Approaches to Determining the Follicle Reserve

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Overview

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• Non-growing follicles
  – normative models
  – populations at birth and menopause
• Model validation
• Indirect measures of ovarian reserve

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• Equations
• Statistics
  – p-values
  – Correlation coefficients
  – Confidence intervals
• Derivation details
Non-Growing Follicles

• Ovarian reserve
  – Born with a population that declines until menopause
  – NGFs are selected for maturation

• Impossible to measure *in vivo*
  – Using current technologies

• Populations are counted *in vitro*
  – Histological examination of stained tissue
  – Micro-CT and/or High-Power MRI may improve the process
• All based on histology
• Ovaries are sliced, stained and either photographed or observed directly
• NGFs counted in only a sample of the tissue
  – if 10% is sampled (say)
  – then multiply the count by 10 to get the population
• This assumes that NGFs are distributed evenly in the tissue
  – which we know not to be the case
• We like our model
  – but we’re biased, of course
• Uses all the data we know about
• Includes the population increase phase
• Does not assume an age at menopause
  – other studies forced the curve through an end-point
• Has a lower population at birth than the others
  – 300,000 NGFs per ovary
How many NGFs at birth?

• “In other words, primary oocytes reach their maximum development at ~20 weeks of gestational age, when approximately seven million primary oocytes have been created; however, at birth, this number has already been reduced to approximately 1-2 million”

• This is now thought to be incorrect

• Where did the number come from?
How many NGFs at birth?


• He counted (or estimated) one ovary at birth to have 1,011,800 NGFs

• He counted (or estimated) one ovary at 22 weeks gestation to have 3,415,800 NGFs

• When other studies are taken into account, Bakers numbers become outliers
How many NGFs at birth?
How many NGFs at birth?

- 2010, Wallace & Kelsey: 300,000
- 2008, Hansen et al.: 520,000
- 1992, Faddy & Gosden: 1,000,000
- 1963, Baker: 1,000,000
- 1932, Simkins: 143,000
- 1930, Schröder: 36,000 to 300,000
- 1912, von Hansemann: 31,000
- 1879, Sappey: 422,000

Primary reference: Simkins CS. Development of the human ovary from birth to sexual maturity, Am J Anat. 1932; 51(2)
How many NGFs at birth?

• One million per ovary is probably way too high
• My view is that Sappey was about right 146 years ago
  – no preconceptions, no comparisons with other mammals
• A long periods of underestimates
  – why should so many be present when so few mature into eggs?
• High estimates or outliers then dominate
• Data-driven studies show 300,000 to 400,000
  – but with wide variation from the average number
How many NGFs at menopause?

• 1,000 was an educated guess used by everyone
  – no direct evidence from histology studies
  – the assumption is that below this number there is insufficient to support recruitment towards full maturation.

• Recent results suggest a lower number
  – 2008, Hansen et al.: 750
  – 2010, Wallace & Kelsey: 790
  – 2015, Depmann et al.: 500
NGF Model Validation

• “All models are wrong, but some are useful”

• Does the model accurately predict
  1. age at menopause?
  2. Mean Follicle Densities in ovarian tissue?
  – both from subjects not used to derive the model

• If so, we can consider the model externally validated
  – unless and until new data arrives that contradicts the model predictions
Age at Menopause

• Predicted NGF numbers and distribution of menopausal ages from the Prospect-EPIC cohort (n = 4,037) were compared

• The distributions of observed age at natural menopause and predicted age at natural menopause showed close conformity
  
Mean NGF density values were obtained from 13 ovarian cortical biopsies (16-37 years). These values were compared to age-matched model generated densities.

Age-related NGF and ovarian volume models were combined, assuming that a large ovary contains more NGFs than a small one.

• Ideally, we’d have a machine that counted NGFs safely *in vivo*
  – this machine isn’t going to exist any time soon
• There are currently three things that **can** be measured that suggest a high, average or low ovarian reserve
  1. Ovarian volume (OV)
  2. Antral follicle counts (AFC)
  3. Anti-Müllerian Hormone (AMH)
• Each has strengths and weaknesses
• From about age 19, ovarian volume declines in line with the decline in NGF population

• Claim: “An average-sized ovary contains an average number of NGFs. Moreover, a small ovary contains a small number of NGFs, and a large ovary contains a large number of NGFs”

• There is no evidence for this claim
  – apart from the J Assist Reprod Genet. 2015;32(7) paper, which gives indirect support

• If the claim is true in general, then OV is a very good indirect marker of ovarian reserve
• Again, AFC declines as NGF numbers decline for ages 16 through 50 years
  – and there is a good normative model
  – the average 30 year old has 11 antral follicles
  – 50% have between 8 and 15
  – 90% have between 5 and 22
  – so a 30 year old with fewer than 5 is in the low 2.5 percentile
  – indicating likely POI

• This model is wrong, but appears to be useful

• From about 25 years, AMH declines in line with the rate of recruitment of NGFs towards maturation
  – the assumption is that a low rate of recruitment is caused by a low ovarian reserve, and this is reflected in the low serum AMH
  – again, direct evidence for this is lacking
  – but indirect evidence is that AMH is produced by granulosa cells of the developing pre-antral and antral follicles
• My primary research interest is oncofertility
  – so I’m interested in young ages as well as 25 years plus
  – and I need models for zero chemo- and radiotherapies
• I like OV, AMH and AFC (in that order)
• For a more general perspective, I’ve stolen from a recent review by a colleague
- Good predictive value for the number of oocytes retrieved and stimulation response.
- May help guide protocol and other treatment decisions.
- Easy to perform and personalize.
- Fairly non-invasive.
- Provides immediate results.

- Must be done at the beginning of a cycle due to intra-cycle variation.
- Inter-centre variations.
- May be overestimated owing to inclusion of atretic follicles.
- Inappropriate for many juvenile and adolescent individuals.
- Greater inter-cycle variation with overweight women.
- Costs of ultrasound technician machine.
AMH

- Good predictive value for the number of oocytes retrieved and stimulation response.
- May help guide protocol and other treatment decisions.
- Well-characterized across adolescent and reproductive ages.
- Can be performed at any point during a cycle (low intra-cycle variability).
- Good inter-cycle consistency.
- Good inter-operator and inter-centre consistency.
- Relatively low cost (depending upon the specific assay).

- Labour intensive, requiring several hours (note: a new fully automated assay will take minutes and thus eliminate this disadvantage).
- Requires careful sample preparation and storage.
- No standardization across assays.
Summary

• We know very little about the human ovarian reserve – both for specific individuals and for the general population
• This due to the wide natural variations and the technical difficulties – it’s a hard problem!
• We do have data-driven models that show some utility
• OV, AFC and AMH can be used as indirect indicators of ovarian reserve
  – for ages 25 and older
  – for younger ages we have nothing reliable as yet
• Much more collaborative research is needed
Any questions?