Video Article

Inactivation of mTor: A Tool to Investigate Meiotic Progression and Translational Control During Bovine Oocyte Maturation

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URL: http://www.jove.com/video/53689
DOI: doi:10.3791/53689

Keywords: Bovine oocyte, *in vitro* maturation, meiotic maturation, mTor, inhibitors, translational control, 4E-BP1

Date Published: 8/31/2016

Citation: Kreißelmeier, M., Mayer, S., Wrenzycki, C., Pöhland, R., Tomek, W. Inactivation of mTor: A Tool to Investigate Meiotic Progression and Translational Control During Bovine Oocyte Maturation. *J. Vis. Exp.* (), e53689, doi:10.3791/53689 (2016).

Abstract

Although routinely used in breeding programs, *in vitro* maturation (IVM) of bovine oocytes and *in vitro* production (IVP) of embryos are nevertheless still the subject of basic research owing to suboptimal IVM conditions and variations in the developmental competence of the starting oocytes. In the present study we provide a method to inhibit the Ser/Thr kinase mTor during IVM using two independent inhibitors, Torin2 and Rapamycin. Both substances have different effects on meiotic progression and translational control and may allow discrimination between the mTorC1 and mTorC2 complex functions. The effects of the inhibitors are monitored by inspection of the chromatin configuration using aceto-orcein-staining as well as Western blotting and immunohistochemical analysis of the phosphorylation state of the translational repressor 4E-BP1, which is a prominent mTor target. Whereas Torin2 arrests bovine oocytes in the M I stage and inhibits 4E-BP1 phosphorylation, Rapamycin inhibits asymmetric division and does not influence 4E-BP1. Investigations utilizing these reactions can provide deeper insights into the regulatory events involved in meiotic maturation. Moreover, special focus can be placed on the temporal and spatial regulation of translational control. Such findings can contribute to the definition of the developmental competence of oocytes and to an improvement of IVM conditions.

Introduction

Fully grown bovine oocytes (arrested at prophase I; germinal vesicle -GV- stage) resume meiosis spontaneously when they are released from their follicles and transferred to a suitable culture medium. Previous investigations using the application of different inhibitors to *in vitro* culture media[1, 2] revealed that activation of protein kinases and *de novo* protein synthesis trigger the maturation of mammalian oocytes and arrest them in metaphase II (M II), the stage suitable for fertilization. In the present study we describe a method to inhibit the Ser/Thr kinase mTorduring IVM of bovine oocytes. This approach might provide deeper insights into the complex processes involved in the regulation of protein synthesis in the context of meiotic maturation (transition from GV-stage to M II), because mTor links the phosphorylation of specific factors directly to translational control[3, 4].

The focus on the investigation of translational control reflects the importance of this process; fully grown oocytes are transcriptionally silent and protein synthesis relies on the activation of stored, dormant mRNAs[5]. In this context, mTor plays a predominant role. The kinase directly phosphorylates and inactivates repressors of the mRNA cap-binding protein eIF4E, the so-called 4E-binding proteins (4E-BP1-3), and thereby allows the formation of the 5´-mRNA-cap binding complex eIF4F (composed of eIF4E, the scaffold protein eIF4G and the RNA helicase eIF4A). Together with other factors it also stimulates ribosome binding and translation initiation[6].

mTor, however, exists as two complexes: mTorC1 and mTorC2. Each complex is composed of different major regulators, differs in sensitivity to Rapamycin and has different cellular targets[7]. The major regulator of mTorC1, Raptor (regulatory-associated protein of mTOR), phosphorylates components of the translational machinery, namely ribosomal proteins (for instance RPS6 at Ser235/36) and the translational repressor 4E-BP1 (at Thr37/46/65/70). The major regulator of mTorC2, Rictor (Rapamycin-insensitive companion of mTOR), is Rapamycin- resistant and phosphorylates Akt (PKB) which in turn phosphorylates mTorC1. Preliminary investigations in bovine oocytes during IVM revealed different transient activities of mTorC1 and mTorC2 during IVM. In the GV-stage of oocytes the mTorC2 is active[3] ; it is inactivated in the course of IVM. In contrast, mTorC1 shows the opposite behavior[3]. These results correspond with findings showing that 4E-BP1 phosphorylation is lower in the GV-stage, continuously increases during IVM, and is highest in the M II stage[8, 9].

However, mTorC1 and C2 both respond to the active site inhibitor Torin2[10] and might have other (yet unknown) targets. Candidates are meiotic spindle-forming or regulatory proteins, since mTor associates with meiotic spindles during chromatin segregation.

From a practical point of view, it should be noted that *in vitro* systems yield only 30-40 % transferable embryos in the bovine species [11]. The causes for this could be suboptimal *in vitro* conditions and/or differences in the developmental competence of the starting oocytes which occur despite their selection from follicles of a defined size. However, detailed investigations of meiotic maturation on a molecular level can contribute to the optimization of IVM systems. Furthermore, oocytes might be selected according to their developmental competence, for instance by IVM systems under inhibitory conditions (see discussion). Hence, in the procedure presented here, we used two independent mTor inhibitors, Torin2

and Rapamycin, which resulted in different chromatin statuses and differential phosphorylation of 4E-BP1. Interestingly, approximately 20 % of the oocytes overcame the Torin2 block and might thus be candidates which possess a high developmental competence.

Protocol

All experiments were performed in accordance with the guidelines of the local ethics committee (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei, Mecklenburg-Vorpommern, Germany). The ovaries were obtained from a commercial slaughterhouse. The remaining carcass was used for meat production.

1. Solutions to be prepared.

1. Prepare PBS for gaining oocytes by weighing Glucose (500 mg), Pyruvate (18 mg), Penicillin (10 mg), Streptomycin (20 mg), Heparin (5.6 mg) and BSA (150 mg) and add these substances to 500 mL PBS pH 7.4, supplemented with 1mM Ca^{2+}/Mg^{2+}. Store the solution until needed at 4°C (up to 2-3 days).
2. Prepare TCM as the maturation medium by weighing $NaHCO_3$ (220 mg), Gentamycin (5 mg), Pyruvate (2.2 mg) and TCM-199 Hepes modification (1510 mg) and add those substances to 100 mL ddH2O. Mix the medium for 40 min on a magnetic stirrer. Adjust the pH-value to 7.4. Use a sterile filter to fill the solution into a small, sterile bottle. Store the solution until needed at 4°C (up to 2 weeks).
3. Prepare the Rapamycin working solution with a concentration 800 µM by dissolving 18.2 µg Rapamycin in 25 µL DMSO. Store the solution in aliquots of 5 µL at -20°C.
4. Prepare the 20 mM Torin2 stock solution by dissolving 10 mg Torin2 in 1.16 mL DMSO. Store aliquots of 10 µL at -20°C. Just before performing an experiment, take 3 µL of this stock solution and dilute it in 247 µL DMSO to prepare a working solution with a concentration of 240 µM.
5. Prepare 10 mL of the PBS as a washing buffer for immunohistochemistry. Supplement PBS with 0.5 % BSA and 0.05 % Triton-X-100.
6. Prepare 10 mL of the permeabilization buffer by weighing 0.46 g Hepes, 10.27 g sucrose, 0.29 g NaCl, 0.061 g $MgCl_2$ and 0.5 mL Triton-X-100. Fill up with ddH_2O and adjust to pH 7.4.
7. Prepare 10 mL of the blocking solution (BS) by diluting the commercial blocking solution 1:50 with ddH_2O.
8. Prepare the working solution for antibody incubation by diluting the BSsolution with PBS, pH 7.4, 0.05 % Tween (1:1).
9. Fix and store the oocytes in 3 % paraformaldehyde, 2 % sucrose.

2. Source of oocytes and preparations before going to the slaughterhouse.

1. Prepare a NaCl-solution (0.9 %) by dissolving 9 g NaCl in 1 L ddH_2O. Warm this solution up to 37°C and fill it into a thermally insulated vessel.
2. Recover the ovaries at the slaughterhouse.Remove the ovaries with a pair of scissors from the carcasses of healthy cows immediately after the abdominal cavity is opened by a knife cut of about 40 cm in the ventral median line. Put the ovaries into the vessel with the NaCl-solution. Transport the ovaries back to the laboratory within 3 h after slaughter.
3. Puncturing of the follicles and gaining of the cumulus-oocyte complex's (COCs).
    1. Use a 5 mL syringe to aspirate 1 mL warm PBS (37°C) first and then puncture the follicles (estimated follicle diameter: 4-8 mm). Remove the needle from the syringe and transfer the liquid in a plastic tube (50 mL) with a screwtop and keep it warm. Repeat the previous steps until all follicles sized 4-8 mm are aspirated.
    2. Allow the aspirated liquid to stand for 10 min. The developing sediment contains approximately 400 COCs aspirated from 50 to 80 ovaries.
    3. Use a Pasteur pipette to transfer sedimented COCs into screened Petri dishes with a diameter of 90 mm. Add 5 mL PBS to each of the Petri dishes and store the dishes on a hotplate (37°C).
    4. Take the COCs out of the Petri dishes with a pipette controller (use micropipettes with a volume of 20 µL) with the help of a stereomicroscope with 15x magnification and transfer them to smaller Petri dishes (diameter of 30 mm) filled with PBS.
4. Classification of the COCs[12]
    1. Use only COCs categorized as oocytes with a dark, homogenous cytoplasm and a compact (or only slightly expanded) cumulus oophorus that shows more than five layers of cumulus cells.

3. *In vitro* maturation and inhibitor treatment.

1. Preparing the maturation dishes.
    1. Weigh 60 mg BSA and add it to 20 mL TCM and put it into the incubator for at least 1 h at 39°C, 5 % CO_2 and saturated humidity. Use a syringe with a sterile filter to distribute the TCM equally into two 30 mm Petri dishes.
    2. Transfer 400 µL TCM from the two Petri dishes in each well of four 4-well dishes (these are the washing dishes), so that an equal amount of TCM remains in the Petri dishes.
    3. Take 2760 µL of TCM from one Petri dish and add 40 µL of Rapamycin (800 µM) or Torin2 (240 µM)-working solution (these are the treatment media). Take 2760 µL of TCM from the other Petri dish and add 40 µL of DMSO (this is the control medium).
    4. Add 350 µL of the treatment media and the control medium to each well of two 4-well dishes.
    5. Transfer 25 COCs from the Petri dishes with PBS from point 2.3.5 / 2.4.1 after classification and put them into each well of the four 4-well washing-dishes.
    6. Transfer the COCs from two 4-well-washing-dishes with a pipette using a volume of 50 µL to the two 4-well-dishes containing Rapamycin/Torin2 working solution, so that the total volume in each well is 400 µL and the final concentration is 3 µM for Torin2 and 10 µM for Rapamycin.

7. Do the same with the other washing dishes and the 4-well dishes containing DMSO for the control group. Put the dishes with the COCs into the incubator at 39°C, 5 % CO_2 and saturated humidity. Let the COCs mature there for 24 h.

4. Morphological inspection by Aceto-Orcein staining.

1. Fixation of the oocytes.
    1. Prepare two 4-well-dishes with 400 µL PBS per well.
    2. Transfer 100 oocytes of the Torin/Rapamycin group to well 1 of one of the 4-well dishes with PBS and 100 oocytes of the control group to the other well.
    3. Denude the oocytes by repeated pipetting with 200 µL pipette tips so that the cumulus cells are completely removed. Use the remaining 3 wells to wash the oocytes.
    4. Prepare microscope slides as follows:
        1. Apply two vertical parallel lines of silicone of medium viscosity with the help of a needle attached to a 2 mL-syringe spaced about 1.5 cm apart to each side of the microscope slide.
        2. Use a pipette to transfer 10 oocytes in about 4 µL PBS to the microscope slide. NOTE: The drop with the oocytes should be placed in the center between the two lines of silicone at one side of the microscope slide. Take a microscope cover glass (18x18 mm) and place it carefully onto the lines of silicone and the drop with the oocytes in between.
        3. Press the cover glass lightly and carefully so that the oocytes do not burst. Put a drop of ethanol-glacial acetic acid (3:1) at one edge of the cover glass, so that the liquid may flow through the whole space in between cover glass and microscope slide and enclose the oocytes completely.
        4. Put the microscope slides in a cuvette filled with 40 mL ethanol (absolute) glacial acetic acid (3:1). Ensure that the lower edges of the cover glasses are immersed in the liquid.
        5. Seal the cuvette air tight with self-sealing lab film and store it at 4°C until staining. Keep the slides for no more than two weeks.

2. **Staining of the oocytes.**
    1. Take the microscope slides out of the cuvette and dab them gently with a tissue. Apply 4 drops (approx. 40 µL) of Aceto-Orcein-solution (2 %) to the upper edge of one cover glass so that the liquid is soaked under the cover glass.
    2. Place a strip of filter paper at the lower edge of the cover glass to get the liquid completely soaked through the space between microscope slide and cover glass. Let the staining solution incubate for 10 min.
    3. Apply drops of acetic acid (30 %) to the lower edge of one cover glass and use a strip of filter paper at the upper edge of the cover glass to get the liquid soaked through until the interstice is completely clear.

5. Evaluation.

1. Examine the stained slides under a phase-contrast microscope at 100-400x magnification. Classify the oocytes by reference to the chromatin configuration in metaphase II, anaphase II, telophase II, metaphase I, GVBD-stage, GV-stage and degenerate oocytes[1,5].

6. Western blotting was performed according to standard procedures[13].

1. Subject 50 oocytes per lane to SDS-PAGE on 13 % (w/v) acrylamide gels with an acrylamide: bisacrylamide ratio of 30:0.8. After transferring, block membranes with 5 % fat-free dry milk powder in TTBS for 1 hour at room temperature.
2. Dilute the primary antibodies as follows: 4E-BP1, 1:800; p4E-BP1-Thr37/46, 1:500; RPS6, 1:1000; pRPS6-Ser235/36, 1:1000. NOTE: In all cases, the HRP-labeled secondary antibody is four times more highliy diluted than the primary. Perform ECL detection with a commercial kit.

7. Immunohistochemistry and Confocal Laser Scanning Microscopy (LSM)

1. Wash the fixed oocytes (1.9) once with PBS washing solution at room temperature. Incubate for 5 min in permabilization solution at 0°C. Wash four times, 15 minin washing buffer. Incubate 2 h in blocking solution at room temperature.
2. Incubate the primary antibody (4EBP1, 1:100; p4E-BP1 Ser37/46, 1:200) overnight at 4°C without washing. Wash four times, 15 minin washing buffer. Incubate the secondary, fluorescence dye labeled antibody (1:200) for 5 h in the dark at room temperature. Wash four times, 15 minin washing buffer.
3. Stain the chromatin with SYBR green in the dark for 1 h. Wash four times, 15 minin washing buffer. Fix the oocytes in 2 % paraformaldehyde overnight at 4°C. Mount the oocytes in glycerin gelatin on microscopy slides.
4. Perform fluorescence analysis using a confocal laser scanning microscope. Track1: Laser: Argon 30mW (458/488/514nm) 488nm/12%; HFT/NFT: 488/545nm; Filter: BP 505-530nm Channel1 (Channel2 closed). Track2: Laser: HeNe 1mW (543) 543/60%; HFT/NFT: 543/545nm; Filter: LP 560-nm Channel2 (Channel1 closed). Pinhole: 1AU, gain constant, veraging: 2, Resolution 1024x1024. Stacks: 1 µm between slices.

Representative Results

Source of oocytes, *in vitro* maturation and mTor inhibition

Figure 1 illustrates the collection of cumulus oocyte complexes (COCs) and the analysis of the chromatin configuration. Only healthy ovaries (Figure 1A) obtained from a local slaughterhouse were used as oocyte sources. COCswere aspirated from follicles sized 4-8 mm (Figure 1B). Only COCs with compact layers of cumulus cells (Figure 1C) were used for IVM and inhibitor studies. After IVM COCs were denuded and morphologically analyzed by aceto-orcein-staining. Before IVM (0 h, Figure 1D), oocytes were in the GV-stage. After 24 h IVM, ooctytes reached the M II stage (Figure 1E) and after 10 h they were in M I stage (Figure 1F).Rapamycin treatment (10 µM) for 24 h, however, inhibited asymmetric cell division, i.e. polar body formation (Figure 1G). In contrast, application of Torin2 (3 µM) arrested oocytes in a stage comparable to M I (Figure 1H). However, in Torin2 treated samples, approximately 20 % of the oocytes overcame the mTor inhibition and reached M II stage[3].

Analysis of the effects of Torin2 and Rapamycin on 4E-BP1 using Western blotting

Western blot analysis of the abundance and phosphorylation of 4E-BP1 and RPS6 are depicted in Figure 2. IVM in the control medium or treatment with Rapamycin (24R) or Torin2 (24T) did not alter the 4E-BP1 and RPS6 abundances (Figure 2A-C left panels). However, phosphorylation and probably other posttranslational modifications of 4E-BP1 are accompanied by a pronounced band shift on SDS-gels. These findings are in accordance with previous observations[8, 14]. Rapamycin-treated oocytes (24R; Figure 2A right panel) show the same level of phosphorylation of 4E-BP1 at Thr 37/46 as the untreated control (24; Figure 2A right panel). In contrast, Torin2 treatment clearly reduces the 4E-BP1 phosphorylation at these sites (Figure 2B, right panel, 24 vs 24T). RPS6 phosphorylation at Ser 235/36, however, is not reduced by Torin2 (Figure 2C right panel, 24 vs 24T).

Immunohistochemical analysis of Torin2 and Rapamycin effects on 4E-BP1

In general, SYBR green staining and immunohistochemical analysis of oocytes confirmed the results obtained by aceto-orcein-staining and Western blotting with regard to chromatin configuration, 4E-BP1 abundance (Figure 3A) and phosphorylation at Thr 37/46 (Figure 3B). For instance, the lowest phosphorylation of the protein is observed in the GV-stage (Figure 3B upper panel). A highly phosphorylated form is found in the M II stage and in Rapamycin-treated samples (Figure 3B second and third panel), whereas Torin2 treatment strongly reduced 4E-BP1 phosphorylation (Figure 3B lowest panel). Together, these approaches allow an overview of the distribution of 4E-BP1 in the oocyte. It is likely that some portions of phosphorylated 4E-BP1 are located at the metaphase I plate in Torin2-treated oocytes (Figure 3B, lowest panel).

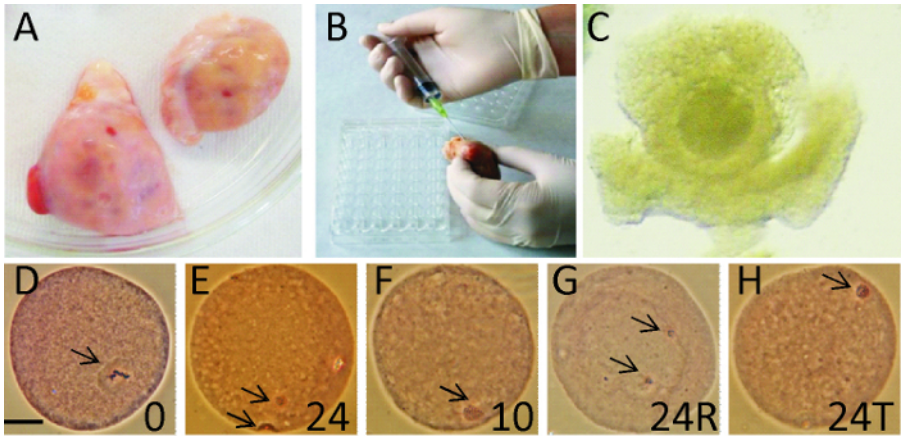

Figure 1: Source of oocytes, recovery of COCs and morphological evaluation. Only healthy ovaries were used as an oocyte source (**A**). COCs were aspirated from follicles of 4-8 mm in diameter with the help of a syringe (**B**). Only COCs with compact layers of cumulus cells were used for further investigations (**C**). After IVM, COCs were denuded by repeated pipetting. The chromatin status was inspected by morphological evaluation after aceto-orcein-staining. Control oocytes are in the GV-stage before IVM (**D**), more than 90 % have reached the M II-stage after 24 h **(**E) or the M I-stage after 10 h **(**F).Rapamycin treatment for 24 h prevents asymmetric division and polar body formation (**G**). Treatment with Torin2, however, arrests approximately 60 % of the oocytes in the M I-stage (**H**), whereas approximately 20 % enter the M II stage, also under inhibitory conditions. Arrows point to chromatin, scale bar 30 µM. Please click here to view a larger version of this figure.

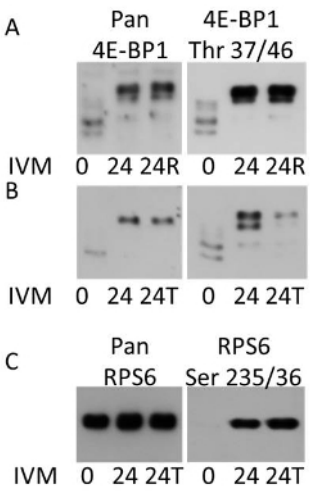

Figure 2: Analysis of the effects of the mTor inhibitors Rapamycin and Torin2 on the phosphorylation of specific target proteins by Western blotting. Depicted are Western blots revealing the abundance of 4E-BP1 (**A**) and (**B**) and RPS6 (**C**) obtained by Pan-antibodies (always left panel) and the corresponding blot analyzing the phosphorylation status of 4E-BP1 at Thr 37/46 and of RPS6 at Ser 235/36 (always right panels). The oocyte samples are analyzed before IVM (0), after 24 h (24) and after 24 h treatment with Rapamycin (24R) or Torin2 (24T), respectively. Please click here to view a larger version of this figure.

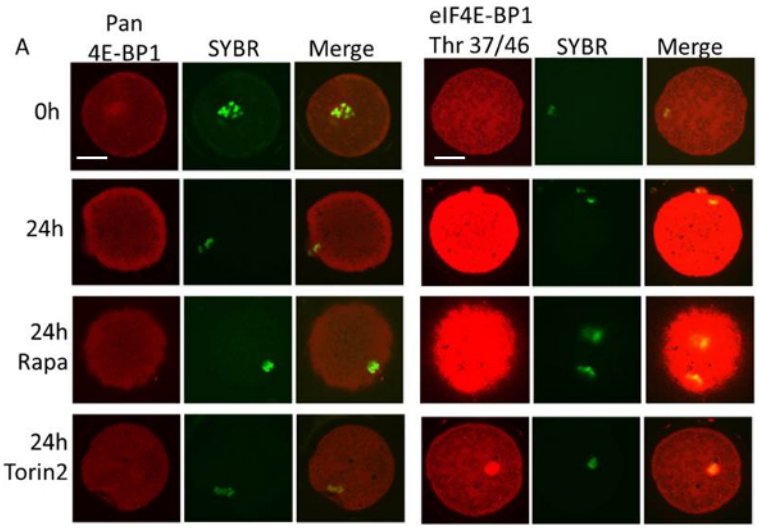

Figure 3: Analysis of the effects of mTor inhibition on 4E-BP1 abundance and phosphorylation by confocal laser-scanning-microscopy (LSM). The results obtained by the Pan-antibody for 4E-BP1 (A) and the Thr 37/46 phosphorylated form of 4E-BP1 (B) are shown here. Controls (0 h and 24 h) and Rapamycin- (Rapa) or Torin2-treated samples are arranged as indicated. The left row (red) shows the antibody staining, the middle row (green) shows the chromatin stained by SYBR green and the right row is the merged image. Scale bar: 50 µm. Please click here to view a larger version of this figure.

Discussion

In vitro maturation (IVM) of bovine oocytes is an important technique because it is an integral part of the in vitro production of embryos in specific breeding programs. However, a critical limitation is the fact that no method exists to assess the developmental competence of fully grown oocytes directly after follicular release. Furthermore, when the chromatin status of bovine oocytes is analyzed morphologically, more than 90 % have reached the M II stage after 24 h of IVM under standard conditions1. However, transferable blastocysts can be produced from only 30-40 % of these oocytes 11. These results have led to the concept that the cytoplasmic maturation state may not correspond to the actual nuclear maturation state. Thus, IVM is also the subject of basic research, investigating the mechanisms of meiotic progression.

The asynchrony of nuclear and cytoplasmatic maturation can be investigated by specific inhibition of mTor because the chromatin configuration and phosphorylation of cytoplasmic factors is differently influenced by Torin2 and Rapamycin. The application of specific, membrane-permeable inhibitors may help to facilitate the identification of distinct signaling cascades without the need for sophisticated devices like micro manipulators for the injection of macromolecules. On the other hand, the cytoplasmic maturation can be assessed by molecular and/or biochemical analysis, but in most cases the oocytes are lost for embryo production thereafter. Knockdown experiments are the gold standard to study critical control points in signal transduction pathways. However, in the early process of oocyte maturation large amounts of mRNA are stored in the oocyte to cover the translation during early embryonic development until the the embryonic genome is activated (cattle: 8-cell stage). However, during the

final maturation of the oocytes, de novo transcription is dramatically reduced. Therefore, knockdown experiments during this phase would not lead to relevant results. In addition, these knockdowns would result in unwanted effects in the later embryos. In contrast, the method shown here is able to provide significant results.

The most important modification of the standard technique for IVM in our experiments was the renunciation of the use of serum in the culture medium. Instead, we used BSA as a protein source. This resulted in lower rates of development to the metaphase II stage compared to the standard protocol3. However, the modification is necessary since serum components can interact with the inhibitors used. The modified IVM method can be regarded as a critical step in the methodology.

Several studies have been performed in an attempt to characterize cytoplasmic maturation specific kinase inhibitors1, 2, 16, 17. However, when the function of the mTor axis was analyzed by specific inhibition, it was revealed that a portion of oocytes overcame the mTor block and reached the M II stage. For instance, the phosphatidylinositol analogue SH6 (which acts on Akt, PKB and therefore on mTorC1) leads to the M I arrest of approximately 60 % of the oocytes, whereas 30 % overcame the block and reached the M II stage16. A comparable result was achieved by applying the mTor inhibitor Torin23. From these results, it can be concluded that subclasses of oocytes exist which differ in their sensitivity to the inhibitor. The inhibitor-resistant oocytes may possess higher basal kinase activities in the GV stage18. Preliminary results from our pilot experiments suggest that these oocytes have high developmental competence with regard to blastocyst formation in vitro. Therefore, an IVM-system under Torin2 inhibitory conditions, followed by standard embryo production in vitro should enable the selection of more competent blastocysts and improve the yield of offspring after embryo transfer. However, this suggestion must be verified by additional experiments.

In the process of a possible troubleshooting, an exact definition of inhibitory but not toxic doses of the inhibitors is important. However, the inhibitors have to enter the relatively large oocyte via the zona pellucida. Therefore, when compared to somatic cells, much higher concentrations (10-100 fold) are required to obtain an effect. This increases the risk of side effects. For instance, we analyzed Torin2 effects by measuring the substrate phosphorylation of distinct kinases3 and found, in addition to the expected mTor and PKB inhibition, minor effects on CDKs. For this reason it is critical to determine the lowest concentration which produces clear and reproducible inhibitory effects.

In contrast to Torin2, were we could show a dose-response relationship for both the morphological effects and the BP1 phosphorylation3, the situation for Rapamycin is different. Other investigators showed a clear morphological effect and reduced 4E-BP1 phosphorylation in mouse oocytes treated with 100 nM Rapamycin5. It is generally known that oocytes from mice and cattle are different in size and particularly in the nature of the zona pellucida. Therefore the situation is not directly comparable. This could be the main reason that, in the application of 100 nM to 5 µM Rapamycin, we did not observe any stable and reproducible effects in cattle either on morphological level or on the phosphorylation of any targets. However, from 10 µM on we observed the described alterations in the chromatin configuration. Nevertheless, even this high concentration of the inhibitor did not result in any reduced phosphorylation of distinct mTor targets (4E-BP1; RPS6). This concentration (10 µM) was used to show that mTor is required for asymmetric division in mouse oocytes15. This is the reason why we also used this concentration of rapamycin in our investigation. In conclusion it must be noted that the described effects of distinct inhibitors are species-specific. This represents a clear limitation of the technique. Nevertheless, the inhibition of mTor by small inhibitors might be a valuable tool to investigate nuclear as well as cytoplasmic maturation of oocytes.

Investigations using mouse oocytes revealed that, shortly after nuclear envelope breakdown, translational hotspots were found in the chromosomal area and in a region which was previously surrounded by the nucleus5. Moreover, actively translated mRNAs co-localize with meiotic spindles19. In other words, translational reprogramming of the oocyte occurs at the resumption of meiosis in a spatially and temporally regulated manner. Hence, one main challenge would be to characterize the mRNAs subjected to such a control. In this context, polysome profiling in combination with the inhibition studies as presented here can provide valuable results. Polysome profiling, where translationally active, ribosome-bound mRNAs are separated from translationally repressed, free mRNAs20 is also possible with scarce samples like mammalian oocytes or early embryos21. Keeping in mind the different effect of the mTor inhibitors Rapamycin and Torin2 on the chromatin status, it is likely that the application of these substances also leads to a different pattern of actively translated mRNA. Together with, for instance, RNA-sequence approaches22, such comprehensive investigations can provide information about proteins which attend nuclear envelope breakdown, spindle regulation, M I to M II transition, asymmetric division and polar body formation.

In conclusion, basic inhibition studies as presented here can be valuable in practical terms for selecting oocytes according to their developmental competence and can provide tools for detailed basic research on a molecular level.

Disclosures

The authors have nothing to disclose.

Acknowledgements

Manuela Kreißelmeier and Sophia Mayer were scholarship holders of the Dr. Dr. Karl-Eibl-Stiftung. We wish to thank Gesine Krüger and Petra Reckling for excellent technical assistance.

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