

Anti-Müllerian hormone (AMH) and its multiple purposes in fertility preservation

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Ovarian function is regulated by a complex interaction of hypothalamic, pituitary and ovarian hormones and cytokines. The ovarian cortex is the region that contains the ovarian follicles in different stages of maturation, from primordial to primary, secondary and tertiary follicles. Cyclic ovulation of a tertiary, or antral, follicle is mainly under hypothalamic-pituitary regulation through follicle stimulating hormone (FSH) and luteinizing hormone (LH), however, the activation and development of primordial follicles is under paracrine regulation of mostly unidentified substances produced by the oocyte itself and by the granulosa cells, as well as by the surrounding follicles. The process that transforms a primordial follicle into a primary follicle is irreversible, and the primary follicle is forced to continue its development into a secondary and tertiary follicle, which is destined to ultimately ovulate or undergo apoptosis. As this process carries on throughout a woman's life it gradually depletes the number of primordial follicles available for maturation.

One of the paracrine substances is a member of the transforming growth factor-beta superfamily, AMH, produced by the granulosa cells. AMH exerts an inhibitory role in the recruitment and development of primordial follicles into developing follicles [1, 2]. In fact, in absence of AMH the pool of primordial follicles is rapidly depleted. In engineered AMH-null mice the ovaries contain almost three-fold greater number of growing secondary follicles and a decreased number of primordial follicles [3]. In addition, AMH has been seen to influence the FSH-dependent follicular growth and the cyclic selection for dominance [4]. However, the mechanism by which AMH exerts its regulatory effect on the ovarian follicles is not thoroughly understood.

In the human, AMH directly correlates with the number of antral follicles assessed by ultrasound and FSH, inhibin-B and estradiol serum levels [5, 6]. In addition, AMH serum concentration can predict the age of menopause. Women with polycystic ovary syndrome (PCOS) have high serum AMH levels, which are correlated with the follicle number and the length of the menstrual cycle and ovulation pattern [7-9]. AMH also appears to have a role in stalling follicular development in women with PCOS [10]. In addition, it could have a role in the development of Müllerian anomalies, such as uterine subseptations, in the offspring of PCOS women [11]. While AMH has been used for diagnostic purposes as a biomarker for over 15 years, new potential therapeutic applications have emerged in recent years.

The use of AMH for ovarian tissue cryopreservation and transplant.

During the first week after transplant there is massive depletion of primordial follicles [12]. This phenomenon has been explained with the slow vascularization of the transplanted tissue and hypoxia-driven follicular damage. In transplant studies of previously frozen/thawed tissue, poor vascularization and hypoxia are still the most plausible causes, rather than the cryopreservation process itself [13]. However, recent experiments showed that follicle loss after ovarian cortex transplantation is unlikely due to ischemic apoptosis, but rather to a burst of primordial follicle recruitment [14, 15, 16]. Premature massive activation of primordial follicles, would result in early apoptosis and depletion of the follicular reservoir and early exhaustion of the transplanted cortex's function.

Even though AMH is supposed to prevent activation of ovarian follicles, AMH supplementation during ovary tissue cryopreservation/ thawing has been demonstrated to reduce follicle apoptosis in a mouse model [17]. This was more recently confirmed by Dr. Meirou and his group, who suggested a role of AMH in protecting follicular depletion and activation during treatment with cyclophosphamide, in a mouse model [16]. In addition, our group found that *in vitro* AMH administration was able to prevent ovarian cortex tissue activation by inhibiting its stemness potential (=direct reprogramming of differentiated somatic cells to create induced pluripotent stem cells that are indistinguishable from embryonic stem cells) and direct activation of the oocyte [18]. Further *in vivo* studies showed that exposure to rAMH during the peri-transplant period inhibited the ovarian cortex activation and the subsequent apoptosis after transplant, thus protecting the tissue until vascularization was established and its own granulosa cells' AMH production restarted [19]. However, rAMH was not able to preserve the primordial follicle loss previously caused by the vitrification-warming process, or by the transplant process itself.

These study results provide evidence that administration of exogenous recombinant AMH at the time of ovarian cortex transplant could protect the ovarian follicles from activation and premature depletion, and expand AMH's clinical applications.

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