Neuregulin 1 modulates meiotic resumption during amphiregulin-induced IVM in cattle

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BACKGROUND

✓ Oocyte *in vitro* maturation (IVM) has been recognized as a major bottleneck of assisted reproductive technologies (ART) applied in animal production.

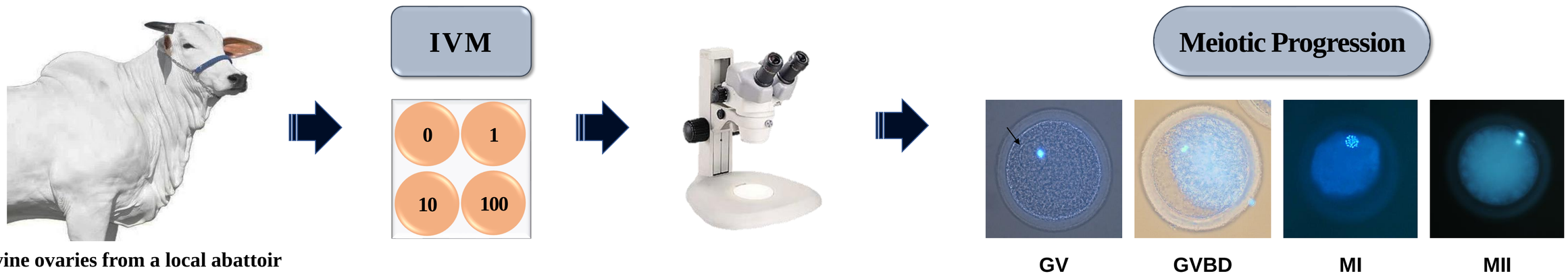
✓ The induction of the ovulatory cascade with EGF-*like* ligands instead of supraphysiological concentrations of FSH during IVM has provided promising results in cattle IVP, although it may accelerate meiotic resumption potentially compromising nuclear/cytoplasmic tuning.

✓ Previous studies in mice suggest that the addition of neuregulin 1 (NRG1), a particular EGF-*like* factor, modulates EGFR-induced pathways during oocyte maturation [1,2].

✓ No previous study has tested the NRG1- amphiregulin (AREG) combination during IVM in cattle.

✓ **Objective:** To assess the effects of NRG1 on meiotic dynamics of bovine oocytes undergoing IVM using amphiregulin (AREG) as the maturation trigger.

MATERIAL AND METHODS



Bovine ovaries from a local abattoir

Figure 1: Experimental design. Three graded concentrations of NRG1 (1, 10, or 100 ng/mL) were added to IVM media containing FSH and steroids at approximately physiological levels and AREG [follicular system (FS); 3], and outcomes were compared with the respective control (0). The FS medium consisted of TCM199 with Earle's salts, BSA, amikacin and pyruvate, supplemented with rhFSH (10^{-2} UI/mL), AREG (100 ng/mL), 17β-estradiol (50 ng/mL) and progesterone (150 ng/mL). Ovaries were obtained from a slaughterhouse and COCs recovered by aspiration were submitted to IVM for 6, 9, 12, 20, and 24h in controlled and humidified air. All the oocytes were denuded by pipetting, fixed in 60% methanol and stained with 1 µg/mL Hoechst 33342. Chromatin status and meiotic staging were determined by epifluorescence microscopy. Oocytes at the germinal vesicle (GV) and germinal vesicle breakdown (GVBD) stage were classified according to rupture of GV and the meiotic staging was classified in metaphase I (MI), metaphase II (MII), or degenerated. Five biological replicates were performed with 20-25 COCs in each group/replica. Data were arcsine transformed and compared with Tukey test using JMP software (SAS Institute Cary, NC). Data are presented by mean ± SEM and differences were considered significant when $P \leq 0.05$.

RESULTS

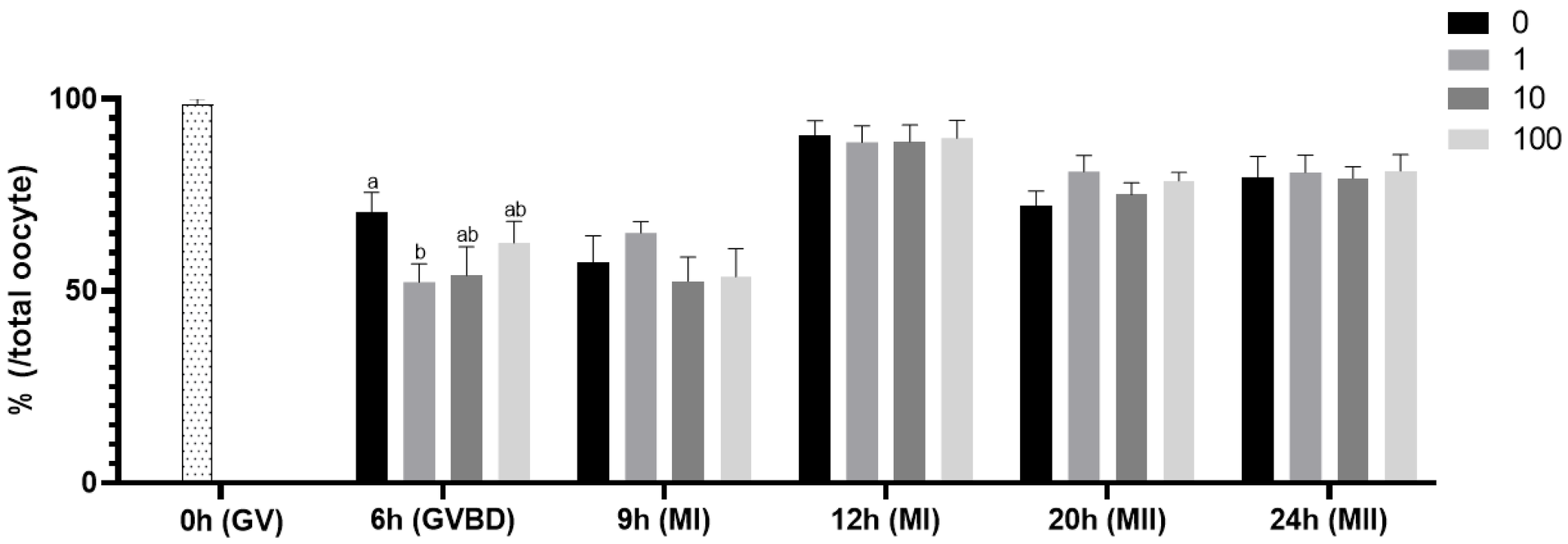


Figure 2: Effects of NRG1 during IVM induced with AREG (FS medium) on meiosis progression. COCs were subjected to IVM with FS medium in presence of 1, 10, 100 ng/mL NRG1 or not (0) for 6, 9, 12, 20, or 24 hours. 0h: immature oocyte; GV: germinal vesicle; GVBD: germinal vesicle breakdown; MI: metaphase I; MII: metaphase II. Data are represented by mean ± S.E.M. of five biological replicates. Different letters represent statistical differences ($P < 0.05$).

CONCLUSION

The present study provides novel evidence that NRG1 is involved in the mechanisms that control meiotic resumption in cattle, being a potential useful tool to improve the efficiency of current IVM/IVP protocols.

REFERENCES

[1] Noma *et al.*, 2011. Molecular Endocrinology. Vol. 25 (1), pp. 104-116.
[2] Kawashima *et al.*, 2014. Molecular Endocrinology. Vol. 28 (5), pp. 706-721.
[3] Soares *et al.*, 2017. Reproduction, Fertility and Development. Vol. 29 (11), pp. 2217-2224.

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