyprinus carpio* L.). The results showed the presence of vitamin D receptors and metabolic enzymes in various tissues of carp embryos and larvae, suggesting that vitamin D may play a role in the embryogenesis of cyprinids. This study marks a preliminary step towards understanding the potential mechanisms by which vitamin D may affect fish development and reproduction.