VALIDATION OF THE ESTRADIOL III ASSAY IN THE OVULATORY PHASE

Dr. A.J. Laskewitz[1], Dr. I. Homminga[2], L. Wagenmaker[3], Dr. A.C. Muller Kobold[3]

1 Laboratory of Clinical chemistry and Hematology, Certe KCL, Leeuwarden, The Netherlands, contact: a.laskewitz@znb.nl
2 Department of Obstetrics and Gynaecology, Section Reproductive Medicine, University of Groningen, University Medical Center Groningen, The Netherlands
3 Department of Laboratory Medicine , University of Groningen, University Medical Center Groningen, The Netherlands.

INTRODUCTION

- Estradiol (E2) is an important parameter in the evaluation of female fertility, especially in determining the right moment for oöcyte retrieval in Modified Natural Cycle In Vitro Fertilization (MNC-IVF).
- E2 is routinely measured by an immunochemical method.
- In our hospital we changed in 2014 from the AutoDELFIA assay (AD, PerkinElmer, fluorescence immunoassay) to the Estradiol II assay (E2[II], Roche Elecsys, chemiluminescence immunoassay) to be able to perform the assay 24hr/7. Within a year after the introduction of E2[II], the manufacturer introduced the Estradiol III assay (E2[III]) using two recombinant monoclonal antibodies instead of the former polyclonal antibodies. However, this change would lead to another change in reference values, which is disturbing continuity in patient care.
- This study was aimed to validate the E2[III] for use in determining E2 for the right moment of oöcyte retrieval in MNC-IVF.

Advantages E2[III] (Roche Elecsys)
- Fast, small sample volume
- Recombinant antibodies
- Broad measuring range
- Standardized to ID-GC-MS

METHODS

- Estradiol levels related to follicle size from patients followed in the MNC-IVF in 2013 (using AD) were compared with levels from patients from 2015 (using E2[II]) by status research.
- E2[III] was compared to E2[II] according to the CLSI EP9 and EP5 protocols.
- Samples from 23 patients in the MNC-IVF were compared by measuring with both E2[II] and E2[III] assays.
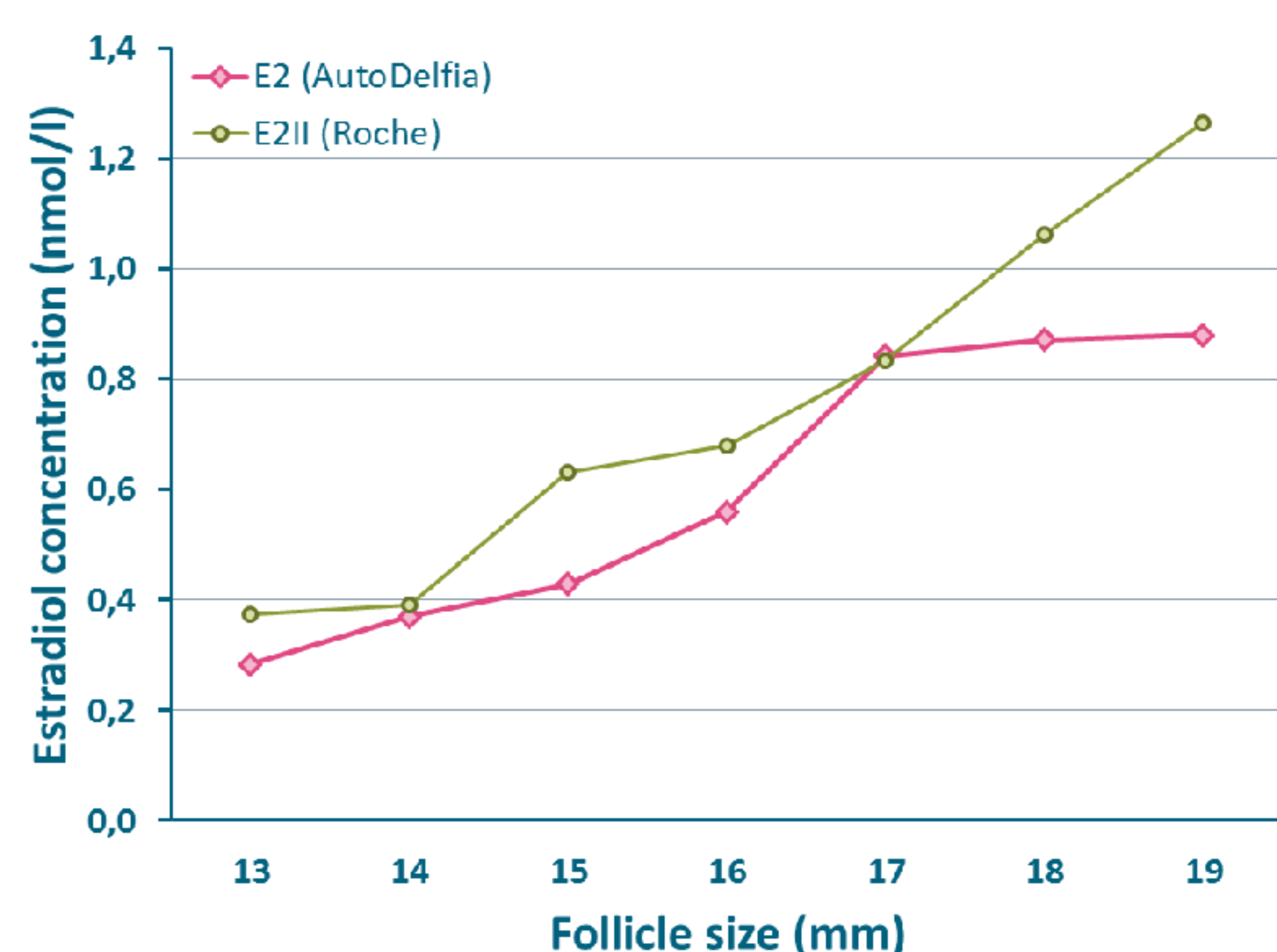
RESULTS

Figure 1 – Estradiol levels related to follicle size
The E2[II] assay results in ~25% higher estradiol levels compared to AD assay, which was also the conclusion of the previous performed comparison. At follicle size 18mm average estradiol levels were 0,87 nmol/l (AD) *vs.* 1,06 nmol/l (E2[II]).

Table 1	E2[II]			E2[III]		
	n	5% - 95%	median	n	2.5% - 97.5%	median
Female						
- Fol	88	0.05 - 0.61	0.228	146	0.045 - 0.854	0.196
- Ov	49	0.32 - 1.83	0.812	150	0.151 – 1.461	**0.462**
- Lut	83	0.16 - 0.77	0.389	151	0.082 – 1.251	0.370
- pMP	32	0.00 - 0.20	0.044	142	0.000 - 0.505	<0.018
Male	109	0.028 - 0.156	0.076	146	0.0994 - 0.192	0.133

Table 1 – Old and new reference values
Table 1 shows the reference values according to manufacturer for the E2[II] and E2[III] assay. The differences in the values can not be explained by the 15% difference in the assay comparison (see Figure 2 and 3).
Fol = follicular phase (day 1-12), Ov = Ovulation (dag 12-14), Lut = Lutheal phase (14-28), pMP = postmenopausal.

RESULTS (CONTINUED)

Table 2	Intra assay		
(nmol/l)	ctrl low	ctrl medium	ctrl high
GEM	0,310	0,631	1,304
SD	0,0071	0,0101	0,0169
CV	2,3% (3,7)	1,6% (1,6)	1,3% (1,1)

Table 3	Inter assay		
	ctrl low	ctrl medium	ctrl high
GEM	0,300	0,617	1,267
SD	0,017	0,021	0,033
CV	5,8% (5,7)	3,4% (2,5)	2,6% (1,9)

Although not at all levels of the intra- and inter assay CV's of the E2[III] meet the claim from the manufacturer (between brackets), they all do meet the medical requirements.
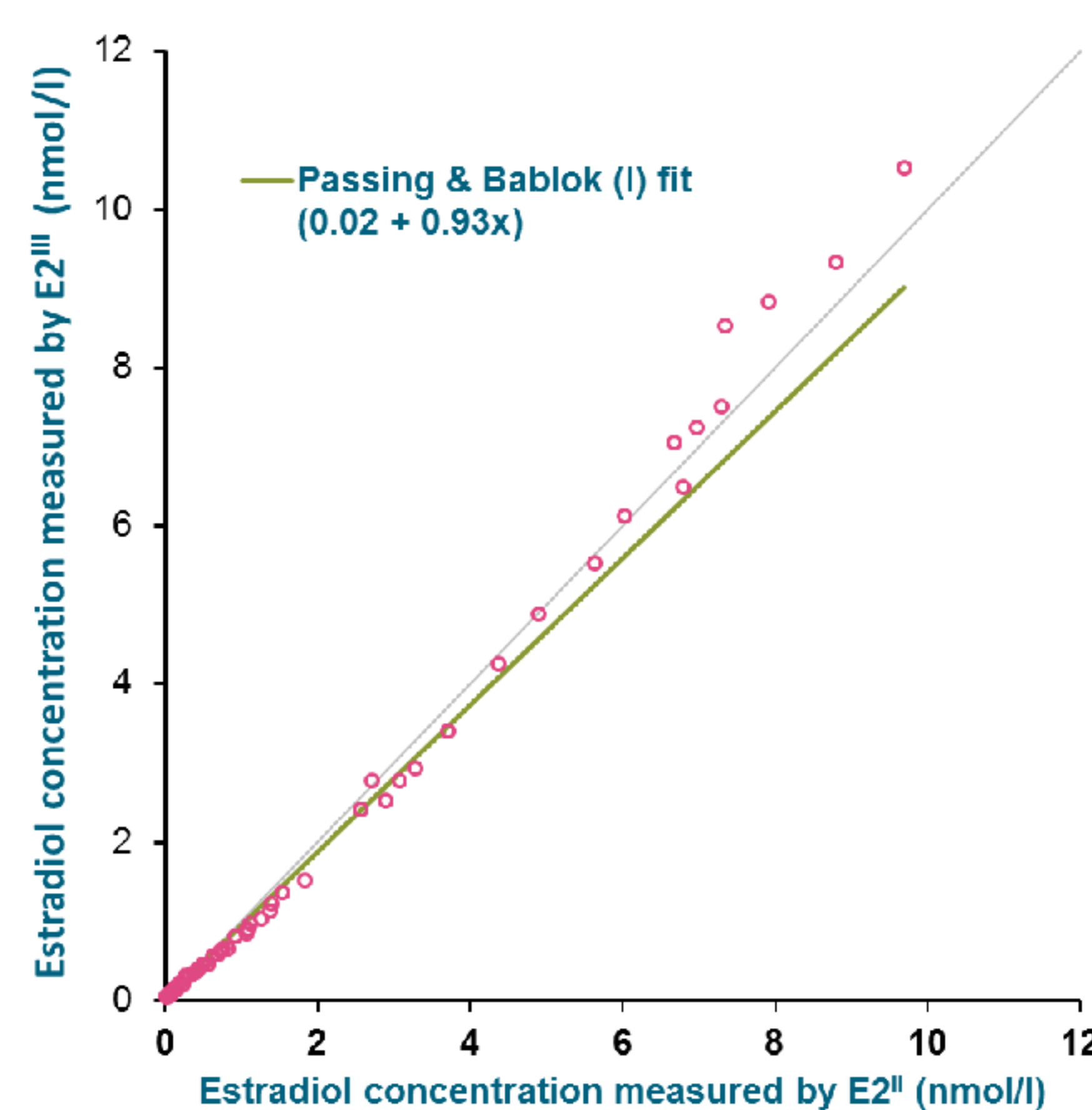

Figure 2 – Comparison E2[II] to E2[III] – whole range
The E2[III] assay results in a ~7% lower estradiol concentration compared to the E2[II] assay, however the manufacturer reported a correlation of E[III] = 0,03 + 0.86 x E2[II], n=60.
When the data was split into two ranges (below and above 4 nmol/l) the correlation was as shown in Table 4, showing a 15% lower level measured with the E2[III] assay for the lower, more clinical important range, like the manufacturer did.

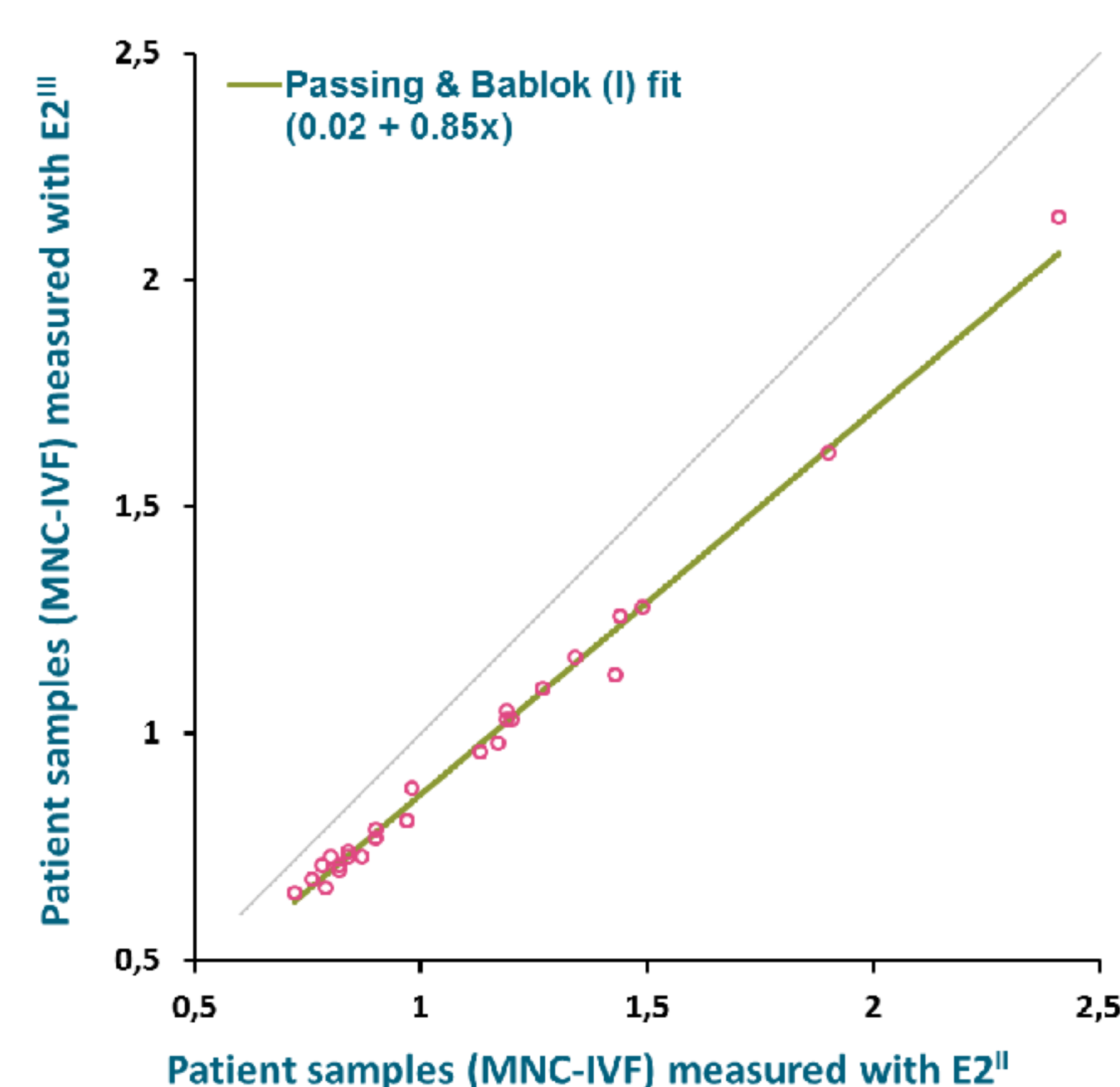| Table 4	Comparison E2[II] *vs.* E2[III]	
Range	0.020 to 3.710	4.370 to 9.690
PB fit	E[III] = **0.03 + 0.85 x** E2[II]	E[III] =-1.14 + 1.20 x E2[II]
N	48	12

Figure 3 – Comparison of patient samples in the modified natural cycle - IVF
In order to verify the results from the comparison, samples from patients followed in the MNC-IVF (stored at -80°C) were used for an extra comparison (N=23).
At this clinical important range (0,720 to 2,410 nmol/l) the results were comparable with the manufacturer and our study in the lower range. Estradiol measured by E2[III] assay are on average 15% lower than the E2[II] assay.

CONCLUSION

- The estradiol E2[III] assay is fit for purpose for routine measurements.
- Results of the E2[III] assay are approximately 15% lower than the current used E2[II] assay, but are more comparable to the formerly used AD assay.
- Changes between immunoassays usually comes with a change in reference values due to differences in antibodies used and the lack of harmonization.
- The reference ranges between the two assays varied more than could be explained by comparison, especially in the ovulatory phase, therefore the by Roche proposed new reference ranges should be more extensively verified.