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Adverse effects of polymeric nanoparticle poly(ethylene glycol)-*block*-polylactide methyl ether (PEG-*b*-PLA) on steroid hormone secretion by porcine granulosa cells

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Objectives. Development of nanoparticles (NPs) for biomedical applications, including medical imaging and drug delivery, is currently undergoing a dramatic expansion. Diverse effects of different type NPs relating to mammalian reproductive tissues have been demonstrated. The objective of this study was to explore the in vitro effects of polymeric nanoparticle poly(ethylene glycol)-*block*-polylactide methyl ether (PEG-*b*-PLA NPs) on functional state and viability of ovarian granulosa cells (GCs), which play an important role in maintaining ovarian function and female fertility.

Methods. The GCs isolated from porcine ovarian follicles were incubated with the different concentrations of PEG-*b*-PLA NPs (PEG average Mn=350 g/mol and PLA average Mn=1000 g/mol; 0.2–100 μg/ml) or poly(ethylene glycol) with an average molecular weight of 300 (PEG-300; 0.2–40 mg/ml) in the presence or absence of stimulators, follicle-stimulating hormone (FSH; 1 μg/ml), androstenedione (100 nM), forskolin (10 μM) or 8Br-cAMP (100 μM), for different time periods (24, 48, 72 h). At the end of the incubation, progesterone and estradiol levels produced by GCs were measured in the culture media by radioimmunoassay. The viability of GCs was determined by the method using a colorimetric assay with MTT.

Results. Treatment of GCs with PEG-*b*-PLA NPs induced a significant decrease in basal as well as FSH-stimulated progesterone secretion above the concentration of 20 and 4 μg/ml, respectively. Moreover, PEG-*b*-PLA NPs reduced forskolin-stimulated, but not cAMP-stimulated progesterone production by GCs. A dose-dependent inhibition of androstenedione-stimulated estradiol release by GCs was found by the action of PEG-*b*-PLA NPs. Incubation of GCs with PEG-300 significantly inhibited basal as well as FSH-stimulated progesterone secretion above the concentration of 40 mg/ml. PEG-*b*-PLA NPs and PEG-300 significantly reduced the viability of GCs at the highest tested concentrations (100 μg/ml and 40 mg/ml, respectively).

Conclusions. The obtained results indicate that polymeric NPs PEG-*b*-PLA might induce alterations in steroid hormone production by ovarian GCs and thereby could modify reproductive functions.

Key words: polymeric nanoparticles, reproductive nanotoxicology, steroidogenesis, granulosa cells

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The development of specialized nanomaterials (NMs)/nanoparticles (NPs) for use in medicine for diagnosis and treatment of many disease entities is increasing. Applications of nanotechnology-based drugs include use in biotechnology, healthcare, pharmaceuticals, drug delivery and skincare. Over the past few decades, polymeric micelles have attracted increasing attention as the carriers for various biologically active substances such as drugs, proteins and nucleic acids (De Jong and Borm 2008; Moritz and Geszke-Moritz 2015; Wang and Rempel 2015; Wang et al. 2017). Polymeric micelles exhibited good biodegradability and biocompatibility; they offer numerous attractive features such as targeted drug delivery, sustained release or prolonged circulation half-life (Semete et al 2010; Moritz and Geszke-Moritz 2015).

An amphiphilic block copolymer (ABC) poly(ethylene glycol)-*block*-poly(lactic acid) (PEG-*b*-PLA), a U.S. Food and Drug Administration (FDA)-approved material, has been designed as a carrier for poorly water-soluble drugs to improve their pharmacokinetics and overcome multidrug resistance (Kedar et al. 2010; Xiao et al. 2010; Shin et al. 2012; Shen et al. 2015). Polymeric NPs, including PEG-*b*-PLA, represent one of the most promising approaches for CNS drug delivery, due to their ability to cross the blood brain barrier (BBB) (Tosi et al. 2008; Patel et al. 2012). PEGylated liposomal doxorubicin (PLD) has been already approved for treatment of advanced ovarian cancer patients failing first-line platinum-based treatment (Ferrandina et al. 2010).

Polyethylene glycols (PEG) have extensive medical applications, many with significant relevance to gastroenterology. During polymeric NP preparation, PEG can be used as a stabilizer or applied as a linker for conjugation of targeting ligands to the surface of NPs. The modification of drug carriers with water-soluble PEG can contribute to a significant increase in the circulation half-life *in vivo* of as-prepared nanovehicles (Moritz and Geszke-Moritz 2015; Wang and Rempel 2015). PEG is not known to be metabolized in humans and there are no known pharmacological actions or confirmed toxicity resulting from the limited absorption. Pelham et al. (2008) confirmed that orally administered PEG-3350 was minimally absorbed, rapidly excreted and primarily eliminated via faeces in healthy young and elderly subjects. However, Gajdova et al. (1993) reported that Tween 80 with PEG-350 as an active ingredient has the potential to behave as a hormone/estrogen active agent. Neonatal exposure of female rats to PEG-350 significantly accelerated their sexual maturation, prolonged the estrous cycle, and induced persistent vaginal estrus. Moreover, ovaries were without corpora lutea and had degenerative follicles (Gajdova et al. 1993).

For safe application of NPs in biomedicine, it is crucial to exclude their potential toxic effects (De Jong and Borm 2008; Oberdoster 2009; Rollerova et al. 2011; Sharma et al. 2012). The association between the exposure to NMs/NPs and their adverse effects on reproductive/neuroendocrine development and function is apparent from a number of *in vivo* and *in vitro* studies (Ema et al .2010; Campagnolo et al. 2012; Taylor et al. 2012; Lu et al. 2013). The adverse effects of polymeric NPs have not been extensively studied and only few studies have focused on their effects on reproductive system to date. We have previously shown that neonatal exposure to PEG-*b*-PLA NPs (20, 40 mg/kg b.w.) may induce delayed adverse effects on hypothalamic-pituitary-ovarian axis development (accelerated onset of vaginal opening) and function [reduced number of regular estrous cycles, altered course of hypothalamic gonadotropin-releasing hormone (GnRH)-stimulated luteinizing hormone (LH) secretion, and increased progesterone serum levels] in 6-month-old female rats (Rollerova et al. 2015a). In addition, neonatal treatment with PEG-*b*-PLA NPs (20 mg/kg b.w.) significantly increased basal and LHRH-induced *in vitro* LH release from anterior pituitary cells of infantile and adult female rats (Scsukova et al. 2015). The molecular mechanisms of the action of PEG-*b*-PLA NPs on female reproductive system are still unknown.

Granulosa cells (GCs) represent the major cell population in ovarian follicles; they play an essential role in the development of maturation of follicles, and thereby in maintaining ovarian function and female fertility. The growth and proliferation of GCs are one of the significant signs of follicular development. To address the impact of polymeric PEG-*b*-PLA NPs on possible toxicity concerning the reproductive system, we examined the effect of PEG-*b*-PLA NPs and the bulk form of PEG with an average molecular weight of 300 (PEG-300) on ovarian steroidogenesis using *in vitro* model of primary culture of porcine GCs.

Materials and Methods

Reagents. Copolymer PEG-*b*-PLA [CH3O(CH2CH2O)×(COCHCH3O)yH, PEG average Mn = 350 g/mol, PLA average Mn = 1000 g/mol, CAS 9004-74-4], poly(ethylene glycol) with an average molecular of 300 (PEG-300), tetrahydrofuran (THF; anhydrous, inhibitor free, purity ≥99.9%), medium M199, fetal bovine serum (FBS), 4-Androstene-3,17-dione (androstenedione), 8-Bromoadenosine 3′,5′-cyclic

monophosphate (8Br-cAMP), 3-(4, 5)-dimethylthiazol-2, 5-diphenyltetrazolium bromide (MTT) and all other chemicals were purchased form Sigma-Aldrich (Steinheim, Germany). Forskolin was from ICN Biochemicals (Aurora, OH, U.S.A.). Antibiotic-Antimycotic solution (100x) was obtained from Biosera (Nualle, France). Porcine follicle-stimulating hormone (pFSH; USDA-pFSH-I-2, AFP-10640B) was from Dr. A.F. Parlow, NIDDK s National Hormone & Pituitary Program (Torrance, CA, U.S.A.).

Preparation and characterization of PEG-*b*-PLA NPs. Fresh NP micelles of PEG-*b*-PLA were prepared by modified solvent evaporation method according to Du et al. (2009) and Shin et al. (2009). Briefly, copolymer PEG-*b*-PLA (20 mg) was dissolved in 2 ml of THF and stirred for 2 h at room temperature (RT). Under moderate stirring (100 rpm, MR Hei-Standard Heidolph, Germany), the ultrapurified water (10 ml) (MiliporeMili-Q Synthesis, 18.5MΩ) was added dropwise. Two hours later, THF was evaporated under mild vacuum (rotating evaporator LABOROTA 4010-digital, Heidolph, Germany) for 1 h at 48°C to obtain polymer micelles. After THF evaporation, water was added to the suspension to obtain the final PEG-*b*-PLA concentration of 2 mg/ml. Immediately before administration, PEG-*b*-PLA suspension was vortexed at the highest speed for 1 min.

Physical particle size, general state of agglomeration/aggregation and morphology of PEG-*b*-PLA suspension were determined by transmission electron micrography (TEM) using JEM 1200 microscope (JOEL, Tokyo, Japan) with 120 kV voltage. Zeta potential was measured by Nicomp Submicron Particle Sizer Autodilute Model 380 (Santa Barbara, CA, USA) using the electrophoretic light scattering (ELS) method. Size distribution of PEG-*b*-PLA was evaluated by dynamic light scattering (DLS) with a NICOMP TM 380 ZLS Particle Sizer (Santa Barbara, CA, USA) (for details see Rollerova et al. 2015a).

Isolation and culture of GCs. Ovaries of noncycling pubertal gilts, about 90 days of age, were obtained after slaughter at a local abattoir. They were washed several times in sterile 0.9% NaCl. GCs were aspirated by sterile syringe and needle from follicles 3–5 mm in diameter and isolated by centrifugation for 10 min at 1000 x g. Cells were then washed, resuspended in culture medium M199 (with Earle salt, 1 mM L-glutamine, and HEPES buffer) supplemented with 1% antibiotic-antimycotic solution and 10% FBS at final density 1 x 10^7 cells/ml medium and cultured in 5% CO_2 incubator at 37°C. The density of cells was measured by a handheld automated cell counter Scepter (Millipore, Billerica, MA, USA) and cell viability was determined in a hemocytometer by trypan blue exclusion, the cell viability ranged from 80 to 90%.

Treatment of GCs for steroid hormone measurement. GCs were seeded in 24-well plates (TPP AG, Trasadingen, Switzerland) at a density 10^6 cells/well (4 wells/group/experiment), recovered for 24 h and then cultured with different concentrations of PEG-*b*-PLA NPs (0.2–100 μg/ml) or PEG-300 (0.2–40 mg/ml) in the absence/presence of stimulators, pFSH (1 μg/ml), androstenedione (100 nM), forskolin (10 μM) or8Br-cAMP (100 μM), for different time periods (24, 48, and 72 h). Control cells were treated with an equal volume of vehicle alone (ultrapurified water after evaporation of THF). After exposure to the tested agents, the culture media were collected and stored at –20°C until assayed for radioimmunoassay. The experiments were repeated from three to five times.

Determination of cell viability. GCs were seeded in 96-well plates (TPP AG, Trasadingen, Switzerland) at a density 10^5 cells/well (6 wells/group/experiment), recovered for 24 h and then cultured with different concentrations of PEG-*b*-PLA NPs (1–100 μg/ml) or PEG-300 (0.2–40 mg/ml) for next 48 and 72 h. Control cells were treated with an equal volume of vehicle alone. The cell viability was determined by the method using a colorimetric assay with MTT. Briefly, after treatment with tested agents, the culture medium was replaced with 100 μl of the fresh medium containing 0.5 mg/ml MTT and the cells were incubated for 4 h at 37°C. The medium was removed and replaced with 100 μl of DMSO. The absorbance was measured on microplate reader at 560 nm. Cell viability was expressed as the relative formazan formation in treated samples compared to control cells after correction for background absorbance. The experiments were repeated three times.

Hormone determination. Progesterone concentration in culture media was determined by commercial radioimmunoassay (RIA) test kit according to the manufacturer's instructions (Institute of Izotopes Ltd, Budapest, Hungary). The assay sensitivity was 0.14 ng/ml. The intra-assay and inter-assay CVs were <10.2% and <11.8%, respectively. The concentration of estradiol in culture media was measured by commercial enzyme immunoassay (EIA) test kit according to the manufacturer's instructions (MP Biomedicals, Orangeburg, NY, USA). The assay sensitivity was 10 pg/ml. The intra-assay and inter-assay CVs were <24.1% and <26.7%, respectively.

Statistical analysis. The data are expressed as the mean ± standard error of the mean (SEM) of 3–5 independent experiments. Statistical analysis was performed with a One-way Analysis of Variance

(ANOVA) followed by Tukey-Kramer or Bonferroni multiple comparisons test. Values of $p<0.05$ were considered significant. Sigma Plot 11.0 software (Systat Software, GmbH, Erkhart, Germany) was used for statistical analysis.

Results

Characterization of PEG-*b*-PLA NPs. TEM demonstrated spherical shape of NPs and the average primary particle size about 50 nm. Zeta potential value was 28.73±1.44 mV. The micelle dispersion resulted in size distribution with two main peaks of secondary particle sizes, averaged as 64.9±10.5 nm and 911.4±177.6 nm, what indicates that the PEG-*b*-PLA micelles were aggregated in solution. The PEG-*b*-PLA micelles were stable for 24 h at ambient temperature as monitored by TEM (for details see Rollerova et al. 2015a).

Effect of PEG-*b*-PLA NPs on progesterone secretion. Initially, we examined time- and dose-dependent effects of PEG-*b*-PLA NPs on FSH-stimulated progesterone release by GCs (Figure 1). Supplementation of culture medium with FSH (1 μg/ml) stimulated progesterone secretion by GCs into the culture medium after 24, 48 and 72 h treatment compared to corresponding basal levels (increase by 33, 140 and 244 times, respectively; $p<0.001$; Figure 1). After 24 and 48 h culture, PEG-*b*-PLA NPs at concentrations of 50 and 100 μg/ml decreased FSH-stimulated progesterone release when compared to control levels ($p<0.001$; Figure 1). After 72 h treatment of GCs with PEG-*b*-PLA NPs, a significant decrease in FSH-induced progesterone secretion was observed at concentrations of 5 μg/ml ($p<0.01$), 50 and 100 μg/ml ($p<0.001$) compared to FSH treatment alone (Figure 1). At the highest tested concentration (100 μg/ml), the inhibition in progesterone release was associated with a decreased viability of GCs (decrease by 15%; $p<0.001$; Figure 2).

Based on the above presented results, we examined the effects of lower concentrations of PEG-*b*-PLA NPs on basal and FSH-induced progesterone release by GCs after 72 h culture (Figure 3). PEG-*b*-PLA NPs at concentrations of 20 and 40 μg/ml induced a significant decrease in basal progesterone secretion by GCs compared to controls ($p<0.001$; Figure 3A). Supplementation of culture medium with FSH (1 μg/ml) significantly stimulated progesterone release by GCs compared to basal levels ($p<0.001$; Figure 3B). PEG-*b*-PLA NPs significantly inhibited FSH-stimulated progesterone secretion by GCs at doses of 4 μg/ml ($p<0.01$), 20 and 40 μg/ml ($p<0.001$) when compared to FSH treatment alone (Figure 3B).

To explore possible effects of PEG-*b*-PLA NPs on FSH-induced signaling pathway, we examined dose-dependent effects of PEG-*b*-PLA NPs on forskolin- and 8Br-cAMP-stimulated progesterone production by GCs after 72 h culture (Figure 4). Supplementation of culture medium with forskolin (10 μM) and 8Br-cAMP (100 μM) increased progesterone secretion by GCs after 72 h culture (increase by 31 and 3 times, respectively; $p<0.001$; Figure 4). Treatment of GCs with PEG-*b*-PLA NPs reduced forskolin-stimulated progesterone release by GCs at concentration of 4 μg/ml ($p<0.05$) and 20 μg/ml ($p<0.001$) compared to forskolin treatment itself (Figure 4). PEG-*b*-PLA NPs had no significant effect on 8Br-cAMP-stimulated progesterone secretion by GCs (Figure 4).

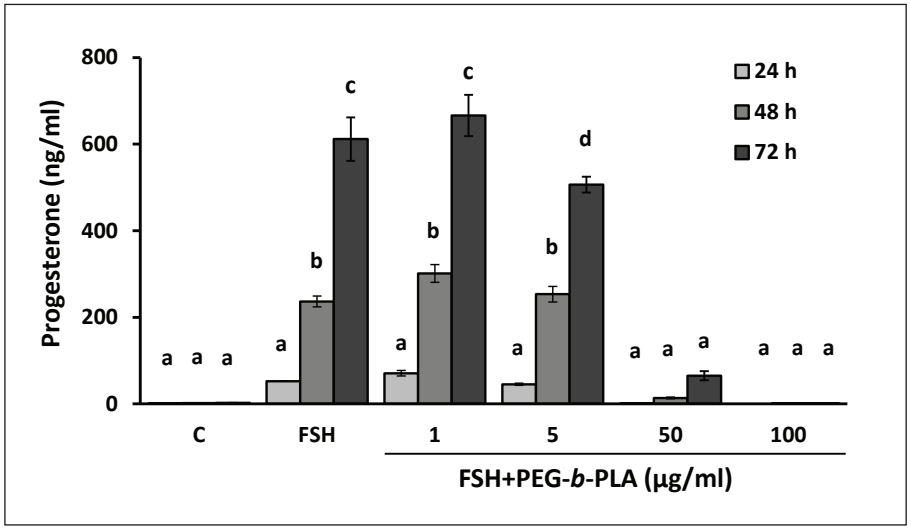

Figure 1. Time course effects of PEG-*b*-PLA nanoparticles (1–100 μg/ml) on follicle-stimulating hormone (FSH)-induced progesterone secretion by porcine ovarian granulosa cells. Data are presented as mean ± SEM (n=12). Data were analyzed by one-way ANOVA followed by Tukey post-test. Different superscripts indicate significant differences among groups.

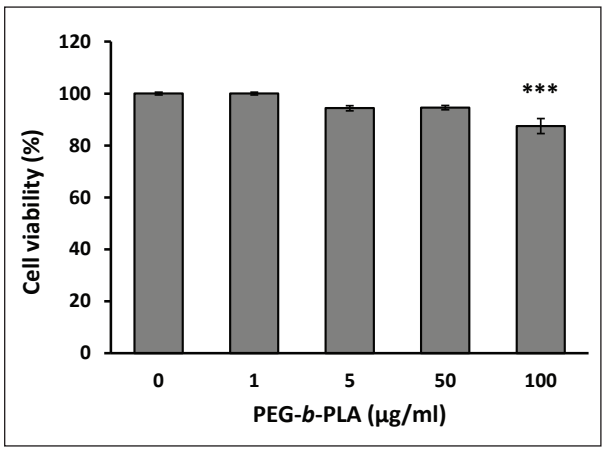

Figure 2. Effects of PEG-*b*-PLA nanoparticles (1–100 µg/ml) on cell viability of porcine ovarian granulosa cells after 72 h culture. Data are presented as mean ± SEM (n=18). Data were analyzed by one-way ANOVA followed by Bonferroni post-test. ***p<0.001 vs. control (0 µg/ml)

Effect of PEG-*b*-PLA NPs on estradiol secretion. Supplementation of culture medium with androstenedione (100 nM) stimulated estradiol release by GCs compared to basal levels (increase by 160 times; p<0.001; Figure 5). Treatment of GCs cells with PEG-*b*-PLA NPs induced a dose-dependent reduction in androstenedione-stimulated estradiol release by GCs after 72 h culture (Figure 5).

Effect of PEG-300 on progesterone secretion by GCs. We further examined the effects of PEG-300 on basal and FSH-induced progesterone release by GCs after 72 h culture (Figure 6). Treatment of GCs with the highest tested concentration of PEG-300

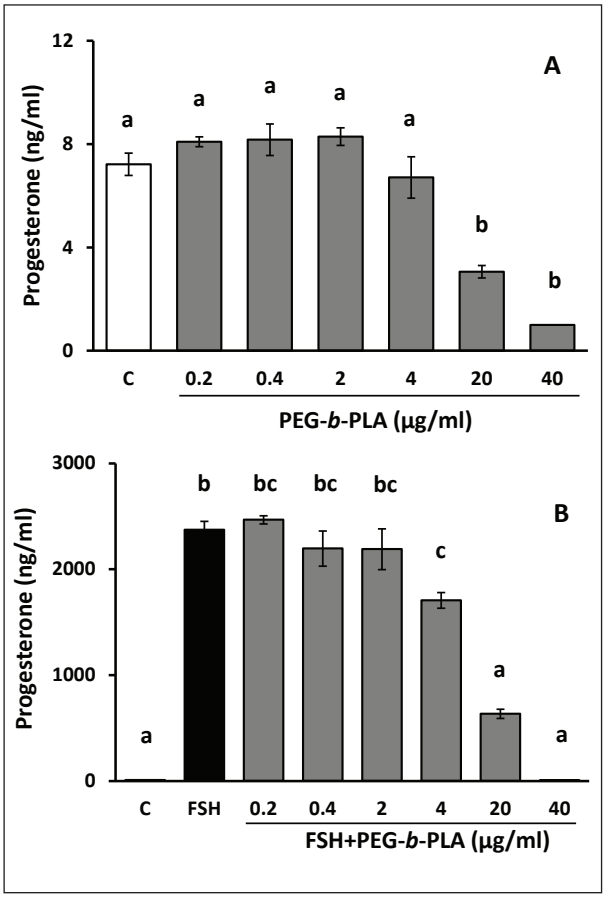

Figure 3. Effects of PEG-*b*-PLA nanoparticles (0.2–40 µg/ml) on basal (**A**) and follicle-stimulating hormone (FSH)-stimulated (**B**) progesterone secretion by porcine ovarian granulosa cells after 72 h culture. Data are presented as mean ± SEM (n=20). Data were analyzed by one-way ANOVA followed by Tukey post-test. Different superscripts indicate significant differences among groups.

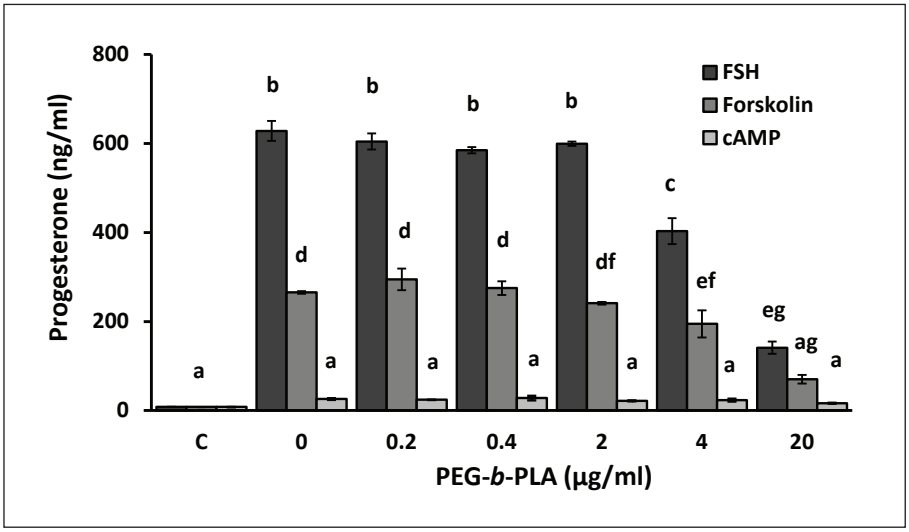

Figure 4. Effects of PEG-*b*-PLA nanoparticles (0.2–20 µg/ml) on follicle-stimulating hormone (FSH)-, forskolin-, and 8Br-cAMP-stimulated progesterone secretion by porcine ovarian granulosa cells after 72 h culture. Data are presented as mean ± SEM (n=12). Data were analyzed by one-way ANOVA followed by Tukey post-test. Different superscripts indicate significant differences among groups.

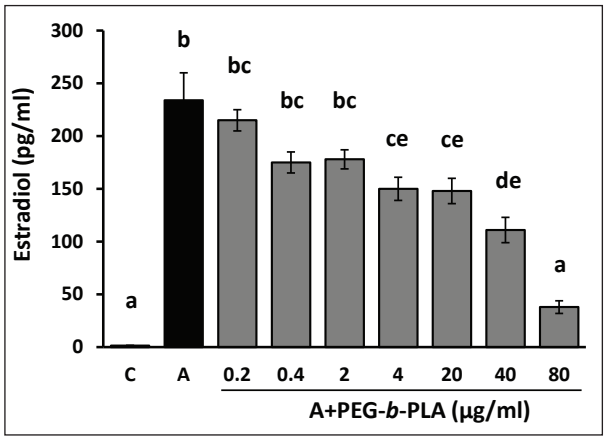

Figure 5. Effects of PEG-*b*-PLA nanoparticles (0.2–80 µg/ml) on androstendione (A)-induced estradiol secretion by porcine ovarian granulosa cells after 72 h culture. Data are presented as mean ± SEM (n=20). Data were analyzed by one-way ANOVA followed by Tukey post-test. Different superscripts indicate significant differences among groups.

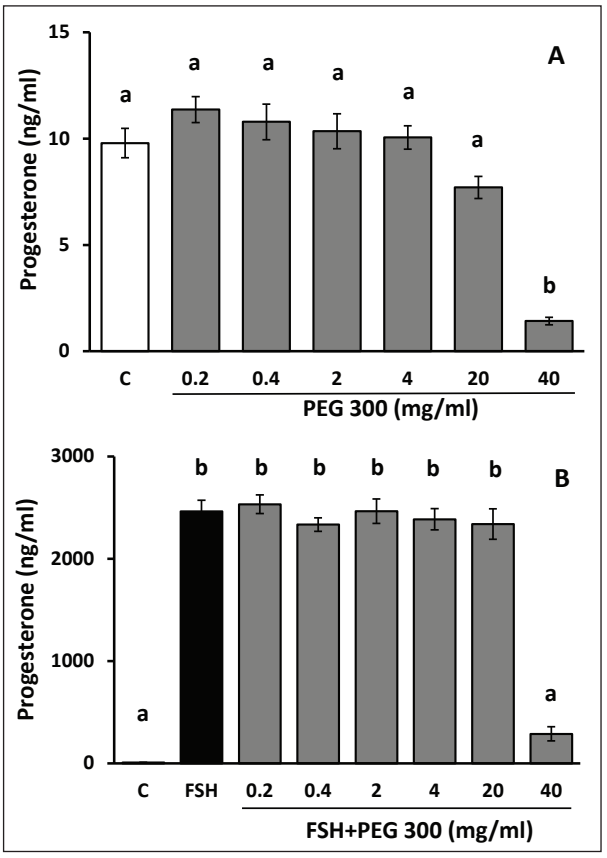

Figure 6. Effects of polyethylene glycol with a molecular weight of 300 (PEG-300) (0.2–40 mg/ml) on basal (**A**) and follicle-stimulating hormone (FSH)-stimulated (**B**) progesterone secretion by porcine ovarian granulosa cells after 72 culture. Data are presented as mean ± SEM (n=20). Data were analyzed by one-way ANOVA followed by Tukey post-test. Different superscripts indicate significant differences among groups.

(40 mg/ml) significantly decreased basal ($p<0.001$; Figure 6A) as well as FSH-stimulated ($p<0.001$; Figure 6B) progesterone secretion by GCs compared to control levels or FSH treatment itself. The reduced progesterone production was associated with the decreased viability of GCs after 48 and 72 h culture (decrease by 60%; $p<0.001$; Figure 7).

Discussion

Nanotechnology-based drugs, nanodrugs, have been developed and tested for many diagnostic and therapeutic applications. Natural and synthetic polymers (liposomes, dextrans, PLA, PLGA, and dendrimers) have been used to prepare a wide variety of nanocarriers for delivering therapeutic and imaging agents to reduce toxicity and side effects associated with particular drugs. Despite a lot of merits of these NMs/NPs, their fate in biological systems and associated side effects on living organisms should be evaluated. The adverse effects of nanovesicles have not been extensively studied, possibly because the components of these NPs are mainly biodegradable, biocompatible and U.S. FDA-approved materials (Semete et al. 2010). However, several recent findings have reported possible harmful effects and unexpected toxicities associated with nanocarriers on organ systems (De Jong and Borm 2008; El-Ansary and Al-Daihan 2009; Oberdoster 2009; Sharma et al. 2012). It has been demonstrated that NPs can pass through biological membranes (Wang et al. 2008; Rollerova et al. 2015b); raising fears that they can affect the physiology of any cell in the body.

In recent years, reproductive and developmental toxicity has increasingly become recognized as an important part of overall toxicology. Findings from the recent *in vivo* and *in vitro* studies have demonstrated that metal and metal oxide NPs can interfere with normal female reproductive functions including cytotoxic effects on ovarian somatic cells, impairing oogenesis and follicle maturation, and altering normal sex hormone levels (Iavicoli et al. 2013; Pottler et al. 2015; Rollerova et al. 2015b; Zhang et al. 2016).

In the present study, primary porcine ovarian GCs in culture have been used to determine the possible adverse effects of polymeric PEG-*b*-PLA NP, designed as a carrier for poorly water-soluble drugs, on female reproductive tissue. For the first time, we have examined the *in vitro* effects of PEG-*b*-PLA NPs (0.2–100 µg/ml) and bulk form of PEG-300 (0.2–40 mg/ml) on proliferation a steroid hormone secretion by GCs. We found that treatment of GCs with PEG-*b*-PLA NPs induced a significant decrease in basal as well as FSH-stimulat-

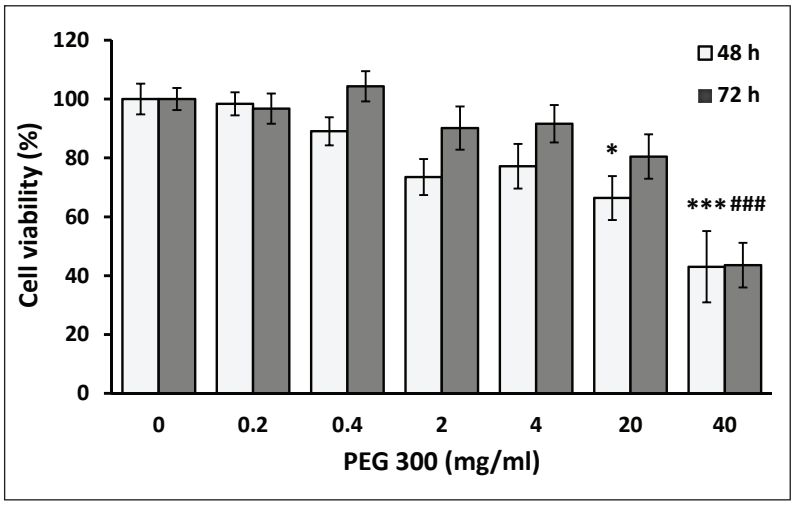

Figure 7. Effects of polyethylene glycol with a molecular weight of 300 (PEG-300) (0.2–40 mg/ml) on cell viability of porcine ovarian granulosa cells after 48 and 72 h culture. Data are presented as mean ± SEM (n=18). Data were analyzed by one-way ANOVA followed by Bonferroni post-test.*p<0.05 and ***p<0.001 vs. 48 h control (0 mg/ml); ###p<0.001 vs. 72 h control (0 mg/ml)

ed progesterone secretion above the concentration 20 and 4 μg/ml, respectively. A dose-dependent inhibition of androstenedione-stimulated estradiol release by GCs was found by the action of PEG-*b*-PLA NPs. Incubation of GCs with PEG-300 significantly inhibited basal as well as FSH-stimulated progesterone secretion above the concentration of 40 mg/ml. PEG-*b*-PLA NPs and PEG-300 significantly decreased the viability of GCs at the highest tested concentrations (100 μg/ml and 40 mg/ml, respectively) suggesting that PEG-*b*-PLA NPs and PEG-300 inhibited proliferation of GCs and thereby reduced secretion of steroid hormones by GCs. Using *in vivo* experimental model, we have previously shown that neonatal exposure female rats to PEG-*b*-PLA NPs (20 mg/kg b.w.) resulted in the elevated progesterone serum levels associated with a significantly increased number of irregular estrous cycles and prevalence of the diestrus stage in adult animals, and an alteration of pituitary LH response to LHRH stimulation (Rollerova et al. 20015a).

FSH receptor (FSHR) when activated by its ligand FSH couple to Gαs protein, which in turn stimulates adenylyl cyclase leading to an elevation in intracellular cAMP and subsequent activation of protein kinase A (PKA). Elevation of intracellular cAMP in GCs by gonadotropins, forskolin (an adenylyl cyclase activator), or membrane-permeable 8Br-cAMP promotes steroidogenesis by increasing expression and activity of enzymes involved in steroid hormone synthesis (Jamnongjit and Hammes 2006). To explore possible effects of PEG-*b*-PLA NPs on FSH-induced signaling pathway, we examined dose-dependent effects of PEG-*b*-PLA NPs on progesterone production by GCs stimulated by forskolin and 8Br-cAMP. PEG-*b*-PLA NPs reduced forskolin-stimulated, but not cAMP-induced progesterone production by GCs indicating that the tested NPs might affect FSH signaling at membrane level. Xiao et al. (2011) investigating the cellular uptake mechanisms of PEG-*b*-PLA micelles demonstrated that they firstly interacted with cell membrane, induced cell membrane depolarization and enhance membrane microviscosity in a dose-dependent manner. Previously, we have reported that cell membrane microenvironment plays an essential role in regulation of structure-function relationship of gonadotropin receptors (Kolena et al. 1995). The obtained results might indicate that understanding of interaction of NPs with cell membranes and their internalization into the cells is important in determining their diverse biological responses.

The present *in vitro* results together with our previous *in vivo* data indicate that polymeric PEG-*b*-PLA NPs might induce adverse effects on steroid hormone production by ovarian cells and thereby could modify reproductive functions. However, the molecular mechanisms of polymeric NPs PEG-b-PLA action remain unclear and needs further investigation.

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