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ORIGINAL ARTICLE

PROSTAGLANDIN F2A DISTURBS OOGENESIS BY CAUSING MEIOTIC SPINDLE DAMAGE

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Abstract. *According to recent data, prostaglandin F2 alpha can have a negative influence on meiosis during oogenesis. Previously, we have found that this prostaglandin may accelerate in a dosage-dependent way the postovulatory aging in ovulated mature oocytes, compromising the integrity of their meiotic spindles.* ***Aim.*** *The study aimed to investigate the effects of prostaglandin F2α on the course and outcome of oocyte meiosis in a mouse model.* ***Materials and Methods.*** *Mouse oocytes were matured in vitro in the presence of prostaglandin F2α in a concentration of 100 ng/ml. Their meiotic stage, spindle morphology and chromosome arrangement were assessed by immunofluorescent labeling of tubulin and fluorescent staining of DNA.* ***Results.*** *We obtained a higher percentage of immature oocytes in metaphase I after the treatment than in untreated control oocytes. In addition, there were specific morphological changes in the meiotic spindles of oocytes exposed to prostaglandin F2α associated with a reduced number of fibers.* ***Conclusion.*** *It is probable that prostaglandin F2α has an impact on the microtubule dynamics of the meiotic spindle that can prevent the transition of maturing oocytes to the second meiotic division, most likely by triggering the spindle assembly checkpoint.*

Key words: *prostaglandin F2 alpha, meiosis, oogenesis, oocytes, female infertility, meiotic spindle*

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INTRODUCTION

Prostaglandins have a complex and multifaceted influence on female reproductive functions. In recent years, inflammatory response mediated at least partly by prostaglandins has been shown to underlie the tissue remodeling that is a prerequisite for successful ovulation. According to current theories, luteinizing hormone surge leads to production of prostaglandins (mostly prostaglandin E2), cytokines and other mediators of inflammation by granulosa and theca cells. Acting in paracrine fashion, these molecular signals trigger secretion of proteases by both ovarian cells and infiltrating immune cells, weakening the extracellular matrix to allow rupture of the follicle [1]. Evidence for such function of prostaglandins includes reports that inhibitors of their synthesis, applied within the proper time window, inhibit oocyte meiotic maturation and ovulation in both women and animal models [2]. According to some authors, prostaglandin supplementation of oocyte culture medium increases oocyte quality and developmental potential [3, 4]. Other researchers, however, have disputed these findings [5].

At the same time, there is evidence of deleterious effects of prostaglandins on oogenesis. In patients with polycystic ovary syndrome, a condition reducing female fertility and associated with low-level inflammation, a negative correlation has been found between prostaglandin levels in follicular fluid and success of *in vitro* fertilization [6]. In particular, prostaglandin F2α has been found to compromise the developmental potential of bovine oocytes subjected to it during their meiotic maturation [7]. However, the mechanism of this deleterious action is not known. In a previous study, we have found that prostaglandin F2α may accelerate in a dosage-dependent way the postovulatory aging in cultured ovulated mature mouse oocytes, compromising the integrity of their meiotic spindles [8]. To investigate the effects of prostaglandin F2α on the course and outcome of oocyte meiosis, we exposed to it mouse oocytes undergoing *in vitro* maturation.

MATERIALS AND METHODS

Animals

ICR female mice (8 to 10-week old) were used for this study. The experiments were performed in accordance with the European Union and Bulgarian legislation concerning humane treatment of animals. Ovarian stimulation was carried out by intraperitoneal injection with 7.5 IU follicle-stimulating hormone and 7.5 IU luteinizing hormone (Meriofert, IBSA Farmaceutici Italia, Lodi, Italy). The mice were euthanized 40 h later and their ovaries were isolated.

In vitro maturation of oocytes

The ovaries were placed in Leibowitz medium (Sigma-Aldrich, Steinheim am Albuch, Germany). Under stereomicroscopic observation, antral follicles were punctured to retrieve immature oocytes. They were separated into two groups: control and prostaglandin-treated. Control oocytes were *in vitro* matured by cultivation in a Hera cell 150 incubator (Heraeus, Hanau, Germany) for 24 h at 37°C and 5% CO_2 in αMEM medium (Sigma-Aldrich) supplemented with 50 μg/ml ascorbic acid, 10 μg/ml transferrin, 5 μg/ml insulin, 5% foetal calf serum, 10 IU/ml penicillin/0.01 mg/ml streptomycin (Sigma-Aldrich) and 0.075 IU/ml FSH (Serono, Modugno Bari, Italy). Prostaglandin-treated oocytes were treated similarly, except that their culture medium was supplemented also with 100 ng/ml prostaglandin F2α.

Immunofluorescence

Immunofluorescent staining of oocytes was performed as described in [9] with small modifications. Fixation was carried out in phosphate-buffered saline (PBS), pH 7.2, with 2% paraformaldehyde (Sigma-Aldrich) and 0.04 % Triton X-100 for 45 min at 37°C. For labeling of microtubules, oocytes were incubated for 45 min at 37°C with mouse anti-α-tubulin monoclonal antibody (clone DM1A; Sigma-Aldrich, Germany) as 1st antibody, diluted 1:1000 in PBS (pH 7.2) supplemented with 0.3% BSA and 0.04% Triton X-100. After it, cells were washed twice for 10 min in PBS (pH 7.2) with 0.3 % BSA and 0.1% Tween 20. Then they were treated with FITC-labeled anti-mouse IgG antibody (Sigma-Aldrich) as 2nd antibody, diluted 1:200 in PBS (pH 7.2) with 0.3% BSA, 0.04% Triton X-100 and 5 μg/ml Hoechst 33342 (Sigma-Aldrich) to visualize DNA. After this incubation, oocytes were washed twice in PBS (pH 7.2) with 0.3% BSA and 0.1% Tween 20. They were transferred to a series of increasingly concentrated solutions of polyvinyl alcohol (Mowiol, Sigma-Aldrich) in PBS (pH 7.2) with 0.3% BSA and 0.02% sodium azide. Finally, the cells were mounted in pure Mowiol on microscopic slides. Oocytes were observed and documented using fluorescent microscope Axioskop 20 (Zeiss, Jena, Germany).

Statistical methods

After recognizing the meiotic stage of each cell and calculating the maturation rates of prostaglandin-treated and control oocytes, the statistical significance of the difference between the two groups was estimated using Fisher's Exact Test.

RESULTS

The maturation rate, i.e. the proportion of oocytes that had successfully reached metaphase II, was substantially lower in prostaglandin-treated oocytes (42.11%) than in controls (63.44%). The difference was statistically significant ($p < 0.05$). Of the immature oocytes, most were in metaphase I, and a small proportion represented earlier stages (germinal vesicle or germinal vesicle breakdown).

Metaphase oocytes from the control group were characterized by well-visible barrel-shaped meiotic spindles with chromosomes arranged at the equator. This morphology was observed both in immature oocytes in metaphase I (Figure 1A) and mature oocytes in metaphase II (Figure 2A).

In prostaglandin-treated metaphase oocytes, meiotic spindles were visibly thinner, with reduced number of fibers and narrow, sharp, concentrated poles reminiscent of mitotic spindle poles. Again, this was observed both in metaphase I (Figure 1B) and metaphase II (Figure 2B). Chromosome arrangement at the spindle equator appeared normal.

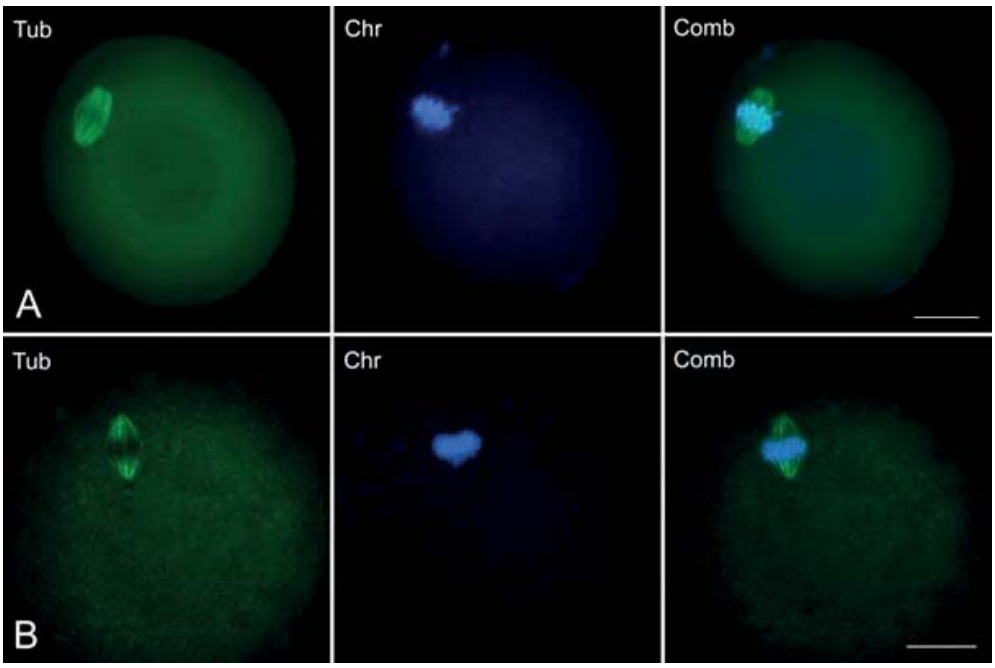

Fig. 1. Localization of meiotic spindles and chromosomes in metaphase I *in vitro* matured mouse oocytes: A) Control oocyte; B) Prostaglandin-treated oocyte. Tub, staining for tubulin; Chr, staining for chromatin; Comb, combined image. Bars = 20 μm

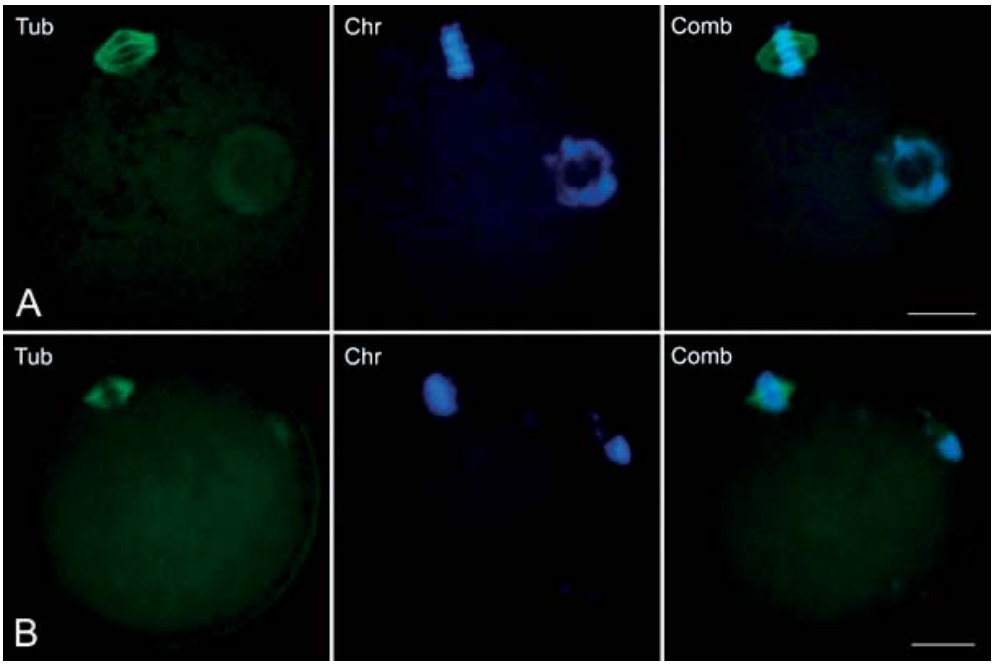

Fig. 2. Localization of meiotic spindles and chromosomes in metaphase II *in vitro* matured mouse oocytes: A) Control oocyte; B) Prostaglandin-treated oocyte. Tub, staining for tubulin; Chr, staining for chromatin; Comb, combined image. Polar bodies are seen at right. Bars = 20 μm

DISCUSSION

The oocyte meiotic spindle is characterized by barrel-shaped morphology, with relatively broad poles. This is due to its acentrosomal nature, making oocytes an exception among dividing animal cells [10]. The absence of centrosomes necessitates alternative pathways of spindle assembly based on recruitment, activation and concentration of small gamma-tubulin containing microtubule-organizing centers in the ooplasm. This process is complex and very sensitive to a variety of potentially disturbing factors, such as the genetic background of the individual [11] and the presence of compounds acting directly or indirectly on the cytoskeleton [12]. Our previous study addressing the effects of prostaglandin F2α on ovulated oocytes [8] showed that this mediator of inflammation, applied in a dose of 100 ng/ml, greatly accelerated the postovulatory aging of oocytes, causing spindle degeneration and chromosome spreading within a few hours. The maturing oocytes observed in the present study sustained less damage, having visible spindles with apparently well-arranged chromosomes. This shows that during meiotic maturation, when the spindle is actively reorganizing, it is more resistant to disturbances and has a better capacity to react to unforeseen events. However, spindles of prostaglandin-treated cells had visibly abnormal morphology: they were thin, with narrow, sharp poles. This finding shows that prostaglandin, through a cur-

rently unknown mechanism, can impair microtubule assembly and/or increase microtubule disassembly. The concentrated, sharp appearance of spindle poles was presumably due to the reduced number of spindle microtubules rather than to formation of abnormal microtubule-organizing centers, because no astral microtubules were observed.

It should be noted that chromosome arrangement at the spindle equator in prostaglandin-treated cells seemed normal. During oocyte meiotic resumption, chromosome organization is partly achieved through a microtubule-independent mechanism – clustering of the chromosomes around the so-called surrounded nucleolus or karyosphere [13]. However, preservation of chromosome arrangement in metaphase I and its reorganization during the metaphase I – metaphase II transition is fully dependent on the meiotic spindle. Hence, the well-organized metaphase plates of mature prostaglandin-treated oocytes proved that the thin, narrow-poled spindles were nevertheless able to successfully perform their function. On the other hand, the lower maturation rate in the prostaglandin-treated group (42.11% versus 63.44% in controls) showed that in a substantial proportion of these cells, prostaglandin-mediated damage to the spindle either rendered it unable to segregate the chromosomes in anaphase I or, more likely, activated the spindle assembly checkpoint and caused arrest in metaphase I. It is known that the spindle assembly checkpoint is functional in oocytes, though its activation requires a substantial level of errors because the enormous cytoplasmic volume makes it less sensitive than in typical-sized cells [14].

The damaging impact of prostaglandin on oocytes observed by us is in accordance with some published studies [7, 6], while other authors have found no effect [5] or have even reported a beneficial influence [3, 4]. A possible explanation of this discrepancy could be sought in the details of the experimental settings. Similarly to other molecular signals, prostaglandins could have vastly different effects depending on the dose and timing of their action [15]. Further studies should also address the possibility of species-specific differences and influence of the age and overall condition of the individual. Given the paramount importance of the oocyte meiotic spindle for female fertility and the aneuploidy rate in the human [16, 17], any relevant factor that can affect the spindle in a negative way should be investigated in detail.

CONCLUSION

Based on the observed lower maturation rate and thin spindles of oocytes exposed to prostaglandin F2α, it is probable that this prostaglandin has an impact on the microtubule dynamics of the meiotic spindle that can prevent the transition of maturing oocytes to the second meiotic division, most likely by triggering the spindle assembly checkpoint.

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