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A STUDY ON THE EFFECTS OF SIMULATED MICROGRAVITY ON PROLIFERATIVE AND CYTOSKELETON OF PORCINE GRANULOSA CELLS

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INTRODUCTION

1. The reasons for choosing research topic

In the recent decases, many experiments have been carried out with various types of simulated micrgravity along with a wide variety of organism. Microgravity simulation system are designed to simulated near zero gravity or freefall in ground-based laboratories to study the effectsof microgravity on change in the phisiological of astronault during space mission in out space. These studies were performed not only to examine physiologycal changes, but also to explores further changes at cullular level in order to gradually understand the influence of microgravity on various species or specialized cell in difference tissues. Many studies by authors around the world have shown that, there are changes, effects on physiology and health reproduction in general including altered fluid and electrolyte balance, cardiovascular changes, decreased bone mineral density, muscle wasting, increased percentage body fat, insulin resistance, sensorimotor changes in vision and vestibular function, changes in lung capacity, increase glomerular filtration rate and decreased sweating, cardiovascular effects include decrease left ventricular volume, decreased blood pressure and inceased prevalence of cardiac arrhythmias, increase oxidative stress. However, due to the diversity of cells types, accompanied by sex-related changes, the effects of microgravity on cell will be diverse and comples. To better understand those effects and influence, we also need to understand how microgravity affects organisms at cellular level. Recently, studies on the influences of microgravity on cell growth at the level of changes of molecular level inside cells which is one of the interesting topics that researcher focus on and special attention to study. Base on PubMed search on March 20th 2023, around 9,798 results regarding the term “weightlessness”, 12,722 results regarding the term “microgravity” and 4,219 results microgravity can cell ”. However in Viet Nam, apart from research group of Hoang Nghia Son

and reseacher in Institutes Biological Tropical, there have not been many studies related this term or topic and the results very limited. In order unserstood further investigate the role of microgravity in the proliferation of cell growth in microgravity, as well as the possible effects to leads the changes of inside of cell structure under microgravity compare normal condition.

2. Objectives

The aim of this thesis was to gain an understanding of how the simulated microgravity condition affects the development of pGC by investigating the changes in cell morphology, proliferation, and cytoskeleton structure of pGCs under *in vitro* condition. (Porcine granulosa cells, pGCs).

3. The main research contents of the thesis

– Isolate and cell culture of pGCs.
– Accession of the effect of simulated microgravity on the proliferation of pGCs.
– Determination of the effect of simulated microgravity on the apoptosis of pGCs.
– Specification of the effect of simulated microgravity on the morphology of nucleus and cytoplasm of pGCs.
– Demonstration of the effect of simulated microgravity on the cytoskeleton of pGCs.

CHAPTER 1. LITERATURE REVIEW

1.1. Introduction of microgravity and simulated microgravity

This section introduces an overview and gives the concepts of simulated microgravity and microgravity.

1.2. Space experiments

Challenging of space experiment when astronaults are in around earth or in space, they also facing with economic problem.

1.3. Effects of space flight on an astronaults

Microgravity during space flight causes numerous phisiological affect to health on an astronaults, including health reproduction.

1.4. The effects of microgravity on fertilization and pregnancy

This section showed affected on simulate microgravity leds abnormal on fertilization and pregnancy.

1.5. Microgravity platforms

Introduction the list of microgravity platform which is using frequently in microgravity research.

1.6. Overview of oocytes and granulosa cell.

This section was introduce about definition of formation and development of granulosa cell.

1.7. Cell proliferation and Cell cycle

This section was introduce about definition and characteric of cell proliferation.

This section covers the definition and stages of the eukaryotic cell cycles, also the cyclin dependent kinases that control the cell cycles.

1.8. Cytoskeleton

Introduce the cytoskeleton structure during cell cycles.

CHAPTER 2. MATERIALS AND METHODS

2.1. Materials

– Porcine granulosa cells were isolate from ovaries which in collect from slaughterhouse in Thu Duc.

– Simulated microgravity system Gravite using for project "Study on changes in skeleton structure and on the proliferation of porcine granulosa cell under simulated microgravity" Code ĐL.03/20-21.

2.2. Methods

2.2.1. Cell culture

2.2.1.1. Ovaries isolation

Porcine ovaries were collected from the slaughterhouse and transported to the laboratory within 2h.

2.2.1.2. Single follicles isolation

Single follicles were collected by a dissection method under stereomicroscope. The follicles were washed in PBS. The fresh pGCs were aspirated from single follicles and the oocyte – cumulus complexes were removed.

2.2.1.3. pGCs isolated and culture

pGCs were cultured in DMEM/Ham's F-12 with L-Glutamine (DMEM-12-A, Capricorn Scientific, Germany) with 15% FBS (FBS-HI-22B, Capricorn Scientific, Germany) and 1% Pen/Strep (15140-122, Gibco, USA) 1% Pen/Strep (PS-B, Capricorn Scientific, Germany).

2.2.1.4. pGCs sub-culture

pGCs were added with 1 ml culture medium containing DMEM/Ham's F-12 with L-Glutamine (DMEM-12-A, Capricorn Scientific, Germany) supplemented with 15% FBS and 1% Pen/Strep, then centrifuged at 1500 rpm for 5 minutes at room temperature to collect cell precipitate. The precipitate was re-suspended in the above culture medium and transferred to T25 flasks. The cells then were cultured at 37°C, 5% CO_2.

2.2.2. Staining of pGCs nuclei

pGC were treat with Triton X-100 0,1% (Merck,) overnight at 4ºC. Stain with Hoechst 33342 (14533 Sigma Alrdrich, Munich) 2µg/ml within 30 minutes and perform under fluorescence microscopes.

2.2.3. Microgravity simulation

Cells were then divided into two groups: simulated microgravity group (SMG) in Gravite and control group (Control). pGCs were induces in SMG for 72h. Each experients were repeat 3 times.

2.2.4. Evaluation of cell proliferation

2.2.4.1. Cell density by WST-1

pGC were treated with WST-1 solution (11644807001, Roche, Switzerland) for 3.5 hours at 37ºC, 5% CO_2. OD values per well were measured by GloMax® Explorer Multimode Microplate Reader spectrometer (Promega, USA) at 450 nm.

2.2.4.2. Evaluation of Cell cycle and transcript expression

Cell harvesting: pGCs were detached by Trypsin 0.25% (TRY-2B, Capricorn Scientific, Germany) and centrifuged at 1500 rpm for 5 minutes at room temperature to obtain cell precipitation. Cell precipitates were resuspended and cultured at 37°C, 5% CO_2.

Evaluation of proportion of phase in cell cycle by Cytell: pGCs were culture in DMEM/Ham's F12 with 15% FBS và 1% Pen/ Strep 96-well plates (161093, Thermo Scientific, USA) with densities of 1×10^3 cells/well in simulated microgravity group (SMG) and control group (Control). Cells were observed under the Cytell fluorescence microscope system (GE Healthcare, USA).

Transcript expression of cell cycle-regulated genes *cdk4, cdk6, cyclin A, cyclin D1*: The transcript expression of *cdk4, cdk6, cyclin A, cyclin D1* were evaluated by real-time qRT-PCR method, *gapdh* gene was used as a control.

The total RNA of pGC extracted*:* used ReliaPrep™ RNA Cell miniprep RNA system kit (Z6011, Promega, Mỹ). RNA samples are store under liquid nitrogen before running real-time qRT-PCR.

Real-time qRT-PCR: A genes were evaluated by real-time qRT-PCR method using 2x qPCR kit SyGreen 1-Step Go Hi-ROX kit (PB25.32.03, PCRBiosystem, UK). Each reagent has total volume is 20µl including 1µl RNA sample, 2µl primer, 10µl 2X Mix Hi-ROX, 1µl RTAse, and 6µl dH_2O. The thermal cycle was as follows: 45°C for 15 min, 95°C for 2 min, 40 cycles of 95°C for 10 sec and 62°C for 15 sec, 71 steps of 60°C for 30 sec, 4°C for 30 min. The sequences of these primers were as follows:

Gene	Primer
cdk4	F 5'- TTC GAG CAT CCC AAT GTT GTC -3'
	R 5'- GTC TCG ATG AAC GAT GCA GTT G-3'
cdk6	F 5'-TTG GCT TTG GTG GGT AGT TCT -3
	R 5'-TGA ATG TCG GGT AGG AAG ATT G-3'
cyclin D1	F 5'-TGC ATC TAC ACC GAC AAC TCC A-3'
	R 5'-GTT GGA AAT GAA CTT CAC GTC TGT-3
cyclin A	F 5'-GAT TTA CAT CTT AGA AAA CAA AGG-3
	R 5'-GGT GAT CCC GCC GTC CAC T-3'
gapdh	F 5'-ATG GTG AAG GTC GGA GTC AAC-3'
	R 5'-CTC GCT CCT GGA AGA TGG T-3'

Translate expression of cell cycle-regulated genes CDK4, CDK6, Cyclin A, Cyclin D1:

The expression level of Cdk4, Cdk6, Cyclin A1, Cyclin A2 proteins was evaluated by Western Blot. Gapdh protein was used as a control. The process following: Sample lysis → SDS-PAGE electrophoresis → PVDF transferred membrane→ Membrane block → Incubated with primary antibodies→ Incubated with secondary antibodies.

2.2.5. Evaluation of apoptosis

2.2.5.1. Flow cytometry

pGCs were treated with FITC Annexin V Apoptosis Detection Kit I (BD Biosciences, USA) and analyzed by BD Accuri C6 flow cytometer system (BD Biosciences, USA).

2.2.5.2. Transcript expression of apoptosis related genes

The transcript expression of *bax* and *bcl-2* genes were evaluated by real-time qRT-PCR method. The process of collecting total RNA and real-time qRT-PCR reaction was performed as in section *2.2.4.2*. The thermal cycle was as follows: 45°C for 15 min, 95°C for 2 min, 40 cycles of 95°C for 10 sec and 62°C for 15 sec, 71 step of 60°C for 30 sec, 4°C for 30 min. The sequences of these primers were as follows:

Gene	Primer
bax	F 5'- CCA GGA GTT CCC AAT GTT GTC -3'
	R 5'- TTC ATC CAG GGG AGG TAC AAC -3'
bcl-2	F 5'-TCT TCA TTC ACA CCG AGT AG TGC -3
	R 5'- TGA GGT TAG AGC CAT CTG GAA A -3'
gapdh	F 5'- CAT GAG AAG TAT GAC AAC AGC CT -3'
	R 5'- AGTCCTTTCCACGATACCAAAGT -3'

2.2.6. Evaluation of the morphological changes in nucleus and cytoplasms

2.2.6.1. Evaluation of the morphological changes in

pGC were stain with Hoechst 33342 (14533, Sigma-Aldrich, Mỹ) and perform under Cytell fluorescence microscopes (GE Healthcare, Mỹ). Cell Cycle App was used to evaluate morphology, including the nuclear area, the nuclear intensity, and the nuclear shape value.

2.2.6.7. Evaluation of the morphological changes in cytoplas

ImageJ software (National Institutes of Health, Bethesda, USA) was used to evaluate cell area. The size of pGCs was also assessed by measuring FSC (Forward Scatter) index using flow cytometry method.

2.2.7. Evaluation of changes in cytoskeleton structure

2.2.7.1. Transcript expression of genes encoding microtubles and microfilaments

The transcript expression of *α-tubulin 3* and *β-actin* genes were evaluated by real-time qRT-PCR method reaction was performed as in Section *2.2.4.2*. The thermal cycle was as follows: 45°C for 15 min, 95°C for 2 min, 40 cycles of 95°C for 10 sec and 62°C for 15 sec, 71 step of 60°C for 30 sec, 4°C for 30 min. The sequences of these primers were as follows:

Gene	Primer
α-tubulin	F 5'- CATTGAAAAGTTGTGGTCTGATCA -3'
	R 5'- GCTTGGGTCTGTAACAAAGCAT -3'
β-actin	F 5'- CACGCCATCCTGCGTCTGGA -3
	R 5'- AGCACCGTGTTGGCGTAGAG -3'
gapdh	F 5'- CATGAGAAGTATGACAACAGCCT -3'
	R 5'- AGTCCTTTCCACGATACCAAAGT -3'

2.2.7.2. Translate expression of genes encoding microtubles and microfilaments

The expression level of α-tubulin và β-actin proteins was evaluated by Western Blot and performed as in Section *2.2.4.2.* Gapdh protein was used as a control. Antibody were used: anti-beta actin antibody (ab8226, Abcam, USA), Anti-alpha Tubulin antibody (ab52866, Abcam, USA), Anti-Gapdh antibody (ab181602, Abcam, USA).

2.2.8. Stastitical analysis

Sigma Plot software (SYSTAT Software, USA) was used to analyze data in the study. One-way ANOVA method was used to evaluate differences between experimental groups, where $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ were statistically significant difference.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Results of isolation and cell culture pGCs.

3.1.1. Results of single follicles collect and pGCs.

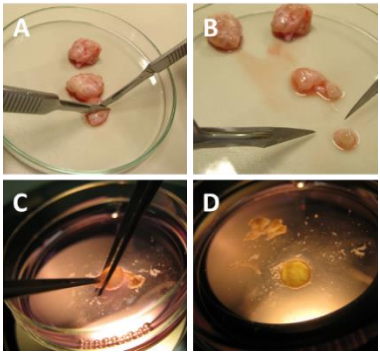


Figure 3.1. The process collects single follicles from porcine ovaries. **A**. Ovarian tissue is carefully divided into small pieces. **B**. single follicles were cut from ovarian tissue. **C**. Follicle shell were dissected from single follicle. **D**. Porcine single follicles.

Figure 3.1 show the process collect single follicles from porcine ovaries by divided into small small pieces. Single follicle will using for isolate porcine granulosa cells and cell culture and sub cell culture in microgravity simulation.

pGC were collected by using capillary tube to aspirate follicular fluide and granulosa cells. pGC transferred to T25 flasks flasks. Culture medium containing DMEM/Ham's F-12 with L-Glutamine (supplemented with 10% FBS and 1% Pen/Strep The cells then were cultured at 37°C, 5% CO_2. After 24h, pGC growth and adhere to the surface of flasks (figure 3.2). pGCs have a rhomboid shape after 3h and 12h of culture. pGC proliferate in normal, no evident of cytoplasmic fragment.

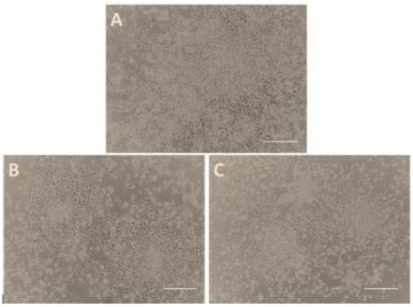


Figure 3.2. Result of primary culture of pGC A, B, C: pGC growth and adhere to the surface of flasks after 24 hour (X 200).

3.1.2. Results of cell culture, subculture and proliferate of pGCs.

In primary culture, pGCs adhere and growth on the surface in flask. In subculture from 1st to 9th pGCs still show strong proliferate during the culture.

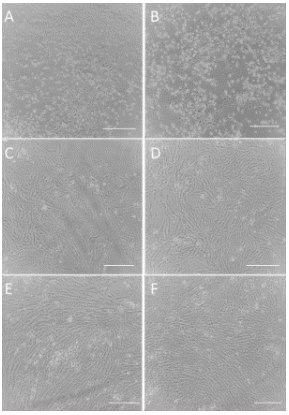


Figure 3.3. pGC morphology the number of time of subculture.
A, B. The 1st subculture of pGC. C, D. The 3rd and 5th subculture of pGC
E, F. The 7th and 9th subculture of pGC (X 200).

3.2. Evaluation of pGCs proliferation under microgravity simulated.

The optical density of pGCs evaluated by WST-1 and observed under the Cytell fluorescence microscope system.

3.2.1. Result of cell density by WST-1

After the microgravity silmuated experiment, the results show that a difference in pGC density between two group control and simulated gravity. Through observation under microscope pGC in SMG lower density than pGCs in control group (figure 3.4).

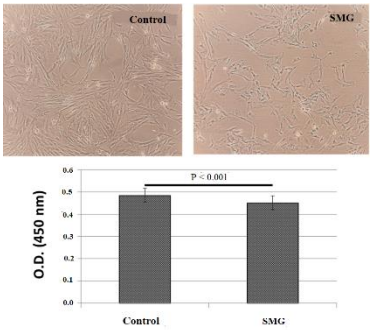


Figure 3.4. Evaluation of pGC proliferation by WST-1

Optical density of pGCs in two experimental groups was calculated and shown in Figure 3.4. Accordingly, the average optical density in SMG group was 0,45 ± 0,03, significantly lower than the control group, which was 0,49 ± 0,03; P <0.001). This result indicated that the proliferation of pGCs decreased when cultured in simulated microgravity condition.

3.2.2.1.Cell ratio in each phase of the cell cycle

The results of cell cycle analysis in figure 3.5 confirm that pGCs proliferation from SMG group was inhibited. In addition, the proportion of pGCs in SMG group is 3,095 ± 1108 cell/well also decreased compared with the control group 3,995 ± 815 cell/well (P = 0.022). This data shows that simulated microgravity can reduce the division and tends to induce the arrest phase of pGCs.

These differences were statistically significant when analyzed by One-way ANOVA with $p \leq 0.01$.

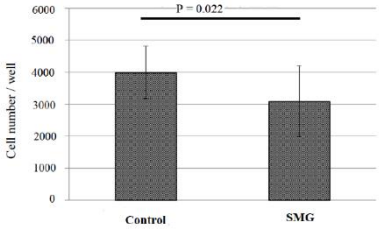


Figure 3.5. The optical density of pGCs evaluated by Cell Cycle App

3.2.3. Result of evaluation of *cell cycle*

3.2.3.1.Cell ratio in each phase of the cell cycle

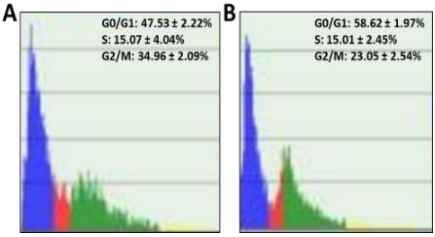


Figure 3.6. Cell cycle analysis by Cell cycle app of Cytell flourescent microscope. G0/G1 phase represent by blue color, S phase represent by red color, G2/M phase represent by green .

A. Control group. B. Simulated Microgravity group SMG

3.2.3.2.Transcript expression of cell cycle-regulated cdk4, cdk6

Real-time qRT-PCR result from show that expression of *cdk4* of pGCs in control group was higher pGCs in simulated microgravity group (P = 0.03). pGCs in control group also show that expression of *cdk6* of pGCs in control group was higher pGCs in simulated microgravity group (figure 3.7) (P = 0.023). The decrease in the transcript expression of *cdk6* of pGCs in SMG was higher transcript expression of *cdk4.*

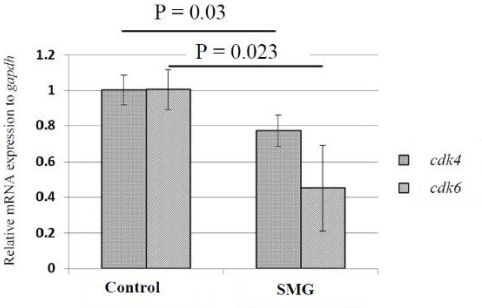


Figure 3.7. Results of real-time qRT-PCR of *cdk4* and *cdk6* in pGCs.

3.2.3.3. Transcript expression of cell cycle-regulated Cyclin A, and Cyclin D

Real-time qRT-PCR result show that expression of *cyclinA* of pGCs in control group was higher than pGCs in simulated microgravity group (P <

0.001). However, expression of *cyclinD* of pGCs in control group and simulated microgravity no significant difference (figure 3.8) (P = 0.821).

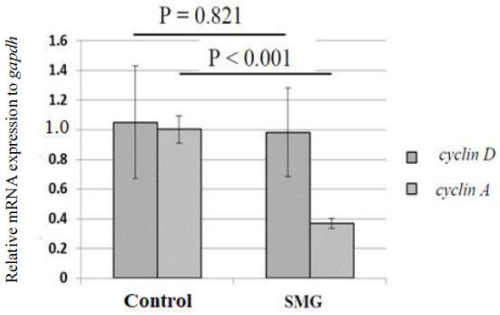


Figure 3.8. Results of real-time qRT-PCR analysis of *cyclin D* and *cyclin A* in pGCs

3.2.3.4. Translate expression of protein Cdk4 and Cdk6.

Western blot analysis show that the expression of protein Cdk4 and Cdk6 are displayed in figure 3.9. pGCs expression of Cdk4 and Cdk6 proteins in pGCs tended to decrease when cultured in simulated microgravity condition. Changes in protein expression Cdk4 and Cdk6 causes the reduction of change during cell division, the effects of ratio changes on pGCs cell cycle pGCs under Cell cycle app analysis.

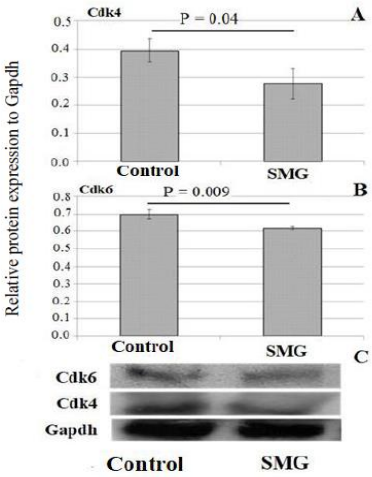


Figure 3.9. The expression of protein cdk4 và cdk6.
A. Result of expression analysis of cdk4. B. The expression analysis of protein CDK6. C. The expression analysis protein CDK4 và CDK6 by western blot

3.2.3.5.Translate expression of cell cycle-regulated Cyclin A, Cyclin D.

The expression of Cyclin A1+A2, and cyclin D in pGC were show in figure 3.10. Western blot analysis show that expression of cyclin D in pGCs under the control group and the SMG group no significant difference (P = 0.568). Meanwhile pGCs under SMG decrease expression protein cyclin A1 and A2. This is proves simulated microgravity alters the expression of cell cycles regulatory protein cyclin A1 and A2, leading to an effects on percentage of cell in the phase of pGC.

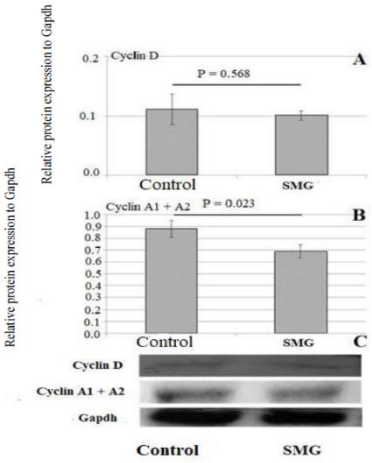


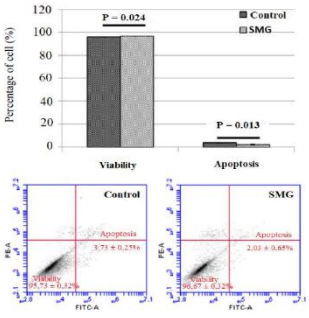
Figure 3.10. The expression of protein cyclin D and cyclin A1 + A2.

A. The expression of protein cyclin D.
B. B The expression of protein cyclin D and cyclin A1 + A2.
C. C. The expression analysis protein Cyclin D and cyclin A1 + A2 by western blot.

3.3. Evaluation of apoptosis

3.3.1.Apoptosis ratio

To evaluate the viability and apoptosis of pGCs using flow cytometry analysis. The flow cytometry showed that a slight increased in the viability ratio of pGCs from the SMG group. The viability ratios of pGCs from the control and SMG group were 95,73 ± 0,32% and 96,67 ± 0,32%, respectively (P = 0.024). The apoptosis ratios of pGCs from the SMG group was lower than control group (2,03 ± 0,65% vs 3,73 ± 0,25%, P = 0.013) (figure 3.11). The results suggested that SMG could enhance the viability of pGCs.



Hình 3.11. Viability and apoptosis analysis in pGC

3.3.2. Results of transcript expression of apoptosis related genes

Real-time qRT-PCR result from figure 3.12 showed that there was no significant difference in mRNA expression of *bcl-2* and *bax* in pGCs between the control group and SMG group. This suggests that apoptosis did not lead to a decrease in proliferation of pGCs.

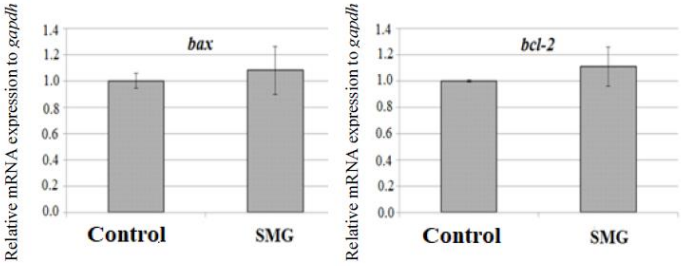


Figure 3.12. Real-time qRT-PCR analysis in gene *bax*, *bcl-2* in pGCs.

3.4. Results of the morphological changes in pGCs

3.4.1. Nuclear morphology

Nuclear morphology was average nuclear intensity which was generated by the Cell Cycle app of Cytell flourescence microscope to perform the nuclear indicator between two experiments group including: nuclear area, nuclear intensity, and nuclear shape (figure 3.13 and 3.14).

pGCs under the SMG condition exhibited higher nuclear area than control group after cell culture 3 days was 184,27 ± 0,90 μm^2 vs 187,81 ± 1,20 μm^2; (P = 0.028) (figure 3.13A). The average nuclear intensity of pGCs from SMG group was 987 ± 62, which was lower than the group control (1,404 ± 128) (P = 0.008). Results showed SMG condition affected to low nuclear intensity in pGCs after 3-days cell culture. Figure 3.13 B also addional for this proves and illustrates that the nuclear of pGC under control group smaller than simulated microgravity group. Simultaneously observation in figure 3.13 C, the fluorescence

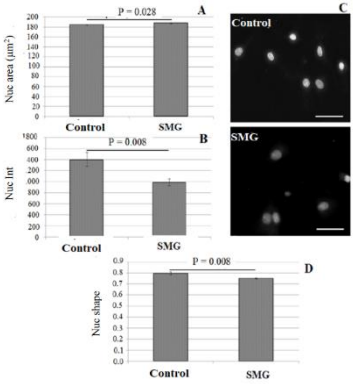


Figure 3.13. Evaluate nuclear morphology by software A. Nuclear area. B. Nuclear intensity. C. Nuclear staining with H33342. D. Nuclear shape

insentity in pGC under SMG lower than control group.

In this study, we perform the value of nuclear shape in pGCs by Cell cycle app. Results from 3.13D showed value of pGCs under control was 0,795 ± 0,014, higher than SMG group (0,750 ± 0,005, P = 0.008). This indicates the deformation of pGCs nuclei in SMG conditions over the 3-days culture. It mean under simulated

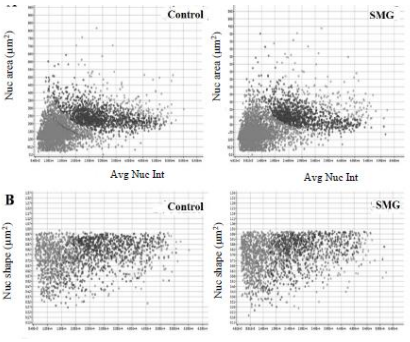


Figure 3.14. Nuclear morphology analysis of pGCs.
A. The distribution of the nuclear area in relation to nuclear intensity.
B. The distribution of the nuclear shape value in relation to nuclerar

microgravity nuclei of pGCs tends to be more elongated and asymetrial than normal condition in the earth.

3.4.2. Results of evaluate cytoplasmic morphology.

Observation cytoplsmic morphologies in pGCs revealed that the control group showed higher nuclear intensity and spread compare SMG group (figure 3.15) pGCs from control group showed a uniform like fibroblast like shape (figure 3.15A, 3.15B). However pGCs from SMG group showed fibroblast like shape more variety of morphologies to dectect in pGCs under SMG condition. In addition, rhomboid and tapered like were also found in this group (figure 3.15C, 3.15D). Results indicated that SMG conditions were alters the morphological of pGCs. Figure 3.15A demontrating the distribution of microfibrillar in pGCs from control group. the distribution of microfibrillar affect to the morphological changes of pGCs under SMG condition.

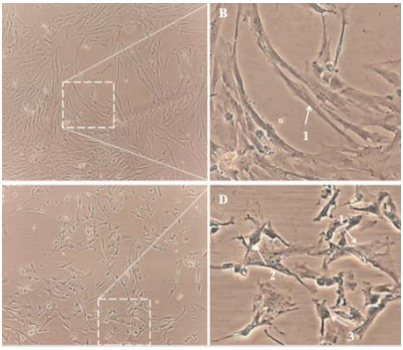


Figure 3.15. Morphology of pGCs

(A), (B) pGCs from control group showed the fibroblast-like shape. (C), (D) pGCs from SMG group exhibited various morphology, including (1) fibroblast-like shape, (2) rhomboid shape, and (3) pebble-like shape. Original magnification x 100 (A, C).

3.5. Results of changes in cytoskeleton structure

3.5.1. Results of evaluation effected of microgravity on microtubule.

3.5.1.1.Transcript expression of genes encoding microtubule.

In this study, changes in mRNA expression of tubulin were describle by realtime RT-PCR (figure 3.16). The results show that, under condition simulated microgravity, the change of *α-tubulin* was even more pronounced when the expression level in the SMG group was decreased compared to the control group after 3-days culture. The decrease in tubulin gene expression in the simulated microgravity group was 23% compare with control group. This is demontrates that SMG induction of the decrease in tubulin gene expression at translate expression.

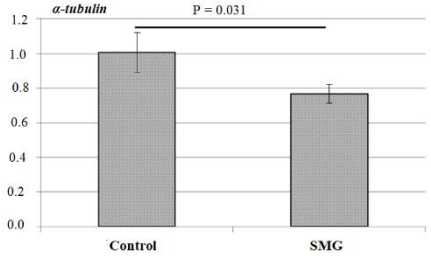


Figure 3.16. Transcript expression of α-tubulin in pGCs.

3.5.1.2 Evaluate of changes in microtubule structure

In this study, we evaluate of the distributon of microtubules in pGC under simulated microgravity compare with control group were perform flourescence stain tubuline display as figure 3.17, microtubule more densely distributed in the control group than in simulated microgravity group.

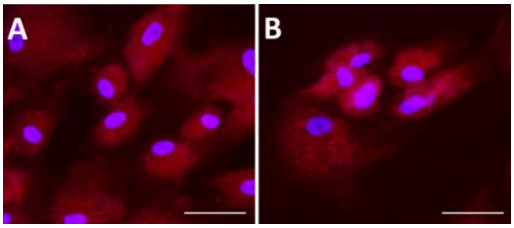


Figure 3.17. Microtubule fluorescence staining of pGCs.
A.Control group, B. Simulated microgravity group. Scale bar = 100 μm

3.5.1.3 Evaluate translate expression of genes encoding microtubules

The expression analysis protein α-tubulin by western blot, α-tubulin is microtubule structure in pGCs in two experiments group. The results to compare with GAPDH in figure 3.18 showed pGC protein strongly declined in simulated microgravity condition.

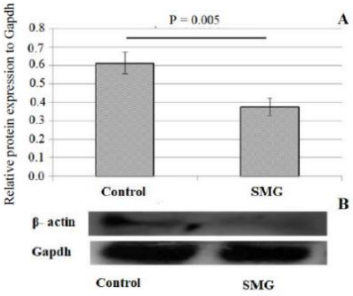


Figure 3.18. Expression of α-tubulin protein
A. Results of analysis the level of expression α-tubulin protein
B. Protein α-tubulin analysis by western blot

3.5.2. Results of evaluation effected of microgravity on microfilaments.

3.5.2.1.Transcript expression of genes encoding microfilaments

Changes in mRNA expression of actin pGCs were describle in figure 3.19. Realtime qRT-PCR analysis showed pGCs were decreased mRNA actin expression under SMG. The expression level of *β-actin* in SMG group much lower than 70% to compared with the control group. This results a remark structural changes of pGCs under SMG.

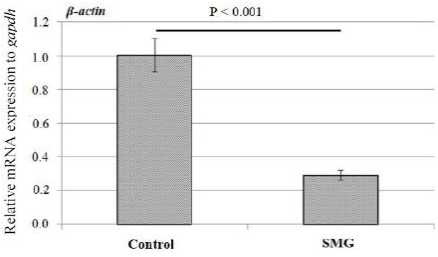


Figure 3.19. Expression of genes encoding *β-actin*.

3.5.2.2. Evaluate of changes in microfilaments structure

Results of actin microfilament staining in figure 3.20 showed that in pGCs under control group was parallel distribution of microflaments which extent the lenght of cells. In simulated microgravity group, actin was shown at lower densities . This results also show that pGC tend to expand rather than elongate under SMG condition.

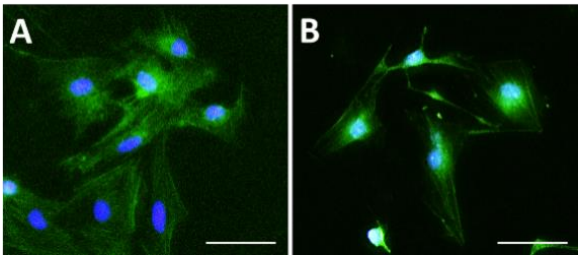


Figure 3.20. Microfilament fluorescence staining of pGCs.
A. Control group. B. Smulated microgravity group. Scale bar = 100µm.

3.5.2.3.Evaluate translate expression of genes encoding microfilaments

In this study, actin expression was analyzed to evaluate the effect of simulated microgravity on microfilaments in pGCs. Western blot results showing that the decrease in actin expression in pGCS from SMG group (figure 3.21). The degree of down regulation of actin expression is stronger than tubulin expression in pGCs. Results of western blot was reinforce the analysis result by real-time RT-PCR and flourescence staining of microfilament. Actin

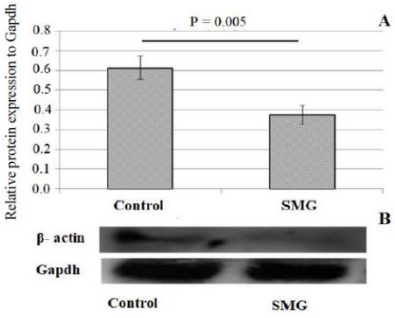


Figure 3.21. The expression of β-actin protein
A. Results of analysis the level of expression β-actin protein.
B. Western blot analysis of β-actin protein

expression was reduced at transcript and translate in pGCs under simulated microgravity. It show that in the SMG condition not only affects the actin expression, but also affects the composition content of actin in the pGCs. Thereby changing the microfilament structure as well as the distribution of microfilament in pGCs.

CONCLUSION AND RECOMMENDATION

Conclusion

This study revealed that SMG inhibited the proliferation of pGCs demonstrated by the reduced OD valuea and cell density after 3 days of culture.

The obtained results showed that the reduction of cell cycle-regulated protein expression as Cyclin A1, A2, Cdk4 and Cdk6 on pGCs. Thereby, captures the cells in the G0/G1 phase and prevents the cells from entering the G2/M phase.

Furthermore, the study also demonstrated that simulated microgravity reduces the expression of structural protein such as β-actin and α-tubulin in PGCs, leads to changes in the synthesis of microtubule microfilaments and inducing of cytoskeleton remodeling.

Depletion of cell cycle- related regulatory proteins and cytoskeleton structural proteins is responsible for the inhibition of pGCs proliferation under SMG.

Recommendation

There is a need for further research with human ovarian granulosa cells to compare and evaluate the physiological functions of this cell, especially the similarities and physiological effects on female astronaut.

Continue to research the hormone regulation mechanism of pGC and human granulosa cells.

The experiment should be able to have different time and others cell line as controls, also different pressure condition should be evaluated.

Study the interaction between granulosa cells and oocyte cells as well as other cells in the ovary.

NEW CONTRIBUTIONS OF THE THESIS

The thesis is the first study to evaluate the changes at the morphological and molecular level on porcine granulosa cells in Viet Nam and in the world (as of the time the study was published). Thereby adding to a better understanding of the characteristics and effects of microgravity on pGC, which is lead to change a series of factors related cell density, cell cycle and apoptosis. In addition, it also effected molecular marker of transcription and translation of cytoskeleton on pGC.

The obtained results showed that the reduction of cell cycle-regulated protein expression as Cyclin A1 and A2, Cdk4 and Cdk6 on pGC. In the study, it was also demonstrated that simulated microgravity reduces the expression of proteins structural such as β-actin and α-tubulin on pGC, in the synthesis of microtubule microfilaments and inducing of cytoskeleton remodeling.

The study has revealed that decrease in expression of microfilament and microtubule genes, which resulted in the inhibition of the formation of mitotic spindle and cleavage furrow during cell division, and leads to reorganization of the cytoskeleton.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Truong Xuan Dai, Hoang Nghia Son, Ho Nguyen Quynh Chi, Hoang Nghia Quang Huy, Nguyen Thai Minh, Nguyen Thi Thuy Tram, Nguyen Thi Thuong Huyen, To Minh Quan, Doan Chinh Chung, Truong Hai Nhung, Tran Hong Diem, Nguyen Thi Phuong Mai, Le Thanh Long, 2021, Simulated microgravity induces the proliferative inhibition and morphological changes in porcine granulosa cells, *Current Issues in molecular biology,* 43(3), pp. 2210-2219.
   doi: 10.3390/cimb43030155.
2. Truong Xuan Dai, Hoang Nghia Son, Le Thanh Long, 2022*,* Simulated microgravity altered the cell cycle progression of porcine granulosa cells, *Vietnam Journal of Biotechnology,* 20(3), pp. 445-451.
   doi.org/10.15625/1811-4989/16510.