**International XXII EVSSAR Congress in Berlin (Germany), 28-29 June 2019**

**GUIDELINES FOR ABSTRACTS**

**Submission deadline: March 15th, 2019**

**1.** TheScientific Committee of the 22nd EVSSAR Congress invites all veterinarians to submit abstracts (related to clinical and basic research, including clinical research, laboratorial trials, retrospective studies, case reports etc.) on small, laboratory, and exotic animal reproduction.

**2.** All delegates are encouraged to submit abstracts for oral or poster presentation which will be reviewed by the Scientific Committee.

**3.** The abstract should describe the objectives and results and must include the following points:

**1.** Introduction and aim

**2.** Materials and methods: brief description of procedures including statistical analysis

**3.** Results

**4.** Conclusions

**5.** Clinical cases should include the following parts: clinical case and discussion

**4.** Abstract preparation: See the enclosed example.

Extended abstracts: must not exceed THREE pages

All other abstracts: must not exceed ONE single page.

Single-spaced 12.0 Times New Roman font and 2 cm margins (top and bottom, right and left). Title in bold small caps (Times New Roman 12.0), one blank line before author names and institution. (Please include e-mail of the corresponding author), followed by one blank line and text. Figures and tables should be minimal in abstracts.

**5.** The abstracts should be **submitted online** **before March 15th, 2019.**

The link to access the system is  <https://evssar2019.exordo.com>. Once you go to this page, you are asked for the email, first name, last name and password, then you can create your account. If you can't access your account, you should try [changing your password](https://evssar2017.exordo.com/%5B%5BForgottenPasswordURL%5D%5D). Otherwise, you can contact our [support center](http://support.exordo.com) or email support@exordo.com. We'll do our best to get back to you as soon as we can. While submitting your abstract you will find questions that have to be answered: Please indicate in the online submission form if you prefer poster or short communication presentation and whether you are a PhD/doctoral student or an ECAR resident, and whether you are an invited speaker.

Abstracts received by regular mail cannot be accepted.

**6.** Instructions for posters presentation.

Posters will remain exhibited throughout the duration of the congress. Poster board size will be 150 cm high x 120 cm wide. Posters must be written in English.

**7.** Authors will be informed about acceptance or rejection of their abstracts within 4 weeks after the submission deadline. If the reviewers require a revision of the abstract please submit a corrected version within the given period indicated in the answer Email. Abstracts that are not re-submitted in this given time will not be included in the program or the proceedings of the congress.

**8.** Abstracts will not be accepted in the following cases:

**a.** If it is submitted later than March 15th, 2019.

**b.** If no results are included.

**c.** If the abstract is not prepared on the basis of the guidelines.

**9.** Short communications will be presented in English and last 15 minutes (10 minutes presentation and 5 minutes discussion).

**Preparation of karyoplasts and cytoplasts from feline oocytes**

**at the germinal vesicle stage**

S. Chigioni, L. Perego and G.C. Luvoni

Department of Veterinary Clinical Sciences, Obstetrics and Gynaecology,

University of Milan, Italy. E-mail: cecilia.luvoni@unimi.it

**Introduction and aim**. The homologous transfer of oocyte nucleus stage I or germinal vesicle (GV-karyoplast) into an enucleated mouse oocyte (cytoplast) at the same developmental stage, resulted in resumption of meiosis (1) and embryo development in vitro (2) of the reconstructed GVoocytes. The transfer of GVs derived from cryopreserved oocytes also resulted in a normal progression to metaphase II (MII) in vitro of the reconstructed mouse and bovine oocytes (3,4). It has been shown that the GV is potentially more resilient to cooling than the spindle of MII, because chromosomes are decondensed and enclosed within the nuclear membrane. However, thawed immature cat oocytes show a poor developmental competence in vitro (5), probably due to the occurrence of chilling-induced damages to the cytoplasm. This compartment has a pivotal role in the resumption and completion of oocyte maturation, which is essential for the developmental competence of the embryo. Since the integrity of the oocyte nucleus may be better preserved than the cytoplasm after cryopreservation, the transfer of cryopreserved GVs into fresh enucleated oocytes could improve the chance of embryo development in culture. In the feline species there are no reports in the literature concerning GV transfer. Hence, the purpose of this work was to make a preliminary evaluation of the feasibility of enucleating immature oocytes in order to produce GVkaryoplasts and cytoplasts for GV transfer in cat oocytes.

**Materials and methods.** A total of 156 immature (GV) cat oocytes collected from anestrous queens after ovariectomy were mechanically deprived of cumulus cells with a small-bore pipette. The oocytes were centrifuged at 14000 rpm for 16 min to obtain the polarization of the cytoplasm and a better visualization of the GV. The nucleus was measured in order to prepare adequate microtools for manipulation. Prior to enucleation, the oocytes were incubated for 30 min at room temperature in a specific medium containing 7.5 µg/mL of cytochalasin B (Sigma Chemical Co., USA) for inducing an increase of the oolemmal elasticity and 50 µg/mL of 3-isobutyl-1- methylxanthine (IBMX, Sigma) in order to prevent the GV breakdown (1). Following lancing of the zona pellucida with a sharp-tipped pipette, GV nuclei were extruded using a bevelled glass pipette with a diameter adequate to the size of GV in cat oocytes. The GV was surrounded by a small amount of cytoplasm and encapsulated by a membrane (GV-karyoplast). The enucleated oocytes were considered as cytoplasts.

**Results.** The mean average of GV diameter in oocytes > 120 µm of diameter was 35.4 ±5.3µm. A bevelled glass pipette, with inner diameter of 40-45 µm, allowed the extrusion of intact and morphologically normal karyoplasts and related cytoplasts in 17.3% (27/156) of micromanipulated oocytes. However, the lancing of the zona pellucida or the extrusion of the karyoplast resulted in rates of 40.4% (63/156) and 42.3% (66/156) of severely damaged oocytes, respectively.

**Conclusions.** These results suggest that is possible to prepare karyoplasts and cytoplasts from felineoocytes, although the efficiency of the technique is low compared to what has been obtained in mouse (around 90%, 1). This is likely due to the thickness and hardness of zona pellucida, and to the larger diameter of the GV of cat oocytes compared to that of mouse (15 µm) or bovine (25-30 µm) oocytes. Further experiments based on the partial dissection of the zona pellucida with an acidic solution in order to reduce the oocyte damage and to improve the efficiency of GV transfer in feline oocytes, are in progress in our laboratory.

**References** 1) Liu et al., Human Reprod 1999; 14:2357-61. 2) Takeuchi et al., Hum Reprod 2004;19:975–81. 3) Moffa et al., Human Reprod 2002;17:178-83. 4) Luciano et al., Reprod Fertil Dev 2006;18:138. 5) Luvoni and Pellizzari, Theriogenology 2000;53:1529-40.