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EXPRESSION OF ER β INDUCES BOVINE OVARIAN GRANULOSA CELL AUTOPHAGY VIA THE AKT/MTOR PATHWAY

Abstract: *The aim of our study was to determine whether silencing or overexpression of estrogen receptor β (ER β) regulates cell proliferation, steroidogenesis, autophagy and signalling pathways in bovine ovarian granulosa cells in vitro. In this study, bovine ovarian granulosa cells (BGCs) were cultured and transfected with ER β siRNA (si-ER β) or a plasmid overexpressing ER β (oe-ER β), and CCK-8 kit was used to assess cell proliferation. Real-time PCR was used to measure gene transcription. Western blotting was used to measure protein expression, and a specific kit was used to measure the production of steroid hormones. The results showed the expression level of ER β affects BGC proliferation according to the gene transcription levels of FSHR, CYP19A1, HSD3 β 1 and STAR and the production of E2 and P4. ER β was identified as an important nuclear receptor that induced BGC autophagy based on the mRNA and protein expression of autophagy-related genes. Furthermore, the role of ER β in BGC autophagy was confirmed through treatment with rapamycin (RAPA) or 3-methyladenine (3-MA) in BGCs by cotransfection with si-ER β or oe-ER β in BGCs. The results related to AKT/ mTOR signalling and phosphorylation suggested that ER β induces BGC autophagy through attenuating AKT/mTOR signalling. In summary, this study demonstrates that silencing or overexpression of ER β regulates BGC proliferation and function and induces BGC autophagy by targeting AKT/mTOR signalling. These data reveal a novel regulatory mechanism of autophagy via ER β and provide insights into the role of autophagy in BGCs.*

Key words: *cattle, cell death, estrogen receptor β , ovarian development, signalling transduction*

Introduction

Ovarian follicular granulosa cells play a critical role in follicle ovulation or atresia through steroid hormone synthesis and communication with oocytes during follicular development (Adhikari & Liu, 2009), which is affected by many factors, including hormones (Hulas-Stasiak & Gawron, 2011). Numerous studies have indicated that apoptosis of granulosa cells (GC) results in follicular atresia, while cumulative evidence indicates that cell autophagy is involved in follicular atresia (Krysko et al., 2008; Liu et al., 2022; Ma et al., 2019; Zheng et al., 2019). Hence, it is necessary to understand the destiny and regulatory mechanism of granulosa cells to provide a new view of follicular ovulation or atresia. As two members of the nuclear receptor superfamily, estrogen receptors (ER α and ER β) are ligand-dependent receptors and respond to the presence of oestrogen (Korach, 1994). ER α and ER β are highly homologous in structure, are distributed in different tissues and have different biological effects with ligand binding (Krege et al., 1998). Both ER α and ER β are expressed in mammalian ovaries but localize to distinct cell types. Generally, ER α is expressed in the interstitium and theca cells, while ER β is only expressed in the granulosa cells of growing follicles (Katherine et al., 2017). It has been confirmed that ER α knockout female mice are infertile because they are anovulatory, have disruption in LH regulation and have uteri that are insensitive to oestrogen. In contrast, ER β knockout female mice are sub-fertile and primarily lack efficient ovulatory function (Couse et al., 2000; Hewitt & Korach, 2003). Several studies have indicated that autophagy is also triggered by ER β through lysosome formation (Ruddy et al., 2014). For instance, in Hodgkin's lymphoma cells, autophagy is induced by ER β -mediated autophagosome formation and results in high expression of LC3-II even when the lysis function of lysosomes is inhibited (Pierdominici et al., 2017). In addition, as a selective agonist of ER β , arctigenin has been shown to inhibit autophagy in T-cell lines through the restriction of mTORC1 expression (Yang et al., 2020). In castrated male mice, the expression of autophagosome formation was upregulated by testosterone to activate AMPK α , demonstrating that ER β

induces autophagy (Pierdominici et al., 2017). In bovine, activin A attenuates BGC apoptosis in atretic follicles by ER β -mediated autophagy signalling (Liu et al., 2022). However, how ER β activates autophagy and signalling pathways remain unknown. In the ovary, autophagy plays an important role during follicular development and constant expression of ATG7 was observed in oocytogenesis stages as a key autophagy-related gene involved in autophagosome formation (Zhou et al., 2019). Our previous study indicated that bovine ovarian granulosa cells (BGCs) undergo autophagy, and VEGFA and FGF2 are a target of BGC autophagy that is inhibited by miR-21-3p (Ma et al., 2019; Ma et al., 2020). Nevertheless, it remains unknown whether ER β is involved in autophagy regulation in ovarian granulosa cells. In this study, we hypothesized that BGC autophagy is regulated by ER β . To test this hypothesis, ER β small interference RNA (si-ER β) and ER β overexpression (oe-ER β) were transfected into BGCs, and the effects of ER β on BGC proliferation and autophagy were determined

Materials and methods

Chemicals and reagents. All chemicals and reagents used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise stated.

Granulosa cell isolation and culture. The isolation and culture of granulosa cells were described by Jiang et al. (2013). Briefly, ovaries were collected from a local abattoir from dairy cattle aged 3–6 years, irrespective of the stage of the oestrous cycle. The ovaries were transported to the laboratory at 30°C in saline with penicillin (100 IU/ml) and streptomycin (100 mg/ml) within 2 h. The 2- to 6-mm follicles filled with clear liquid were selected, and GCs with follicular fluid were aspirated from follicles using a 10-ml syringe. At least 10 ovaries were collected to obtain enough GCs. A 150-mesh steel sieve was used to filter the cell suspension, and the mixture was centrifuged at 1000 g for 5 min to remove the follicular fluid. DMEM/F12 medium was used to resuspend the granulosa cell pellet, and the number of cells was counted using Trypan blue dye exclusion. To culture the cells, a 24-well tissue culture plate was used to seed the cells at a density of 5×10^5 viable cells in 1 ml of DMEM/ F12 including 10 mM sodium bicarbonate, 4 ng/ml sodium selenite, 0.1%, W/V of bovine serum albumin (BSA), 100 U/ml of penicillin, 100 μ g/ml streptomycin, 2.5 μ g/ml transferrin, 1.1 mmol/L non- essential amino acid mix, 10 ng/ml bovine insulin, 10^{-7} M androsten- edione and 10 ng/ml bovine FSH (BIONICHE Inc. Ontario, Canada). Cultures were maintained at 37°C in 5% CO₂ and 95% air for 2 days. On day 2, the medium was replaced using 70% fresh medium in each well, and then, the treatments were applied.

Plasmid construction. Bovine specific ER β siRNA (si-ER β) and ER β overexpression (oe-ER β) plasmids were synthesized by and purchased from RiboBio Company (Guangzhou, China). Sequences were cloned into pcDNA3.1 (+) eu- karyotic expression vectors.

Cell transfection and treatments. Lipofectamine™ 3,000 (Life Technologies) was used in this study to transfect si-ER β or oe-ER β constructs into BGCs according to the manufacturer's instructions. Negative control (50 nM), ER β siRNA (si-ER β , 50 nM), overexpression ER β (oe-ER β , 50 nM) plasmids, rapamycin (autophagy inducer, 10 nM) and 3-methyladenine (3-MA, autophagy inhibitor, 10 nM) were applied to the cells on day 2 for 6 h at 37°C in 5% CO₂ and 95% air, respectively, after which the medium was replaced with fresh medium, and the cells were cultured for 42 h before they were harvested for the next step of the experiment. The cell culture medium was stored at –20°C for steroid hormone determination.

Assessment of cell proliferation. A MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide) kit was used to assess the proliferation of GCs according to the manufacturer's guidelines. Briefly, after transfection for 6 h and another 42 h of culture, 20 μ l MTT solution (5 mg/ml) was added to the medium and mixed gently. The mixture of cells with MTT solution was then incubated for 4 h at 37°C and 5% CO₂. A microplate reader (BioTek, Winooski, VT, USA) was used to read the absorbance of samples at 450 and 620 nm. The cell viability was calculated according to a standard curve, which was prepared by culturing and measuring GC at different plating densities in quintuplicate (from 5×10^3 to 5×10^6 /ml) for 4 h.

Analysis of steroid hormone production. The secretion of 17 β -oestradiol (E2) and progesterone (P4) in cultured granulosa cells was determined using specific ELISA kits (Diasource, Louvain-la-Neuve, Belgium) according to the manufacturer's guidelines. Briefly, based on competition between free hormone and hormone linked to an acetylcholinesterase, the immunoassay was performed for a limited number of antibody-binding sites. A spectrophotometer was used to determine the intensity of acetylcholinesterase

to determine the production of hormones. The inter- and intra-assay CVs averaged 15% and 10.5%, respectively, and the minimum detectable concentration were 5 pg/ml for E2 and 0.2 ng/ml for P4.

RNA extraction and real-time PCR. After transfection, the cell culture medium was removed and TRIzol reagent was used to extract total RNA from BGCs. Total RNA concentration was measured by absorbance at 260 nm. cDNA was synthesized from purified total RNA (1 µg) using 5× All-In-One RT Master Mix (Abm, Richmond, BC, Canada). The ABI real-time PCR system (Applied Biosystems 7900) was used to quantify gene expression using SYBR Green I PCR Master Mix in a 20-µl reaction volume. To amplify each transcript, the thermal cycling parameters were as follows: 3 min at 95°C, 40 cycles of 15 s at 95°C, 30 s at 59°C and 30 s at 72°C. The housekeeping gene GAPDH was used for normalization of the mRNA expression levels. The $2^{-\Delta\Delta C_t}$ method was used to calculate the relative expression of mRNA. All samples were run in triplicate. The specific primers for cattle quantitative real-time PCR for the target genes are listed in Table 1.

Western blotting. After treatment, RIPA buffer containing protease inhibitors was used to lyse the cultured granulosa cells. The protein concentrations were determined by BCA assays (Pierce Inc., IL, USA). The protein samples (20 µg) were resolved on 10% SDS-PAGE gels and electrophoretically transferred to polyvinylidene difluoride (PVDF, Millipore) membranes using a Bio-Rad wet Blot Transfer Cell apparatus (transfer buffer containing 20% methanol, 48 mM Tris-base, 39 mM glycine and 1% SDS, pH 8.3). At room temperature, blocking buffer containing 5% BSA, 25 mM Tris, 150 mM NaCl, 2 mM KCl and 0.05% Tween 20, pH 7.4 was used to block the membranes for 2 h, and then, the membranes were incubated with primary antibodies against LC3 (anti-LC3/II, # L8918, 1:2000; Abcam, UK), P62 (anti-P62, # ab101266, 1:2000; Abcam), AKT (anti-AKT, # ab64148,

1:3000; Abcam), phospho-AKT (anti-pAKT, # ab8932, 1:3000; Abcam), mTOR (anti-mTOR, #ab2372, 1:2000; Abcam), phospho-mTOR (anti-pmTOR, 1:2000, #ab84400, phospho S2448; Abcam) and β -actin (anti- β -actin, 1:5000; #4970, Cell Signalling Technology, USA) diluted in BSA blocking buffer at 4°C. Membranes were then incubated for 2 h at room temperature with 1:4,000 anti-rabbit HRP-conjugated IgG (LK2003, Sungen Biotechnology, Tianjin, China) diluted in QuickBlock™ Secondary Antibody Dilution Buffer (Beyotime Biotechnology, China). Membranes were washed in blocking buffer, and protein bands were revealed by enhanced chemiluminescence (ECL, Millipore, Billerica, USA). Semi-quantitative analysis was performed using NIH ImageJ software.

Double staining with MDC and DAPI. MDC (monodansylcadaverine) has been used to stain autophagosomes in vitro, and an autophagosome was labelled as a clear punctum by fluorescence microscopy. In this experiment, BGCs were cultured in 12-well plates and transfected with si-ER β or oe-ER β , or treated with rapamycin or 3-MA on day 2 for 6 h. Then, the cells were grown with 0.05 mM MDC and/or 1 µg/ml

Table - 1 Specific primer sequences of cattle target genes

Gene	(5'-3') Forward	(5'-3') Reverse	ID
GADPH	CACCCTCAAGATTGTCAGCA	GGTCATAAGTCCCTCCACGA	NM_001034034.2
ER β	CTTCGTGGAGCTCAGCCTGT	TCTCCACATCAGACCCACCA	XM_027554293.1
ATG7	ATTGCTGCATCAAGAGACCCA	CCTTCTGGCGATTATGGTCA	XM_027523157.1
ATG3	GGTTGTTCCGGCTATGATGAG	GGGAGATGAGGGTGATTTTC	XM_027519324.1
LC3	TTATCCGAGAGCAGCAGCATCC	AGGCTTGATTAGCATTGAGC	XM_027513856.1
P62	AGGACTGAAGGAAGCTGCAC	GAGAGGGACTCAATCAGCCG	XM_027548457.1
FSHR	GCCCCTTGTCACAACTCTATGTC	GTTCCCTCACCGTGAGGTAGATGT	NM_174061.1
BECN1	AGTTGAGAAAGGCGAGACAC	GATGGAATAGGAACCACCAC	NM_001033627.2
CYP19A1	GTGGACGTGTTGACCCTCAT	GGCACTTTTCATCCAAGGGGA	NM_174305.1
HSD3B1	ACAATCTGACCGCATCGTCCT	CCACTTGCACCAAGTGTCTTG	NM_174343.1
STAR	GCCCAGAAACCTCAGCTCTTA	AGCTTTCCTGCTCCTAAGCAA	NM_174189.1

DAPI (4'6-diamidino-2-phenylindole) at 37°C for 15 min and fixed immediately with paraformaldehyde (4%) in PBS for 20 min. After washing three times with PBS, a fluorescence microscope (Nikon company, Japan, excitation: 390 nm, emission: 460 nm) was used to visualize the cells immediately. The percentage of cells with puncta in total cells showed the ratio of cell autophagy. A total of 200 cells in each sample were counted.

Statistical analysis In this study, each experiment was performed in triplicate. GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. Independent t-tests were used to evaluate the significance of the results between groups. ANOVA was used to test the main effects among treatments, and culture replicates were included as a random variable in the F-test. If the data were not normally distributed (Shapiro–Wilk test), they were transformed to logarithms. Data were presented as the means \pm SEM.

Results

Effects of expression of ER β on proliferation and steroidogenesis in BGCs. To investigate the role of ER β in BGC function, small interfering RNAs for ER β (si-ER β) and an ER β overexpression construct (oe-ER β) were transfected into BGCs to determine the effects of level of ER β on cell proliferation and steroid hormone production in BGCs. The effects of level of ER β on BGC proliferation were assessed using an MTT kit in this experiment, and the results indicated that transfection of si-ER β promoted, while transfection of oe-ER β inhibited BGC proliferation (Figure 1a). We then measured the mRNA abundance levels of *FSHR*, *CYP19A1*, *HSD3 β 1* and *STAR* in cultured granulosa cells, which are involved in BGC survival and function (Figure 1b). The results showed that si-ER β transfection upregulated *FSHR*, *CYP19A1*, *HSD3 β 1* and *STAR* mRNA abundance, and oe-ER β transfection downregulated *CYP19A1*, *HSD3 β 1* and *STAR* mRNA abundance; however, oe-ER β transfection did not change the *FSHR* mRNA level. To study the effects of ER β on BGC function, E2 and P4 production levels in BGCs transfected with si-ER β or oe-ER β were detected (Figure 1c). The results showed that si-ER β transfection significantly increased E2 production but did not change P4 production. The oe-ER β transfection significantly decreased E2 production but increased P4 production. These data indicated that expression of ER β regulates BGC proliferation and steroid hormone production.

Expression of ER β induces BGC autophagy. To study the role of ER β in BGC autophagy, si-ER β or oe-ER β was transfected into BGCs, MDC staining was used to label the autophagic vacuole, and gene mRNA abundance and protein expression were evaluated in this experiment. BGC autophagy was detected using a fluorescence microscope (Figure 2a), and the statistical results showed that the percentage of autophagy was significantly lower in BGCs transfected with si-ER β and higher in BGCs transfected with oe-ER β (Figure 2b). Quantitative real-time PCR was used to determine gene mRNA abundance in BGCs following transfection with si-ER β or oe-ER β , and *LC3*, *BECN-1*, *ATG3*, *ATG7* and *P62* mRNA abundance levels are shown in Figure 2c. The *LC3*, *BECN-1*, *ATG3* and *ATG7* mRNA levels were significantly downregulated in the cells transfected with si-ER β , while *P62* mRNA levels were upregulated; however, the mRNA levels of *LC3*, *BECN-1*, *ATG3* and *ATG7* were upregulated in the cells transfected with oe-ER β , while *P62* mRNA level was downregulated. Furthermore, Western blotting was used to determine the LC3 and P62 protein levels (Figure 2d), and the results indicated that the expression patterns were similar to the mRNA levels. LC3 protein level was decreased in BGCs transfected with si-ER β , while it was increased in BGCs transfected with the oe-ER β plasmid. Conversely, the p62 protein level was increased in BGCs transfected with si-ER β but was decreased in BGCs transfected with oe-ER β (Figure 2e). Together, these results indicate that silencing or overexpression of ER β induces BGC autophagy.

ER β knockdown rescues BGC autophagy induced by rapamycin. It has been confirmed that autophagy is induced by rapamycin (RAPA). To investigate the role of ER β knockdown in BGC autophagy, a rescue experiment was designed in which 10 nM RAPA and/or 10 nM si-ER β was used to challenge cultured BGCs. Gene expression analysis revealed that the expression levels of *LC3*, *BECN*, *ATG3* and *ATG7* mRNAs were significantly upregulated in BGCs by RAPA challenge, while those genes were downregulated by transfection with si-ER β . Meanwhile, in BGCs treated with both RAPA and si-ER β , the mRNA abundance levels of *LC3*, *BECN*, *ATG3* and *ATG7* were lower than those of cells challenged with RAPA alone but higher than those of cells transfected with si-ER β alone, in addition to the *ATG3* gene (Figure 3a). The *P62* mRNA abundance was significantly downregulated in BGCs in the single treatment

and in both the RAPA and si-ER β treatments (Figure 3a). It is likely that the protein levels of LC3 and P62 in BGCs treated with RAPA and/or transfected with si-ER β had similar patterns as the mRNAs (Figure 3b). These results indicate that ER β knockdown rescues BGC autophagy induced by rapamycin. ER β overexpression accelerates BGC autophagy inhibited by 3-methyladenine. The 3-Methyladenine (3-MA) has been confirmed as an inhibitor of autophagy. To study the role of ER β overexpression in BGC autophagy, rescue experiments were designed in which 10 nM 3-MA and/or 10 nM oe-ER β were used to challenge cultured BGCs. The gene expression results showed that the mRNA abundance levels of *LC3*, *BECN*, *ATG3* and *ATG7* were significantly upregulated in BGCs transfected with oe-ER β , while those genes were downregulated by challenge with 3-MA. In addition, the mRNA abundance levels of *LC3*, *BECN*, *ATG3* and *ATG7* were lower after treatment with both 3-MA and oe-ER β than after single oe-ER β transfection, but higher than after 3-MA challenge (Figure 4a). *P62* mRNA abundance was significantly downregulated in BGCs transfected with oe-ER β but upregulated by 3-MA challenge. However, the *P62* mRNA level was higher after treatment with both 3-MA and oe-ER β than after single oe-ER β transfection but lower than that after 3-MA treatment (Figure 4a). In the same way, the LC3 and P62 mRNAs and proteins had similar expression patterns (Figure 4b). These results suggest that oe-ER β accelerates BGC autophagy inhibited by 3-MA.

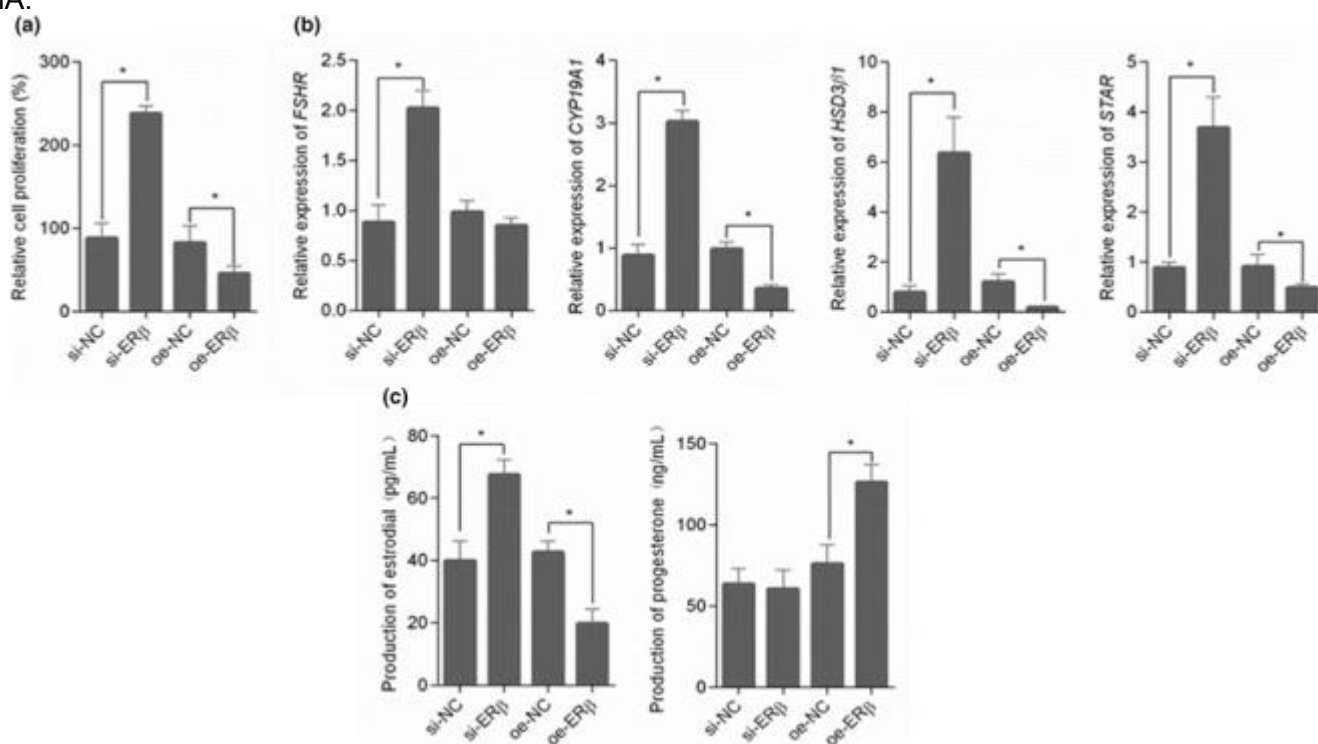


FIGURE - 1 Effects of ER β on BGC proliferation and steroidogenesis. BGC proliferation was measured by MTT kit (a), qRT-PCR was used to measure mRNA abundance of *FSHR*, *CYP19A1*, *HSD3B1* and *STAR* genes (b), and E2 and P4 concentration levels in BGCs transfected with si-ER β or oe-ER β (c). NC means the negative control. Data are means \pm SEM of three independent replicates (* $p < .05$)

ER β overexpression inhibits AKT/mTOR signalling in BGCs. The AKT/mTOR pathway has been confirmed as one of the important signalling pathways for autophagy (Krege et al., 1998). To determine whether expression of ER β regulates the AKT/mTOR pathway, phosphorylated AKT (p-AKT) and mTOR (p-mTOR) were detected by western blotting in BGCs following transfection with si-ER β or oe-ER β . As shown in the results, p-AKT expression was significantly higher in BGCs transfected with si-ER β (Figure 5a), while it was significantly lower in BGCs transfected with oe-ER β than in negative control (Figure 5b). The p-mTOR in the BGCs transfected with si-ER β and oe-ER β had a similar expression pattern as p-AKT in the BGCs transfected with si-ER β and oe-ER β (Figure 5c, d). These data suggest that overexpression

of ER β inhibits the phosphorylation of AKT and mTOR in BGCs.

Discussion.

It is well understood that follicular atresia is closely related to the apoptosis of granulosa cells; however, granulosa cell autophagy has been confirmed in a previous study during follicular atresia Ma et al., 2019. In rat granulosa cells, serum starvation induces the accumulation of autophagosomes, leading to apoptosis activation by reducing Bcl-2 expression and resulting in caspase activation (Choi et al., 2011). In mice, autophagy of granulosa cells was induced by FSH, which is important to maintain follicular atresia and granulosa cell proliferation (Shen et al., 2016). In porcine, the early induction of autophagic flux contributes to oxidative stress- induced apoptosis in granulosa cells (Zhang et al., 2021). Moreover, autophagy of granulosa cells is suppressed by activation of the mTOR signalling pathway mediated by AKT during follicular atresia (Zhang et al., 2019). In our laboratory, a previous study indicated that BGC autophagy is involved in follicular atresia (Ma et al., 2019; Zheng et al., 2019). Although studies have elucidated the function of GC autophagy in follicular atresia and ovarian primordial follicular reserves (Song et al., 2015), the regulatory mechanism of autophagy in GC is indistinct. In the present study, we demonstrate that (a) ER β inhibits BGC proliferation and (b) ER β induces BGC autophagy by decreasing AKT/mTOR phosphorylation. Together, the current results demonstrate that ER β plays an important role in BGC autophagy.

As a nuclear estrogen receptor, ER β is highly expressed in mammalian ovaries but is limited to granulosa cells (Katherine et al., 2017). A previous study in ER β knockout mice treated with pregnant mare serum gonadotropin (PMSG) demonstrated that ER β plays an important role in FSH-mediated GC differentiation and maturation of the whole follicle (Deroo & Korach, 2006). In addition, estrogen receptor β -null (β ERKO) mice exhibit subfertility, and β ERKO female mice treated by super ovulation exhibited a significantly lower percentage of ovulations and trapped oocyte follicles (Hsieh et al., 2015). In this study, BGCs were treated with si-ER β and oe-ER β , and the mRNA levels and production of E2 and P4 indicated that ER β inhibits BGC proliferation and regulates steroid hormone production.

As a form of programmed cell death, autophagy is involved in the regulation of intracellular homeostasis (Doherty & Baehrecke, 2018).

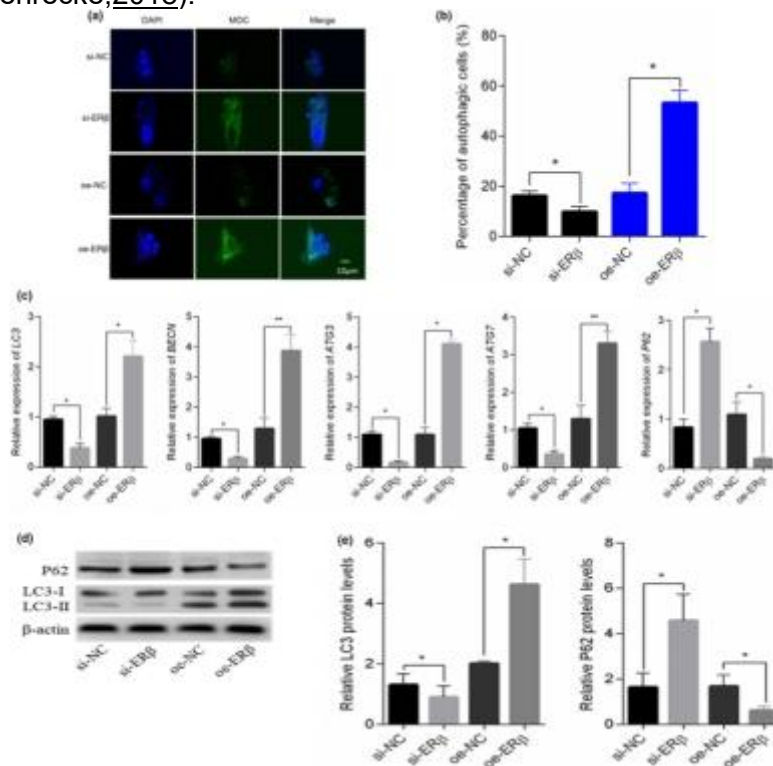


FIGURE-2 ER β induces BGC autophagy. The autophagy was labelled by MDC in BGCs transfected with si-ER β or oe-ER β , bars = 10 μ m (a), the statistical results of percentage of autophagic BGCs (b), gene expression of *LC3*, *BECN-1*, *ATG3*, *ATG7* and *P62* in BGCs transfected with si-ER β or oe-ER β (c), protein expression levels of LC3 and P62 in BGCs transfected with si-ER β or oe-ER β (d), and the statistical results were shown in (e). NC means the negative control. Data are means \pm SEM of three independent replicates (* p < .05, ** p < .01)

Conclusion.

In conclusion, the results above show for the first time that expression of ER β inhibits BGC proliferation and contributes to BGC autophagy by attenuating PI3K/AKT/mTOR signalling. These findings further our understanding of the mechanisms of follicular development and atresia in cattle ovaries.

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ВЫРАЖЕНИЕ ER β ИНДУЦИРУЕТ АВТОФАГИЮ ГРАНУЛЁЗНЫХ КЛЕТОК ЯИЧНИКА КРС ЧЕРЕЗ ПУТЬ АКТ/МТОР

Цель нашего исследования заключалась в изучении того, влияет ли снижение или сверхэкспрессия рецептора эстрогена β (ER β) на пролиферацию клеток, стероидогенез, аутофагию и сигнальные пути в гранулёзных клетках яичника крупного рогатого скота *in vitro*. В данном исследовании гранулёзные клетки яичника крупного рогатого скота (ГККРС) культивировали и трансфицировали с использованием siRNA для ER β (si-ER β) или плазмиды с сверхэкспрессией ER β (oe-ER β). Для оценки пролиферации клеток использовали набор ССК-8. Количественная ПЦР в реальном времени применялась для измерения уровня транскрипции генов. Экспрессию белков определяли методом вестерн-блоттинга, а для оценки продукции стероидных гормонов использовали специализированный набор. Результаты показали, что уровень экспрессии ER β влияет на пролиферацию ГККРС, что подтверждается изменениями уровней транскрипции генов FSHR, CYP19A1, HSD3 β 1 и STAR, а также продукцией эстрадиола (E2) и прогестерона (P4). ER β был идентифицирован как важный ядерный рецептор, индуцирующий аутофагию ГККРС на основании уровней мРНК и белков, связанных с аутофагией. Дополнительно роль ER β в аутофагии ГККРС была подтверждена обработкой клеток рапамицином (RAPA) или 3-метиладенином (3-MA) при сопутствующей трансфекции с si-ER β или oe-ER β . Данные по сигнальному пути АКТ/мТОР и уровню его фосфорилирования свидетельствуют, что ER β индуцирует аутофагию ГККРС путем подавления сигнального пути АКТ/мТОР. В заключение, настоящее исследование демонстрирует, что снижение или

сверхэкспрессия ERβ регулируют пролиферацию и функцию ГККРС и индуцируют аутофагию за счет воздействия на сигнальный путь АКТ/mTOR. Полученные данные раскрывают новый механизм регуляции аутофагии посредством ERβ и предоставляют новые представления о роли аутофагии в гранулёзных клетках яичника.

Ключевые слова: крупный рогатый скот, гибель клеток, рецептор эстрогена β, развитие яичника, сигнальная трансдукция.

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ERβ ЭКСПРЕССИЯСЫ АКТ/MTOR ЖОЛЫ АРҚЫЛЫ ІРІ ҚАРА АНАЛЫҚ БЕЗ ГРАНУЛОЗА ЖАСУШАСЫНЫҢ АУТОФАГИЯСЫН ИНДУКЦИЯЛАЙДЫ.

Зерттеуіміздің мақсаты - эстрогеннің β рецепторының (ERβ) төмендеуі немесе жоғары экспрессиясының сиыр овариінің гранулярлық жасушаларының пролиферациясына, стероидогенезіне, аутофагиясына және сигналдық жолдарына әсерін зерттеу болды. Зерттеуде сиыр овариінің гранулярлық жасушалары (СОГЖ) in vitro жағдайда өсіріліп, ERβ-ге қарсы siRNA (si-ERβ) немесе ERβ-нің жоғары экспрессиясын тудыратын плазмидалармен (oe-ERβ) трансфекцияланды. Жасушалардың пролиферациясын анықтау үшін ССК-8 жинағы қолданылды. Ген транскрипциясының деңгейін анықтау үшін сандық нақты уақыттағы ПТР қолданылды. Белоктардың экспрессиясын вестерн-блот әдісімен бағалады, ал стероид гормондарының өндірісін арнайы жинақпен өлшеді. Нәтижелер ERβ экспрессиясының деңгейі гранулярлық жасушалардың пролиферациясына әсер ететінін көрсетті, бұл FSHR, CYP19A1, HSD3β1 және STAR гендерінің транскрипция деңгейінің өзгерістерімен, сондай-ақ эстрадиол (E2) және прогестерон (P4) өндірісімен расталды. ERβ-гранулярлық жасушалардағы аутофагияны индуциялайтын маңызды ядролық рецептор ретінде анықталды, бұл аутофагияға қатысты мРНҚ және белоктардың деңгейлеріне негізделген. Сонымен қатар, ERβ-ның гранулярлық жасушалардағы аутофагиядағы рөлі рапамицин (RAPA) немесе 3-метиладенин (3-MA) өңдеулері кезінде si-ERβ немесе oe-ERβ трансфекциясымен бірге расталды. АКТ/mTOR сигналдық жолы мен оның фосфорлану деңгейі бойынша алынған мәліметтер ERβ гранулярлық жасушалардағы аутофагияны АКТ/mTOR жолының тежелуі арқылы индуциялайтынын көрсетті. Қорытындылай келе, бұл зерттеу ERβ төмендеуі немесе жоғары экспрессиясының гранулярлық жасушалардың пролиферациясы мен функциясын реттеп, аутофагияны АКТ/mTOR сигналдық жолы арқылы индуцирлейтінін көрсетті. Алынған деректер ERβ арқылы аутофагияны реттеудің жаңа механизмін ашып, овари гранулярлық жасушаларындағы аутофагияның рөлі туралы жаңа түсінік берді.

Түйінді сөздер: сиыр, жасуша өлімі, эстроген β рецепторы, овари дамуы, сигналдық трансдукция.

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СРАВНИТЕЛЬНЫЙ АНАЛИЗ НАЧАЛЬНЫХ ЭТАПОВ РОСТА СОРТОВ КАРТОФЕЛЯ XS1, XS4, XS5 И ГАЛА В ЗАСУШЛИВОМ КЛИМАТЕ АБАЙСКОЙ ОБЛАСТИ

Аннотация: В данной статье проведена сравнительная оценка показателей всхожести сортов картофеля XS1, XS4, XS5 (Китай) и отечественного сорта Гала в условиях засушливой почвы Абайской области. Исследование проводилось в весенний период 2025 года, в рамках которого определялись процент всхожести семенных клубней, сроки прорастания и средняя высота начальных побегов. Согласно полученным результатам, сорт Гала продемонстрировал наивысшую всхожесть (91%) и интенсивный начальный рост. Сорт XS4 также показал хорошие адаптационные способности (86%). В то же время сорта XS1 и XS5 показали относительно низкие показатели. По результатам исследования установлено, что в засушливых климатических условиях Абайской области наиболее эффективными являются сорта Гала и XS4.

Картофель является одной из важнейших продовольственных культур, широко выращиваемых в Казахстане. В связи с разнообразием агроклиматических условий в разных регионах страны, способность сортов к адаптации и их всхожесть могут значительно различаться. В последние годы среди отечественных сортов наибольшим спросом пользуется сорт Гала, который активно применяется в хозяйствах.

Абайская область характеризуется континентальным, засушливым климатом с малым количеством осадков, особенно в летний период, когда наблюдается дефицит влаги. Эти климатические особенности существенно влияют на всхожесть семенного картофеля и скорость начального роста растений. Настоящая статья направлена на сравнительную оценку уровня всхожести указанных сортов в условиях засушливой почвы Абайской области.

Методика проведения научных исследований разработана в сотрудничестве с Северо-Западным университетом сельского и лесного хозяйства Китая, в рамках продолжающегося изучения способности адаптации образцов пшеницы к природно-климатическим условиям региона.

В целом, полученные результаты будут использованы в дальнейших исследованиях для более полного и объективного анализа изучаемого материала.

Ключевые слова: картофель, сорт, фенологический анализ, адаптация, почва, всхожесть.