

# **Effect of cortisol on bovine oocytes maturation and further embryonic development after in vitro fertilization**

Dissertação de Mestrado em Engenharia Zootécnica

Aya Khaled Hassan Mahdy

Mestrado em

**Engenharia Zootécnica**



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Prepared by

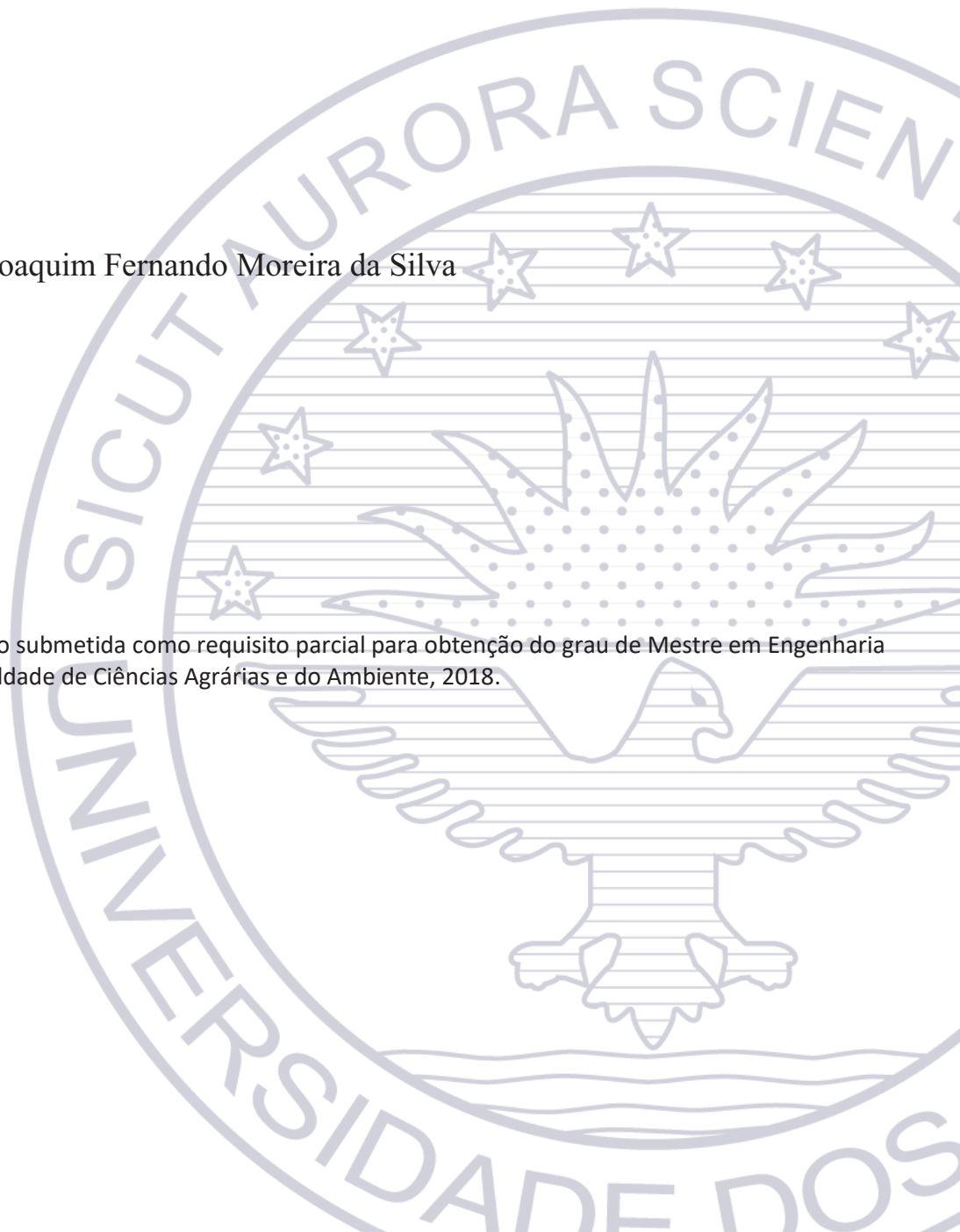
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*To my beloved family*

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## ABSTRACT

Oocyte meiotic maturation and further embryonic development after fertilization is the important physiological requirements for species survival. Herein, the aim of the study was to evaluate the effects of the stressful hormone, cortisol, on the nuclear maturation and embryo development of bovine oocytes after *in vitro* fertilization (IVF). This hormone (C<sub>21</sub>H<sub>30</sub>O<sub>5</sub>) is a corticosteroid of the steroid family, produced by the upper part of the adrenal gland released when an organism is stressed. Therefore, several studies demonstrated that cortisol plays a vital role inhibiting the extracellular signal-regulated kinases, necessary for meiotic prophase progression, essential for onset of early events of meiotic maturation oocyte maturation (resumption of meiosis), ovulation and further embryo development. In the present study, to evaluate the effect of cortisol on bovine oocyte maturation and further embryonic development, a total of 1439 immature oocytes were collected from slaughtered cows and matured *in vitro* for 24 hours with different concentrations of cortisol (0 (control); 50 µM; 150 µM ;250 µM). Afterwards, 412 oocytes were denuded, dyed with aceto-orcein and evaluated for meiotic development. The other 1027 were submitted to IVF and cultured for 9 days, being evaluated on day 2, 6 and 9, for cleavage, morula and blastocyst, respectively.

In the control, 85 % of oocytes reached Metaphase II, decreasing to 49, 32 and 15 % for the concentration of the cortisol (50, 150, and 250 µM, respectively). For the embryos, obtained from the oocytes submitted to IVF, in the control group, 28.3 ± 4.8% reached the stage of blastocyst, while for the concentrations of cortisol this value decreased to 22.1 ± 5.4%, 15.4 ± 6.0% and 6.5 ± 2.1% for 50, 150 and 250 µM of cortisol, respectively). Results of the present study clearly demonstrated that animal's stress and particularly high concentrations of cortisol impair bovine nuclear maturation as well as the further embryonic development after IVF.

Keywords: Cortisol, *In Vitro* Oocyte Nuclear Maturation, IVF, Embryo development.

## RESUMO

A maturação meiótica dos ovócitos e o posterior desenvolvimento embrionário após a fertilização são importantes requisitos fisiológicos para a sobrevivência das espécies. Desta forma, o objetivo do presente estudo foi avaliar os efeitos da hormona relacionada com o stress, cortisol, na maturação nuclear e desenvolvimento embrionário de oócitos bovinos após fecundação *in vitro*. Esta hormona ( $C_{21}H_{30}O_5$ ) é um corticosteroide da família de esteroides, produzido pela parte superior da glândula supra-renal libertada quando um organismo está sob stress. Vários estudos demonstraram que o cortisol desempenha um papel vital inibindo as quinases extracelulares reguladas por sinal, necessárias para a progressão da prófase meiótica, essenciais para o início de eventos iniciais de maturação do ovócito de maturação meiótica (retomada da meiose), ovulação e posterior desenvolvimento embrionário. No presente estudo, para avaliar o efeito do cortisol na maturação dos ovócitos bovinos e desenvolvimento embrionário, foram recolhidos um total de 1439 óculos de vacas e novilhas púberes, abatidas em matadouros e maturados *in vitro* durante 24 horas com diferentes concentrações de cortisol (0 (controlo); 50  $\mu$ M; 150  $\mu$ M; 250  $\mu$ M). Posteriormente, 412 oócitos foram desnudados, corados com aceto-orceína, sendo avaliado o desenvolvimento meiótico. Os outros 1027 foram submetidos à fecundação *in vitro* (FIV) e cultivados durante 9 dias, sendo avaliados nos dias 2, 6 e 9, para clivagem, mórula e blastocisto, respetivamente.

No controlo, 85% dos oócitos atingiram a metáfase II, diminuindo para 49, 32 e 15% para a concentração do cortisol (50, 150 e 250  $\mu$ M, respetivamente). Para os embriões obtidos a partir dos oócitos submetidos à FIV, no grupo controlo,  $28,3 \pm 4,8\%$  atingiram o estágio do blastocisto, enquanto que para as concentrações de cortisol esse valor diminuiu para  $22,1 \pm 5,4\%$ ,  $15,4 \pm 6,0\%$  e  $6,5 \pm 2,1\%$  para 50, 150 e 250  $\mu$ M de cortisol, respetivamente). Os resultados do presente estudo demonstraram claramente que o stress do animal e particularmente altas concentrações de cortisol prejudicam a maturação nuclear bovina, bem como o desenvolvimento embrionário posterior após a FIV.

Palavras-chave: Cortisol, maturação nuclear *in vitro* de oócito, FIV, desenvolvimento embrionário.

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# LIST OF ABBREVIATIONS

**11HSD1 – 11 $\beta$ -Hydroxysteroid Dehydrogenase type 1**

**11HSD2 – 11 $\beta$ -Hydroxysteroid Dehydrogenase type 2**

**ACTH – Adrenocorticotrophin**

**AI – Anaphase I**

**BI – Blastocyst**

**BMP-15 – Bone Morphogenetic Protein 15**

**C<sub>21</sub>H<sub>30</sub>O<sub>5</sub> – Cortisol**

**cAMP – Cyclic Adenosine Monophosphate**

**CB<sub>1</sub> – Cannabinoid type 1**

**CB<sub>2</sub> – Cannabinoid Receptor type 2**

**CDC25 – Cell Division Cyclin 25**

**CDC25B – Member of the CDC25 family of phosphatases**

**CGs – Cortical Granules**

**CL – Corpus Luteum**

**COCs – Cumulus oocyte complexes**

**CRF – Corticotrophin Releasing Factor**

**CRH – Corticotrophin Releasing Hormone**

**Cyclin B – CDK1, also known as Cdc2 or p34 kinase**

**DPBS – Dulbecco's Phosphate Buffered Saline**

**E2 – Estradiol**

**eCB – Endocannabinoid**

**ERKs – Extracellular Signal Regulated Kinases**

**ET – Embryo Transfer**

**FasL – Fas Ligand**

**FBS – Fetal Bovine Serum**

**GCs – Granulosa Cells**

**GDF-9 – Growth Differentiation Factor-9**

**GnRH – Gonadotropin Releasing Hormone**

**GPCRs – G-protein Coupled Receptors**

**GV – Germinal Vesicle**

**GVBD – Germinal Vesicle Break Down**

**HPA – Hypothalamic pituitary adrenal**

**HPO – Hypothalamus Pituitary Ovarian**

**HSPs – Heat Shock Proteins**

**IVC – *In Vitro* Culture**

**IVEP – *In Vitro* Embryo Production**

**IVF – *In Vitro* Fertilization**

**IVM – *In Vitro* Maturation**

**KITL – KIT Ligands**

**LH – Luteinizing Hormone**

**MAPK – Mitogen Activated Protein Kinases**

**MI – Metaphase I**

**MII – Metaphase II**

**MOS – c-Mos Pro- Oncogene**

**MPF – Maturation/Metaphase Promoting Factor**

**mRNA – Messenger RNAs**

**MTOCs – Microtubule Organizing Centres**

**Myt1 – Myelin transcription factor 1**

**NCM – Non-Capacitating HEPES-buffered Medium**

**NMR – Nuclear Maturation Rate**

**PGCs – Primordial Germ Cells**

**PHE – Phenylalanine**

**Phospho-ERK– p-ERK**

**PI – Prophase I**

**PVN – Paraventricular Nucleus**

**RNA – Ribonucleic Acid**

**rRNA – Ribosomal Ribonucleic Acid**

**SNS – Sympathetic Nervous System**

**SO – Superovulation**

**TALP – Tyroide's Albumin Lactate**

**TC – Theca Cells**

**THC – Tetrahydrocannabinol**

**TI – Telophase I**

**Tm – Tight morula**

**Wee1B – Wee1-like protein kinase 1B**

**ZP– Zona Pellucid**

# I INTRODUCTION

Stress is a process which activates the entire system and produces an organic response generating negative effects on animal health and production. The hormone mainly produced during stress is the cortisol ( $C_{21}H_{30}O_5$ ) which is secreted by the upper part of the adrenal gland, being an useful indicator as a biomarker to detect stress on the animals (Martínez-Miró *et al.*, 2016). Besides, cortisol plays an important role during the catabolic phase and its negative effect on several metabolism has been well described, it is not yet clear the role of this hormone in ovaries and particularly on oocyte nuclear maturation and further embryo development after fertilization (González *et al.*, 2010a). The immature oocytes begin to develop in the ovaries, possessing a large nucleus referred to as germinal vesicle (GV), in which a sequence called germinal vesicle breakdown (GVBD), initiates the process of nuclear oocyte maturation, finishing at the stage of metaphase II just before ovulation (Chen *et al.*, 2010). Brunet and Maro (2005) reported that the maturation promoting factor (MPF) is activated at GVBD and increases until it reaches a plateau at the end of the Metaphase I. A transient decline in MPF activity takes place during the transition between meiosis I, arresting at metaphase II. During oocyte maturation the extracellular signal-regulated kinases (ERKs) are activated and a comprehensive, extensive rearrangement of the cytoskeleton and associated proteins occurs involving a spindle pole close to the cortex (Kwon *et al.*, 2011). After polar body extrusion, chromosomes realign progressing to metaphase II (Sojung and Hyunjung, 2011). All meiosis developmental stages occur when follicles are growing from preantral to antral follicles. Moreover, the ovulation occurs when oocyte is in the metaphase II stage (Palma *et al.*, 2012). At the endocrine level, folliculogenesis is regulated by a central nervous system, anterior pituitary, and ovary cascade mechanism (Christensen *et al.*, 2012). Specialized hypothalamic neurons secrete pulses of gonadotropin-releasing hormone (GnRH) into the portal blood vessels, which acts on the

gonadotrophs to cause a pulsatile release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which act on ovarian follicle cells to control folliculogenesis. Although GnRH, FSH, and LH are critically important in regulating folliculogenesis, hormones and growth factors, which are themselves products of the follicle, can act locally to modulate (amplify or attenuate) FSH and LH action (Kumar and Sharma, 2014). This is the autocrine/paracrine system of developing follicles. It is believed that this local regulatory system plays an important role in the complex mechanisms governing the timing of folliculogenesis and whether a follicle becomes dominant or atretic. An important point is that estradiol produced by the dominant follicle rises production of GnRH, FSH and LH, increasing follicular growth, leading to its rupture/ovulation (Hsueh *et al.*, 2000; Gittens *et al.*, 2005; McGee and Raj, 2015). Studies developed by Macfarlane and collaborators (2000), have shown that stress-like levels of cortisol suppress follicular growth and development and block or delay the preovulatory surge of LH when cortisol is present during the late luteal and early follicular phases of the oestrous cycle. In fact, since the last century it has been postulated that stressful stimuli reduce fertility in domestic species (Welsh and Johnson, 1981; Wilson *et al.*, 1998), such climatic extremes (Doney *et al.*, 1973; Mahdy *et al.*, 2017), transportation (Ehnert and Moberg, 1991; Smart *et al.*, 1994) or laparoscopy (Martin *et al.*, 1981) as well as psychological stress (Prasad *et al.*, 2016) suppress or delay expression of behavioural oestrus and ovulation. In addition to reducing fertility, these stressors also stimulate the activity of the hypothalamic-pituitary-adrenal (HPA) axis, and a marked increase in serum concentration of cortisol is commonly associated with management-related stressors (Martin *et al.*, 1981; Ehnert and Moberg, 1991; Komesaroff *et al.*, 1998). Furthermore, Wagenmaker and collaborators (2009) pointed out that cortisol reduces amplitude of GnRH and LH secretion and lowers plasma estradiol levels in follicular-phase. Therefore, the high cortisol levels can inhibit the reproduction physiology (De Graaf-Roelfsema *et al.*, 2007). Lotfi and Mendonca (2016), observed that the effect of cortisol inhibition in the protein ERKs

disrupting their functions in meiotic maturation of full-grown oocytes and/or arrest at metaphase of meiosis II prior to fertilization (Lee *et al.*, 2007). Although the causal link between stress and infertility has not been precisely defined, several studies indicate that glucocorticoids in general and cortisol in particular may contribute to the anti-gonadal effect of stress (Dobson and Smith, 1995; Chrousos *et al.*, 1998). Prasad *et al.* (2016) suggested the increased level of cortisol reduces estradiol production possibly by affecting the granulosa cell functions within the follicle, which results deterioration in oocyte quality, leading to a poorest ability to develop after fertilization.

Therefore, taking into account results obtained in field situations in which stress affects the reproductive performance in domestic animals, the objective of the present study was to evaluate the effect of different cortisol concentrations on meiosis development during oocyte's maturation as well as the capacity of embryonic development after *in vitro* fertilization.