Melatonin Receptor 2 Promotes Lipid Metabolism of Cumulus-oocyte Complexes via the cAMP/PKA Pathway

Jun-Xue Jin (junxue-jin@hotmail.com)
Northeast Agricultural University https://orcid.org/0000-0003-0932-2105
Hong-Di Cui
Northeast Agricultural University
Chao-Qian Jiang
Northeast Agricultural University
Zi-Cheng Qi
Northeast Agricultural University
Ya Bian
Northeast Agricultural University
Jia-Hui Liu
Northeast Agricultural University
Xi-Qing Jiang
Northeast Agricultural University
Jing-Tao Sun
Northeast Agricultural University
Zhong-Hua Liu
Northeast Agricultural University

Research

Keywords: Melatonin, MT2 receptor, Protein kinase A, Lipolysis, Cumulus cells, Oocytes

Posted Date: June 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-547303/v1

Abstract

Background: The importance of the processes of lipogenesis and lipolysis in providing an essential energy source during oocyte maturation is increasingly being recognized. Recent our studies have demonstrated that melatonin up-regulated lipid metabolism during oocyte maturation. Nevertheless, there is still limited information regarding the underlying molecular mechanisms of action of melatonin on lipid metabolism in porcine cumulus-oocyte complexes (COCs). Here, our aim was to investigate the effect of melatonin on COCs, and the melatonin receptor-mediated lipid metabolism signaling pathway.

Materials/methods: To determine the melatonin-mediated lipolysis pathway in cumulus cells, COCs were treated with melatonin and the correlated metabolic responses were assessed using melatonin receptor-mediated signaling.

Results: The results showed that exposure of COCs to melatonin during *in vitro* maturation significantly increased cumulus expansion index, blastocyst formation rate and total cell numbers/blastocyst, although nuclear maturation was no significant difference. The levels of proteins MT1, MT2, $G_s\alpha$, PKA, and lipolysis-related factors (AGTL, HSL, PLIN A+B) were significantly increased by melatonin supplementation, and this effect was inhibited by simultaneous treatment with melatonin antagonists (luzindole or 4P-PDOT), although 4P-PDOT treatment did not completely block the effect of melatonin on MT1. Further, the gene expression patterns reflected their relevant protein levels in cumulus cells. Melatonin-mediated lipolysis could significantly reduce lipid droplets (LDs) numbers and increase fatty acid (FA) production and ATP levels by increasing the β-oxidation-related gene expression in cumulus cells. Simultaneously, melatonin significantly increased the amount of LDs, FAs, ATP, and enhanced the lipid metabolism-related gene expression in oocytes. Finally, the oocyte quality was improved by increasing GDF9, BMP15 and GSH and decreasing ROS levels.

Conclusion: These findings revealed that the MT2-mediated cAMP/PKA signaling pathway promotes intracellular lipolysis and FA production in cumulus cells, which provided an essential energy source for COCs development.

1. Background

Oocyte development is particularly sensitive to the alterations in nutritional and chemical environments; Well-balanced and timed metabolism is essential for making a high-quality oocyte [1, 2]. Emerging evidence indicates that impaired oocyte quality represents a critical mechanism mediating the inter/transgenerational phenotypes induced by maternal metabolic syndrome, such as obesity, diabetes, and polycystic ovary syndrome (PCOS) [3]. In particular, women with obesity or poorly controlled diabetes have an increased risk of infertility, miscarriage, obstetric complications, neonatal morbidity and mortality, and birth defects in their offspring [4]. In fact, compromised oocyte quality including meiotic abnormalities, mitochondrial dysfunction, redox imbalance and oxidative stress has been widely reported

in obese mice [5–8]. Therefore, metabolic balance in oocytes may contribute to the improved reproductive potential experienced by women with metabolic disorders.

Oocyte maturation is an extremely complicated process, controlled by numerous molecular factors, and accompanied by the morphological transformation, redistribution and migration of organelles. Lipid droplets (LDs) are crucial organelles, providing indispensable energy substrates for oocyte development, especially in species with lipid-rich oocytes [9, 10]. During oocyte maturation, transportation of fatty acids (FAs) from the cumulus cells into the oocyte is mediated by transzonal projections and fatty acid binding protein 3 (FABP3) or/and CD36 [11, 12]. The oocyte then *de novo* synthesizes LDs from the available FAs through the physical process of liquid-liquid phase separation and the accumulation of lipids in the membrane of the endoplasmic reticulum [13, 14]. Consequently, a major role of cumulus cells is the coordination of complex communications with the oocyte, to support its development [15]. Simultaneously, the activation of lipases dramatically reduces the size of LDs through triglyceride (TG) hydrolysis, and the released FAs are transported into mitochondria where they undergo β-oxidation and produce ATP [9, 16]. Therefore, dynamic change in the LDs, mediated by the processes of lipolysis and lipogenesis, is critical for their potential ability to support and promote oocyte development.

Melatonin (N-acetyl-5-methoxytryptamine) is a natural hormone mainly produced by the pineal gland [17]. It is also released from reproductive tissues, including the ovary, uterus, and testis [18–21]. Melatonin is participated in physiological processes including endocrine regulation, disease resistance and cellular metabolism [9, 22–25]. The functions of melatonin are also considered essential to oocyte and embryo development [9]. Interestingly, we have previously demonstrated that melatonin promoted lipogenesis and lipolysis in porcine oocytes, thereby providing a indispensable energy source to support and improve oocyte development [9]. Additionally, He *et al.* also indicated that melatonin facilitated LD accumulation (as well as TG content) in porcine oocytes [25]. Nevertheless, several aspects of the underlying mechanism by which melatonin-mediated lipid metabolism in porcine cumulus-oocyte complexes (COCs) remain to be elucidated, such as: (i) melatonin receptor-mediated intracellular signal transduction in cumulus cells; (ii) the relationship between the signaling pathway and FA production; and (iii) the origin and development of FAs in oocytes. Therefore, the aim of this study was to investigate the relationship between the effects of melatonin and lipid metabolism in porcine COCs during *in vitro* maturation (IVM).

2. Methods

2.1 Chemicals and ethics

All drugs and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. Porcine ovaries were obtained at a local slaughterhouse for all experiments, and no experiments were performed on live animals.

2.2 Porcine oocyte *in vitro* maturation

Porcine ovaries were transported to laboratory from a local slaughterhouse at 30–37°C, and using a syringe aspirated 3–6 mm antral follicles. The COCs together with numerous layers of cumulus cells were collected and washed with tissue culture medium-199 (TCM-199; Invitrogen) containing 0.3% polyvinyl alcohol (PVA), 10 mM HEPES and 1% Pen-Strep (Invitrogen), and transferred into culture dish with IVM medium (TCM-199 contained 10 IU/mL luteinizing hormone, 10 IU/mL follicle stimulating hormone, 10% porcine follicular fluid, and 0.91 mM sodium pyruvate). The pooled COCs were cultured under an incubator with 5% CO_2 at 100% humidity and 38.5°C for 42 h (with or without 10^{-9} M melatonin/10^{-9} M antagonist).

2.3 Assessment of cumulus cells expansion degrees

The cumulus expansion index (CEI) was distinguished to five grades after 42 h IVM. Briefly, Briefly, a CEI grade of 0 indicated no expansion, characterized by detachment of cumulus cells from the oocyte to assume a flattened monolayer of fibroblastic appearance, leaving a partially or fully denuded oocyte. A CEI grade of 1 indicated no expansion, but the cumulus cells were spherical and remained compacted around the oocyte. For CEI grade 2 complexes, only the outermost layers of cumulus cells had expanded, while CEI grade 3 complexes had all cell layers except the corona radiata (cells most proximal to the oocyte) prominently expanded, and a CEI grade of 4 indicated the maximum degree of expansion, including the corona radiata.

2.4 Nuclear maturation assessment

Using a pipette removed cumulus cells from oocyte with 0.1% hyaluronidase. Nuclear maturation was classified into immature, metaphase II (MII) or others (degenerated) under a microscope.

2.5 Parthenogenetic activation (PA) and *in vitro* culture (IVC)

The denuded porcine MII oocytes were suffered an electrical activation with a single direct current pulse of 1.5 kV/cm for 60 μs. After that, the activated oocytes were placed into culture dish containing 500 μL porcine zygote medium 3 (PZM3) per well and incubated at 38.5°C under 100% humidity and an atmosphere of atmosphere of 5% CO_2 in air for 7 days. The rate of cleavage and blastocyst calculated on Day 2 and Day 7.

2.6 LDs, FAs and ATP staining

Using 4% paraformaldehyde (PFA) fixed COCs or denuded oocytes for 4 h. After that, COCs or oocytes were transferred into phosphate-buffered saline (PBS) supplemented with 10 μg/mL BODIPY-LD (BODIPY 493/503; D3922; Molecular Proves, Eugene, OR), 6 μM BODIPY-FA (BODIPY 558/568 C12; D3835; Molecular Probes, Eugene, OR) and 500 nM BODIPY-ATP (BODIPY FL ATP; A12410; Molecular Probes, Eugene, OR) for 1 h, respectively. Then, COCs or oocytes were washed and mounted on cover slips. The fluorescence intensities were measured by Image J software (version 1.46r; National institutes of Health, USA).

2.7 Reactive Oxygen Species (ROS) and Glutathione (GSH) staining in oocytes

Using H2DCFDA (Invitrogen) and CellTracker Blue CMF2HC (Invitrogen) indicated green fluorescence (ROS) and blue fluorescence (GSH) in porcine oocytes. Each group was treated for 30 min in 10 μM H2DCFDA and 10 μM CellTracker Blue. Then oocytes were moved to 4 μL droplets of PBS, and intensity of fluorescence was measured by Image J software. The excitation and emission wavelengths were 371/464 nm for CMF2HC, and 492 ~ 495/517 ~ 527 nm for H2DCFDA, respectively.

2.8 Immunofluorescence staining

Using 4% PFA fixed porcine COCs or oocytes for 4 h, after then the samples placed into 1% Triton X-100 for 30 min. Under 2% bovine serum albumin (BSA)-PBS blocked non-specific sites, and incubated with primary antibody overnight at 4°C. After three times washes of 5 min each in the 2% BSA-PBS solution, COCs/oocytes were placed into goat-anti-rabbit secondary antibody for 1 h. The information about antibodies is presented in Table S1.

2.9 Western blots

Cumulus cells were collected and washed several times with 1% PVA-PBS from each group, lysed in 60 μL of lysis buffer (20 mM glycerol phosphate, 1 mM EDTA, 150 mM NaCl, 10% glycerol, 20 mM HEPES ,1% Triton X-100 and 2 mM EGTA) containing 0.6 μl PMSF (100 mM, Beyotime) for 3 h. Then, the sampling tube contained samples boiled in the water for 5 min at 100°C. Using 12% ExpressPlus™ PAGE Gels (GenScript) distinguished proteins, and placed into nitrocellulose (NC) membranes (Millipore). After that, using TBST with 5% BSA blocked NC membranes, and incubated with primary antibody overnight at 4°C. The NC membranes were washed three times in TBST and incubated with horseradish peroxidase-conjugated secondary antibodies. The information about antibodies is presented in Table S1.

2.10 Detection of gene expression by real-time PCR

Total RNA was extracted by mRNA Purification Kit (Invitrogen), and the cDNA was generated using cDNA Synthesis Kit (Takara). Real-time PCR carried out with SYBR Green using a StepOne™ Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). Experiments were performed at least in triplicate. Pre-mRNA processing factor3 (RP18) was used as an internal control.

2.11 Statistical analysis

The data were described as mean values ± standard error of the mean (SEM) with univariate analysis of variance (ANOVA) followed by Tukey's test using SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant. Each experiment was repeated at least 3 times.

3. Results

3.1 Effects of melatonin on cumulus cell, oocyte and embryo development.

The effects of melatonin on cumulus expansion were evaluated using a total of 3612 COCs in sixteen replicates. As shown in Fig. 1A-F, melatonin significantly increased the proportion of COCs exhibiting complete cumulus expansion (CEI grade 4) with a significant reduction in the proportion of COCs with CEI grade 3 compared to other groups (control, luzindole and 4P-PDOT, $P < 0.05$). However, luzindole and 4P-PDOT inhibited the increased cumulus expansion resulting from melatonin treatment.

The nuclear maturation was evaluated using a total of 3815 COCs in seventeen replicates after IVM. We found that there were no significant differences on nuclear maturation between the groups (Fig. 1G). We then assessed the accepted indicators for oocyte development, and found that melatonin treatment significantly enhanced GDF9 and BMP15 expression as well as intracellular GSH in oocytes than control group ($P < 0.05$), while significantly reducing ROS levels ($P < 0.05$, Figure S2A-G).

A total of 2308 oocytes in twelve replicates were used to estimate the effects of melatonin on embryo development after PA (Fig. 1H). Melatonin significantly enhanced the PA-derived blastocyst formation rate compared to others ($P < 0.05$). According to these results, we chose control, melatonin, melatonin with luzindole (MTn + Lu), and melatonin with 4P-PDOT (MTn + 4P) for the following experiments.

3.2 Melatonin-mediated PKA signaling pathway in cumulus cells

The expression of melatonin receptors (MT1 and MT2), G-protein stimulatory subunit alpha ($G_s\alpha$) and PKA mRNA and protein were detected in cumulus cells by real-time PCR, immunofluorescence (IF) staining or western blot (WB). All these proteins were significantly enhanced in cumulus cells following melatonin supplementation compared to the control ($P < 0.05$), and these effects were inhibited by melatonin antagonists (luzindole and 4P-PDOT, Fig. 2). Further, the mRNA expression and WB results were similar to the IF staining results (Figure S1). Interestingly, since 4P-PDOT is a selective MT2 antagonist (> 300 fold selectivity for the MT2 vs. the MT1 subtype), our results showed that the mRNA and protein expression of MT1 were more completely repressed in the MTn + Lu group than the MTn + 4P group.

3.3 Expression of lipolysis- and β-oxidation-related factors in cumulus cells

To determine the activities of lipases, the lipolysis-related proteins adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL) and perilipin (PLIN A + B) were detected in cumulus cells by IF staining. The protein expression of ATGL, HSL and PLIN A + B were significantly enhanced in the melatonin group compared to the control ($P < 0.05$), and this melatonin-induced increase was inhibited by its antagonists (Fig. 3). As shown in Figure S1A, the lipolysis-related protein expression results obtained by IF staining were supported by our WB results. Moreover, our results showed that the mRNA expression of *ATGL* and

comparative gene identification 58 (*CGI58*), were higher in the melatonin group than others. While *HSL* was also increased in melatonin group, there was no significant difference in *PLIN2*, monoacylglycerol lipase (*MGL*) and lipoprotein lipase (*LPL*) between the groups (Figure S1B).

We carried out additional mRNA detection in the cumulus cells and found that the β-oxidation-related genes (*ACADS*, *CPT1B* and *CPT2*) and mitochondrial biogenesis genes (*TFAM, PGC1α*and *PRDX2*) were more highly expressed following melatonin treatment (Figure S1B).

3.4 Detection of LDs, FAs and ATP in cumulus cells

To confirm the process of lipolysis and β-oxidation, we examined the contents of LDs, FAs and ATP in cumulus cells. The number of LDs was significantly reduced by melatonin supplementation than others ($P < 0.05$; Fig. 4), which implied that the utilization of LDs was catalyzed by the cumulus cell lipases. Additionally, the levels of FAs and ATP increased in melatonin supplementation than control group ($P < 0.05$), but this was inhibited by melatonin antagonists.

3.5 Regulation of lipid metabolism in oocytes

In our previous studies [9], we only determined the lipid metabolism-related mRNA expression in oocytes. To test our hypothesis and expand upon our previous results, we performed IF staining in oocytes to measure PPARγ, SREBP1, ATGL, HSL, PLIN A + B and PGC1α protein expression. All measured indicators were significantly enhanced by melatonin supplementation ($P < 0.05$), with these increases being inhibited by melatonin antagonists (Figs. 5 and 6). The lipases were detected in the ooplasm, and were localized to the surface of the LDs (which are seen as black dots in Fig. 6). Moreover, the contents of LDs, FAs and ATP were also significantly enhanced in oocytes by melatonin treatment, with this increase blocked by melatonin antagonists (Fig. 7). Remarkably, while the mRNA expression of the fatty acid transportation protein *CD36* was higher following melatonin treatment compared to the control, this was not the case for *FABP3* and *FABP5* (Figure S2H).

4. Discussion

It is well known that G protein-coupled receptors (GPCRs) are the most functional cell surface proteins and mediate a series of physiological processes [26]. The melatonin receptors, MT1 and MT2, are GPCRs that receive external melatonin signals and are also involved in a vast array of physiological processes [27–29]. Nevertheless, the beneficial effects of melatonin on the reproductive system have been explained simply as being due to indirect, antioxidant effects, and so little information is available on the underlying mechanism by which melatonin concretely acts on porcine COC development. Therefore, a better understanding of the melatonin receptor-mediated intracellular signaling pathway in cumulus cells is required.

Oocyte maturation is important step for the ovum to reach competence for successful insemination and early embryonic development, and is mainly regulated by bilateral interactions of cumulus cells and oocyte [11, 30]. The oocytes without cumulus cells exert negatively influences to oocyte nuclear

maturation, cytoplasmic maturation and early embryonic development after fertilization [31]. Hence, cumulus cells are critical in supporting oocyte maturation during IVM. In the previous study, we have observed the melatonin receptors (*MT1* and *MT2*) expression in porcine cumulus cells [32]. In the present study, we found that protein of MT1 and MT2 could be detected in cumulus cells by IF and WB, and that their expressions were significantly enhanced by melatonin supplementation. Additionally, the melatonin-mediated increase in MT2 expression was blocked by luzindole or 4P-PDOT, while melatonin-mediated MT1 expression was completely eliminated by luzindole, but only partially by 4P-PDOT. Indeed, 4P-PDOT plays its own specific role in COC development, so these results supported our subsequent experiments to research the function of MT1 and/or MT2 in cumulus cells.

Adenylate cyclase (AC) is primarily activated downstream of GPCRs by interaction with the α subunit of the G_s protein [33]. The second messenger (cAMP) was generated by AC activation, it could regulate several effectors, the most well studied of which is cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) [34]. The enzyme of PKA is participated numerous pathological and physiological actions in mammals and control homeostasis in eukaryotes [35, 36]. It has been indicated that melatonin receptors (MT1 and/or MT2) could regulate intracellular processes *via* AC, which accumulates cAMP concentration, stimulating PKA activity in human granulosa-lutein cells [37]. In addition, HSL is translocated from the ooplasm to the LD surface following PKA stimulation, where it interacts with the PLIN family in stimulated lipolysis [38, 39]. After activation of PKA, ATGL also interacts with PLIN, despite this most probably takes place indirectly through the co-factors CGI58 in adipocytes [40]. The lipolysis of LDs in COCs is also catalyzed by lipolytic enzyme including HSL and ATGL [41]. Moreover, HSL also catalyzes the lipolysis of diglycerides to monoglycerides, suggesting that it has a stronger lipolytic function than ATGL [42–44]. In the current study, melatonin significantly increased G_sα expression, which up-regulated the cAMP/PKA pathway, and then activated the lipolytic processes with promoting lipases (ATGL and HSL) and their co-factors (PLIN and CGI58) in cumulus cells. Consequently, melatonin significantly decreased the number of intracellular LDs, and increased FA and ATP levels, and enhanced genes transcripts related to β-oxidation in cumulus cells. Nevertheless, these melatonin-mediated effects were completely blocked by luzindole or 4P-PDOT. Based on these results, it appeared that the melatonin-mediated G_sα-PKA signaling pathway could facilitate the lipolytic processes *via* MT2, producing metabolic substrates as essential energy for cumulus cell development during IVM. In our previous study, we elucidated that the cumulus cells expansion and embryonic development were increased by melatonin treatment *via* the sonic hedgehog signaling activation [32]. It is extremely likely that the sonic hedgehog signaling was regulated by MT2, because the activation of PKA leads to phosphorylation and stimulation of Smoothened (Smo) [45]. He *et al.* have demonstrated that the function of porcine granulosa cells was modulated by MT2 receptor [46], and it is suggested that MT2 is likely to mediate porcine cumulus cell expansion by melatonin effects.

As reported previously, a porcine oocyte contains 161 ± 18 ng of endogenous lipid content, the majority of which is in the TG form, much more than for other species [13, 47]. Therefore, porcine oocytes are widely recognized as an excellent model to study the role of lipid metabolism in mammalian female germ cells.

Generally, the intracellular lipids in the oocyte are mainly stored in LDs and released FAs by activation of lipolysis, which provide energy for oocyte maturation and development [9, 13, 48, 49]. Additionally, it is recognized that FAs have powerful anti-inflammatory property [50] such as specialized proresolving lipid mediators (resolvins and protectins) which combined with specific G-protein-coupled receptors and reduced hydrogen peroxide-induced DNA damage and oxidative stress [51–53]. We have demonstrated previously that melatonin enhanced lipogenesis- and lipolysis-related mRNA expression in the porcine oocyte [9]. Continuing from these results, we detected several lipogenetic and lipolytic proteins in oocytes, the levels of which were significantly increased by melatonin treatment, leading to increased FA and ATP generation. Thus, melatonin-mediated lipid metabolism dramatically improved oocyte quality and blastocyst formation rate by decreasing ROS and increasing GSH levels, though not nuclear maturation. Further, mRNA expression of *CD36*, but not *FABP3* and *FABP5*, was significantly increased by melatonin treatment. Thus, the FA transportation-mediated lipid accumulation was mediated by the CD36 and lipogenesis process; and that this was the energy source generated to support oocyte and embryo development. Moreover, the removal of cumulus cells during IVM induced an imbalance in lipid metabolism, affecting the developmental competence of the oocyte [54, 55]. Therefore, these results indicated that the cumulus cells not only protected the developing oocyte but also maintained lipid homeostasis by supporting intracellular lipogenetic and lipolytic processes in the oocyte.

5. Conclusion

In summary, for the first time, we evidenced that the melatonin-mediated cAMP/PKA signaling pathway promotes intracellular lipolysis and FA production *via* MT2 in cumulus cells, reducing oxidative stress and providing an essential energy source for COC development (Fig. 8). These findings advised that melatonin receptor 2 could be potential therapeutic target for female reproduction.

Abbreviations

MT1, melatonin receptor 1; MT2, melatonin receptor 2; G_s, G-protein stimulatory subunit alpha; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; MGL, monoacylglycerol lipase; PLIN, perilipin; CGI58, comparative gene identification 58; CD36, fatty acid transport protein; ACACA, acetyl-CoA carboxylase alpha; FASN, fatty acid synthase; PPARγ, peroxisome proliferator activated receptor gamma; SREBP1, sterol regulatory element binding protein 1; CPT1a, carnitine palmitoyltransferase 1A; CPT1b, carnitine palmitoyltransferase 1B, CPT2, carnitine palmitoyltransferase 2.

Declarations

ACKNOWLEDGEMENTS

Not applicable

AUTHORS' CONTRIBUTIONS

JXJ conceived the study, designed, and conducted the experiment. HDC, CQJ, ZCQ and YB mainly provided practical assistance with the experiment. JHL, XQJ and JTS assisted with the project and revised the manuscript. ZHL and JXJ supervised the research and supplied the funding. All authors read, revised, and approved the final manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (grant numbers 32002179 and 31872360), the National Key Research and Development Program of China-Stem cell and Translational Research (grant numbers 2016YFA0100200), the Excellent Youth Project of Heilongjiang Province Natural Science Foundation of China (grant numbers YQ2020C007), and the Heilongjiang Human Resources and Social Security Bureau Project (grant numbers [2019]277).

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Porcine ovaries were obtained at a local slaughterhouse for all experiments, and no experiments were performed on live animals. Thus, it is not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that there is no conflict of interest associated with this manuscript.

AUTHOR DETAILS

Key Laboratory of Animal Cellular and Genetics Engineering of Heilongjiang Province, College of Life Science, Northeast Agricultural University, Harbin, 150030, China.

References

1. Li L, Zhu S, Shu W, Guo Y, Guan Y, Zeng J, et al. Characterization of Metabolic Patterns in Mouse Oocytes during Meiotic Maturation. Molecular cell. 2020;80(3):525-40 e9.
2. Han L, Chao R, Ling L, Li X, Ge J, Wang H, et al. Embryonic defects induced by maternal obesity in mice derive from Stella insufficiency in oocytes. Nature Genetics. 2018;50(3).
3. Risal S, Pei Y, Lu H, Manti M, Stener-Victorin E. Prenatal androgen exposure and transgenerational susceptibility to polycystic ovary syndrome. Nature Medicine. 2019;25(11).
4. Gu L, Liu H, Gu X, Boots C, Moley KH, Wang Q. Metabolic control of oocyte development: linking maternal nutrition and reproductive outcomes. Cellular and molecular life sciences : CMLS. 2015;72(2):251-71.
5. Wu LL, Russell DL, Wong SL, Chen M, Tsai TS, St John JC, et al. Mitochondrial dysfunction in oocytes of obese mothers: transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. Development. 2015;142(4):681-91.
6. Jungheim ES, Schoeller EL, Marquard KL, Louden ED, Schaffer JE, Moley KH. Diet-induced obesity model: abnormal oocytes and persistent growth abnormalities in the offspring. Endocrinology. 2010;151(8):4039-46.
7. Grindler NM, Moley KH. Maternal obesity, infertility and mitochondrial dysfunction: potential mechanisms emerging from mouse model systems. Mol Hum Reprod. 2013;19(8):486-94.
8. Wang H, Cheng Q, Li X, Hu F, Han L, Zhang H, et al. Loss of TIGAR Induces Oxidative Stress and Meiotic Defects in Oocytes from Obese Mice. Molecular & cellular proteomics : MCP. 2018;17(7):1354-64.
9. Jin JX, Lee S, Taweechaipaisankul A, Kim GA, Lee BC. Melatonin regulates lipid metabolism in porcine oocytes. Journal of pineal research. 2017;62(2):e12388.
10. Martin S, Parton RG. Lipid droplets: a unified view of a dynamic organelle. Nature reviews Molecular cell biology. 2006;7(5):373-8.
11. Del Collado M, da Silveira JC, Sangalli JR, Andrade GM, Sousa L, Silva LA, et al. Fatty acid binding protein 3 and transzonal projections are involved in lipid accumulation during in vitro maturation of bovine oocytes. Scientific reports. 2017;7(1):2645.
12. Uzbekova S, Elis S, Teixeira-Gomes AP, Desmarchais A, Maillard V, Labas V. MALDI mass spectrometry imaging of lipids and gene expression reveals differences in fatty acid metabolism between follicular compartments in porcine ovaries. Biology. 2015;4(1):216-36.
13. Prates EG, Nunes JT, Pereira RM. A role of lipid metabolism during cumulus-oocyte complex maturation: impact of lipid modulators to improve embryo production. Mediators of inflammation. 2014;2014:692067.
14. Zoni V, Khaddaj R, Campomanes P, Thiam AR, Schneiter R, Vanni S. Pre-existing bilayer stresses modulate triglyceride accumulation in the ER versus lipid droplets. eLife. 2021;10.

15. Diaz FJ, Wigglesworth K, Eppig JJ. Oocytes determine cumulus cell lineage in mouse ovarian follicles. Journal of cell science. 2007;120(Pt 8):1330-40.
16. Dunning KR, Cashman K, Russell DL, Thompson JG, Norman RJ, Robker RL. Beta-oxidation is essential for mouse oocyte developmental competence and early embryo development. Biology of reproduction. 2010;83(6):909-18.
17. Lopez-Gonzalez MA, Guerrero JM, Rojas F, Delgado F. Ototoxicity caused by cisplatin is ameliorated by melatonin and other antioxidants. Journal of pineal research. 2000;28(2):73-80.
18. Tan DX, Reiter RJ. Mitochondria: the birth place, battle ground and the site of melatonin metabolism in cells. Melatonin Research. 2019;2(1):44-66.
19. Frungieri MB, Calandra RS, Rossi SP. Local actions of melatonin in somatic cells of the testis. International journal of molecular sciences. 2017;18(6).
20. Reiter RJ, Tamura H, Tan DX, Xu XY. Melatonin and the circadian system: contributions to successful female reproduction. Fertility and sterility. 2014;102(2):321-8.
21. Stehle JH, Saade A, Rawashdeh O, Ackermann K, Jilg A, Sebesteny T, et al. A survey of molecular details in the human pineal gland in the light of phylogeny, structure, function and chronobiological diseases. Journal of pineal research. 2011;51(1):17-43.
22. He C, Ma T, Shi J, Zhang Z, Wang J, Zhu K, et al. Melatonin and its receptor MT1 are involved in the downstream reaction to luteinizing hormone and participate in the regulation of luteinization in different species. Journal of pineal research. 2016;61(3):279-90.
23. Markus RP, Buckeridge MS, Nakaya HI, Moraes CB, Fujita A, Muxel SM, et al. Melatonin-Index as a biomarker for predicting the distribution of presymptomatic and asymptomatic SARS-CoV-2 carriers. Melatonin Research. 2021;4(1):189-205.
24. Zhang R, Wang X, Ni L, Di X, Ma B, Niu S, et al. COVID-19: Melatonin as a potential adjuvant treatment. Life sciences. 2020;250:117583.
25. He B, Yin C, Gong Y, Liu J, Guo H, Zha R. Melatonin-induced increase of lipid droplets accumulation and in vitro maturation in porcine oocytes is mediated by mitochondrial quiescence. Journal of cellular physiology. 2017.
26. Yen HY, Hopper JTS, Liko I, Allison TM, Zhu Y, Wang D, et al. Ligand binding to a G protein-coupled receptor captured in a mass spectrometer. Science advances. 2017;3(6):e1701016.
27. Boutin JA, Witt-Enderby PA, Sotriffer C, Zlotos DP. Melatonin receptor ligands: A pharmaco-chemical perspective. Journal of pineal research. 2020;69(3):e12672.
28. Cecon E, Liu L, Jockers R. Melatonin receptor structures shed new light on melatonin research. Journal of pineal research. 2019;67(4):e12606.
29. Stauch B, Johansson LC, Cherezov V. Structural insights into melatonin receptors. FEBS Journal. 2019;287(8).
30. Sutton ML, Cetica PD, Beconi MT, Kind KL, Gilchrist RB, Thompson JG. Influence of oocyte-secreted factors and culture duration on the metabolic activity of bovine cumulus cell complexes.

Reproduction. 2003;126(1):27-34.
31. Wongsrikeao P, Kaneshige Y, Ooki R, Taniguchi M, Agung B, Nii M, et al. Effect of the removal of cumulus cells on the nuclear maturation, fertilization and development of porcine oocytes. Reproduction in domestic animals = Zuchthygiene. 2005;40(2):166-70.
32. Lee S, Jin JX, Taweechaipaisankul A, Kim GA, Ahn C, Lee BC. Melatonin influences the sonic hedgehog signaling pathway in porcine cumulus oocyte complexes. Journal of pineal research. 2017;63(3).
33. Sassone-Corsi P. The cyclic AMP pathway. Cold Spring Harbor perspectives in biology. 2012;4(12).
34. Pierce KL, Premont RT, Lefkowitz RJ. Seven-transmembrane receptors. Nature reviews Molecular cell biology. 2002;3(9):639-50.
35. Filteau M, Diss G, Torres-Quiroz F, Dube AK, Schraffl A, Bachmann VA, et al. Systematic identification of signal integration by protein kinase A. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(14):4501-6.
36. Taylor SS, Ilouz R, Zhang P, Kornev AP. Assembly of allosteric macromolecular switches: lessons from PKA. Nature reviews Molecular cell biology. 2012;13(10):646-58.
37. Cheng JC, Fang L, Li Y, Wang S, Li Y, Yan Y, et al. Melatonin stimulates aromatase expression and estradiol production in human granulosa-lutein cells: relevance for high serum estradiol levels in patients with ovarian hyperstimulation syndrome. Experimental & molecular medicine. 2020;52(8):1341-50.
38. Miyoshi H, Souza SC, Zhang HH, Strissel KJ, Christoffolete MA, Kovsan J, et al. Perilipin promotes hormone-sensitive lipase-mediated adipocyte lipolysis via phosphorylation-dependent and -independent mechanisms. The Journal of biological chemistry. 2006;281(23):15837-44.
39. Granneman JG, Moore HP, Granneman RL, Greenberg AS, Obin MS, Zhu Z. Analysis of lipolytic protein trafficking and interactions in adipocytes. The Journal of biological chemistry. 2007;282(8):5726-35.
40. Granneman JG, Moore HP. Location, location, location: protein trafficking and lipolysis in adipocytes. Trends in endocrinology and metabolism: TEM. 2008;19(1):3-9.
41. Dunning KR, Russell DL, Robker RL. Lipids and oocyte developmental competence: the role of fatty acids and beta-oxidation. Reproduction. 2014;148(1):R15-27.
42. Townsend KL, Tseng YH. Brown fat fuel utilization and thermogenesis. Trends in endocrinology and metabolism: TEM. 2014;25(4):168-77.
43. Thiele C, Spandl J. Cell biology of lipid droplets. Current opinion in cell biology. 2008;20(4):378-85.
44. Walther TC, Farese RV, Jr. The life of lipid droplets. Biochimica et biophysica acta. 2009;1791(6):459-66.
45. Chen Y, Jiang J. Decoding the phosphorylation code in Hedgehog signal transduction. Cell research. 2013;23(2):186-200.

46. He YM, Deng HH, Shi MH, Bodinga BM, Chen HL, Han ZS, et al. Melatonin modulates the functions of porcine granulosa cells via its membrane receptor MT2 in vitro. Animal reproduction science. 2016;172:164-72.
47. McEvoy TG, Coull GD, Broadbent PJ, Hutchinson JS, Speake BK. Fatty acid composition of lipids in immature cattle, pig and sheep oocytes with intact zona pellucida. Journal of reproduction and fertility. 2000;118(1):163-70.
48. Sturmey RG, O'Toole PJ, Leese HJ. Fluorescence resonance energy transfer analysis of mitochondrial:lipid association in the porcine oocyte. Reproduction. 2006;132(6):829-37.
49. Zhuan Q, Ma H, Chen J, Luo Y, Fu X. Cytoplasm lipids can be modulated through hormone-sensitive lipase and are related to mitochondrial function in porcine IVM oocytes. Reproduction, Fertility and Development. 2020;32.
50. Beaudoin-Chabot C, Wang L, Smarun AV, Vidovic D, Shchepinov MS, Thibault G. Deuterated Polyunsaturated Fatty Acids Reduce Oxidative Stress and Extend the Lifespan of C. elegans. Frontiers in physiology. 2019;10:641.
51. Spite M, Clària J, Serhan C. Resolvins, Specialized Proresolving Lipid Mediators, and Their Potential Roles in Metabolic Diseases. Cell metabolism. 2014;19(1):21-36.
52. Quiros M, Feier D, Birkl D, Agarwal R, Zhou DW, Parkos CA, et al. Resolvin E1 is a pro-repair molecule that promotes intestinal epithelial wound healing. Proceedings of the National Academy of Sciences. 2020;117(17):201921335.
53. Krashia P, Cordella A, Nobili A, La Barbera L, Federici M, Leuti A, et al. Blunting neuroinflammation with resolvin D1 prevents early pathology in a rat model of Parkinson's disease. Nature communications. 2019;10(1):3945.
54. Khan R, Jiang X, Hameed U, Shi Q. Role of Lipid Metabolism and Signaling in Mammalian Oocyte Maturation, Quality, and Acquisition of Competence. Frontiers in cell and developmental biology. 2021;9.
55. Auclair S, Uzbekov R, Elis S, Sanchez L, Kireev I, Lardic L, et al. Absence of cumulus cells during in vitro maturation affects lipid metabolism in bovine oocytes. American journal of physiology Endocrinology and metabolism. 2013;304(6):E599-613.

Figures

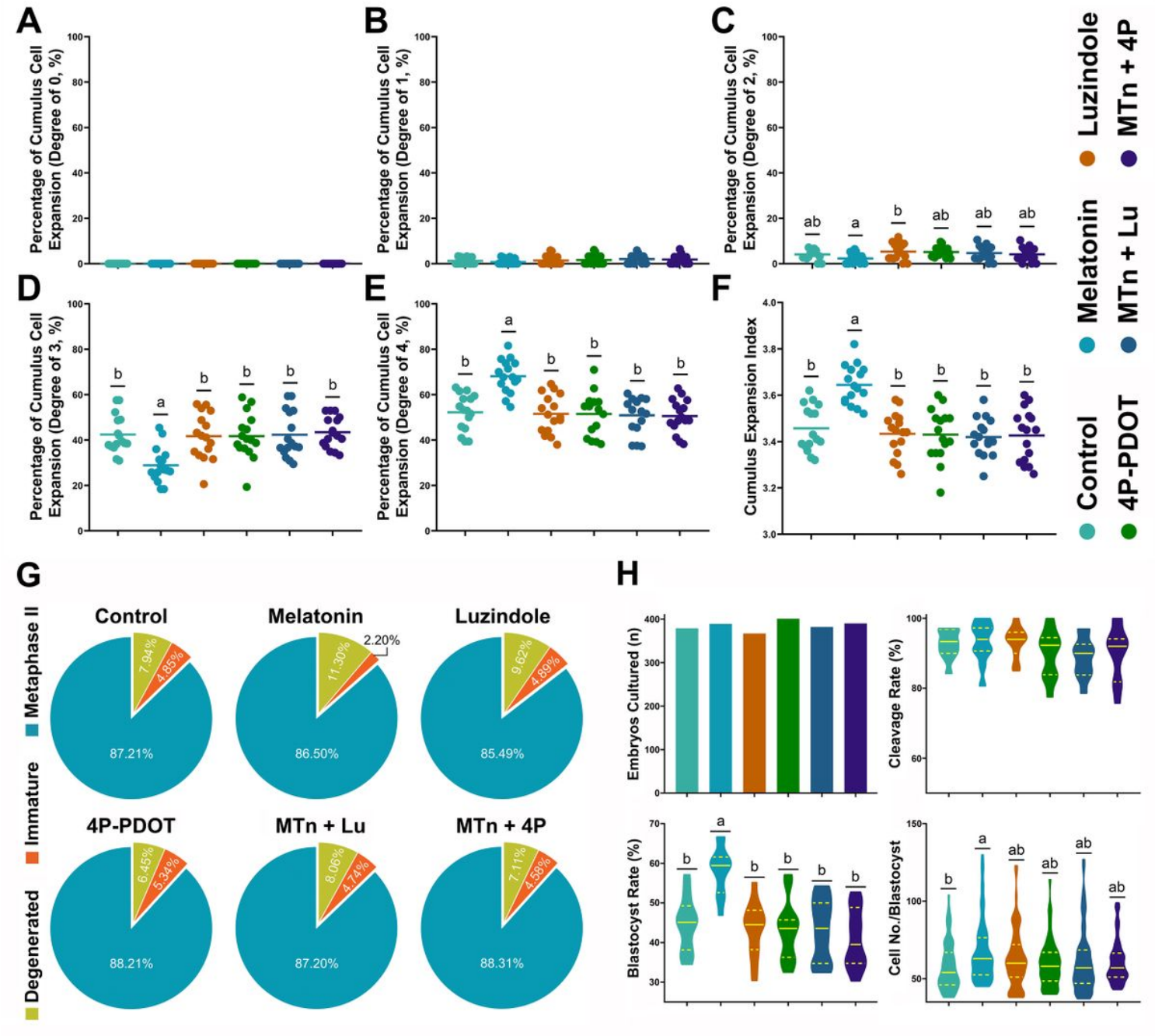

Figure 1

Effects of melatonin on cumulus cells expansion, nuclear maturation and embryo development after parthenogenetic activation. (A-F) Cumulus expansion index. (G) Oocyte nuclear maturation. (H) Embryo development. Melatonin/MTn, 10-9 mol/L melatonin; Luzindole/Lu, 10-9 mol/L Luzindole; 4P-PDOT/4P, 10-9 mol/L 4P-PDOT. Different letters denote significant difference ($P < 0.05$).

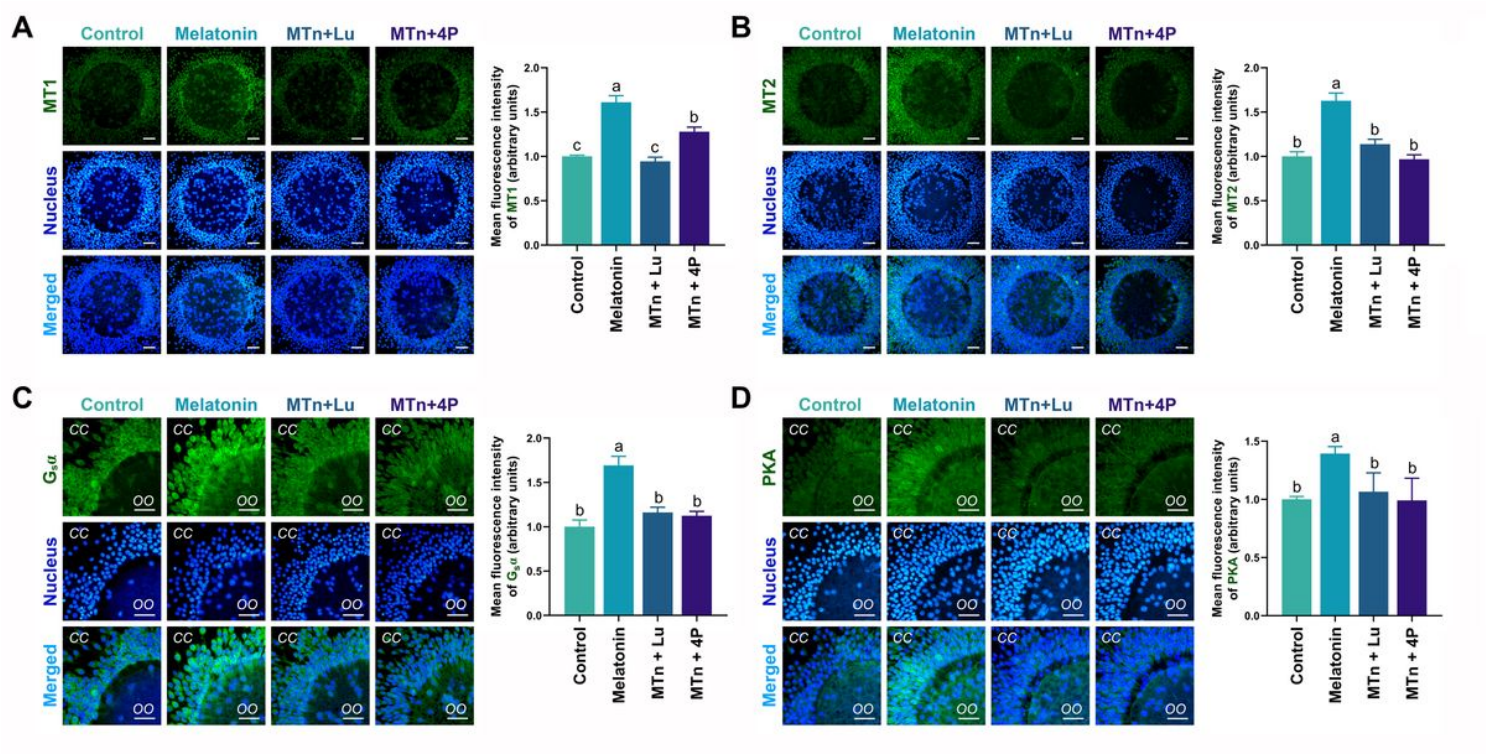

Figure 2

Immunofluorescence analysis of melatonin receptors (A and B), Gsα (C) and PKA (D) proteins in cumulus cells. Melatonin/MTn, 10-9 mol/L melatonin; Luzindole/Lu, 10-9 mol/L Luzindole; 4P-PDOT/4P, 10-9 mol/L 4P-PDOT. Scale bar = 30 μm. Different letters denote significant difference ($P < 0.05$).

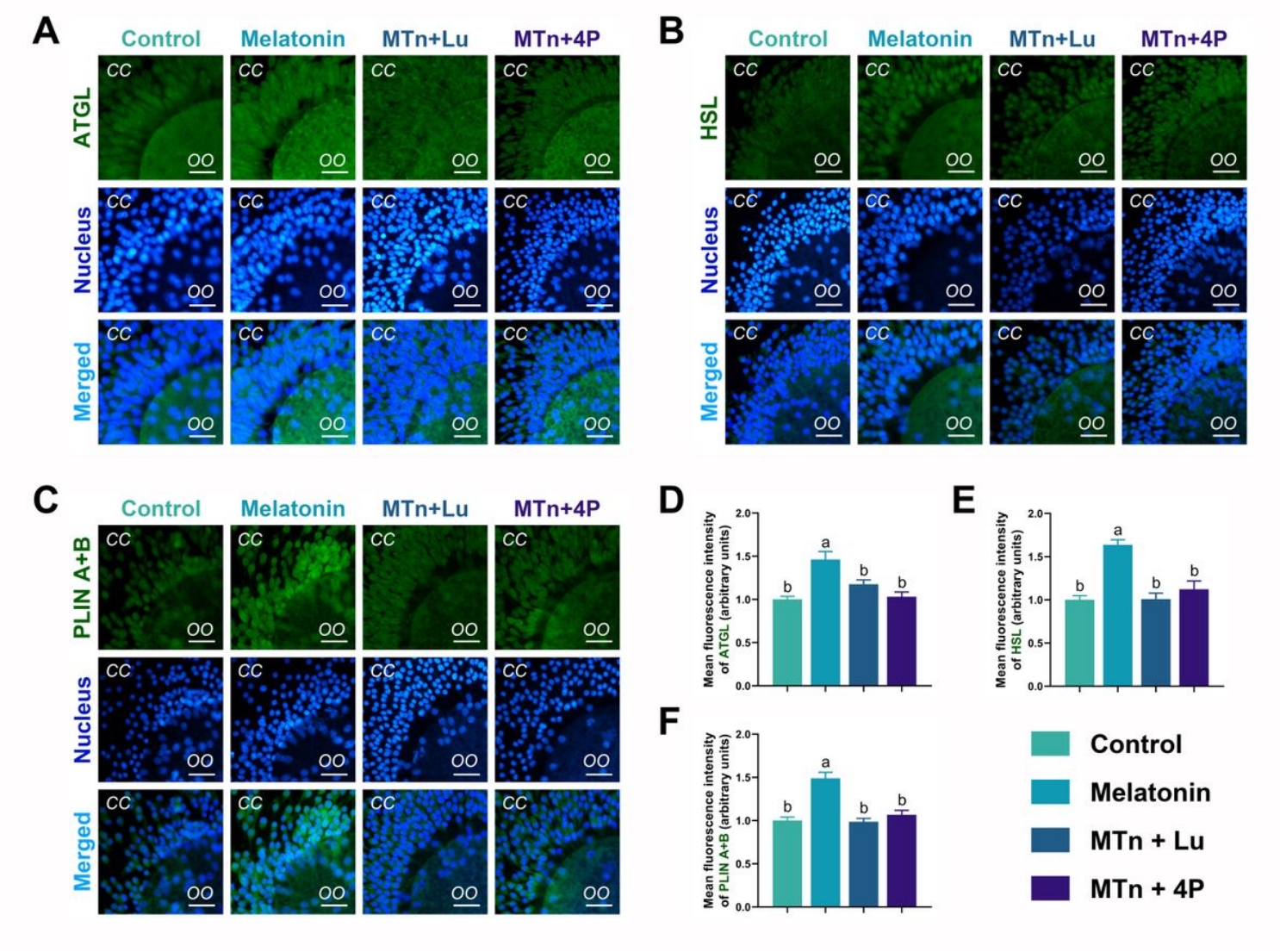

Figure 3

Immunofluorescence analysis of lipolysis proteins in cumulus cells. (A and D) ATGL, (B and E) HSL and (C and F) PLIN A+B. Melatonin/MTn, 10-9 mol/L melatonin; Luzindole/Lu, 10-9 mol/L Luzindole; 4P-PDOT/4P, 10-9 mol/L 4P-PDOT. Scale bar = 30 μm. Different letters denote significant difference ($P < 0.05$).

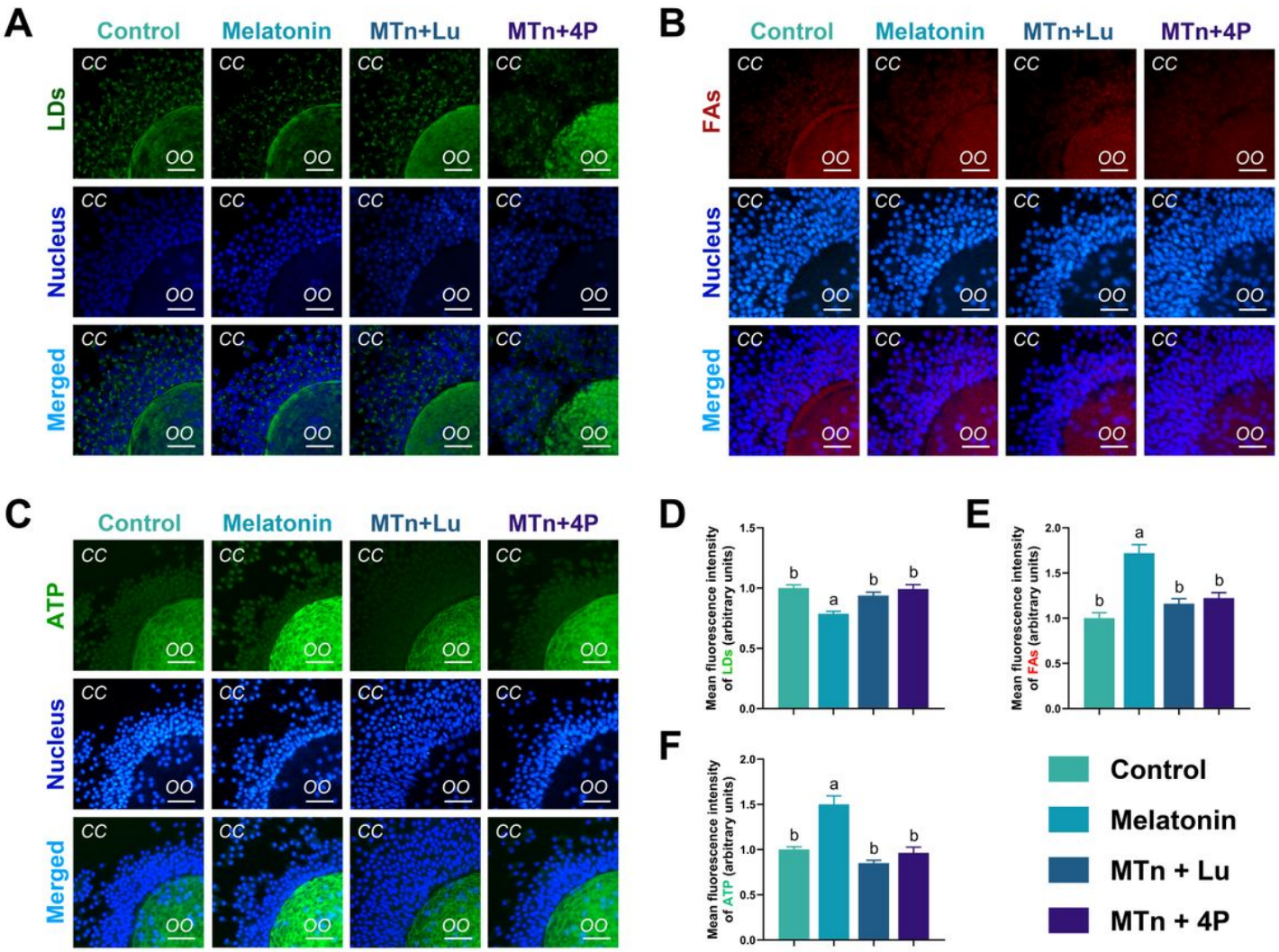

Figure 4

The contents of LDs, FAs and ATP in cumulus cells (A-F). Melatonin/MTn, 10-9 mol/L melatonin; Luzindole/Lu, 10-9 mol/L Luzindole; 4P-PDOT/4P, 10-9 mol/L 4P-PDOT. Scale bar = 30 μm. Different letters denote significant difference ($P < 0.05$).

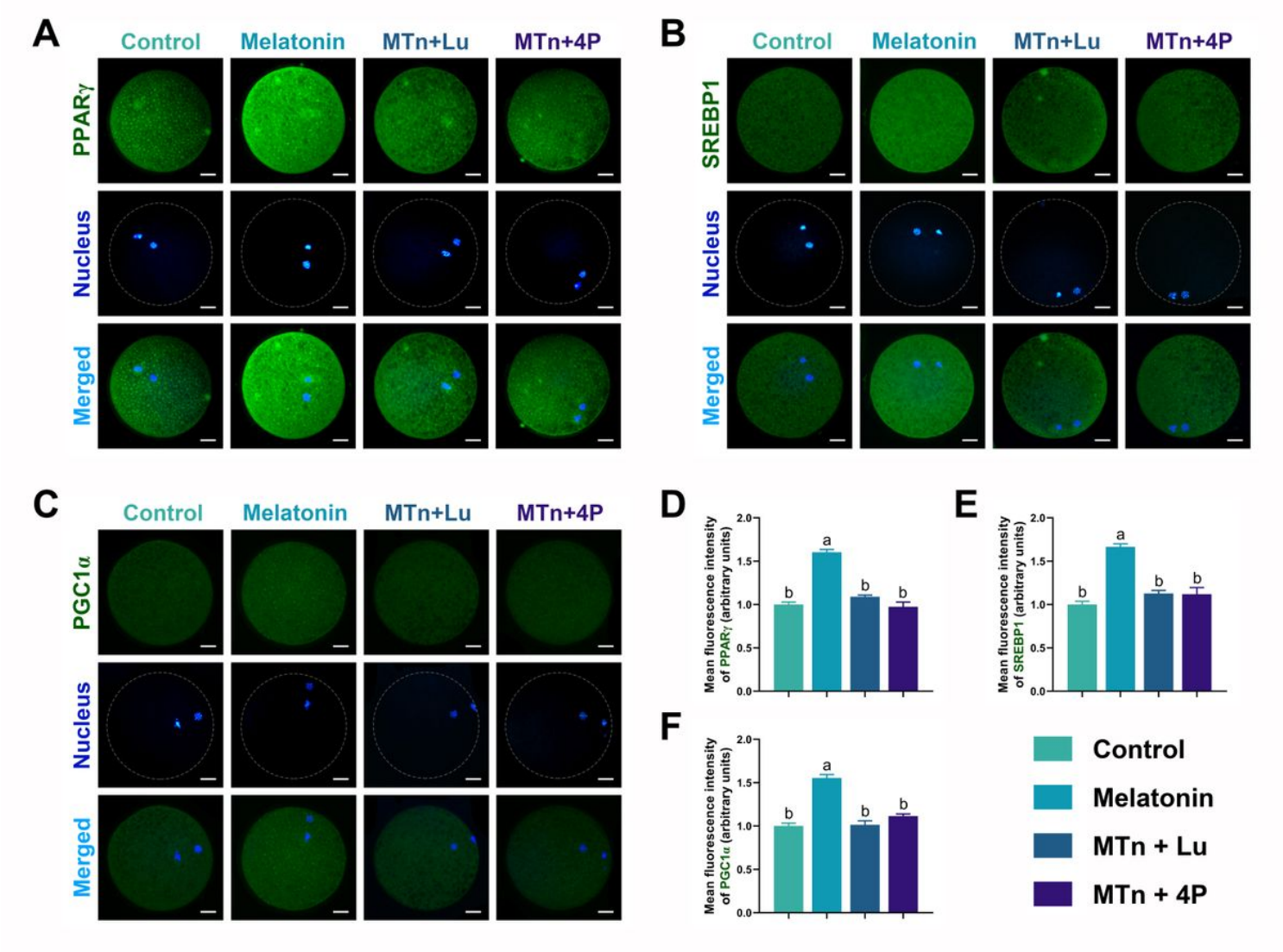

Figure 5

Lipogenesis and mitochondrial biogenesis-related protein expression in oocytes. (A and D) PPARγ, (B and E) SREBP1 and (C and F) PGC1α. Melatonin/MTn, 10-9 mol/L melatonin; Luzindole/Lu, 10-9 mol/L Luzindole; 4P-PDOT/4P, 10-9 mol/L 4P-PDOT. Scale bar = 25 μm. Different letters denote significant difference ($P < 0.05$).

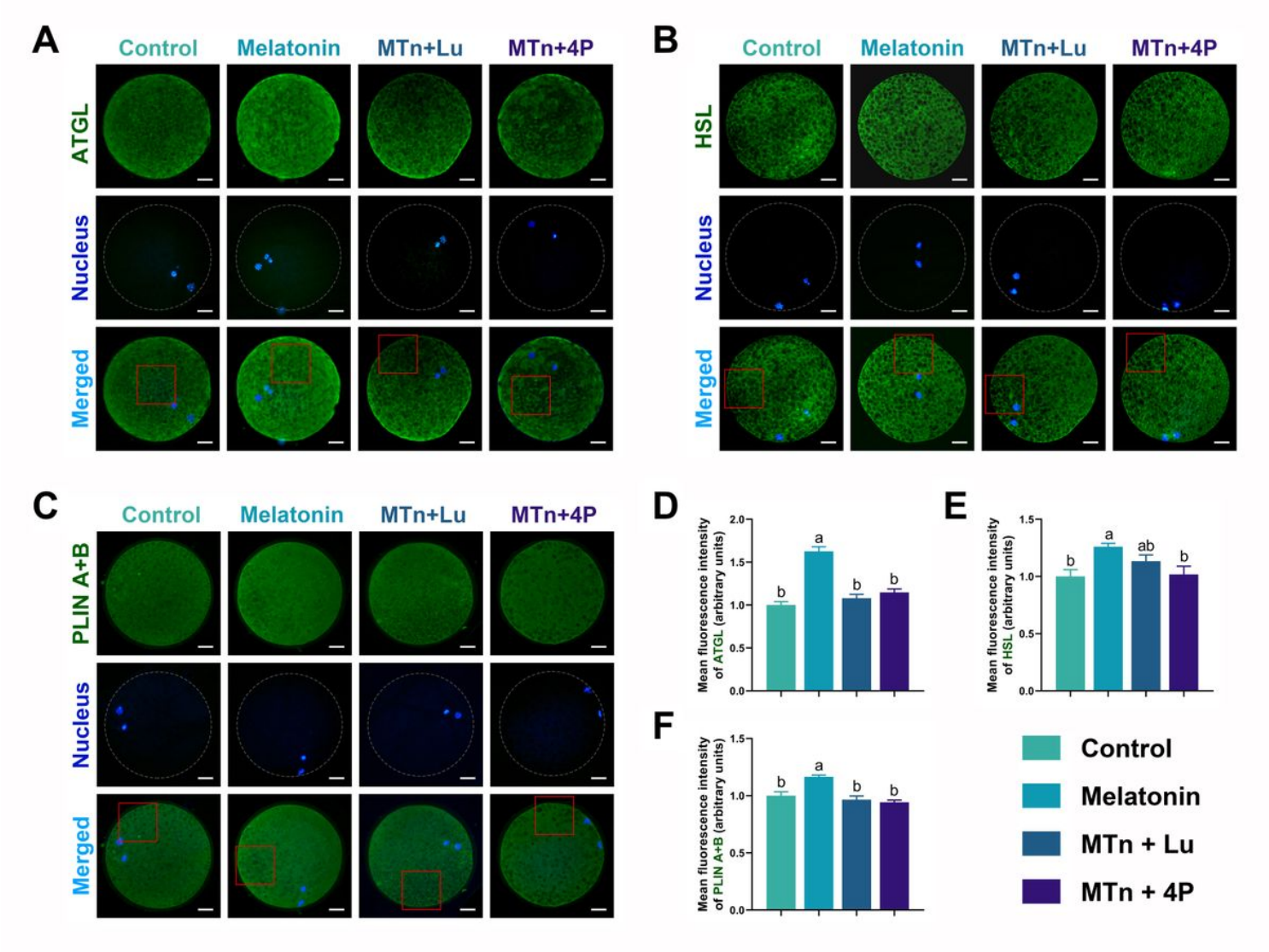

Figure 6

Expression of lipolysis-related proteins in the porcine oocyte. (A and D) ATGL, (B and E) HSL and (C and F) PLIN A+B. Melatonin/MTn, 10-9 mol/L melatonin; Luzindole/Lu, 10-9 mol/L Luzindole; 4P-PDOT/4P, 10-9 mol/L 4P-PDOT. Scale bar = 25 μm. Different letters denote significant difference ($P < 0.05$).

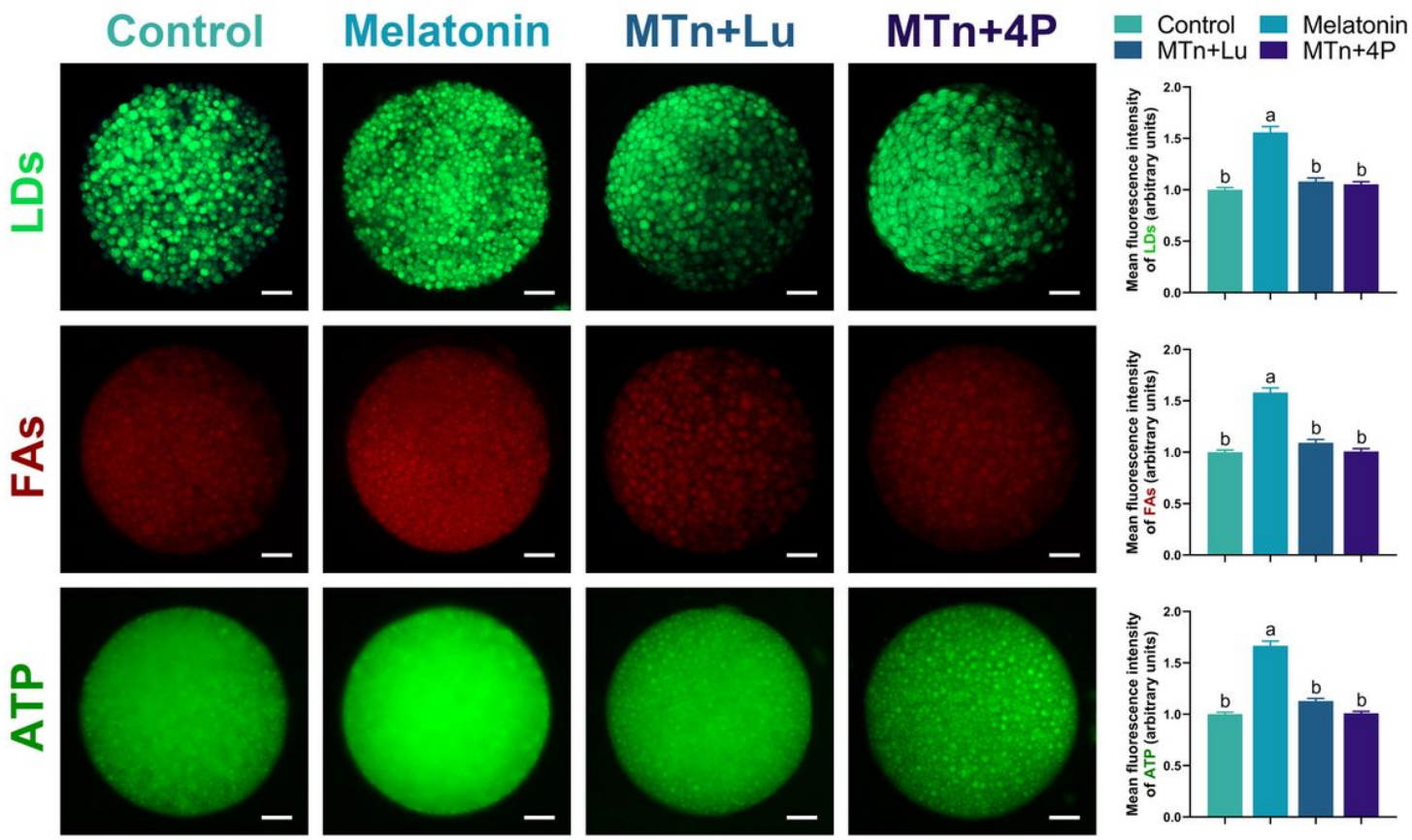

Figure 7

The levels of LDs, FAs and ATP in oocyte. Melatonin/MTn, 10-9 mol/L melatonin; Luzindole/Lu, 10-9 mol/L Luzindole; 4P-PDOT/4P, 10-9 mol/L 4P-PDOT. Scale bar = 25 μm. Different letters denote significant difference ($P < 0.05$).

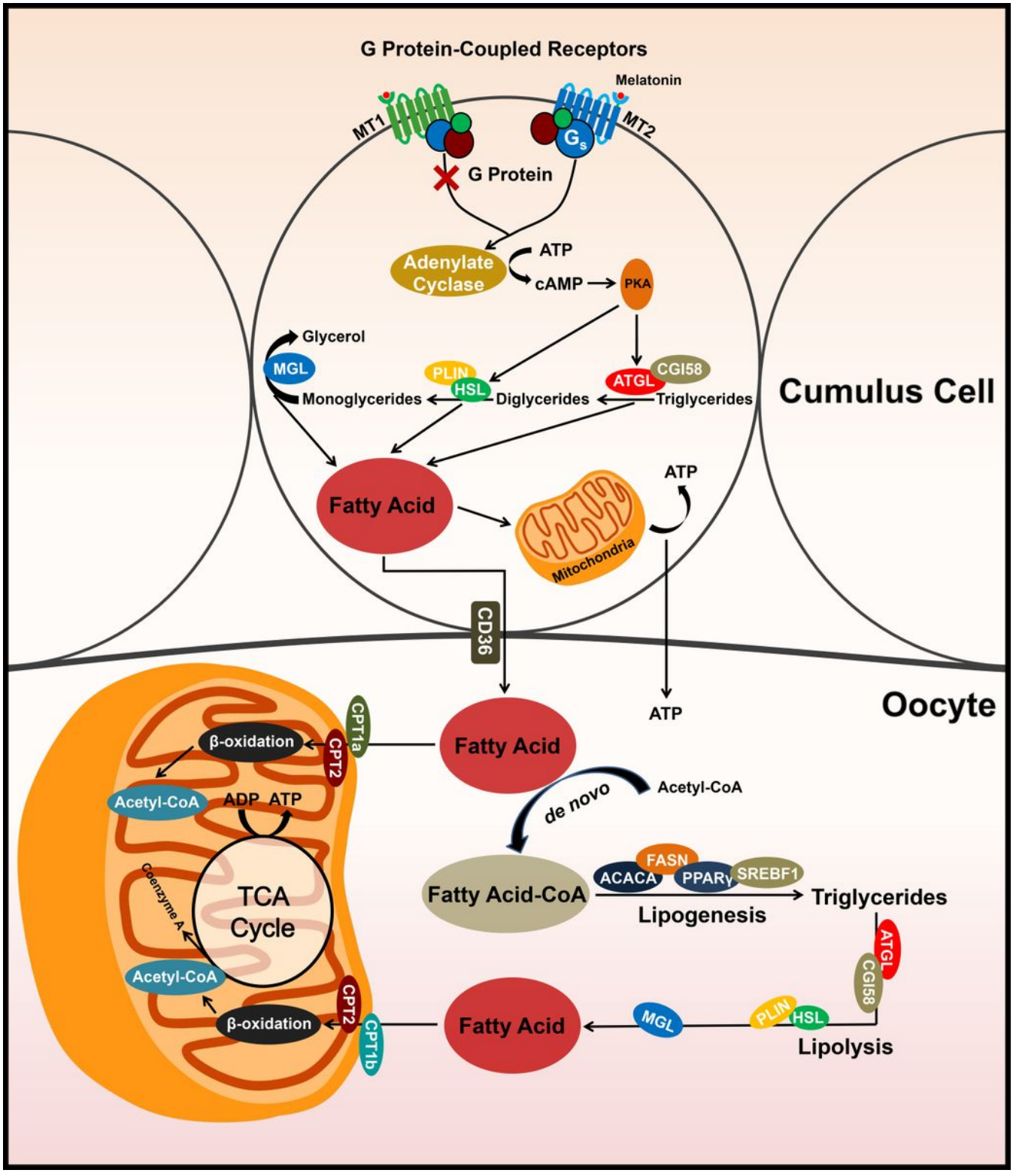

Figure 8

The schematic diagram. A model for melatonin and melatonin receptor-mediated lipid metabolism in porcine COCs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.jpg
- SupplementaryFigure2.jpg
- SupplementaryTable1.docx