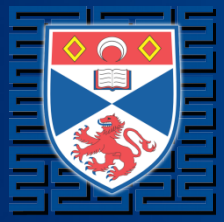


University of St Andrews
School of Computer Science

Approaches to Determining the Follicle Reserve

CFAS 2015

Halifax, Nova Scotia

Tom Kelsey PhD

Colleagues & Competing Interests

- W Hamish B Wallace, Oncology, Edinburgh, UK
- Richard A Anderson, OBGYN & Endocrinology, Edinburgh, UK
- Scott M Nelson, OBGYN & Endocrinology, Glasgow, UK
- Richard Fleming, Biochemistry, Glasgow, UK
- Claus Yding Andersen, Reproductive Physiology, Copenhagen, DK

- I have no competing financial interests to declare
- My research is funded exclusively by UK Research Councils and Cancer Charities

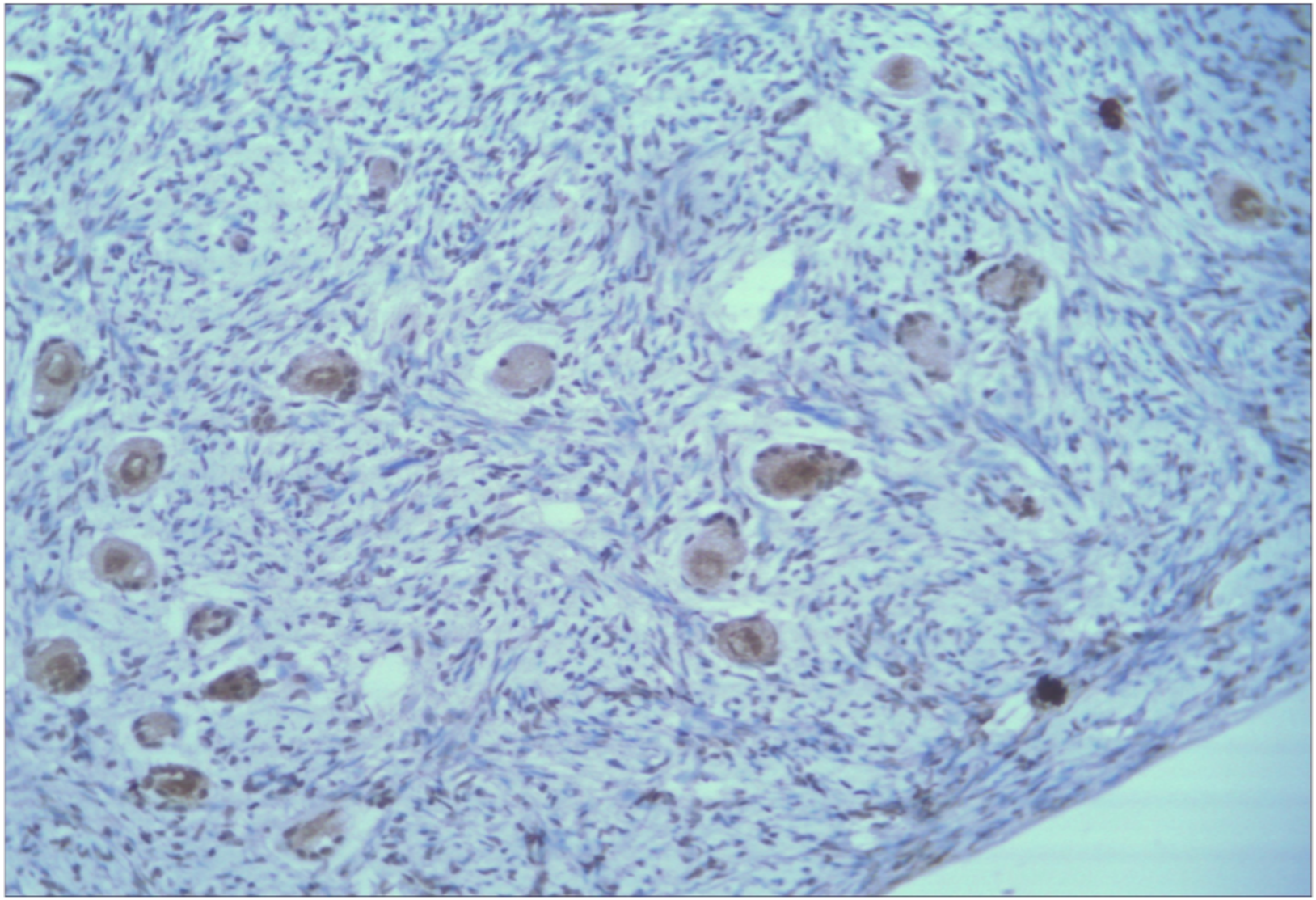
Overview

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

- Equations
- Statistics
  - p-values
  - Correlation coefficients
  - Confidence intervals
- Derivation details

Non-Growing Follicles

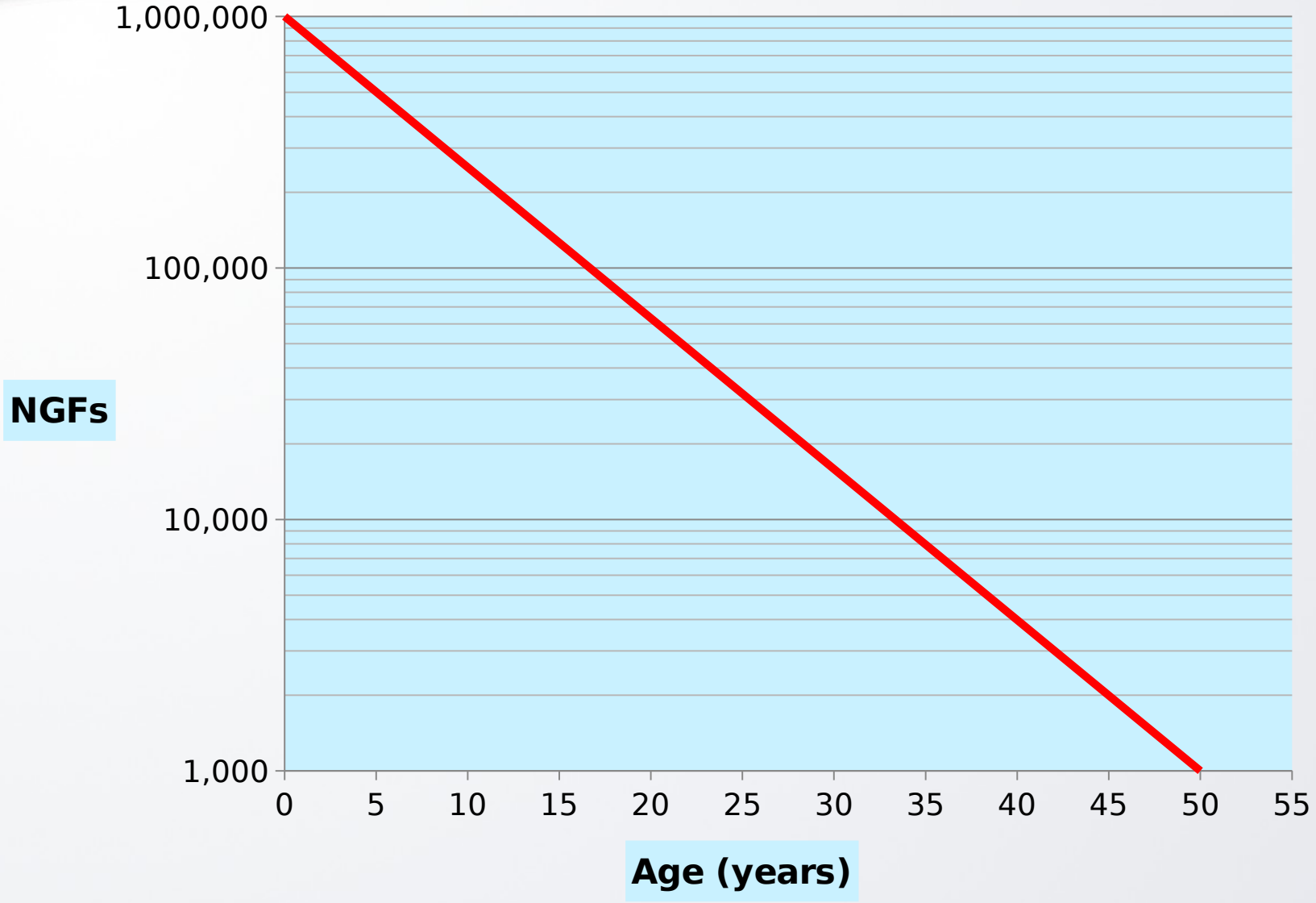
- Ovarian reserve
  - Born with a population that declines until menopause
  - NGFs are selected for maturation
- Impossible to measure *in vivo*
  - Using current technologies
- Populations are counted *in vitro*
  - Histological examination of stained tissue
  - Micro-CT and/or High-Power MRI may improve the process



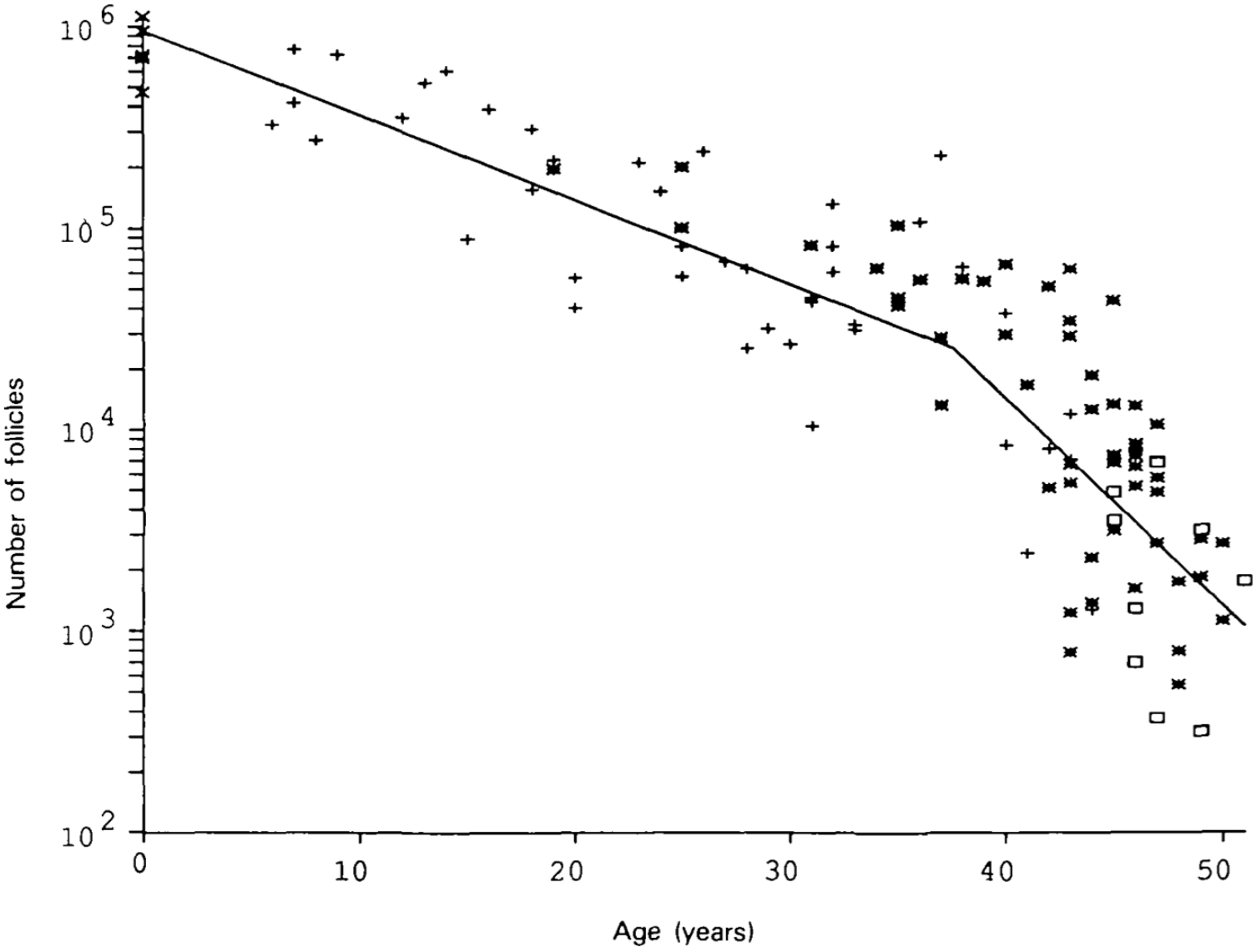
Models of NGF population with age

- 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

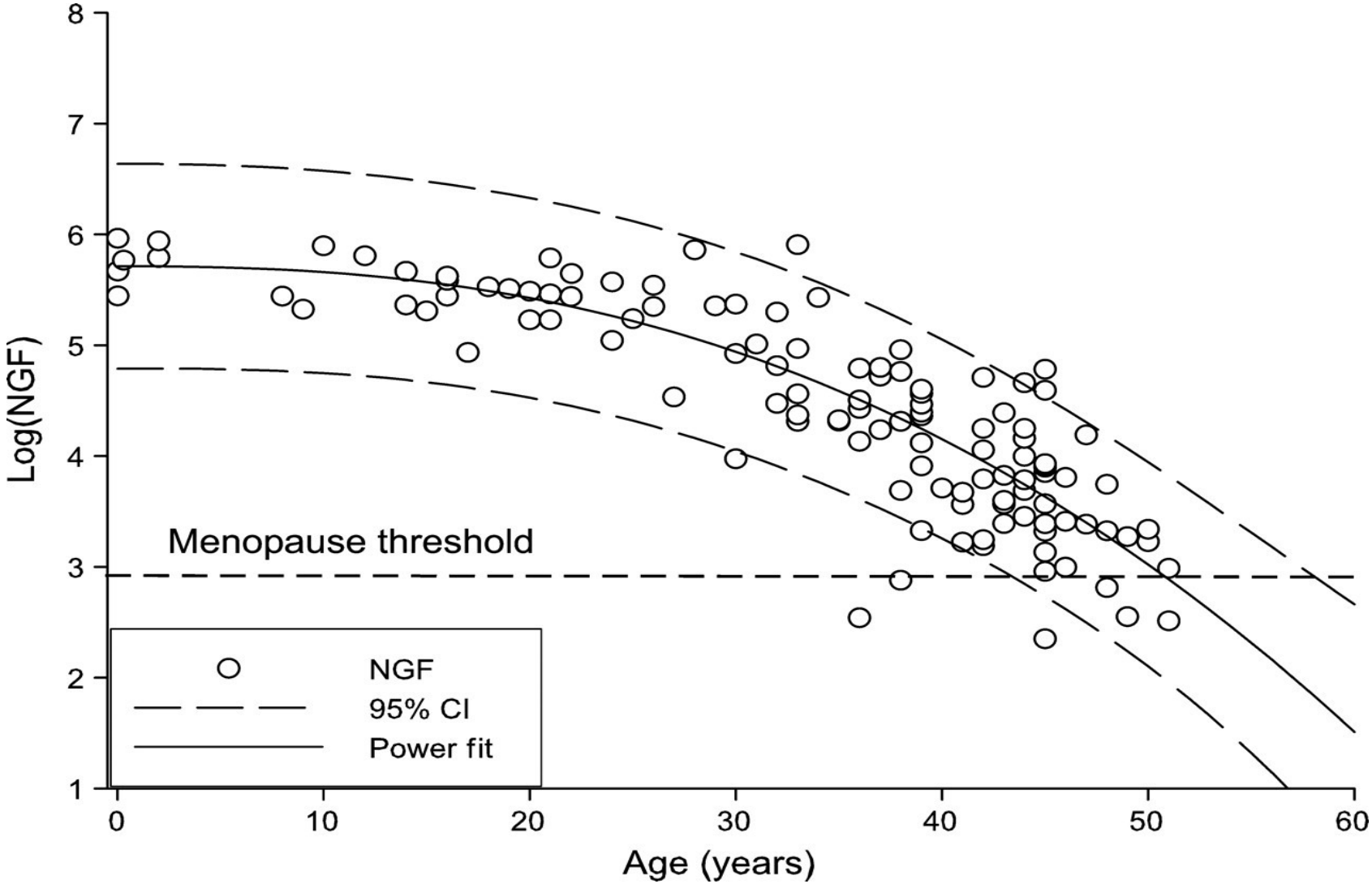
NGFs - WHB Wallace, MD Thesis 1989



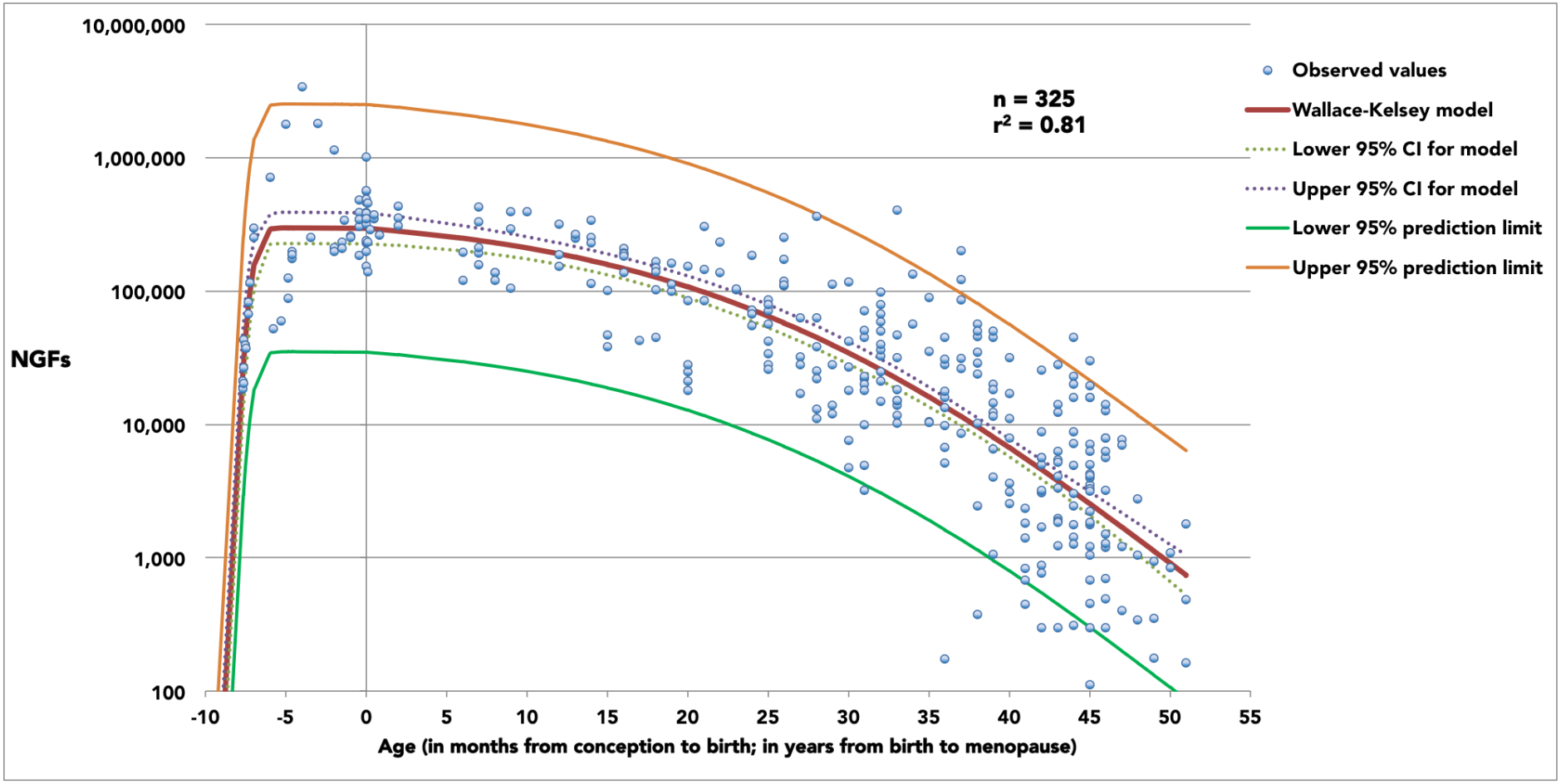
NGFs - Faddy & Gosden 1992



NGFs – Hansen et al. 2008



NGFs – Wallace & Kelsey 2010



NGFs – Wallace & Kelsey 2010

- 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

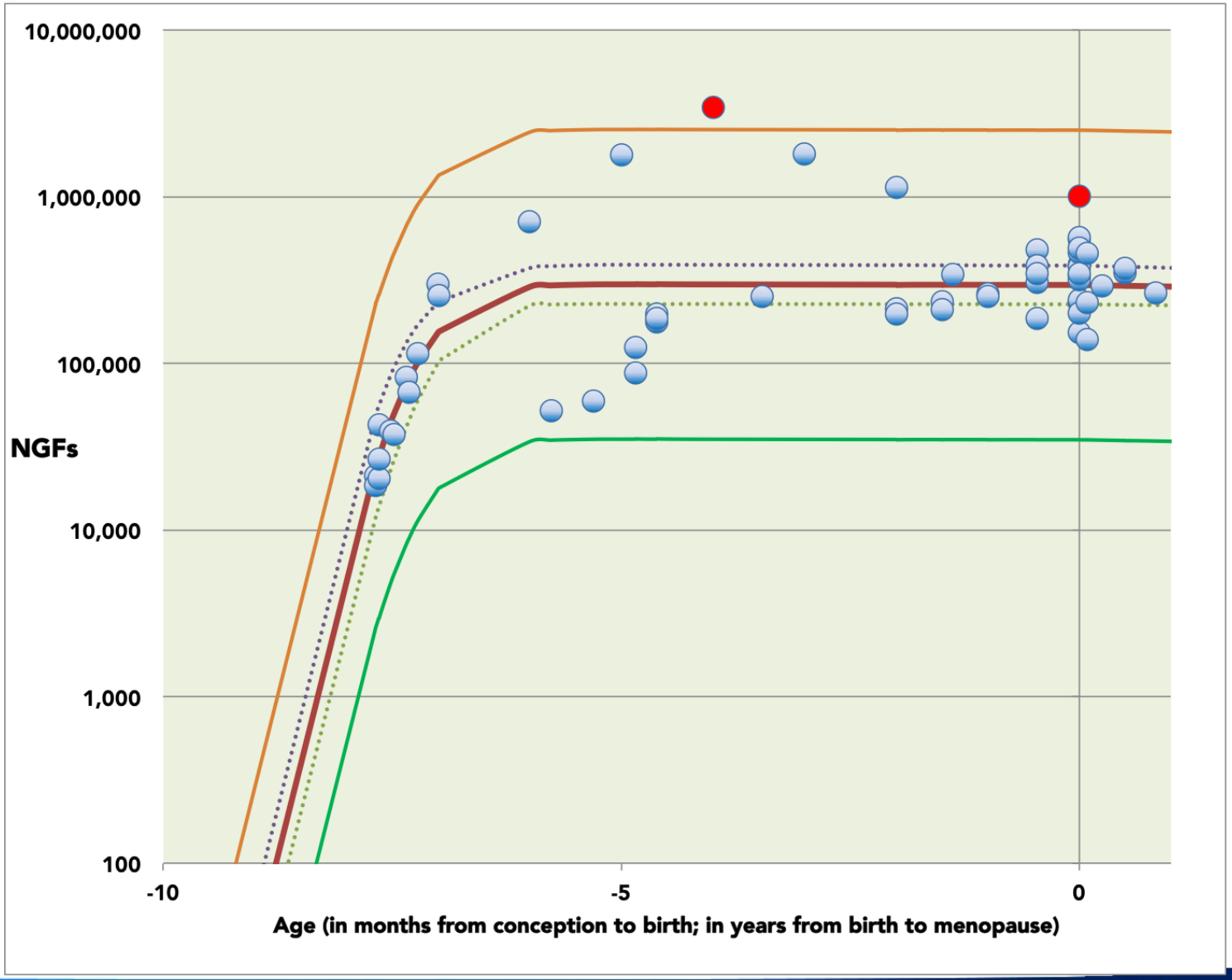
How many NGFs at birth?

- "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"
  - https://en.wikipedia.org/wiki/Oogenesis
- This is now thought to be incorrect
- Where did the number come from?

How many NGFs at birth?

- Baker T (1963) A quantitative and cytological study of germ cells in human ovaries. Proceedings of the Royal Society of London Series B, Biological Sciences 158: 417-433.
- 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"
  - Box, G. E. P., and Draper, N. R., (1987), *Empirical Model Building and Response Surfaces*,
- 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

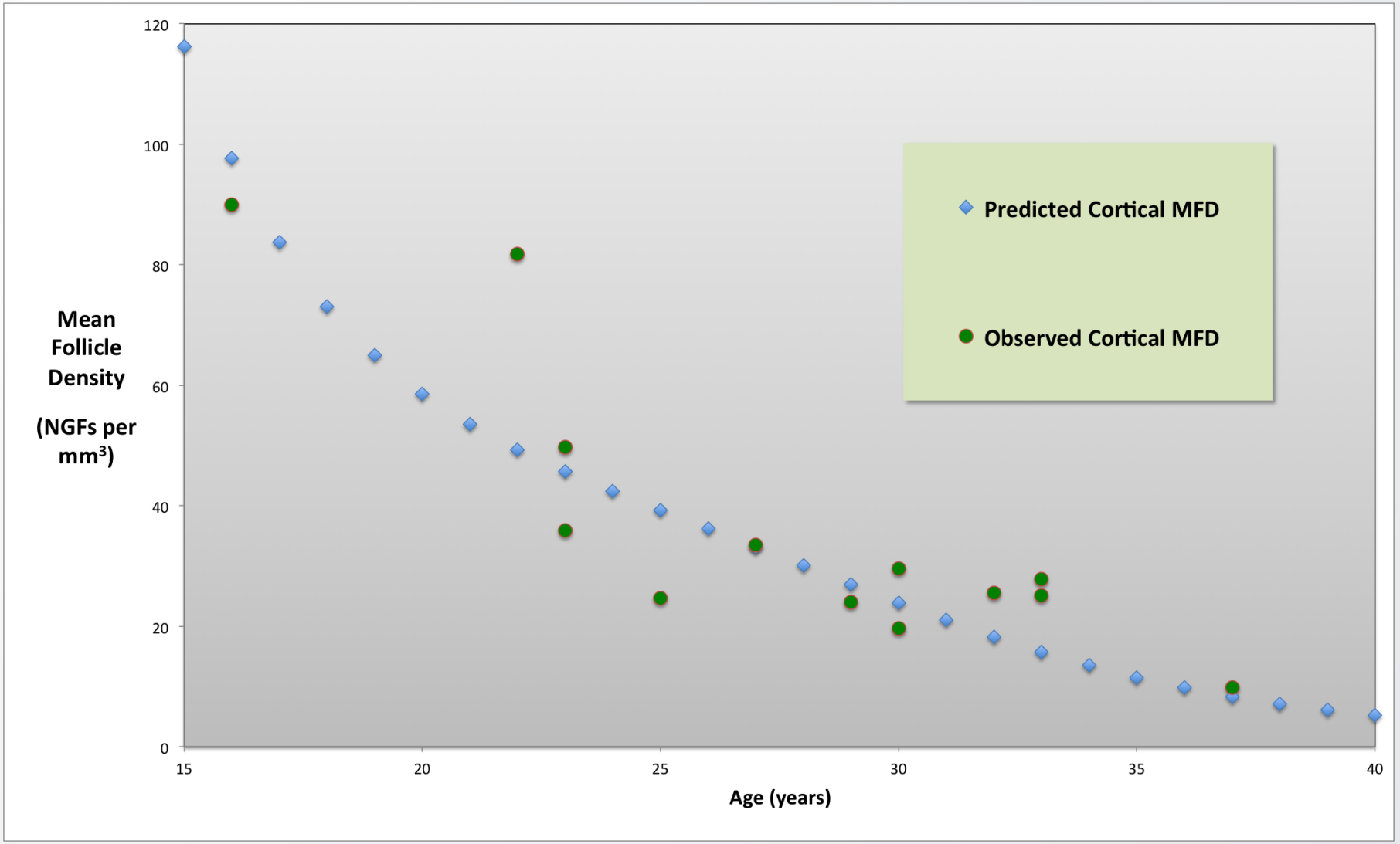
Age at Menopause

- 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
  - Depmann M, Faddy MJ, Van der Schouw YT, et al. *The Relationship Between Variation in Size of the Primordial Follicle Pool and Age at Natural Menopause*. J Clin Endocrinol Metab. 2015;100(6):E845-51.

Mean Follicle Density

- 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
  - Mclaughlin M, Kelsey TW et al. *An externally validated age-related model of mean follicle density in the cortex of the human ovary*. J Assist Reprod Genet. 2015;32(7):1089-95.

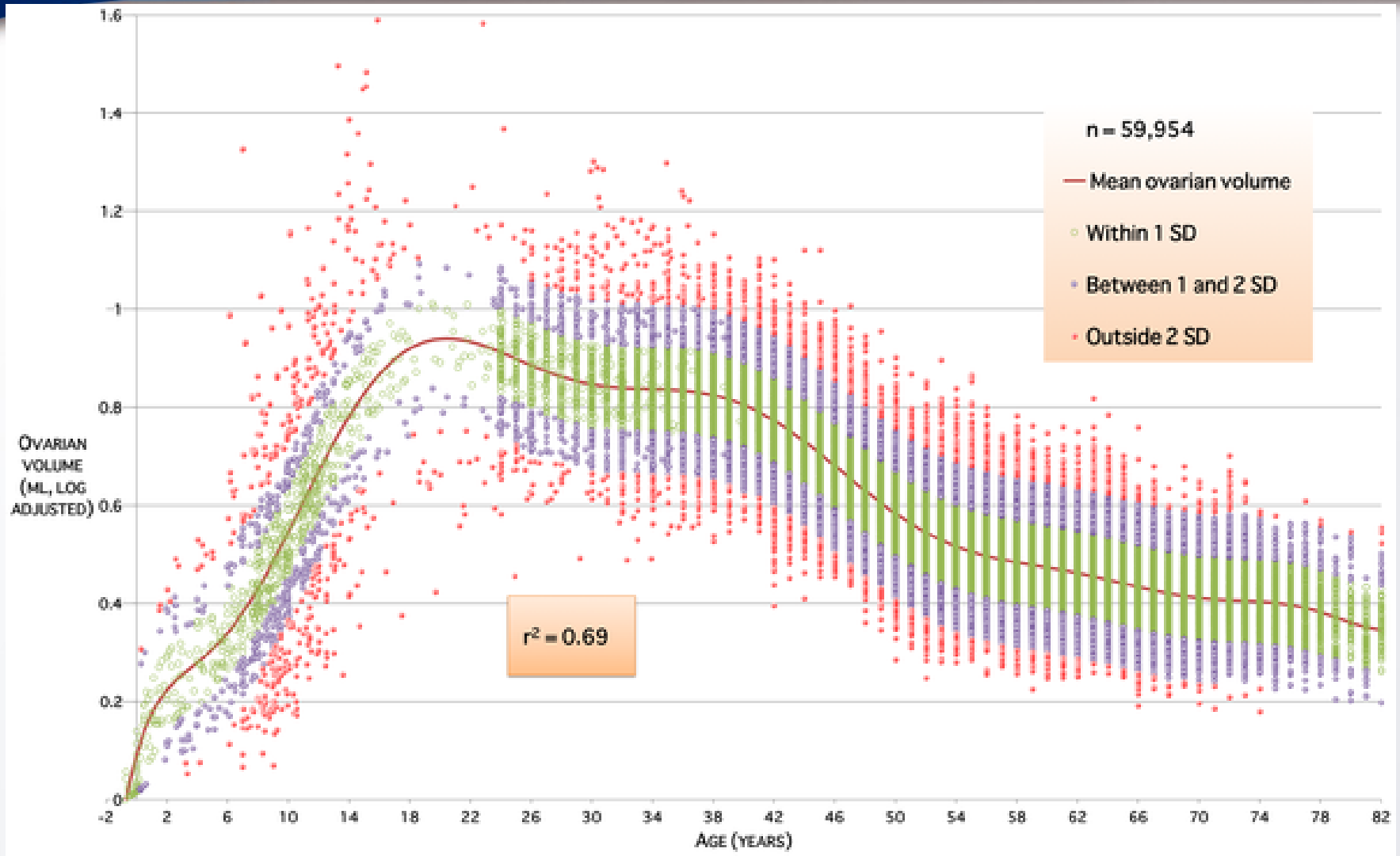
MFD Model


120
100
80
60
40
20
0
15
20
25
30
35
40
Mean Follicle Density (NGFs per mm³)
Age (years)
Predicted Cortical MFD
Observed Cortical MFD

Indirect Measures of Ovarian Reserve

- 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

Age-related model of Ovarian Volume

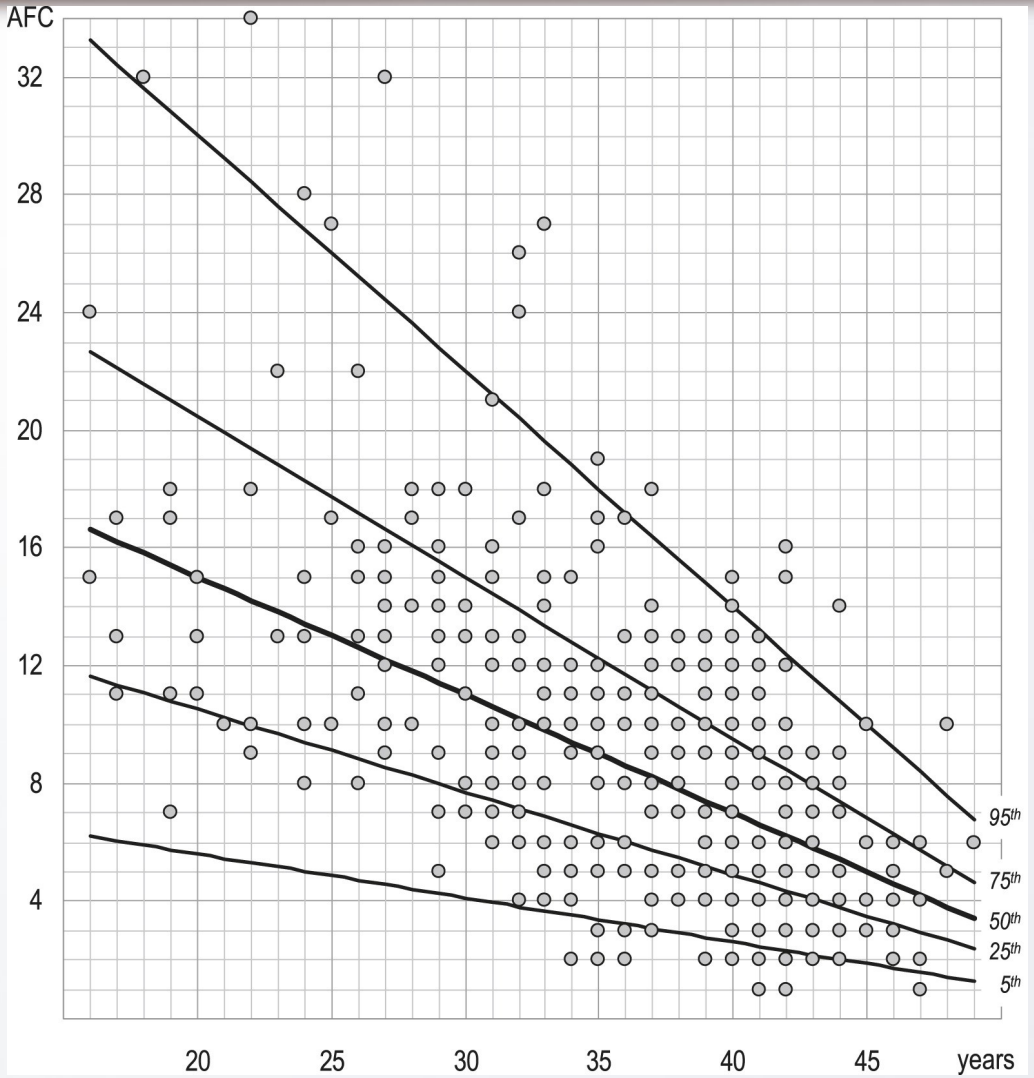


Kelsey TW et al. Ovarian volume throughout life: a validated normative model. PLoS ONE. 2013;8(9):e71465.

Ovarian Volume

- 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

Antral Follicle Counts

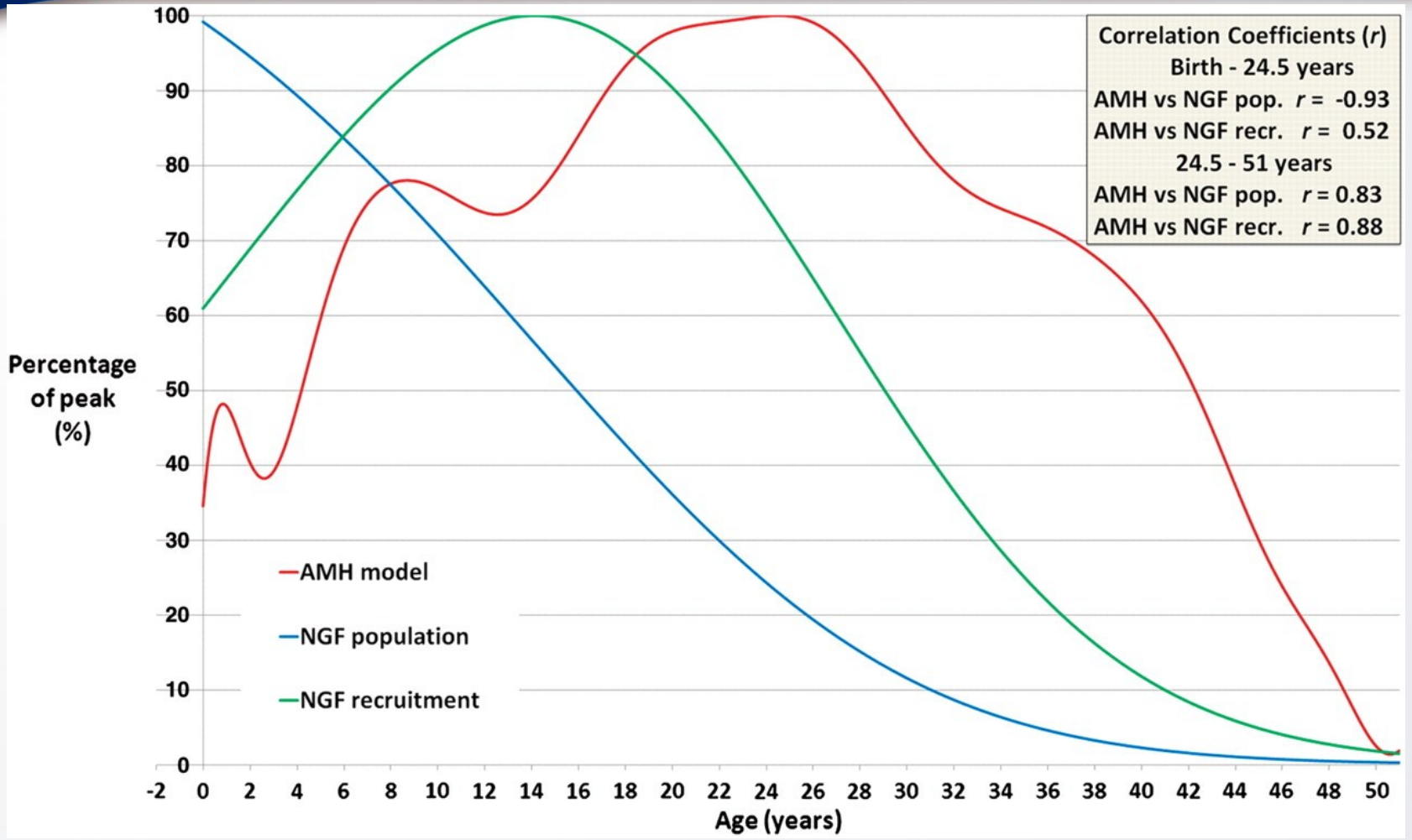


La Marca A et al. Age-specific nomogram for the decline in antral follicle count throughout the reproductive period. Fertil Steril. 2011;95(2):684-8.

Antral Follicle Counts

- 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

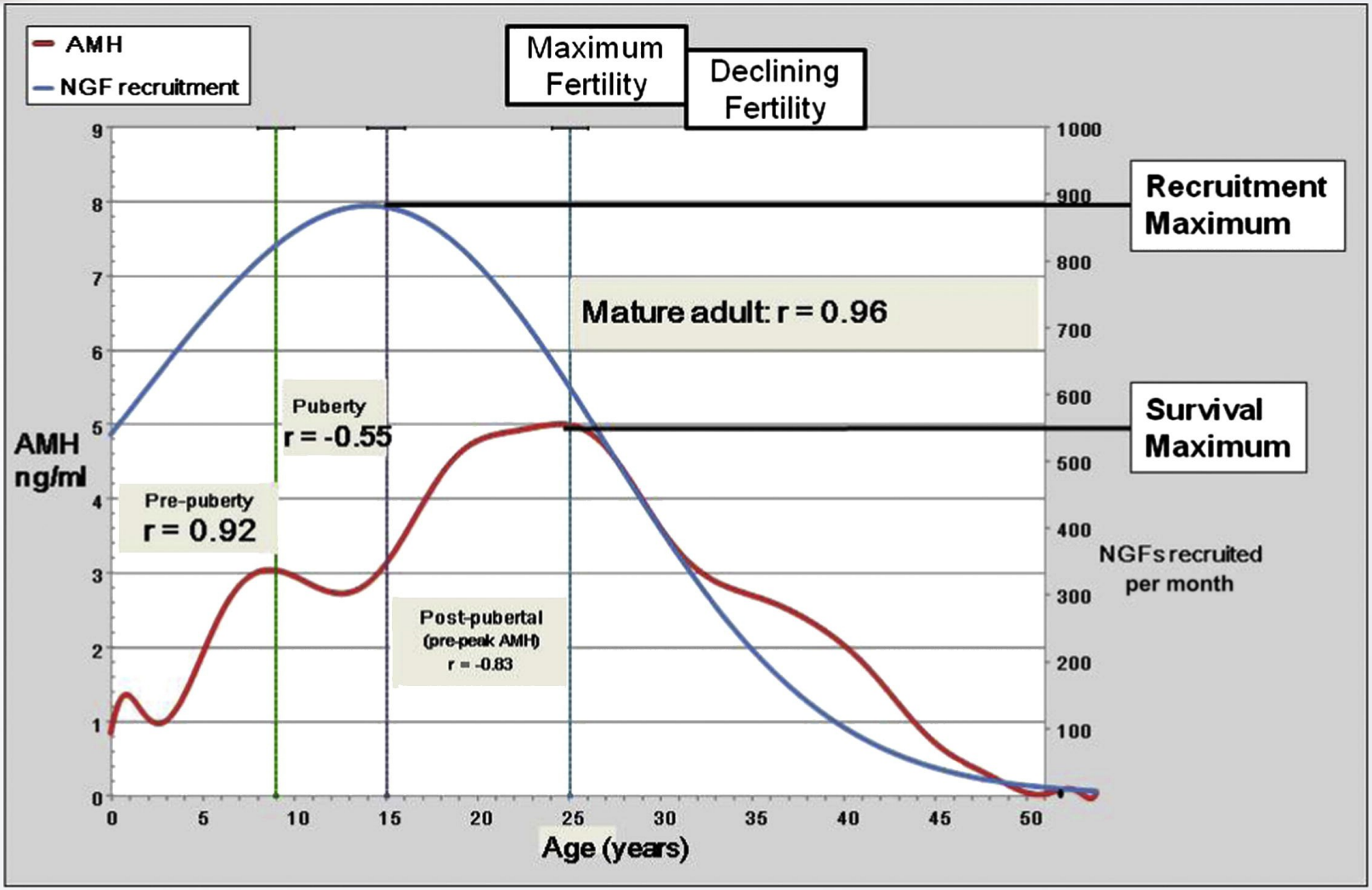
Anti-Müllerian Hormone



Kelsey TW et al. Data-driven assessment of the human ovarian reserve. Mol Hum Reprod. 2012;18(2):79-87.

Kelsey TW et al. A validated model of serum anti-müllerian hormone from conception to menopause. PLoS ONE. 2011;6(7):e22024.

Anti-Müllerian Hormone



Fleming R, Kelsey TW et al. Interpreting human follicular recruitment and antimüllerian hormone concentrations throughout life. Fertil Steril. 2012;98(5):1097-102.

Anti-Müllerian Hormone

- 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

Which is the best marker?

- 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
  - Fleming R et al. Assessing ovarian response: antral follicle count versus anti-Müllerian hormone. Reprod Biomed Online. 2015; epub

AFC

- 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

Thank You

Any questions?