J Assist Reprod Genet (2015) 32:1031–1042
DOI 10.1007/s10815-015-0488-0

EMBRYO BIOLOGY

Unaltered timing of embryo development in women with polycystic ovarian syndrome (PCOS): a time-lapse study

Linda Sundvall[1,2] · **Kirstine Kirkegaard**[1,2] · **Hans Jakob Ingerslev**[1,2] · **Ulla Breth Knudsen**[1,2]

Received: 2 March 2015 / Accepted: 13 April 2015 / Published online: 1 May 2015

Abstract
Purpose Polycystic ovarian syndrome (PCOS) is a common cause of female infertility. Factors other than anovulation, such as low embryo quality have been suggested to contribute to the infertility in these women. This 2-year retrospective study used timelapse technology to investigate the PCOS-influence on timing of development in the pre-implantation embryo (primary endpoint). The secondary outcome measure was live birth rates after elective single-embryo transfer.
Methods In total, 313 embryos from 43 PCOS women, and 1075 embryos from 174 non-PCOS women undergoing assisted reproduction were included. All embryos were monitored until day 6. Differences in embryo kinetics were tested in a covariance regression model to account for potential confounding variables: female age, BMI, fertilization method and male infertility.
Results Time to initiate compaction and reach the morula stage as well as the duration of the 4th cleavage division was significantly shorter in PCOS embryos compared with non-PCOS embryos. No other kinetic differences were found at any time-points annotated. The proportion of multi-nucleated cells at the 2-cell stage was significantly higher in PCOS embryos compared with non-PCOS embryos. The live birth rates were comparable between the two groups.
Conclusion The findings suggest that the causative factor for subfertility in PCOS is not related to timing of development in the pre-implantation embryo.

Keywords Polycystic ovarian syndrome · Embryo development · Assisted reproductive techniques · Fertility, Time-lapse

Capsule By the use of time-lapse analysis, we demonstrate that the timing of development in the pre-implantation embryo from women with PCOS is comparable to that of non-PCOS embryos. The findings suggest that the causative factor for subfertility in PCOS is not related to timing of development in the pre-implantation embryo.

Trial registration number: NCT01953146

Electronic supplementary material The online version of this article (doi:10.1007/s10815-015-0488-0) contains supplementary material, which is available to authorized users.

✉ Linda Sundvall
linda.karin.sundvall@post.au.dk

[1] The Fertility Clinic, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark

[2] Health, Aarhus University, Vennelyst Boulevard 9, DK-8000 Aarhus C, Denmark

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common causes of female infertility. This endocrinological disorder affects 5–10 % of women in the reproductive age [1]. PCOS is traditionally defined by the Rotterdam criteria involving *polycystic ovaries*, *biochemical or clinical signs of androgen excess* and *ovulatory dysfunction* [2].

The ultimate choice of treatment of infertility when ovulation induction fails is In Vitro Fertilization (IVF). Traditionally, PCOS patients are reported to produce an increased number of oocytes of poor quality, which leads to lower fertilization rate [3–5]. Furthermore, PCOS women are at increased risk for early pregnancy loss, and more often suffer from pre-eclampsia and preterm-birth [4, 6–10]. Mechanisms responsible for these adverse outcomes are unclear, but inherited or metabolism-induced factors in the oocyte have been suggested. While the pregnancy rates after IVF for women with PCOS are generally satisfactory compared with infertile patients with normal ovaries [10–12], ovarian stimulation protocols must be tailored

to avoid their increased risk of developing ovarian hyperstimulation syndrome (OHSS).

Studies of ovarian growth factors in tissue from PCOS ovaries and gene expression profiles in MII oocytes suggest that PCOS is associated with disturbed oocyte maturation [13–17].

Most efforts to evaluate embryo quality in PCOS have been based on static evaluation of embryo morphology, which is correlated only weakly with viability [18, 19]. In contrast time-lapse analysis has proven to be a sensitive method of detecting reduced viability in mouse embryos [20], and several studies suggest that timing of development is related to clinical outcome in IVF patients [21–24]. A recent study using time-lapse technology showed that embryos from hyperandrogenic PCOS women were significantly delayed at early stages compared with embryos from non-PCOS regularly cycling women [25]. Another recent paper documented that embryos derived from women undergoing ovarian stimulation with the flexible GnRH antagonist protocol underwent the earliest cleavage faster than embryos derived from women undergoing a protocol consisting of long GnRH agonist [26]. We hypothesized that differences between embryos from an unselected group of PCOS women and non-PCOS women would be detectable with time-lapse imaging. Accordingly, in order to assess the impact of PCOS on embryo viability, the aim of this study was to analyze timing of time-lapse parameters and pregnancy outcome from a cohort of infertile women, with and without PCOS.

Methods

A consecutive cohort of 249 infertile women undergoing IVF or ICSI treatment at the Fertility Clinic, Arhus University Hospital was recruited from February 2011 to May 2013. During this period, couples undergoing IVF/ICSI treatment were offered blastocyst culture to day 6 and time-lapse imaging (TLI) as part of a study evaluating parameters for embryo selection [27]. Indications for ICSI were male infertility or three previously failed IVF-fertilization attempts. Only women regarded as good prognosis patients were included: women aged <38 years without endometriosis and with ≥8 oocytes.

Diagnosis of polycystic ovarian syndrome

Patients were categorized in two groups: Women fulfilling the diagnostic criteria for PCOS detected by the presence of oligomenorrhea or anovulatory cycles and polycystic ovaries, diagnosed by trans-vaginal ultrasound, and women with normal ovarian morphology and regular cycles. Women with polycystic ovaries or oligo-amenorrhea were tested for biochemical androgen excess. The diagnosis of PCOS was based upon the *Rotterdam criteria*, i.e., the presence of two out of the three characteristics (polycystic ovaries, oligo-amenorrhea or signs of androgen excess) [2]. In case biochemical parameters were not available and the patient fulfilled only one clinical parameter (polycystic ovaries or oligo-amenorrhea), the patient was *excluded* from the study. Patients with non-elevated total testosterone (total testosterone ≤2.5 nmol) were classified as "norm-androgenic".

Ethical approval

Written informed consent was obtained from all patients before inclusion. The Central Denmark Region Committees on Biomedical Research Ethics and the Danish Data Protection Agency approved the study. The study was registered at ClinicalTrials.gov (no. NCT01953146).

Ovarian stimulation

Ovarian stimulation and oocyte retrieval were performed according to standard procedures as previously described [28, 29]. Patients were treated with individualized doses of gonadotropin, based on serum AMH and/or antral follicle count and BMI. Patients were stimulated by either rec-FSH or HMG in a GnRH agonist- or antagonist protocol according to clinical guidelines. A dose of 10,000 IU of hCG was administered when at least three follicles measured ≥17 mm, and ultrasound guided oocyte retrieval was conducted 36 h later. Biochemical pregnancy rate was confirmed by serum hCG measurement 16 days after aspiration. Trans-vaginal ultrasound was performed 5 weeks after embryo transfer (at gestational week 8) to confirm intrauterine clinical pregnancy. Live birth rate were collected after birth.

Embryo culture

After oocyte retrieval, ICSI embryos were transferred to 37 ° C, 5 % O_2 and 6 % CO_2 in the time-lapse incubator (EmbryoScope™) immediately after fertilization. IVF embryos were cultured for approximately 18 h in 20 % O_2 in a conventional incubator, followed by removal of adhering cumulus cells to ensure optimal image acquisition, before transfer to the time-lapse incubator. On day 6 embryos were removed from the time-lapse incubator and placed under an inverted microscope for morphological evaluation. A single embryo was selected for transfer based on conventional measures of morphological quality according to the Gardner criteria [30].

Time-lapse imaging

Images were recorded automatically in seven focal planes every 20 min (15 μm intervals, 1280×1024 pixels, 3 pixels per μm, monochrome, 8-bit<0.5 s per image, using single 1 W red LED). A time-point was automatically assigned to each image reported as hours after t0. For ICSI embryos t0 was defined as the time of injecting the sperm into the oocyte. For IVF embryos t0 was defined as the time of adding the sperm to the dish. The t0 was programmed into the EmbryoScope when the slide was loaded.

Embryo assessment

Manual annotation of time-lapse images was performed at an external workstation (Embryo Viewer™). Only normal fertilized (PN=2) embryos completing the 1st division were evaluated. The time-point of the following events were annotated: 1st - 7th division, appearance and disappearance of 1st and 2nd nucleus after 1st division, the Final divisions, start of Compaction, Morula stage, Early and Full expanded blastocyst stage, start of hatching and fully hatched blastocyst. *Full definitions* of annotated time-points are shown in (Table 5). The parameters were annotated according to definitions previously described [28, 29, 31]. Two observers preformed the time-lapse annotations. Inter observer variability of the analysis have been evaluated in another study [32] showing average value of intra-class correlation coefficients (ICC) of 0.8 and the median value ICC of 0.9, which indicate strong and almost perfect agreement, respectively. All time-points were normalized to first cleavage and treated as durations for further analysis in order to overcome the limitation of inexact starting points, and facilitate comparison between IVF and ICSI populations. For the same reason, no parameters before first cleavage were investigated. Durations of cell cycles and cell stages were subsequently calculated as the interval between two time-points. Two parameters (multi-nucleation and direct cleavage to 3-cell stage) were assessed by binary values yes and no. Embryo transfer took place at day 6. No time-lapse parameters were used in the selection process. The observer was blinded to the patient's treatment data and medical history.

Statistics

Baseline and time-lapse data were tested for the assumption of normality by histograms, QQ plots and Shapiro-Wilk test.

Baseline and treatment characteristics data (Tables 1 and 2): Continuous data were non-normal distributed and expressed as medians with lower (Q1) and upper quartiles (Q3). Wilcoxon rank-sum test was used to test the hypothesis of no difference between the PCOS and non-PCOS group. Categorical data were expressed as exact numbers and percentages and Fisher's test was used to test the hypothesis of no difference between the PCOS and non-PCOS group, using bivariate analysis of two-way tables of percentages. A proportional odds model (logistic function) was applied to test the hypothesis of no difference in proportions of normal fertilized and cleaved oocytes between groups.

Since several embryos were derived from each woman (dependent observations) the regression analysis was clustered to patient.

Time-lapse data (Tables 3 and 4) and (Table 1 in Online resource material): Only size variables were normal distributed and therefore expressed as mean ± SD. Continuous time-lapse data were non-normal distributed and expressed as medians with lower (Q1) and upper quartiles (Q3). Linear regression with clustered data was applied to estimate the influence of PCOS (independent variable) on time-lapse data (response variable), reported as odds ratio different to one with 95 % CI. Potential confounding factors that may have impact on embryonic development or that were significantly different between the PCOS and non-PCOS group consisted of: female age (continuous variable), BMI (continuous variable), male infertility (dichotomous variable) and fertilization method (IVF or ICSI) (dichotomous variable). These were enclosed in a covariance regression model to create an "adjusted" odds ratio. The adjusted odds ratio was reported as odds ratio different to one with 95 % CI. Significance levels of the crude and adjusted odds ratios were reported by the STATA output expressed as *p*-value of the Z score of Beta coefficient. Binary parameters (multi-nucleation, direct cleavage to 3-cell stage, survival rates) were expressed as exact numbers and percentages and a proportional logistic regression clustered by patient was applied to test the hypothesis of no difference between PCOS and non-PCOS group. Chi-squared test was used to test for differences between groups in biochemical pregnancy rate and live birth rate. A two-sided *P*-value of <0.05 was considered significant.

Results

In total, 313 oocytes from 43 women diagnosed with PCOS, and 1075 oocytes from 174 women categorized as non-PCOS were included in the data analysis. According to the protocol, another 31 oligo-amenorrheic patients without PCO, where a biochemical test of androgen excess was not available, were excluded at baseline since they did not fulfill the Rotterdam criteria. One woman from the non-PCOS group was excluded from the cohort because she had a double embryo transfer.

For demographics, see (Table 1). PCOS women were significantly younger than non-PCOS women (29 vs. 32 years). Serum levels of total testosterone 1,4(0.9–2.1) nmol/L, AMH 37.2 (28.0–57.0) nmol/L and LH 10.5 (7.0–17.3) nmol/L were significantly higher for women with PCOS. In 4 women diagnosed with PCOS (both PCO and oligomenorrhea) no data

Table 1 Baseline characteristics

Biochemical data	$n_{pcos\ cycles}/n_{non\text{-}pcos\ cycles}$	PCOS	non-PCOS	*P*-value
LH nmol/L	39/68	10.5(7.0–17.3)	6.1(5.0–8.5)	0.001
FSH nmol/L	40/129	5.4(4.5–6.7)	6.1(5.2–7.8)	0.065
E_2 nmol/L	33/33	0.2(0.14–0.40)	0.3(0.14–0.38)	0.763
Testosterone nmol/L	39/36	1.4(0.9–2.1)	1.0(0.78–1.5)	0.026
Testosterone ≥2.5 nmol/L n (%)		6(14)	0	0
Androstenedione nmol/L	25/35	10.1(7.5–12.5)	6.9(4.7–10.4)	0.016
DEHAS μmol/L	23/34	5.9(3.7–6.9)	4.6(3.2–6.1)	0.684
SHBG nmol/L	27/34	65.9(44.0–80.0)	66.0(45.0–93.0)	0.542
TSH nmol/L	40/109	1.8(1.4–2.3)	1.8(1.5–2.3)	0.993
prolactin	29/40	247.3(182.0–283.0)	300.9(211.0–403.0)	0.046
AMH pmol/L	26/90	37.2(28.0–57.0)	16(9.5–23.0)	0.001
Clinical data		PCOS (n_{cycles}=43)	non-PCOS (n_{cycles}= 174)	*P*–value
BMI (kg/m2)		23.1(21.3–27.4)	22.7 (20.9–27.3)	0.867
Age		29.0(27.0–30.9)	32.0(29.0–34.0)	0.001
PCO (follicle count ≥12) n (%)		42(97.7)	12(6.9)	0.001
Oligomen. n(%)		28(65.1)	0	
Amenorrhea n(%)		15(34.9)	0	
Acne n(%)		12(27.9)	NA	
Alopecia n(%)		4(9.3)	NA	
Hirsutism n(%)		12(27.9)	NA	
Metformin therapy (%)		18(41.2)	0	
Metformin dose per day (mg)		1500.0(1000.0–1500.0)		
Main cause of couples infertility according to medical record		PCOS (n_{cycles}=43)	non-PCOS (n_{cycles}=174)	*P*-value
Male infertility n(%)		10(23.3)	98(58.0)	0.04
Anovulation n(%)		11(25.6)	0	0
PCOS n(%)		12(27.9)	0	0
Tubal n(%)		0	12(7.1)	0.066
Unspecified n(%)		10(23.3)	48(28.4)	0.365
Other n(%)		0	16(6.5)	0

Continuous data (Biochemical data, age, BMI, Metformin dose/day) expressed as median and lower (Q1) and upper quartiles (Q3)

Categorical variables (Clinical data, Reason for infertility, serum testosterone ≥2.5 nmol/L) were expressed as exact numbers and percentages

Wilcoxon sum-rank test was used to test the hypothesis of no difference in between PCOS and non-PCOS, for continuous data

Fisher's test was used to test the hypothesis of no difference of proportions between PCOS and non-PCOS, for categorical variables

Two-sided *p*-values less than 0.05 were considered significant. *BMI* body mass index, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *E2* estradiol, *AMH* anti mullerian hormone, *DEHAS* Dehydroepiandrosterone sulfate, *SHBG* Sex Hormone-Binding Globulin, *PCO* polycystic ovary. *NA* Not Available, data not collected for non-PCOS

on androgen excess were available. Thirty-three (n=33) out of 39 PCOS women had serum levels of total testosterone below 2.5 nmol/L.

Cycle characteristics are shown in (Table 2). Treatment procedure (ICSI or IVF) was equally distributed between the PCOS and non-PCOS group. Total dosage of recombinant gonadotropins was significantly lower and proportion of GnRH antagonist protocols was significantly higher in PCOS group compared with non-PCOS group. There was no difference in number of oocytes retrieved per cycle between the PCOS and non-PCOS group. The proportion of 2PN and cleaved oocytes was equally distributed between the PCOS and non-PCOS group after adjustment of potential confounding variables (age, BMI, fertilization method and male infertility). In the PCOS group, three embryo transfers were cancelled because of ovarian hyper- stimulation syndrome and two because of poor embryo quality. In the non-PCOS group, three transfers were cancelled because of ovarian hyper- stimulation syndrome and five because of poor embryo quality. A higher proportion of non-PCOS women had a negative pregnancy test. Three women in the non-PCOS group had a missed abortion. The implantation failure rate, fetal heartbeat (clinical pregnancy rate) and live birth rate did not differ significantly between the two groups.

Table 2 Treatment cycle characteristics

Fertility method	PCOS (n_{cycles} =43)	non-PCOS ($n_{cycles=}$ 174)	*P*-value
ICSI procedures n(%)	23 (53.5)	101(58.1)	0.355
IVF procedures n(%)	20(46.5)	73(41.9)	
Treatment protocol			
Long GnRH agonist n(%)	22(51.2)	145(82.9)	0.001
GnRH antagonist n(%)	21(48.8)	30(17.1)	
Type of gonadotropins used			
Puregon n(%)	30(69.8)	133(76.9)	0.255
Menopur n(%)	13(30.2)	34(19.7)	0.108
Other: Clomifen, Elonva, Fostimon n(%)	0	6(3.4)	
Cumulative gonadotropin dose used (IU)	1430.7(1149.9–1900.0)	1800(1300.0–2625.0)	0.005
Oocyte retrieval			
Normal fertilized and cleaved oocytes/ retrieved oocytes n(%)	313/541(0.56)	1075/2158(0.50)	0.069(0.081*)
No. retrieved oocytes/cycle	13.4(10.0–15.5)	12.5(9.9–14.0)	0.401
No. normal fertilized and cleaved oocytes/ cycle	7.3(6.0–14.0)	6.5(5.0–10.0)	0.136
Fertility outcome			
Negative pregnancy test n(%)	18/38(47.37)	106/165(64.24)	0.051
Biochemical pregnancy n(%)	20/38 (52.63)	59/165(35.76)	0.054
Implantation failure n(%)	5/38(13.16)	11/165(6.67)	0.181
Fetal heart sound n(%)	15/38(39.47)	45/165(27.27)	0.137
Live-birth n(%)	15/38(39.47)	45/165(27.27)	0.131
Missed abortion n(%)	0	3/165(1.82)	
No. cycle cancellation before embryo transfer	5/43	9/174	0.283

Continuous data (, Cumulative FSH dose used, No retrieved oocytes/cycle, No normal fertilized and cleaved oocytes/cycle) expressed as median and lower (Q1) and upper quartiles (Q3)

Categorical variables (Fertility method, Treatment protocol, Type of FSH used, Normal fertilized and cleaved oocytes/ retrieved oocytes) were expressed as exact numbers and percentages

Wilcoxon sum-rank test was used to test the hypothesis of no difference in between PCOS and non-PCOS, for continuous data

Fisher's test was used to test the hypothesis of no difference of proportions between PCOS and non-PCOS, for categorical variables

*p-valueadj.: Hypothesis of no difference between PCOS and non-PCOS, with adjustment for age, BMI, male infertility and fertilization method (IVF/ICSI)

Two-sided p-values less than 0.05 were considered significant

Early cleavage events

Timing of early cleavage events showed significant differences between PCOS embryos and non-PCOS embryos in two events only (unadjusted data) (Table 3). The time between the disappearance of 1st and 2nd nuclei at the two-cell stage was longer (less synchronized) in PCOS embryos compared to non-PCOS embryos. The proportion of multi-nucleated cells at the two-cell stage was significantly higher in the PCOS group compared with non-PCOS group. After adjustment for potential confounders (age, BMI, male infertility and fertilization method) only the proportion of multi-nucleated cells showed a statistically significant difference. From the 2-cell stage up to the 8-cell stage, the PCOS embryos and non-PCOS embryos displayed almost identical cleavage times. Similarly, the proportion of embryos with direct cleavage to 3-cell stage was comparable between the PCOS and non-PCOS groups.

Post-compaction cleavage events

Time of compaction and reaching the morula stage was significantly earlier in PCOS embryos compared with non-PCOS embryos after controlling for potential confounders (Table 3).

Duration of cleavage stages

The analysis of durations showed comparable lengths of 2-, 3- and 4-cell stages as well as the 2nd cleavage division and 3rd cleavage division between PCOS and non-PCOS embryos.

Table 3 Time-points of embryonic stages (hours)

Parameter	$n_{pcos}/n_{non\text{-}pcos}$	Median $(IQR)_{pcos}$	Median $(IQR)_{non\text{-}pcos}$	OR_{crude}	[95 % CI]$_{crude}$	p-value$_{crude}$	$OR_{adj.}$	[95 % CI]$_{adj.}$	p-value$_{adj.}$
1st nucleus appearance	233/712	1.1(0.7–2.0)	1.0(0.6–1.7)	1.09	(0.92–1.29)	0.304	1.03	(0.86–1.20)	0.731
2nd nucleus appearance	228/696	1.5(0.9–2.4)	1.3(0.7–2.1)	1.13	(0.95–1.35)	0.17	1.06	(0.87–1.30)	0.537
1st nucleus disappearance	245/759	9.6(8.7-10.4)	9.7(9.0–10.4)	0.96	(0.91–1.01)	0.13	0.97	(0.91–1.02)	0.21
2nd nucleus disappearance	236/730	10.4(9.3–11.1)	10.5(9.5–11.3)	1.01	(0.96–1.04)	0.84	1	(0.95–1.05)	0.884
Synchrony of nuclei appearance at 2–cell stage	142/389	0.6(0.3–1.0)	0.6(0.3–0.7)	1.03	(0.89–1.24)	0.75	1.12	(0.92–1.36)	0.392
Synchrony of nuclei disappearance at 2-cell stage	172/570	0.8(0.4–1.4)	0.7(0.3–1.2)	1.18	(1.05–1.33)	0.01	1.18	(0.97–1.43)	0.102
Division to 5 cells (t5)	269/854	24.6(22.4–27.1)	25.0(22.0–27.9)	1.01	(0.96–1.06)	0.676	1.03	(0.97–1.10)	0.371
Division to 7/8 cell stage (t7/t8)	232/738	29.7(26.0–32.1)	29.4(26.3–34.8)	0.97	(0.92–1.02)	0.236	0.98	(0.93–1.03)	0.471
Compaction	253/755	51.7(47.4–59.9)	55.4(49.8–61.4)	0.94	(0.88–0.99)	0.097	0.95	(0.90–1.00)	0.046
Morula	233/703	60.0(55.5–67.9)	63.9(58.3–69.8)	0.95	(0.90–1.00)	0.038	0.95	(0.92–1.00)	0.045
Early blastocyst	231/696	70.5(64.7–74.2)	71.9(66.1–77.9)	0.98	(0.95–1.01)	0.13	0.99	(0.96–1.01)	0.45
Full expanded blastocyst	228/665	81.3(75.8–85.6)	82.0(75.9–88.6)	0.98	(0.96–1.01)	0.148	1	(0.97–1.02)	0.785
Hatching blastocyst	137/380	96.5(90.6–101.3)	96.5(91.6–101.7)	0.99	(0.97–1.02)	0.626	0.99	(0.97–1.01)	0.339
Hatched blastocyst	18/60	107.0(105.1–111.1)	107.6(103.9–111.0)	1	(0.98–1.03)	0.89	1	(0.97–1.02)	0.785
Multinucleation (%)	57/111	18.2	11	1.81	(1.25–2.57)	0.025	1.83	(1.05 –3.21)	0.031
Direct cleavage to 3-cell stage (%)	23/115	8.6	12.3	0.63	(0.37–1.02)	0.105	0.67	(0 0.34–1.24)	0.241

All time-points are normalized to 1st division (t2). Time-point of embryonic stages (hours) expressed as median and lower (Q1) and upper quartiles (Q3)

OR_{Crude} / 95 % CI_{crude}: The influence of PCOS on time-lapse data (response variable), expressed by odds ratio different to one with 95 % CI and p-values

$OR_{adj.}$/ 95 % $CI_{adj.}$: The influence of PCOS on time-lapse data (response variable) with adjustment for female age, BMI, fertilization method(IVF/ICS) and male infertility, expressed by odds ratio difference to one and p-value-values. Data were treated as dependent by the regression model clustering by patient

Two-sided p-values less than 0.05 were considered significant. n = embryos, bl1 = early blastocyst, bl3 = full expanded blastocyst, bl5 = hatching blastocyst, bl6 = fully hatched blastocyst

Table 4 Duration of the embryonic stages (hours)

Parameter	$n_{pcos}/n_{non\text{-}pcos}$	Median $(IQR)_{pcos}$	Median $(IQR)_{non\text{-}pcos}$	OR_{crude}	[95 % $CI]_{crude}$	$p\text{-value}_{crude}$	$OR_{adj.}$	[95 % $CI]_{adj.}$	$p\text{-value}_{adj.}$
Duration of the 2-cell stage	276/899	10.4 (10.6–12.3)	9.9(10.7–12.5)	1.05	(0.96–1.14)	0.306	1.05	(0.93–1.20)	0.426
Duration of the 3-cell stage	226/769	1.1(0.4–2.0)	1.0(0.4–2.3)	0.89	(0.72–1.09)	0.249	0.83	(0.63–1.08)	0.159
Duration of the 4-cell stage	265/839	12.8(10.6–14.5)	12.7(10.7–15.0)	1.04	(0.93–1.16)	0.529	1.06	(0.93–1.21)	0.374
Duration of 2nd cleavage division	302/907	12.3(11.3–13.6)	12.3(11.4–13.7)	0.99	(0.94–1.05)	0.742	0.99	(0.92–1.05)	0.689
Duration of 3rd cleavage division	285/963	16.6(14.2–19.0)	17.8(14.6–22.3)	0.95	(0.88–1.03)	0.212	0.97	(0.90–1.06)	0.539
Duration of 4th cleavage division	252/757	18.4(14.8–22.7)	19.7(15.3–23.8)	0.84	(0.69–1.03)	0.086	0.84	(0.73–0.98)	0.027
Synchrony of the 3rd cleavage division	262/840	5.5(2.3–14.7)	9.7(2.7–16.7)	0.8	(0.67–0.96)	0.023	0.85	(0.68–1.05)	0.138
Blastulation (bl3-bl1)	230/689	10.1(7.3–13.4)	10.4(7.3–13.5)	0.97	(0.88–1.08)	0.615	1	(0.90–1.01)	0.984
Duration of 2-cell stage to full expanded blastocyst	228/665	81.3(75.8–85.6)	82.4(75.9–88.5)	0.98	(0.96–1.01)	0.148	0.99	(0.96–1.02)	0.472

Duration of embryonic stages (hours) expressed as median and lower (Q1) and upper quartiles (Q3)

OR_{Crude} / 95 % CI_{crude}: The influence of PCOS on time-lapse data (response variable), expressed by odds ratio different to one with 95 % Confidence intervals and *p*-values

$OR_{adj.}$ / 95 % $CI_{adj.}$: The influence of PCOS on time-lapse data (response variable) with adjustment for female age, BMI, fertilization method(IVF/ICS) and male infertility, expressed by odds ratio difference to one and *p*-values. Data were treated as dependent by the regression model clustering by patient

Two-sided *p*-values less than 0.05 were considered significant. n = embryos, bl1 = early blastocyst, bl3 = full expanded blastocyst

After adjustment for potential confounders, the time intervening the 8-cell stage and the final divisions (duration of 4th cleavage division) was significantly shorter in the PCOS group compared with non-PCOS group (Table 4). Full definitions of annotated time-points are shown in (Table 5).

Survival to blastocyst stage

Analysis of total percentages of embryos reaching a developmental stage, showed a comparable chance for survival up to the 8-cell stage between PCOS embryos and non-PCOS embryos. Although a higher proportion of embryos reached the early- and full-expanded blastocyst stage in the PCOS-group, this was only significant for unadjusted estimates (Table 1 in Online Resource material). To evaluate whether the delay in cleavage times in non-PCOS embryos (morula stage and synchrony of 3rd cleavage division) was related to subsequent embryo-arrest 450 embryos (371 embryos from the non-PCOS group and 79 embryos from the PCOS group) not reaching the full-expanded blastocyst stage were excluded and an additional, comparable analysis were performed for all parameters. The results were unchanged.

Discussion

This study investigated the details of early development of in-vitro fertilized embryos from women with PCOS compared to non-PCOS patients with use of time-lapse imaging. The total analysis of almost 1400 embryos (313 from PCOS affected women/1075 from non-PCOS women) indicate that the viability of embryos from women with PCOS is comparable to, or even better, than that of embryos from the non PCOS patients. The live birth rate was similar between the two groups. The reports of disturbed oocyte maturation in women with PCOS have suggested these women have embryos are of poor quality [14–16, 33]. The here-presented results, however, challenge such a statement.

Comparison of embryo kinetics between PCOS embryos and non-PCOS embryos adjusted for female age, BMI, male infertility and fertilization method showed statistical significant difference between three parameters. Time to reach compaction and morula stage and the duration of the 4th cleavage division was significantly shorter in PCOS embryos compared with non-PCOS embryos. There was no difference in the proportion of oocytes with normal fertilisation ([2]PN) and no difference in cleaved embryos per treatment cycle and no difference in the early- or full-expanded blastocyst rates per treatment cycle between PCOS and non-PCOS group. Selection of embryos for transfer based on conventional criteria of morphological quality (Gardner criteria) resulted in a non-

Table 5 Definitions of investigated parameters

Parameters	Definition	Assessme
1st and 2nd nuclei appearance	Appearance of 1st and 2nd nucleus at 2-cell stage.	Time-point
1st and 2nd nuclei disappearance	Disappearance of 1st and 2nd nucleus at 2-cell stage.	
Divisions from 2 cells to 8 cells	Each division is a complete separation of two daughter cells enclosed by their own cytoplasmic membrane	
Final division	The final cleavage division to ≥9 cells before initiation of compaction	
Compaction	The first fusion of two cell boundaries followed by a decrease in embryo diameter	
Morula	The compaction process is complete for all cells. All cell boundaries are unclear.	
Early blastocyst	The initiation of blastulation when a blastocoel is visible (bl1)	
Full expanded blastocyst	The blastocoel cavity fills out ≥50 % of the embryo and an inner cell mass i well defined (bl3)	
Hatching blastocyst	The trophectoderm herniation (bl5) through the zona Pellucida.	
Hatched blastocyst	The embryo has full escaped the zona Pellucida (bl6)	
Multinucleation	Multinucleate at 2-cell stage	Binary
Direct cleavage to 3 cell stage	t3-t1 <5 h	
First cytokinesis	Appearance of cleavage furrow or considerable elongation (>15 %) at one-cell stage until complete separation.	Duration
Synchrony of nuclei appearance at 2-cell stage	Time from the appearance of 1st and 2nd nuclei at 2-cell stage	
Synchrony of nuclei disappearance at 2-cell stage	Time from the disappearance of 1^{st} and 2^{nd} nuclei at 2-cell stage	
Duration of 2-cell stage	t3-t2	
Duration of 3-cell stage	t4-t3	
Duration of the 4-cell stage	t5-t4	
Duration of 2nd cleavage division	t4-t2	
Duration of 3rd cleavage division	t8-t4	
Synchrony of the 3rd cleavage division	t8-t5	
Duration of 4th cleavage division	$t \geq 9$-t8	
Blastulation (bl3-bl1)	Duration from early to full expanded blastocyst (bl3-bl1)	

The time-point assessment was applied to the dynamic parameters. Measurement of a certain duration was subsequently calculated as the interval between two time-points. Assessment of binary values yes and no was applied to morphologic parameters

significant higher number of biochemical pregnancies in the PCOS group compared with non PCOS group.

Live birth rates between PCOS and non-PCOS group were comparable.

Nevertheless multi-nucleation at the 2-cell stage was seen significantly more frequent in PCOS embryos compared with non-PCOS embryos. Since multi-nucleated embryos are often chromosomally abnormal the increased risk of miscarriage in PCOS patients may be associated to this finding [34] and may reduce the implantation rate [35]. De Vincentiis et al. recently reported that multinucleation is associated with oocyte immaturity [36]. It may be speculated that the more frequent observation of multi-nucleated cells in PCOS blastomeres compared with non-PCOS blastomeres could result from the influence of elevated LH and testosterone on oocyte growth. Hyperandrogenism and elevated concentrations of LH in women with PCOS have been associated with alterations in accumulation of messenger RNAs necessary for completion of meiosis [13], which may affect the chromosomal constitution of the oocyte [4]. Still, multinucleation is sometimes difficult to accurately observe owing to presence of fragmentation or cytoplasmic inclusions. We have previously shown that manual annotation of multi-nucleation at the 2-cell stage have potential for inter/intra-observer differences [32]. With the chosen level of significance and the number of analyzed events, the reported difference in multi-nucleation could be a product of chance. Nevertheless an increased frequency of multinucleated, chromosomal abnormal embryos may contribute to a lowered fertility in PCOS women.

Timing of cell divisions and cell cycle length, achievable with time-lapse imaging (TLI), has been regarded as a promising tool for embryo selection and de-selection in IVF treatments [21, 22, 37–43]. Previous research with time lapse has proposed predictive parameters of blastocyst formation and clinical pregnancy, as reviewed by Kirkegaard et al. 2015 [24]. Lemmen et al. 2008 found synchronicity in appearance of nuclei after 1st division to differ between implanted and non-implanted embryos [44]. Azzarello et al. 2012, detected an association between pronuclei breakdown and pregnancy. Meseguer et al. 2011 observed a correlation between timing of

the 5–cell stage, and duration of 2-cell stage and 3-cell stage and pregnancy. We found a similar blastocyst formation rate in PCOS and non-PCOS embryos.

In contrast to the present results, a recent time-lapse study comparing 25 hyper-androgenic PCOS patients (110 embryos) and 20 non-PCOS women (97 embryos) found a significant delay in time of pronuclei breakdown, 1st division and 2nd 3rd and 6th division in embryos from hyper-androgenic PCOS [21]. A graphic plot of their data showed that the hyper-androgenic PCOS embryos are offset from pronuclei breakdown and throughout the earliest cleavage by a constant time interval, compared with control embryos. Therefore, the observed delay for the hyper-androgenic PCOS may originate in an error in the oocyte activation stage, rather than an error in the cleavage stage embryo. Thus, in agreement with the present data, Wissing et al., found no difference in embryo kinetics between embryos from normo-androgenic PCOS patients compared with controls. There are several significant differences between our study and the study by Wissing et al. First, the fraction of patients with hyperandrogenism in the two studies may be different due to differences in assay methods (mass spectroscopy MS/MS vs. immunoassay), and two different definitions of hyperandrogenism. Secondly, different stimulation protocols were used. Third, in our study all cleavage times were normalized to first cleavage and treated as durations to facilitate comparison between IVF and ICSI embryos. Probably Wissing et al. would have obtained a smaller difference between PCOS and control embryos, if all cleavage time-point were adjusted to a common event, since the delay in PCOS embryos seemed to be constant from the pronuclei breakdown and onward.

Apart from anovulation, PCOS patients have several additional possible fertility-reducing factors. The increasing understanding of the syndrome links PCOS to the metabolic syndrome and diabetes [45, 46], both of which have been linked to poor reproductive outcomes [47, 48]. Essentially, obesity is more common among women with PCOS and is known to exacerbate the symptoms of PCOS [49, 50]. With use of an oocyte donation model, a subgroup of women with PCO, obesity and insulin resistance with poor embryo quality have been identified [51]. It may be that some phenotypes of PCOS are more susceptible to changes in the embryo quality or in the endometrial receptivity than others. One recent study has reported that obesity and PCOS independently resulted in smaller oocyte size. The importance of oocyte size to developmental competence is yet unknown [52]. In our study, there was no difference in BMI values between PCOS and non-PCOS women and median BMI was in the normal range of weight for both groups. Furthermore 33 out of 39 PCOS women tested for biochemical androgen excess, in our study, had only polycystic ovaries and anovulation, previously described as a "mild" presentation of the disorder [9]. The fact that there was no difference between PCOS patients and the control group in terms of number of oocytes harvested could support such an assumption. Therefore it should be stressed that PCOS combined with androgen excess and more severe metabolic abnormalities possibly could have produced different results.

The evidence for adverse effects of PCOS in the developing embryo has been vividly questioned [7, 33, 53–55]. PCOS women are characterized by having high response to gonadotropins, resulting in higher number of oocytes obtained [3]. Consequently, there is greater choice in the selection of embryos based on morphological criteria for transfer. This may explain the significantly higher cumulative embryo score (calculated by multiplying the number of blastomeres in each embryo by the morphologic quality score of that embryo and adding the values for the embryos transferred in each patient) reported for PCOS embryos compared to controls in previous studies [54, 55]. Furthermore, there are some fundamental differences among the comparative studies on PCOS pre-implantation embryos (female BMI and PCOS phenotype, fertilization method and stimulation protocols) that may also account for some of the conflicting results obtained.

Factors related to fertilization method, culture oxygen tension, gonadotropin doses and ovarian stimulation protocol have been reported to affect cleavage kinetics of IVF embryos. Previously high oxygen tension was demonstrated to influence pre-compaction development, with embryos cultured in atmospheric (20 %) oxygen completed the third cell cycle later than those cultured in 5 % oxygen [29]. In present study we detected no differences in pre-compaction intervals between PCOS and non-PCOS embryos. By controlling for fertilization method in regression modeling we minimized bias from oxygen exposure. Both gonadotropin doses and ovarian stimulation protocol have been reported to affect timing of embryo development. Embryos from women receiving higher doses of gonadotropins or on GnRH agonist +HCG triggering protocols underwent the embryonic stages later than those derived from women receiving the lower doses or an GnRH antagonist + GnRH agonist triggering protocols [26, 56]. In the present study, the treatment protocol and gonadotropin doses were administered according to expected and actual treatment response of the patient. Consequently, PCOS patients were more often treated with GnRH antagonist protocols and given lower gonadotropin doses compared with non-PCOS women. This may have accounted for some of the kinetic differences found at late embryonic stages, with non-PCOS embryos reaching post-compaction stages later than PCOS embryos.

Inflammation and dysregulation of local growth factors responsible for oocyte maturation have been described in PCOS ovaries and may contribute to an aberrant embryo development [14–16, 33, 50]. Gene expression profiling studies have identified over one hundred genes with altered expression in unfertilized PCOS oocytes [13] and cumulus cells of

mature MII oocytes [14]. Putative nuclear receptor binding sites have been described in the same genes, suggesting that metabolic and epigenetic interactions might affect oocyte quality. Nevertheless, it has been unclear whether the alterations reported in the oocyte actually are manifest in later embryo development. Our data may indicate that the causative factor for subfertility in PCOS women after IVF is not related to timing of development of the pre-implantation embryo. PCOS in combination with androgen excess have been suggested to lead to endometrial dysfunction and/or impair endometrial receptivity, and may, on the contrary, be a more important causal factor behind subfertility in women with PCOS [57, 58].

It may be speculated that some of the women seeking medical assistance for anovulatory infertility might have been incorrectly classified as having the disease. The measure of 12 follicles per ovary for polycystic ovaries has been suggested as a normal variant of ovarian morphology (especially in younger women under 30 years of age) [59–62]. In our data, such misclassification is possible of women diagnosed with PCOS (both PCO and oligomenorrhea) without androgen excess, which may lead to an underestimation of the correlation between PCOS and developmental timing (Type II error). Furthermore, our control group includes some women with polycystic ovarian morphology according to the Rotterdam criteria. Although all controls with PCO were tested for androgen excess this may have increased the risk of a Type II error. The fact that data were not restricted to first cycles might be important too. Thus, certain patients with other risk factors for failed cycles might be over-represented in the dataset and unevenly distributed between groups. The younger age for the women with PCOS probably reflects earlier visits to the fertility clinic due to menstrual irregularity. Therefore, the non-PCOS women may have a longer duration of infertility and more severe infertility, which may potentially lead to Type II errors also. However, for several reasons we consider this unlikely. Firstly, all patients enrolled in our study were regarded as good-prognosis patients. Secondly, male infertility was the main reason for the couple's infertility in the non-PCOS group.

We conclude from these observations that the viability, i.e., developmental kinetics and blastocyst formation of embryos from women with PCOS seem to be comparable to that of embryos from other non-PCOS regularly cycling women. The present data indicate that it is less likely that embryonic factors contribute to subfertility in PCOS patients, and further research is needed to elucidate the role of endometrial receptivity in this respect.

Acknowledgments The authors wish to thank the patients and staff at the Fertility Clinic, Aarhus University Hospital. The authors are grateful for statistical support provided by Morten Frydenberg, University of Aarhus.

Authors' roles J.I and U.B.K conceived the study and designed the project. L.S. drafted the manuscript. L.S. and K.K. did the acquisition and interpretation of data.

K.K., J.I and U.B.K performed critical revision of the manuscript. All authors have given their final approval of present version to be published.

Funding Funding for the present study was provided by Aarhus University. Research at the Fertility Clinic, Aarhus University Hospital is supported by an unrestricted grant from MSD and Ferring.

Conflict of interest The authors declare no conflict of interest.

References

1. Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. J Clin Endocrinol Metab. 1998;83:3078–82.
2. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81:19–25.
3. Dor J, Shulman A, Levran D, Ben-Rafael Z, Rudak E, Mashiach S. The treatment of patients with polycystic ovarian syndrome by in-vitro fertilization and embryo transfer: a comparison of results with those of patients with tubal infertility. Hum Reprod. 1990;5:816–8.
4. Sengoku K, Tamate K, Takuma N, Yoshida T, Goishi K, Ishikawa M. The chromosomal normality of unfertilized oocytes from patients with polycystic ovarian syndrome. Hum Reprod. 1997;12:474–7.
5. Urman B, Fluker MR, Yuen BH, Fleige-Zahradka BG, Zouves CG, Moon YS. The outcome of in vitro fertilization and embryo transfer in women with polycystic ovary syndrome failing to conceive after ovulation induction with exogenous gonadotropins. Fertil Steril. 1992;57:1269–73.
6. Sahu B, Ozturk O, Ranierri M, Serhal P. Comparison of oocyte quality and intracytoplasmic sperm injection outcome in women with isolated polycystic ovaries or polycystic ovarian syndrome. Arch Gynecol Obstet. 2008;277:239–44.
7. Weghofer A, Munne S, Chen S, Barad D, Gleicher N. Lack of association between polycystic ovary syndrome and embryonic aneuploidy. Fertil Steril. 2007;88:900–5.
8. Roos N, Kieler H, Sahlin L, Ekman-Ordeberg G, Falconer H, Stephansson O. Risk of adverse pregnancy outcomes in women with polycystic ovary syndrome: population based cohort study. BMJ. 2011;343:d6309.
9. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet. 2007;370:685–97.
10. Heijnen EM, Eijkemans MJ, Hughes EG, Laven JS, Macklon NS, Fauser BC. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome. Hum Reprod Update. 2006;12:13–21.
11. Kim YJ, Ku SY, Jee BC, Suh CS, Kim SH, Choi YM, et al. A comparative study on the outcomes of in vitro fertilization between women with polycystic ovary syndrome and those with sonographic polycystic ovary-only in GnRH antagonist cycles. Arch Gynecol Obstet. 2010;282:199–205.
12. Vause TD, Cheung AP, Sierra S, Claman P, Graham J, Guillemin JA, et al. Ovulation induction in polycystic ovary syndrome: No. 242, May 2010. Int J Gynaecol Obstet. 2010;111:95–100.

13. Wood JR, Dumesic DA, Abbott DH, Strauss 3rd JF. Molecular abnormalities in oocytes from women with polycystic ovary syndrome revealed by microarray analysis. J Clin Endocrinol Metab. 2007;92:705–13.
14. Haouzi D, Assou S, Monzo C, Vincens C, Dechaud H, Hamamah S. Altered gene expression profile in cumulus cells of mature MII oocytes from patients with polycystic ovary syndrome. Hum Reprod. 2012;27:3523–30.
15. Qiao J, Feng HL. Extra- and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. Hum Reprod Update. 2011;17:17–33.
16. Dumesic DA, Padmanabhan V, Abbott DH. Polycystic ovary syndrome and oocyte developmental competence. Obstet Gynecol Surv. 2008;63:39–48.
17. Zhao SY, Qiao J, Chen YJ, Liu P, Li J, Yan J. Expression of growth differentiation factor-9 and bone morphogenetic protein-15 in oocytes and cumulus granulosa cells of patients with polycystic ovary syndrome. Fertil Steril. 2010;94:261–7.
18. Lane M, Gardner DK. Selection of viable mouse blastocysts prior to transfer using a metabolic criterion. Hum Reprod. 1996;11:1975–8.
19. Lane M, Gardner DK. Differential regulation of mouse embryo development and viability by amino acids. J Reprod Fertil. 1997;109:153–64.
20. Wolff HS, Fredrickson JR, Walker DL, Morbeck DE. Advances in quality control: mouse embryo morphokinetics are sensitive markers of in vitro stress. Hum Reprod. 2013;28:1776–82.
21. Meseguer M, Rubio I, Cruz M, Basile N, Marcos J, Requena A. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. Fertil Steril. 2012;98:1481–9.e10.
22. Rubio I, Galan A, Larreategui Z, Ayerdi F, Bellver J, Herrero J, et al. Clinical validation of embryo culture and selection by morphokinetic analysis: a randomized, controlled trial of the EmbryoScope. Fertil Steril. 2014;102:1287–1294.e5.
23. VerMilyea MD, Tan L, Anthony JT, Conaghan J, Ivani K, Gvakharia M, et al. Computer-automated time-lapse analysis results correlate with embryo implantation and clinical pregnancy: a blinded, multi-centre study. Reprod Biomed Online. 2014;29:729–36.
24. Kirkegaard K, Ahlstrom A, Ingerslev HJ, Hardarson T. Choosing the best embryo by time lapse versus standard morphology. Fertil Steril. 2015;103:323–32.
25. Wissing ML, Bjerge MR, Olesen AIG, Hoest T, Mikkelsen AL (2013) Impact of PCOS on early embryo cleavage kinetics. Reprod Biomed Online.
26. Munoz M, Cruz M, Humaidan P, Garrido N, Perez-Cano I, Meseguer M. The type of GnRH analogue used during controlled ovarian stimulation influences early embryo developmental kinetics: a time-lapse study. Eur J Obstet Gynecol Reprod Biol. 2013;168:167–72.
27. Kirkegaard K, Kesmodel US, Hindkjaer JJ, Ingerslev HJ (2013) Time-lapse parameters as predictors of blastocyst development and pregnancy outcome in embryos from good prognosis patients: a prospective cohort study. Hum Reprod
28. Kirkegaard K, Hindkjaer JJ, Ingerslev HJ. Human embryonic development after blastomere removal: a time-lapse analysis. Hum Reprod. 2012;27:97–105.
29. Kirkegaard K, Hindkjaer JJ, Ingerslev HJ (2012) Effect of oxygen concentration on human embryo development evaluated by time-lapse monitoring. Fertil Steril.
30. Gardner DK, Surrey E, Minjarez D, Leitz A, Stevens J, Schoolcraft WB. Single blastocyst transfer: a prospective randomized trial. Fertil Steril. 2004;81:551–5.
31. Kirkegaard K, Agerholm IE, Ingerslev HJ. Time-lapse monitoring as a tool for clinical embryo assessment. Hum Reprod. 2012;27:1277–85.
32. Sundvall L, Ingerslev HJ, Breth Knudsen U, Kirkegaard K (2013) Inter- and intra-observer variability of time-lapse annotations. Hum Reprod.
33. Artini PG, Ruggiero M, Parisen Toldin MR, Monteleone P, Monti M, Cela V, et al. Vascular endothelial growth factor and its soluble receptor in patients with polycystic ovary syndrome undergoing IVF. Hum Fertil (Camb). 2009;12:40–4.
34. Staessen C, Van Steirteghem A. The genetic constitution of multinuclear blastomeres and their derivative daughter blastomeres. Hum Reprod. 1998;13:1625–31.
35. Saldeen P, Sundstrom P. Nuclear status of four-cell preembryos predicts implantation potential in in vitro fertilization treatment cycles. Fertil Steril. 2005;84:584–9.
36. De Vincentiis S, De Martino E, Buffone MG, Brugo-Olmedo S. Use of metaphase I oocytes matured in vitro is associated with embryo multinucleation. Fertil Steril. 2013;99:414–21.
37. Cruz M, Garrido N, Herrero J, Perez-Cano I, Munoz M, Meseguer M. Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality. Reprod Biomed Online. 2012;25:371–81.
38. Dal Canto M, Coticchio G, Mignini Renzini M, De Ponti E, Novara PV, Brambillasca F, et al. Cleavage kinetics analysis of human embryos predicts development to blastocyst and implantation. Reprod Biomed Online. 2012;25:474–80.
39. Azzarello A, Hoest T, Mikkelsen AL. The impact of pronuclei morphology and dynamicity on live birth outcome after time-lapse culture. Hum Reprod. 2012;27:2649–57.
40. Hashimoto S, Kato N, Saeki K, Morimoto Y. Selection of high-potential embryos by culture in poly(dimethylsiloxane) microwells and time-lapse imaging. Fertil Steril. 2012;97:332–7.
41. Hlinka D, Kalatova B, Uhrinova I, Dolinska S, Rutarova J, Rezacova J, et al. Time-lapse cleavage rating predicts human embryo viability. Physiol Res. 2012;61:513–25.
42. Wong C, Chen AA, Behr B, Shen S. Time-lapse microscopy and image analysis in basic and clinical embryo development research. Reprod Biomed Online. 2013;26:120–9.
43. Conaghan J, Chen AA, Willman SP, Ivani K, Chenette PE, Boostanfar R, et al. Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: results from a prospective multicenter trial. Fertil Steril. 2013;100:412–9.e5.
44. Lemmen JG, Agerholm I, Ziebe S. Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes. Reprod Biomed Online. 2008;17:385–91.
45. Gluszak O, Stopinska-Gluszak U, Glinicki P, Kapuscinska R, Snochowska H, Zgliczynski W, et al. Phenotype and metabolic disorders in polycystic ovary syndrome. ISRN Endocrinol. 2012;2012:569862.
46. Han AR, Kim HO, Cha SW, Park CW, Kim JY, Yang KM, et al. Adverse pregnancy outcomes with assisted reproductive technology in non-obese women with polycystic ovary syndrome: a case-control study. Clin Exp Reprod Med. 2011;38:103–8.
47. Tian L, Shen H, Lu Q, Norman RJ, Wang J. Insulin resistance increases the risk of spontaneous abortion after assisted reproduction technology treatment. J Clin Endocrinol Metab. 2007;92:1430–3.
48. Wittemer C, Ohl J, Bailly M, Bettahar-Lebugle K, Nisand I. Does body mass index of infertile women have an impact on IVF procedure and outcome? J Assist Reprod Genet. 2000;17:547–52.
49. Franks S, Stark J, Hardy K. Follicle dynamics and anovulation in polycystic ovary syndrome. Hum Reprod Update. 2008;14:367–78.
50. Norman RJ. Obesity, polycystic ovary syndrome and anovulation–how are they interrelated? Curr Opin Obstet Gynecol. 2001;13:323–7.
51. Cano F, Garcia-Velasco JA, Millet A, Remohi J, Simon C, Pellicer A. Oocyte quality in polycystic ovaries revisited: identification of a

particular subgroup of women. J Assist Reprod Genet. 1997;14: 254–61.
52. Marquard KL, Stephens SM, Jungheim ES, Ratts VS, Odem RR, Lanzendorf S, et al. Polycystic ovary syndrome and maternal obesity affect oocyte size in in vitro fertilization/intracytoplasmic sperm injection cycles. Fertil Steril. 2011;95:2146-9–2149.e1.
53. Ludwig M, Finas DF, al-Hasani S, Diedrich K, Ortmann O. Oocyte quality and treatment outcome in intracytoplasmic sperm injection cycles of polycystic ovarian syndrome patients. Hum Reprod. 1999;14:354–8.
54. Hardy K, Robinson FM, Paraschos T, Wicks R, Franks S, Winston RM. Normal development and metabolic activity of preimplantation embryos in vitro from patients with polycystic ovaries. Hum Reprod. 1995;10:2125–35.
55. Child TJ, Abdul-Jalil AK, Gulekli B, Tan SL. In vitro maturation and fertilization of oocytes from unstimulated normal ovaries, polycystic ovaries, and women with polycystic ovary syndrome. Fertil Steril. 2001;76:936–42.
56. Munoz M, Cruz M, Humaidan P, Garrido N, Perez-Cano I, Meseguer M. Dose of recombinant FSH and oestradiol concentration on day of HCG affect embryo development kinetics. Reprod Biomed Online. 2012;25:382–9.
57. Shang K, Jia X, Qiao J, Kang J, Guan Y. Endometrial abnormality in women with polycystic ovary syndrome. Reprod Sci. 2012;19:674–83.
58. Cermik D, Selam B, Taylor HS. Regulation of HOXA-10 expression by testosterone in vitro and in the endometrium of patients with polycystic ovary syndrome. J Clin Endocrinol Metab. 2003;88: 238–43.
59. Duijkers IJ, Klipping C. Polycystic ovaries, as defined by the 2003 Rotterdam consensus criteria, are found to be very common in young healthy women. Gynecol Endocrinol. 2010;26:152–60.
60. Johnstone EB, Rosen MP, Neril R, Trevithick D, Sternfeld B, Murphy R, et al. The polycystic ovary post-rotterdam: a common, age-dependent finding in ovulatory women without metabolic significance. J Clin Endocrinol Metab. 2010;95:4965–72.
61. Kristensen SL, Ramlau-Hansen CH, Ernst E, Olsen SF, Bonde JP, Vested A, et al. A very large proportion of young Danish women have polycystic ovaries: is a revision of the Rotterdam criteria needed? Hum Reprod. 2010;25:3117–22.
62. Lauritsen MP, Bentzen JG, Pinborg A, Loft A, Forman JL, Thuesen LL, et al. The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-Mullerian hormone. Hum Reprod. 2014;29: 791–801.