Anti-Müllerian hormone levels, numbers and sizes of antral follicles in regularly menstruating women referenced to true ovulation day

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Introduction

- Anti-Müllerian hormone (AMH) may vary throughout the menstrual cycle, though evidence is conflicting, due to differences in assay performance and methodologies used to assign menstrual cycle phase in previous studies[1]
- In experimental settings, AMH appears only to be produced substantially by small antral follicles, but this has not been confirmed in a healthy population of regularly menstruating women.[2]

Objectives

- To establish whether AMH levels vary throughout the menstrual cycle in women of reproductive age and whether AMH production is linked to follicle size.

Methods

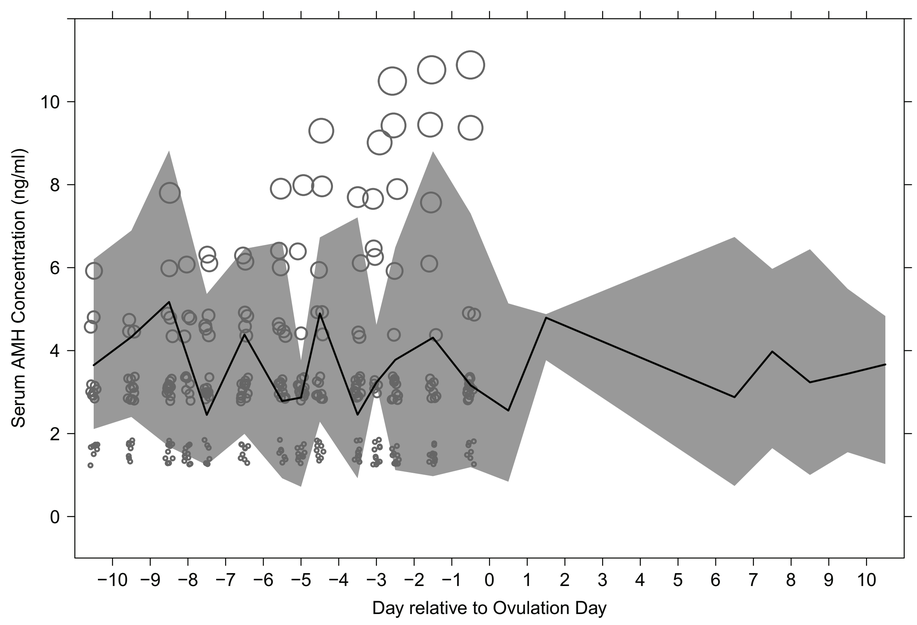
- Daily urine samples were collected from complete menstrual cycles of volunteers in the **Me**nstrual Cycle **Mo**nitoring Study (MeMo; NCT01802060)[3,4]
  - o N=40; all regularly menstruating with documented ovulation; aged 18–37 years, mean age 28.9 years
- Volunteers visited the study centre for transvaginal ultrasound during one complete menstrual cycle
  - o Visits were every 2 days, or daily from follicle size >16mm until post-ovulation
- Urinary AMH was measured using the Beckman Coulter Generation II AMH assay (Beckman Coulter, Inc., Webster, Texas)[2]
- Poisson mixed-effects models were used to analyse AMH variability and correlation of follicle counts and size classes with AMH levels.

Results

Mean AMH concentrations vary across ovulatory menstrual cycles

- The behaviour of AMH concentration across the menstrual cycle, aligned to true day of ovulation is shown in Figure 1.

Figure 1: AMH fluctuations and antral follicle counts by day, referenced to ultrasound-observed ovulation.

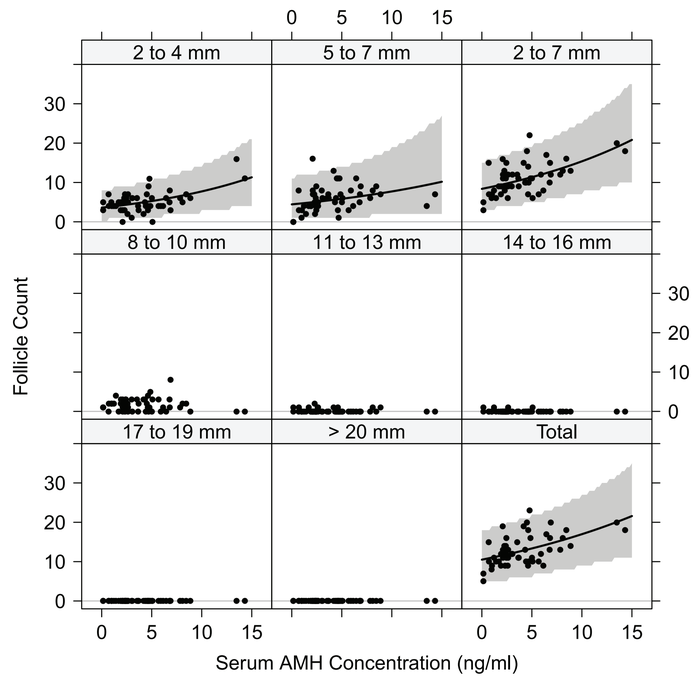


Median AMH level with 10th to 90th percentile range and 90th percentiles of the antral follicle count in seven size classes by day relative to ultrasound-observed ovulation for all days with n>2 samples. The number of circles represents the 90th percentile of the antral follicle count, while their diameter indicates the size class (2–4, 5–7, 8–10, 11–13, 14–16, 17–19, ≥20 mm, represented by size of circle [from smallest to largest]).

AMH is only produced by small antral follicles (2–7 mm)

- Figure 2 maps the AMH production to the size of follicles, showing that AMH is predominantly produced by follicles of size 2–7mm whereas there is virtually no production from follicles of greater size.

Figure 2. Antral follicle counts for AMH levels measured on cycle days 4–7 per size class and in total.

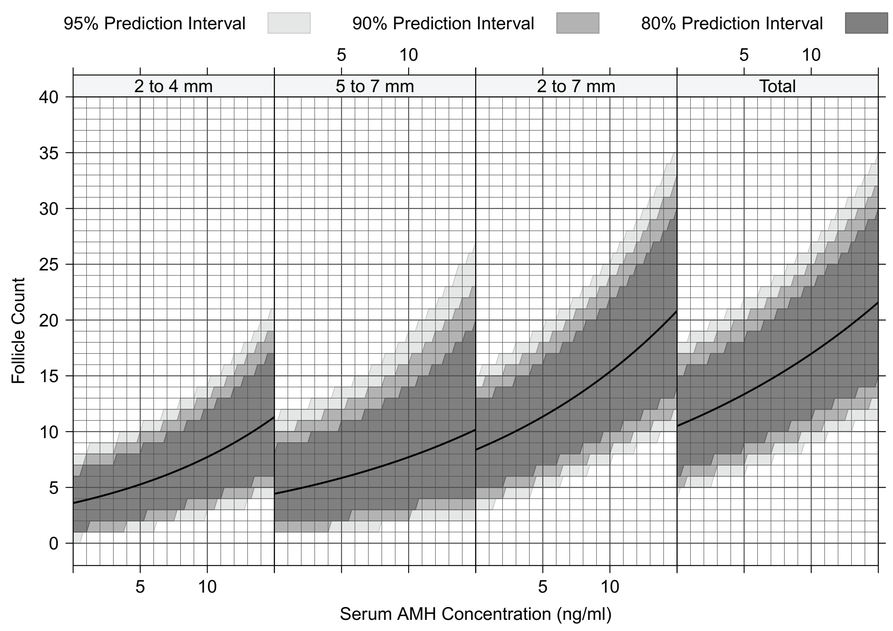


The regression lines correspond to the coefficients; grey bands represent pointwise 95% prediction intervals which cover the follicle count corresponding to the measured AMH level on cycle days 4–7 with a probability of 95%.

The number of small antral follicles can be estimated from the level of follicular phase AMH

- Poisson mixed-effects models to estimate the number of antral follicles from AMH levels were created using days 4–7 (Figure 3) and days 4–ovulation
- Both approaches led to nearly identical predictions for the number of small antral follicles
- Prediction intervals for the days 4–ovulation model were wider, due to the larger variance of the random effects.

Figure 3. Antral follicle counts per size class to AMH levels for cycle days 4–7; regression lines and prediction intervals for three common coverage probabilities (80, 90 and 95%).



Conclusions

- **AMH shows slight variation across the menstrual cycle according to its physiological role in follicular maturation**
- **The findings reported here provide conclusive evidence that AMH is only produced in substantial quantities by small antral follicles measuring 2–7 mm**
- **The number of antral follicles may be estimated from AMH levels when measured prior to ovulation, with more precise prediction if sampling is conducted on days 4–7 of the menstrual cycle**
- **These findings may have an impact on dosage in ovarian stimulation, prediction of ovarian response, and diagnosis in cases of ovulation disorders associated with difficult or impossible vaginal ultrasound. In the future, facilitate comparison of different AMH assays as a step to standardisation.**

References

1.Rustamov O, *et al*. J Clin Endocrinol Metab (2014) 99: 723–732. 2.Kumar A, *et al*. J Immunol Methods (2010) 362: 51–59. 3.Johnson S, *et al*. Clin Chem Lab Med (2015) 53:1099–1108. 4.Roos J, *et al*. Eur J Contracept Reprod Health Care (2015). doi:10.3109/13625187.2015.1048331.

Disclosures

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