BACK IN TIME: FISH OOCYTE AS A SUPERIOR MODEL FOR HUMAN REPRODUCTION? A REVIEW*

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The progress of reproductive biotechnology is dependent on the amount, quality, and availability of female gametes – oocytes. The proper selection of a suitable model organism is vital to ensure effective research of the signal pathways that regulate oogenesis and meiotic maturation. Many factors are involved in meiosis regulation and some of them are evolutionarily conserved. *Xenopus laevis* is a traditional model for cell cycle research, which has become a background for a more detailed study of models that are similar to humans. In contrast to mammalian models, water-living vertebrates are appropriate models for studying effects of environmentally occurring pollutants such as endocrine-disrupting chemicals (EDCs). The triploid gynogenetic Prussian carp is a unique biological model for reproduction studies. The ability of clone production in combination with alternative sexual mode of reproduction brings advantages for the testing of sensitiveness to the effects of EDCs in terms of studying the alternative molecular pathways in meiosis regulations. The aim of this review is to compare meiosis regulating pathways among various animal models, and to suggest the possible utilization of these models in researching EDCs. A comparison of the currently recognized oocyte signalization and the endocrine disruptor effect points out the need for their molecular target identification and introduces some in water living vertebrates as suitable study models.

meiotic maturation; oocyte; *Xenopus*; pig; mouse; *Carassius*; biological model

INTRODUCTION

Recently, sufficient reproductive abilities of farm animals and other mammals including humans have been the object of current reproductive biotechnologies. The quality of fertilized oocytes is a key factor for adequate interaction between an oocyte and a spermatozoon, which results in successful embryonic development and progress in reproductive biotechnology. However, reproductive disorders, based on faults in hormonal release, transport, metabolism and interactions, affect the quality of gametes. Endocrine disrupting chemicals (EDCs), occuring ubiquitously in the environment, are frequently suspected as the cause of these failures (C o l b o r n , 2004) result-ing in decreases in reproductive potential in animals and humans exposed to EDCs (H u a n g et al., 2014; R o m a n i et al., 2014). The origin of EDCs has various sources including pesticides, plastic compounds, medicines, and heavy metal residues.

The assignment of advanced reproductive biotechnologies involves relieving the EDCs' negative effect. A complete understanding of the reproduction physiology of both male and female is a necessary condition for reproductive biotechnology success. In this review, a summary of known mechanisms in female reproduction focusing on oogenesis is submitted. A comparison of common reproductive models stressing their utilization for the study of the effect of potential endocrine disruptors has been performed.

Oogenesis and meiotic maturation

Generally, oogenesis is female gamete production and it begins as early as during female embryonic development. Oocyte progression takes place throughout meiotic maturation, and is blocked in dictyotene of the first meiotic prophase in the germinal vesicle stage (GV). This state is established in the follicle of the female embryonic ovary as the first meiotic arrest. The blocked oocyte persists into puberty onset without significant changes. Under hormonal stimulation in adult females, the oocyte is stimulated to grow as necessary for oocyte maturation (W a s s a r m a n , 1988; Y a n a g i m a c h i , 1988).

In growing oocytes, increasing amounts of cell organelle such as mitochondria, endoplasmic reticulum, Golgi complex, and cortical granules occur. Moreover, *zona pellucida*, a glycoprotein membrane covering the oocyte, separating it from the surrounding follicular cells, is created. The cell organelles of the growing oocyte are important for the synthesis of key meiosis regulators (W a s s a r m a n , 1988; Y a n a g i m a c h i , 1988). Key regulators of meiotic division are accumulated as pro-enzymes and they are necessary for meiotic competence acquisition, complete meiotic progress, and correct chromosome segregation (W a s s a r m a n , A l b e r t i n i , 1994).

Meiotic division of the fully grown oocyte predestined for ovulation and fertilization is known as oocyte maturation, which begins with germinal vesicle breakdown (GVBD). After that, the oocyte achieves the first meiotic metaphase (MI) and the meiotically component oocyte undergoes the 2nd meiosis. The 2nd meiosis of fish, amphibian, and some mammalian oocytes, including mouse, pig, bovine, and human, is spontaneously blocked in the metaphase (MII). Meiosis completion occurs after fertilization by sperm or spontaneous parthenogenetic activation (W a s s a r m a n , 1988; Y a n a g i m a c h i , 1988). Oocyte maturation takes place in a tertiary follicle just before ovulation. Meiotic maturation of oocytes is also inducible under *in vitro* conditions and usable for cell cycle study. Oocyte maturation is a key process for the acquisition of developmental competence and thus for a successful early embryonic development (H a n et al., 2006; A u c l a i r et al., 2013).

One of the key regulators of oocyte meiotic maturation is M-phase/maturation promoting factor (MPF), consisting of regulatory and catalytic subunits – cyclin B and cyclin-dependent kinase 2 (CDC2), respectively. Both compounds are synthesized, aggregated and accumulated in an inactive form of proenzyme called pre-MPF. Inhibitory phosphorylation of Thr-14/Tyr-15 of CDC2 by WEE1 and MYT1 kinases is
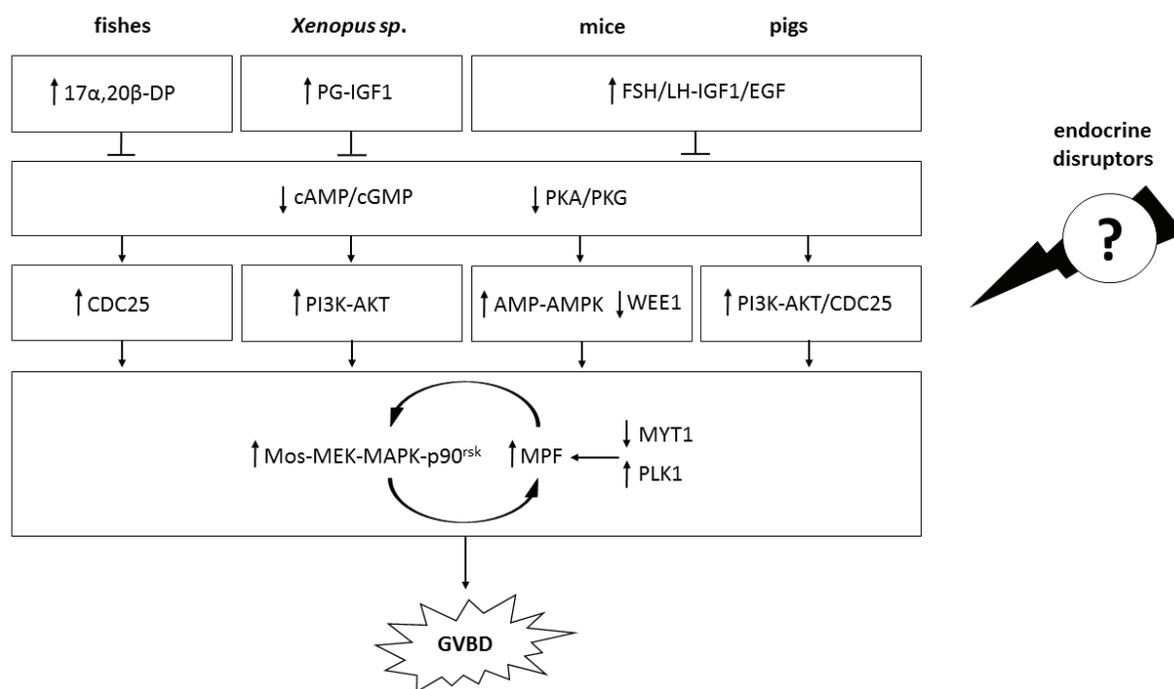

Fig. 1. General signal pathways leading to germinal vesicle breakdown and meiotic re-iniciation as a crucial point of gametogenesis of females under endocrine disruptor pressure. Some of signalizations considering species differences are selected
AKT = protein kinase B, AMP = adenosine monophosphate, AMPK = AMP-activated protein kinase, cAMP = cyclic adenosine monophosphate, CDC25 = cell division cycle 25 phosphatase, cGMP = cyclic guanosine monophosphate, 17α,20β-DP = 17α,٢٠β-dihydroxy-٤-pregnen-٣-one, EGF = epidermal growth factor, FSH = follicle-stimulating hormone, IGF = insulin-like growth factor ١, LH = luteinising hormone, MAPK = mitogen-activated protein kinase, MPF = M-phase/maturation promoting factor, PG = progesterone, PI٣K = phosphatidylinositol -٣kinase, PKA = cAMP-dependent protein kinase, PKG = cGMP-dependent protein kinase, PLK1 = polo-like kinase 1

responsible for the maintenance of inactive pre-MPF (Taieb et al., 1997). MPF activation depends not only on CDC2 dephosphorylation, but also on activating phosphorylation of cyclin B on Ser-94/Ser-96 (Izumi, Maller, 1991).

Biochemical changes of MPF leading to meiosis re-initiation and completion are induced by hormonal stimuli. The hormonal stimulus is 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP) for fish such as zebrafish (*Brachydanio rerio*) and goldfish (*Carassius auratus*), progesterone (PG) for frogs *Xenopus* sp. (Masui, Market, 1971; Nurse, 1993; Kobayashi et al., 2000), 17β-estradiol and gonadotropins luteinizing hormone (LH) and follicule stimulating hormone (FSH) for mammals (Dekel, Beers, 1978; Fukui et al., 1982; Mattioli et al., 1991; Funahashi et al., 1994) (Fig. 1). Oocyte receptors are sensitive to certain hormones. Hence, estrogene receptors in maturing oocytes are responsible for regulating second messengers and key kinase pathways (Prossnitz et al., 2007; Pang et al., 2008). Therefore, the above-mentioned hormones induce concentration changes of second messengers, simple molecules with signal transduction ability, including cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Metabolites of follicular cells flow through gap junctions into the oocyte and influence various enzymes with kinase activity, such as cAMP-dependent and cGMP-dependent protein kinases (PKA, PKG) (Levesque, Sirard, 1995; Mori et al., 2000; Zhang et al., 2005). In addition to cyclic nucleotides, gasotransmitters are another group of small signalling molecules involved in the regulation of oocyte maturation (summarized in Šmelcová, Tichovská, 2011). Nitric oxide, which is enzymatically released directly in the oocyte, is the most commonly observed gasotransmitter with a physiological role in oocyte maturation. Nitric oxide signalization is essential for meiosis re-initiation and the completion of oocyte maturation (Jablonka-Shariff, Olson, 2000; Chmelíková et al., 2010; Tichovská et al., 2011).

Another second messenger, a Ca^{2+} ion, acts outright in the oocyte. The signal pathway of Ca^{2+} includes down-stream regulated kinases: calmodulin-dependent kinase (CaMKII) (Fan et al., 2003) and some Ca^{2+}-dependent protein kinase (PKC) isoforms (Fan et al., 2002a; Fan, Sun, 2004). These kinases cause the activation of Cell Division Cycle 25 phosphatase (CDC25) with dephosphorylating activity responsible for removing inhibitory phosphates of CDC2 (Taieb et al., 1997). Changes of MPF phosphorylation pattern and auto-accelerating positive feedback lead to an intense increase in MPF activity, resulting in GVBD (Lee et al., 1999; Fan et al., 2002b; Kishimoto, 2003).

Increasing MPF activity in the oocyte reaches a maximum in the MI stage, and MPF decreases together with the arrival of anaphase I and telophase I (Verlhac et al., 1993, 1994). Decreasing MPF activity is up-regulated by anaphase promoting factor/cyclosome (APC/C), degrading the regulatory subunit of MPF – cyclin B. This degradation is based on polyubiquitination, and cyclin B is destined to proteolysis in proteasome S26 (Peters, 2002; Yi et al., 2008). APC/C inactivation in meiosis II is followed by re-activation of MPF (Taieb et al., 1997; Kubelka et al., 2000).

Another key meiotic regulator is activated around GVBD – mitogen-activated protein kinase (MAPK), a member of Ser/Thr protein kinase family (Inoue et al., 1998; Fan et al., 2002b; Ohashi et al., 2003; Tao et al., 2005). The MAPK signal pathway includes a few up-stream factors – Mos and MEK (Kyriakis, Avruch, 2001). In addition, G protein estrogen receptors are involved in MAPK activity (Li et al., 2013). Due to the action of these factors, MAPK activity is nearly constant over the whole oocyte maturation (Lee et al., 2000; Villa-Diaz, Miyano, 2004). This activity profile is necessary for meiosis I followed immediately by meiosis II without an interphase between them (Kishimoto, 2003; Fan, Sun, 2004; Sasaki, Chiba, 2004).

Maturation signal pathways and the correct MPF dynamics with interaction with Mos-MEK-MAPK determine chromatin condensation, nuclear lamin phosphorylation and depolymerisation, correct chromatid segregation, and extrusion of homologous chromosomes in first polar body (Lüscher et al., 1991; Hampl, Eppig, 1995; Inoue et al., 1995, 1998; Lee et al., 2000; Takakura et al., 2005). Persistent high MPF and MAPK activity in MII maintains the haploid oocyte in the so-called second meiotic arrest (Taieb et al., 1997).

Multi-enzyme complex cytostatic factor (CSF) is accountable for second meiotic arrest maintenance (Li et al., 2002; Reimann, Jackson, 2002). The above-mentioned Mos is one of its compounds. The role of CSF is suppression of cyclin B proteolytic degradation, MPF stabilization, and maintaining constant MPF and MAPK activity in a matured MII-oocyte (Maller et al., 2001, 2002; Kyriakis, Avruch, 2001; Fan, Sun, 2004). A fall in CSF activity follows the release and oscillation of Ca^{2+} ions after fertilization and oocyte activation (Fan et al., 2002b; Fan, Sun, 2004).

We can assume that endocrine disruptors influence the above-mentioned signal pathways (Fig. 1). However, their molecular mechanisms still remain unclear and certain common model organisms are sufficient for the preliminary elucidation of endocrine disruptor action.

Xenopus oocytes: a traditional model for cell cycle study

The reasons for introducing *Xenopus laevis* as a biological model are its especially easy keeping and

handling, resistance to disease, short reproductive cycle, and constant response to hormonal stimulation regardless of the season (K a y, P e n g, 1991). Another advantage is simple preparation and manipulation with a large amount of fairly big oocytes.

The mitotic division of amphibian oogonies occurs throughout the whole lifetime. Primary oocytes enter first meiosis, which is physiologically disrupted in the diplotene of first prophase, in first meiotic arrest. The arrested oocytes grow intensively, accumulating organels and proteins, acquiring meiotic competence and encapsulating in the thin-walled follicle closely surrounding the oocyte. Follicular cells communicate with the oocyte throughout gap junctions, participating in egg yolk cumulation and meiosis regulation (B r o w n e et al., 1979). Oocyte growth is not a fully synchronized process and oocytes in various developmental stages occur in both ovary lobes. The stage of oocyte growth is evaluable and classifiable into one of six growth stages (D u m o n t, 1972).

Fully grown oocytes in final stage VI achieve 1.3 mm in diameter with visible animal and vegetative poles. The non-pigmented equatorial band is enclosed by both of the poles. The germinal vesicle is situated in the middle of oocyte and includes a few nucleoli with a large amount of mRNA. Just fully grown oocytes are meiotically competent and able to re-initiate and finalize meiotic maturation (R a s a r, H a m m e s, 2006).

The oocyte maturation of *Xenopus laevis* (herafter referred as *Xenopus*) is regulated by epiphyseal gonadotropins. They give an incentive to progesterone production by follicular cells. Progesterone is in usage for *in vitro* maturation of *Xenopus* oocytes. Under *in vitro* conditions, only fully grown oocytes respond to progesterone treatment (S m i t h, E c k e r, 1970).

Progesterone acts as a ligand for receptors enclosed in the oocyte cytoplasmic membrane as well as for soluble receptors in ooplasm. By binding to both receptors, it inhibits adenylate cyclase (AC) activity and reduces the intracellular concentration of cAMP suppressing meiosis re-initiation. Therefore, a decrease in cAMP-dependent protein kinase (PKA) activity responsible for the first meiotic arrest takes place in the oocyte. Low PKA activity is an adequate inducement for GVBD (P a l m e r, N e b r e d a, 2000).

G-protein coupled receptors are one of the progesterone membrane receptors. They are able to activate factors leading to oocyte maturation re-initiation – Mos, MEK, and subsequently mitogen activated protein kinase (MAPK) (B l u m e r, J o h n s o n, 1994). The Mos-MEK-MAPK signal pathway induces activity of p90^{rsk}, ribosomal protein s6 kinase (E r i k s o n, 1991). This protein is responsible for MYT1 inhibition and dephosphorylation of inhibiting phosphate residua on CDC2, the catalitic subunit of m-phase/maturation promoting factor (MPF) (P a l m e r et al., 1998).

Moreover, the activation of other regulating factors of MPF occurs there: polo-like kinase 1 (PLK1) and CDC25 (K a r a ï s k o u et al., 1998).

Subsequently to this, MAPK and MPF activity increases. The activation of the mentioned signal pathways leads to GVBD induction and re-initiation of oocyte maturation, also called M-phase for *Xenopus* oocytes. While MPF is essential for GVBD and meiosis, MAPK is dispensable and its lack causes at most MAPK dynamics and meiotic maturation disruption (S c h m i t t, N e b r e d a, 2002).

The meiotic division of *Xenopus* oocytes has become a process appropriately describing biochemical changes of MPF, a key factor in cell cycle regulation. The G-phase to M-phase transition in *Xenopus* oocyte offers an opportunity for MPF activity and dynamics understanding as background for the further study of somatic cells' cell cycle and also for meiosis study in higher vertebrate biological models more similar to farm animals and human.

Mus musculus: mammalian model with *Xenopus* advantages

Mouse (*Mus musculus*) represents a more human-like genetic and biological model than amphibians, including *Xenopus*. Mice have a high reproductive ability and there are also a number of options for genetic modifications and the study of their influence on reproductive efficiency.

Oocyte capability of GVBD and meiosis I to II transition is conditioned by age and puberty achievement (W a s s a r m a n, 1988). During all post-partal stages of ontogenesis, ovarian follicles and oocytes are developing. During the growth stage, oocyte attains 30, 60, and 80 μm in diameter in 5, 15, and 21 days after the birth of the female, respectively. Only 60 μm oocytes are able to initiate meiotic maturation. A rapid increase in the number of oocytes capable of GVBD takes place in ovaries around the 17th day of age (S o r e n s e n, W a s s a r m a n, 1976). Oocytes with less than 60 μm in diameter are retained in the dictyate stage unable of meiosis. However, small and meiotic incompetent mouse oocytes are able to mature into fully-grown competent oocytes (F u l k a J r. et al., 1985).

A fully grown mouse oocyte is maintained in the first meiotic arrest by a high concentration of cAMP (S c h u l t z et al., 1983). The molecules of cAMP are generated by 3-adenyl cyclase in the oocyte (H o r n e r et al., 2003) or follicular cells followed by cAMP flow into the oocyte (D e k e l et al., 1981). Follicular cells closely surrounding the oocyte, which are known as cumulus cells, are stimulated by gonadotropins LH and FSH to produce a large amount of extracellular matrix compounds and mucify, a process known as cumulus expansion (D e k e l, 1988). As such, gap junctions connecting cumulus cells and oocyte are

disrupted and cAMP flow into the oocyte is prevented (D e k e l, 1988). However, a transient rise in cAMP concentration in mouse cumulus-oocyte complexes occurs due to FSH action (S a l u s t r i et al., 1985; W e b b et al., 2002; E d r y et al., 2006; C h e n et al., 2009). Thereafter, enzymatic cAMP degradation by phosphodiesterase (PDE) follows (S h i t s u k a w a et al., 2001). The PDE activity is stimulated by phosphorylation through protein kinase b (also called akt) participating in oocyte maturation (H a n et al., 2006). the metabolic product of PDE-induced cAMP degradation, adenosine monophosphate, is an important regulating factor of AMP-activated protein kinase (AMPK), supporting GVBD and oocyte maturation (C h e n et al., 2006). Before this process, a sufficient amount of cAMP in oocyte determines adequate stimulation of meiosis resumption by AMPK (C h e n et al., 2009).

One of the cAMP-dependent factors regulating meiosis in oocytes is protein kinase A (PKA). PKA maintains a high activity of WEE1 and MYT1, responsible for enzymatically inactive pre-MPF maintenance. The fall in cAMP concentration and PKA activity enables CDC25 activation, a further regulating kinase activating MPF (H a n, C o n t i, 2006; P i r i n o et al., 2009; O h et al., 2010). In addition to MPF activation, cyclin B proteosynthesis is ongoing throughout the whole meiotic maturation (W i n s t o n, 1997). The result of MPF activation is GVBD occurring at around 3 h of oocyte maturation (S z ö l l ö s i et al., 1972; J u n g et al., 1993). With meiosis I to II transition, MPF activity decreases and re-achieves an activity peak in MII-oocytes (A b r i e u et al., 1991).

The high cAMP concentration in immature GV-oocytes suppresses MAPK activity. When MAPK activity increases, MAPK remains active and independent of further concentration of cAMP (S u n et al., 1999). MAPK is necessary for spindle formation, correct chromosome segregation, and MPF reactivation in meiosis II; rather, MAPK signal pathway is not essential for GVBD and first polar body extrusion in the mouse oocyte (A r a k i et al., 1996).

Endocrine disruptors likely affect key signal pathways of oocyte maturation. Published data regarding bisphenol A point to the influence of estrogen receptors in mouse oocytes (C h a o et al., 2012). Subsequently, DNA methylation and epigenetic inheritance, as well as nitric oxide production, are regulated (C h a o et al., 2012; T r a p p h o f f et al., 2013; P a n d e y, D e s h p a n d e, 2015). Oocyte signalization failure results in faulty spindle microtubular organization and an increased incidence in aneuploidy (H u n t et al., 2003; C a n et al., 2005). However, current knowledge lacks the complete explanation of EDCs action.

Obtaining and cultivating mouse oocytes are less difficult than in some other models (e.g. cows, pigs), and for this reason they have been utilized as a suitable biological model for the study of factors involved in MPF activation and meoisis resumption, such as

PLK1, MYT1, WEE1, and CDC25. On the other hand, following maturation, mouse oocytes have a few differences in comparison with bovine, porcine, and human oocytes. The application of endocrine disruptor studies based on mouse model seems to be lees suitable for human. For greater resemblance, porcine oocytes are used as a more appropriate biological model offering applications in human transplantation and reproductive medicine.

Sus scrofa: just like a human

Pig (*Sus scrofa domestica*) is a multiparous species with high fertility and its ovaries are well available immediately after slaughtering. Pig is a more advisable model for research result applications in human medicine than frogs or mice.

Early oogenesis in pigs occurs in a similar manner to mouse. In the prenatal stage of ontogenesis, meiotic division of oogonies and their entry to meiosis take place in ovarian follicles. Meiosis is arrested in the first meiotic block consisting of prophase I maintenance until sex maturity is achieved. Ovaries of newborn gilt contain about 210 thousand immature oocytes with the established first meiotic arrest (P r a t h e r, D a y, 1998). Oocytes of matured gilts and swines are destined to re-initiation of growing and meiosis as a result of hormonal stimuli.

The duration of meiotic maturation under *in vitro* condition is comparatively long (44–48 h), and thus the porcine oocyte is suitable for the study of GVBD and particular meiotic stages. The meiotic maturation of fully grown GV-oocyte arrested in prophase I with 120–125 µm in diameter is initiated by GVBD. This process includes four different stages: GV I, GV II, GV III, and GV IV. In GV I, the nucleoplasm is intact and chromatin is established in a ring. The GV II is specified by recognizable nucleus containing heterochromatin missing its ring shape. The chromatin of the GV III oocyte is fully diffused and not recognizable by staining. Chromatin of the GV IV oocyte is highly condensed in bivalents and the nuclear envelope is disaggregated and not visible. Under *in vitro* condition, completed GVBD takes 16–24 h (M o t l í k, F u l k a, 1976; M e i n e c k e, M e i n e c k e - T i l l m a n n, 1979).

GVBD is immediately followed by the MI stage. Anaphase I and telophase I are difficult to distinguish and they are jointly evaluated as AI/TI stage where homologue chromosomes are segregated and diverged to spindle poles. Meiosis I continuously passes into meiosis II when the first polar body is extruded, meiotic division is stopped in MII and 2nd meiotic arrest is established. MII achievement is considered the termination of oocyte maturation. Meiosis completion is allowed after fertilization and oocyte activation by sperm where the sister chromatids are excluded as second polar body (T h i b a u l t et al., 1987; W a s s a r m a n, 1988).

GVBD in *in vivo* matured oocytes occurs after hormonal stimulation by gonadotropins – follicle stimulating hormone (FSH) and luteinizing hormone (LH). Gonadotropins cause suppression of meiosis inhibiting factors, such as cAMP and cAMP-dependent kinase (PKA), in cumulus cells and oocytes (M a t t i o l i et al., 1994). Under *in vitro* conditions, usage of gonadotropins and their synthetic analogs commonly used with blood serum proteins and growth factors is possible for the induction of oocyte maturation (S i n g h et al., 1997; U h m et al., 1998).

The cAMP/PKA signal pathway in cumulus cells and the oocyte is responsible for the first dictyate arrest in porcine oocytes. The cAMP generation is enzymatically catalyzed by adenylate-cyclase localized in the oocyte cytoplasmic membrane (L i a n g et al., 2005). Falling cAMP concentration is a result of inhibited adenylate-cyclase activity and concurrent activation of phosphodiesterases cleaving cyclic bounds of cAMP (M a t t i o l i et al., 1994). In addition to cAMP role, another cell second messenger participates in meiosis regulation, cyclic guanosine monophosphate (cGMP), followed by cGMP-dependent protein kinase (PKG) activity decreasing. Falling amounts of cGMP in cumulus cells allow phosphodiesterase activation and decrease of cAMP concentration in the oocyte (L a P o l t et al., 2003). Further concentration of cAMP in the oocyte also decreases due to the gap junction disconnecting between the oocyte and cumulus cells following meiosis induction (L i a n g et al., 2007). The initial cAMP and PKA suppression is essential for meiosis re-initiation and GVBD (R a c o w s k y, 1983; M a t t i o l i et al., 1994). Low cAMP concentration persists throughout oocyte maturation (M a t t i o l i et al., 1994).

At the same time as the decrease in the concentration of inhibitory factors, Ca^{2+} ions are released from endoplasmic reticulum by inositol-triphosphate receptors (IP3R) and ryanodine receptors (RyR) into the oocyte cytoplasm (M a c h a t y et al., 1997). The Ca^{2+} ions activate protein kinases calmodulin-dependent kinase (CaMKII) (F a n et al., 2003) and some isoforms of Ca^{2+}-dependent protein kinases (PKC) (F a n et al., 2002a; F a n, S u n, 2004). Together with CDC25 (T a i e b et al., 1997), PLK1 (A n g e r et al., 2004), and phosphatidylinositol 3-kinase (PI3K)-up-regulated AKT (K a l o u s et al., 2009), these kinases are involved in the regulation of key maturation factors, MPF, and MAPK (M o t l í k, K u b e l k a, 1990).

MPF activity decreases with entry into AI/TI. This decrease is important for meiosis I to II transition, chromosome segregation, and first polar body extrusion (G l o t z e r et al., 1991). Particular MPF inactivation is based on proteolytical degradation of cyclin B, the regulator compound of MPF. Ubiquitination through anaphase-promoting complex/cyclosome (APC/C) is required for cyclin B proteolysis by proteasome S26 (P e t e r s, 2002). As such, ubiquitin-proteoasome system (UPS) is necessary for the correct progression of porcine oocyte maturation (Yi et al., 2008). Subsequent to this, MPF activity increases again and achieves maximum activity in MII where it is essential for the 2nd meiotic arrest maintenance (Y a n a g i m a c h i, 1988).

The signal pathway of Mos-MEK-MAPK is involved in porcine meiotic maturation through FSH (Li et al., 2002), cAMP (L i a n g et al., 2005), and MPF action (F a n et al., 2002b). W e h r e n d, M e i n e c k e (2001) observed increasing MAPK activity immediately before MPF-induced GVBD. In spite of MPF, MAPK activity during oocyte maturation persists almost constantly (L e e et al., 2000; V i l l a - D i a z, M i y a n o, 2004). The important target of MAPK is ribosome S6-kinase p90[rsk] (F a n et al., 2002b; K i s h i m o t o, 2003; F a n, S u n, 2004; R o u x, B l e n i s, 2004) and MAPK participates in cyclin B re-synthesis and MPF re-activation through this target molecule during meiotic maturation (O h a s h i et al., 2003). In addition, MAPK suppresses MYT1 activity through PLK1 and prevents inhibitory phosphorylation of Cdk2 and MPF to pre-MPF conversion (F a n et al., 2002b; K i s h i m o t o, 2003; O h a s h i et al., 2003). Moreover, MAPK is required for CDC25 to remain stable and thus ensures MPF activity (K i s h i m o t o, 2003). Regulatory factors, such as the above-mentioned PKA, PKC, and CaMKII are other MAPK target systems (K i s h i m o t o, 2003; F a n, S u n, 2004).

MAPK has a non-essential role in GVBD induction (F a n et al., 2002b), however it participates in meiosis resumption through MPF activation (O h a s h i et al., 2003). Active MAPK induces spindle migration in the ooplasm during meiosis II, asymmetric cytokinesis, and excluding of the first polar body (T o n g et al., 2003). MAPK remains active until MII achievement and holds 2nd meiotic arrest through highly active CSF keeping (F a n et al., 2002b; M a l l e r et al., 2002; K i s h i m o t o, 2003; O h a s h i et al., 2003).

CSF is an important factor for 2nd meiotic arrest maintenance in the matured MII oocyte because CSF inhibits APC/C activity and cyclin B degradation. Concurrently, active MPF promotes CSF activity through Emi1, one of the CSF compounds, activating phosphorylation (K i s h i m o t o, 2003). The second compound of CSF – Mos, is involved in Mek and MAPK activity keeping (K y r i a k i s, A v r u c h, 2001).

The comprehensive and exact mechanism of oocyte maturation is key for the so-called developmental competence acquisition, determining success following early embryogenesis (L i et al., 2002; R e i m a n n, J a c k s o n, 2002). Understanding regulatory mechanisms under *in vitro* conditions will be of help in studying various artificial substances possibly present in the environment and endangering the reproductive health of farm animals and humans, including their gametogenesis and oocyte maturation.

Regulatory mechanisms of porcine oocyte maturation are largely similar to the human oocyte. For long onset of GVBD, the porcine oocyte offers a suitable

way to study the dynamic regulatory factors responsible for meiosis reinitiation. In addition to GVBD study, a long duration of further meiosis is convenient for understanding meiosis I to II transition. Moreover, porcine meiosis is an appreciated model for testing the antioxidant or negative effect of environmental pollutants on oocyte maturation and embryonic development. Recently there have been developments in research of pollutants occurring at very low concentrations. Findings include the fact that EDCs are able to negatively influence human health, manifested especially in reproduction failures (M a c h t i n g e r et al., 2013; M a c h t i n g e r, O r v i e t o, 2014).

There is a logical assumption that organisms living in water are permanently exposed to certain concentrations of substances that may act as endocrine disruptors. This is mainly due to the fact that aquatic environment (rivers, lakes, and seas) serve as recipient of sewage. Although the sewage treatment is a common practice in many countries, it does not influence many substances that may act as endocrine disruptors (G o l o v k o et al., 2014).

Comeback to lower vertebrates

Aquatic lower vertebrates are confronted with a water environment usually over their whole life-time. In these conditions, pollutant accumulations take place. Therefore e.g. fishes are considered as an appreciated model for the study of EDCs and other pollutants since they may significantly influence their reproduction (Z h a n et al., 2000; T o k u m o t o et al., 2004, 2005).

The zebrafish (*Brachydanio rerio*) is a traditional model for molecular and developmental biology. Its advantages include quick ontogenetic development and short life cycle, as well as embryonic development outside the female body and availability to observe organogenesis in *in vitro* cultured embryos. Therefore, this model is utilized as a simple biological indicator for endocrine disruptor effects (H o l b e c h et al., 2006; S a n t o s et al., 2014; K i n c h et al., 2015).

Goldfish (*Carassius auratus*) and common carp (*Cyprinus* carpio) are other fishes commonly used as model organisms suited for studies on the effect of EDCs (W a n g et al. 2015). On the other hand, they are mainly utilized for the study of male reproduction disorders (Y a n et al., 2013; G o l s h a n et al. 2014) and female reproduction affection remains often unexplained.

Fish oocytes are maintained in the prophase of first meiotic division through high cAMP concentration just like in the previously mentioned mammalian model organisms. The cAMP concentration decline induces meiotic maturation. Meiosis regulation is determined by three key factors: gonadotropins, the maturation inducing hormone, identified as 17α,20β-dihydroxy-4-pregnen-3-one, and M-phase promoting factor (Y a m a s h i t a, 1998).

Pituitary gonadotropins act on thecal cells surrounding the oocyte. Hormone-induced thecal cells produce testosterone under receptor-mediated adenylate cyclase-cAMP control (W a n g, G e, 2003; Z h o u et al., 2000). Testosterone production is regulated through other intracellular signal pathways such as Ca^{2+} ions, calmodulin, and arachidonic acid. Testosterone is converted into estradiol-17β by aromatases directly in the thecal cells, where it is involved in oocyte growth. In addition to estradiol-17β production, 17α-hydroxyprogesterone is created by thecal cells and thereafter 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP) is produced by 20β-hydroxysteroid dehydrogenase. 17α,20β-DP is specific for fish females and the ooplasm is equipped with appropriate 17α,20β-DP receptors. After 17α,20β-DP binding onto its receptors, MPF activation and subsequent GVBD take place in the fish oocyte (N a g a h a m a, 1997; Y a m a s h i t a, 1998).

In contrast to the previously-mentioned species, pre-MPF is not present in fish oocytes (T a n a k a, Y a m a s h i t a, 1995). Meiotic incompetent oocytes do not include cyclin B, but these oocytes dispose of the inactive form of CDC2. Just before meiotic maturation, an increase in the amount of cyclin B and its conjugation with CDC2 and MPF creation is induced by 17α,20β-DP as a necessary condition of oocyte maturation. The cyclin B presence in a sufficient amount is an adequate stimulus for meiosis induction. For CDC2 activation, phosphorylation of CDC2 by p40[MO15] is required, unlike cyclin B phosphorylation on serine residuum (K a t s u et al., 1993; T o k u m o t o et al., 1997; Y a m a s h i t a, 1998).

The incompetent oocyte has not enough Mos protein as an up-stream regulator of MAPK. Mos occurs around GVBD, and it achieves a peak in the MII-oocyte. MAPK is activated by Mos and is followed by MPF activity increasing. However, the Mos-MEK-MAPK signal pathway is not necessary for induction of meiotic maturation. The main MAPK action is based on participating in the 2nd meiotic arrest stabilization in matured oocytes by CSF (Y a m a s h i t a, 1998; K a j i u r a - K o b a y a s h i et al., 2000; K h a n, M a i t r a, 2013).

Capability of a uniparental inheritance induced by gynogenesis or androgenesis is an advatage of fish model organisms (K o m e n, T h o r g a a r d, 2007). Clonal lines of fish are of great interest since they can be used in a variety of research areas, including toxicology. Homozygous offspring in the first generation is the main benefit of gynogenetic reproduction (S a r d e r et al., 1999). Although the induction of gynogenesis requires human intervention, natural gynogenesis also appears sporadically within the fish taxa (P i f e r r e r et al., 2009). The Prussian carp (*Carassius gibelio*) as a related species to goldfish may reproduce by gynogenesis (K a l o u s, K n y t l, 2011; K a l o u s et al. 2013) and such ability greatly facilitates the

availability of a great number of presumably clonal individuals. Moreover, the reproductive mechanism of Prussian carp is more complicated allowing also sexual reproduction when homologous sperm and egg meet (G u i , Z h o u , 2010; K n y t l et al., 2013). Such unique alternative system of reproduction comprising gynogenesis in combination with sexual reproduction determines this model organism for studying the alternative molecular pathways in meiosis regulations, which then could be used for more accurate research on the negative effects of pollutants acting in very low doses.

From the above mentioned reasons we consider oocytes of triploid Prussian carp as a potential model for evidence of negative effects of EDCs, which occur in the environment and could be potentially responsible for disorder in reproductive functions of humans.

CONCLUSION

The oocyte is an unrivalled material for reproductive biotechnology. The size, simplicity of manipulation, and opportunity to study the cell cycle and embryonic development are all advantages of oocyte use. For *in vitro* oocyte cultivation and usage of the organism as a biological model, an understanding of oogenesis regulatory signal pathways is important.

Re-initiation of oocyte meiosis is induced by hormones. Subsequently, second messenger molecules responsible for meiotic block are regulated and signal transduction requiring CDC25, MYT1, MPF, and MAPK is involved in oocyte meiotic maturation. MPF activity increases, resulting in lamin phosphorylation and subsequent GVBD. MPF and MAPK signal pathways are responsible for the complete and correct meiotic maturation and 2nd meiotic arrest maintenance.

For MPF activation and studying its function, *Xenopus* oocytes providing a lot of large oocytes are suitable. They supply a background for further research of the cell cycle of higher vertebrates including mammals. Mouse or pig biological models are used for the study of molecular reproduction. The great similarities of the pig model to humans are utilized for result applications in human disease healing, assisted reproduction, and xenotransplantaion.

Besides regulatory mechanisms of gametogenesis, recent applied reproductive biology has focused on the negative effects of some environmental pollutants, such as phytoestrogens, mycotoxins or endocrine-disrupting chemicals (EDCs). The act of the last appointed consists of very low and hardly-detectable concentrations expected in water environment. Recent understanding of endocrine disruptor action is often limited to hormonal failures (C a s e r t a et al., 2014; G o l s h a n et al., 2014; P a n d e y , D e s h p a n d e , 2015).

Due to simple way of obtaining eggs, natural gynogenesis, and alternative sexual reproduction, Prussian carp could be suggested as appropriate model for the study of key signal pathways of oocyte maturation. Anyway, the proper genetic indentification of individulas taken to the experiments is essential due to different ploidy levels and various genome combinations within *Carassius gibelio* complex (W o u t e r s et al. 2012; R y l k o v á et al. 2013).

In the future the unique oocyte of Prussian carp could be an eligible object of reproductive biology studies focusing on pollutants dangerous for health and reproduction in humans due to its alternative mode of reproduction, which may unreveal what still stays hidden in other animal models.

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LIST OF ABBREVIATIONS:

17α,20β-DP = 17α,20β-dihydroxy-4-pregnen-3-one, AC = adenylate cyclase, AMPK = AMP-activated protein kinase, APC/C) = anaphase-promoting complex/cyclosome, CaMKII = calmodulin-dependent kinase, cAMP = cyclic adenosine monophosphate, CDC2 = cyclin-dependent kinase 2, CDC25 = cell division cycle 25 phosphatase, cGMP = cyclic guanosine monophosphate, CSF = multi-enzyme complex cytostatic factor, EDCs = endocrine disrupting chemicals, FSH = follicule stimulating hormone, GV = germinal vesicle stage, GVBD = germinal vesicle breakdown, LH = luteinizing hormone, MAPK = mitogen-activated protein kinase, MI = the first meiotic metaphase, MII = the second meiotic metaphase, MPF = M-phase/maturation promoting factor, PG = progesterone, PI3K = phosphatidylinositol 3-kinase, PKA = cAMP-dependent protein kinase, PKA = protein kinase A, PKC = Ca2+-dependent protein kinase, PKG = cGMP-dependent protein kinase,PLK1 = polo-like kinase 1, UPS = ubiqiutin-proteoasome system.

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