High-Resolution Ultrasonography of Xenografted Domestic Cat Ovarian Cortex

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Abstract. Transplantation of ovarian tissue has high potential for female gamete conservation. However, optimal timing of oocyte recovery for in vitro maturation and fertilization is still critical. Therefore, the aim of the present study was to use high-resolution transcutaneous ultrasonography to monitor follicular development within xenografted ovarian tissue. Ovarian cortex fragments (n=44) from domestic cats were transplanted into athymic nude rats (n=12). Graft development in the animals was assessed weekly by high frequency ultrasound (10–22 MHz) under two different FSH regimes. Blood collection for serum estradiol determination and vaginal smears were performed simultaneously. The xenografts were removed at different time points according to the ultrasound findings. The survival rate of the transplants 4 weeks after surgery was 54.5% and antral follicular growth was observed within 10 grafts from 5 different hosts (8.6 ± 6.43 follicles per graft). Early follicle antrums could be detected from 0.4 mm onwards. The growth rate of the antral cavity was calculated from weekly measurements (0.56 ± 0.44 mm per week). Although vaginal cells and estradiol levels followed a cyclic pattern, no correlation was found between follicular diameter, estradiol and keratinized vaginal cells. We recovered 5, 1 and 4 cumulus oocyte complexes from three different individuals during weeks 19, 21, and 23 respectively. Extrusion of a polar body (1 oocyte) and germinal vesicle breakdown (7 oocytes) indicated progression of maturation after in vitro culture.

We conclude that ultrasonography provided a reliable method to examine xenograft survival and follicular development within the grafts. Furthermore, this technique is suitable for assessment of the efficiency of hormonal treatment and narrowing of the optimal time frame for oocyte retrieval. To our knowledge, this is the first report of the in vivo development of early antral follicles in mammalian species.

Key words: Cat, Ovary, Ultrasonography, Xenotransplantation

Application of advanced reproductive techniques (e.g., in vitro fertilization and embryo transfer) to assisted breeding program for domestic and endangered zoo and wild animals is often limited by the availability of mature female germ cells. The mammalian ovary contains a huge primordial pool of oocytes representing a rich source of genetic material. Female gametes finish mitotic multiplication during the perinatal phase and arrest enclosed in primordial follicles in the first of two reduction

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divisions. During the reproductive lifespan follicles are constantly recruited from this dormant pool to enter the growth and developmental phase until the large antral stages with mature oocytes at the time of ovulation [1–3]. Collection of gametes from preantral follicle stages and maturation in vitro would be an enormous advantage. Full-term culture of primordial follicles to a mature stage and birth of viable offspring after in vitro fertilization and embryo transfer has only been achieved in mice (first described by Eppig and O’Brien, [4]). The follicle growth period is estimated to last up to several months in large animals, such as the pig (84 days [5]) and cow (200–220 [6]), and culture systems for them are difficult to develop. In vitro oocyte growth has been established for several species, such as the pig [7], pig [8–10], sheep [11] and cow [12–15], but these previous studies only covered a limited phase of the oocyte and follicle development.

Since severely compromised immunodeficient mouse (SCID) and athymic rat strains are available [16–18], transplantation of ovarian tissue into a xenogeneic host has been discussed as an alternative long-term system. Gosden et al. [19] first reported xenotransplantation of fresh ovarian domestic cat and sheep cortical slices in SCID mice. Autopsy showed transplant survival and development of antral follicles for up to nine month after grafting. More recently, Bosch et al. [20] demonstrated antral follicle stages following xenografting of cortex fragments from frozen/thawed domestic cat ovaries in SCID mice by post-mortem histology two months after grafting. During the last decade, it has been shown in a range of species, including humans that small follicles in fresh and frozen/thawed ovarian cortical xenogenic tissue transplants survive and develop to antral stages [21–24]. The full potential of xenografting has already been demonstrated to produce in vitro fertilized healthy and fertile offspring after graft of mouse ovaries into athymic nude rat recipients and in vitro culture of collected cumulus-oocyte complexes [25]. Valuable evidence has been obtained in livestock animals showing that the combination of xenografting and in vitro culture can successfully produce fertilization competent oocytes. Senbon et al. [26] reached the 5–8 cell stage after in vitro culture and fertilization of xenografted bovine secondary follicles. In pigs, a blastocyst was obtained from in vitro fertilized oocytes after recovery from xenotrans-
able tool (i) for monitoring transplant survival of cat ovarian tissue xenografted into female athymic nude rats and (ii) for identification of the optimal time for xenograft and oocyte removal.

Materials and Methods

All chemicals were obtained from Sigma (Sigma-Aldrich, St. Louis, MO, USA) unless stated otherwise and were of the highest purity available.

Xenografts

Domestic cats served as model species for other felids because their ovaries were easy to obtain after routine ovariectomy at local animal shelters. The female cats (n=9) were between 10 months and approximately three years of age. Immediately after removal, the ovaries were placed in Dulbecco’s phosphate buffered saline solution (D-PBS) containing 1% (v/v) antibiotic/antimycotic solution at room temperature (RT) and stored within a refrigerator (4°C). Transport to the laboratory was always within 4 to 6 h. They were then freed from connective tissue and washed in sterile D-PBS. The ovarian cortex was sliced to rupture visible follicles and was further cut into small pieces (1–2 mm³). Luteal tissue was discarded. Ovary pieces were kept in sterile D-PBS until transplantation. All procedures were conducted as fast as possible at RT.

Recipients

Female athymic nude rats (HsdRHFoxn 1 rnu; Harlan Winkelmann, Berlin, Germany) were selected as recipients for ovarian xenografts. The rats were housed at the laboratory of Harlan Winkelmann in filter-topped cages in an isolated positive pressure cupboard. They had ad libitum access to gamma-irradiated food pellets and sterile water. The light-cycle was set to 12 h light /12 hrs dark.

Ethics

All examinations were approved by the Landesamt für Arbeitsschutz, Gesundheitsschutz und Technische Sicherheit, Berlin, Germany.

Xenotransplantation

Transplantation was performed for a total of 12 nude rats. Each animal received 0.25 mg/kg Fina-dyne® (Essex Tierarznei, Munich, Germany) and 5 mg vitamin E (Sanum-Kehlbeck, Hoya, Germany). The rats were anaesthetized with isoflurane (1.0–1.5 Vol. %, Forene; Abbott, Wiesbaden, Germany). Surgery was performed in lateral recumbence. The abdominal cavity was opened by an approximately 1.5 cm horizontal incision in an angle between the last rib and the longitudinal back muscles. For ovariectomy, ligature of ovarian vessels was performed using a titan clip (Ligaclip®; Mca 6 mm, Ethicon Endo-Surgery, Norderstedt, Germany), and the ovaries were removed. Both kidneys were exteriorized. Then, 3 or 4 cat ovarian cortex pieces (total of 44 transplants) were carefully pushed beneath the capsule. The incision was glued with a fibrin adhesive (Tissucol Duo S; Baxter, Unterschleißheim, Germany). The kidney was repositioned, and the abdominal cavity was closed.

Hormone stimulation and experimental design

Hormonal treatment began 5 days after surgery for all animals. Initially, half of the animals (n=6 rats; treatment group A) received weekly injections of 5 IU porcine FSH (pFSH; Sioux Biochemical, Sioux Centre, IA, USA) encapsulated in liposomal vesicles (see below). For administration, liposome-pFSH was diluted in 0.1 ml incomplete Freund’s adjuvant. The rest of the animals (n=6 rats; treatment group B) received daily injections of 1.25 IU pFSH (in 0.1 ml 0.9% NaCl). All injections were applied subcutaneously. After a 6 week period, all animals received a single injection of equine chorionic gonadotropin (eCG; 30 IU, s.c.) to restart follicular growth. From then on, all rats were treated weekly with injections of liposome-pFSH until they were euthanized. If oocyte recovery was planned, the rats were treated with 30 IU eCG 36 h prior to euthanasia.

Incorporation of pFSH within liposome vesicles

The pFSH was incorporated into large multilamellar liposomes (MLV) consisting of soybean lecithin (Phospholipon G90; Nattermann, Cologne, Germany) and cholesterol at a total lipid concentration of 315 mM. The molar ratio of the lipids was lecithin/cholesterol = 10/1 (w/w). Vesicles were prepared by transferring both chloroform-dissolved components into a glass tube and drying them with nitrogen. Then pFSH (100 IU) was dissolved in 1mL PBS and added to the lipids. This lipid-protein suspension was shaken intensively at 55 C for 1 h and was then subjected to five freeze-
thaw-cycles (−70 C, 10 min; 55 C, 5 min). The non-encased pFSH was left with the MLV to serve as initial hormone stimulants. The liposome pFSH was stored in aliquots of 100 µL at −70 C for further use.

**Ultrasonography**

Prior to each ultrasound examination, the skin surface was prepared by manual shaving. Alcohol and coupling gel were used. A high frequency ultrasound system equipped with linear transducers ranging from 10–28 MHz (Diasus; Dynamic Imaging, Livingston, Scotland, UK) was used to image the presence, structure and size of the ectopic xenografts. All ultrasound examinations were recorded on digital video tapes and evaluated retrospectively.

**Vaginal cytology**

Sterile cotton swabs (3 mm; Hain Diagnostika Lifescience, Nehren, Germany) were inserted in the vestibule and vagina. The swabs were smeared on a Poly-L-lysine slide, fixed immediately with an ethanol-polyethylenglycol spray (Merckofix®; Merck, Darmstadt, Germany), stained (Papanicolaou method) [35] and evaluated.

**Serum hormone levels**

Blood samples were collected once per week from the tail vein by puncture with a 22G needle (0.5 ml per individual). The blood was collected into microtubes (Microvette CB 300®; Sarstedt, Nümbrecht, Germany) and centrifuged (6000 g, 15 min). The serum was frozen at −20 C.

Serum samples were analysed for estradiol using in-house micro titre plate enzyme immunoassay procedures as described previously [36]. The antibody (provided by Prof. Meyer, Weihenstephan, Germany) was raised in rabbits immunized against 1,3,5 (10) estradien-3,17β diol-17-hemisuccinate-BSA (bovine serum albumin). The cross-reactivities of the antibody to different estrogens and gestagens were as follows: 100% for 17β estradiol, 69.2% for 17α estradiol, 11.4% for estrone, 5.9% for ethylestradiol and <0.1% for estratrien, estrone sulphate, estriol, pregnandiol and progesterone. We used 5α-pregnandiol, 1,3,5 (10) estradien-3, 17β diol-17-hemisuccinate-HP (horse radish peroxidase) as an enzyme conjugate. The intra-assay coefficients of variation were 10.4 and 6.3% for 0.3 and 1.7 ng/ml, respectively. The inter-assay coefficients of variation were 17 and 26% for 1.1 and 2.5 ng/ml respectively.

Transabdominal ultrasound, blood collection and vaginal cytology were performed weekly under general anaesthesia. All examination procedures were carried out in a laminar flow hood under aseptic conditions.

**Removal of ovarian grafts**

Xenografts were removed according to ultrasound findings. In the case of visible follicles within the grafts and planned oocyte recovery, each recipient received 30 IU eCG s.c. 36 h prior to euthanasia.

The rats were euthanized by cervical dislocation under general anaesthesia. After removal of the kidney, the number of visible xenografts was counted. The transplants were then carefully lifted from the kidney tissue under a stereomicroscope. If no oocyte recovery was planned, the xenotransplant was fixed in Bouin’s solution, and histological analysis of the grafts was performed using HE-stained serial sections (3 µm).

**Oocyte collection and in vitro maturation**

Xenotransplants containing antral follicles were dissected in a Petridish (50 mm, Nunc, Roskilde, Denmark) containing M199 and Earle’s salts supplemented with 3 mg BSA ml⁻¹, 0.6 mg sodium lactate/ml, 1.4 mg HEPES/ml, 0.25 mg sodium pyruvate/ml and 0.055 mg gentamicine/ml. All cumulus-oocyte complexes (COCs) were collected by gentle disruption of the antral follicles. Oocytes with complete compact cumulus and uniform, dark cytoplasm were cultured as described previously [37], with one variation in the method: about 20 companion COCs obtained from domestic cats ovariectomized the same day were added to each well in a Tissue Culture Insert (10 mm, NUNC) to keep them separated from the recovered eggs. After in vitro maturation for 24 h the oocytes were analysed under a phase contrast microscope for polar body extrusion and were stained with aceto-orcein to confirm nuclear maturation.

**Data and statistical analysis**

Means, standard deviations (SD) and coefficients of determination were calculated. The means of independent samples were compared using the unpaired t-test or Mann-Whitney Test (GraphPad InStat 3.0).
Results

The surgical procedures form ovariectomy and ectopic transplantation of cat ovarian cortex tissues were performed successfully for all 12 nude rats. The animals recovered within a few hours after surgery. During the whole experiment, no disturbances were observed in relation to general health.

Graft survival and follicular growth

The grafts were successfully identified in vivo using the high resolution ultrasound system (Fig. 1 A–D). The optimal patient position was lateral recumbence, as the best ultrasound window was often closed to the costal arch. The kidneys were identified easily by their ultrasonographic parenchyma structure. The echogenic white capsule line allowed for differentiation of subcapsular-positioned grafts and surrounding tissue. Tests of several probes showed that the 10–22 MHz probe was most suitable for identification and assessment of the ovarian transplants. 24 of the 44 grafts were classified as viable by ultrasound after 4 weeks, representing a total survival rate of 54.5%. The criteria for viability of grafts were their size and growth in consecutive examinations, shape (oval), middle echogenic texture and transplant margins (well definable). In comparison, the regressed grafts (45.5%) could barely be detected by ultrasound. These remnants mostly appeared at the kidney surface as small, flat, high echogenic areas with indistinct borders. Post-mortem, they were identified as whitish dots on the kidney surface. This material, which was recovered for histological examination, contained predominantly dense connective tissue and few remaining primordial and primary follicles.

Viable grafts were easy to re-identify during the weekly follow-up examinations. This allowed for
evaluation of the development of each transplant separately with emphasis on follicle antrum growth. Formation of small anechogenic, fluid filled cavities was an indicator of enhanced transplant growth and antral follicular development. These were found during the fourth week after surgery (Fig. 1A–D). Early antrum formation could be detected from 0.4 mm in diameter onwards. Overall, follicle antrum development was observed in 10 grafts from 5 of the monitored hosts. During the 19 weeks of the experiment, a total number of 86 antral follicles were counted. This represents 8.6 ± 6.43 antral follicles (mean ± SD) per graft monitored. The largest antrum measured had a diameter of 3.3 mm.

During the period of study, we were able to follow six antral follicles during three consecutive weekly examinations and two antral follicles during four consecutive weekly examinations. The antral cavity diameter of each follicle was measured during examination, the data were pooled and a growth curve was calculated (Fig. 2). The differences in the size of antra from the same follicle between the two examinations ranged from 0.16 to 1.63 mm, and the mean was 0.56 ± 0.44 mm (± SD).

There was no significant difference in antral follicular size among the two hormonal treatment groups (16 follicles measured for A and 11 follicles measured for B; P=0.3943; Mann-Whitney Test).

Vaginal cytology and serum estradiol
All 5 rats for which follicular growth was detected showed weekly changes in their cell profiles of basophile- and acidophil-stained superficial epithelial cells and increasing and decreasing serum estradiol concentrations. However, there were no significant relationships between follicle diameter, percentage of keratinized vaginal cells and serum estradiol, as demonstrated in Fig. 3, which shows the follicular growth dynamics (panel A), vaginal cytology (panel B) and serum estradiol (panel C) for nude rat No. 2. The follicle growth in this rat was characterized by two separate waves. The first growth wave occurred after eCG injection between weeks 6 and 11, and second a second wave occurred between weeks 16 and 21 (panel A). The percentage of keratinized (acidophil stained) superficial vaginal epithelial cells (panel B) seemed to follow the follicular growth pattern, but it did not correlate with follicle diameter or the number of antral follicles (data not shown). The serum estradiol concentration showed a clear increase and decrease pattern (panel C).
diol levels (panel C) expressed a triple cyclic pattern.

Overall, the mean distance between estradiol peaks was 24.2 ± 3.5 days (11 cycles in 5 animals), but the estradiol profiles did not coincide with either vaginal cell cytology or follicular diameter gain in any of the animals.

The serum estradiol values of the two pFSH treatment groups were nearly balanced (65.1 ± 8.6 ng/ml for A versus 65.8 ± 9.26 ng/ml for B, mean ± SD; P=0.9621, unpaired t-test).

Oocyte recovery and in vitro maturation

Antral follicular development within xenotransplants was detected in five of the nude rats by ultrasonography. Two of these rats showed irregular antrum formation. Nude rat No. 6 had several single antral follicles until week 15; afterwards only dense transplant tissue was visible. Nude rat No. 7 was euthanized at week 10 because an antral follicle had grown to 3.3 mm in diameter; however, no oocyte was recovered. Nude rats No. 2, No. 4 and No. 12 showed regular antral follicle development. The recovery of oocytes was timed according to the antrum diameter of the largest follicle within the graft (Fig. 4). Whenever the largest antral follicle reached approximately 2 mm in diameter, the recipient was treated with eCG and then euthanized 36 h later. Nude rat No. 2 had one graft with five follicles at week 21; nude rat No. 4 had two grafts with 4 follicles per graft at week 19; and nude rat No. 12 had one graft with 4 follicles at week 23. We attempt to collect COCs from not only the largest antral follicles, but also from all other antral follicles that we observed. The results of ultrasonography, number and size of the antral follicles and the corresponding number of successfully recovered COCs are listed in Table 1. All 10 collected COCs were transferred to 24-h in vitro culture in the presence of companion oocytes. Extrusion of a polar body was observed in one oocyte (recovered from nude rat No. 4; antrum diameter 2.17 mm). After orcein staining, 7 oocytes showed germinal vesicle break down, one oocyte was fragmented and the other one reached the 4-cell stage, presumably by spontaneous parthenogenetic activation.

Discussion

We investigated the survival of cat ovarian cortex tissue transplanted under the kidney capsule of ovariectomized athymic nude rats. Graft development and follicular growth was visualized by transcutaneous high-resolution ultrasonography (22 MHz). This technique allowed selection of animals for successful post-mortem puncture of follicles and recovery of oocytes followed by in vitro culture in order to demonstrate the feasibility of good qual-
ity oocyte retrieval. The results of monitoring ovarian tissue activity by vaginal smears and/or serum estradiol levels did not correlate with in vivo follicular diameter gain. Thus, in this study, these methods were considered to be insufficient for determination of the optimal recovery time.

In the present study, 54.5% of the xenotransplants were categorized as viable by ultrasonography four weeks after surgery. Bosch et al. [20] previously found proliferating cells in all studied transplants using post-mortem immunohistochemistry tissue staining. These higher survival rates might be explained by the different methods applied. Histological analysis generally reflects the actual state at the time of euthanasia and may therefore overestimate long-term survival outcome. On the other hand, ultrasonography is used to monitor follicular dynamics over time, not cell survival per se. Although, the remnants of the ovarian tissue (45.5%) were assumed to have undergone regression or more likely ischemic necrosis, some of the pieces still contained small follicles. Overall, transplantation success remains less predictable because transplant fate is influenced by many factors. Aside from specific graft rejection mechanism, local physiological and infectious immune defenses must be overcome. Post-surgical blood supply might be improved by perioperative treatment of the recipient with FSH and LH, presumably through the action of vascular endothelial growth factors [28]. In addition, reduction of ischemic perfusion injury by antioxidants (such as vitamin E) has a beneficial effect on follicles [38]. It has been shown that graft position also influences survival and development. More grafts are recovered and more follicles are present if xenografts are posi-

Table 1. Summary of the ultrasonographical findings prior to post-mortem follicular puncture and number of successfully recovered oocytes

<table>
<thead>
<tr>
<th>Nude rat No.</th>
<th>Week and position</th>
<th>Follicle number</th>
<th>In vivo</th>
<th>Ex vivo</th>
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<tr>
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<td>Graft size (mm)</td>
<td>Follicle size (mm)</td>
<td>Oocytes recovered</td>
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<td>2</td>
<td>21</td>
<td>6.87 × 4.33</td>
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The number of transplants and antral follicles detected by ultrasonography correspond in all cases. (ki: kidney. t: transplant. Follicles are marked as black circles)
tioned under the kidney capsule compared to peripheral sites [29]. The kidney capsule is very well vascularized and contains a high concentration of angiogenic growth factors [39]. Therefore, we applied a commercially available two-component fibrin glue to the grafting site. These fibrin glues have been widely used in human surgery and have been proven to expedite hemostasis and the process of wound healing [40]. If the xenografting pathway is considered to rescue genetic potential, graft survival of 50% could be seen as satisfying results. In particular, ovaries obtained from other felids or humans are larger in size and thus the number of potential pieces for transplantation would be higher.

To our knowledge, this is the first study in which ultrasonography has been applied to monitor transplant fate in a xenogeneic host. The use of live animals for research purposes is intensively discussed and is subject to rigorous legal restrictions. Ultrasonography could help to reduce the number of required animals because it is considered to be non-invasive and therefore repeatable. Up to now, no negative clinical side effects have been shown for high-resolution ultrasonography [41, 42].

The applied high-resolution ultrasound system has previously been used successfully in other small rodents to image internal reproductive organs, and it was considered to be qualified to replace the former inevitable carcass dissection [43]. We were able to detect antral follicle formation from 0.4 mm onwards, which is a very early antrum state according to Jewgenow and Pitra [44], who described the beginning of antrum growth from 300 µm onwards. Our study documented the formation and growth dynamics of cat ovarian antral follicles. Such data is not available in situ because high frequency ultrasound probes providing the resolution required for imaging microanatomical structures depend on a very short distance between the scan head and the region of interest. These in vivo measurements of antrum growth after ovarian cortex tissue transplantation in a small host could be the basis for future assessments of follicle growth in cats and other species.

Comparison of the two different FSH application regimes did not reveal any difference between the experimental groups. Administration of drugs in liposomes has already been described for immunocontraceptive vaccines in different seal species [45], but has yet to be examined for slow release of reproductive proteohormones. Since weekly liposome-FSH-delivery reduces disturbance of the animals, we suggest that this method be chosen for future experiments. The animals were treated with eCG to restart their follicular development after six weeks. All follicles that were visible in the rats before eCG treatment were lost after the injection. New antral follicular growth was monitored within at least 9 days (Fig. 1 B).

We compared the in situ follicular growth with the pattern of serum estradiol and cornification of the vaginal epithelium. Although we found no significant correlation between any of these parameters, vaginal cornification appeared to coincide with antrum formation, as shown in Fig. 3. Vaginal smears also reflected serum estradiol peaks, but not every estradiol peak was accompanied by cornification and/or follicle development. Furthermore, the observed interval between two estradiol peaks (24.2 days) did not correspond to the physiological cycle length in the rats (4–5 days) [46] nor did it correspond to the interestrus duration of domestic cats [47]. In regard to our data, we conclude, that serum estradiol and vaginal cornification, in particular, can be used to monitor graft survival and follicular development; but we suggested the use of ultrasonography as final proof for follicle growth to the antral state.

Bosch et al. [20] euthanized all the animals in their experiments randomly on one day without having any detailed information about graft or follicular stage. Xenotransplants were evaluated post-mortem using immunochemical staining. In contrast the ultrasound imaging technique offers the possibility to follow graft fate at any time and to adapt the experimental protocol immediately according to intermediate findings.

In the present study COCs were collected during weeks 19, 21 and 23 from three different individuals. In total, 10 COCs were collected from 4 grafts including 17 antral follicles. From the published literature, it is very difficult to get any information about harvesting rates. Bosch et al. [20] described histomorphological and immunohistochemical results, but they did not comment number of recovered oocytes. Kaneko et al. [30], who transplanted porcine ovary slices into nude mice, reported a mean number of 2.4 and 10 successfully recovered oocytes per graft. In a follow-up study, the same authors found 0.6 oocytes per graft [27].

The largest follicle we observed achieved a size
of 3.3 mm, but no oocytes were found inside the antral cavity. We assumed that the fluid-filled structure was a modified cystic follicle rather than a healthy follicle.

We were able to in vitro mature the recovered COCs. However, the results were unsatisfactory; only one oocyte showed an extruded polar body, one oocyte was parthenogenetically activated and one oocyte was fragmented. We attempt to support the in vitro maturation by adding companion oocytes to the maturation drop. This may have a supporting effect on the development of immature oocytes, if an insufficient number of gametes are retrieved from xenografts. It has been proven [48] that con- and heterospecific (mouse and cattle) companion embryos confer benefit to further development of single two-cell stage embryos in regard to cell number per embryo and in vitro co-culture of blastocysts. The maturation system with companion oocytes still requires improvement. Based on our limited data, we assume that the best time for oocyte recovery of COCs from xenotransplanted tissue in cats for further IVM and potential IVF trials is when they are between 2 and 3 mm in diameter.

In conclusion, application of a non-invasive imaging technique is a valuable tool for narrowing the time frame for oocyte retrieval from xenotransplants, and it helps to avoid euthanasia of animals at an inappropriate time. Thus, host animals can be selected for oocyte collection and further IVM/IVF trials depending on the size of follicles present in the graft.

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