

**An addition of nicotinamide mononucleotide (NMN) into oocyte maturation medium improves the developmental competence of bovine oocytes after IVF.**

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**Study question:** Does an increase of NAD<sup>+</sup> level in oocytes lead to an improvement of blastulation after IVF?

**Summary answer:** The addition of NAD<sup>+</sup> precursor to the culture medium increased NAD levels in the oocytes, resulting in a decrease in ROS levels and an increase in ATP levels in oocytes, and an increase in blastocyst formation rate after IVF.

**What is known already:** With maternal ageing, the enzyme that degrades NAD<sup>+</sup> (CD38) increases in cells and the amount of NAD<sup>+</sup> in cells, including oocytes, decreases. The degradation of NAD<sup>+</sup> is also increased when cells are isolated from tissues.

**Study design, size, duration:** This study is a prospective study using bovine oocytes retrieved from ovaries obtained from a local slaughterhouse in Osaka city. A total of 300 bovine oocytes were used in this study.

**Participants/materials, setting, methods:** Bovine cumulus-oocyte-complexes (COCs) were cultured for 21 h in IVM medium supplemented with FSH, estradiol-17 $\beta$ , and 0-10  $\mu$ M NMN at 39°C under 5% CO<sub>2</sub> in air with high humidity. Matured oocytes were inseminated with the washed spermatozoa. Presumptive zygotes were cultured for 168 h. Matured oocytes were also examined its NAD<sup>+</sup>, reactive oxygen(ROS) and ATP levels

**Main results and the role of chance:** The addition of NMN to the culture medium increased intracellular NAD<sup>+</sup> levels at 1  $\mu$ M and above, increased intracellular ATP levels at 10  $\mu$ M and above, decreased ROS levels, and increased blastocyst formation rates at 100  $\mu$ M.

**Limitations, reasons for caution:** This study was conducted in vitro using bovine oocytes. Thus, studies using human samples should be performed before clinical application

**Wider implications of the findings:**

**Study funding/Competing interest(s):** Part of this work was supported by a grant from the Japan Society for the Promotion of Science (KAKENHI 20K09674 to S.H.). No other competing interests are declared.

**Trial registration number:** None.