Challenges and Solutions For Female Fertility Preservation in Mammals

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Editorial

Reduced or impaired reproductive functions – namely, hypofertility and infertility – used to be regarded as issues that impact the fulfillment and wellbeing of individuals or couples struggling in childbearing. Modern life style, longer life expectancy, postponed childbearing, and environmental pollution, concurred to increase the occurrence of hypofertility and infertility, which is currently perceived as a public health problem. The development of assisted reproductive technologies (ART), in many cases associated with minimally invasive surgical procedures, holds the promise of restoring, at least in part, the reproductive functions and is gaining increasing interest for fertility conservation in oncological patients, even of pediatric age. Furthermore ART found considerable applications outside the biomedical field. For instance, modern farming has evolved with the possibility of efficiently amplifying the offspring of animals selected for productive traits, significantly impacting on livestock sustainability and profitability. Also wildlife conservation programs often rely on a broad range of assisted reproduction strategies. Among the most notable examples is the BioRescue project, that applies 'advanced reproductive technologies for saving critically endangered mammals like the northern white rhinoceros'. Extinction, however, threatens many indigenous domestic breeds that are globally replaced by the widespread of more productive cosmopolite breeds. Fertility preservation, therefore, has become essential also for safeguarding biodiversity of livestock genetic resources, to keep them available for future generations of producers and consumers. In this view, cryobanking of gametes and embryos is suggested as an *ex situ* conservation strategy since it holds the potential of reviving threatened or even extinct species or domestic breeds. However, while embryo and sperm cryopreservation is quite well established in many species, the preservation of the maternal heritage, comprising mitochondrial DNA, is not as efficient. The limited source of gametes, the lack of adult progenitor cells, together with the bio-physical complexity of the oocytes are among the main limiting factors.

The aim of this collection is to provide insights into several methodological approaches, chosen among the most successful, that have been implemented in order to overcome some of the described limits, spanning different mammalian species.

Traditionally, *in vitro* embryo production (IVP) makes use of oocytes that are grown *in vivo* and are extracted from the ovarian context as immature, fully grown oocytes - that then undergo *in vitro* maturation (IVM) - or as mature oocytes. The second approach is typical of medically assisted reproduction in humans and requires hormonal stimulation. However, only a very limited amount of gametes can be recruited this way and the vast majority of the ovarian reserve remains untapped. Here, methodological approaches to access, at least in part, the unexploited resources are described by Barros and colleagues[1], focusing on cow growing oocytes, collected from developmental stages that precede the fully grown. Specifically, gametes collected from small antral follicles are cultured *in vitro* and can be then used for IVM, as in traditional schemes of bovine IVP.

As mentioned above, the IVM step is not normally applied in humans, due to a lower efficiency of the process compared to other species. However, when a hormonal stimulation cannot be scheduled before chemotherapy, radiotherapy, or surgery, for instance, in patients of prepubertal age, or when the intervention must be immediate, or there is a known susceptibility to the ovarian stimulation, IVM constitutes a fertility preservation option. In this scenario, Liu and colleagues[2] suggest a combined surgical and biotechnological approach for patients undergoing benign gynecological surgery, whereby oocytes for IVM are retrieved contextually with the gynecological operation (OP-IVM). The preservation of the patient's fertility is completed by oocyte cryopreservation or fertilization.

Actually, whenever a partner is available, embryo cryopreservation is preferred due to the higher standardization and success compared to oocyte's freezing. The oocyte is in fact a large cell, having a disadvantaged surface/volume ratio, an important lipid content, and enveloped by a protein shelf, all characteristics that make this cell poorly amenable to cryoprotectant permeation and even rates of temperature decrement during freezing and ultra-rapid freezing. However, fertility preservation schemes, in human patients as much as animal specimens, would greatly benefit by such approach. In this view, two protocols are presented that promote oocyte ultra-rapid freezing, also known as vitrification, in feline and ovine oocytes[3,4]. While Succu and colleagues highlight the importance of post-warming culture conditions and timing for the restoration of oocyte developmental potential in juvenile donors[4] that might have implications also in prepubertal human patients, Colombo and Luvoni focus their attention on the development of field-friendly protocols, mainly aiming at safeguarding wildlife biodiversity[3].

An alternative option to oocyte freezing is the cryopreservation of ovarian fragments. These fragments can eventually be previously depleted of larger oocytes to be destined to other procedures as described by Brouillet et al.[5]. Ovarian fragments will then have to be cultured in order to allow for follicles and enclosed oocytes growth, until suitable stages are reached. Alternatively, they can be re-grafted[6]. This approach, however, remains largely experimental and, despite some success providing a proof of principle of the feasibility, it still requires considerable efforts not just from a research and development point of view but also at the basic biology/physiology level, as pointed out by the contribution provided by Winship and collaborators[7].

Last but not least, fertility preservation mandatorily involves the possibility of the formed embryo to implant and develop to term. In this view, an approach to ensure appropriate maternal-fetal recognition making use of minimally invasive surgical interventions in the horse is reported[8].

A comprehensive collection of biotechnological and surgical approaches is presented herein for the research and medical groups that are looking for cutting-edge methodologies for fertility preservation, with the hope that the thorough protocol sharing will be useful for the amelioration of existing practice and for critical thinking of relevant challenges in the field and how they might be overcome.

Disclosures

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