# Exposure of pig oocytes to PCBs during *in vitro* maturation: effects on developmental competence, cytoplasmic remodelling and communications with cumulus cells

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Polychlorinated biphenyls (PCBs) are one of the most persistent and widespread groups of endocrine disrupting compounds in the ecosystem. These substances are present in sewage sludge that is spread in increasing amounts on arable land and pasture as fertilizer, and are ingested by farm animals with food and drinking water. This study investigated the effect of different PCB concentrations on pig oocyte *in vitro* maturation and developmental competence as well as examined the possible mechanisms involved. A concentration ranging from 0 to 1 μg/mL of Aroclor 1254 (A1254), a pool of more than 60 PCB congeners, was added to the maturation medium, as its composition is considered environmentally relevant. A1254 had no effect on maturation of pig oocytes and on the number of oocytes that cleaved following parthenogenetic activation at any of the doses tested. By contrast, a significant decrease in the number of zygotes that developed to blastocyst stage became evident at a concentration of 10 ng/mL. The number of blastocysts obtained decreased significantly, and in a dose response manner with higher concentrations. Exposure to PCBs altered mitochondria relocation during maturation and this was associated with the lack of a cytoplasmic microtubule network. No effect on mitochondria activity was observed. A1254 exposure also perturbed gap-junction mediated communications between oocytes and cumulus cells. In conclusion, PCB exposure of pig oocytes during *in vitro* maturation significantly decreased oocyte developmental competence, altered both their cytoplasmic remodelling and the communication with the somatic compartment. These data indicated that accumulation of PCBs in the pig organism may have a detrimental effect on the reproductive efficiency in this species.

Key words: pig, oocyte, embryo, development, PCB, mitochondria, cytoskeleton, gap-junctions.

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We have recently become aware that some synthetic chemical compounds can interfere with normal reproductive functions in humans and animals, acting as endocrine disrupters (Colborn *et al.*, 1993; Peterson *et al.*, 1993). Amongst them, polychlorinated biphenyls (PCBs), mixtures of chlorinated hydrocarbons that were synthesized from the early 1920s, constitute a main threat even if they were banned in industrialized countries during the late 1970s (Toppari *et al.*, 1996). In fact, the properties of PCBs characterizing their industrial benefits — stability and resistance towards degradation — also made them one of the most persistent and widespread group of endocrine disrupting compounds in the ecosystem (Safe, 1994). High concentrations of these substances are known to be present in sewage sludge from industrial, agricultural and domestic origin that is spread in increasing amounts on arable land and pasture as fertilizer (Wild and Jones, 1992) and is found in water (Abbassy *et al.*, 1999; Fingler *et al.*, 1992). Compounds typically present in sewage include phthalates, alkylphenols, organochlorine pesticides and PCBs (Giger *et al.*, 1984; Wild and Jones, 1992). These are ingested by farm animals with food and drinking water and it is likely that the rates of ingestion will increase in the future, as increasing amounts of sewage sludge are recycled onto agricultural land. At present neither the rates of uptake, retention, degradation, modification and excretion of these compounds, nor their effects on animal health and reproductive system have been fully determined.

We have recently demonstrated that a complex mixture of PCBs adversely affects bovine oocyte maturation, fertilization and early embryo development *in vitro* (Pocar *et al.*, 2001b). Limited data are currently available on the effects of these compounds on pig oocyte maturation and developmental competence (Campagna *et al.*, 2002; Campagna *et al.*, 2001); this study was undertaken to investigate the

effect exerted by exposure to different concentrations of PCBs on pig oocyte *in vitro* maturation and developmental competence. Since PCBs are a family of 209 molecules, which are present in the environment as a mixture, Aroclor 1254 (A1254), a pool of more than 60 congeners, was used in these experiments, as its composition is considered to be environmentally relevant (Stack *et al.*, 1999).

Moreover, we investigated the possible mechanisms through which PCBs act on the oocyte. In particular, we studied the activity and cytoplasmic distribution of mitochondria as an easily detectable marker of cytoplasm compartmentalization. Microtubules were also studied as the recognized responsible for the establishment and maintenance of cellular asymmetry during oocyte maturation (Gard *et al.*, 1997). Furthermore, based on previous evidences showing that A1254 perturbs post-transcriptional activity of gap-junction mRNAs in bovine oocytes (Pocar *et al.*, 2001a), as well as in different experimental models (Brevini et al, 2004), we used Lucifer Yellow microinjection to study functional changes in gap junction-mediated cumulus cell-oocyte communications.

## Materials and Methods

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise stated.

### *Recovery and maturation of cumulus oocyte complexes (COCs)*

Ovaries of animal were transported to the laboratory and rinsed in Dulbecco Modified Phosphate Balance Solution at pH 7.5 supplemented with Antibiotic-Antimicotic solution (Sigma 9909).

Cumulus oocyte complexes (COCs) were obtained from ovaries of slaughtered gilts (240±15 days of age). Follicles with a diameter of 2-7 mm were aspirated with an 18-gauge needle and vacuum pressure of 50 mL/min. The follicles aspirate was collected in 15 mL tubes and COCs were washed twice in pre-warmed (37°C) aspiration medium. The medium was composed of TCM199 supplemented with 6.5 mg/mL HEPES, 1.1. mg/mL sodium bicarbonate, 4 mg/mL bovine serum albumin (BSA), 75 μg/mL penicillin and 50 μg/mL streptomycin. Only COCs with large, compact cumulus and homogeneous oocyte cytoplasm were selected for IVM. IVM was performed as previously described (Grupen *et al.*, 2003) with minor modifications. Briefly COCs were cultured in TCM199 supplemented with 25% pig follicular fluid, 1.1 mg/mL sodium bicarbonate, 0.1 μg/mL sodium piruvate, 0.5 mM cysteamin, 8.2 μg/mL insulin, 10 ng/mL EGF, 1 mM dcAMP, 0.5 I.U./mL porcine FSH:LH (Pluset, Serono, Rome, Italy), 1.0 μg/mL 17 β-estradiol, 75 μg/mL penicillin and 50 μg/mL streptomycin. COCs were matured in this medium for 22h at 38.5°C in an atmosphere of 5% $CO_2$ in air. COCs were washed in fresh medium and matured for an additional 24h in the same medium without dcAMP.

### *Exposure to Aroclor 1254*

A1254 was a kind gift by Prof. S. H. Safe, Texas Veterinary College. The compound was dissolved in absolute ethanol (Merck, Germany) at 100 mg/mL and serially diluted in maturation medium to obtain the desired concentration with the maximal alcohol content of 0.01% (v/v). Control culture dishes contained medium with the same amount of alcohol.

A-1254 was added to the maturation medium described above in various concentrations, ranging from 0 to 1 μg/mL. Between 25 and 35 COCs were matured in 500 μL of medium; incubation was performed for 46 h, in four-well dishes (Nunc, Roskilde, DK) at 38.5°C in a humid atmosphere of 5% $CO_2$ in air.

### *Meiotic assessment*

At the end of maturation, oocytes were denuded by gently pipetting in aspiration medium containing 0.1% hyaluronidase at 38.5°C, washed in medium without enzyme and mounted on microscope slides. Samples were fixed for 48h in acetic acid:ethanol (1:3) and stained with acetic orcein 0.1% (v/v) for 30 min. Samples were destained in glycerol:acetic acid:water (1:1:3) and meiotic stage was evaluated using a Nikon Eclipse E600 microscope at 200x and 400x magnification.

### *Parthenogenetic activation and in vitro culture*

Since *in vitro* fertilization of pig oocytes is affected by a high degree of polyspermy (Prather and Day, 1998), parthenogenetic activation has been used in order to obtain a more homogeneous rate of development.

After IVM, oocytes were denuded as described above, washed for 10 min in TCM199 supplemented with 20% (v/v) foetal calf serum and then incu-

bated in TALP medium for 30 min at 38.5°C. Parthenogenetic activation was performed according to Boquest *et al.* (2002). Oocytes were sequentially exposed to 5 μM ionomycin in TALP for 5 min at 38.5°C in the dark and to 2 mM 6-DMAP in medium NCSU-23 (Petters and Wells, 1993) for 3 h at 38.5°C in an atmosphere composed of 5% $CO_2$, 5% $O_2$ and 90% $N_2$. Presumptive parthenotes were washed thoroughly in medium NCSU-23 and cultured in groups of 25-35 in 50 μl NCSU-23 drops under mineral oil at 38.5°C in the same atmosphere as above. Embryonic cleavage was recorded 48 hours post activation. On day 5 post activation, half of the medium was replaced with fresh NCSU-23 containing 20% (v/v) FCS, to reach a final FCS concentration of 10% (v/v) in the drop. At day 7-post activation, culture was interrupted and embryonic development evaluated.

### *Staining of mitochondria and microtubules*

Mitochondria localization was studied in oocytes at different stages of maturation. Organelles were stained either with MitoTraker Green FM, which preferentially accumulate in mitochondria regardless of their membrane potential, making it possible to determine the whole mitochondrial mass, or with MitoTracker Orange CMTM-Ros, which stains selectively active mitochondria and is well retained after fixation (Molecular Probes Europe, Leiden, The Netherlands).

A stock solution of both dyes was prepared according to manufacturer specifications. MitoTraker Green was used at the concentration of 400 nM and MitoTracker Orange at 250 nM in maturation medium for 30 min at 38.5°C in the dark. After staining, oocytes were briefly rinsed in PBS and fixed in 3.7% para-formaldehyde in PBS for 30 min at 37°C. Samples were mounted on glass slides in a glicerol-based mounting medium. Oocytes were stored below 0°C until confocal microscopy was performed.

Immunolocalization of microtubules was carried out as previously described (Sun *et al.*, 2001a), with minor modifications; briefly, oocytes were fixed in 3.7% para-formaldehyde in PBS for 30 min at 37°C. They were permeabilized in PBS containing 0.5% Triton X-100 for 20 h at 37°C and then incubated in PBS containing 115 mM glycine and 1% Triton X-100 for 30 min. After washing for 15 min in PBS, oocytes were incubated with a FITC-conjugated anti-α-tubulin antibody (Sigma, F-2168) diluted 1:50 for 90 min at room temperature. After two washes in PBS, DNA was stained with DAPI and oocytes were mounted as described above. When mitochondria and microtubules were stained in the same oocytes, the two protocols described here were performed in sequential order.

### *Confocal microscopy*

Stained samples were examined using a TCS-NT laser scanning confocal microscope (Leica Microsystems, Heidelberg, Germany) equipped with Ar/Kr and He/Ne lasers. Blocking filters used were: BP 530±30 (microtubules and total mitochondria); LP 590 (active mitochondria) and LP 450 (DNA). Mitochondria distribution was assessed through one equatorial optical section of 6.7 μm thickness. Laser intensity was 1.5 mV. Objective (10x and 40x Leica Floutar, Leica Microsystems, Heidelberg, Germany), pinhole (1 Airy unity), filters, offset, gain and PMT settings were kept constant throughout the experiments. Microtubules distribution was assessed by sequential scanning of at least 40 μm of sample with 2.6 μm step size and controlled over sampling.

### *Dye coupling assay*

To detect cumulus-oocyte communications through gap junctions, cumulus-enclosed oocytes were injected with a 4.5% Lucifer Yellow (LY) solution in 5 mM lithium chloride. Control and A1254 (0.1 μg/mL) treated oocytes were microinjected (n = 125) at Germinal Vesicle (GV), at first metaphase (MI) and at second metaphase (MII) stages respectively. The COCs were placed in 50μl droplets of hepes-buffered TCM 199 under mineral oil on the heated stage (37°C) of an inverted microscope (Nikon Diaphot, Japan) equipped with an epi-fluorescent light (excitation 490nm, emission 510nm) and a microinjection apparatus (Narishige CO LTD, Tokyo, Japan) to guide the holding and injecting micropipettes. This apparatus was coupled with an Eppendorf Femtojet injector that ensured quantitative reproducibility through pressure and time standardization. Holding pipettes were made from borosilicate glass capillaries without inner filament (outer diameter, OD, 1 mm, inner diameter, ID, 0.58 mm. Clark, England); they were hand-pulled and then fire-polished with a microforge to have an ID of 20-30 μm. Injection needles were pulled with a micropipette puller from borosilicate glass capillaries with inner filament (OD 1 mm, ID 0.78 mm.

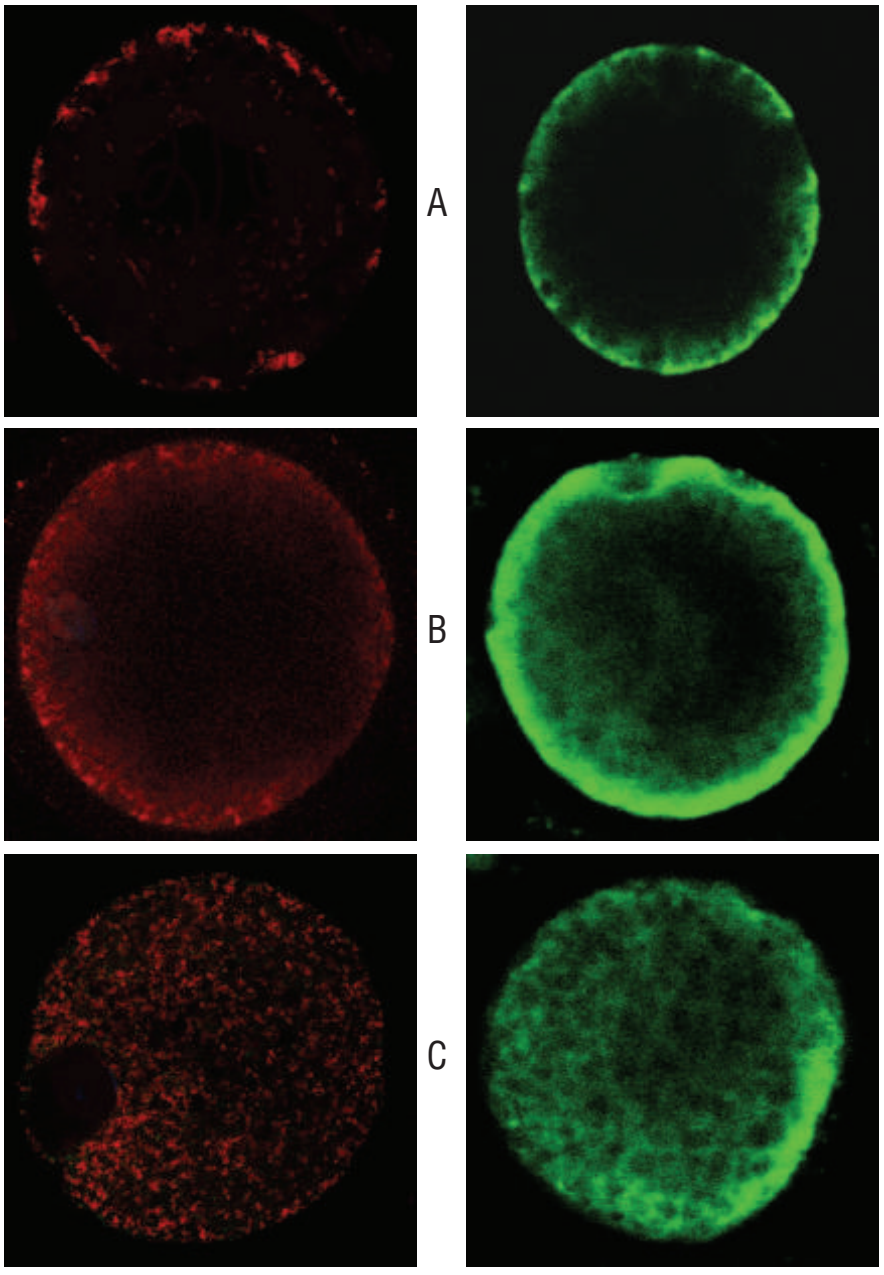



**Figure 1. Representative pictures of mitochondria localisation in pig oocytes. Mitochondria were stained either with MitoTracker Orange (left), which stains selectively active mitochondria or with MitoTraker Green (right), which determines the whole mitochondrial mass. Regardless of the dye used, mitochondria showed three distributions: peripheral (A), intermediate (B) or diffused (C). Original magnification 200×.**

Clark, England); final ID was <1 μm. Both holding and injection pipette were bent to an angle of 30°.

The diffusion of LY from the oocyte cytoplasm to the cumulus cells was evaluated 10 minutes after injection. COCs were classified as "open" when at least 80% of corona radiata cells were fluorescent, as "intermediate" when only a limited number of cells showed signs of dye diffusion between ooplasm and corona radiata cells and as "closed" when the LY was confined in the cytoplasm or only few cells showed fluorescence (Figure 3).

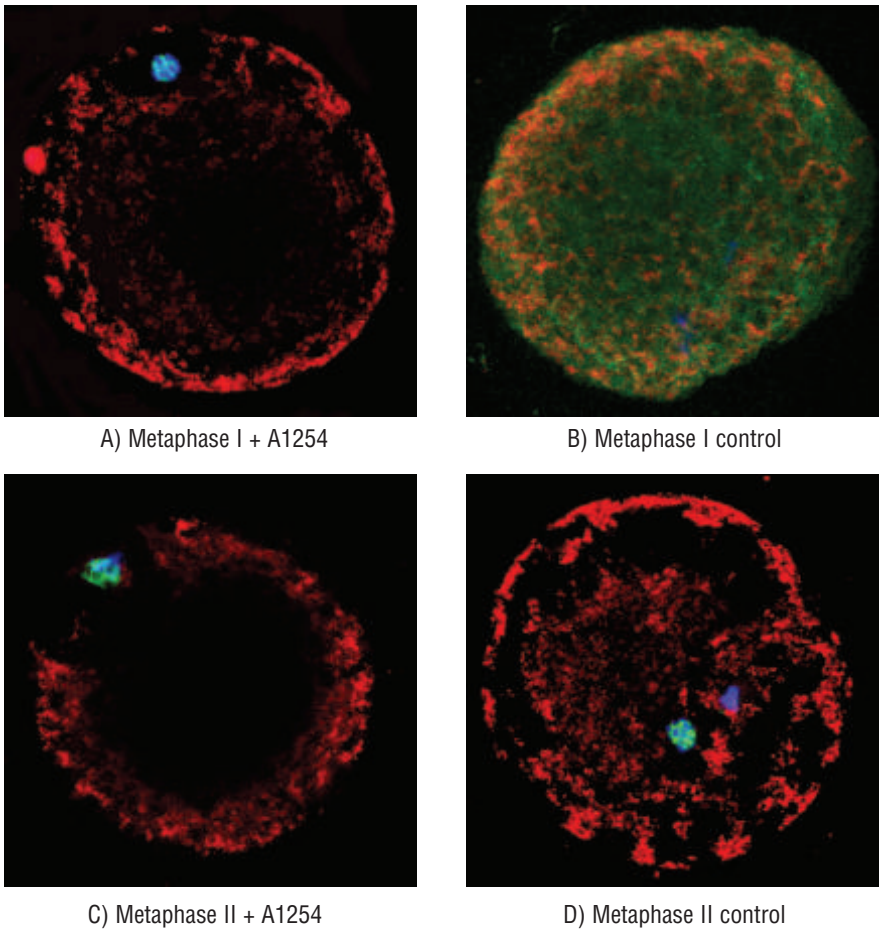
### *Statistical analysis*

All experiments were replicated at least three times. Oocyte distribution within the different categories was analyzed with chi square analysis and the criterion for significance was set at $p < 0.05$. Results are expressed as total numbers and/or percentages.

## Results

### *Effects of A1254 on oocyte maturation and developmental competence*

As summarised in Table 1, A1254 had no effect on maturation of pig oocytes at any of the doses tested.



A) Metaphase I + A1254 B) Metaphase I control

C) Metaphase II + A1254 D) Metaphase II control

**Figure 2. Effect of A1254 on microtubule assembly during pig maturation. While microtubules (green) are associated with DNA (Blue) in the meiotic spindles with and without A1254, a clear cytoplasmic microtubule network is formed only around the time of metaphase I, in the control group. The cytoplasmic network is not visible at the end of maturation (metaphase II) but is required for mitochondria (red) relocation during in vitro maturation. Original magnification 200×.**

**Table 1. Effect of different concentrations of A1254 during *In vitro* oocyte maturation on meiotic resumption and parthenogenetic development.**

| *A1254 concentration* | *Cultured oocytes* | *Matured oocytes (%)* | *Activated oocyte* | *Cleaved embryos (%)* | *Blastocyst (%)* |
|---|---|---|---|---|---|
| Control | 45 | 43 (95.5) | 193 | 169 (87.6) | 107[a] (55.4) |
| 0.1 ng/mL | 21 | 19 (90.5) | 34 | 23 (67.6) | 17[a] (50.0) |
| 1 ng/mL | 21 | 20 (95.2) | 49 | 38 (77.6) | 27[a] (55.1) |
| 10 ng/mL | 35 | 33 (94.3) | 108 | 74 (68.5) | 23[b] (21.3) |
| 100 ng/mL | 24 | 23 (95.8) | 67 | 51 (76.1) | 14[b] (20.9) |
| 1 µg/mL | 22 | 21 (95.4) | 57 | 33 (57.9) | 7[c] (12.3) |

*Data with different superscripts are statistically different as established using $\chi^2$ test ($p < 0.05$).*

Similarly, the number of oocytes that cleaved following parthenogenetic activation was comparable in every group considered and was not significantly different from control oocytes. By contrast, a significant decrease in the number of parthenotes that developed to blastocyst stage became evident at a concentration of 10 ng/mL (21.3% vs. 55.4% in the control group). The number of blastocysts obtained decreased significantly and in a dose response manner with higher concentrations of A1254.

### *Effect of A1254 on ooplasm remodelling during maturation*

Three mitochondria distributions were identified: peripheral, semi-peripheral and diffused (Figure 1), regardless of the dye used. At collection (time 0), most oocytes presented a peripheral distribution of total as well as of active mitochondria, while a smaller proportion had a semi-peripheral or diffused pattern of distribution (Table 2). At the end of *in vitro* maturation, after 46h of culture (time 46), oocytes in the treated group maintained the predominantly peripheral distribution, with no significant changes compared with the initial time point. Conversely, in the control group, a significant increase in proportion of the oocytes that presented a diffused distribution was observed. Again, both total and active mitochondria followed the same pattern (Table 2).

No microtubules were detected at collection in any of the oocytes analyzed (10/10, data not shown). While maturation proceeded, in all oocytes, disregarding of treatment, small asters of microtubules were detected in conjunction with DNA

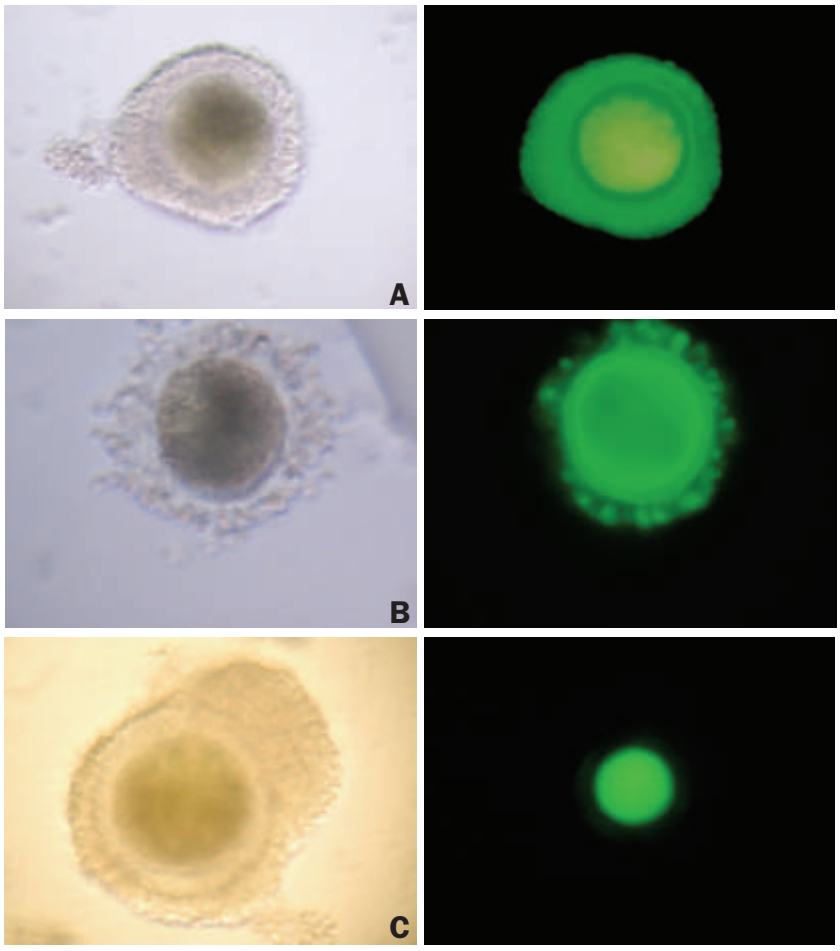



**Figure 3. Representative pictures of oocyte-cumulus cell communications mediated by gap-junctions. Pig oocytes were injected with Lucifer Yellow and the status of the gap-junctions connecting the oocyte with the surrounding cells was described as: Open (A), Intermediate (B) or Close (C). Original magnification 100×.**

**Table 2. Effects of exposure to 100 ng/mL of A1254 during *In vitro* maturation on mitochondria distribution in pig oocytes. Both total mitochondria and active mitochondria were considered. Treatments with different superscripts, within the same experiment, are statistically different as established using chi-square test ($p < 0.05$).**

| | | | *Total Mitochondria distribution (%)* | | |
|---|---|---|---|---|---|
| *Treatment* | *time* | *n* | *Peripheral* | *Intermediate* | *Diffused* |
| Control[a] | 0 | 23 | 16 (72.7) | 4 (18.2) | 3 (13.6) |
| Control[b] | 46 | 25 | 2 (8.0) | 6 (24.0) | 17 (68.0) |
| 100 ng/mL A1254[a] | 46 | 22 | 17 (77.3) | 5 (22.7) | 0 (0) |

| | | | *Active Mitochondria distribution (%)* | | |
|---|---|---|---|---|---|
| *Treatment* | *time* | *n* | *Peripheral* | *Intermediate* | *Diffused* |
| Control[a] | 0 | 26 | 20 (76.9) | 5 (19.2) | 1 (3.8) |
| Control[b] | 46 | 34 | 7 (20.6) | 8 (23.5) | 19 (55.9) |
| 100 ng/mL A1254[a] | 46 | 25 | 14 (56.0) | 9 (36.0) | 2 (8.0) |

until the end of maturation (Figure 2). Independently from the distribution of this DNA-linked microtubule population, at 24 h of maturation, the oocytes in the control group presented a cytoplasmic mesh of microtubules. Conversely, in the A1254 exposed group, no oocytes presented a diffused pattern of distribution of cytoplasmic microtubules. In both groups, no cytoplasmic microtubule network was visible at the end of maturation.

The contemporary staining of the samples for active mitochondria, tubulin and DNA allowed us to time the relative relocation of mitochondria with respect of cytoskeletal formation. Mitochondria relocation and a visible cytoplasmic network of microtubules occur at the same time. The absence of such network corresponded to a lack of mitochondria relocation.

### *Effect of A1254 on communications between oocytes and cumulus cells*

As expected, Lucifer yellow microinjection demonstrated that gap junction communications between pig oocytes and cumulus cells were open and functional at GV stage, with dye migration starting about three minutes following injection.

Gap junction communications gradually closed along maturation with 54.5% of control oocytes

**Table 3. Effect of A1254 (100ng/mL) on gap-junction mediated pig oocyte-cumulus cell communications. Treatments within the same time, with different superscripts, are statistically different as established using chi-square test ($p < 0.05$).**

| Time | Treatment | Gap-Junctions (%) Open | Intermediate | Close |
|---|---|---|---|---|
| GV | Control | 27 (90.0) | 0 | 3 (10.0) |
| MI | Control[a] | 2 (9.1) | 8 (36.4) | 12 (54.5) |
| MI | A1254[b] | 17 (60.7) | 6 (21.4) | 5 (17.9) |
| MII | Control | 0 | 2 (8.7) | 21 (91.3) |
| MII | A1254 | 5 (22.7) | 2 (9.1) | 15 (68.2) |

having closed gap junctions at MI and 91.3% at MII (Table 3). Interestingly, A1254-exposed oocytes displayed a delay in this maturation-related gap junction closing process with a significantly higher number of oocytes that displayed functionally opened gap junctions at MI. By MII, though the trend persisted, no significant differences were present between A1254 treated and control oocytes.

## Discussion

The present study indicates that a very low concentration of a PCB mixture representative of what is present in the environment is sufficient to alter the developmental competence of pig oocyte. However, even the highest dose tested in this study (1 μg/mL) had no effect on oocyte maturation and embryo cleavage after parthenogenetic activation. Our results are consistent with a previous study on the effect of a complex mixture of contaminants on the developmental competence of pig oocytes where levels of 4.2 μg of PCB/mL were required before an effect on maturation was visible (Campagna *et al.*, 2001). These exposure levels are similar to those required in the mouse, where a negative effect on oocyte maturation was observed starting from concentrations of 10 and 1.0 μg/mL (Kholkute *et al.*, 1994). This is in contrast with our previous study in cattle where A-1254 concentrations ranging between 1.0 and 0.01 μg/mL were enough to significantly decrease the percentage of oocytes able to reach metaphase II (Pocar *et al.*, 2001b) and clearly demonstrates that the effects of PCB exposure are highly species-dependent. Caution should therefore be used in drawing inferences among different species.

Changes in mitochondrial organization are known to be a faithful indicator of oocyte capability to sustain embryonic development (Bavister and Squirrell, 2000) and to occur during *in vitro* maturation in many species including mouse (Calarco, 1995; Van Blerkom and Runner, 1984), cow (Stojkovic *et al.*, 2001) and human (Wilding *et al.*, 2001). Pig mitochondria migrate during maturation from the peripheral part of the oocyte to the inner region of the cell both *in vitro* (Sun *et al.*, 2001c) and *in vivo* (Torner *et al.*, 2004), even though there is scarce information about the correlation between this phenomenon and subsequent oocyte development. Our results indicate that exposure to A1254 during oocyte maturation prevents mitochondria relocation, suggesting that the lack of mitochondria distribution through the ooplasm is associated with the low developmental ability caused by the exposure to PCBs. No effect on mitochondria activity was visible in our experiments since the distribution of total mitochondria was identical to that of the active ones. This suggests that PCBs have no effect on the energy levels available to the oocytes during maturation and is consistent with the lack of effect on meiotic completion and cleavage observed in this study.

The transfer of mitochondria within different areas of the cytoplasm is mediated by the cytoskeletal network of microtubules in the mouse oocyte (Van Blerkom, 1991). While there is a general agreement on the involvement of microtubules in forming the meiotic spindle, little is known on the dynamics of cytoplasm-associated microtubules in pig oocytes. Some studies failed to identify a cytoplasmic microtubule network altogether (Kim *et al.*, 1996; Sun *et al.*, 2001b), while other reports describe only a finely distributed mesh of cytoplasmic microtubules surrounding the GV nucleus (Ju *et al.*, 2003; Lee *et al.*, 2000; Rozinek *et al.*, 1995). Therefore the question remains open whether the missed distribution of mitochondria during the maturation of defective oocytes is due to the lack of formation of an appropriate microtubule network or is caused by an uncoupling between mitochondria and microtubules, possibly related to the lack of specific motor molecules such as dynein and kinesin (Cohen, 2002; Schliwa and Woehlke, 2003). Here we described the formation of a microtubule network in the cytoplasm of control oocytes, which, at 24h of IVM, extended from the periphery of the oocyte to the inner parts of the cytoplasm. At

the end of IVM, microtubules were not detectable anymore in the cytoplasm, but persisted in correspondence of the DNA. The contemporary staining of active mitochondria, DNA and microtubules allowed us to notice that the lack of relocation of active mitochondria to the inner part of the oocyte is related to the absence of an appropriate and timely formation of the microtubule network in the cytoplasm. Oocytes exposed to PCBs did not show a diffused pattern of distribution of cytoplasmic microtubules suggesting that the lack of mitochondria relocation observed in these same oocytes might be due to their inability to form a cytoplasmic microtubule network rather than to the inability of mitochondria to migrate along the tubules. The formation of a normal meiotic spindle, observed in both groups of oocytes, explains why the rate of meiotic progression to the second metaphase was not altered in the presence of A1254. This observation illustrates clearly the hypothesis that defective oocyte developmental competence is due to the uncoupling of nuclear and cytoplasmic maturation. Indeed while the normal formation of nucleus-associated microtubules allows the correct segregation of chromosomes during the reductive meiotic divisions, the lack of a microtubule cytoplasmic network prevents a correct relocation of mitochondria, which is likely to reflect a more generally altered compartmentalization of the ooplasm.

A1254 detrimental effect on the regulation of gap junction communication between oocyte and cumulus cells during the maturation process itself further supports this hypothesis. Such de-regulation is likely to be one of the possible factors responsible for the observed reduction of oocyte developmental competence. Small molecules like amino acids, nucleotides and glucose are transferred from the cumulus cells to the oocytes through the gap junctions along the entire folliculogenesis (Eppig, 1991). When the oocyte has reached its full development, these communicating channels are used for the passage of the signals that regulate meiotic maturation (Downs and Hunzickerdunn, 1995). Therefore, the uncoupling between maturation progression and cumulus-oocyte communications, in fact, may alter several downstream events and may explain A1254 adverse impact on development.

We conclude that oocyte exposure to A1254 during *in vitro* maturation causes a decrease of developmental competence that is associated with the lack of a microtubule cytoplasmic network. This in turn, prevents a correct relocation of mitochondria and is likely to reflect a more generally altered compartmentalization of the ooplasm. Altered communications between the oocyte and the somatic compartment induced by the exposure to PCBs further prevent a correct cytoplasmic maturation. All this can occur independently from the formation of the microtubule machinery required for the completion of chromosome disjunctions explaining why meiotic resumption was not affected even at the highest doses tested in this study.

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