Mind the gap

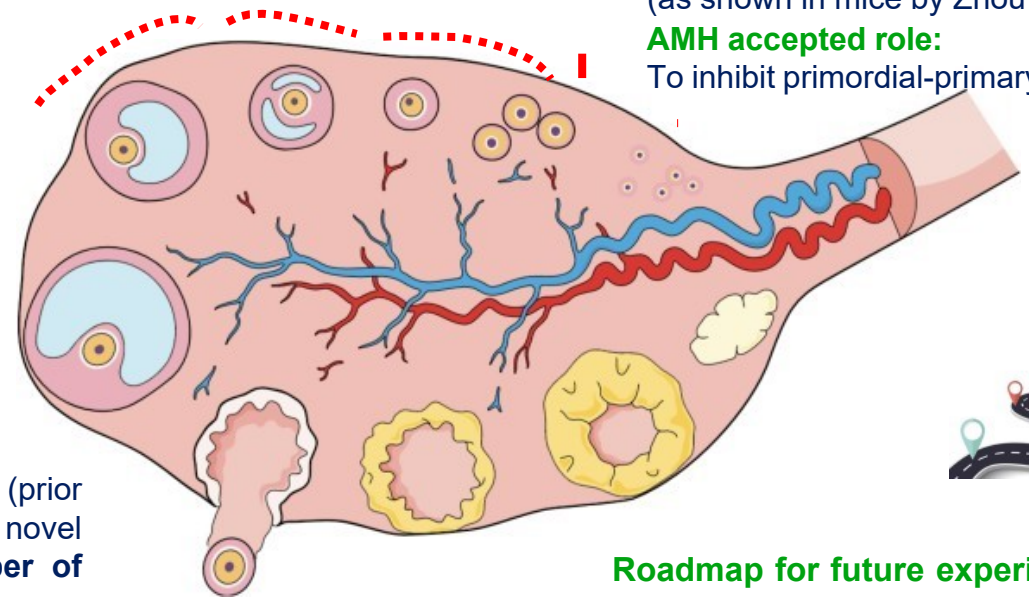
"Mind the gap: Deciphering the role of AMH in follicular development - from animal studies towards clinical application"

What are the new implications of AMH-induced atresia in a clinical context ?

- on AMH concentration levels
- on FSH role rescuing follicles
- on AMH variations during ovarian supression

AMH novel role:
Induce atresia in preantral follicles to prevent follicle pool from becoming too large
(as shown in mice by Zhou *et al*, 2022)

AMH accepted role:
To inhibit primordial-primary follicle transition



Prevention of atresia in **humans** (prior to FSH dependency) could be a novel method to **increase the number of antral follicles for stimulation**

Roadmap for future experimental studies:

- ✓ Alternate ***in vivo* models** to study AMH induced atresia at different time points
- ✓ ***in vitro* or *ex vivo* models** to describe cellular and molecular pathways and biomechanical ovarian tissue features

“Mind the gap: Deciphering the role of AMH in follicular development - from animal studies towards clinical application”

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Anti-Müllerian hormone (AMH) is produced by granulosa cells during follicular development where it inhibits the transition from primordial to primary follicles. It also regulates the follicle-stimulating hormone (FSH)-sensitivity of early antral stages during dominant follicle selection (Broer et al, 2014). The inhibitory effect of AMH on primordial follicle activation has been described in several mammals (Durlinger et al, 2002; Nilsson et al, 2007; Yang et al, 2017) and has been considered the main mechanism maintaining the pool of primordial follicles from early exhaustion (Moolhuijsen and Visser, 2020). Other than the established role of AMH as an inhibitory molecule on follicle activation and the regulation of FSH sensitivity, it is important to decipher if AMH has any other functions. The December edition of the ESHRE Journal Club discussed a basic research paper by Zhou *et al*, (2022), in which an additional role of AMH was suggested, as the results showed experimental evidence of AMH-induced atresia in murine preantral follicles. Zhou et al, used transgenic mice to determine the number of follicles in mice lacking the AMH gene (*Amh*$^{-/-}$) and observed that these animals had less primordial follicles, but more primary, secondary, and small antral follicles compared to wild type mice. The authors observed that this difference was due to a higher survival ratio of primordial follicles transitioning into primary follicles. In mice, overexpressing AMH in central nervous system neurons led to higher AMH levels in circulating blood. These mice had higher rates of follicle atresia as evidenced by apoptosis markers and empty primordial follicles in the ovaries, thus leading the authors to conclude that the role of AMH is not to maintain the ovarian reserve but rather to prevent the antral follicle pool becoming too large through AMH-induced atresia of preantral follicles. If these findings can be generalized to humans, they would represent a paradigm shift on the role of AMH and possibly affect clinical

practice. The Journal Club discussion focused on the different implications of AMH-induced atresia both in biological and clinical contexts. These included the importance of AMH concentration levels, the role of FSH in rescuing follicles from AMH induced atresia, the variation of AMH levels during ovarian suppression in humans as well as the translation of the findings into clinical practice. The Graphical abstract summarises the key points discussed during the ESHRE journal club.

Does the concentration of AMH play a role on follicular atresia?

The systemic function of AMH has not been fully explored and we have very limited knowledge regarding its role other than the primordial-to-primary follicle transition and the regulation of FSH sensitivity. Research in the past decade has shown that AMH acts beyond gonadal expression through the Hypothalamic–Pituitary–Gonadal (HPG) axis, therefore, increased concentration in serum is anticipated (Silva et al., 2021). In the article discussed, authors used transgenic mice overexpressing AMH in neurons with a subsequent increase of the serum levels of AMH. In the discussed paper, AMH concentration in serum increased to ~1nmol/l, which is higher than normal serum concentration but lower than that found in follicular fluid (~6nmol/l). Increasing AMH levels to 6 nmol/L would be anticipated to manifest a stronger effect on follicle atresia. As it has been previously shown, high AMH expression induced by adenovirus blocks 50% of primordial follicle activation in mice, but most importantly, all follicle progression to the secondary stage comes to a halt (Kano et al., 2017). Therefore, by altering AMH concentration we would expect that the concentration of other paracrine factors would also be affected. Such factors include members of the TGF beta family, in particular

Activin that promotes structural integrity of follicles (McLaughlin et al., 2010). Balance between FSH and Activin is critical to maintain connexin 43 expression and oocyte-granulosa cell interactions (El-Hayek and Clarke., 2015). However, knowledge is lacking on the effects of AMH overexpression on cell-to-cell communication, cytoskeleton stability and extracellular matrix composition, important variables that can dictate the physiological state of the ovaries (Amargant et al, 2020; Chan et al, 2021; Ouni et al, 2021).

Can FSH rescue follicles from AMH induced atresia?

The potential of FSH, as a survival molecule, to reduce the AMH induced atresia in preantral follicles was then explored. The main question is whether FSH can rescue follicles from AMH induced atresia or whether AMH can control the number of follicles responsive to FSH. Since both actions likely occur at different stages of follicular development, there is no definite answer to this question and it is hypothesised that AMH could have different roles at different stages of follicular development. Interactions between FSH and AMH are most likely to occur at the early antral stage when FSH sensitivity ramps up, before AMH expression drops rather than at the preantral stage. Oocyte derived factors such as growth differentiation factor 9 (*GDF9*) and bone morphogenetic protein 15 (*BMP15*) might also be involved in AMH-FSH interactions during follicular development (Juengel and McNatty, 2005). It is well known that preantral follicles require FSH to mature in both poly and mono-ovulatory species of rodents and primates (Zeleznik, 2004; Kreeger et al, 2005; Xu et al, 2010). However, it is also known that FSH sensitivity varies among species (Baird., 1987; Ginter et al., 2001) and it has been suggested that the difference between the two ovulation strategies results from

molecular variations in BMP15 and GDF9 (Monestier et al, 2014). Thus, understanding the cellular and molecular interactions in follicle development might involve an interdisciplinary work where physiology, cell biology and evolutionary perspective could control all aspects of follicle maturation and growth in humans for clinical purposes.

Variation in AMH levels during ovarian suppression in humans.

Transitioning from the FSH independent to the FSH dependent phase in humans, the small preantral follicles that survived atresia should mature to antral follicles (Orisaka et al, 2021). This transition is probably gradual with an overlap in the small antral follicles phase: they produce AMH, but are already FSH sensitive (Nelson., 2013). However, there are published results showing temporary temporarily reduced AMH levels in patients using hormonal contraception or with hypogonadotropic hypogonadism (Hariton et al, 2021). These data are collected from large registries, but reported without a clear rationale, so there is still uncertainty in the scientific community about the role of low FSH and LH on circulating AMH levels. The Journal Club participants were given a poll on the role of low FSH on AMH levels: 31.8% of the voters stated that we cannot exclude that the lower levels of AMH observed in patients with low FSH and LH are a case of association and not causation, 36.4% that there is strong evidence of a causal link between low FSH and AMH levels and 31.8% remained unsure on whether there is a connection between low FSH and reduced AMH levels. The results are emblematic of the uncertainty around the topic.

Another example of influence of suppression of ovarian activity on AMH levels can be seen in cancer patients: immediately following chemotherapy, very low or not delectable

levels of AMH are common, although they increase marginally over time (Anderson et al, 2022). Temporary ovarian suppression with GnRH analogues (GnRHa) for ovarian function preservation during chemotherapy (Lambertini et al, 2018) may lead to consequent temporary AMH suppression by reducing the number of small AMH producing preantral follicles. However, the same deflection in AMH levels is seen also when GnRHa are not used and there is temporary chemotherapy-induced amenorrhea and FSH and LH levels are not low, but high (Bala et al, 2016). This observation seems to point to ovarian function suppression, for whichever reason, as the *primum movens* for the observed suppression in AMH levels.

The causal link between the aforementioned causes of ovarian suppression and the observed temporary reduced levels of AMH cannot be questioned. It is logical that prolonged ovarian suppression would lead to a reduced number of AMH producing small antral follicles, hence reduced serum levels of AMH (La Marca et al., 2010). Assuming therefore that AMH plays a role in inducing atresia in humans, what could that role be? We know that the transient decline in AMH levels does not reduce atresia nor preserve ovarian reserve *per se,* so the most rational hypothesis would be that AMH expression declines differently in follicles at different stages, with the acquisition of FSH dependency as a turning point.

Translation into clinical practice

Could prevention of atresia prior to FSH dependency be a novel avenue to increase the number of antral follicles that can be stimulated in ART cycle? There are conflicting results

using AMH in human culture experiments. Follicular development is exceptionally complex with follicles developing in different time frames, in different systems and involving a network of different molecular mechanisms that are not yet unraveled.

What are therefore the required experiments for exploring applying the findings by Zhou et al, into clinical practice? It is necessary that a variety of models and approaches are employed due to the complexities of follicle development. An *in vivo* model can be employed to study atresia by treating with AMH at different time points (using multiple mice of similar age and characteristics) during follicular development, but study design would need to utilise a multi-variate approach. An *in vitro* model without serum or a xenotransplantation model can also be used depending on the question being asked. Using human ovarian tissue in culture in well-designed experiments with sufficient power and clear outcomes will improve our knowledge of the role of AMH. However bovine and ovine ovarian tissue can also be utilised a mono-ovulatory model that is more readily available.

Regardless of the experiment however, careful consideration should be given to structural factors within the ovary and follicle density affecting follicular interactions. Studying cell to cell communication and cytoskeleton stability in the AMH overexpressing mice, mainly the matrix composition such as collagen and Fibrin, can provide insights into cell death pathways since they could be affected by biomechanical characteristics of the tissue (Ouni et al, 2021). Furthermore, there is not enough research on changes in AMH levels when follicle development is disrupted or altered. Does the drop in AMH occur due to fewer antral follicles when FSH is low or are the granulosa cells responsible for switching

off AMH production? Further exploration is essential to fully understand these molecular interactions.

Conclusion

Follicular development is exceptionally complex and involves a net of different mechanisms that are not yet unraveled. AMH appears to have a more central role in early follicle development than what was originally thought. Deciphering the precise role of AMH could be pivotal and exploring applications for humans could be done by combining various approaches such as *ex vivo* models, xenotransplantation, and evolutionary biology.

Data availability

No datasets were generated or analyzed in the current manuscript.

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Conflicts of interest

All authors report no conflicts of interest to declare**.**

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