Supplementary Appendix

SUPPLEMENTARY S1: FOLLOW-UP PROTOCOL

Day	Scr	1 – 7	8 - 14	15 - 21	22 – 28	29 - 35	36 - 42	43 – 49	50 – 56	57 – 63	71 - 77	78 – 84	85 – 91	113 - 119	246 – 252	330 – 336
Week		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**11**	**12**	**13**	**17**	**36**	**48**
Informed Consent	x															
Anamnesis	x															
Clinical examination	x	X				x				X			X	x	x	X
Transvaginal ultrasound	x	X				x				X			X	x	x	X
Administration of r-hCG		3 x per week[1]	3 x per week	3 x per week	3 x per week	3 x per week	3 x per week	3 x per week	3 x per Week	3 x per week	3 x per week	3 x per week				
Biopsy		X											x		x	
Blood test (hematology & biochemistry)		X				x				X			x	X	x	X
Blood test (E_2, progesterone, βhCG)		X				x				X			x	X	x	X
Questionnaire “Side effects of Ovidrel®”						x				X			x	X		

Supplementary S1: Flow chart of the study from screening (scr) until the end of the study and the safety follow up. Participants were excluded if they were receiving any other agents, investigational or otherwise, for the purpose of primary prevention; if they had a history of allergic reactions attributed to compounds of similar chemical or biologic composition to r-hCG preparations or one of its excipients; if they were receiving medications that could interfere with the study protocol objectives such as prednisone, thyroid hormones, or insulin; if they had previous treatment with follicle-stimulating hormone (FSH) for assisted reproduction; if they had uncontrolled intercurrent illness including, but not limited to ovarian enlargement of undetermined origin, ongoing or active infection, NYHA ≥ class 1 congestive heart failure, unstable angina pectoris, cardiac arrhythmia, severe cognitive deficit or psychiatric illness/social situations that could make the participant unable to give informed consent or would limit compliance with study requirements; or if they were HIV-positive, or had an infection with hepatitis B or C.

SUPPLEMENTARY S2: MATERIALS AND METHODS

SPIROTOME® BIOPSY

The Spirotome® biopsy was performed on the right lower inferior quadrant of the breast. The site was chosen to give the least esthetic impact of the small scan scar that may originate from the biopsy. An area with enough glandular tissue was selected by breast ultrasound (12-15 Hz probe, Medison, Germany). After disinfection of the skin a disposable drape with an 8-cm round opening was attached to the biopsy area. First a local anesthetic (0.5 mL 1% xylocaine) was injected into the skin using a 26-gauge needle. The future trajectory of the Spirotome® biopsy was then anaesthetized using 10 mL of the anesthetic injected via a 22-gauge needle. A small 4-mm cut in the skin was performed using a pointed bistoury. Subsequently the Spirotome® trocar was inserted. The Spirotome® helix was gently used to remove tissue. After the removal of the first sample, a second insertion of the Spirotome® helix was performed through the cutting cannula/coax to remove a second tissue specimen. After the biopsy, the skin was covered with 3M Steri-Strips™.

Breast specimens were divided into 2 parts. One fragment was placed in 70% alcohol and the other tissue fragment was divided in different fragments and stored in RNA*later*. All biopsies were performed by H. Depypere and sent to Prof. Jose Russo, MD, at the Irma H. Russo, MD-Breast Cancer Research Laboratory at the Fox Chase Cancer Center (FCCC) Temple Health in Philadelphia, USA, in a special box provided by Bioncise (Professor Janssens). The biopsies were always taken on Monday, Tuesday, or Wednesday so that the shipment with chemical icepack, in special containers, was done during the week. This avoided delays in shipments to FCCC due to weekends or holidays. Special attention was given to sending an email after the biopsy, to notify the staff at the FCCC that a shipment was on its way.

The shipment of breast biopsies from Belgium to Philadelphia usually takes 3-4 days. Breast tissues fixed in 70% ethanol were processed using a Modular Vacuum Processor (manufactured by Instrumentation Laboratory) upon receipt at FCCC. Breast tissue biopsy fragments in RNA*later* were immediately stored in a freezer at -80°C upon receiving.

HEMATOXYLIN & EOSIN (H&E) STAINING

Paraffin blocks were prepared using a Leica EG1160 Embedding Station. Paraffin sections at 4 µm thickness were sectioned using a Microm HM300 Microtome. The H&E staining was performed following a standard protocol.

IMMUNOHISTOCHEMISTRY (IHC)

Paraffin sections at 4 µm were stained with primary antibodies using a i6000 BioGenex Autostainer following a standard protocol. The antibodies used were as follows: purified mouse anti-E-cadherin (BD Biosciences, #610182) at a dilution of 1:200, Tri-methyl-Histone (Lys27) (C36B11) Rabbit mAb (Cell signaling, #9733S) at a dilution of 1:800. A Super Sensitive TM Polymer-HRP Detection System (BioGenex, #QD430-XAKE) was used to detect the staining. Tissues were counterstained with hematoxylin. The images were acquired using an Olympus DP72 microscope.

RNA ISOLATION AND SEQUENCING

Total RNA was extracted within a month after all samples were received using the RNeasy Lipid Tissue Mini kit (Qiagen, US) according to the manufacturer’s protocol. The RNA quality was measured by a Nanodrop™- Nd-1000 Spectrophotometer (Thermo Fisher Scientific, US) and integrity was evaluated using a 2100 Bioanalyzer Instrument (Agilent Technologies, US) with an RNA 6000 Pico kit (Agilent Technologies, US) according to the manufacturer's protocol. RNA samples with an RNA integrity number (RIN) less than 4.8 were discarded. Library construction was performed using PE100 strand-specific library preparation for eukaryote (BGI, CA, US) as described in Supplementary S1 to generate DNA nanoball (DNB), which had more than 300 copies of one molecule. The DNBs were loaded into the patterned nanoarray and pair end 100 bases reads were generated by combinatorial Probe-Anchor Synthesis (cPAS) on the BGISEQ-500 platform (BGI, CA, US) with more than 60 million reads delivered to each of the samples. The library construction and sequencing were carried out by the BGI Company in Hong Kong.

PRIMARY RNA-SEQ ANALYSIS

All the raw sequences were quality checked using FastQC (Babraham Institute, USA) prior to alignment. The raw reads were quality filtered to remove low-quality reads using Genomic Workbench version 12.0 (USA). Then the cleaned reads were used for mapping against the Homo_sapiens. GRCh38 reference genomes (Esemble GRCh38/hg38) using CLC Genomics Workbench version 12.0.3 (Qiagen, US). In total, there were 166 files sequenced with each containing from 128-199 million reads. The mapping rate ranged from approximately 98% to 99% for all the samples. For analyses, only the reads aligned to 23 pairs of human chromosomes were considered. To estimate the expression level, the number of exon reads mapped per kilobase per million mapped reads, RPKM, for each gene was measured using CLC Genomics Workbench version 12.0.3 (Qiagen, US)[1,2]. Each gene was modeled by a separate Generalized Linear Model (GLM). The Robinson and Smyth's Exact Test[3], implemented in the CLC Genomics Workbench version 12.0.3 (Qiagen, US), which assumes a Negative Binomial distribution of the data and takes into account the overdispersion caused by biological variability, was used to compare expression levels between each time point for treated group and controls. Fold changes were calculated from the GLM, which corrects for differences in library size between the samples. A false discovery rate (FDR)-adjusted p-value of (FDRp) ≤0.05 was chosen to indicate statistical significance. The genes with absolute fold change (FC) larger than 1.5 and with an FDR p less than 0.05 were considered as differentially expressed genes (DEGs).

1. Tong M, Chan KW, Bao JYJ, et al. Rab25 Is a Tumor Suppressor Gene with Antiangiogenic and Anti-Invasive Activities in Esophageal Squamous Cell Carcinoma. Cancer Res 2012;72:6024-35.
2. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nature methods 2008;5:621-8.
3. Robinson MD, Smyth GK. Small-sample estimation of negative binomial dispersion, with applications to SAGE data. Biostastics 2008;9:321-32.

SUPPLEMENTARTY 3: LIBRARY CONSTRUCTION AND RNA SEQUENCING:

For PE100 strand-specific library preparation the first step in the workflow involved purifying the poly-A containing mRNA molecules using poly-T oligo-attached magnetic beads. Following purification, the mRNA was fragmented into small pieces using divalent cations under elevated temperature. The cleaved RNA fragments were copied into first strand cDNA using reverse transcriptase and random primers. This was followed by second strand cDNA synthesis using DNA Polymerase I and RNase H. This process removes the RNA template and synthesizes a replacement strand, incorporating dUTP in place of dTTP to generate dscDNA. The incorporation of dUTP quenched the second strand during amplification. These cDNA fragments were added with a single ‘A’ base and subsequently ligated to the adapter. The resultant product was purified and enriched with PCR amplification to yield the final cDNA library. The PCR yield was quantified and was subjected to single strand circularized DNA molecule (ssDNA circle) preparation for final library construction. DNA nanoballs (DNBs) were generated with the ssDNA circle by rolling circle replication (RCR) to intensify the fluorescent signals during the sequencing process. The DNBs were then loaded into the patterned nanoarrays and pair-end reads of 100 bp were read through on the BGISEQ-500 platform for subsequent data analysis.

SUPPLEMENTARY S4, PARTICIPANTS QUALIFIED FOR RNA-SEQ OR HISTOLOGY ANALYSIS

Group without contraceptives---responders

Participant ID	RNA-seq	Histology
101	Y	Y
103	Y	Y
104	Y	**N**
105	Y	**N**
107	Y	Y
108	Y	Y
109	Y	Y
110	Y	Y
114	Y	Y
121	Y	**N**
124	Y	Y
129	**N**	Y

Group with contaceptives---low responders

Participant ID	RNA-seq	Histology
102	Y	Y
106	Y	**N**
111	**N**	Y
113	Y	Y
115	Y	Y
116	Y	Y
117	**N**	Y
118	Y	Y
119	Y	Y
120	**N**	Y
122	**N**	Y
123	**N**	Y
125	Y	**N**
126	Y	Y
127	Y	Y
128	Y	Y
130	**N**	Y
131	Y	Y
132	Y	Y
133	Y	Y

Y: tissue used for analysis
N: tissue not used for analysis due to short of material at some time points

SUPPLEMENTARY S5: SEQUENCE READS PER SAMPLE AND READS STATISTICS

Q20(%): The number of nucleotide with quality higher than 20/total nucleotide.

GC(%): GC number/total nucleotide

Sample Name	Total Reads	Total bases	Read length (bp)	Q20(%)	GC(%)
101---01	143.991.168	14.399.116.800	100	96,66%	48,31%
101---02	138.194.028	13.819.402.800	100	97,21%	48,35%
101-03	154.515.884	15.451.588.400	100	97,45%	50,23%
101-04	153.386.238	15.338.623.800	100	97,07%	49,67%
102---01	149.790.736	14.979.073.600	100	96,72%	48,38%
102---02	130.168.692	13.016.869.200	100	97,12%	48,07%
102-03	154.098.226	15.409.822.600	100	97,29%	49,17%
102-04	152.637.118	15.263.711.800	100	97,12%	49,31%
103---01	154.002.070	15.400.207.000	100	96,74%	47,82%
103---02	153.358.184	15.335.818.400	100	97,16%	48,60%
103---03	142.644.022	14.264.402.200	100	96,81%	48,42%
103-04	152.845.046	15.284.504.600	100	97,28%	49,16%
104---01	156.145.510	15.614.551.000	100	96,60%	48,07%
104---02	129.155.544	12.915.554.400	100	96,80%	48,32%
104---03	135.231.130	13.523.113.000	100	96,91%	48,18%
105---01	154.591.948	15.459.194.800	100	96,73%	48,10%
105---02	153.366.614	15.336.661.400	100	97,25%	48,63%
105-03	151.585.202	15.158.520.200	100	97,21%	49,25%
106---01	138.942.698	13.894.269.800	100	96,02%	48,28%
106---02	152.625.474	15.262.547.400	100	97,09%	47,79%
106---03	182.285.912	18.228.591.200	100	96,39%	48,43%
107---01	146.916.572	14.691.657.200	100	96,02%	47,48%
107---02	168.878.200	16.887.820.000	100	96,45%	47,52%
107---03	199.580.496	19.958.049.600	100	96,33%	48,45%
108---01	156.248.640	15.624.864.000	100	97,06%	47,51%
108---02	155.092.526	15.509.252.600	100	96,68%	48,23%
108---03	154.992.088	15.499.208.800	100	95,99%	47,65%
108-04	151.095.294	15.109.529.400	100	97,11%	49,46%
109---01	199.514.766	19.951.476.600	100	95,90%	47,83%
109---03	132.635.004	13.263.500.400	100	96,00%	48,17%
109-02	151.667.322	15.166.732.200	100	97,11%	49,64%
109-04	152.261.604	15.226.160.400	100	97,22%	48,92%
110---01	200.618.464	20.061.846.400	100	96,21%	49,03%
110---02	137.148.764	13.714.876.400	100	96,91%	48,42%
110---03	128.309.514	12.830.951.400	100	96,07%	47,92%
113---01	142.422.860	14.242.286.000	100	96,98%	48,03%
113---02	142.404.814	14.240.481.400	100	96,81%	48,23%
113-03	152.335.808	15.233.580.800	100	96,96%	50,02%
113-04	152.210.202	15.221.020.200	100	97,06%	48,84%

114---01	154.269.654	15.426.965.400	100	97,17%	47,86%
114---02	130.012.576	13.001.257.600	100	96,83%	48,18%
114---03	154.079.896	15.407.989.600	100	95,91%	48,60%
115---01	141.405.550	14.140.555.000	100	96,80%	47,66%
115---02	160.798.294	16.079.829.400	100	96,83%	48,00%
115---03	122.507.962	12.250.796.200	100	95,84%	48,09%
116---01	153.316.018	15.331.601.800	100	97,14%	47,96%
116---02	193.361.616	19.336.161.600	100	97,21%	47,73%
116-03	150.671.982	15.067.198.200	100	97,04%	49,41%
116-04	152.784.560	15.278.456.000	100	97,00%	48,75%
118---01	157.952.978	15.795.297.800	100	95,71%	48,49%
118---02	155.257.552	15.525.755.200	100	96,82%	48,56%
118-03	152.049.572	15.204.957.200	100	96,88%	48,94%
119---01	126.820.316	12.682.031.600	100	96,67%	48,36%
119---02	142.142.572	14.214.257.200	100	96,97%	48,26%
119---03	144.509.820	14.450.982.000	100	96,43%	48,10%
119-04	152.657.166	15.265.716.600	100	97,10%	48,44%
121---01	134.550.074	13.455.007.400	100	97,28%	47,82%
121---02	202.806.818	20.280.681.800	100	96,29%	48,61%
121---03	148.559.214	14.855.921.400	100	96,36%	48,50%
124---01	153.762.190	15.376.219.000	100	97,27%	47,76%
124---02	162.904.852	16.290.485.200	100	96,61%	48,48%
124-03	131.288.674	13.128.867.400	100	97,94%	47,04%
125---01	156.258.488	15.625.848.800	100	97,29%	48,30%
125---02	144.602.166	14.460.216.600	100	97,00%	48,39%
125-03	154.119.620	15.411.962.000	100	97,50%	49,72%
126---01	156.250.386	15.625.038.600	100	97,24%	48,00%
126---02	121.850.198	12.185.019.800	100	96,82%	47,83%
126-03	140.812.394	14.081.239.400	100	97,22%	49,83%
127---01	153.199.030	15.319.903.000	100	97,34%	48,29%
127---02	138.172.606	13.817.260.600	100	97,16%	48,45%
127-03	153.655.730	15.365.573.000	100	97,29%	48,91%
128---01	150.606.992	15.060.699.200	100	97,37%	47,68%
128-02	152.945.874	15.294.587.400	100	97,25%	49,59%
128-03	152.605.622	15.260.562.200	100	97,20%	48,66%
131---02	120.601.400	12.060.140.000	100	97,10%	48,39%
131-01	154.461.850	15.446.185.000	100	97,20%	49,42%
131-03	148.824.798	14.882.479.800	100	97,17%	49,45%
132---01	157.557.148	15.755.714.800	100	97,34%	48,14%
132---02	160.913.456	16.091.345.600	100	96,72%	48,00%
132-03	152.918.778	15.291.877.800	100	97,08%	49,79%
133---01	151.692.278	15.169.227.800	100	97,18%	48,24%
133-02	153.367.878	15.336.787.800	100	97,25%	49,28%
133-03	152.144.032	15.214.403.200	100	96,99%	48,90%

SUPPLEMENTARY S6: STATISTICS OF READS GENERATED FROM FORWARD AND REVERSE SEQUENCING FOR EACH SAMPLE

Sample Name	% Dups	% GC	M Seqs
101---01_1	61,20%	48%	72
101---01_2	60,00%	48%	72
101---02_1	65,00%	48%	69,1
101---02_2	63,30%	48%	69,1
101-03_1	70,90%	50%	77,3
101-03_2	69,10%	50%	77,3
101-04_1	70,40%	49%	76,7
101-04_2	66,80%	49%	76,7
102---01_1	66,50%	48%	74,9
102---01_2	64,50%	48%	74,9
102---02_1	71,30%	48%	65,1
102---02_2	69,90%	48%	65,1
102-03_1	75,20%	49%	77
102-03_2	72,20%	49%	77
102-04_1	50,50%	49%	76,3
102-04_2	78,30%	49%	76,3
103---01_1	67,10%	47%	77
103---01_2	65,20%	47%	77
103---02_1	68,80%	48%	76,7
103---02_2	67,50%	48%	76,7
103---03_1	64,50%	48%	71,3
103---03_2	60,80%	48%	71,3
103-04_1	48,40%	49%	76,4
103-04_2	74,80%	49%	76,4
104---01_1	67,70%	48%	78,1
104---01_2	66,20%	48%	78,1
104---02_1	70,40%	48%	64,6
104---02_2	67,60%	48%	64,6
104---03_1	62,20%	48%	67,6
104---03_2	58,80%	48%	67,6
105---01_1	59,60%	48%	77,3
105---01_2	59,00%	48%	77,3
105---02_1	64,70%	48%	76,7
105---02_2	63,10%	48%	76,7
105-03_1	67,60%	49%	75,8
105-03_2	67,10%	49%	75,8
106---01_1	64,70%	48%	69,5
106---01_2	65,70%	48%	69,5

106---02_1	66,10%	47%	76,3
106---02_2	63,70%	47%	76,3
106---03_1	69,70%	48%	91,1
106---03_2	67,70%	48%	91,1
107---01_2	67,50%	47%	73,5
107---02_1	64,90%	47%	84,4
107---02_2	62,50%	47%	84,4
107---03_1	61,20%	48%	99,8
107---03_2	68,50%	48%	99,8
108---01_1	65,40%	47%	78,1
108---01_2	63,30%	47%	78,1
108---02_1	61,20%	48%	77,5
108---02_2	61,30%	48%	77,5
108---03_1	61,50%	47%	77,5
108---03_2	56,50%	47%	77,5
108-04_1	52,60%	49%	75,5
108-04_2	78,60%	49%	75,5
109---01_1	66,20%	47%	99,8
109---01_2	64,50%	47%	99,8
109---03_1	64,20%	48%	66,3
109---03_2	58,40%	48%	66,3
109-02_1	48,70%	49%	75,8
109-02_2	73,70%	49%	75,8
109-04_1	49,70%	48%	76,1
109-04_2	75,70%	48%	76,1
110---01_1	67,90%	48%	100,3
110---01_2	66,20%	49%	100,3
110---02_1	61,10%	48%	68,6
110---02_2	61,30%	48%	68,6
110---03_1	62,70%	47%	64,2
110---03_2	57,50%	47%	64,2
113---01_1	62,60%	48%	71,2
113---01_2	60,70%	48%	71,2
113---02_1	59,10%	48%	71,2
113---02_2	59,30%	48%	71,2
113-03_1	51,30%	49%	76,2
113-03_2	76,90%	50%	76,2
113-04_1	50,20%	48%	76,1
113-04_2	75,40%	48%	76,1
114---01_1	62,20%	47%	77,1
114---01_2	60,40%	47%	77,1

114---02_1	57,10%	48%	65
114---02_2	56,80%	48%	65
114---03_1	60,80%	48%	77
114---03_2	55,00%	48%	77
115---01_1	68,90%	47%	70,7
115---01_2	66,40%	47%	70,7
115---02_1	72,00%	47%	80,4
115---02_2	70,80%	48%	80,4
115---03_1	70,10%	48%	61,3
115---03_2	64,20%	48%	61,3
116---01_1	66,20%	47%	76,7
116---01_2	63,60%	47%	76,7
116---02_1	71,80%	47%	96,7
116---02_2	71,60%	47%	96,7
116-03_1	51,90%	49%	75,3
116-03_2	75,40%	49%	75,3
116-04_1	52,60%	48%	76,4
116-04_2	76,90%	48%	76,4
118---01_1	67,80%	48%	79
118---01_2	67,70%	48%	79
118---02_1	70,50%	48%	77,6
118---02_2	67,60%	48%	77,6
118-03_1	54,90%	48%	76
118-03_2	77,60%	49%	76
119---01_1	67,40%	48%	63,4
119---01_2	68,90%	48%	63,4
119---02_1	61,80%	48%	71,1
119---02_2	50,30%	48%	71,1
119---03_1	64,00%	48%	72,3
119---03_2	60,40%	48%	72,3
119-04_1	66,00%	48%	76,3
119-04_2	66,10%	48%	76,3
121---01_1	66,10%	47%	67,3
121---01_2	64,30%	47%	67,3
121---02_1	65,30%	48%	101,4
121---02_2	75,10%	48%	101,4
121---03_1	71,80%	48%	74,3
121---03_2	68,00%	48%	74,3
124---01_1	60,90%	47%	76,9
124---01_2	58,90%	47%	76,9
124---02_1	64,00%	48%	81,5

124---02_2	62,20%	48%	81,5
124-03_1	67,10%	46%	65,6
124-03_2	66,80%	47%	65,6
125---01_1	65,30%	48%	78,1
125---01_2	63,20%	48%	78,1
125---02_1	66,60%	48%	72,3
125---02_2	63,70%	48%	72,3
125-03_1	71,70%	49%	77,1
125-03_2	70,40%	49%	77,1
126---01_1	70,60%	47%	78,1
126---01_2	68,50%	48%	78,1
126---02_1	68,50%	47%	60,9
126---02_2	66,00%	47%	60,9
126-03_1	70,90%	49%	70,4
126-03_2	68,60%	49%	70,4
127---01_1	61,20%	48%	76,6
127---01_2	59,50%	48%	76,6
127---02_1	64,00%	48%	69,1
127---02_2	60,10%	48%	69,1
127-03_1	75,20%	48%	76,8
127-03_2	73,00%	48%	76,8
128---01_1	65,40%	47%	75,3
128---01_2	64,00%	47%	75,3
128-02_1	72,20%	49%	76,5
128-02_2	69,60%	49%	76,5
128-03_1	73,60%	48%	76,3
128-03_2	72,60%	48%	76,3
131---02_1	64,80%	48%	60,3
131---02_2	61,40%	48%	60,3
131-01_1	73,50%	49%	77,2
131-01_2	70,40%	49%	77,2
131-03_1	76,70%	49%	74,4
131-03_2	74,00%	49%	74,4
132---01_1	67,50%	48%	78,8
132---01_2	65,90%	48%	78,8
132---02_1	57,30%	47%	80,5
132---02_2	61,30%	48%	80,5
132-03_1	74,50%	49%	76,5
132-03_2	70,70%	49%	76,5
133---01_1	64,20%	48%	75,8
133---01_2	62,30%	48%	75,8

133-02_1	74,90%	49%	76,7
133-02_2	72,30%	49%	76,7
133-03_1	53,40%	48%	76,1
133-03_2	77,70%	48%	76,1

SUPPLEMENTARY S7

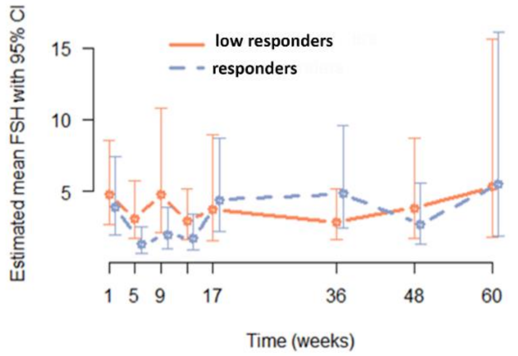
Part	BRCA	Age	Contraceptive group	Remarks (name of medication, if Y)
101	BRCA1	25	A	None
102	BRCA1	23	C	None
103	BRCA1	21	A	Pain killer for headache at week 48
104	BRCA2	24	A	None
105	BRCA2	19	A	Pain killer for headache after week 5. Several medications at week 13 (Pantomed, Aerius, Imodium). Antibiotics at week 48
106	BRCA1	25	52 mg LNG over 5 y	Sinutaben pain killer for headache at week 13
107	BRCA2	26	A	Movicol for obstipation
108	BRCA2	18	A	Pain killer for menstrual pain
109	BRCA1	24	A	None
110	BRCA2	22	A	Gyno-Daktarin for Candida
111	BRCA1	25	C	Pain killer for abdominal pain at week 5. Pain killer for intermittent headache
112	BRCA1	21	A	Pain killer for intermittent headache
113	BRCA1	24	52 mg LNG over 5 y	None
114	BRCA1	24	A	Pain killer for abdominal pain at week 5.
115	BRCA1	22	13.5 mg LNG over 3 y	Sipralexa for depression at week 9
116	BRCA2	20	C	Anti-histaminica, pain killer for intermittent headache
117	BRCA2	22	C	Anti-histamine for household dust allergy
118	BRCA1	19	13.5 mg LNG over 3 y	None
119	BRCA1	24	C	Diclofenac for abdominal pain
120	BRCA1	20	C	Medication for diarrhea at week 9. Hepatitis A vaccine at week 17 + medication in preparation for a holiday to Costa Rica. Morning-after pill in September 2017. Pain killer for abdominal pain at week 36
121	BRCA1	26	A	None
122	BRCA1	26	C	Pain killer for headache and sore throat at week 5. Sostilar after week 48
123	BRCA1	24	C	Pain killer for intermittent headache
124	BRCA2	24	A	None
125	BRCA2	19	EE 0.04 mg + DSG 0.025 mg	Cortisone + antibiotics for boop lungs after week 17. Subject restarted with oral contraceptive on June 14, 2018 (after second biopsy, before third biopsy)
126	BRCA1	20	17βE2 1.5 mg + Nomac 2.5 mg	Pain killer for breast hematoma after week 1. Medication for intermittent migraine and intermittent abdominal pain after week 1. Medication for cold and nausea after week 5. Anti-histamine for allergy for dogs and cats
127	BRCA2	23	C	Medication for asthma and allergy for cats and dogs. Medication for abdominal pain, headache and cold after week 1. Medication for viral infection in August, September and October 2018. Vaccination for tetanus, hepatitis A and flu in September and October 2018

128	BRCA1	22	19.5 mg LNG over 5 y	Pain killer for abdominal pain and medication for bladder infection after week 1
129	BRCA1	25	A	Pain killer for intermittent headache after week 13
130	BRCA1	22	19.5 mg LNG over 5 y	NA
131	BRCA1	18	19.5 mg LNG over 5 y	Ventolin for exercise asthma
132	BRCA2	26	C	Pain killer for migraine after week 1. NSAID for muscular inflammation in shoulder after week 17. Temesta for depressive thoughts since week 17
133	BRCA2	21	etonogestrel 68 mg over 3 y	Pain killer for headache after week 13

Supplementary S7. A summary of all medications used during the trial.

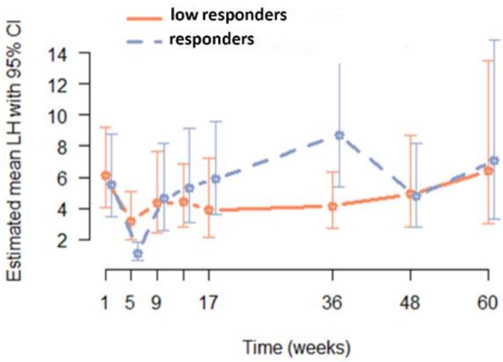
SUPPLEMENTARY S8: FIGURES

A



	Mean in low responders (95% CI)	Mean in responders (95% CI)	Mean ratio	p value
WEEK 1	4.78 (2.67-8.56)	3.85 (1.99-7.44)	1.24	0.63
WEEK 5	3.12 (1.7-5.71)	1.32 (0.68-2.54)	2.37	0.059
WEEK 9	4.76 (2.09-10.8)	1.94 (0.97-3.86)	2.45	0.1
WEEK 13	2.9 (1.62-5.2)	1.75 (0.91-3.38)	1.66	0.259
WEEK 17	3.71 (1.53-8.98)	4.39 (2.2-8.74)	0.85	0.768
WEEK 36	2.88 (1.61-5.16)	4.83 (2.42-9.62)	0.6	0.259
WEEK 48	3.82 (1.68-8.67)	2.7 (1.31-5.58)	1.41	0.532
WEEK 60	5.32 (1.81-15.63)	5.49 (1.87-16.12)	0.97	0.968

B



	Mean in low responders (95% CI)	Mean in responders (95% CI)	Mean ratio	p value
WEEK 1	6.1 (4.04-9.19)	5.54 (3.49-8.8)	1.1	0.76
WEEK 5	3.19 (2.01-5.05)	1.1 (0.66-1.82)	2.91	**0.003**
WEEK 9	4.31 (2.44-7.63)	4.61 (2.6-8.16)	0.94	0.872
WEEK 13	4.4 (2.83-6.84)	5.34 (3.12-9.13)	0.82	0.582
WEEK 17	3.89 (2.11-7.19)	5.91 (3.64-9.59)	0.66	0.293
WEEK 36	4.15 (2.71-6.35)	8.73 (5.38-14.15)	0.48	**0.024**
WEEK 48	4.9 (2.77-8.67)	4.78 (2.8-8.19)	1.02	0.952
WEEK 60	6.41 (3.05-13.49)	7.04 (3.34-14.84)	0.91	0.86

Supplementary S8-1. Mean FSH and LH with 95% confidence intervals according to visit and response. Mean FSH level at weeks 5, 9, and 13 in low responders is 3.26 (2.15-4.95) and is significantly (p= 0.028) higher the mean value 1.64 (1.05-2.56) of the responders at week 5, 9, 13. Mean LH level at weeks 5, 9, and 13 of the low responders was not different from the responders (p=0.204).

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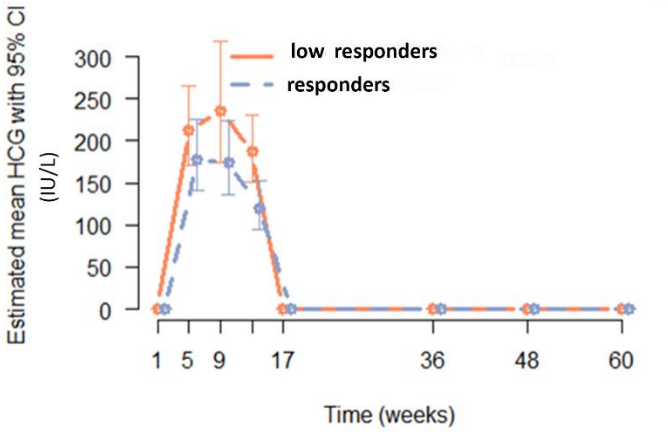
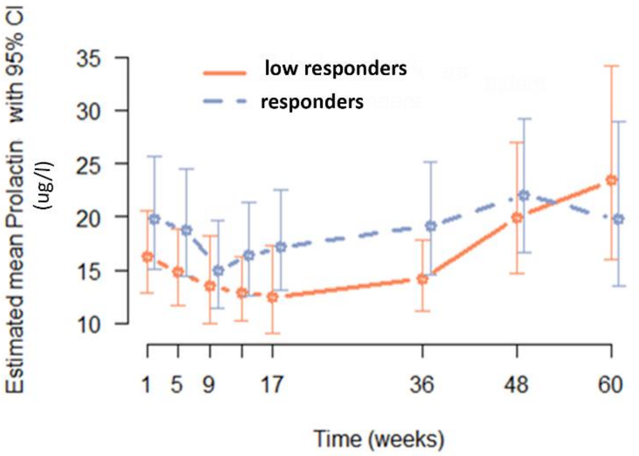


	Mean in low responders (95% CI)	Mean in responders (95% CI)	Mean ratio	p value
WEEK 1	0.5 (0.4-0.62)	0.5 (0.39-0.63)	1	1
WEEK 5	212.94 (170.97-265.2)	178.26 (140.44-226.27)	1.19	0.28
WEEK 9	235.84 (174.63-318.51)	174.42 (135.79-224.04)	1.35	0.129
WEEK 13	186.94 (151.32-230.94)	119.65 (94.27-151.88)	1.56	**0.006**
WEEK 17	0.5 (0.36-0.69)	0.5 (0.39-0.64)	1	1
WEEK 36	0.5 (0.4-0.62)	0.69 (0.53-0.88)	0.73	0.057
WEEK 48	0.5 (0.37-0.68)	0.5 (0.38-0.65)	1	1
WEEK 60	0.5 (0.34-0.75)	0.5 (0.34-0.75)	1	1

B



	Mean in low responders (95% CI)	Mean in responders (95% CI)	Mean ratio	p value
WEEK 1	16.27 (12.87-20.58)	19.73 (15.14-25.72)	0.82	0.28
WEEK 5	14.81 (11.64-18.84)	18.8 (14.42-24.5)	0.79	0.188
WEEK 9	13.51 (9.99-18.28)	14.95 (11.38-19.65)	0.9	0.623
WEEK 13	12.9 (10.2-16.31)	16.35 (12.55-21.31)	0.79	0.186
WEEK 17	12.52 (9.09-17.25)	17.17 (13.07-22.57)	0.73	0.14
WEEK 36	14.15 (11.19-17.89)	19.17 (14.59-25.19)	0.74	0.097
WEEK 48	19.96 (14.75-27.01)	22.03 (16.61-29.24)	0.91	0.637
WEEK 60	23.42 (16.05-34.17)	19.78 (13.49-29)	1.18	0.536

Supplementary 8-2. Mean estradiol and progesterone with 95% confidence interval according to visit and response.

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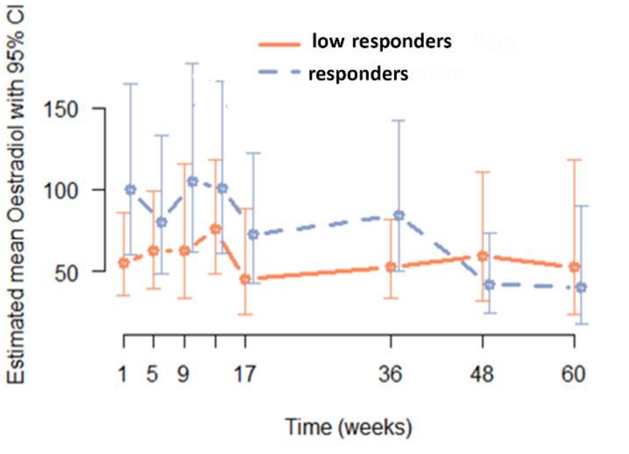

	Mean in low responders (95% CI)	Mean in responders (95% CI)	Mean ratio	p value
WEEK 1	54.81 (35.05-85.72)	99.67 (60.18-165.08)	0.55	0.082
WEEK 5	62.38 (39.25-99.14)	80.33 (48.5-133.04)	0.78	0.466
WEEK 9	62.23 (33.39-115.97)	104.61 (61.72-177.29)	0.59	0.21
WEEK 13	75.51 (48.29-118.1)	100.49 (60.68-166.44)	0.75	0.403
WEEK 17	45.12 (23.1-88.13)	72.29 (42.66-122.52)	0.62	0.276
WEEK 36	52.39 (33.5-81.94)	84.23 (49.72-142.68)	0.62	0.177
WEEK 48	59.35 (31.85-110.61)	42.03 (24.16-73.14)	1.41	0.414
WEEK 60	52.57 (23.31-118.54)	40.05 (17.76-90.31)	1.31	0.641

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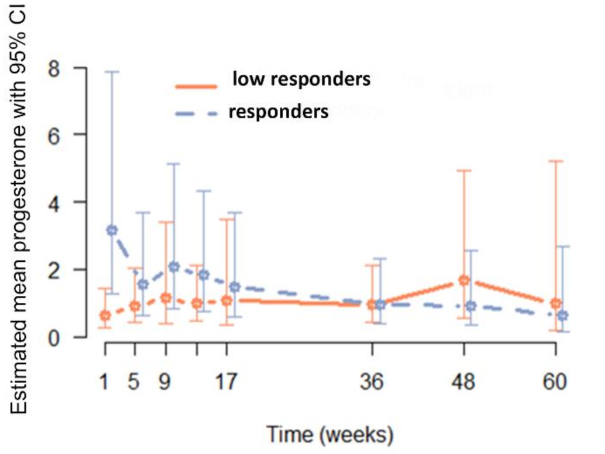

	Mean in low responders (95% CI)	Mean in responders (95% CI)	Mean ratio	p value
WEEK 1	0.64 (0.29-1.42)	3.16 (1.27-7.87)	0.2	**0.01**
WEEK 5	0.92 (0.41-2.04)	1.54 (0.65-3.68)	0.59	0.386
WEEK 9	1.15 (0.39-3.41)	2.06 (0.83-5.13)	0.56	0.417
WEEK 13	0.98 (0.45-2.12)	1.82 (0.76-4.35)	0.54	0.294
WEEK 17	1.08 (0.34-3.5)	1.49 (0.6-3.7)	0.73	0.674
WEEK 36	0.95 (0.43-2.11)	0.94 (0.38-2.34)	1.01	0.987
WEEK 48	1.67 (0.56-4.95)	0.93 (0.34-2.58)	1.79	0.439
WEEK 60	1 (0.19-5.22)	0.64 (0.15-2.68)	1.57	0.686

Supplementary 8-3. Mean hCG with 95% confidence intervals according to visit and response.