ESHRE2025 O-70

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Embryology (incl. IVF - ICSI, oocyte and embryo selection, culture, cryopreservation, developmental biology, quality control)

Oral presentation

Abstract title

NAD+ precursor nicotinamide mononucleotide improves the developmental competence of bovine embryos through enhancement of mitochondrial function in morula

Biography

Dr Hashimoto obtained his PhD in Reproductive Physiology at Kyoto University in 2001.

He also developed assisted reproduction technology in cattle at Snow Brand Milk Products and in human at IVF Namba Clinic. Currently, he is the professor of Osaka Metropolitan University Graduate School of Medicine. He received the JSAR innovative technology Award in 2008, the Japanese Society of Mammalian Ova Research outstanding presentation Award in 2009, 2014 and 2020, the memorial award of World Congress on In Vitro Fertilization in 2015, and the ASRM Star Award in 2016-2019.

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Study question: Does the addition of nicotinamide mononucleotide (NMN), an NAD+ precursor, to the culture medium of fertilized ovum increase their ability to develop into blastocysts?

Summary answer: The addition of NMN to the embryo culture medium increased mitochondrial function at morula stage and increased the ability to develop into blastocysts.

What is known already: NAD+ and its metabolites function as key regulators to maintain physiological processes, allowing cells to adapt to environmental changes such as nutritional disorders, genotoxic factors, infection, inflammation, and xenobiotics. Culturing isolated cells is predicted to decrease

NAD+ levels below the physiological requirement. We have shown that addition of NMN to bovine oocyte culture medium (1-100 mM) increases oocyte NAD+ levels, increases mitochondrial function and ATP levels, decreases ROS and anaphase lagging, and increases blastocyst formation (Sci Rep 2025). However, it remained unclear whether the addition of NMN to the embryo culture medium would improve the developmental ability of the embryos.

Study design, size, duration: This prospective study was conducted by randomly allocating 2-cell stage embryos obtained after IVF of bovine oocytes retrieved from slaughterhouses ovaries. Two-cell embryos obtained after IVF

were randomly divided into two groups (NMN 0 vs. 100 mM), and the blastulation were examined. Since it was predicted from the morphological changes recorded over time that development would differ after the morula stage, RNA was extracted from the morulae and a comprehensive gene expression analysis was performed.

Participants/materials, setting, methods: Only normal 2-cell stage embryos that did not undergo abnormal cytoplasmic division were used in this experiment. We used 310 two-cell stage embryos to assess the ability to develop into blastocysts, 125 two-cell embryos to assess morphological changes over time, 486 morulae for nextgeneration gene expression analysis, and 56 morulae for mitochondrial function analysis by measurement of oxygen consumption rate of each embryo.

Main results and the role of chance: Addition of NMN significantly increased the blastocyst formation rate (P = 0.0195, NMN: 41% vs. control: 23%) and the percentage of embryos reaching mid-stage blastocyst (P = 0.0018, NMN: 24% vs. control: 11%). No difference was observed in blastocyst formation rate after an additional 24 hours of culture (P = 0.053, NMN: 52.4% vs. control: 32.3%), indicating that NMN improves embryo development rate and embryo quality. We then captured morphological changes of embryos over time at 15-minute intervals using a microscope inside incubator. The morphological changes of embryos over time indicated that developmental retardation of embryos cultured in medium without NMN occurred after morula stage. Therefore, RNA was extracted from morulae cultured with or without NMN, and a comprehensive gene expression analysis was performed, focusing on mitochondria using Mitocarata 3.0. to extract mitochondria-related genes among the differentially-expressed genes. RNA sequence data proposed NMN could significantly affect mitochondrial function in morulae. Therefore, we measured the oxygen consumption rate of individual embryos to evaluate the mitochondrial function of morula, and found that NMN significantly increased the oxygen consumption rate of morulae (P = 0.00046, NMN: 3.3 fmol/sec vs. 2.76 fmol/sec).

Limitations, reasons for caution: Since the data in this study were obtained using bovine fertilized ovum, safety should be verified using donated human embryos before use in clinical practice.

Wider implications of the findings: Our data indicate that the addition of NAD+ precursors to the embryo culture medium improves mitochondrial function and developmental ability to blastocyst. Our data provide a rationale for applying NMN to improve the efficiency of reproductive medicine and IVF zygote production in domestic animals.

COI: I have no potential conflict of interest to disclose Keywords

nicotinamide adenine dinucleotide nicotinamide mononucleotide mitochondria

morula

RNA sequence