Title:
SLOW GROWING EMBRYOS BIOPSIED ON DAY 7: WHAT ARE REALISTIC EXPECTATIONS?

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Objective:

Extended embryo culture to Day 5/6 has become standard procedure in many IVF laboratories internationally. The value of prolonging culture to Day 7 is debated due to concerns regarding the reproductive competence of slowly developing embryos. However, a report published by Su Yu et al. showed Day 7 blastocysts have up to a 36.7% euploid rate and were shown to have a 20% pregnancy rate at transfer. Still, there is limited published data to support routinely prolonging blastocyst culture to Day 7 for slowly progressing embryos. This study describes observed aneuploidy and pregnancy rates in embryos cultured to Day 7 of development in a single IVF lab.

Design:

Retrospective, cohort analysis.

Materials and Methods:

The study included patients that underwent an IVF cycle with preimplantation genetic testing for aneuploidy (PGT-A) from January 2012 to March 2018. Patients utilizing donor oocytes, requiring surgical sperm retrieval or harboring chromosomal rearrangements on peripheral karyotype testing were excluded. Embryos were routinely cultured until reaching criteria for trophectoderm biopsy (Gardner´s > 4,C,C), which was performed on Day 5, 6 or 7, as appropriate. Trophectoderm biopsies were analyzed by Next-Generation Sequencing or quantitative Polymerase Chain Reaction (qPCR). Embryo cohorts were established based on day of biopsy (Day 5, Day 6, or Day 7). Comparative analysis was performed using ANOVA and chi squared tests. A GEE model adjusting for potential cofounders and repeated measures was used to analyze the odds of aneuploidy using Day 5 as a control group. A sample size of 524 embryos per group was calculated to detect a difference of 10% in aneuploidy rate with a 90% power (alpha = 0.05). A sub analysis was performed to analyze differences in IVF outcomes (i.e. biochemical pregnancy, implantation rate, ongoing pregnancy rate, multiple pregnancy rate and clinical loss rate) among these groups, utilizing a single euploid FET.

Results:

A total of 25,775 biopsied embryos were analyzed (Day 5: 12,535 embryos (48.6%); Day 6: 11, 939 embryos (46.3%); and Day 7: 1,298 embryos (5.10%)). Significant differences were found in patient age, gonadotropin dosage during COH, E2 at trigger, P4 at trigger, number of previous retrievals, baseline AMH, BAFC, number of eggs retrieved, 2PN embryos, and number of embryos biopsied among cohorts. Aneuploidy rates were significantly higher in Day 7 blastocysts compared to Day 5 and 6 cohorts (Day 5= 42.3%; Day 6= 44.1% ;Day 7= 55.1%, p=<0.0001). When using Day 5 as a control group and adjusting for age, AMH, BMI, embryo

quality, and number of biopsied embryos; there was a significant association between aneuploidy and slow growing embryos biopsied on Day 7 (β= -0.79, CI 95%=-0.3 - -0.09, OR=0.79, CI 95% 0.68 – 0.91, p=0.001). Sub analysis of 3,556 single, euploid FET cycles (Day 5: 2,211 cycles (62.1%); Day 6: 1,263 (35.5%); and Day 7: 82 (23.05%)) showed significant differences in age, BMI, AMH, endometrial thickness at ET, embryo quality, biochemical pregnancy, implantation rate, ongoing pregnancy rate and clinical loss rate among cohorts (Table 2). Using a GEE model controlling for endometrial thickness at ET, age, BMI and embryo quality, there were a significant association with the odds of lower biochemical pregnancy, implantation, and ongoing pregnancy rates, yet no association with clinical loss rates, in patients who had extended embryo culture to Day 7 (Table 3).

Conclusions:

Developmental stage, morphological grade and ploidy status are paramount factors affecting the implantation potential of blastocysts. The study showed embryos cultured to Day 7 have a 21% higher aneuploidy rate when compared to Day 5/6 embryos. However, 40.5% of Day 7 embryos were found to be euploid and suitable for transfer. This study sub-analysis showed that Day 7 embryos have lower, but acceptable implantation rates (28%). To our knowledge, this is the largest known analysis of embryos cultured to Day 7 evaluating ploidy rates and IVF outcomes. We suggest utilizing high morphologic quality Day 5/6 euploid embryos as a first-line approach during ART treatments. However, patients with slower growing embryos requiring extended culture to Day 7 can be comforted in knowing that these embryos can be used for transfer and that successful pregnancy outcome is achievable.

Support:

None.

Table 1:

Demographic characteristics, ovarian stimulation, embryological parameter comparisons per embryo age group.

	Day 5		Day 6		Day 7		ANOVA	
	n=12535		n=11939		n=1298		p value	
Patients Age	35.32	4.66	35.60	4.72	36.01	4.78	<0.0001	*
BMI	23.62	4.24	23.62	4.27	23.92	4.39	0.0501	
Cumulative Gonadotropine Dose Utilized (IU)	3273.61	1282.32	3443.36	1315.30	3777.54	1277.82	<0.0001	*
Estradiol at trigger (pg/mL)	2641.22	1247.15	2553.73	1232.89	2455.60	1197.60	<0.0001	*
Progesterone at trigger (ng/mL)	0.92	0.54	0.94	0.51	0.94	0.52	0.001	*
Baseline FSH (IU/mL)	5.97	3.26	5.90	3.21	6.09	3.39	0.14	
Baseline AMH (ng/mL)	4.54	5.22	4.03	4.54	3.59	4.13	<0.0001	*
Basal antral follicle count	14.53	7.71	13.65	7.43	13.02	7.05	<0.0001	*
Oocytes Retrieved	19.86	11.2	19.18	11.11	17.30	9.21	<0.0001	*
M2 Oocytes retrieved	15.61	9.15	14.65	9.25	12.98	7.60	<0.0001	*
2PN embryos	13.19	8.11	12.30	8.13	10.68	6.56	<0.0001	*
# Embryos biopsied	8.19	5.21	7.13	4.96	5.65	4.16	<0.0001	*
# Previous Oocyte Retrievals	0.53	0.97	0.64	1.10	0.79	1.47	<0.0001	*
Gravida	1.17	1.40	1.25	1.43	1.02	1.33	<0.0001	*
Para	0.42	0.75	0.47	0.81	0.42	0.75	<0.0001	*
Aneuploid embryos/ Tested embryos (%)	5305/12535	**42.30%**	5271/11939	**44.10%**	716/1298	**55.10%**	<0.0001	*
Euploid embryos/ tested embryos (%)	6867/12535	**54.70%**	6325/11939	**52.90%**	530/1298	**40.50%**	<0.0001	*
Unamplified or inconclusive result embryos/ tested embryos (%)	363/12535	**2.80%**	343/11939	**2.80%**	52/1298	**4.00%**	0.06	NS

Table 2:

Demographic characteristics of populations, stimulation and embryological parameters of FET-SET cycles per embryo age.

	Day 5		Day 6		Day 7			
	n=2211		n=1263		n=82		ANOVA/Chisq	
	Mean	Sd	Mean	SD	Mean	SD	p value	sign
Patient age at retrieval	35.75	3.85	36.37	4.00	37.40	3.54	<.0001	*
Patient age at ET	36.08	3.90	37.02	3.97	37.84	3.51	<.0001	*
BMI	23.53	4.17	23.92	4.55	23.72	4.37	0.03	*
Estradiol at Trigger (pg/mL)	450.50	385.92	420.45	348.73	671.39	690.58	0.008	*
Progesterone at conversion (pg/mL)	0.31	0.18	0.33	0.30	0.32	0.29	0.61	NS
AMH (ng/mL)	4.19	4.87	3.06	3.78	2.79	3.93	0.28	NS
Endometrial Thickness at Transfer	9.38	2.08	9.27	2.00	9.24	2.00	<.0001	*
Endometrial Type 3 at ET	1888/2211	85.30%	1071/1263	84.80%	76/82	92.60%	0.14	NS
Top embryo at ET (>4,B,B)	1747/2525	79.01%	764/1263	60.49%	14/82	17.07%	<.0001	*
Biochemical Pregnancy Rate	1695/2211	**76.66%**	845/1263	**66.90%**	37/82	**45.12%**	<.0001	*
Implantation rate	1425/2211	**64.40%**	682/1263	**53.90%**	23/82	**28.04%**	0.0004	*
Ongoing pregnancy rate	1237/2211	**55.94%**	556/1263	**44.02%**	15/82	**18.20%**	<.0001	*
Clinical loss rate	188/1425	**13.10%**	126/682	**18.40%**	8.0/28	**28.50%**	0.005	*
Multiple pregnancy rate	43/2211	**1.90%**	17/1263	**1.34%**	0/82	**0%**	0.45	NS

Table 3:

Day 7 embryo parameter estimates, using a Generalized Estimating Equation (GEE) adjusting for cofounders for IVF success rates.

GEE estimates controlling for Age, BMI, AMH, Endometrial thickness and Embryo quality at ET.						
	Std. Error	Beta estimate	CI 95%		p value	significance
Biochemical Pregnancy Rate	0.2339	0.5464	0.0879	1.0048	0.0195	*
Implantation rate	0.3648	0.6831	-0.0319	1.3981	0.0611	*

Ongoing pregnancy rate	0.3516	0.9117	0.2227	1.6008	0.0095	*
Clinical loss rate	0.4245	-0.4893	-1.3214	0.3428	0.2491	NS