Effect of Vanadate on the Chromatin Configuration in Pig GV-oocytes

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Abstract. Vanadate, an inhibitor of tyrosine phosphatases, has been reported to prevent germinal vesicle breakdown in mammalian oocytes. We examined the effect of vanadate on the chromatin configuration of fully grown pig oocytes. In the presence of human menopausal gonadotropin (hMG), vanadate (0.5–5 mM) resulted in a dose-dependent change in oocyte chromatin in germinal vesicles from the condensed state to a decondensed filamentous or stringy configuration. The effect of vanadate and hMG on chromatin configuration could be replicated with 2 mM dibutyryl cyclic AMP (dbcAMP) in place of hMG. Western blot analysis showed that vanadate caused a massive accumulation in the oocytes of tyrosine-phosphorylated proteins with a range of molecular weights that was enhanced by both hMG and dbcAMP in a similar manner. These results suggest that inhibition of tyrosine phosphatase(s) in the presence of an effective level of cAMP induces a change in chromatin configuration of pig oocytes.

Key words: Cyclic AMP (cAMP), Oocyte Maturation, Pig, Vanadate

Full grown mammalian oocytes arrested at prophase I resume meiosis spontaneously in vitro when they are released from the inhibitory influences of their surrounding follicles [1]. Because meiotic resumption is inhibited by membrane-permeable cAMP analogs, such as dibutyryl cAMP (dbcAMP), and inhibitors of cyclic nucleotide phosphodiesterase, such as 3-isobutyl-1-methylxanthine (IBMX), it is thought that a sustained high level of cAMP inhibits this process in oocytes within the follicles [2]. In this environment, meiotic resumption is triggered by the preovulatory gonadotropin surge from the pituitary [3]. Gonadotropins promote meiotic resumption of oocytes via an indirect mechanism mediated by surrounding cumulus granulosa cells rather than by a direct action on the oocytes [4].

A burst of phosphorylation of a range of proteins occurs in oocytes during meiotic resumption [5, 6]. The key kinase that phosphorylates many cellular substrates is a maturation-promoting factor (MPF), a serine/threonine protein kinase consisting of a catalytic subunit, Cdc2, and a regulatory subunit, cyclin B [7, 8]. Protein tyrosine phosphatases (PTPases) have also been suggested to be involved in meiotic resumption of oocytes. Vanadate, a potent inhibitor of tyrosine phosphatases, has been used to study the role of PTPases in oocytes and somatic cells [9]. In mouse and rat oocytes, vanadate prevents germinal vesicle breakdown by inhibiting tyrosine dephosphorylation of Cdc2 [10, 11]. In pig oocytes, Aquino et al. have reported that vanadate inhibits germinal vesicle breakdown almost completely at concentrations of 250 μM and 500 μM [12].

We repeated this experiment using pig oocytes and found that vanadate induced changes in chromatin configuration in arrested germinal vesicles. In the present study, we cultured fully grown pig oocytes with vanadate at different concentrations and examined the precise change in chromatin configuration. The results showed that after culturing the oocytes in a medium containing gonadotropin, vanadate induced a change in chromatin configuration from a state of condensed heterochromatin mass to a decondensed state similar to that in small, growing oocytes. To examine if cAMP could be substituted for gonadotropin, the effect of vanadate on chromatin configuration was also examined using dbcAMP. The effect of vanadate on the tyrosine phosphorylation of oocyte proteins was examined by western blotting.

Materials and Methods

Collection and culture of oocytes

Pig ovaries were obtained from prepubertal gilts at a local slaughterhouse. Ovaries were washed once with 0.2% (w/v) cetyltrimethylammonium bromide and twice with Ca²⁺- and Mg²⁺-free Dulbecco’s phosphate-buffered saline (PBS) containing 0.1% (w/v) polyvinyl alcohol (PBS-PVA; Sigma Chemical, St. Louis, MO, USA). To collect growing oocytes, small antral follicles of 0.5–3 mm in diameter were dissected from ovarian cortical slices (1–3 mm thickness) using a surgical blade (No. 21). Under a dissecting microscope, antral follicles were dissected from the cortices, and the tissues surrounding the follicles were removed using forceps. Small growing oocytes were collected from the follicles, fixed and stained with 1% orcein. The nuclear morphology was examined under a differential interference microscope to compare the chromatin configuration with that of fully grown vanadate-treated oocytes.

To collect fully grown oocytes, healthy antral follicles that were 4–6 mm in diameter were dissected from ovaries in PBS-PVA using two surgical blades (No. 11). From these follicles, oocyte-cumulus granulosa cell complexes (OCGs) were collected. The
basic culture medium was bicarbonate-buffered TC-199 (Earle's salts; Nissui Pharmaceutical, Tokyo, Japan) containing 10% (v/v) heat-treated fetal calf serum (Biocell, Carson, CA, USA), 0.1 mg/ml sodium pyruvate, 0.08 mg/ml kanamycin sulfate (Sigma Chemical) and 2.2 mg/ml sodium bicarbonate. OCGs were cultured for 42 h in this basic culture medium supplemented with 0–5 mM sodium meta-vanadate (Nacalai Tesque, Kyoto, Japan) and either 0.1 IU/ml human menopausal gonadotropin (hMG; Pergonal; Teikoku Zoki, Tokyo, Japan) or 0–2 mM dbcAMP (Sigma Chemical). Approximately ten OCGs were cultured in each well of four-well dishes (4-Well Multidish, Nunclon, Roskilde, Denmark) in a CO2 incubator at 38.5°C under humidified air containing 5% CO2.

After culture, the oocytes were fixed and stained with 1% orcein, and the nuclear morphology was examined under a differential interference microscope. The nuclear morphology of the germinal vesicle-stage oocytes was classified into the following six categories, as described by Motlik and Fulka [13] and Hirao et al. [14].

- Filamentous chromatin stage (FC): decondensed filamentous chromatin is distributed throughout the germinal vesicle; the nucleolus is stained with orcein and has many vacuoles.
- Stringy chromatin stage (SC): chromatin has begun to condense, forming thick chromatin strings in the germinal vesicle, and the nucleolus is not stained with orcein.
- Germinal vesicle I stage (GV I): the intact germinal vesicle is characterized by a distinct nuclear envelope and by the chromatin, which is stained only around the nucleolus.
- Germinal vesicle II stage (GV II): chromatin arranges around the nucleolus, and a few orcein-positive zones (chromocenters) appear near the nuclear membrane.
- Germinal vesicle III stage (GV III): the nucleoplasm loses its granularity, but the nuclear membrane remains distinct, and filamentous bivalents appear around the nucleolus.
- Germinal vesicle IV stage (GV IV): the nuclear membrane becomes less distinct, and the nucleolus disappears completely. Individual filamentous bivalents are distinguishable.

Electrophoresis and western blotting

OCGs were cultured for 42 h in a basic culture medium supplemented with 2 mM vanadate and 0.1 IU/ml hMG or 2 mM dbcAMP. After culture, the oocytes were denuded of cumulus cells by pipetting. After being washed twice in PBS-PVA, each group of 40 denuded oocytes was transferred into an Eppendorf tube with 10 μl of SDS sample buffer [15], boiled for 5 min and stored at −20°C until being used for electrophoresis. After thawing, samples were run in 13% SDS-polyacrylamide gels, and proteins were transferred to hydrophobic polyvinylidene difluoride membranes (Immobilon P, Millipore Corporation, Bedford, MA, USA) in a Trans-Blot SD semi-dry transfer cell (Bio-Rad Laboratories, Hercules, CA, USA) for 1 h at 2 mA/cm² in transfer buffer. The membranes were blocked with 10% fetal calf serum in PBS containing 0.1% Tween20 (PBS-Tween) for 2 h and then incubated with mouse monoclonal anti-phosphotyrosine antibody 4G10 (1:500; Upstate Cell Signaling Solutions, Charlottesville, VA, USA) for 4 h at room temperature. After three washes in PBS-Tween, the membranes were treated with horseradish peroxidase-labeled anti-mouse immunoglobulin (1:1000, Dako Japan, Tokyo, Japan) in the blocking buffer for 1 h at room temperature. After three washes of 10 min each with PBS-Tween, peroxidase activity was visualized with an ECL western blotting detection system (Amersham Life Science, Buckinghamshire, UK).

Statistical analysis

The frequencies of oocytes at each stage for each replicate experiment were subjected to arcsine transformation. The transformed values were analyzed using a one-way ANOVA (F1-test) followed by Tukey's multiple range test. P values less than 0.05 were considered to indicate statistical significance.

Results

Effect of vanadate on the chromatin configuration of oocytes cultured in medium containing hMG

The effects of vanadate on meiotic resumption and nuclear morphology of oocytes are shown in Table 1 and Fig. 1. Most of the oocytes were at the germinal vesicle I (GVI) stage before culture (95%, 38/40, Fig. 1a). In the absence of gonadotropin, OCGs did not show any expansion, oocytes in OCGs did not resume meiosis and the chromatin configuration did not change significantly at any concentration of vanadate (Table 1). Thus, most of these oocytes were at the GVI stage after culture.

In all OCGs cultured in vanadate-free medium, hMG induced cumulus expansion (Fig. 2b), and 82% of oocytes matured to metaphase II (MII) after 42 h (Table 1 and Fig. 1b). By contrast, vanadate at all tested concentrations (0.5–5 mM) significantly inhibited both cumulus expansion (Fig. 2c) and meiotic resumption of oocytes (Table 1). The highest rate of oocyte chromatin decondensation from the GVI stage to the stringy configuration (SC stage) was obtained with 2 mM of vanadate, and the highest rate of oocyte decondensation from the GVI stage to the filamentous configuration (FC stage) was obtained with 5 mM vanadate (Figs. 1c and d). Thus, vanadate induced chromatin decondensation in the oocytes in a dose-dependent manner. The characteristics of these configurations were the same as those observed in growing oocytes. Growing oocytes from 2–3-mm follicles showed a stringy configuration (58/70, Fig. 1e), and oocytes from 0.5–1-mm follicles had a filamentous configuration and orcein-positive nuclei in the germinal vesicles (60/69, Fig. 1f). However, the nuclei of the fully grown vanadate-treated oocytes did not change in morphology after treatment, even though their chromatin showed a filamentous configuration.

Effect of vanadate on the chromatin configuration of oocytes in the medium containing dbcAMP

Since gonadotropins induce an increase in the cAMP concentrations of cumulus cells and oocytes [16], we examined the effects of substitution of hMG with membrane-permeable dbcAMP. In this experiment, we chose a concentration of 2 mM vanadate because this concentration induced a change in chromatin configuration to the stringy or filamentous state in more than half of the treated oocytes. In the absence of vanadate, most of the oocytes (87–93%) remained at the GVI stage after 42 h of culture at any concentration of dbcAMP (0.1–2 mM; Table 2). In the presence of vanadate,
Table 1. Effect of vanadate on the chromatin configuration in pig oocytes in the presence of hMG

<table>
<thead>
<tr>
<th>Concentration of vanadate (mM)</th>
<th>hMG</th>
<th>No. of oocytes examined</th>
<th>No. (%) of oocytes at GV stage</th>
<th>No. (%) of oocytes after GVBD</th>
<th>No. (%) of oocytes degenerated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>FC</td>
<td>SC</td>
<td>SC-GVI</td>
</tr>
<tr>
<td>0</td>
<td>48</td>
<td>48(100)a</td>
<td>0</td>
<td>0</td>
<td>2(4)a</td>
</tr>
<tr>
<td>0.5</td>
<td>47</td>
<td>47(100)a</td>
<td>0</td>
<td>0</td>
<td>1(2)a</td>
</tr>
<tr>
<td>1 – 4</td>
<td>42</td>
<td>42(100)a</td>
<td>0</td>
<td>0</td>
<td>4(9)a</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>44(98)a</td>
<td>0</td>
<td>0</td>
<td>2(4)a</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>43(96)a</td>
<td>0</td>
<td>0</td>
<td>7(17)b</td>
</tr>
<tr>
<td>0</td>
<td>50</td>
<td>5 (10)b</td>
<td>0</td>
<td>0</td>
<td>0(0)a</td>
</tr>
<tr>
<td>0.5</td>
<td>40</td>
<td>40(100)b</td>
<td>0</td>
<td>0</td>
<td>1(2)b</td>
</tr>
<tr>
<td>1</td>
<td>41</td>
<td>41(100)c</td>
<td>2</td>
<td>5</td>
<td>10(24)c</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>46(100)c</td>
<td>8</td>
<td>17</td>
<td>26(57)c</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>40(98)c</td>
<td>19</td>
<td>47</td>
<td>16(39)c</td>
</tr>
</tbody>
</table>

Oocyte-cumulus-granulosa cell complexes collected from follicles 4-6 mm in diameter were cultured in the basic culture medium supplemented with vanadate and 0.1 IU/ml hMG for 42 h. GVBD, germinal vesicle breakdown; FC, filamentous chromatin stage; SC, stringy chromatin stage; GV I, GV II, GV III and GV IV, germinal vesicle I, II, III and IV stages; M I, metaphase I; M II, metaphase II. ** Values with different superscripts in the same column differ significantly (P<0.05).

Fig. 1. Effect of vanadate on chromatins configuration in pig oocytes. Fully grown oocytes collected from 4-6-mm follicles had a condensed chromatin ring around the nucleus in the germinal vesicle (GVI stage) before culture (a). After 42 h of culture in the control medium containing hMG, oocytes matured to metaphase II (b). On the other hand, oocytes cultured in the medium supplemented with 2 mM vanadate and hMG showed stringy (c) or filamentous chromatin (d) in the germinal vesicles. In the oocytes, chromatin was decondensed and distributed throughout the germinal vesicles (GVs). Growing oocytes from 2-3-mm follicles had a stringy configuration (e), and oocytes from 0.5–1-mm follicles had a filamentous configuration of chromatin and orcein-positive nucleoli in the GVIs (f). The bar represents 10 μm.

Fig. 2. Effect of vanadate on cumulus expansion of pig oocyte-cumulus-granulosa cell complexes (OCGs). OCGs before culture (a). OCGs cultured in the control medium containing hMG expanded after 42 h of culture (b), whereas OCGs cultured in the medium supplemented with 2 mM vanadate and hMG showed no expansion (c). The bar represents 250 μm.
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dbcAMP at the higher concentrations changed the oocyte chromatin to a stringy or filamentous configuration. At 2 mM of dbcAMP, the chromatin configurations of 93% of oocytes changed to a diffuse configuration as observed in growing oocytes. However, the morphologies of the nucleoli of the oocytes did not change after the treatment.

**Effect of vanadate and dbcAMP on the chromatin configuration of denuded oocytes**

It has been suggested that companion granulose cells play a role in modulating chromatin configuration and subsequent transcriptional activity in the oocyte genome [17]. We examined the cooperative effect on denuded oocytes to assess the role of companion cumulus granulosa cells. OCGs were denuded completely of cumulus cells by gently pipetting and referred to as denuded oocytes. Both of OCGs and denuded oocytes were cultured in basic medium containing vanadate or vanadate +dbcAMP (Table 3).

Culture of OCGs in the medium containing both vanadate and dbcAMP resulted in the chromatin changing to a diffused configuration in a high percentage of oocytes (Table 3). On the other hand, denuded oocytes did not show a change in chromatin configuration after culture in the medium containing dbcAMP and vanadate and were in the GV I stage (78%). There was no significant difference in chromatin configuration between denuded oocytes and OCGs when they were cultured in the medium containing only vanadate. Most of them were arrested at the GV I stage after 42 h of culture.

### Table 2. Effect of dibutyryl cAMP (dbcAMP) on the chromatin configuration of pig oocytes in the presence of vanadate

<table>
<thead>
<tr>
<th>dbcAMP (mM)</th>
<th>Vanadate (2 mM)</th>
<th>No. of oocytes examined</th>
<th>No. (%) of oocytes at GV stage</th>
<th>No. (%) of oocytes degenerated</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>42</td>
<td>0 (0)%</td>
<td>0 (0)%</td>
</tr>
<tr>
<td>0.1</td>
<td>0</td>
<td>39</td>
<td>0 (0)%</td>
<td>0 (0)%</td>
</tr>
<tr>
<td>0.5</td>
<td>–</td>
<td>43</td>
<td>0 (0)%</td>
<td>0 (0)%</td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>0 (0)%</td>
<td>0 (0)%</td>
<td>0 (0)%</td>
</tr>
<tr>
<td>2</td>
<td>44</td>
<td>0 (0)%</td>
<td>0 (0)%</td>
<td>0 (0)%</td>
</tr>
<tr>
<td>0.1</td>
<td>0</td>
<td>42</td>
<td>0 (0)%</td>
<td>0 (0)%</td>
</tr>
<tr>
<td>0.5 +</td>
<td>45</td>
<td>0 (0)%</td>
<td>0 (0)%</td>
<td>0 (0)%</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>2 (5)%</td>
<td>9 (23)%</td>
<td>15 (37)%</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>8 (20)%</td>
<td>11 (27)%</td>
<td>19 (46)%</td>
</tr>
</tbody>
</table>

Oocyte-cumulus-granulosa cell complexes collected from follicles 4–6 mm in diameter were cultured in the basic culture medium supplemented with dbcAMP and 2 mM vanadate for 42 h. FC, filamentous chromatin stage; SC, stringy chromatin stage; GV I, germinal vesicle I stage; GV II - GV IV, germinal vesicle II, III, IV stages. Values with different superscripts in the same column differ significantly (P<0.05).

### Table 3. Effects of vanadate and dbcAMP on the chromatin configuration in denuded pig oocytes

<table>
<thead>
<tr>
<th>Oocytes</th>
<th>Treatment</th>
<th>No. of oocytes examined</th>
<th>No. (%) of oocytes at GV stage</th>
<th>No. (%) of oocytes degenerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denuded oocyte</td>
<td>Vanadate + dbcAMP</td>
<td>50</td>
<td>0 (0)%</td>
<td>39 (78)%</td>
</tr>
<tr>
<td>Denuded oocyte</td>
<td>Vanadate</td>
<td>53</td>
<td>0 (0)%</td>
<td>44 (83)%</td>
</tr>
<tr>
<td>OCGs</td>
<td>Vanadate + dbcAMP</td>
<td>48</td>
<td>10 (21)%</td>
<td>42 (81)%</td>
</tr>
<tr>
<td>OCGs</td>
<td>Vanadate</td>
<td>52</td>
<td>0 (0)%</td>
<td>42 (81)%</td>
</tr>
</tbody>
</table>

OCGs and denuded oocytes were cultured for 42 h in the basic medium supplemented with 2 mM vanadate in the presence or absence of 2 mM dibutyryl cAMP (dbcAMP). FC, filamentous chromatin stage; SC, stringy chromatin stage; GV I, germinal vesicle I stage; GV II-GV IV, germinal vesicle II, III, IV stages. Values with different superscripts in the same column differ significantly (P<0.05).

**Effect of vanadate and dbcAMP on the chromatin configuration of denuded oocytes**

It has been suggested that companion granulose cells play a role in modulating chromatin configuration and subsequent transcriptional activity in the oocyte genome [17]. We examined the cooperative effect on denuded oocytes to assess the role of companion cumulus granulosa cells. OCGs were denuded completely of cumulus cells by gently pipetting and referred to as denuded oocytes. Both of OCGs and denuded oocytes were cultured in basic medium containing vanadate or vanadate +dbcAMP (Table 3).

**Change in tyrosine-phosphorylated proteins in the vanadate-treated oocytes**

Immunoblot analyses using an anti-phosphotyrosine antibody confirmed the effect of vanadate on the tyrosine phosphorylation of oocyte proteins. Germinal vesicle oocytes showed several bands of tyrosine-phosphorylated proteins (Fig. 3, lane a), of which a band with an apparent molecular weight 50 kDa disappeared after maturation and vanadate treatment. After vanadate treatment, a 60-kDa band increased in intensity, and several bands with higher molecular weights appeared (Fig. 3, lane c). The effects of hMG and dbcAMP on vanadate-treated oocytes were similar, with the occurrence of a massive accumulation of tyrosine-phosphorylated proteins.
The chromatin configuration of the oocyte undergoes a dynamic change during oocyte growth, from a decondensed chromatin typically found in the nucleoplasm of growing oocytes toward progressive condensation and redistribution of chromatin around the nucleolus. The present study demonstrates that culture of fully grown pig oocytes in a medium containing vanadate in the presence of dbcAMP (or hMG) results in conversion of the chromatin configuration from GVI to FC/SC. This result is unexpected because it has been suggested that gonadotropin increases the level of cAMP in oocytes and granulosa cells [16, 19]. This in turn activates the cyclic AMP-dependent protein kinase to modify the biochemical activity of the granulosa cells. Therefore, in the next experiment, dbcAMP was substituted for hMG. The results showed that dbcAMP in the presence of vanadate clearly changed the chromatin to a stringy or filamentous configuration in a dose-dependent manner. By contrast, the appearance of the nucleoli of the oocytes did not change after treatment. It is known that, in GVI oocytes, the nucleolus is compacted to a dense fibrillar sphere attached to a fibrillar center, and it is most likely that this morphology of the nucleolus was not affected by the vanadate treatment. These results suggest the possibility that tyrosine phosphatase(s) induces the change in chromatin configuration from FC/SC to GVI during the final growth phase of pig oocytes, and that the role of this phosphatase(s) is dependent on an effective level of cAMP. It is possible that this putative tyrosine phosphatase(s) is not involved in the change in oocyte nucleolus morphology.

The results for the effect of vanadate and dbcAMP on oocytes that had been denuded of granulosa cells demonstrated that vanadate with dbcAMP induced a change in the chromatin configuration from GVI to FC/SC in the presence of companion granulosa cells. This result is consistent with the study of De La Fuente and Eppig in mouse oocytes [17]. These authors suggested that companion granulosa cells might have a role in modulating some aspects of chromatin modification and transcriptional activity during oocyte growth. Moreover, granulosa cells have also been shown to regulate the phosphorylation of several oocyte proteins in the mouse [20, 21]. Indeed, denuded oocytes can resume meiosis spontaneously in vitro because they are released from the inhibitory influences of their surrounding follicles [1]. However, most denuded oocytes stopped at the GVI stage due to the effects of vanadate (Table 3). This result reinforces that the trigger to resume meiosis of hMG in the vanadate treatment is promotion of oocyte-secreted factors. It has been suggested that oocyte-secreted factors can act on adjacent cumulus-granulosa cells via pathways to induce expression of genes [22]. It is possible that some soluble factors from granulosa cells, or direct oocyte-granulosa cell communication through gap junctions, are necessary for configurational change of pig oocyte chromatin by inhibition of tyrosine phosphatase(s).

The western blot analysis showed that vanadate caused a massive accumulation of tyrosine-phosphorylated proteins of different molecular weights (Fig. 3, lanes d and e).

**Discussion**

The chromatin configuration of the oocyte undergoes a dynamic change during oocyte growth, from a decondensed chromatin typically found in the nucleoplasm of growing oocytes toward progressive condensation and redistribution of chromatin around the nucleolus. The present study demonstrates that culture of fully grown pig oocytes in a medium containing vanadate in the presence of dbcAMP (or hMG) results in conversion of the chromatin configuration from GVI to FC/SC. This result is unexpected because hMG stimulates the meiotic resumption of oocytes under normal culture conditions. However, this showed that vanadate is unable to alter the chromatin configuration in OCGs unless oocyte maturation is stimulated (Table 1). It has been suggested that vanadate does not block meiotic progression after GVBD until the second metaphase in mouse and pig oocytes [12, 18]. Obviously, a marked increase in phosphorylation of oocyte protein occurs before GVBD. Therefore, vanadate would potently disrupt the normal balance of the enzymes only after the enzymes augment its activity. In addition, the effect of vanadate was dose dependent. A dose of 1 or 2 mM of vanadate effectively changes the chromatin configuration in GV pig oocytes. At a concentration of 0.5 mM, vanadate just inhibits GVBD, and oocytes remain at the GV stage.

In addition to the stimulatory effect of hMG on oocyte maturation, it has been suggested that gonadotropin increases the level of cAMP in oocytes and granulosa cells [16, 19]. This in turn activates the cyclic AMP-dependent protein kinase to modify the biochemical activity of the granulosa cells. Therefore, in the next experiment, dbcAMP was substituted for hMG. The results showed that dbcAMP in the presence of vanadate clearly changed the chromatin to a stringy or filamentous configuration in a dose-dependent manner. By contrast, the appearance of the nucleoli of the oocytes did not change after treatment. It is known that, in GVI oocytes, the nucleolus is compacted to a dense fibrillar sphere attached to a fibrillar center, and it is most likely that this morphology of the nucleolus was not affected by the vanadate treatment. These results suggest the possibility that tyrosine phosphatase(s) induces the change in chromatin configuration from FC/SC to GVI during the final growth phase of pig oocytes, and that the role of this phosphatase(s) is dependent on an effective level of cAMP. It is possible that this putative tyrosine phosphatase(s) is not involved in the change in oocyte nucleolus morphology.

The results for the effect of vanadate and dbcAMP on oocytes that had been denuded of granulosa cells demonstrated that vanadate with dbcAMP induced a change in the chromatin configuration from GVI to FC/SC in the presence of companion granulosa cells. This result is consistent with the study of De La Fuente and Eppig in mouse oocytes [17]. These authors suggested that companion granulosa cells might have a role in modulating some aspects of chromatin modification and transcriptional activity during oocyte growth. Moreover, granulosa cells have also been shown to regulate the phosphorylation of several oocyte proteins in the mouse [20, 21]. Indeed, denuded oocytes can resume meiosis spontaneously in vitro because they are released from the inhibitory influences of their surrounding follicles [1]. However, most denuded oocytes stopped at the GVI stage due to the effects of vanadate (Table 3). This result reinforces that the trigger to resume meiosis of hMG in the vanadate treatment is promotion of oocyte-secreted factors. It has been suggested that oocyte-secreted factors can act on adjacent cumulus-granulosa cells via pathways to induce expression of genes [22]. It is possible that some soluble factors from granulosa cells, or direct oocyte-granulosa cell communication through gap junctions, are necessary for configurational change of pig oocyte chromatin by inhibition of tyrosine phosphatase(s).
after removal of dbcAMP, these oocytes exhibit enhanced meiotic resumption and embryonic quality after fertilization [24, 25]. This indicates that synchronizing meiotic resumption by dbcAMP treatment improves the developmental capacity of the embryo. Actually, protein tyrosine phosphorylation is an essential element in the control of fundamental cellular signaling events involved in growth, proliferation and differentiation, including regulation of exocytosis. It has been suggested that both tyrosine kinases and phosphatases play a central role in sperm exocytosis [26]. In porcine nuclear transfer embryos, it has been suggested that vanadate protein kinase without preceding phosphorylation during meiotic cell cycle in mouse oocytes. BioMed Res Int 2012; 12: 423–427.

In conclusion, our findings in the present study suggest that inhibition of tyrosine phosphatase(s) by vanadate induces the change in chromatin configuration in oocytes and that the role of the putative phosphatase(s) might be dependent on an effective level of cAMP. Much remains to be learned about the mechanisms involved in this effect of vanadate.

Acknowledgments

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