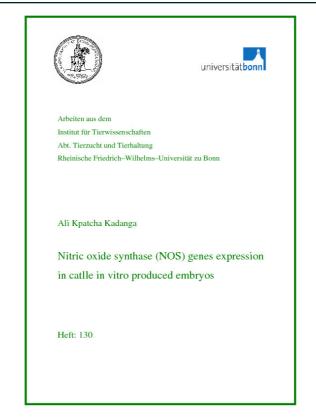


Ali Kpatcha Kadanga (Autor) Nitric oxide synthase (NOS) genes expression in cattle in vitro produced embryos



https://cuvillier.de/de/shop/publications/2425

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentzsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen, Germany Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: https://cuvillier.de

Contents		Page				
Abstract		Ι				
List of abbreviations						
List of tables						
List of figures						
1	Introduction	1				
2	Literature review	3				
2.1	Cattle embryo production	3				
2.1.1	Developmental competence	3				
2.1.2	Oocyte maturation	4				
2.1.3	Nitric oxide in embryo grouth and development					
2.1.4	Genetic control of early embryonic development	6				
2.2	Nitric oxide synthase genes	7				
2.2.1	Nitric oxide synthase structure	8				
2.2.2	Other NOS: mitochondrial NOS	9				
2.2.3	Regulation of NOS	9				
2.2.3.1	Regulation of NOS activity	9				
2.2.3.2	Regulation of NOS localization	13				
2.2.3.2	Nitric oxide synthase inhibition					
2.3	NOS expression in female reproductive tract and gonad tissue					
2.3.1	Uterus and vagina					
2.3.2	Ovary					
2.4	NO/NOS expression in embryo					
2.5	The role of nitric oxide	24				
2.5.1	Nitric oxide and infertility					
2.5.2	Nitric oxide and hypothalamic- pituitary- gonadal axis	26				
2.5.3	Nitric oxide in oocyte maturation, fertilization and embryo	26				
	development					

2.5.4	Nitric oxide and apoptosis in embryos	28
3	Materials and methods	30
3.1	Materials	30
3.1.1	Media used for IVP	30
3.1.2	Reagents and other media	32
3.1.3	Buffers and solutions	34
3.1.4	Equipments	38
3.1.5	Software programms	38
3.1.6	Biological material	39
3.2	Methods	39
3.2.1	Biotechnology methods	40
3.2.1.1	Oocyte collection and maturation	40
3.2.1.2	In vitro fertilization	40
3.2.1.3	In vitro culture	41
3.2.1.4	Nitric oxide inhibition and embryo development study	41
3.2.1.5	Statistical analysis	42
3.2.2	Molecular genetics methods	42
3.2.2.1	RNA isolation	43
3.2.2.2	cDNA synthesis	44
3.2.2.3	Nitric oxide synthase genes primer design	44
3.2.2.4	Polymerase chain reaction (PCR)	45
3.2.2.5	DNA extraction from agarose gel	46
3.2.2.6	Cloning and sequencing of PCR fragments	46
3.2.2.7	Plasmid isolation	48
3.2.2.8	Plasmid serial dilutions	49
3.2.3	Real-time quantitative PCR	49
3.2.3.1	Optimization of the PCR conditions	49
3.2.3.2	Quantification: precision/reproductibility of the replicates	50
3.2.3.3	Statistical analysis	51

3.3	NOS proteins detection and localization in bovine oocytes and					
	embryos					
3.3.1	Immunohistochemistry	51				
3.3.2	Immunofluorescence					
3.3.2.1	Pre-treatment of samples					
3.3.2.2	Incubation with specific NOS antibodies					
3.3.2.3	Identification of antigen-antibody complex by FITC secondary					
	antibody					
3.3.2.4	Propidium iodide staining	54				
3.3.2.5	Image capture and analysis	54				
3.4	Nitric oxide synthase proteins immunoblotting	54				
3.4.1	Protein isolation	55				
3.4.2	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis	55				
	(SDS-PAGE)					
3.4.3	Semi dry blotting and blocking	56				
3.4.4	Immunodetection (antibody affinity)	56				
3.4.5	Bands identification	57				
4	Results	58				
4.1	Nitria avide and having amhress development in vitue	58				
	Nitric oxide and bovine embryo development <i>in vitro</i>					
4.2 4.3	Qualitative identification of endothelial and inducible NOS genes					
	Quantitative expression profile of NOS in oocytes and embryos	62				
4.4	Expression and localization of NOS during preimplantation development	65				
4.5	Western blot analysis for endothelial NOS	67				
4.5	western blot analysis for endothenal NOS	07				
5	Discussion	68				
5.1	Inhibitory effects of L-NAME on development of embryos in vitro	68				
5.2	Expression profile of NOS genes	70				

5.3	Expression	and	localization	of	eNOS	and	iNOS	during	72
	preimplantation development								
5.4	Nitric oxide	role							74
5.5	Future prosp	oects							77
6	Summary								79
7	References								83
									0.