New strategy developed for cryopreservation of mouse follicles

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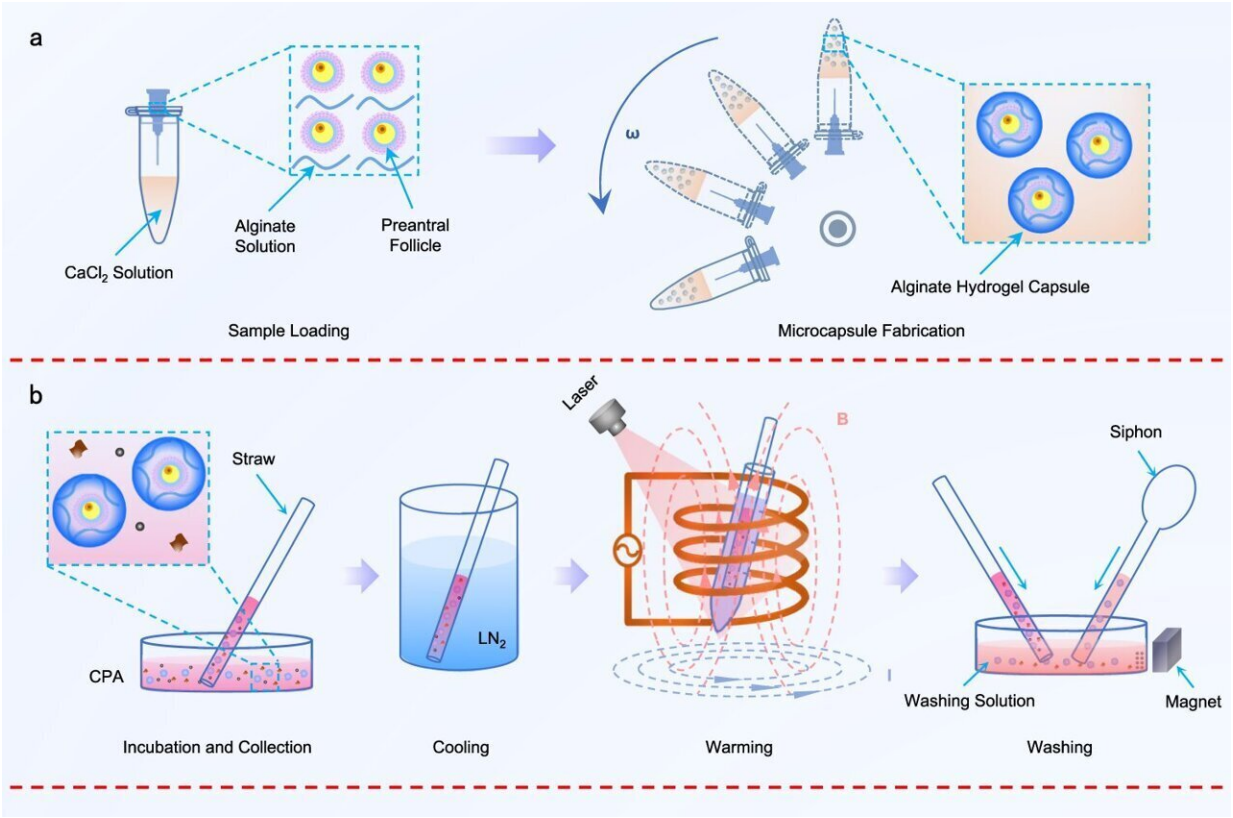

Schematic illustration of microencapsulation, cryopreservation, development of preantral follicles (PAFs) in vitro and subsequent in vitro fertilization (IVF) and transplant. a Fabrication of PAF-alginate hydrogel microcapsules by centrifugal microfluidic technology. CaCl2 solution: 0.15 M CaCl2 solution; Alginate solution: 1% (w/v) alginate solution. b Cryopreservation and nanowarming [combined with magnetic induction heating (MIH) and laser-induced heating (LIH)] of PAFs. CPA: 0.75 M ethylene glycol (EG) + 0.75 M 1, 2-propanediol (PROH) + 1 M trehalose; Washing solution: α-MEM + 10% FBS. c In vitro

culture of PAFs after warming. d IVF and the birth of the next generation of mice after in vivo transplantation of 2-cell zygotes. NPs nanoparticles, GO graphene oxide, COCs cumulus-oocyte complex, LN2 liquid nitrogen, CPA cryoprotective agent. Credit: *Nature Communications* (2022). DOI: 10.1038/s41467-022-34549-2

Recently, a research team led by Prof. Zhao Gang, Prof. Shi Qinghua from the University of Science and Technology (USTC) of the Chinese Academy of Sciences, and Prof. Cao Yunxia from Anhui Medical University, realized the high-quality cryopreservation of mouse preantral follicles (PAFs) based on biomimetics and physical field-assisted ice inhibition. This work was published in *Nature Communications*.

Fertility preservation is the only method to overcome the decrease in female fertility. The cryopreservation of follicles plays an important role in fertility preservation because follicles are rich in resources, easy to obtain and do not involve ethical issues. However, the existing methods for follicle cryopreservation are complex and ineffective due to the high concentration of toxic cryoprotective agents.

To solve this problem, the research team developed the low-concentration cryoprotective agent vitrification cryopreservation of mouse PAFs based on active ice inhibition approach. They combined physical field-assisted space heating techniques such as electromagnetic and laser heating with hydrogel microencapsulation, thus reducing the concentration of penetrating cryoprotective agents by 75%.

The physical field-assisted space heating techniques effectively improved the speed of heating and the uniformity of temperature distribution, suppressing the possible devitrification or recrystallization that could cause fatal damage to cells during the warming process.

Hydrogel microencapsulation provided biomimetic cryopreservation and critical biochemical and mechanical microenvironment, reducing the demand for toxic cryoprotective agents and supporting the in vitro development of follicles. It also isolated the follicles from external magnetothermal and photothermal materials, which improved biosecurity.

The results showed that the survival rate of follicles preserved by this method was improved by about a third. Furthermore, the team managed to obtain oocytes after 3D in vitro culture of the cryopreserved PAFs. The oocytes were then fertilized in vitro and transplanted into surrogate mice, which gave birth to healthy offspring.

This study builds a platform that integrates microencapsulation, cryopreservation and 3D in vitro culture, providing a unique solution for the preservation and utilization of follicles.

More information: Conghui Tian et al, Microencapsulation and nanowarming enables vitrification cryopreservation of mouse preantral follicles, *Nature Communications* (2022). DOI: 10.1038/s41467-022-34549-2

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