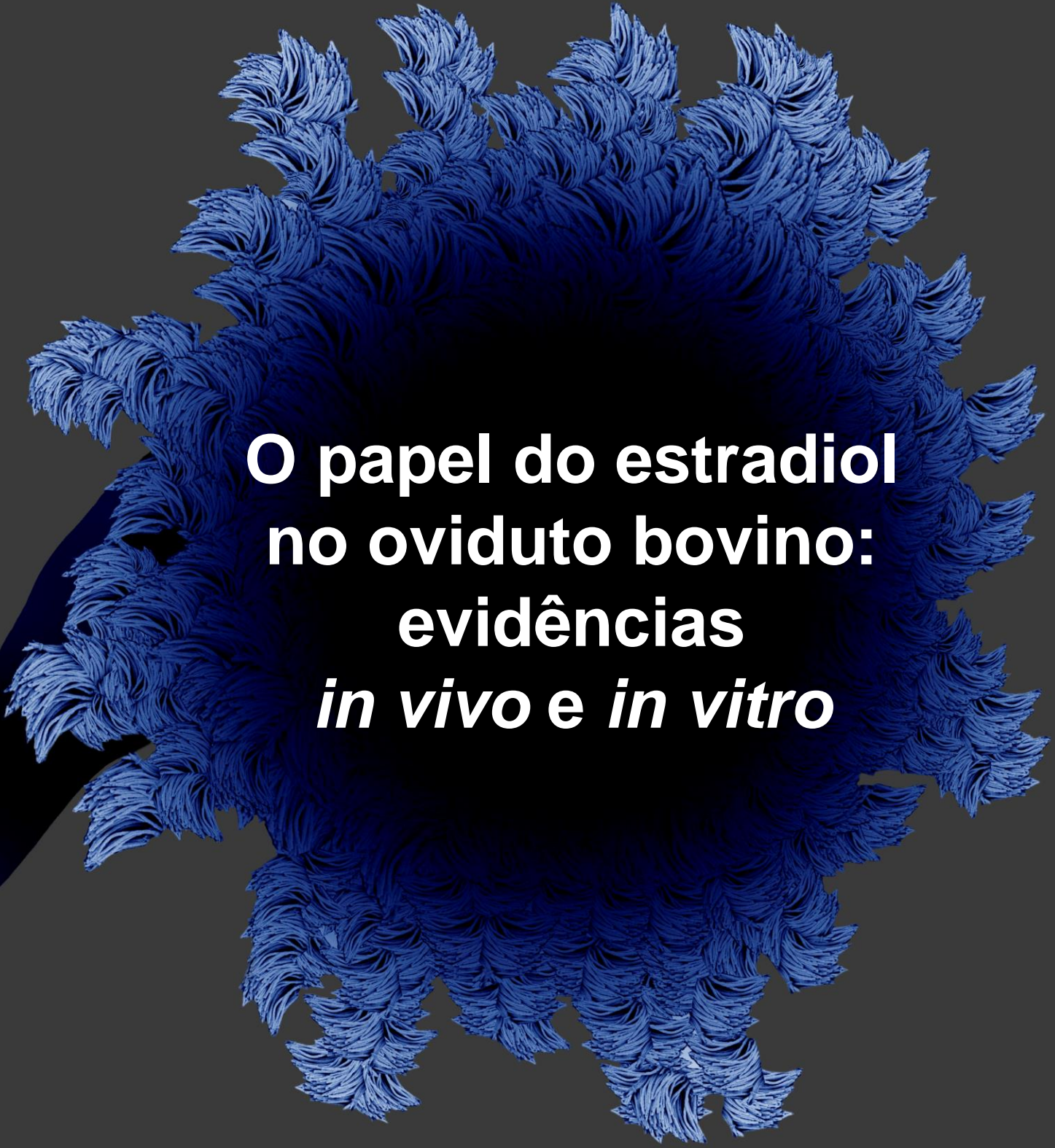


# RESSALVA

Atendendo solicitação do(a)  
autor(a), o texto completo desta  
tese será disponibilizado  
somente a partir de 15/10/2022.

Universidade Estadual Paulista “Júlio de Mesquita Filho”  
Instituto de Biociências de Botucatu  
PPG – Farmacologia e Biotecnologia



**O papel do estradiol  
no oviduto bovino:  
evidências  
*in vivo e in vitro***

Patricia Kubo Fontes  
2020

UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”  
INSTITUTO DE BIOCÊNCIAS DE BOTUCATU

# **O PAPEL DO ESTRADIOL NO OVIDUTO BOVINO: EVIDÊNCIAS *IN VIVO* E *IN VITRO***

**Patricia Kubo Fontes**

Orientador: Anthony César de Souza Castilho

Co-orientador: Mário Binelli

Tese apresentada ao Programa de Pós-graduação  
do Instituto de Biociências de Botucatu,  
Universidade Estadual Paulista (UNESP), para  
obtenção do título de doutora em Farmacologia e  
Biotecnologia

Botucatu | São Paulo | Brasil

Outubro, 2020

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.  
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP  
BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Fontes, Patricia Kubo.

O papel do estradiol no oviduto bovino : evidências in vivo e in vitro / Patricia Kubo Fontes. - Botucatu, 2020

Tese (doutorado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências de Botucatu

Orientador: Anthony César de Souza Castilho

Coorientador: Mário Binelli

Capes: 20100000

1. Bovino Reprodução. 2. Estradiol. 3. Indução da ovulação. 4. Técnicas in vitro. 5. Células epiteliais. 6. Falópio, Trompas de.

Palavras-chave: Células epiteliais do oviduto bovino; Cultivo 3D in vitro; Estradiol; Oviduto; Superstimulação ovariana.

Autora: Patricia Kubo Fontes

Título: O papel do estradiol no oviduto bovino: evidências in vivo e in vitro

### **COMISSÃO EXAMINADORA**

**Prof. Dr. Anthony César de Souza Castilho**

Presidente e orientador

Universidade do Oeste Paulista (UNOESTE) – Presidente Prudente, São Paulo, Brasil.

**Prof. Dra. Claudia Maria Bertan Membrive**

Membro

Universidade Estadual Paulista (UNESP) – Dracena, São Paulo, Brasil.

**Prof. Dr. Felipe Perecin**

Membro

Universidade de São Paulo (USP) – Pirassununga, São Paulo, Brasil.

**Profa. Dra. Angela Maria Gonella Diaza**

Membro

Universidade da Florida (UF) – Flórida, Estados Unidos da América.

**Profa. Dra. Marcella Pecora Milazzotto**

Membro

Universidade Federal do ABC (UFABC) – Santo André, São Paulo, Brasil.

Data da defesa: 15 de Outubro de 2020

Local da defesa: Instituto de Biociências de Botucatu, UNESP (Botucatu, SP, Brasil)

*“Science and everyday life cannot and should not be separated”*  
(A ciência e a vida cotidiana não podem e não devem ser separadas)

— Rosalind Franklin

Dedico esse trabalho aos meus pais, **Luis** e **Neide**, e minhas irmãs, **Daiane** e **Luisa**. Onde sempre encontro paz, segurança, incentivo e amor.

# CONTEÚDO | CONTENTS

<b>AGRADECIMENTOS   ACKNOWLEDGMENTS</b>	<b>1</b>
<b>RESUMO</b>	<b>3</b>
<b>ABSTRACT</b>	<b>5</b>
<b>INTRODUCTION</b>	<b>7</b>
<b>Capítulo 1   Chapter 1</b> Revisão de Literatura   Literature Review	<b>9</b>
<b>Capítulo 2   Chapter 2</b> Equine chorionic gonadotropin increases estradiol levels in the bovine oviduct and drives the transcription of genes related to fertilization in superstimulated COWS	<b>36</b>
<b>Capítulo 3   Chapter 3</b> Should the phase of the estrous cycle be considered when harvesting bovine oviduct epithelial cells for application in <i>in vitro</i> coculture systems?	<b>62</b>
<b>CONSIDERAÇÕES FINAIS   FINAL REMARKS</b>	<b>86</b>
<b><i>CURRICULUM VITAE</i></b>	<b>91</b>

## AGRADECIMENTOS | ACKNOWLEDGMENTS

Agradeço aos meus pais por serem minha base, minha motivação e me incentivarem incondicionalmente. Às minhas irmãs, Daiane e Luisa, por me fazerem acreditar em mim mesma, e um agradecimento extra à Daiane, que sempre materializa as mais malucas ideias de esquemas, incluindo a capa dessa tese. Ao Anthony Castilho, por me dar asas do mundo da pesquisa e ainda me ensinar a voar. Ao Mario Binelli, por me ensinar a pensar além de barreiras pré-existentes e a ver o mundo por ângulos diferentes. Ao Marcelo Nogueira, pela confiança e filosofias de vida. Ao Di Stasi, pelo abrigo e carinho diário. Ao professor Ciro Barros, por ser exemplo de ética, respeito e humanidade. À Fernanda Franchi e Priscila dos Santos, por compartilharem frustrações, tristezas, alegrias e comemorações. Ao Eduardo Razza, por ser meu guia no início dessa vida acadêmica e pela amizade perene. À Ângela Gonella, por ser um exemplo de pesquisadora e por muitas discussões científicas. À Márcia Ferraz, te conhecer foi um dos prazeres que o doutorado me promoveu, obrigada pela ajuda em estabelecer meu estágio da Holanda e ser um exemplo de pesquisadora. Agradeço aos professores Cláudia Membrive, Wellerson Scarano e Luis Justulin Júnior pela disponibilidade e ensinamentos na etapa de qualificação do meu doutorado. Agradeço ao professor Juliano Silveira, Ana Clara e Jorge por tentarem até o último momento encontrar exossomos nas minhas amostras. Agradeço à Marcella Milazzotto, Érika e Jéssica, pelo auxílio com análises de RAMAN, discussões científicas e bom convívio. Agradeço ao professor José Rybarczyk Filho e André Molan pelas análises de bioinformática. Um agradecimento extra à Ângela Gonella, Márcia Ferraz, Cláudia Membrive e Felipe Perecin, por prontamente se disponibilizarem a participar como banca examinadora desta tese de doutorado.

Agradeço a confiança dos professores, Gisele Mingotti, Mateus Sudano, Yeda Watanabe, Fabíola Paula-Lopes, Fernanda Landin, Flávio Meirelles, Lindsay Gimenes, Mayra Assumpção, Ester Caixeta, Felipe Perecin e Marcelo Seneda, pessoas incríveis que tive a feliz oportunidade de estabelecer colaborações durante meu doutorado. Assim como queridos colegas de trabalho, Rafael Satrapa, Barbara Loureiro, Antonio Pupulim, Mauricio Favoreto, Vinicius Pinehiro, Ramon Botigelli, Raquel Puelker, Ronaldo Ereno, Luiz Domigues, Alan Giroto, Leonardo Mendes, Elisa Pioltine, Gabriella Mamede, Mariana Sponchiado, Maite del Collado, Ana Carolina Soares, Amanda Zangirolamo, Camila Bortoliero, Camila Rosa, Geovana Nunes, Tamie Almeida, Lais Latorraca, Flavia Barros, Thaisy Dellaqua, Felipe Dalanezi, Andressa Carvalho, Raquiel Rodrigues, Talita Sebastião, Thainá Manssur e Ana Paula Marques. E também a todos do Laboratório de Fitomedicamentos, Farmacologia e Biotecnologia (FitoFarmaTec).

I want to thank all the people that made my one-year internship in the Netherlands the most special experience in my Ph.D. Bart Gadella, I appreciate this unique opportunity you gave me to be in your lab, and more than that, you are my inspiration of what kind of person (in the full meaning of the word) I aspire to be. I also want to thank Tom Stout for teaching me how to become more confident

and trust in my abilities. And Heiko Henning for making me become a better scientist, I very much admire your way of guiding people in developing a critical opinion and I would like to thank you very much for teaching me this. I also want to thank Peter Vos, Hilde Aardema, Bernard Roelen, Arend Rijnveld, Christine Oei, Elly Zeinstra for all the help and knowledge shared with me. A special thanks to Leni van Tol, for teaching me more than she can imagine and also to prove to me that I will never be able to do a Western Blotting better than her! Thank you to my dear friend Bart Leemans, who was great and fun company for any moment in- and outside the lab. Also for dear colleagues from the lab: Claudia Deelen, Inge Bertijn, Romi Hoogendijk, Minjie Tam, Bo Yu, Josephine Marchand, Liz Bromfield, Maxime Kromwijk, Nikola du Preez, and Paula Maitan. E claro, minha família brasileira de Utrecht: Cris, Will, Rodrigo, Nanda, Hedley, Samantha, Daniel, Widney, Bel e Paula.

Agradeço essas pessoas que são mais que especiais: Adriana Del Ben, umas das pessoas que mais admiro nesse mundo, obrigada por ser quem você é. Camila de Lima, só você para me aguentar na vida pessoal, acadêmica, e qualquer outra que existir, não tem para onde escapar, acho que significa que essa amizade será eterna, e agradeço muito por isso. Bianca Balbino, obrigada por conseguir estar presente mesmo com um oceano entre nós, sua positividade e sua personalidade me inspira, obrigada. Agradeço também às queridas amigas pelo companheirismo de sempre, Ana Elisa Quaglio, Tainan Curimbaba, Livia Branisso, Marcela Gonçalves, Mariana Ide, Liliana Rosa, Marília Carneiro e todas da eterna república Abstinência (Cristiane, Júlia, Luana, Caroline, Débora, Cíntia, Paula), Sarah Nunes, Cristiane Mori e Vanessa Giorgi. Agradeço à Patricia Garcia, por me dar o prazer do ensinar e pela agradável amizade. And I also want to thank Joachim Valk, for being the best chapter that this thesis could have given to me.

Agradeço aos funcionários do departamento de farmacologia, Cris, Paulão, Luiz e Hélio, em especial um agradecimento a Janete, pela descontração diária. À seção de pós-graduação do Instituto de Biociências de Botucatu, em especial ao Davi, por todo o suporte com as burocracias da vida. Aos professores do programa de pós-graduação em Farmacologia e Biotecnologia pelos ensinamentos e dedicação nas disciplinas. À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela bolsa de pesquisa durante o primeiro ano de doutorado, Código de Financiamento 001. À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), pela Bolsa de doutorado (2016/25685-4), pela Bolsa no exterior (2017/13481-8) e auxílio financeiro regular (2018/06674-7).

## RESUMO

O oviduto desempenha diversas funções até a formação do embrião, como maturação e transporte final dos gametas, processo de fertilização, transporte do embrião e desenvolvimento inicial do embrião. No entanto, nenhuma dessas etapas precisa ocorrer obrigatoriamente no oviduto. A capacidade de desenvolver embriões até o estágio de blastocisto sem qualquer contato com o trato reprodutivo materno gerou a hipótese de que o oviduto seria secundário e não essencial no estágio inicial da vida. Hoje, no entanto, é amplamente conhecido que a interação embrião-oviduto, ou interação com produtos de células ovidutais, pode impactar o embrião de diferentes maneiras, especialmente em relação às características epigenéticas, metabolismo e vários marcadores de qualidade. Para reconectar o embrião em desenvolvimento às células ovidutais (OC), muitas abordagens artificiais foram delineadas. Nesta tese, investigamos as CO diante de duas dessas abordagens: os protocolos de superestimulação ovariana (SOV) e a cocultura *in vitro* de embrião-CO. No primeiro estudo, as vacas foram submetidas à: i) protocolo SOV apenas com hormônio folículo-estimulante (FSH), ii) protocolo SOV usando FSH com gonadotrofina coriônica equina (eCG), ou iii) ovulação única por sincronização hormonal. O uso de protocolos SOV permite o desenvolvimento de múltiplos embriões dentro do oviduto, possibilitando a interação dos embriões com as CO. Este primeiro estudo demonstrou que as CO de vacas submetidas à SOV com FSH + eCG apresentam níveis mais elevados de estradiol, que também modulou a expressão gênica de genes relacionados ao processo de fertilização. Em seguida, esse perfil hormonal foi aplicado em um sistema de cultivo *in vitro* de OC. As CO podem ser cultivadas para aplicação em sistema de cocultura com embriões, o que permite que o embrião produzido *in vitro* interaja com as CO. A cultura de OC ainda não possui protocolo padrão ouro. Portanto, para analisar o efeito dos diferentes perfis hormonais obtidos como resultado do primeiro estudo, foi necessário incluir outra variação no segundo estudo: diferentes perfis hormonais x CO coletadas em diferentes fases do ciclo estral. Ao analisar os dados obtidos na análise de RNAseq, após o cultivo *in vitro*, as OC coletadas durante a fase folicular apresentam um perfil de transcriptoma muito diferente do perfil das células coletadas na fase lútea, caso nenhum tratamento hormonal for aplicado. Porém, quando altos níveis de estradiol são aplicados na cultura *in vitro* dessas células, o perfil do transcriptoma das CO torna-se muito semelhante, independentemente do ciclo estral em que as CO foram coletadas. Em conclusão, essas duas abordagens artificiais para possibilitar a interação dos embriões com o oviduto modulam as CO em função dos perfis hormonais. Portanto, o perfil hormonal é um fator importante a ser considerado nas estratégias para estabelecer a interação de embriões e células ovidutais.

## ABSTRACT

The oviduct plays many roles in the embryo formation, as gametes final maturation and transport, fertilization process, embryo transport, and embryo early development. However, none of these steps need to occur in the oviduct mandatorily. The ability to develop embryos up to the blastocyst stage without any contact with the maternal reproductive tract hypothesized that the oviduct would be minor and inessential at the early stage of life. Today, however, it is widely known that the embryo-oviduct interaction, or interaction with oviductal cells' products, can impact the embryo in different ways, especially regarding epigenetic characteristics, metabolism, and several quality markers. To reconnect the embryo in development to the oviductal cells (OC), many artificial approaches have been designed. In this thesis, we investigated the OC from two of these approaches: the ovarian superstimulation protocols (OVS) and the embryo-OC *in vitro* coculture. In the first study, cows were submitted to: i) OVS protocol only with follicle-stimulating hormone (FSH), ii) OVS protocol using FSH with equine chorionic gonadotrophin (eCG), or iii) single ovulation by hormone synchronization. The use of OVS protocols allows the development of multiples embryos inside the oviduct, making possible the interaction of the embryos with the OC. This first study demonstrated that OC from cows submitted to OVS using FSH+eCG has higher levels of estradiol, which also modulated gene expression of genes related to the fertilization process. Next, this hormone profile was applied in an *in vitro* culture system of OC. The OC can be cultured for application in a coculture system with embryos, which enable the *in vitro* produced embryo to interact with the OC. The culture of OC has still no gold standard protocol. Therefore, to analyze the effect of different hormone profiles obtained as a result of the first study, it was necessary to include another variation in the second study: different hormone profiles x OC collected in a different phase of the estrous cycle. When analyzing the data obtained from the RNAseq analysis, after *in vitro* culture, OC collected during the follicular phase presents a very different transcriptome profile than cells collected during the luteal phase, if no hormone treatment is applied. However, when high levels of estradiol are applied in the *in vitro* culture, the transcriptome profile of the OC become very similar to each other, independently of the estrous cycle when OC was collected. In conclusion, these two artificial approaches to enable the interaction of embryos with the oviduct modulate the OC depending on the hormone levels presented. Therefore, the hormone profile is an important factor to be considered in strategies to establish the interaction of embryos and oviductal cells.

## INTRODUCTION

After reproductive biotechnologies have been developed, exponential growth was observed in embryo production numbers. Currently, the total number of bovine embryos produced by *in vitro* and *in vivo* techniques exceeds 1.5 million per year worldwide. Associated with numerical expansion, many studies investigate how to improve embryo quality and increase the chance of establishing a successful pregnancy after embryo transfer. The oviduct is the organ responsible for establishing a suitable microenvironment for the final oocyte maturation, sperm reserve formation and capacitation, fertilization, gamete/embryo transport, and initial embryo development. Although the interaction with the oviduct does not impede bovine embryo formation and initial development, when the embryo is in natural circumstances with oviduct cells, its quality is unquestionably superior. Thus, the improvement of embryonic quality and consequently increasing pregnancy success may lie in the interaction of the embryo with oviduct cells, or oviductal cells products. To provide this embryo-oviduct contact, several artificial methods have been developed and are constantly evolving. A general selection of these methods is presented in **Chapter 1**. This literature review addresses the main *in vivo* and *in vitro* methods available for providing interaction between the embryo and the oviduct, their limitations, and perspectives.

Superovulation protocol is one of these methods. Although cattle are monovulatory animals, hormonal treatments stimulate multiple follicle growth and ovulation, enabling the formation and development of several embryos in the oviduct lumen. This interaction with the oviduct is very beneficial to these embryos, although their quality is still inferior when compared to embryos produced from single ovulation. The cause of this inferior embryonic quality may be multifactorial, and changes in oviductal cells can be one of them. In **Chapter 2**, oviduct tissues were evaluated after cows had been treated with two ovarian superstimulation protocols and one synchronization protocol for single ovulation. The oviducts were evaluated one day before ovulation, to understand the effects of the protocols without interference from the gametes/embryos presence. Cows submitted to the

ovarian superstimulation protocols, specifically treated with follicle-stimulating hormone associated with equine chorionic gonadotropin, had their oviducts with higher estradiol levels and modulation on transcription levels of genes related to the fertilization process in the oviduct.

In **Chapter 3**, another method that provides interaction between the embryo and the oviduct was studied, in this case, an approach for embryos produced *in vitro*. During the *in vitro* culture of embryos, it is possible to establish a coculture system with oviductal cells. Currently, compartmentalized culture systems are more efficient at this task, as it provides cellular nutrition through the basolateral segment, which mimics blood nutrition in oviductal cells and cell apical region free of culture medium, which mimics the organ lumen. In these compartmentalized systems, it is possible to place embryos in the zygote stage in the apical compartment to develop in contact with oviductal cells. At the same time, it is possible to modulate the culture medium in the basolateral compartment to mimic blood supply. This modulation, especially with hormonal treatments, is not yet fully established. For this reason, in **Chapter 3** the data from the analysis of the culture of bovine oviduct epithelial cells submitted to hormonal treatments in a compartmentalized system is present. It was observed that treatment with high levels of estradiol seems to be the major modulator of transcription in oviductal cells and more importantly, the results suggest that the moment of the estrous cycle when the cells are obtained for later *in vitro* culture is an essential aspect that should be considered for the cultivation of bovine oviduct epithelial cells. Finally, in **Chapter 4**, the final considerations of the findings of these studies are presented.

## 8. REFERENCES

- [1] W.C. Buhi, Characterization and biological roles of oviduct-specific, oestrogen-dependent glycoprotein., *Reproduction* 123 (2002) 355-362.
- [2] J.L. Yániz, F. Lopez-Gatius, P. Santolaria, K.J. Mullins, Study of the functional anatomy of bovine oviductal mucosa., *Anat Rec* 260 (2000) 268-278. 10.1002/1097-0185(20001101)260:3<268::AID-AR60>3.0.CO;2-L [pii].
- [3] M. Binelli, A.M. Gonella-Diaza, F.S. Mesquita, C.M.B. Membrive, Sex Steroid-Mediated Control of Oviductal Function in Cattle, *Biology (Basel)* 7 (2018). 10.3390/biology7010015.
- [4] B. Rodríguez-Alonso, J.M. Sánchez, M. Hamdi, M. McDonald, V. Havlicek, U. Besenfelder, P. Lonergan, D. Rizos, Asynchrony between the early embryo and the reproductive tract affects subsequent embryo development in cattle, *Reprod Fertil Dev* 32 (2020) 564-571. 10.1071/RD19360.
- [5] M. Saint-Dizier, J. Schoen, S. Chen, C. Banliat, P. Mermillod, Composing the Early Embryonic Microenvironment: Physiology and Regulation of Oviductal Secretions, *Int J Mol Sci* 21 (2019). 10.3390/ijms21010223.
- [6] Y. Menezo, P. Guerin, The mammalian oviduct: biochemistry and physiology, *Eur J Obstet Gynecol Reprod Biol* 73 (1997) 99-104.
- [7] M.A.M.M. Ferraz, H.S. Rho, D. Hemerich, H.H.W. Henning, H.T.A. van Tol, M. Hölker, U. Besenfelder, M. Mokry, P.L.A.M. Vos, T.A.E. Stout, S. Le Gac, B.M. Gadella, An oviduct-on-a-chip provides an enhanced in vitro environment for zygote genome reprogramming, *Nat Commun* 9 (2018) 4934. 10.1038/s41467-018-07119-8.
- [8] R. Lopera-Vasquez, M. Hamdi, V. Maillo, V. Lloreda, P. Coy, A. Gutierrez-Adan, P. Bermejo-Alvarez, D. Rizos, Effect of bovine oviductal fluid on development and quality of bovine embryos produced in vitro, *Reprod Fertil Dev* 29 (2017) 621-629. 10.1071/RD15238.
- [9] S. Canovas, P.J. Ross, G. Kelsey, P. Coy, DNA Methylation in Embryo Development: Epigenetic Impact of ART (Assisted Reproductive Technologies), *Bioessays* 39 (2017). 10.1002/bies.201700106.
- [10] M.J. Sudano, E.S. Caixeta, D.M. Paschoal, A. Martins, R. Machado, J. Buratini, F.D. Landim-Alvarenga, Cryotolerance and global gene-expression patterns of *Bos taurus indicus* and *Bos taurus taurus* in vitro- and in vivo-produced blastocysts, *Reprod Fertil Dev* (2013). 10.1071/RD13099.
- [11] U. Besenfelder, V. Havlicek, G. Brem, Role of the oviduct in early embryo development, *Reprod Domest Anim* 47 Suppl 4 (2012) 156-163. 10.1111/j.1439-0531.2012.02070.x.
- [12] S.G. Moore, J.F. Hasler, A 100-Year Review: Reproductive technologies in dairy science, *J Dairy Sci* 100 (2017) 10314-10331. 10.3168/jds.2017-13138.
- [13] C.M. Barros, R.A. Satrapa, A.C. Castilho, P.K. Fontes, E.M. Razza, R.L. Ereno, M.F. Nogueira, Effect of superstimulatory treatments on the expression of genes related to ovulatory capacity, oocyte competence and embryo development in cattle, *Reprod Fertil Dev* 25 (2013) 17-25. 10.1071/RD12271.
- [14] A. Gad, U. Besenfelder, F. Rings, N. Ghanem, D. Salilew-Wondim, M.M. Hossain, D. Tesfaye, P. Lonergan, A. Becker, U. Cinar, K. Schellander, V. Havlicek, M. Hölker, Effect of reproductive tract environment following controlled ovarian hyperstimulation treatment on embryo development and global transcriptome profile of blastocysts: implications for animal breeding and human assisted reproduction, *Hum Reprod* 26 (2011) 1693-1707. 10.1093/humrep/der110.

- [15] V. Havlicek, F. Wetscher, T. Huber, G. Brem, M. Mueller, U. Besenfelder, In vivo culture of IVM/IVF embryos in bovine oviducts by transvaginal endoscopy, *J Vet Med A Physiol Pathol Clin Med* 52 (2005) 94-98. 10.1111/j.1439-0442.2005.00694.x.
- [16] M.A. Sirard, R.D. Lambert, Birth of calves after in vitro fertilisation using laparoscopy and rabbit oviduct incubation of zygotes, *Vet Rec* 119 (1986) 167-169. 10.1136/vr.119.8.167.
- [17] K.H. Lu, I. Gordon, M. Gallagher, H. McGovern, Pregnancy established in cattle by transfer of embryos derived from in vitro fertilisation of oocytes matured in vitro, *Vet Rec* 121 (1987) 259-260. 10.1136/vr.121.11.259.
- [18] B.P. Enright, P. Lonergan, A. Dinnyes, T. Fair, F.A. Ward, X. Yang, M.P. Boland, Culture of in vitro produced bovine zygotes in vitro vs in vivo: implications for early embryo development and quality, *Theriogenology* 54 (2000) 659-673. 10.1016/S0093-691X(00)00381-2.
- [19] D. Rizos, F. Ward, P. Duffy, M.P. Boland, P. Lonergan, Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: implications for blastocyst yield and blastocyst quality, *Mol Reprod Dev* 61 (2002) 234-248. 10.1002/mrd.1153.
- [20] D. Rizos, P. Lonergan, M.P. Boland, R. Arroyo-García, B. Pintado, J. de la Fuente, A. Gutiérrez-Adán, Analysis of differential messenger RNA expression between bovine blastocysts produced in different culture systems: implications for blastocyst quality, *Biol Reprod* 66 (2002) 589-595. 10.1095/biolreprod66.3.589.
- [21] D. Tesfaye, P. Lonergan, M. Hoelker, F. Rings, K. Nganvongpanit, V. Havlicek, U. Besenfelder, D. Jennen, E. Tholen, K. Schellander, Suppression of connexin 43 and E-cadherin transcripts in in vitro derived bovine embryos following culture in vitro or in vivo in the homologous bovine oviduct, *Mol Reprod Dev* 74 (2007) 978-988. 10.1002/mrd.20678.
- [22] F. Wetscher, V. Havlicek, T. Huber, M. Gilles, D. Tesfaye, J. Griese, K. Wimmers, K. Schellander, M. Müller, G. Brem, U. Besenfelder, Intrafallopian transfer of gametes and early stage embryos for in vivo culture in cattle, *Theriogenology* 64 (2005) 30-40. 10.1016/j.theriogenology.2004.11.018.
- [23] B.J. Restall, R.G. Wales, The fallopian tube of the sheep. 3. The chemical composition of the fluid from the fallopian tube, *Aust J Biol Sci* 19 (1966) 687-698. 10.1071/bi9660687.
- [24] H.R. Tervit, D.G. Whittingham, L.E. Rowson, Successful culture in vitro of sheep and cattle ova, *J Reprod Fertil* 30 (1972) 493-497. 10.1530/jrf.0.0300493.
- [25] C. Wrenzycki, D. Herrmann, J.W. Carnwath, H. Niemann, Alterations in the relative abundance of gene transcripts in preimplantation bovine embryos cultured in medium supplemented with either serum or PVA, *Mol Reprod Dev* 53 (1999) 8-18. 10.1002/(SICI)1098-2795(199905)53:1<8::AID-MRD2>3.0.CO;2-K.
- [26] H.M. Kubisch, M.A. Larson, A.D. Ealy, C.N. Murphy, R.M. Roberts, Genetic and environmental determinants of interferon-tau secretion by in vivo- and in vitro-derived bovine blastocysts, *Anim Reprod Sci* 66 (2001) 1-13. 10.1016/s0378-4320(01)00086-0.
- [27] B. Algarra, V. Maillo, M. Avilés, A. Gutiérrez-Adán, D. Rizos, M. Jiménez-Movilla, Effects of recombinant OVGP1 protein on in vitro bovine embryo development, *J Reprod Dev* 64 (2018) 433-443. 10.1262/jrd.2018-058.
- [28] R.C. Botigelli, E.M. Razza, E.M. Pioltine, P.K. Fontes, K.R.L. Schwarz, C.L.V. Leal, M.F.G. Nogueira, Supplementing in vitro embryo production media by NPPC and sildenafil affect the cytoplasmic lipid content and gene expression of bovine cumulus-oocyte complexes and embryos, *Reprod Biol* 18 (2018) 66-75. 10.1016/j.repbio.2018.01.004.
- [29] J. Lamy, J. Gatien, F. Dubuisson, L. Nadal-Desbarats, P. Salvetti, P. Mermillod, M. Saint-Dizier, Metabolomic profiling of bovine oviductal fluid across the oestrous cycle using proton nuclear magnetic resonance spectroscopy, *Reprod Fertil Dev* (2018). 10.1071/RD17389.
- [30] E.C. dos Santos, A.M. Fonseca Junior, C.B. de Lima, J. Ispada, K. Annes, J.V.A. da Silva, M.P. Milazzotto, Embryonic Culture Supplementation (ECS) – a new culture system based on oviduct

and uterus fluid composition improves bovine embryo production and quality 33rd Annual Meeting of the Brazilian Embryo Technology Society, Animal Reproduction, 2019, pp. 674.

[31] F. Gandolfi, R.M. Moor, Stimulation of early embryonic development in the sheep by co-culture with oviduct epithelial cells, *J Reprod Fertil* 81 (1987) 23-28. 10.1530/jrf.0.0810023.

[32] W.H. Eyestone, N.L. First, Co-culture of early cattle embryos to the blastocyst stage with oviducal tissue or in conditioned medium, *J Reprod Fertil* 85 (1989) 715-720.

[33] N. Ouhibi, Y. Menezo, G. Benet, B. Nicollet, Culture of epithelial cells derived from the oviduct of different species, *Hum Reprod* 4 (1989) 229-235. 10.1093/oxfordjournals.humrep.a136877.

[34] B. Schmaltz-Panneau, A. Cordova, S. Dhorne-Pollet, C. Hennequet-Antier, S. Uzbekova, E. Martinot, S. Doret, P. Martin, P. Mermillod, Y. Locatelli, Early bovine embryos regulate oviduct epithelial cell gene expression during in vitro co-culture, *Anim Reprod Sci* 149 (2014) 103-116. 10.1016/j.anireprosci.2014.06.022.

[35] A.K. Talukder, M.B. Rashid, M.S. Yousef, K. Kusama, T. Shimizu, M. Shimada, S.S. Suarez, K. Imakawa, A. Miyamoto, Oviduct epithelium induces interferon-tau in bovine Day-4 embryos, which generates an anti-inflammatory response in immune cells, *Sci Rep* 8 (2018) 7850. 10.1038/s41598-018-26224-8.

[36] K. Goto, Y. Kajihara, S. Kosaka, M. Koba, Y. Nakanishi, K. Ogawa, Pregnancies after co-culture of cumulus cells with bovine embryos derived from in-vitro fertilization of in-vitro matured follicular oocytes, *J Reprod Fertil* 83 (1988) 753-758. 10.1530/jrf.0.0830753.

[37] S.E. Ulbrich, K. Zitta, S. Hiendleder, E. Wolf, In vitro systems for intercepting early embryo-maternal cross-talk in the bovine oviduct, *Theriogenology* 73 (2010) 802-816. 10.1016/j.theriogenology.2009.09.036.

[38] R. Rottmayer, S.E. Ulbrich, S. Kölle, K. Prella, C. Neumueller, F. Sinowatz, H.H. Meyer, E. Wolf, S. Hiendleder, A bovine oviduct epithelial cell suspension culture system suitable for studying embryo-maternal interactions: morphological and functional characterization, *Reproduction* 132 (2006) 637-648. 10.1530/rep.1.01136.

[39] M.A. Ferraz, H.H. Henning, T.A. Stout, P.L. Vos, B.M. Gadella, Designing 3-Dimensional In Vitro Oviduct Culture Systems to Study Mammalian Fertilization and Embryo Production, *Ann Biomed Eng* (2016). 10.1007/s10439-016-1760-x.

[40] S. Chen, S.E. Palma-Vera, M. Langhammer, S.P. Galuska, B.C. Braun, E. Krause, A. Lucas-Hahn, J. Schoen, An air-liquid interphase approach for modeling the early embryo-maternal contact zone, *Sci Rep* 7 (2017) 42298. 10.1038/srep42298.

[41] C.A. Simintiras, T. Fröhlich, T. Sathyapalan, G.J. Arnold, S.E. Ulbrich, H.J. Leese, R.G. Sturmey, Modelling oviduct fluid formation in vitro, *Reproduction* (2016). 10.1530/REP-15-0508.

[42] V.A. van der Weijden, S. Chen, S. Bauersachs, S.E. Ulbrich, J. Schoen, Gene expression of bovine embryos developing at the air-liquid interface on oviductal epithelial cells (ALI-BOEC), *Reprod Biol Endocrinol* 15 (2017) 91. 10.1186/s12958-017-0310-1.

[43] R. Lopera-Vásquez, M. Hamdi, B. Fernandez-Fuertes, V. Maillo, P. Beltrán-Breña, A. Calle, A. Redruello, S. López-Martín, A. Gutierrez-Adán, M. Yañez-Mó, M. Ramirez, D. Rizos, Extracellular Vesicles from BOEC in In Vitro Embryo Development and Quality, *PLoS One* 11 (2016) e0148083. 10.1371/journal.pone.0148083.

[44] C. Simon, D.W. Greening, D. Bolumar, N. Balaguer, L.A. Salamonsen, F. Vilella, Extracellular Vesicles in Human Reproduction in Health and Disease, *Endocr Rev* 39 (2018) 292-332. 10.1210/er.2017-00229.

[45] A. Bridi, F. Perecin, J.C.D. Silveira, Extracellular Vesicles Mediated Early Embryo-Maternal Interactions, *Int J Mol Sci* 21 (2020). 10.3390/ijms21031163.

[46] M. Hishinuma, Y. Takahashi, H. Kanagawa, Isolation and monolayer culture of bovine oviduct epithelial cells, *Nihon Juigaku Zasshi* 51 (1989) 1201-1208. 10.1292/jvms1939.51.1201.

- [47] M.A.M.M. Ferraz, H.H.W. Henning, P.F. Costa, J. Malda, F.P. Melchels, R. Wubbolts, T.A.E. Stout, P.L.A.M. Vos, B.M. Gadella, Improved bovine embryo production in an oviduct-on-a-chip system: prevention of poly-spermic fertilization and parthenogenic activation, *Lab Chip* 17 (2017) 905-916. 10.1039/c6lc01566b.
- [48] M.P. Wijayagunawardane, S.P. Kodithuwakku, D. Yamamoto, A. Miyamoto, Vascular endothelial growth factor system in the cow oviduct: a possible involvement in the regulation of oviductal motility and embryo transport, *Mol Reprod Dev* 72 (2005) 511-520. 10.1002/mrd.20379.
- [49] S. Danesh Mesgaran, J. Sharbati, R. Einspanier, C. Gabler, mRNA expression pattern of selected candidate genes differs in bovine oviductal epithelial cells in vitro compared with the in vivo state and during cell culture passages, *Reprod Biol Endocrinol* 14 (2016) 44. 10.1186/s12958-016-0176-7.
- [50] S. Palma-Vera, R. Einspanier, J. Schoen, Bovine oviductal epithelial cells: long term culture characterization and impact of insulin on cell morphology, *Reprod Biol* 14 (2014) 206-212. 10.1016/j.repbio.2014.04.006.
- [51] R. Gualtieri, V. Mollo, S. Braun, V. Barbato, I. Fiorentino, R. Talevi, Long-term viability and differentiation of bovine oviductal monolayers: bidimensional versus three-dimensional culture, *Theriogenology* 78 (2012) 1456-1464. 10.1016/j.theriogenology.2012.06.010.
- [52] R. Gualtieri, V. Mollo, S. Braun, V. Barbato, I. Fiorentino, R. Talevi, Bovine oviductal monolayers cultured under three-dimension conditions secrete factors able to release spermatozoa adhering to the tubal reservoir in vitro, *Theriogenology* 79 (2013) 429-435. 10.1016/j.theriogenology.2012.10.014.
- [53] M.P. Wijayagunawardane, N. Hambruch, J.D. Haeger, C. Pfarrer, Effect of epidermal growth factor (EGF) on the phosphorylation of mitogen-activated protein kinase (MAPK) in the bovine oviduct in vitro: Alteration by heat stress, *J Reprod Dev* 61 (2015) 383-389. 10.1262/jrd.2014-061.
- [54] A. Cordova, C. Perreau, S. Uzbekova, C. Ponsart, Y. Locatelli, P. Mermillod, Development rate and gene expression of IVP bovine embryos cocultured with bovine oviduct epithelial cells at early or late stage of preimplantation development, *Theriogenology* 81 (2014) 1163-1173. 10.1016/j.theriogenology.2014.01.012.
- [55] L. Jordaens, M. Arias-Alvarez, I. Pintelon, S. Thys, S. Valckx, Y. Dezhkam, P.E. Bols, J.L. Leroy, Elevated non-esterified fatty acid concentrations hamper bovine oviductal epithelial cell physiology in three different in vitro culture systems, *Theriogenology* 84 (2015) 899-910. 10.1016/j.theriogenology.2015.05.030.
- [56] M. Hamdi, M.J. Sánchez-Calabuig, B. Rodríguez-Alonso, S. Bagés Arnal, K. Roussi, R. Sturmey, A. Gutiérrez-Adán, P. Lonergan, D. Rizos, Gene expression and metabolic response of bovine oviduct epithelial cells to the early embryo, *Reproduction* 158 (2019) 85-94. 10.1530/REP-18-0561.
- [57] E.V. García, M. Hamdi, A.D. Barrera, M.J. Sánchez-Calabuig, A. Gutiérrez-Adán, D. Rizos, Bovine embryo-oviduct interaction, *Reproduction* 153 (2017) 631-643. 10.1530/REP-16-0654.
- [58] A.V. Carvalho, E. Canon, L. Jouneau, C. Archilla, L. Laffont, M. Moroldo, S. Ruffini, E. Corbin, P. Mermillod, V. Duranthon, Different co-culture systems have the same impact on bovine embryo transcriptome, *Reproduction* 154 (2017) 695-710. 10.1530/REP-17-0449.
- [59] B.P.S. Cometti, R.K. Dubey, B. Imthurn, M. Rosselli, Natural and environmental oestrogens induce TGFB1 synthesis in oviduct cells, *Reproduction* 155 (2018) 233-244. 10.1530/REP-17-0425.
- [60] T. Greve, H. Callesen, Rendez-vous in the oviduct: implications for superovulation and embryo transfer, *Reprod Nutr Dev* 41 (2001) 451-459.
- [61] R. Sartori, M.R. Bastos, M.C. Wiltbank, Factors affecting fertilisation and early embryo quality in single- and superovulated dairy cattle, *Reprod Fertil Dev* 22 (2010) 151-158. 10.1071/RD09221.

- [62] F.C. Dias, M.I. Khan, M.A. Sirard, G.P. Adams, J. Singh, Differential gene expression of granulosa cells after ovarian superstimulation in beef cattle, *Reproduction* 146 (2013) 181-191. 10.1530/REP-13-0114.
- [63] A. García Guerra, A. Tribulo, J. Yapura, J. Singh, R.J. Mapletoft, Lengthening the superstimulatory treatment protocol increases ovarian response and number of transferable embryos in beef cows, *Theriogenology* 78 (2012) 353-360. 10.1016/j.theriogenology.2012.02.010.
- [64] P.H. Santos, R.A. Satrapa, P.K. Fontes, F.F. Franchi, E.M. Razza, F. Mani, M.F.G. Nogueira, C.M. Barros, A.C.S. Castilho, Effect of superstimulation on the expression of microRNAs and genes involved in steroidogenesis and ovulation in Nelore cows, *Theriogenology* 110 (2018) 192-200. 10.1016/j.theriogenology.2017.12.045.
- [65] S. Ghersevich, E. Massa, C. Zumoffen, Oviductal secretion and gamete interaction, *Reproduction* 149 (2015) R1-R14. 10.1530/REP-14-0145.
- [66] B.E. Barton, G.G. Herrera, P. Anamthathmakula, J.K. Rock, A. Willie, E.A. Harris, K.I. Takemaru, W. Winuthayanon, Roles of steroid hormones in oviductal function, *Reproduction* 159 (2020) R125-R137.
- [67] S.E. Ulbrich, A. Kettler, R. Einspanier, Expression and localization of estrogen receptor alpha, estrogen receptor beta and progesterone receptor in the bovine oviduct in vivo and in vitro., *J Steroid Biochem Mol Biol* 84 (2003) 279-289. S0960076003000396 [pii].
- [68] G.J. Killian, D.A. Chapman, J.F. Kavanaugh, D.R. Deaver, H.B. Wiggin, Changes in phospholipids, cholesterol and protein content of oviduct fluid of cows during the oestrous cycle, *J Reprod Fertil* 86 (1989) 419-426. 10.1530/jrf.0.0860419.
- [69] J.W. McDaniel, H. Scalzi, D.L. Black, Influence of ovarian hormones on histology and histochemistry of the bovine oviduct, *J Dairy Sci* 51 (1968) 754-761. 10.3168/jds.S0022-0302(68)87067-5.
- [70] S. Bauersachs, S. Rehfeld, S.E. Ulbrich, S. Mallok, K. Prella, H. Wenigerkind, R. Einspanier, H. Blum, E. Wolf, Monitoring gene expression changes in bovine oviduct epithelial cells during the oestrous cycle, *J Mol Endocrinol* 32 (2004) 449-466.
- [71] K.L. Cerny, E. Garrett, A.J. Walton, L.H. Anderson, P.J. Bridges, A transcriptomal analysis of bovine oviductal epithelial cells collected during the follicular phase versus the luteal phase of the estrous cycle, *Reprod Biol Endocrinol* 13 (2015) 84. 10.1186/s12958-015-0077-1.
- [72] A.M. Gonella-Díaza, S.C. Andrade, M. Sponchiado, G. Pugliesi, F.S. Mesquita, V. Van Hoeck, R.e.F. Strefezzi, G.R. Gasparin, L.L. Coutinho, M. Binelli, Size of the Ovulatory Follicle Dictates Spatial Differences in the Oviductal Transcriptome in Cattle, *PLoS One* 10 (2015) e0145321. 10.1371/journal.pone.0145321.
- [73] J. Lamy, V. Labas, G. Harichaux, G. Tsikis, P. Mermillod, M. Saint-Dizier, Regulation of the bovine oviductal fluid proteome, *Reproduction* 152 (2016) 629-644. 10.1530/REP-16-0397.
- [74] C. Almiñana, G. Tsikis, V. Labas, R. Uzbekov, J.C. da Silveira, S. Bauersachs, P. Mermillod, Deciphering the oviductal extracellular vesicles content across the estrous cycle: implications for the gametes-oviduct interactions and the environment of the potential embryo, *BMC Genomics* 19 (2018) 622. 10.1186/s12864-018-4982-5.
- [75] S. Chen, S.E. Palma-Vera, B. Kempisty, M. Rucinski, A. Vernunft, J. Schoen, In Vitro Mimicking of Estrous Cycle Stages: Dissecting the Impact of Estradiol and Progesterone on Oviduct Epithelium, *Endocrinology* 159 (2018) 3421-3432. 10.1210/en.2018-00567.
- [76] L.B. Ferré, M.E. Kjelland, L.B. Strøbech, P. Hyttel, P. Mermillod, P.J. Ross, Review: Recent advances in bovine, *Animal* 14 (2020) 991-1004. 10.1017/S1751731119002775.
- [77] M.A. Sirard, 40 years of bovine IVF in the new genomic selection context, *Reproduction* 156 (2018) R1-R7. 10.1530/REP-18-0008.