Nº PROYECTO : 1140658  
DURACIÓN : 3 años  
AÑO ETAPA : 2016

TÍTULO PROYECTO : NEW INSIGHTS IN TEMPORAL REGULATION OF FOLLICULAR-OOCYTE INTERACTIONS IN CANINE: FUNCTIONS OF PARACRINE FACTORS ON IN VIVO AND IN VITRO MATURATION EVENTS

DISCIPLINA PRINCIPAL : CIENCIAS VETERINARIAS
GRUPO DE ESTUDIO : SALUD PROD ANIM
INVESTIGADOR(A) RESPONSABLE : MONICA ISABEL DE LOS REYES SOLOVERA
DIRECCIÓN :
COMUNA :
CIUDAD : Santiago
REGION : METROPOLITANA
MODIFICACIONES ACADÉMICAS

El informe no presenta modificaciones académicas.
PROJECT RESULTS:
Describe the results of your research in reference to its original and/or modified Project objectives.
The maximum extension of this section is 5 pages (Arial or Verdana font, size 10).

In this proposal we hypothesized that the endogenous production of GDF-9 and BMP-15 in canine are follicle stage-specific and the temporal influence of these factors will be determinant in different parameters, affecting the follicular the oocyte developmental competence. Therefore, the main objective was to explore the system of GDF-9 and BMP-15, as a transcripts and proteins in ovaries of cycling bitches, through preantral follicles, and at different follicular stage in order to analyze the temporal relationships existing between these factors and the developing follicles and oocyte competence. All objectives were completely finished and the results of this project are presented according the specific goals. The figures and tables are shown in Annexes.

Specific Goals

Goal 1.-In this first objective we proposed to investigate whether levels of GDF-9 and BMP-15 mRNA (by qRT-PCR) and GDF-9 and BMP-15 proteins vary in vivo at different stages of folliculogenesis, and to characterize the relationship of these important factors in canine during follicular development throughout estrus cycle.

Results:
mRNA: Ovarian structures (follicles and corpus luteum) and plasma progesterone concentration were used to confirm the physiological status of each donor. Denuded oocytes and their follicular cells were recovered from follicles (n=675) distributed into four types (preantral, small antral ~0.2-0.39 mm, medium antral ~0.4-5.9 mm and large antral ~6-8 mm). Total RNA was extracted and reverse transcribed and the levels of expression for these two genes were determined using a quantitative real-time polymerase chain reaction (qRT-PCR) technique; the data were evaluated by ANOVA. Relative expressions levels of GDF-9 and BMP-15 transcripts were detected in the oocyte and follicular cells in all follicular stages evaluated, showing differential changes (P<0.05) during development over the estrus cycle (Annexes Fig 1). The expression patterns of both transcripts were highly correlated between follicles cells and oocytes (r >0.8; P<0.05 for GDF-9 and BMP-15), although GDF-9 was expressed at higher levels (P<0.05) in the oocyte compared with the follicle cells (Annexes Fig 2). All cell types showed more GDF-9 mRNA abundance at early developing stages, mainly in the anestrus phase, and declining levels in the later stages (P < 0.05), whereas BMP-15 mRNA levels increased (P< 0.05) in follicular cells and oocytes from the preantral to the later stages, and remained constant during the final preovulatory stage. Therefore, these two genes were detected in dog follicular cells and oocytes and were differentially expressed during the follicular development across the estrus cycle.

Proteins: Dog follicular cells at different stages of development and the estrous cycle were incubated with anti-human GDF-9 polyclonal and anti-mouse BMP-15 monoclonal antibodies. A size and complexity discriminatory gate was used for the cytometry analysis in the initial dot plot and, additionally, a CD45 marker for leukocyte and propidium iodide (PI) were used for erythrocyte and debris discrimination. It was corroborated the presence of both proteins in canine follicle cells, but these proteins were not expressed equally during follicular development (Annexes Fig 3). The results analyzed by ANOVA showed that GDF-9 expression decreased (P<0.05) during follicular growth in anestrus and proestrous/estrous, but increased in diestrus (P<0.05) (Annexes Table1). The expression levels of BMP-15 rose (P<0.05) from small to medium sizes in anestrus without changing at diestrus. Small antral follicles expressed the highest values of GDF-9 at anestrus while only BMP-15 showed higher value in small antral follicles at proestrous-estrous compared to diestrus and anestrus. Both proteins decreased in proestrous/estrous (P<0.05) with increasing follicle size, registering the lowest levels in large follicles.

Products
Goal 2 We proposed in this goal to study the temporal relationships existing between GDF-9 and BMP-15 on LH-R (mRNA and proteins) and thus the possible influence of these factors on the expression of LH-R.

Results

Dog ovaries were obtained from bitches at proestrus/estrus, anestrus and diestrus stages following ovariohysterectomy as describe before. Follicular cells were mechanically recovered from follicles distributed into preantral, small antral, medium antral and large antral. Total RNA extraction was performed and the evaluation of gene expression levels was achieved by relative quantification q-PCR analysis. Intrafollicular amounts of LHR were assessed by western blot method. The expression levels of mRNA LHR in follicular cells were observed in every stage of development, however this gene expression varied over the estrous cycle. LHR transcripts increased (P<0.05) from preantral to antral stage. There were not differences in LHR gene expression among follicles at preantral stages; however, at antral stages the lowest (P<0.05) LHR mRNA expression was found at anestrus and the highest (P<0.05) at proestrues/estrus (Annexes Fig.4) The LHR protein was also detected in dog follicles in all reproductive phases with patterns varying with stage of follicular development over the reproductive cycle (Annexes Fig.5)

Comparing the levels of GDF-9 and BMP-15 with LHR, there was a positive correlation between these paracrine factors and LHR during preantral and early antral follicular stage (P<0.05), but a negative correlation during preovulatory period (P<0.05) (Annexes Fig 6 and 7). These findings obtained from our studies, that BMP15 and GDF9 transcripts did not increase in the preovulatory stages and the decreasing levels of the BMP15 and GDF9 proteins observed in late follicular development in canines, might be related to the preovulatory follicular luteinization process observed in canines. In other species BMP15 and GDF9 can inhibit the early expression of the LH receptor (LHR) on the granulosa cells surrounding the oocyte, indicative of a role in suppression of luteinization. But we found in canine follicles that the LHR and its messenger are present even at preantral stage, as well as GDF-9 and BMP-15, but increasing during follicular growth, mainly during prostrous/estrous, concomitant with GDF-9 and BMP15 decrease at this time. Therefore, our studies suggest that the minor expression of these paracrine factors as the high expression levels of LHR in preovulatory follicles might have an effect on the luteinizing condition in preovulatory canine follicles.

Products

The results of this objective gave rise one paper (De los Reyes M, Palomino M, Parraguez VH, Ramirez F. Analysis of LH receptor in canine ovarian follicles throughout the estrous cycle. Theriogenology 2017, 93:71-77); one undergraduate Thesis (Fernando Ramirez, "Análisis del receptor de la hormona luteinizante (LH-R) durante el desarrollo folículo en la perra ", Fac Cs Vet y Pec. U de Chile); three Congress presentation (Ramírez F, Palomino J, Parraguez VH, De los Reyes M. Luteinizing Hormone receptor RNA gene expression in canine developing follicles at anestrous and diestrous stages. 18th EVSSAR Congress. 11th and 12th September Hannover, Germany 2015; De los Reyes, M, Palomino J, Ramirez F. Expression of luteinizing hormone receptor messenger RNA by canine ovarian follicles during the estrous cycle. 18th International Congress on Animal Reproduction (ICAR) Tours, France. June 26-30, 2016; De los Reyes M, Palomino J, Ramirez F. Evaluation of luteinizing hormone receptor (LHR) over the estrus cycle in canine ovaries. 43rd Annual Conference of the IETS. Austin, Texas USA, 9-12 January, 2017. And one submitted abstract (Palomino J, De los Reyes M. Relationship between TGF-b members and LHR mRNA expression in canine follicular cells over the estrous cycle. Submitted to 50th Annual Meeting of the Society for the Study of Reproduction, Washington DC, July 13-16 2017).
**Goal 3.** This objective was to correlate the GDF-9 and BMP-15 findings in follicles and the oocyte quality.

The ovaries were collected from 9 adult bitches (1-6 y) at different stage of the estrous cycle, following ovariohysterectomy. Antral medium follicles were excised from the ovary. The oocytes and its cumulus cells (COCs) were carefully liberated from antral follicles and approximately 200 oocytes morphologically classified as grade 1 oocytes (oocytes > 100 μm, surrounded by two or more dense layers of cumulus cells, darkly pigmented cytoplasm)(Annexes Fig 8A) or non-grade 1 oocytes (grades 2 or 3)(Annexes Fig 8B) and their corresponding follicular fluid from each follicle was collected by flushing followed by cutting open each follicle with a small scalped blade and gently scraping with a needle into the same flushing fluid to recovery granulosa and theca cells. The GDF-9 and BMP-15 levels were evaluated by Flow Cytometry as previously described (objective 1).

There were different levels (P<0.05) of BMP-15 in follicles with oocytes grade 1 or follicles with oocytes grade 2,3 in all phases of the estrous cycle. In contrast, there was not a significant difference in GDF-9 levels between the different type of oocytes, excepting in anestrus phase. Minor percentage (P<0.05) of BMP-15 was observed in follicular cells from follicles with non-healthy oocytes than those with healthy appearance (Annexes Table 3). Overall results highlight differential effects of these two main TGF-β protein on oocyte characteristics, supporting the concept that an adequate amount of BMP-15 secreted in the follicular cells may be critical for canine fertility.

**Goal 4.** In the same way, in this aim we proposed to determine the incidence of atresia among ovarian follicles at different sizes and estrous cycle, and whether the incidence of atresia is a function of the presence or absence of the paracrine factors GDF-9 and BMP-15.

The follicular cells were collected from the different follicles by scraping the granulosa and theca cells and also by the aspiration of the intrafollicular contents from dissected follicles. Follicular cells were placed into PBS and then fixed in 4% paraformaldehyde-PBS.

a) **Histology**, fixed cells were mounted on glass slides coated with silane (aminoalkylsilane) (Silane-Prep Slides, Sigma Diagnostics, St. Louis, MO). The proportion of apoptotic cells in the granulosa cell compartment was determined by the TUNEL procedure using the In Situ Cell Death Detection Kit (Roche, Mannheim, Germany). Accordingly, follicular cells were labeled with the TUNEL reaction mixture for 60 min at 37 °C in accordance with the manufacturer’s instructions. Scoring for TUNEL-positive cells was carried out by counting the number of positive nuclei per field in 5 randomly using a fluorescent microscopy at x400 magnification. Approximately 100 to 250 nuclei were screened per each field. For each condition, all data from three to five separate bitches obtained from separate experiments were pooled to obtain final cell numbers (Annexes Fig 9).

b) **Flow Cytometry** Determination of the percentage of cells with apoptosis (TUNEL +) (In Situ Cell Death Detection Kit, Roche), and GDF-9 and BMP-15 for follicles cells was carried out using a 6 color Gallius Flow Cytometer (Beckman Coulter, Brea, CA, USA). A size and complexity discriminatory gate was used for the cytometric analysis as previously describe in Objective 1 (Annexes, Fig 10 and Table 4).

The results obtained with both methods were coincident. Surprisingly, we found the greatest number of apoptotic cells in estrous (P<0.05) and the lowest percentage was registered in anestrus phase. Comparing among follicular sizes, the percentage of apoptotic cells increased with follicular size. Both, estrous phases and follicular sizes influenced the levels of paracrine factors, mainly GDF-9, therefore the incidence of atresia among ovarian follicles at different sizes and estrous cycle may be a function of the absence of this paracrine factor.

**Products**

The results of objectives 3 and 4 are considered for one manuscript (not finished yet); one undergraduate research (Tomás Fernandez, Reconocimiento y Extracción de Folículos Ováricos Caninos, Fac Cs Vet y Pec. U de Chile). One undergraduate Thesis, the student will register her thesis in the course of April. And one submitted abstract (De los Reyes, M, Paomino J, Quezada B. Apoptosis in follicular cells during follicular development: relationship with the oocyte morphology. XX Congress of the European Veterinary Society for Small Animal Reproduction EVSSAR, June 29th-July 1st 2017, Vienna, Austria.
Goal 5. In this aim we proposed to analyze in canines the presence and cellular localization of GDF-9 and BMP-15 proteins in follicles cells and oocytes during follicle development.

Results

The presence and localization of GDF-9 and BMP-15 proteins in follicles cells and oocytes during follicle development was determined by immunohistochemistry as previously described in Methodology. In brief, sections of 4% paraformaldehyde in PBS ovaries obtained from 15 bitches at different stage of oestrous cycle (proestrus-oestrus n = 5; diestrus n = 5; anestrus n = 5) were dehydrated (xylene–ethanol solutions) and embedded into paraffin blocks. Individual paraffin sections were inactivated with methanol/H₂O₂. Sections were blocked at 37 °C in the peroxidase reagent and incubated with rabbit anti-human GDF-9 (1:200) or goat anti-human BMP-15 (1:150) antibodies overnight at 4°C and with secondary antibodies goat anti-rabbit IgG and donkey anti-goat IgG, both HRP conjugated. Previously negative control sections received rabbit serum. Sections were stained with 1 mg mL⁻¹ 3,3-diaminobenzidine (DAB) and counterstained with haematoxylin. All sections were examined at magnification × 200 using an IX71 inverted microscope and the intensity of immunostaining in both oocyte and follicular cells of each follicle was evaluated with Image J version 1.45s software. The images were transformed to grey scale and the corrected total cell mark was calculated by subtracting the mean staining of background reading to the integrated density of each sample. The results showed that samples of all slices contained primordial, primary, secondary and pre-antral follicles as well as different sizes of antral follicles showed positive GDF-9 and BMP-15 immunoreactions in the oocyte cytoplasm and in the follicles cells (Annexes Fig 11 and 12). No such labelling was found in the negative controls. Follicle sizes differed in GDF-9 and BMP-15 immunoreactions according to estrous cycle (Annexes Fig 13). The pattern of GDF-9 immunostaining in proestrus, oestrus, and diestrus was more intense within preantral rather than antral follicles/oocytes, whereas BMP-15 immunoreaction was prominent mainly in proestrus stage antral stage. These results indicated temporal pattern of GDF-9 and BMP-15 in canine ovarian follicles that might play important roles in follicular/oocyte development and ovulation during oestrous cycle.

Products

The results of this objective gave rise one undergraduate research Unit (Camila Gomez, Inmunocitoquímica en ovarios de perra para detección de proteínas BMP-15 y GDF-9, Fac Cs Vet y Pec. U de Chile), one undergraduate Thesis (Diane Maupeu, Localisation des facteurs de croissance GDF 9 et BMP 15 dans les follicules et ovocytes canins par immunohistochimie, Ecole Nationale Vétérinaire de Toulouse, France). One Congress presentation (Maupeu D, Palomino J, De los Reyes M. Immunohistochemistry localization of growth differentiation factor 9 (GDF-9) and bone morphogenetic protein 15 (BMP-15) in canine follicles throughout estrus cycle. 41st Annual Conference of the IETS. Versailles, France, 9-13 January, 2015.

Goal 6.- This goal proposed to investigate the function of these factors in oocyte maturation, by evaluating the effect of addition of exogenous GDF-9 and BMP-15, or GDF-9 and BMP-15 antibodies during canine IVM at different time of culture, on subsequent in vitro oocyte development and cumulus function.

For this proposes, ovaries at specific stages of the estrous cycle were collected from normal adult mixed-breed bitches following ovariohysterectomy. The estrous cycle of each bitch was estimated by evaluating the presence of developing follicles or corpus luteum as previously described. The cumulus-oocytes complexes (COCs) were obtained from each follicle via gentle pipetting using a fine bore glass pipette in PBS under a dissecting microscope. COCs were cultured in a tissue culture medium 199 (TCM199-Hepes) supplemented with 10%, FCS, 0.25 mM pyruvate, 10 IU/mL of hCG, 300 IU/mL of penicillin, 20 mg mL⁻¹ of streptomycin and 2 µg mL⁻¹ of estradiol (E₂) as a basal medium at 38 °C, 5% CO₂. Groups of oocytes were arranged in five different experimental groups considering the differentially expression of these factors during the follicular development in vivo (results of objective 1).

- Group 1: IVM oocytes incubated in basal medium (Control) for 72 h
- Group 2: oocytes incubated in basal medium plus 1/100 GDF-9 antibody (Ab) (C-I8, Santa Cruz), for 48h and then transferred to control medium without Ab for 24 h.
- Group 3: oocytes incubated in basal medium for 48 h and then transfer to control medium plus 1/50 anti-human BMP-15 Ab (R&D Systems), for 24 h.
- Group 4: oocytes incubated in basal medium plus 100 or 200 ng/mL recombinant human GDF-9 protein, (R&D Systems), for 48h and then transferred to control medium without protein for 24 h.
- Group 5: oocytes incubated in basal medium for 48 h and then transfer to control medium plus 100 or 200 ng/mL recombinant human BMP-15 protein (R&D Systems), for 24 h.
The DNA of the oocytes was stained with DAPI (10 µg mL⁻¹ in PBS) and visualized on an inverted fluorescence microscope to determine the stage of meiosis. Oocytes in which chromatin was unidentifiable or not visible were considered as non-evaluable oocytes.

**Results**

The presence of both Ab and protein BMP-15 or GDF-9 has an influence over meiotic development (Fig 14; Table 5).

- **Ab**: As it is shown in Table 5, the temporal exposure to Ab against GDF-9 or BMP-15 during IVM has a negative impact on meiotic development. Higher number of oocytes were arrested in GVBD stage when they were incubated with either GDF-9 or BMP-15 Ab in comparison to those in Control group (P<0.05). In contrast more (P<0.05) oocytes in control groups reached MI-MII stages comparing to those groups with GDF-9 or BMP-15 Ab (P<0.05). In anestrus, oocytes cultured with the Abs were not able to reach the MII stage.

- **Recombinant Proteins**: Supplementation with these two growth factors (Annexes Table 5), influenced the rate of GV stage during anestrus and diestrus. Higher percentages of oocytes in control group remained arrested at GV stage when compare to those cultured with either GDF-9 or BMP-15 recombinant proteins (P<0.05). However, there were not differences in MI-MII rates between oocytes cultured with recombinant proteins at any stage of the reproductive cycle or doses. It seems that the effect on oocyte maturation could be more relevant in the meiotic resumption rather than final oocyte maturation. The lack of effect of recombinant GDF-9 or BMP-15 on oocyte competence could indicate that the recombinant proteins need to be specific for dogs, since different species origins of recombinant proteins have different effects on granulosa cell.

When compared the effect of these recombinant proteins among anestrus, proestrus/estrous and diestrus for all meiotic stages, there was no observable dose-response to BMP15 nor GDF-9 from 100 ng mL⁻¹ to 200 ng mL⁻¹.

**In vitro** studies using recombinant GDF-9 protein have clarified the biological roles and importance of GDF-9 actions in oocyte development. The results presented herein potentially have great implications for our understanding of canine oocyte biology as well as for the development of IVM media and the improvement of IVM success rates.

**Products**

# PRODUCTOS

## ARTÍCULOS

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<td>2</td>
<td>Palomino J, De los Reyes M</td>
<td>Theriogenology</td>
<td>Temporal expression of GDF-9 and BMP-15 mRNAs in canine ovarian follicles</td>
<td>ISI</td>
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Nº: 1

Número Completo de la Revista: Revista Científica, FCV-LUZ
Título (Idioma original): EXPRESIÓN DE LA PROTEÍNA MORFOGÉNICA ÓSEA 15 (BMP-15) DURANTE LA MADURACIÓN in vitro DE OVOCITOS CANINOS/Expression of bone morphogenetic protein 15 (BMP-15) during In vitro maturation in canine oocyt
Indexación: ISI

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Número Completo de la Revista: Theriogenology
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THERIO_LH_2017.pdf
Pannexin channels increase propidium iodide permeability in 1 frozen/thawed dog spermatozoa.

Autor (a)(es/as) : Torres, JL.; Palomino, J.; Moreno, RD.; De los Reyes, M.
Título (Idioma original) : Pannexin channels increase propidium iodide permeability in 1 frozen/thawed dog spermatozoa.
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Nº : 2
Autor (a)(es/as) : Mónica De los Reyes, Jaime Palomino, Victor Hugo Parraguez, Claudia Rojas
Título (Idioma original) : FACTORES DE CRECIMIENTO DURANTE EL DESARROLLO FOLICULAR OVÁRICO EN LA PERRA Growth factors during bitch ovarian follicular development
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ISBN :
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Factores_del_Crecimiento_Libro_II_Simposio_Latinoamerica_no_de_ReproduccioI__768___n_Animal.pdf
CONGRESOS

Nº : 1
Autor (a)(es/as) : Fernández T, Palomino J, De Los Reyes M.
Título (Idioma original) : Detection of the Growth Differential Factor 9 (GDF-9) and the Bone Morphogenetic Protein 15 (BMP-15) in the bitch antral follicles by flow cytometry
Nombre del Congreso : II Simposio Latinoamericano de Reproducción Animal
País : CHILE
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II_Simposio_Latinoamericano_de_Reproduccio__769_n_Animal__Tomas.pdf

Nº : 2
Autor (a)(es/as) : Castro R, Palomino, J, De Los Reyes, M.
Título (Idioma original) : M. BMP-15 expression during follicular growth in bitch ovaries.
Nombre del Congreso : II Simposio Latinoamericano de Reproducción Animal
País : CHILE
Ciudad : Santiago
Fecha Inicio : 13/11/2014
Fecha Término : 14/11/2014
Nombre Publicación : Proceeding II Simposio Latinoamericano de Reproducción Animal
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II_Simposio_Latinoamericano_de_Reproduccio__769_n_Animal._Rodrigo_.pdf
3. **Autor (a)(es/as):** Ramirez F, Palmino J, De los Reyes M  
**Título (Idioma original):** ESTUDIO DE LA EXPRESIÓN DE GDF-9 EN CÉLULAS DEL CÚMULO CANINO EVALUADAS MEDIANTE CITOMETRÍA DE FLUJO. (Study of GDF-9 expression in canine cumulus cells assessed by Flow Cytometry)  
**Nombre del Congreso:** II Simposio Latinoamericano de Reproducción Animal  
**País:** CHILE  
**Ciudad:** Santiago  
**Fecha Inicio:** 13/11/2014  
**Fecha Término:** 14/11/2014  
**Nombre Publicación:** Procedings II Simposio Latinoamericano de Reproducción Animal  
**Año:** 2014  
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**Páginas:**  
**Enviar documento en papel:** no  
**Archivo Asociado:**  
II_Simposio_Latinoamericano_de_Reproduccion_Animal._Fernando_.pdf

4. **Autor (a)(es/as):** Maupeu D, J Palomino J De Los Reyes M  
**Título (Idioma original):** Immunohistochemistry localization of growth differentiation factor 9 (gdf-9) and bone morphogenetic protein 15 (bmp-15) in canine follicles throughout estrus cycle.  
**Nombre del Congreso:** 41st Annual Conference of the IETS (International Embryo Transfer Society)  
**País:** FRANCIA  
**Ciudad:** Versailles  
**Fecha Inicio:** 10/01/2015  
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**Nombre Publicación:** Reproduction Fertility and Development  
**Año:** 2014  
**Vol.:** 27  
**Nº:** 1  
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**Enviar documento en papel:** no  
**Archivo Asociado:** IETS_2015.pdf

5. **Autor (a)(es/as):** Ramirez F, Palomino J, Parraguez VH, De los Reyes M
Luteinizing Hormone receptor RNA gene expression in canine developing follicles at anestrous and diestrus stages

18th European Veterinary for Small Animal Reproduction (EVSSAR) Congress

ALEMANIA
Hannover
11/09/2015
12/09/2015
Proceedings 18th European Veterinary Society For Small Animal Reproduction
2015

In vitro matured canine oocytes derived from follicles at different stage of development across the estrus cycle

8th International Symposium on Canine and Feline Reproduction (ISCFR)
FRANCIA
Paris
22/06/2016
24/06/2016
Proceedings of 8th International Symposium on Canine and Feline Reproduction (ISCFR)
2016

Expression of luteinizing hormone receptor messenger RNA by canine ovarian follicles during the estrous cycle.

18th International Congress on Animal Reproduction (ICAR)

Aspee K, Astudillo I, Palomino J, De los Reyes M.

De los Reyes M, Palomino J, Ramirez F.
Nº : 12
Autor (a)(es/as) : García, P.; Ramírez, G., Torres-Fuentes, JL; Palomino, J.; De los Reyes, M.,
Título (Idioma original) : Inhibición de bmp-15 durante la maduración in vitro de ovocitos de perra
Nombre del Congreso : XXVII Reunión Anual de la Sociedad Chilena de Reproducción y Desarrollo.
País : CHILE
Ciudad : Antofagasta
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Título de Tesis: ANÁLISIS DEL FACTOR DE CRECIMIENTO DIFERENCIAL 9 (GDF-9) Y LA PROTEÍNA MORFOGENÉTICA ÓSEA (BMP-15) DURANTE EL DESARROLLO DE FOLÍCULOS ANTRALES EN OVARIOS CANINOS MEDIANTE CITOMETRÍA DE FLUJO

Nombre y Apellidos del(de la) Alumno(a): Tomás Eduardo

Nombre y Apellidos del(de la) Tutor(a): Fernandez Vergara

Título Grado: Pregrado

Institución: Universidad de Chile

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Título de Tesis: MADURACIÓN IN VITRO DE OVOCITOS DE PERRA PROVENIENTES DE FOLÍCULOS POLIOVOCÍTICOS

Nombre y Apellidos del(de la) Alumno(a): Igor Astudillo

Nombre y Apellidos del(de la) Tutor(a): Monica De los Reyes

Título Grado: Pregrado

Institución: Facultad de Ciencias Veterinarias, Universidad de Chile

País: CHILE

Ciudad: Santiago

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Nº : 3
Título de Tesis : Estudio de la maduración in vitro de ovocitos caninos provenientes de folículos ováricos en diferentes estados del desarrollo a través del ciclo estral
Nombre y Apellidos del(de la) Alumno(a) : Karla Aspee Mallanes
Nombre y Apellidos del(de la) Tutor(a) : Mónica De los Reyes
Título Grado : Pregrado
Institución : Facultad de Ciencias Veterinarias, Universidad de Chile
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Ciudad : Santiago
Estado de Tesis : Terminada
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Nº : 4
Título de Tesis : Expresión de BMP-15 durante el desarrollo folicular ovárico y maduración in vitro de ovocitos en la perra
Nombre y Apellidos del(de la) Alumno(a) : Rodrigo Castro
Nombre y Apellidos del(de la) Tutor(a) : Mónica De los Reyes S
Título Grado : Doctorado
Institución : Universidad de Chile, Campus Sur
País : CHILE
Ciudad : Santiago
Estado de Tesis : Terminada
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Nº : 5
Título de Tesis : Análisis de los transcriptos de GDF-9 y BMP-15 en las células del cúmulo en caninos y su relación con la maduración in vitro de los ovocitos
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<td>EVALUACIÓN DEL ROL DE GDF-9 Y BMP-15 EN LA MADURACIÓN IN VITRO DE OVOCITOS DE PERRA</td>
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**Nombre y Apellidos del(de la) Alumno(a):** Georges Andre Ramírez Saboya  
**Nombre y Apellidos del(de la) Tutor(a):** Georges Andre Ramírez Saboya  
**Título Grado:** Pregrado  
**Institución:** Facultad de Ciencias Veterinarias, Universidad de Chile  
**Pais:** CHILE  
**Ciudad:** Santiago  
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**Nombre y Apellidos del(de la) Alumno(a):** Diane Maupeu  
**Nombre y Apellidos del(de la) Tutor(a):** Monica De los Reyes  
**Título Grado:** Pregrado  
**Institución:** Ecole Nationale Vétérinaire de Toulouse  
**Pais:** FRANCIA  
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**Nombre y Apellidos del(de la) Alumno(a):** Pablo Andrés García Badilla  
**Nombre y Apellidos del(de la) Tutor(a):** Mónica De los Reyes S  
**Título Grado:** Pregrado  
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Análisis del receptor de la hormona luteinizante (LHR) durante el desarrollo follicular en la perra
Fernando Ramirez Moyano
Mónica De los Reyes S
Pregrado
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ANEXOS