شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم

HOSSAM MAGHRABY

شبكة المعلومات الجامعية

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

HOSSAM MAGHRABY

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها

علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

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تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

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Cryoprotectants and Gene Expression of Cryopreserved Oocytes and Embryos in Buffalo

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For the Degree of Ph.D.
(Theriogenology)

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APPOVAL SHEET

This is to certify that ,the disserattion enttitled (Cryoprotectants and Gene Expression of Cryopreserved Oocytes and Embryos in Buffalo)submittd by ESRAA ALI SAYED ISMAIL to the Faculty of Veterinary Medicine Cairo Univesity , for the Ph.D Degreee (Theriogenolgy) has been approved.

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ABSTRACT

This work was carried out for four successive years (2018, 2019 , 2020 and 2021) in order to investigate the effect of different cryoprotectant agents on 1) vitrified/thawed buffalo oocytes developmental competence, 2) viability of vitrified/thawed transferable buffalo embryos and 3) gene expression of in vitro vitrified/thawed matured buffalo oocytes and embryos. Excellent and good oocytes were cultured in TCM-199 medium at 38.5 ºC, 5% CO2 for 22 hrs, then fertilized using frozen semen and cultured on SOF media. Matured oocytes or transferable embryos were vitrified in DMSO, EG or combination of DMSO+EG by two steps procedure and stored in LN. The morphologically normal vitrified/thawed in vitro matured oocytes were fertilized using frozen semen and cultured on SOF media for detection the cleavage and transferable embryos rates to assess its viability. Fresh matured buffalo oocytes/embryos and recovered vitrified/thawed buffalo oocytes/ embryos were stained with Hochest stain to assess the stage of maturation of in vitro matured oocytes or cell count of in vitro produced embryos and stained with Mitotricker red for detection the mitochondrial distribution and intensity. mRNA was extracted from vitrified/thawed buffalo in vitro matured oocytes or in vitro produced embryos vs fresh matured oocytes or embryos using Picopure kits, cDNA was synthesized by QuantiTect Reverse Transcription kits. Quantitative analysis of the level of BMP15, IGF1, BCL2 and BAX genes in vitrified/thawed in vitro matured buffalo oocytes vs fresh oocyts and quantitative analysis of the level of OCT4, CDX2, BCL2 and BAX genes in vitrified/thawed in vitro produced buffalo embryos vs fresh embryos by real time PCR was performed. Recovered vitrified/thawed oocytes in DMSO+EG showed a higher significant ($P < 0.05$) percent of morphologically normal recovered oocytes and transferable embryos rates when compared with that vitrified in DMSO or EG. Also, a higher portion of mitochondria of fresh oocytes significantly diffused through the cytoplasm than all vitrified groups. The Mean No. of mitochondrial intensity of recovered mature oocytes

vitrified in EG+DMSO group was significantly higher ($P < 0.05$) than oocytes vitrified in DMSO or EG group. Recovered vitrified/thawed embryos vitrified in DMSO + EG showed a significantly higher ($P < 0.05$) mean cell number in embryos vitrified using EG+ DMSO than embryos vitrified using EG or DMSO. Mitochondria were distributed close to nuclei in blastomeres and were more clearly detected in fresh embryos and vitrified embryos in DMSO+EG group than vitrified group in DMSO or EG. Fresh in vitro matured buffalo oocytes showed a significant higher expression of BMP15, IGF1 and significant lower in BAX level than all vitrified groups. Combination group (DMSO+EG) was highly significant difference ($P < 0.05$) than DMSO or EG group in BMP15, IGF1and BCL2 transcription level and had a lower significant difference ($P < 0.05$) than both DMSO or EG group in BAX transcription level. OCT4 and CDX2 genes were up-regulated ($P < 0.05$) in fresh group than all vitrified groups. BCl2 gene showed no significant difference between fresh and combination group (DMSO+EG) . BAX (pro-apoptotic) gene was down-regulated ($P < 0.05$) in fresh group than all vitrified groups. Results showed no significant difference between combination group and EG group in OCT4 expression. Combination group were significant difference ($P < 0.05$) higher than DMSO or EG group in CDX2 and BCL2 transcription level and with a lower significant difference ($P < 0.05$) in BAX transcription level.

In conclusion, vitrification process affected developmental competence of in vitro matured buffalo oocyte and in vitro produced buffalo embryos. Combination between DMSO and EG was the best cryoprotectant agent for vitrification of in vitro produced buffalo oocytes and embryos on morphological, substructural and transcriptional level.

Keywords: Buffalo, In Vitro Embryo Production, Vitrification, Cryoprotectant, EG, DMSO, Mitochondria, gene expression

Dedication

I would like to dedicate this humble dissertation with lots of love and respect to my father, mother, husband and children lara, kenda and perry. Without their support, love and care, I would not have realized my dreams in life.