

# Dynamic changes of the Golgi apparatus during bovine *in vitro* oocyte maturation

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## Abstract

For successful fertilization by the male gamete, oocyte cytoplasmic organelles such as the Golgi apparatus have to undergo specific changes: the entire process is known as cytoplasmic maturation. The goal of this study was to unravel the dynamics of the Golgi apparatus in bovine oocytes at critical stages of *in vitro* maturation, i.e. germinal vesicle (GV), GV breakdown (GVBD), metaphase I (MI) and metaphase II, and to investigate the role of various molecules critically involved therein. The cytoplasmic distribution of proteins was assessed by immunocytochemistry and laser confocal microscopy. We applied specific inhibitors, including nocodazole to unravel the functional role of the microtubular elements; sodium orthovanadate, which primarily inhibits cytoplasmic dynein ATPase activity; monastrol which inhibits the kinesin EG5; and roscovitine to inhibit the kinase cyclin-dependent kinase 2A (CDC2A). Prior to GVBD, the Golgi apparatus was translocated from the centre of the cytoplasm to the cortical area in the periphery, where it underwent fragmentation. A second translocation was observed between GVBD and MI stages, when the Golgi apparatus was moved from the cortex to the centre of the cytoplasm. Incubation with the specific inhibitors revealed that microtubules played an active role in the final localization at GVBD, while CDC2A was essential for Golgi fragmentation at GVBD stage. This partitioning was a precondition for the second movement. In conclusion, for the first time we show basic mechanisms critically involved in the regulation of the dynamic changes of Golgi apparatus during meiosis of the bovine oocyte.

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## Introduction

The successful acquisition of mammalian oocyte maturation critically depends upon a complex series of nuclear and cytoplasmic changes. Only the fully matured oocyte is able to be normally fertilized to undergo early embryonic development. The Golgi apparatus undergoes substantial re-organization between the germinal vesicle (GV) stage and the metaphase II (MII) stage, which is an important feature of cytoplasmic maturation (Moreno *et al.* 2002, Payne & Schatten 2003). The Golgi apparatus plays a central role in intracellular trafficking, specifically related to protein synthesis and delivery, and the processing of molecules via the endocytotic pathway (Rothman 1994, Glick & Malhotra 1998, Lowe & Kreis 1998, Nichols & Pelham 1998, Allan & Balch 1999, Pfeffer 1999). Any cell division is associated with fragmentation and movement of Golgi vesicles. In somatic cells, fragmentation ensures equal division of this single-copy organelle between the two daughter cells (Nelson 2000). Dispersed fragments are present in GV bovine oocytes (Hyttel *et al.* 1986,

Payne & Schatten 2003) that are distributed into the two blastomeres after first cleavage. Fragmentation appears to be a gradual process that converts stacks into membranous blobs (Nelson 2000, Lowe & Barr 2007). The Golgi apparatus is closely associated with the microtubules in somatic cells (Thyberg & Moskalewski 1999). Microtubule molecular motors, including dyneins and kinesins, are critically involved therein (Lowe 2002). Molecules of different pathways regulate Golgi organization in a redundant regulatory setup (Lowe & Barr 2007). The mechanisms underlying Golgi apparatus fragmentation during mitosis have been extensively studied (Lowe & Barr 2007). However, Golgi fragmentation during female meiosis is only poorly understood.

In mammalian oocytes, meiosis is resumed at puberty and primarily regulated by activation of several kinases (Dekel 2005), among which the cyclin-dependent kinase 2A (CDC2A) plays a pivotal role. CDC2A associates as heterodimer with cyclin B to form the metaphase promoting factor (Masui & Markert 1971). The structural Golgi matrix protein 130 (GM130) is phosphorylated in Ser-25 by CDC2A. Experimental inhibition of the kinase

significantly reduced GM130 phosphorylation and Golgi fragmentation in somatic cells (Lowe *et al.* 1998, 2000). However, GM130 phosphorylation may not be essential for Golgi fragmentation since CDC2A exerts several functions on the Golgi apparatus together with other kinases (Lowe & Barr 2007). Protein kinases such as Polo-like kinase 1 and MAP kinase kinase 1 (MEK1) are involved in Golgi fragmentation but their roles are yet unclear (Lowe & Barr 2007). Whether CDC2A is involved in Golgi fragmentation during meiotic division is unexplored.

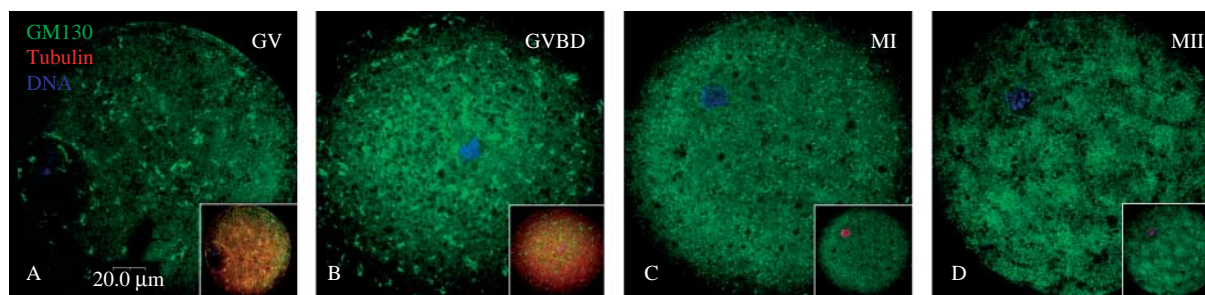
In the present study, we investigated the dynamics of the Golgi apparatus and the effects of CDC2A, microtubules, and the molecular motors (cytoplasmic dynein and the kinesin EG5) on the Golgi apparatus dynamics by treatment with specific inhibitors at defined time-points during bovine oocyte *in vitro* maturation (IVM).

## Results

### *Distribution and morphology of Golgi apparatus during bovine oocyte maturation in vitro*

At GV stage, the majority of the oocytes (77%, 125/163) showed that the GM130 dispersed throughout the cytoplasm and the surrounding GV (Fig. 1A). The Golgi marker appeared as fragments, called mini-Golgis (Payne & Schatten 2003). During oocyte maturation, the Golgi apparatus displayed two distinct movements.

The first movement was observed between the first 5 h of IVM and GV breakdown (GVBD) stage (9 h of IVM), during which the mini-Golgis translocated from the cytoplasm to the cortical area of the egg (86%, 222/259; Fig. 1B), where the first fragmentation was observed. The second movement was determined between GVBD and metaphase I (MI) stages, when the small spots moved from the cortex to the cytoplasm (93%, 140/151; Fig. 1C). This pattern was maintained until the MII stage (89%, 238/268; Fig. 1D).



**Figure 1** Confocal microscopic images showing the dynamics of the Golgi apparatus (marker = Golgi matrix protein of 130 kDa (GM130)) in relation to DNA and tubulin during bovine oocyte IVM. (A, B, C and D) Oocytes recovered during IVM in (A) GV stage, (B) GVBD, (C) MI and (D) MII. Green colour: GM130, red: tubulin and blue: DNA. (A) The Golgi apparatus is distributed throughout the ooplasm and surrounds the nucleus as mini-Golgi structures, (B) at GVBD stage after the first movement it localizes in the periphery of the cell. Fragmentation occurs in the cortical area and mini-Golgis become visible as punctuate foci. (C) The punctuate organelle moves a second time and at MI the Golgi apparatus is found throughout the ooplasm. (D) At MII, GM130 shows the same distribution as at MI, no changes in morphology and no further movement are found. Time-points for each developmental stage were described by Racedo *et al.* (2008). Insets show the merge of the three staining.

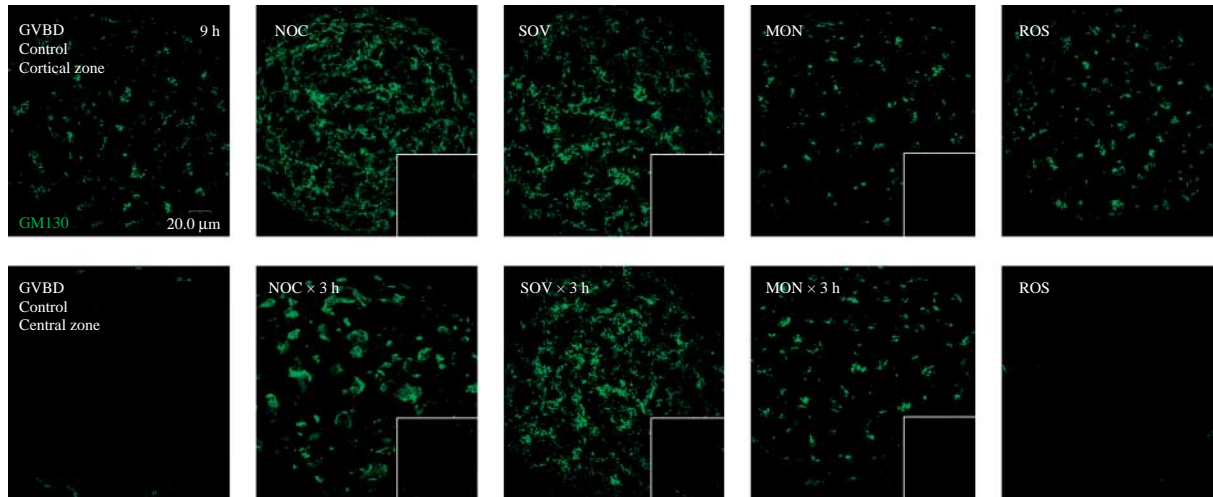
### *Microtubules are not essential for the first movement of the Golgi apparatus but affect localization at GVBD stage*

Starting nocodazole (NOC) treatment 5 h after onset of IVM and with a 0.5 h exposure, oocytes displayed disorganized mini-Golgis in the cortical area (Fig. 2; 67%; 60/90) or during the 3 h prior to GVBD (60%, 67/112). Three hours before GVBD, Golgi vesicles started to move from the cytoplasm to the cortical area of the oocyte, which was independent from the microtubules (note the absence of signal in the central part of the oocytes; Fig. 2 insets). Although movement of Golgi to the cortical area was observed, their localization at GVBD after NOC treatment revealed a role of the microtubules in maintaining mini-Golgis in the cortical area since the pattern found in NOC-treated oocytes was different from that of the GVBD controls as shown by the disorganization of Golgi vesicles.

### *Cytoplasmic dynein plays a role in the localization of Golgi at GVBD*

To explore a possible role of cytoplasmic dynein ATPase activity in the distribution of mini-Golgis during the first movement, oocytes were treated with sodium orthovanadate (SOV). Since SOV inhibits an array of molecules, the combined use of SOV and NOC is useful for unraveling the effects on dynein-ATPase (Carabatsos *et al.* 2000). The majority of oocytes incubated with SOV starting 5 h after onset of IVM had disorganized Golgi vesicles in the cortical area, irrespective of whether exposed for 0.5 h (60%; 58/98; Fig. 2), or 3 h prior to GVBD (60%; 53/88). This pattern was similar to that observed in NOC-treated oocytes (Fig. 2).

Oocytes treated with monastrol (MON) for 1 h to inhibit kinesin EG5 displayed the same pattern of mini-Golgis as the GVBD control group (90%; 106/118; Fig. 2) or the group exposed during 3 h prior to the GVBD stage (91%; 110/121). The insets in Fig. 2 show



**Figure 2** Confocal microscopic images showing the dynamics of the Golgi apparatus (marker: GM130, green colour) at 9 h of IVM (GVBD in control oocytes) after treatment with each inhibitor at 5 h of IVM, prior to the first movement of the organelle. Mini-Golgis move to the cortical area leaving the central area free of vesicles, control cortical and central zones respectively. Insets and ROS in the lower picture show the central part of the oocyte in each treatment.

that the central part of the oocytes was free of vesicles suggesting that dynein and kinesin EG5 are not involved in the first movement of the Golgi vesicles.

### ***CDC2A is essential for Golgi fragmentation and Golgi fragmentation is a prerequisite for the second movement***

When oocytes were treated with roscovitine (ROS) to inhibit kinase CDC2A activity at 5 h after onset of IVM, the cortical area displayed the same pattern as the control group (90%, 114/127; Fig. 2). The mini-Golgis were predominantly found in the cortical area in the majority of the oocytes, suggesting that phosphorylation from this kinase is not essential for the first movement. Incubation of the oocytes in the presence of ROS at GVBD stage for 3 or 4 h prior to the MI stage (Fig. 3) revealed that partitioning of Golgi vesicles into punctate foci was not affected (94%, 92/98; Fig. 3 and 91%; 74/81 respectively). This indicates that fragmentation started immediately after mini-Golgis had reached the cortical area at GVBD stage. The majority of the oocytes incubated for 24 h in the presence of ROS showed formation of mini-Golgis (74%, 66/89; Fig. 4) that were exclusively located in the cortical area (inset) suggesting that fragmentation is a prerequisite for distribution throughout the cytoplasm after GVBD stage.

### ***The second movement of Golgi apparatus is independent of microtubules and microtubule molecular motors***

Treatment from GVBD stage to fixation at MI stage allowed us to explore the role of microtubules, cytoplasmic dynein and kinesin EG5 in the second

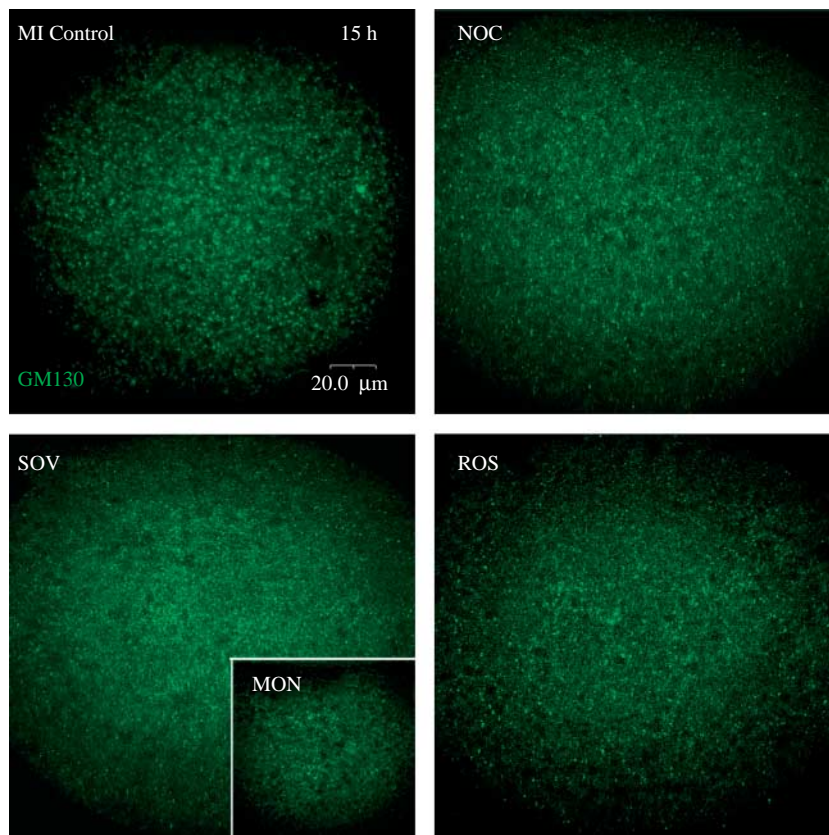
movement of the Golgi apparatus from the cortical area to the central cytoplasm. The majority of the treated oocytes displayed a similar pattern as the controls (Fig. 3), showing that microtubules (96%; 78/81), cytoplasmic dynein (89%; 71/80) and kinesin EG5 (94%; 75/80) were not required for the Golgi apparatus to be moved from the cortical area to the cytoplasm (Fig. 3).

Incubation of oocytes for 24 h with NOC, at 20  $\mu$ M (90%; 125/139) or 100  $\mu$ M (94%; 132/141; Fig. 4) did not affect final distribution of the Golgi apparatus. This lends further support to the finding that the second movement is independent of the function of microtubules.

The Golgi dynamics were independent of the presence of cumulus cells during the maturation period as denuded GV oocytes matured for 24 h showed a similar pattern of vesicles (93%; 37/40) as the controls (Fig. 4). Additionally, the effect of each inhibitor treatment was reversible as the pattern of Golgi vesicles at MII stage was similar to that in the control groups (data not shown).

## **Discussion**

Maturation of the mammalian oocyte encompasses a complex series of molecular and structural nuclear events, culminating in meiotic arrest of the chromosomes at the MII stage. In order to be successfully fertilized by the male gamete, cytoplasmic organelles such as the Golgi apparatus have to undergo specific changes. The dynamics of the Golgi apparatus constitute a hallmark of cytoplasmic maturation. Here, a study of the vesicle movements with the changes in morphology during bovine oocyte maturation was combined with the

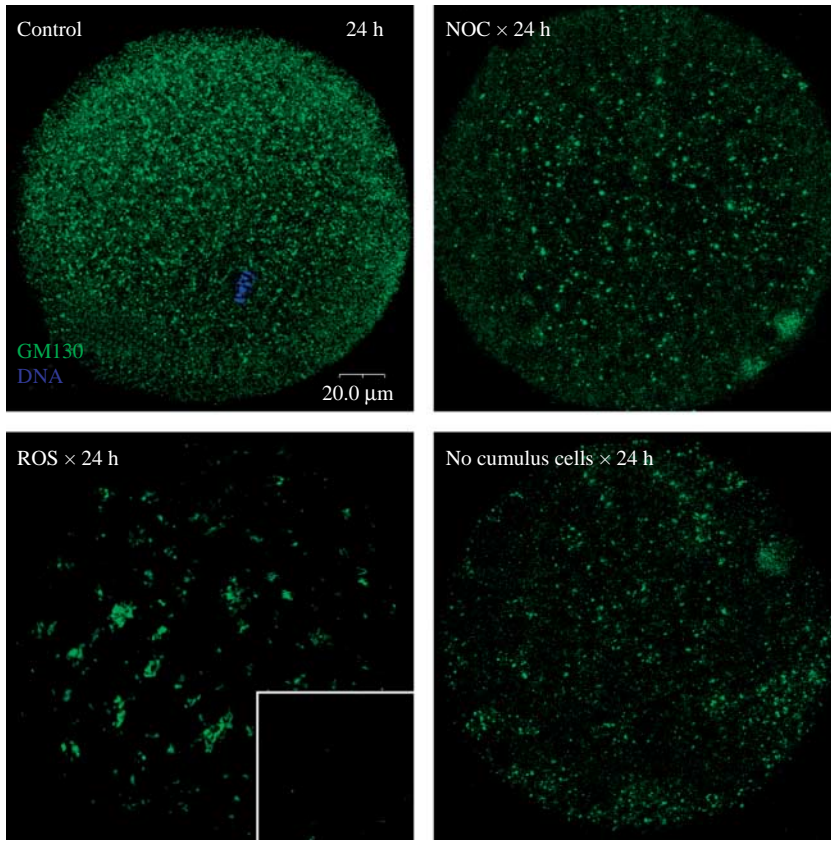


**Figure 3** Confocal microscopic images showing the dynamics of the Golgi apparatus (marker: GM130, green colour) at 15 h of IVM (MI in control oocytes) after treatment with each inhibitor at 9 h of IVM, prior to the second movement of the organelle. Mini-Golgis fragmentize and become punctuate foci concomitant with GVBD stage. After fragmentation, Golgi vesicles move from the cortical area to the ooplasm of control oocytes and treated oocytes.

use of different inhibitors to unravel the physiological function of molecules that could potentially be involved in these changes in morphology and site. Previous studies had shown that the Golgi apparatus is no longer visible when oocyte growth has started while many Golgi stacks appear in the cytoplasm (Wassarman & Josefowicz 1978, Weakley *et al.* 1981). These stacks are called mini-Golgis, which fragmentize and remain in that form in oocytes until arrest at MII (Weakley 1966, Calarco *et al.* 1972, Wassarman & Josefowicz 1978). In GV oocytes from mouse and rhesus monkey, the Golgi apparatus is visible as mini-Golgi stacks which are distributed throughout the cytoplasm (Moreno *et al.* 2002). The same pattern was described in bovine GV oocytes (Payne & Schatten 2003). Mini-Golgis disappear at the GVBD stage and accumulate as dotted structures in the central part of the mouse oocyte while they are distributed throughout the matured cytoplasm of bovine oocytes (Moreno *et al.* 2002, Payne & Schatten 2003). This distribution pattern is maintained after extrusion of the first polar body in mouse and bovine MII oocytes (Moreno *et al.* 2002, Payne & Schatten 2003).

Our results show for the first time that mini-Golgis are predominantly located in the cortical area of GVBD oocytes where they undergo fragmentation (see Fig. 5). The polymerized cytoplasmic network of the microtubules surrounding the nucleus directs the localization

of different cargoes in the bovine oocyte (Racedo *et al.* 2009). The reasons for the specific localization of mini-Golgis in bovine oocytes at GVBD stage and prior to fertilization are unknown. Given the lack of functionality of Golgi during meiotic maturation, it could be that the Golgi follows the changes of endoplasmic reticulum export sites (Payne & Schatten 2003). Additionally, matured bovine oocytes are acentrosomal which could explain the contrasting findings in bovine and mouse oocytes regarding Golgi distribution (Schatten 1994, Zhang *et al.* 2011). Presumably, during early embryonic development Golgi fragmentation ensures equal distribution of the organelle between daughter cells since the division during meiosis is unequal. By applying different inhibitors at distinct time-points during maturation, we were able to unravel important steps of this sequence of events. We found that microtubules do not play an essential role for Golgi dynamics during bovine oocyte maturation, which had not been shown previously. Incubation with NOC during maturation did not interfere with Golgi morphology and localization at MII stage. However, a short incubation for 5 h after the onset of IVM affected the mini-Golgi pattern in the cortical area at GVBD stage. We propose that the microtubules act as anchors at GVBD stage, but are not essential for the final status of this organelle. This is supported by findings in somatic cells where the microtubules act in



**Figure 4** Confocal microscopic images showing the dynamics of the Golgi apparatus (marker: GM130) after 24 h of IVM (MII in control oocytes) and 24 h incubation in 100  $\mu$ M NOC, ROS treated or denuded GV stage oocytes. ROS for 24 h maintained oocytes at GVBD stage, mini-Golgis in the cortical zone and kept the central zone free of vesicles (inset). Green colour: GM130, blue: DNA.

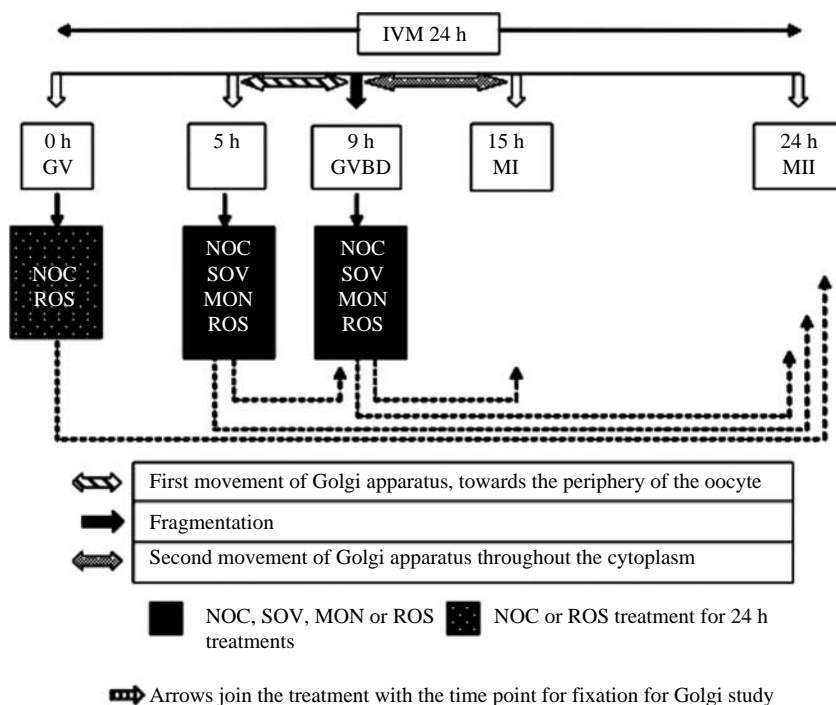
an anchor-like manner for the binding of Golgi elements (Thyberg & Moskalewski 1999). The lack of functionality of the Golgi apparatus during bovine oocyte maturation observed by Payne & Schatten (2003) suggests that the Golgi apparatus is not essential for achieving bovine oocyte nuclear maturation. Instead, it is prominent feature of cytoplasmic maturation.

Microtubules are involved in a variety of physiological processes during cytoplasmic and nuclear maturation (Brunet & Maro 2005). After GVBD stage, the microtubular network and the Golgi vesicles undergo substantial transformation which is independent from each other. A putative interaction between microtubules and Golgi vesicles must occur between the GV stage and GVBD stage; afterwards microtubules rearrange to allow the progress of nuclear maturation.

During mitosis of somatic cells treated with the microtubule disruptor, NOC, the Golgi apparatus is dispersed due to the association of Golgi with the centrosome (Kodani & Sutterlin 2008). This does not occur during meiotic division (Payne & Schatten 2003) because the centrosome is not present prior to fertilization in bovine, human and rhesus monkey oocytes (Sathananthan *et al.* 1991, Navara *et al.* 1994, Schatten 1994, Sutovsky *et al.* 1996). Other motor proteins, such as dynein and kinesin, have been implicated in Golgi movements and bind to Golgi

membranes in somatic cells (Thyberg & Moskalewski 1999). When we applied inhibitors for microtubule motor proteins, we observed that SOV reproduced the effects of NOC at GVBD, suggesting that cytoplasmic dynein acts as anchoring like step proposed for microtubules at GVBD stage. SOV is an inhibitor of phosphatase and ATPase activity with high selectivity for dynein ATPase activity (Niclas *et al.* 1996), however, inhibition of other enzymes and cellular pathways cannot be ruled out. The disturbances in the cortical area reported herein may not reflect a direct effect on dynein motors. Nevertheless, the use of NOC and SOV produced similar effects, reinforcing the idea of a microtubules related effect. Future studies could extend into a selective inhibition of dynein function with either decreased SOV concentration or the use of an even more specific inhibitor. The specific inhibitor for kinesin EG5 did not affect the pattern of Golgi under our incubation conditions.

Critical steps during oocyte maturation are regulated by the cytoskeleton. The phosphorylation of many substrates by kinases, such as CDC2A and the MAP kinases cascade, is crucial for progression of maturation (Dekel 2005). Morphology and function of the Golgi complex in mitotic cells are affected by CDC2A and other protein kinases, irrespective of the integrity of the microtubules (Thyberg & Moskalewski 1999). Inhibitors



**Figure 5** Time-points during bovine IVM when movement and fragmentation of the Golgi apparatus are observed combined with onset of treatments and fixations. NOC, ROS, SOV and MON.

of MEK1 and CDC2A have been successfully used to determine that these kinases act in a stage-specific manner to block Golgi fragmentation in somatic cells (Kano *et al.* 2000). MEK1 and CDC2A may work sequentially to drive re-organization of the Golgi stack into blobs (MEK1 dependent), followed by transformation into a dispersed state throughout the cytoplasm (CDC2A dependent) in somatic cells (Nelson 2000). Otherwise, the role of MEK1 in Golgi fragmentation is unknown (Lowe & Barr 2007).

We applied ROS to study the role of the kinase CDC2A on Golgi dynamics and found that incubation during the entire maturation period reproduced the same pattern of vesicles as found for GVBD stages in the control group. This indicates an essential role of CDC2A in fragmentation of mini-Golgis at GVBD stage of bovine oocytes. These mini-Golgis were located in the cortical area suggesting that the second movement of Golgi vesicles is triggered by phosphorylation of targets of the kinase CDC2A. The lack of effects of the ROS treatment except for the 24 h incubation suggests that fragmentation occurs rapidly at the GVBD stage.

We also demonstrated that the absence of cumulus cells during maturation did not affect Golgi morphology and distribution, suggesting that Golgi dynamics are independent of cumulus cells and that the effects induced by inhibitors are due to a direct function within the oocyte.

In the present study we used inhibitors to unravel the biological function of the target molecules. This approach has limitations as the inhibitors may not always be specific enough and may affect other molecules. However, other approaches such as

microinjection of inhibitory molecules into the cytoplasm are not a good alternative. Any experiment that includes microinjections in immature bovine oocytes can be detrimental for the fragile cytoplasm. This was clearly not in line with the goal of our study in which we tried to avoid any perturbations of the cells to be able to observe the effects on cytoplasmic events during maturation. Moreover, microinjection would have required removing the cumulus cells, which would have further interfered with oocyte maturation and masked the effects on the Golgi compartment.

In summary, for the first time we describe Golgi morphology and movements during bovine oocyte maturation *in vitro* and analysed the physiological role of different molecules within this process by applying specific inhibitors at distinct time-points of maturation. Microtubules and cytoplasmic dynein are critical for the anchoring like step at GVBD stage, the kinase CDC2A plays an essential role in Golgi fragmentation which in turn is required for the second movement. The Golgi dynamics seem to be independent from the kinesin EG5 during bovine oocyte maturation. Future studies will have to show that these observations are also valid for growing oocytes and *in vivo* derived oocytes.

## Materials and Methods

### Oocyte collection and IVM

Bovine ovaries were collected at a local abattoir and transported to the laboratory at 25–30 °C. Ovaries were washed three times in 0.9% NaCl (S5886; Sigma) supplemented with penicillin (PEN-NA; Sigma) and streptomycin (35500; Serva, Heidelberg,

Germany). Cumulus–oocyte complexes (COCs) were isolated by slicing the ovary's surface. COCs were selected and matured *in vitro* as described previously (Racedo *et al.* 2008).

### Fixation at different meiotic stages

The time-points selected for fixation at different meiotic stages were: GV: 0 h; GVBD: 9 h; MI: 15 h; and MII: 24 h after onset of IVM (Racedo *et al.* 2008). Random samples were taken from pools of maturing oocytes at these different time-points for fixation and subsequent analysis as described recently (Racedo *et al.* 2009).

### Antibodies, immunocytochemistry and confocal microscopy

Primary antibodies were applied overnight at 4 °C. GM130 was identified by using the mouse MAB clone 35 (BD Transduction Labs, Franklin Lakes, NJ, USA) at 1:20. Tubulin was studied using a sheep polyclonal antibody (ATN02, Cytoskeleton, Darmstadt, Germany) at 1:150. Negative controls included incubation of primary antibodies with their corresponding antigen for 1 h (specificity of the first antibody) and by using secondary antibodies in the absence of the primary antibodies to detect possible fluorescence due to unspecific binding (specificity of the second antibody).

Alexa-Fluor 488 goat anti mouse IgG (11001; Molecular Probes, Franklin Lakes, NJ, USA) and Alexa-Fluor 568 phalloidin (A12380; Molecular Probes) were used as secondary antibodies and applied for 2 h at 37 °C in the dark. DNA was labelled with 10 µg/ml TOTO-3 (T3604; Molecular Probes) during 30 min in the dark. RNase (R5500; Sigma) was added for avoiding cytoplasmic RNA labelling. Oocytes were mounted in an anti-fade medium (Vectashield H-1000; Vector Laboratories) to minimize photobleaching and were visualized

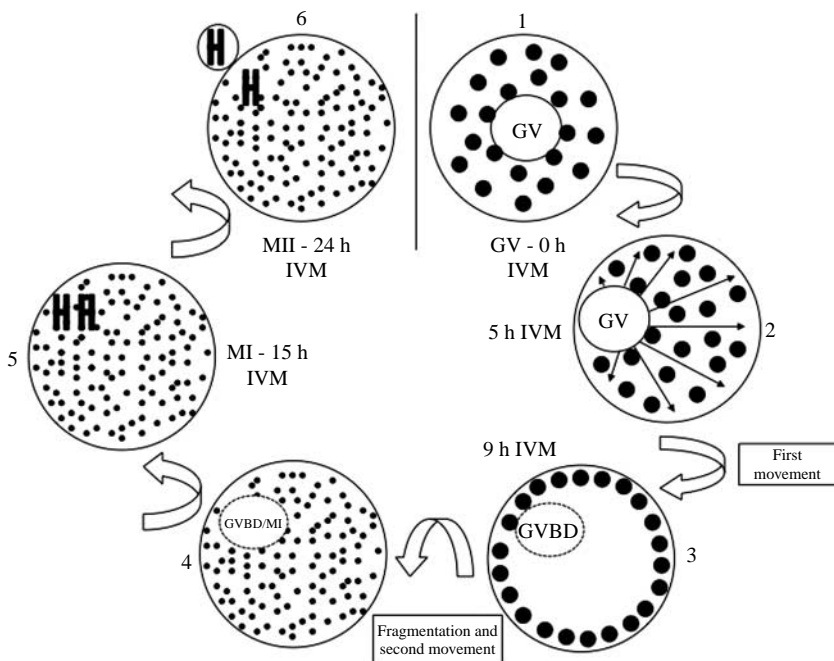
with a Zeiss LSM 510 laser scanning microscope, using laser with 488-, 568-, and 633-nm wavelengths respectively. Images were then processed by using Adobe Photoshop 7.0.

### Oocyte treatment and evaluation of maturation rate: determination of dosage and incubation period for each inhibitor

NOC (M1404; Sigma) was used to disrupt microtubules. It was applied at two different concentrations (20 and 100 µM) associated with a subtle or pronounced perturbation of the cell (Rawe *et al.* 2004, Katayama *et al.* 2006).

SOV (S6508; Sigma), which specifically inhibits different ATPases and phosphatases, is highly selective on dynein ATPase activity (Niclas *et al.* 1996), and was used at a concentration of 400 µM (Wang *et al.* 2004).

MON (M8515) was applied at a concentration of 14 µM to inhibit kinesin protein EG5 (Mayer *et al.* 1999). ROS (R7772; Sigma) was used at 50 µM to inhibit CDC2A kinase activity (Mermillod *et al.* 2000). Treatments were started according to the first observation of morphological changes of the Golgi apparatus during oocyte maturation, either prior to localization at GVBD (5 h of IVM) or after localization at GVBD (9 h of IVM) (see Fig. 6). Inhibitors were applied for the maximum tolerable time, i.e. prior to observing a significant decrease in nuclear maturation. This maximum tolerable exposure was determined by testing each inhibitor for different incubation periods followed by assessing the nuclear maturation rate at 24 h of IVM. Controls included incubation with the vehicle (DMSO or water depending on the inhibitor) for the same time periods as with the respective inhibitors (see Supplementary Figure 1, see section on supplementary data given at the end of this article). The vehicle solution was added to the maturation media in the same concentration as with the specific inhibitor. Maturation



**Figure 6** Schematic representation of Golgi apparatus dynamics during bovine oocyte IVM. Golgi vesicles or mini-Golgis (black circles) are distributed throughout the cytoplasm and are surrounding the nucleus in the GV stage (1). Between 5 h of IVM and GVBD stage, mini-Golgis migrate towards the cortical area (2). The first movement is independent of microtubules and microtubule molecular motors, but these cytoplasmic components affect the distribution and pattern of mini-Golgis at GVBD. Once they have reached the cortical area (3), CDC2A-dependent fragmentation takes place, immediately followed by the second movement. The punctuate foci (4) localize throughout the cytoplasm up to the MII stage (5 and 6). Arrows in (2) show the direction of the movements.

rate or distribution of Golgi was never affected by the presence of the vehicle solution. It was essential for this study to allowing the oocytes to accomplish nuclear maturation so that we could evaluate the role of each molecule and cytoplasmic events in parallel with the endogenous nuclear process. This allowed us to differentiate cytoplasmic events in an accurate manner.

Each inhibitor was added for disrupting cytoplasmic maturation at 5 and 9 h after onset of IVM (see Fig. 6 for inhibitor treatment design). Oocytes were randomly divided and cultured for 0.5 or 1 h in NOC, 0.5 or 1 h in SOV, 0.5, 1 or 2 h in MON and 2, 3 or 4 h in ROS. Subsequently, oocytes were washed and cultured in inhibitor-free maturation medium up to 24 h of IVM (end point). To determine the maturation rate after 24 h of IVM, oocytes were denuded using 0.1% hyaluronidase (H4272; Sigma) for 10 min at 37 °C and vortexed for 4 min. Subsequently, oocytes were washed twice in PBS containing 0.1% polyvinyl alcohol (PVA; P8136; Sigma) and were stained with Hoechst 33342 (B2261; Sigma) in 1 ml/100 ml PBS–PVA for 10–15 min. Extrusion of the first polar body was determined under an inverted microscope at 200× magnification. Stained oocytes were observed under u.v. light at 400× magnification with the filter BX60 F-3 (Olympus Optical, Hamburg, Germany) and the percentage of oocytes in MII was recorded. A total of 80–120 oocytes from at least three different IVM replicates were evaluated for each inhibitor treatment and per time-point (5 and 9 h of IVM), in parallel with the corresponding vehicle controls. In total, 2700 nuclear maturation events were evaluated in this experiment in order to establish the experimental conditions for studying morphology and dynamics of the Golgi apparatus.

### Statistical analysis

The effects of NOC, SOV, MON or ROS on the maturation rate were analysed with SigmaStat 2.0 software package (Jandel Scientific, San Rafael, CA, USA) using  $\chi^2$  and Fisher exact tests. Differences of  $P < 0.05$  were considered statistically significant.

### Oocyte treatments and fixation at selected time-points

In the first experiment, treatments with NOC, SOV, MON or ROS started at 5 and 9 h respectively after onset of IVM (Fig. 6). We evaluated the role of microtubules, cytoplasmic dynein ATPase activity, kinesin EG5 and CDC2A kinase activity on the dynamics of the Golgi apparatus. Oocytes incubated with NOC, SOV, MON or ROS for 3 h prior to GVBD or MI and NOC and ROS for 24 h of IVM were also studied in order to test stronger treatments; in these cases nuclear maturation rates were affected.

The effects of the inhibitors on the distribution of the GM130 that localizes to cis-Golgi apparatus were evaluated immediately after GVBD in oocytes treated at 5 h of IVM and after the second localization (MI) in oocytes treated after 9 h of IVM (Fig. 6). Distribution of the Golgi apparatus at the end of the IVM was additionally determined in a subgroup of the treated oocytes to test the reversibility of the inhibitor effects. These experiments had at least three independent replicates with a total of 2400 oocytes.

### Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/REP-11-0492>.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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