Preparation of Endometrium For Thawed Embryo Transfer

MSRM 2016, İzmir

Umit Goktolga , MD, Assoc. Prof.
Bahceci Health Group, Istanbul

The History of Cryopreservation

How to minimize the risk of OHSS?

Addition of antagonist (to prevent premature LH surge)
Triggering oocyte final maturation by a GnRH agonist

(Resulted in "Luteal-phase defect)

Can it be corrected by deferring ET by vitrification And,

subsequent warming and ET?

(Humaidan et al. 2005; Kolibiniakis et al., 2005; Griesinger et al. 2007;Blockeel et al. 2015)

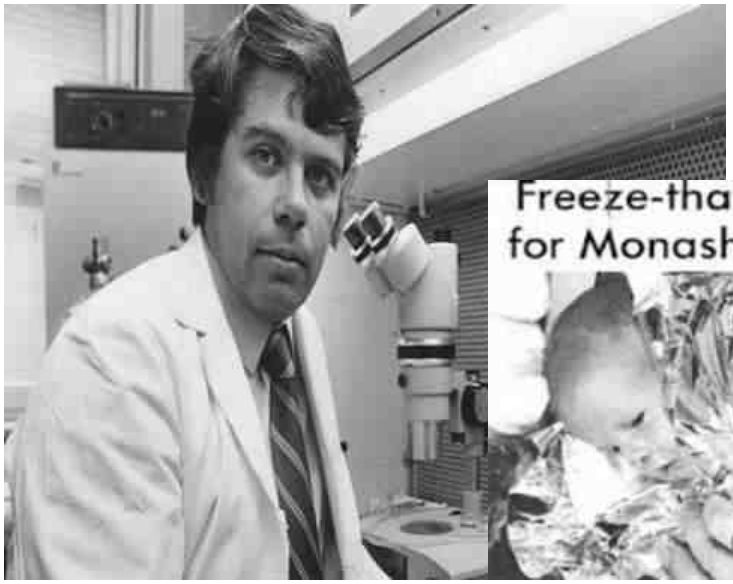
The History of Cryopreservation

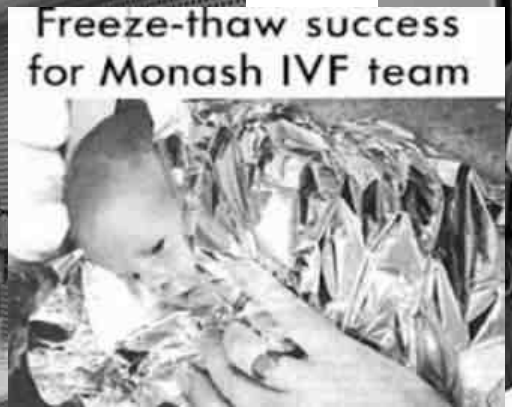
Motility after thawed spermatozoa (1938)

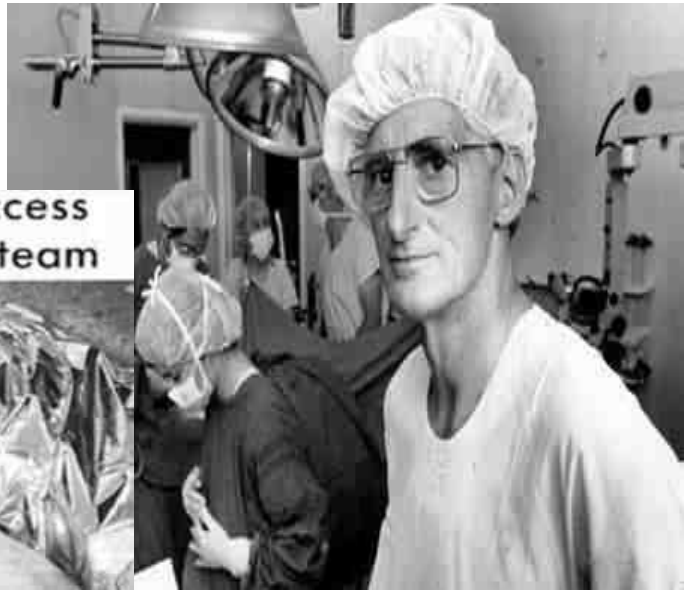
The first fertilization and pregnancy with thawed spermatozoa cells in mouse (1977)

First pregnancy with frosen-thawed human embryo (1983)

First IVF Baby from frozen embryos “Zoe Leyland” was born in Melbourne, in March, 28th, 1984.



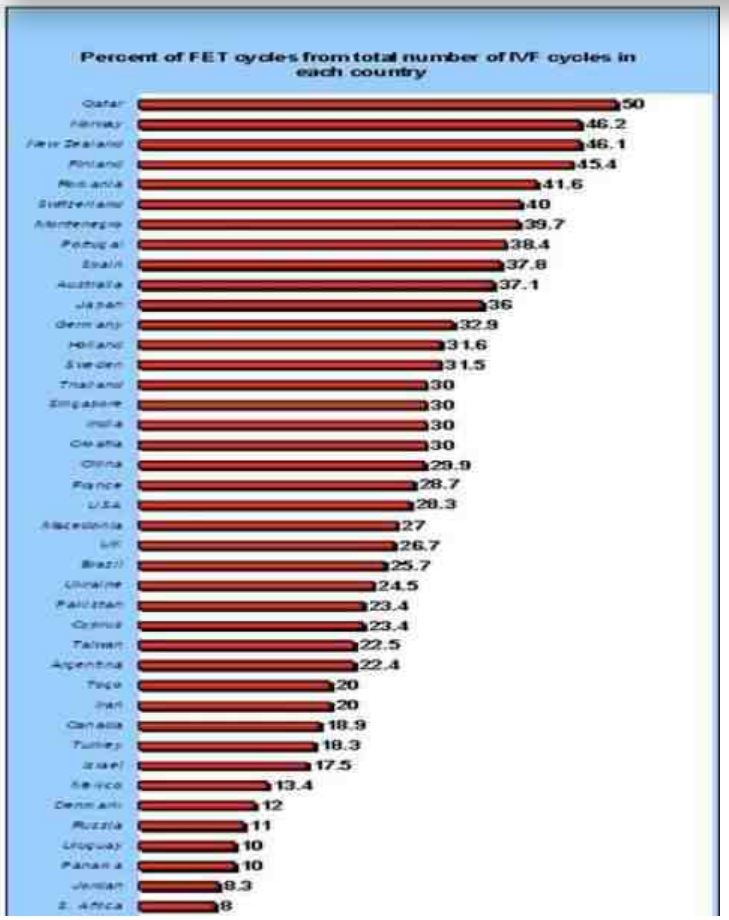




Dr. Alan Trounson

Dr Carl Wood

The History of Cryopreservation



IVF Worldwide Survey 2012

Bahceci Fulya IVF Centre	2012	2013	2014	2015
Num. of total OPU cycles	2284	2680	3149	3733
Freeze-all cycles (%)	37.0	42.9	56.4	70.5

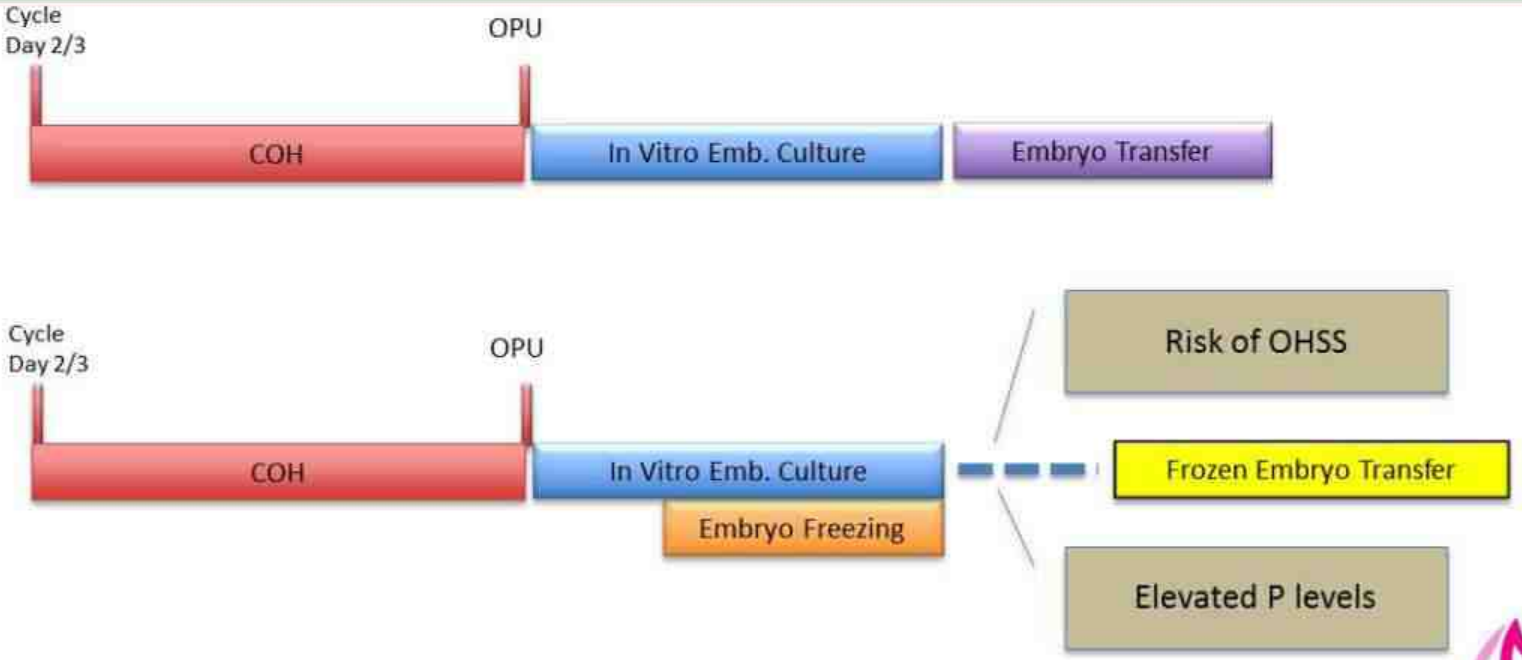
Clinical aspects / Indications

- **Treatment protocol-related reasons**
  - Save the surplus embryos from wastage
  - Reduce the risk of OHSS during COH
  - Improve the endometrial receptivity
- **Procedure/patient-related reasons**
  - Save time for embryo manipulation
  - Create a psychosocial comforting period between OPU – ET
- **Clinical outcome-related reasons**
  - Minimize the risk of multiple pregnancies
  - Maximize the PR by embryo accumulation
  - Increase the cumulative PR rate

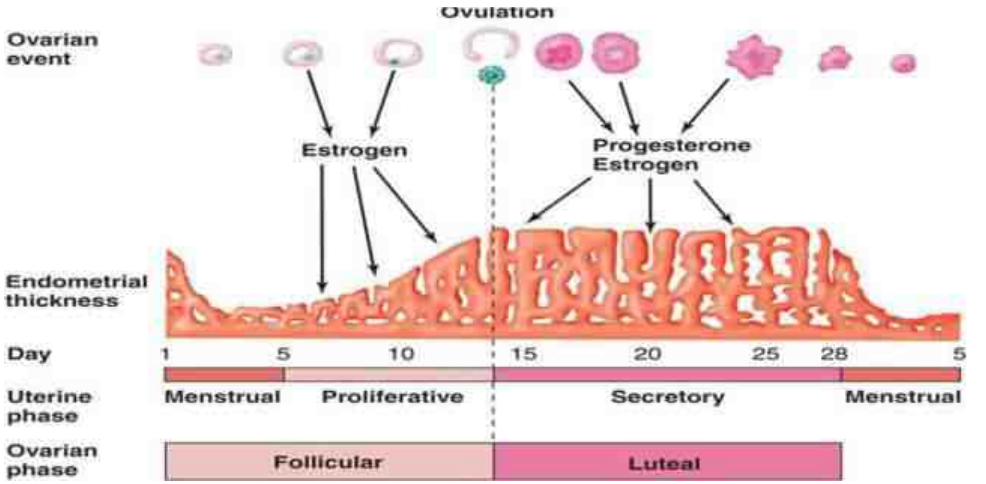
Clinical aspects of Freeze-all

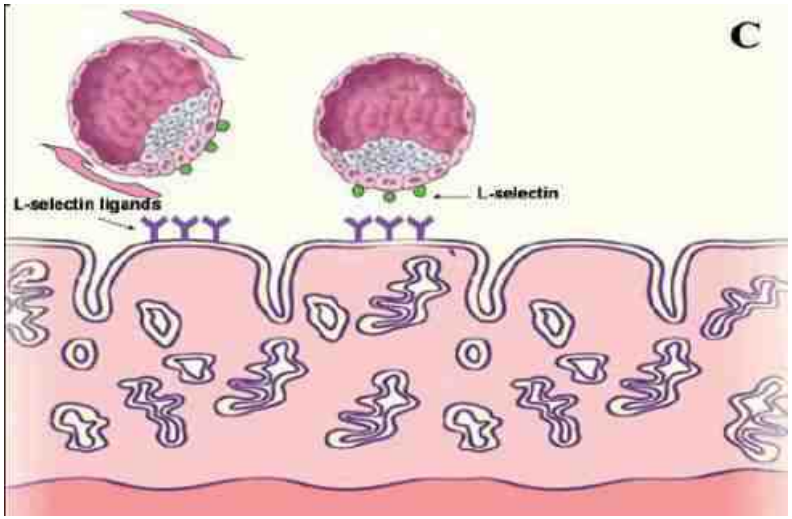
In freeze-all cycles, the primary aim is to improve the endometrial conditions & receptivity"

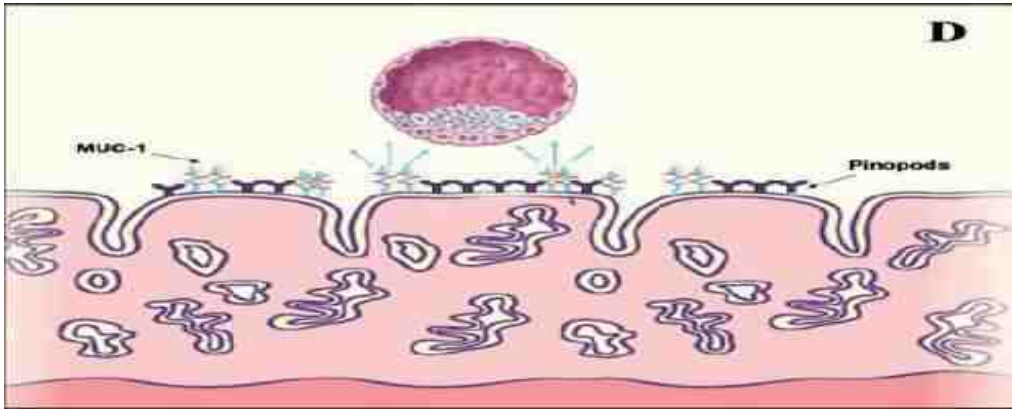
"Segmentation of ovarian stimulation"

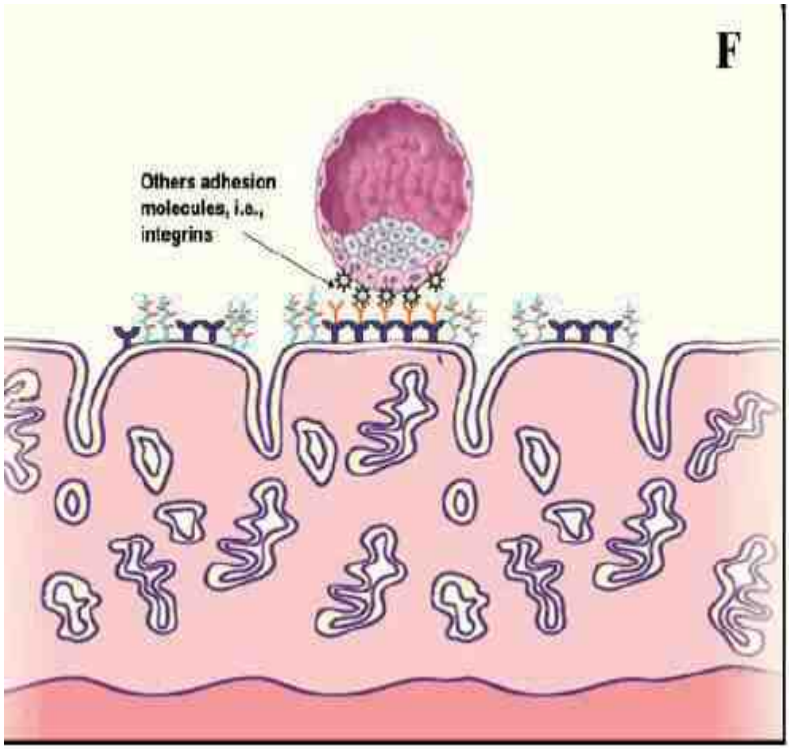


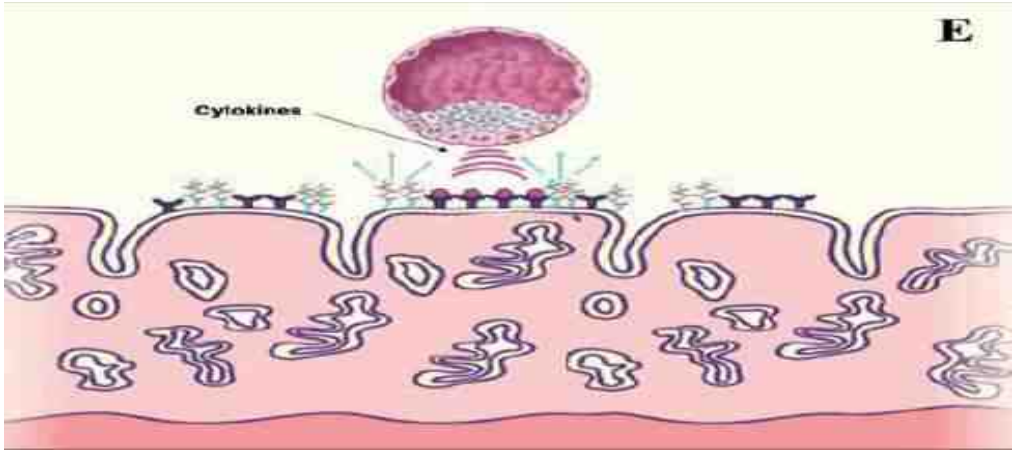
(Devroey et al. 2011)


Ovulation
Ovarian event
Estrogen
Progesterone
Estrogen
Endometrial thickness
Day
1
5
10
15
20
25
28
5
Uterine phase
Menstrual
Proliferative
Secretory
Menstrual
Ovarian phase
Follicular
Luteal


C
L-selectin ligands
L-selectin


D
MUC-1
Pinopods


F
Others adhesion molecules, i.e., integrins

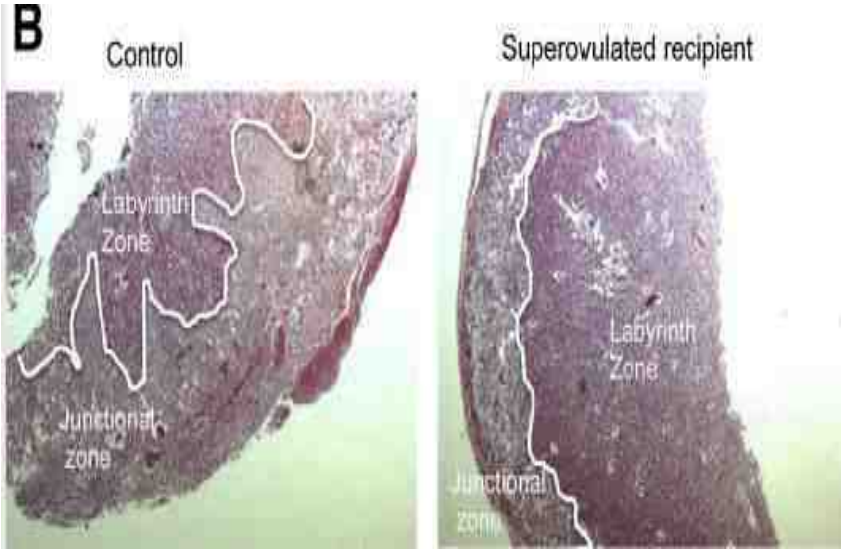

E
Cytokines

Clinical aspects of Freeze-all

Gene expression profiles of simulated and nonstimulated human endometrium during the window of embryo implantation.

Study	No. of samples	Fold change considered to be significant	Number of genes	
			Up	Down
Mirkin et al. (45)	13	≥1.2	5–6[a]	1–6[a]
Horcajadas et al. (46)	19	≥3	281	277
Simon et al. (47)	28	≥2	22–88[a]	24–100[a]
Horcajadas et al. (48)	49	–	69	73
Liu et al. (49)	13	≥2	5–244[a]	2–159[a]
Haouzi et al. (50)	84	≥2	321–657[a]	0–4[a]

- High progesteron and estrogen;
- NK cells, Integrins ⇩
- Changes in gene expression,
- Glandular and stromal changes,
- Lapsing in ‘’implantation window’’



Weinerman et al 2014

Clinical aspects of Freeze-all

Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis

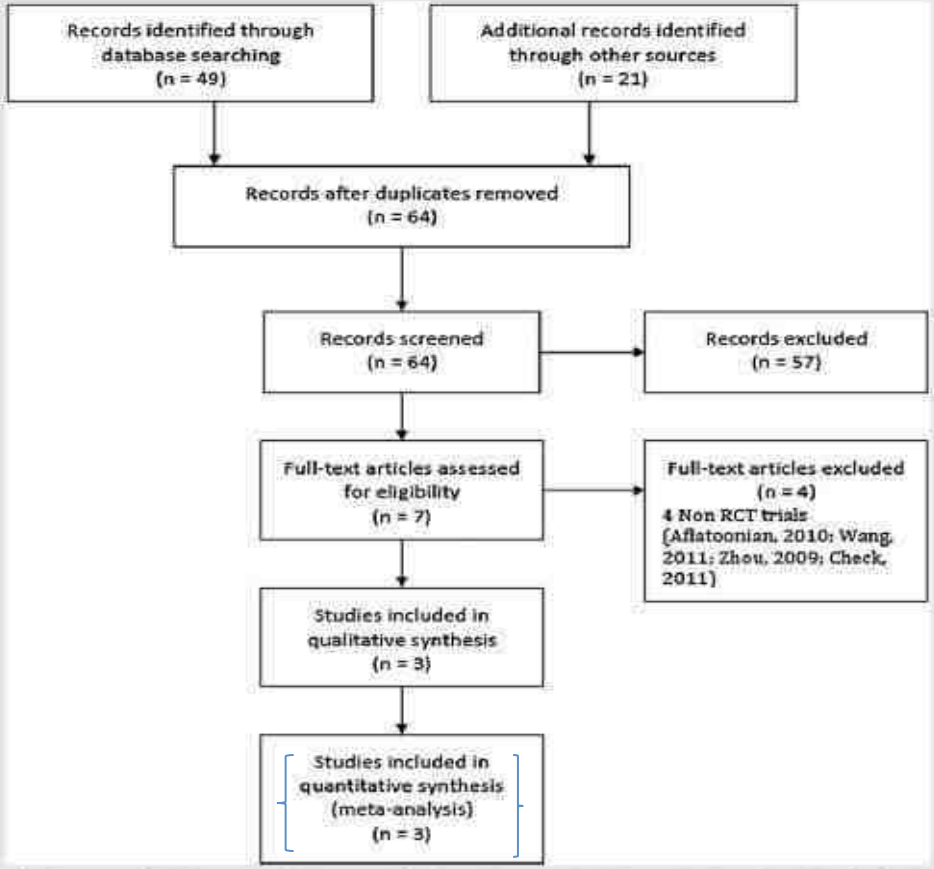
Matheus Roque, M.D.,[a,c] Karinna Lattes, M.D.,[a,d] Sandra Serra, M.Sc.,[a,d] Ivan Solà, B.Psych.,[e,f,g] Selmo Geber, Ph.D.,[c,h] Ramón Carreras, Ph.D.,[b] and Miguel Angel Checa, Ph.D.[b,d]

[a] Máster Internacional Medicina Reproductiva, Hospital del Mar, and [b] Department of Obstetrics and Gynecology, Parc de Salut Mar, Universitat Autònoma de Barcelona, Barcelona, Spain; [c] Origen Center for Reproductive Medicine, Belo Horizonte, Brazil; [d] Centro de Infertilidad y Reproducción Humana, Barcelona, Spain; [e] Iberoamerican Cochrane Center, Barcelona, Spain; [f] Institute of Biomedical Research (IIB Sant Pau), Barcelona, Spain; [g] CIBER Epidemiología y Salud Pública, Barcelona, Spain; and [h] Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Objective: To examine the available evidence to assess if cryopreservation of all embryos and subsequent frozen embryo transfer (FET) results in better outcomes compared with fresh transfer.
Design: Systematic review and meta-analysis.
Setting: Centers for reproductive care.
Patient(s): Infertility patient(s).
Intervention(s): An exhaustive electronic literature search in MEDLINE, EMBASE, and the Cochrane Library was performed through December 2011. We included randomized clinical trials comparing outcomes of IVF cycles between fresh and frozen embryo transfers.
Main Outcome Measure(s): The outcomes of interest were ongoing pregnancy rate, clinical pregnancy rate, and miscarriage.
Result(s): We included three trials accounting for 633 cycles in women aged 27–33 years. Data analysis showed that FET resulted in significantly higher ongoing pregnancy rates and clinical pregnancy rates.
Conclusion(s): Our results suggest that there is evidence that IVF outcomes may be improved by performing FET compared with fresh embryo transfer. This could be explained by a better embryo-endometrium synchrony achieved with endometrium preparation cycles. (Fertil Steril® 2013;99:156–62. ©2013 by American

Clinical aspects of Freeze-all

FIGURE 1



Preferred Outcome Items for Systematic Reviews and Meta-analysis flow diagram detailing selection of studies for inclusion. RCT = randomized controlled study

Roque. Elective frozen-thawed embryo transfer. Fertil Steril 2013.

Clinical aspects of Freeze-all

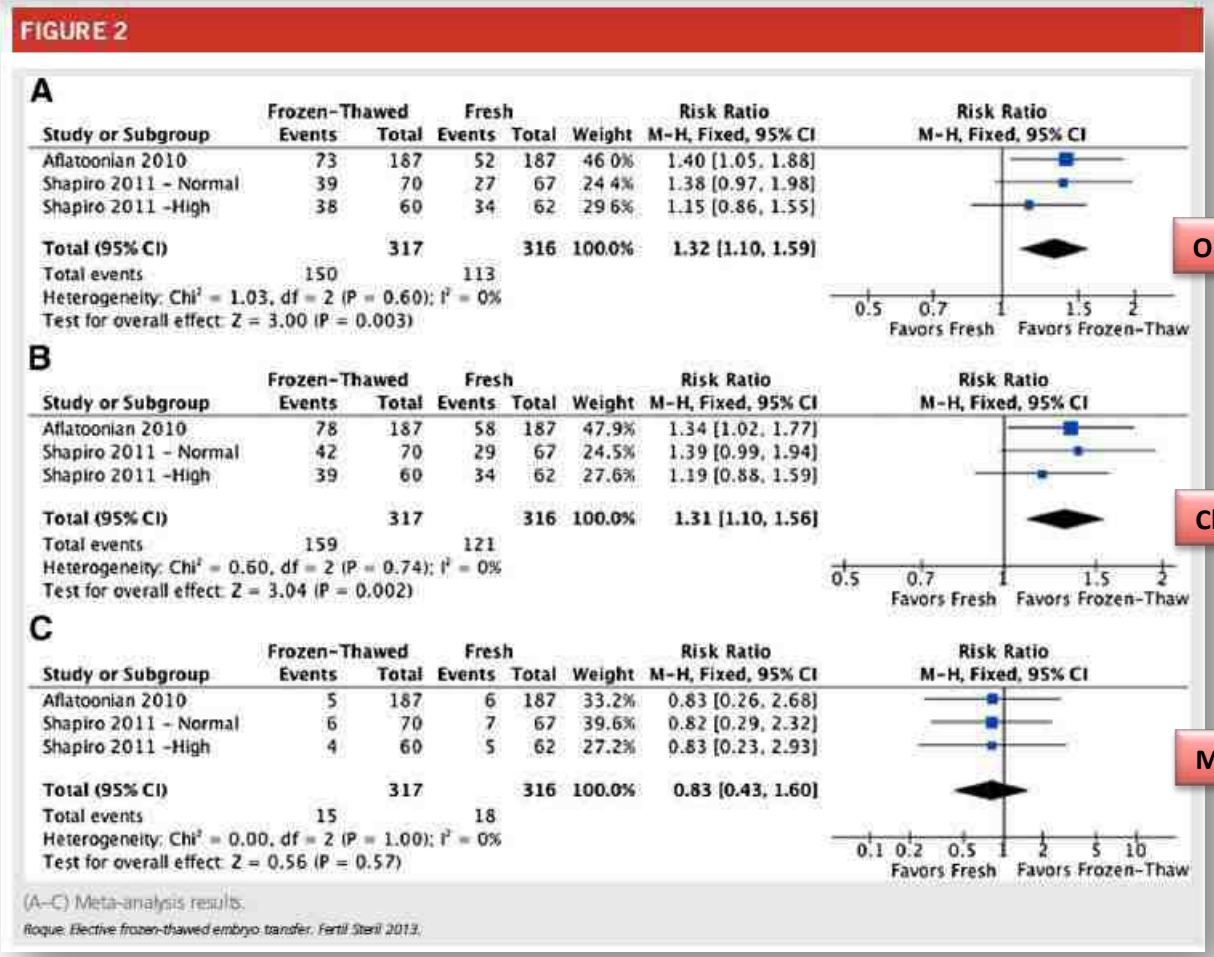


FIGURE 2

A

Study or Subgroup	Frozen-Thawed Events	Total	Fresh Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Aflatoonian 2010	73	187	52	187	46.0%	1.40 [1.05, 1.88]
Shapiro 2011 - Normal	39	70	27	67	24.4%	1.38 [0.97, 1.98]
Shapiro 2011 -High	38	60	34	62	29.6%	1.15 [0.86, 1.55]
Total (95% CI)		**317**		**316**	**100.0%**	**1.32 [1.10, 1.59]**
Total events	150		113			

Heterogeneity: $Chi^2 = 1.03$, $df = 2$ ($P = 0.60$); $I^2 = 0\%$
Test for overall effect: $Z = 3.00$ ($P = 0.003$)

Risk Ratio M-H, Fixed, 95% CI
0.5 0.7 1 1.5 2
Favors Fresh Favors Frozen-Thaw

B

Study or Subgroup	Frozen-Thawed Events	Total	Fresh Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Aflatoonian 2010	78	187	58	187	47.9%	1.34 [1.02, 1.77]
Shapiro 2011 - Normal	42	70	29	67	24.5%	1.39 [0.99, 1.94]
Shapiro 2011 -High	39	60	34	62	27.6%	1.19 [0.88, 1.59]
Total (95% CI)		**317**		**316**	**100.0%**	**1.31 [1.10, 1.56]**
Total events	159		121			

Heterogeneity: $Chi^2 = 0.60$, $df = 2$ ($P = 0.74$); $I^2 = 0\%$
Test for overall effect: $Z = 3.04$ ($P = 0.002$)

Risk Ratio M-H, Fixed, 95% CI
0.5 0.7 1 1.5 2
Favors Fresh Favors Frozen-Thaw

C

Study or Subgroup	Frozen-Thawed Events	Total	Fresh Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Aflatoonian 2010	5	187	6	187	33.2%	0.83 [0.26, 2.68]
Shapiro 2011 - Normal	6	70	7	67	39.6%	0.82 [0.29, 2.32]
Shapiro 2011 -High	4	60	5	62	27.2%	0.83 [0.23, 2.93]
Total (95% CI)		**317**		**316**	**100.0%**	**0.83 [0.43, 1.60]**
Total events	15		18			

Heterogeneity: $Chi^2 = 0.00$, $df = 2$ ($P = 1.00$); $I^2 = 0\%$
Test for overall effect: $Z = 0.56$ ($P = 0.57$)

Risk Ratio M-H, Fixed, 95% CI
0.1 0.2 0.5 1 2 5 10
Favors Fresh Favors Frozen-Thaw

(A–C) Meta-analysis results.

Roque. Elective frozen-thawed embryo transfer. Fertil Steril 2013.

Ongoing Pregnancy

ClinicalPregnancy

Miscarriages

Clinical aspects of Freeze-all

Freeze-all policy: fresh vs. frozen-thawed embryo transfer

Matheus Roque, M.D.,[a] Marcello Valle, M.D.,[a] Fernando Guimarães, B.S.,[a] Marcos Sampaio, M.D., Ph.D.,[b] and Selmo Geber, M.D., Ph.D.[b,c]

[a] ORIGEN, Center for Reproductive Medicine, Rio de Janeiro; [b] ORIGEN, Center for Reproductive Medicine, Belo Horizonte; and [c] Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Objective: To compare in vitro fertilization (IVF) outcomes between fresh embryo transfer (ET) and frozen-thawed ET (the "freeze-all" policy), with fresh ET performed only in cases without progesterone (P) elevation.
Design: Prospective, observational, cohort study.
Setting: Private IVF center.
Patient(s): A total of 530 patients submitted to controlled ovarian stimulation (COS) with a gonadotropin-releasing hormone–antagonist protocol, and cleavage-stage, day-3 ET.
Intervention(s): None.
Main Outcome Measure(s): Ongoing pregnancy rates.
Result(s): A total of 530 cycles were included in the analysis: 351 in the fresh ET group (when P levels were ≤1.5 ng/mL on the trigger day); and 179 cycles in the freeze-all group (ET performed after endometrial priming with estradiol valerate, at 6 mg/d, taken orally). For the fresh ET group vs. the freeze-all group, respectively, the implantation rate was 19.9% and 28.5%, clinical pregnancy rate was 35.9% and 46.4%; and ongoing pregnancy rate was 31.1% and 39.7%.
Conclusion(s): The IVF outcomes were significantly better in the group using the freeze-all policy, compared with the group using fresh ET. These results suggest that even in a select group of patients that underwent fresh ET (P levels ≤1.5 ng/mL), endometrial receptivity may have been impaired by COS, and outcomes may be improved by using the freeze-all policy.
(Fertil Steril® 2015;103:1190–3. ©2015 by American Society for Reproductive Medicine.)
Key Words: Freeze-all, frozen-thawed embryo transfer, delayed frozen-thawed embryo transfer, embryo cryopreservation

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/roquem-fresh-frozen-thawed-embryo-transfer/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

(Roque et al. 2015)

Clinical aspects of Freeze-all

Does a frozen embryo transfer ameliorate the effect of elevated progesterone seen in fresh transfer cycles?

Mae Wu Healy, D.O.,[a] George Patounakis, M.D., Ph.D.,[a] Matt T. Connell, D.O.,[a] Kate Devine, M.D.,[a,b] Alan H. DeCherney, M.D.,[a] Michael J. Levy, M.D.,[b] and Micah J. Hill, D.O.[a,b]

[a] Program in Reproductive and Adult Endocrinology, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland; and [b] Shady Grove Fertility Science Center, Rockville, Maryland

Objective: To compare the effect of progesterone (P) on the day of trigger in fresh assisted reproduction technology (ART) transfer cycles versus its effect on subsequent frozen embryo transfer (FET) cycles.
Design: Retrospective cohort study.
Setting: Large private ART practice.
Patient(s): Fresh autologous and FET cycles from 2011–2013.
Intervention(s): None.
Main Outcome Measure(s): Live birth.
Result(s): A paired analysis of patients who underwent both a fresh transfer and subsequent FET cycle and an unpaired analysis of data from all fresh transfer cycles and all FET cycles were performed. We analyzed 1,216 paired and 4,124 unpaired cycles, and P was negatively associated with birth in fresh but not FET cycles in all analyses. Interaction testing of P and cycle type indicated P had a different association with birth in fresh versus FET cycles. When P was ≥2 ng/mL at the time of trigger, live birth was more likely in FET versus fresh cycles in the paired analysis (47% vs. 10%), in the unpaired analysis (51% vs. 14%), and in unpaired, good blastocyst only transfer was lower in fresh cycles, with P ≥2 ng/mL versus P <2 ng/mL (15% vs. 45%).
Conclusion(s): Elevated P levels on the day of trigger during the initial fresh cycle were negatively associated with live birth in the fresh transfer cycles but not in subsequent FET cycles. Freezing embryos and performing a subsequent FET cycle ameliorates the effect of elevated P on live-birth rates. (Fertil Steril® 2016;105:93–9. ©2016 by American Society for Reproductive Medicine.)

Use your smartphone to scan this QR code

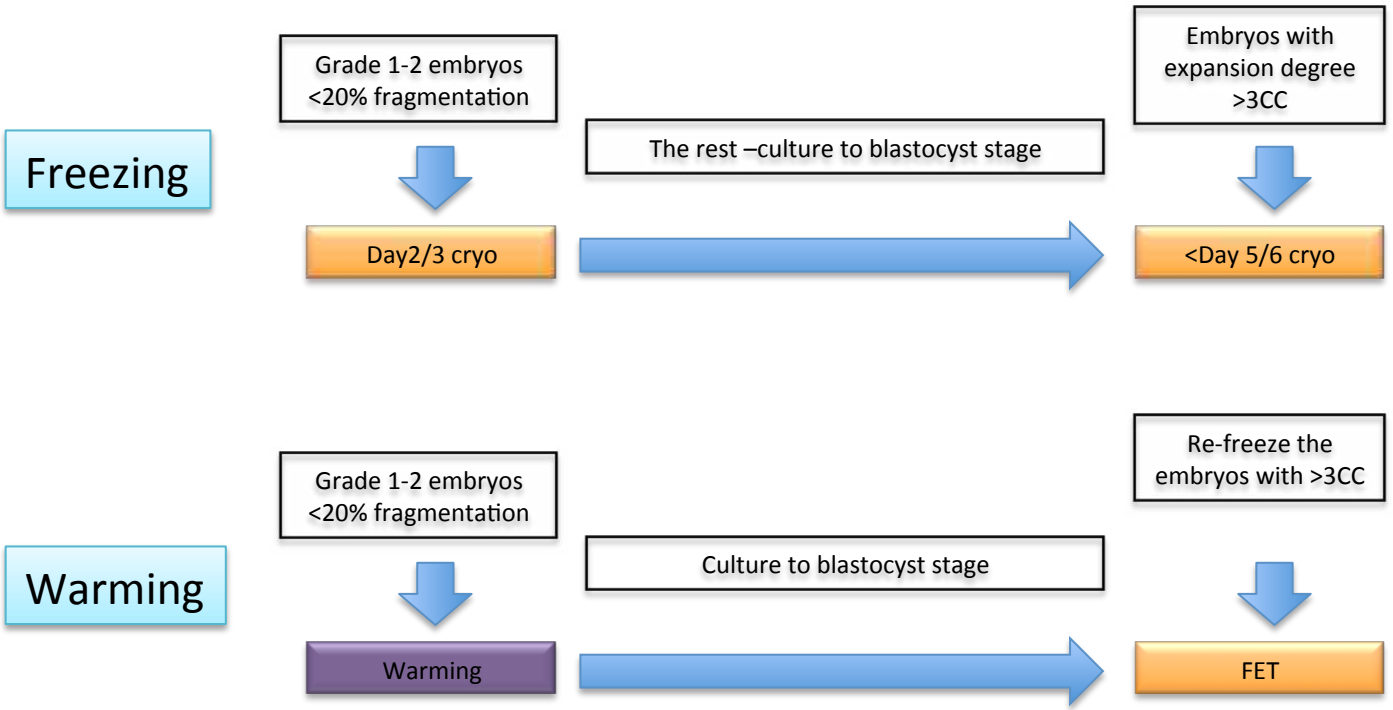
Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/healym-elevated-progesterone-fet-cycles/

this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Laboratory aspects of Freeze-all

"Optimal strategy"



Clinical aspects of Freeze-all

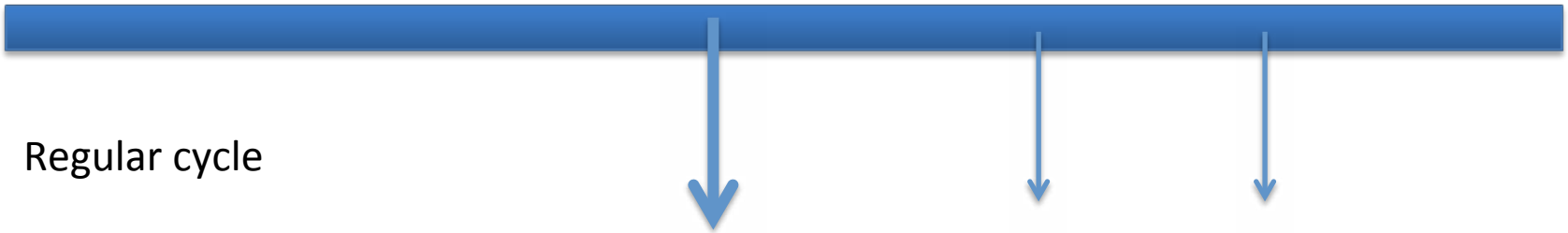
A. Natural Cycle

B. Artificial Cycle

C. Modified natural cycle (OI)

1. Gonadotrophins
2. Letrozol
3. Clomiphene Citrate

Natural Cycle

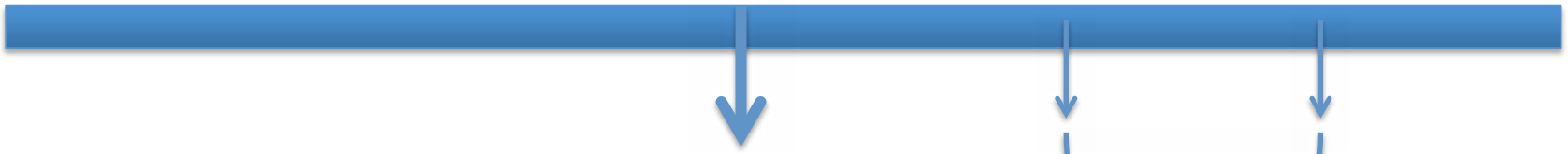


Regular cycle

- Urine LH kits (30% false +)/ patient orientation?
- Blood Test (?)

- **OVULATION**
- (About 16 hr)
- (About 36-40 hr)

ET on 3th / 5th day



Irregular cycle

USG, dom.foll. >17 mm , **hCG** triggering, ovulation in 36-40 hours

ET on 3th / 5th day

Natural Cycle

ORIGINAL PAPER

Natural cycle cryo-thaw transfer may improve pregnancy outcome

Vadim Morozov · Jane Ruman · Daniel Kenigsberg · Glenn Moodie · Steven Brenner

	HRT	Natural cycle
Number of cycles	174	68
Number of pregnancies (pregnancy rate)	41 (22.99 %)*	25 (36.76 %)*
Mean no of embryos transferred	2.73 ± 0.08	2.86 ± 0.09
Mean age (years)	37.19 ± 0.38	35.46 ± 0.42
Mean AES at freezing	25.15 ± 0.56*	22.63 ± 0.79*
Mean AES at transfer	26.39 ± 0.57	25.88 ± 0.75
Mean endometrial thickness (mm)	8.89 ± 0.14*	9.95 ± 0.26*
Mean E_2 level (pg/ml)	526.1 ± 16.90*	103.8 ± 6.75*

Morozov et al 2007

Natural Cycle

Natural cycle is superior to hormone replacement therapy cycle for vitrificated-preserved frozen-thawed embryo transfer

Zhuoni Xiao[1]*, Xin Zhou[2], Wangming Xu[1], Jing Yang[1], and Qingzhen Xie[1]

	Subgroup A (Three 8-cell embryos transferred)			Subgroup B (Three good-quality embryos transferred)			Subgroup C (Intact and mitosis recovered embryo transferred)		
	Natural Cycles	HRT Cycles	*P* Value	Natural Cycles	HRT Cycles	*P* Value	Natural Cycles	HRT Cycles	*P* Value
Total No. of ET Cycles	90	142		158	246		194	384	
Implantation Rate(%)	23.70 (64/270)	13.62 (58/426)	0.001*	18.98 (90/474)	14.63 (108/738)	0.045*	18.82 (96/510)	18.95 (188/992)	0.908
Biochemical Pregnancy Rate per ET(%)	2.22 (2/90)	11.27 (16/142)	0.012*	3.80 (6/158)	9.76 (24/246)	0.026*	4.12 (8/194)	8.33 (32/384)	0.060
Clinical Pregnancy Rate per ET(%)	48.89 (44/90)	30.99 (44/142)	0.006*	41.77 (66/158)	32.52 (80/246)	0.059	37.11 (72/194)	35.93 (138/384)	0.781
Ongoing Pregnancy Rate per ET(%)	40.00 (36/90)	21.83 (31/142)	0.003*	32.28 (51/158)	20.33 (50/246)	0.007*	30.42 (59/194)	28.65 (110/384)	0.659

Xiao et al 2012

Natural Cycle / hCG Trigger?

Spontaneous ovulation versus HCG triggering for timing natural-cycle frozen—thawed embryo transfer: a randomized study

Ariel Weissman *, Eran Horowitz, Amir Ravhon, Zohar Steinfeld, Ravit Mutzafi, Avraham Golan, David Levran

Clinical pregnancy rate per cycle	8/25 (32.0)	8/30 (26.7)	NS
Clinical pregnancy rate per transfer	8/24 (33.3)	8/27 (29.6)	NS
Live-birth rate per cycle	8/25 (32.0)	5/30 (16.7)	NS
Live-birth rate per transfer	8/24 (33.3)	5/27 (18.5)	NS
Implantation rate	9/55 (16.4)	8/60 (13.3)	NS

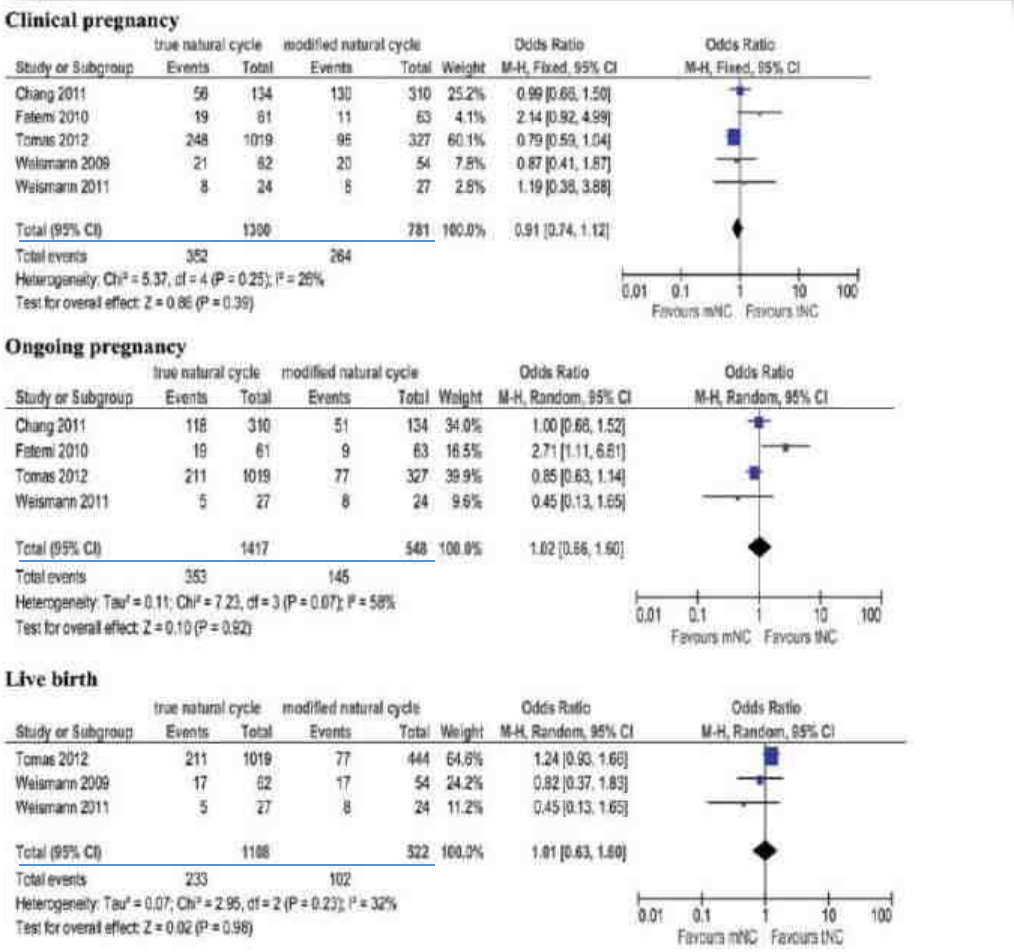
Table 3 Number of monitoring visits in trials comparing spontaneous ovulation with human chorionic gonadotrophin (HCG) triggering.

Study	*Spontaneous ovulation*	*HCG triggering*	*P-value*
Weissman et al. (2009)	4.4 ± 1.4	3.5 ± 1.8	<0.0001
Fatemi et al. (2010)	4.1 ± 1.4	2.6 ± 1.1	0.001
Current study	4.7 ± 1.6	3.2 ± 1.4	0.002

Values are mean ± standard deviation.
HCG = human chorionic gonadotrophin.

Weissman et al 2011

Natural Cycle / hCG Trigger?



Groenewoud et al 2013

Natural Cycle / P monitoring?

The frozen–thawed embryo transfer timing determined by serum progesterone level: a retrospective follow-up study

Zhe Dong [a,1], Ling Sun [a,1,*], Hanwang Zhang [b], Zhiheng Chen [a], Yuehong Jian [a]

[a] Reproductive Medicine Center, Guangzhou Women and Children's Medical Center, No. 9, Jinsui Road, Guangzhou 510623, Guangdong, People's Republic of China
[b] Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, The People's Republic of China

Clinical outcomes after FET in the MOR group and MPR groups.

	MOR group	MPR group	Odds ratio	95% CI	p Value
Clinical pregnancy, *n*	43	85			
Rate without adjustment (%)	48.3	63.0	1.819[a]	1.057–3.130[a]	0.030[a]
Rate with adjustment (%)	48.1[b]	61.6[b]	1.996[c]	1.123–3.549[c]	0.019[c]
Ongoing pregnancy rate, *n*(%)	37(41.6)	73(54.1)	1.655[a]	0.964–2.841[a]	NS[a]
Implantation rate (%)	66/188 (35.1)	113/265(42.6)			0.001[a]

Monitoring Ovl. (USG+LH kits)
USG; D8-10,
Urine LH Kits,
D3 - ET

Monitoring P ;
D10 USG,
Ovl. (D0),
D0 - P≤3 ng/mL
D1 - P3-6 ng/mL
D2 - P 6-8 ng/mL
D3 - P 8-10 ng/mL
D3 - ET

Dong et al 2014

Natural Cycle / P supporting?

Luteal phase progesterone increases live birth rate after frozen embryo transfer

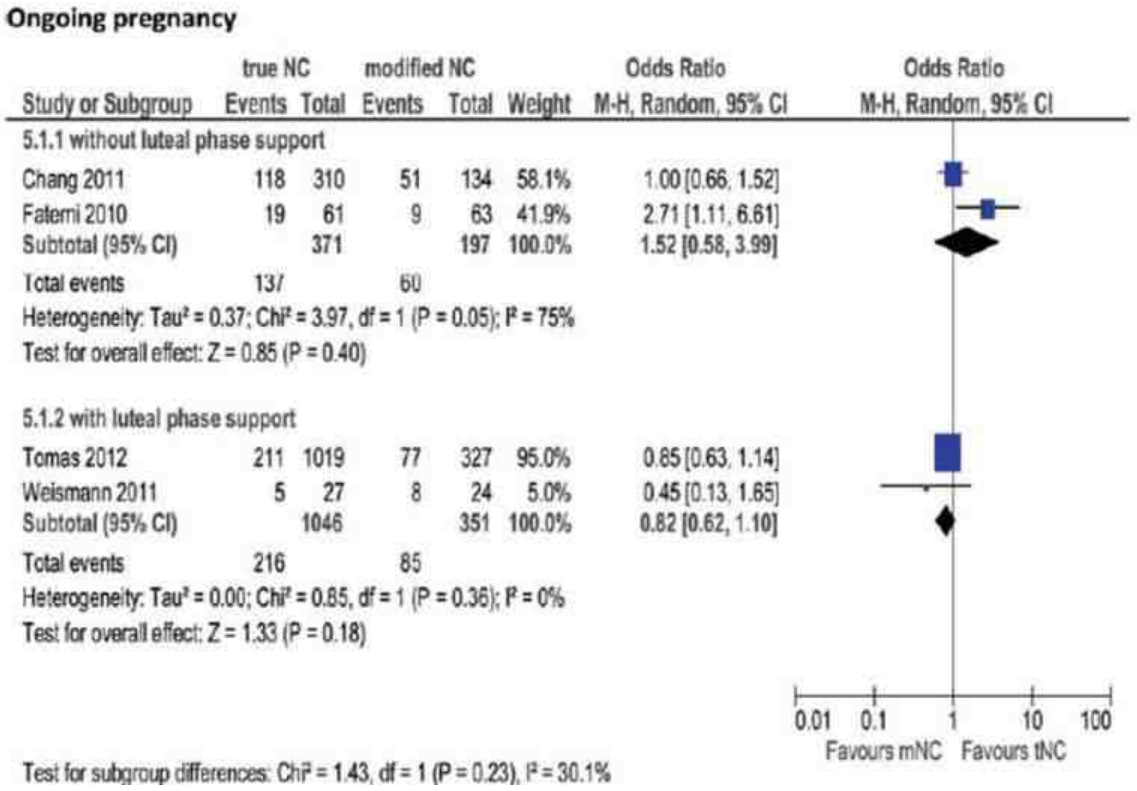
Kerstin Bjuresten, B.S.,[a] Britt-Marie Landgren, M.D., Ph.D.,[a] Outi Hovatta, M.D., Ph.D.,[a] and Anneli Stavreus-Evers, Ph.D.[b]

Pregnancy outcome in the two treatment groups.

	Progesterone	No progesterone	*P* value
No. of transfers	n = 219	n = 216	.8921
No. of embryos transferred	n = 290	n = 293	.9067
No. of embryos transferred (mean)	n = 1.32	n = 1.36	—
No. of single embryo transfers	n = 148	n = 139	.5423
No. of transfers with good-quality embryos	n = 164	n = 178	.3706
No. of transfers with lower-quality embryos	n = 126	n = 116	.3706
No. of blastocyst transfers	n = 3	n = 9	.1497
No. of IVF transfers	n = 110	n = 105	.7728
No. of ICSI embryos	n = 109	n = 112	.7728
Positive hCG rate	0.35 (76 of 219)	0.28 (60 of 216)	.1458
Miscarriage rate	0.03 (7 of 219)	0.03 (6 of 216)	.7977
Clinical pregnancy rate	0.32 (69 of 219)	0.25 (54 of 216)	.1614
Clinical abortion rate	0.02 (4 of 219)	0.05 (10 of 216)	.1105
Live birth rate (at least one live infant)	0.30 (65 of 219)	0.20 (44 of 216)	.0272*

Bjuresten et al 2010

Natural Cycle / P supporting?



Groenewoud et al 2013

Artificial Cycle

Advantages ;

- Timing of ET,
- No need for Reg.Cycle,
- Cheaper? USG,LH Kits ↓
- Patient Orientation

Disadvantages;

Pregnancy rates?,
Abortion rates?

Artificial Cycle

(GnRHa?) 17 β Estradiol 4 mg/ day

Micronisated P , 800 mg/ day

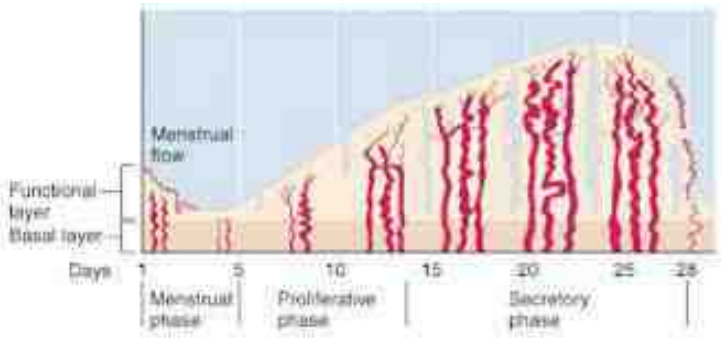
D11 USG;

Echo > 7mm

P <1.5 ng/mL

D3 ET

D5 ET



Clinical aspects of NC v AC

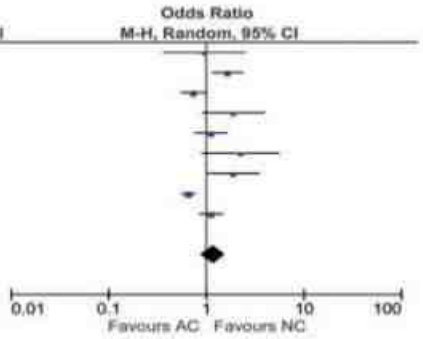
- Hum Reprod Update. 2013 Sep-Oct;19(5):458-70. doi: 10.1093/humupd/dmt030. Epub 2013 Jul 2.
- **What is the optimal means of preparing the endometrium in frozen-thawed embryo transfer cycles? A systematic review and meta-analysis.**
- Groenewoud ER[1], Cantineau AE, Kollen BJ, Macklon NS, Cohlen BJ

Clinical aspects of NC v AC

Clinical pregnancy

Study or Subgroup	NC Events	NC Total	AC Events	AC Total	Weight	Odds Ratio M-H, Random, 95% CI
Catolli 1994	9	50	12	64	6.2%	0.95 [0.37, 2.48]
Chang	186	444	62	204	13.0%	1.65 [1.16, 2.35]
Givens 2009	288	862	106	262	13.9%	0.74 [0.58, 0.98]
Hancke 2012	51	148	12	55	8.4%	1.88 [0.91, 3.89]
Kawamura 2007	310	720	55	136	12.8%	1.11 [0.77, 1.62]
Loh 1999	9	51	14	161	6.6%	2.25 [0.91, 5.56]
Morozov 2007	25	68	41	174	9.8%	1.89 [1.03, 3.45]
tomas 2012	343	1346	854	2492	15.2%	0.66 [0.57, 0.76]
Xiao 2011	144	380	228	646	14.1%	1.12 [0.86, 1.45]
Total (95% CI)		4069		4194	100.0%	1.17 [0.86, 1.58]
Total events	1365		1384			

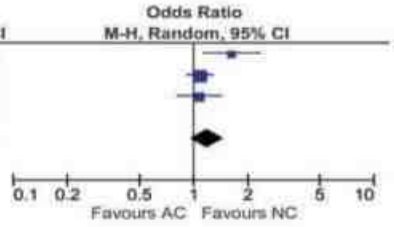
Heterogeneity: $Tau^2 = 0.15$; $Chi^2 = 48.08$, df = 8 ($P < 0.00001$); $I^2 = 83\%$
Test for overall effect: $Z = 0.99$ ($P = 0.32$)



Ongoing pregnancy

Study or Subgroup	NC Events	NC Total	AC Events	AC Total	Weight	Odds Ratio M-H, Random, 95% CI
Chang	169	444	56	204	22.8%	1.62 [1.13, 2.33]
tomas 2012	288	1346	500	2492	46.5%	1.08 [0.92, 1.28]
Xiao 2011	112	380	181	646	30.7%	1.07 [0.81, 1.42]
Total (95% CI)		2170		3342	100.0%	1.19 [0.95, 1.47]
Total events	569		737			

Heterogeneity: $Tau^2 = 0.02$; $Chi^2 = 4.20$, df = 2 ($P = 0.12$); $I^2 = 52\%$
Test for overall effect: $Z = 1.54$ ($P = 0.12$)



Live birth

Study or Subgroup	NC Events	NC Total	AC Events	AC Total	Weight	Odds Ratio M-H, Random, 95% CI
Givens 2009	245	862	77	262	27.7%	0.95 [0.70, 1.29]
Hancke 2012	31	148	7	55	7.9%	1.82 [0.75, 4.41]
Kawamura 2007	263	720	42	136	22.6%	1.29 [0.87, 1.91]
Loh 1999	8	51	6	161	5.4%	4.81 [1.58, 14.60]
Tomas 2012	288	1346	500	2492	36.3%	1.08 [0.92, 1.28]
Total (95% CI)		3127		3106	100.0%	1.23 [0.93, 1.62]
Total events	835		632			

Heterogeneity: $Tau^2 = 0.05$; $Chi^2 = 9.44$, df = 4 ($P = 0.05$); $I^2 = 58\%$
Test for overall effect: $Z = 1.46$ ($P = 0.15$)

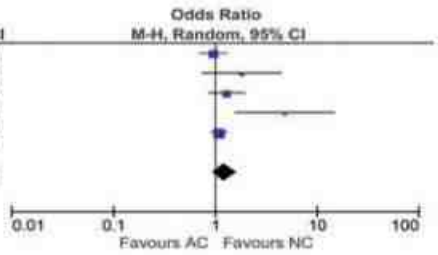


Figure 4 NC versus AC.

Clinical aspects of NC v AC

Pregnancy loss after frozen-embryo transfer—a comparison of three protocols

Candido Tomás, M.D., Ph.D.,[a] Birgit Alsbjerg, M.D.,[b] Hannu Martikainen, M.D., Ph.D.,[c] and Peter Humaidan, M.D., D.M.Sc.[d]

- Retrospective analyses of ; 4470 FET cycles

NC (LH urine kits) + luteal P support
(N=1168)

NC + hCG trigger
(No luteal P support)
(N=444)

AC
(N=2858)

Tomas et al 2012

Clinical aspects of NC v AC

Treatment outcome after FET in natural cycles with P, natural cycles with hCG, and substituted cycles.

	Natural cycle with luteal P	Natural cycle with hCG induction	Substituted cycles	*P* value
Started cycles, n	1,168	444	2,858	
ET, n (%)	1,019 (87.2)	327 (73.9)	2,492 (87.2)	NS
Positive pregnancy test/ET, n (%)	272 (26.7)	116 (35.5)	854 (34.3)	.0001
Clinical pregnancy/ET, n (%)	248 (24.3)	95 (29.1)	691 (27.7)	NS
Deliveries/ET, n (%)	211 (20.7)	77 (23.5)	500 (20.1)	NS
Deliveries/started cycle, %	18.1	17.3	17.5	NS

Pregnancy loss after FET in natural cycles with P, natural cycles with hCG, and substituted cycles.

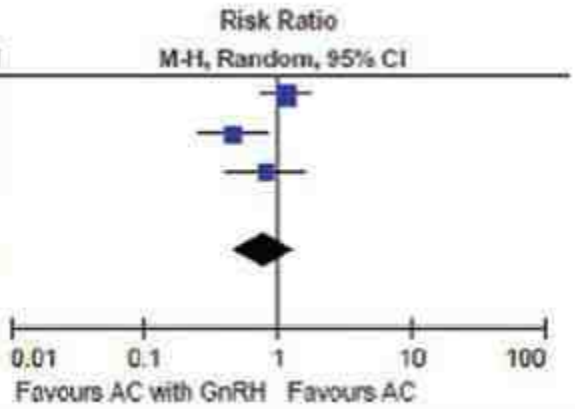
	Natural cycle with luteal P	Natural cycle with hCG induction	Substituted cycles	*P* value
ET	1,019	327	2,492	
Pregnancy test/ET, n (%)	272 (26.7)	116 (35.5)	854 (34.3)	< .0001
Clinical pregnancy/ET, n (%)	248 (24.3)	95 (29.1)	691 (27.7)	NS
Deliveries/ET, n (%)	211 (20.7)	77 (23.5)	500 (20.1)	NS
Preclinical pregnancy loss, n (%)	24 (8.8)	21 (18.1)	163 (19.1)	< .0001
Clinical pregnancy loss, n (%)	37 (13.6)	18 (15.5)	191 (22.4)	< .005
Total pregnancy loss, n (%)	61 (22.4)	39 (33.6)	354 (41.5)	< .0001

Clinical aspects of NC v AC

Study or Subgroup	Artificial cycle Events	Total	AC with GnRH Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Dal Prato 2002	34	150	28	142	39.0%	1.15 [0.74, 1.79]
El Toukhy 2004	13	117	28	117	32.1%	0.46 [0.25, 0.85]
Simon 1998	11	52	14	53	28.8%	0.80 [0.40, 1.60]
Total (95% CI)		319		312	100.0%	**0.77 [0.44, 1.35]**
Total events	58		70			

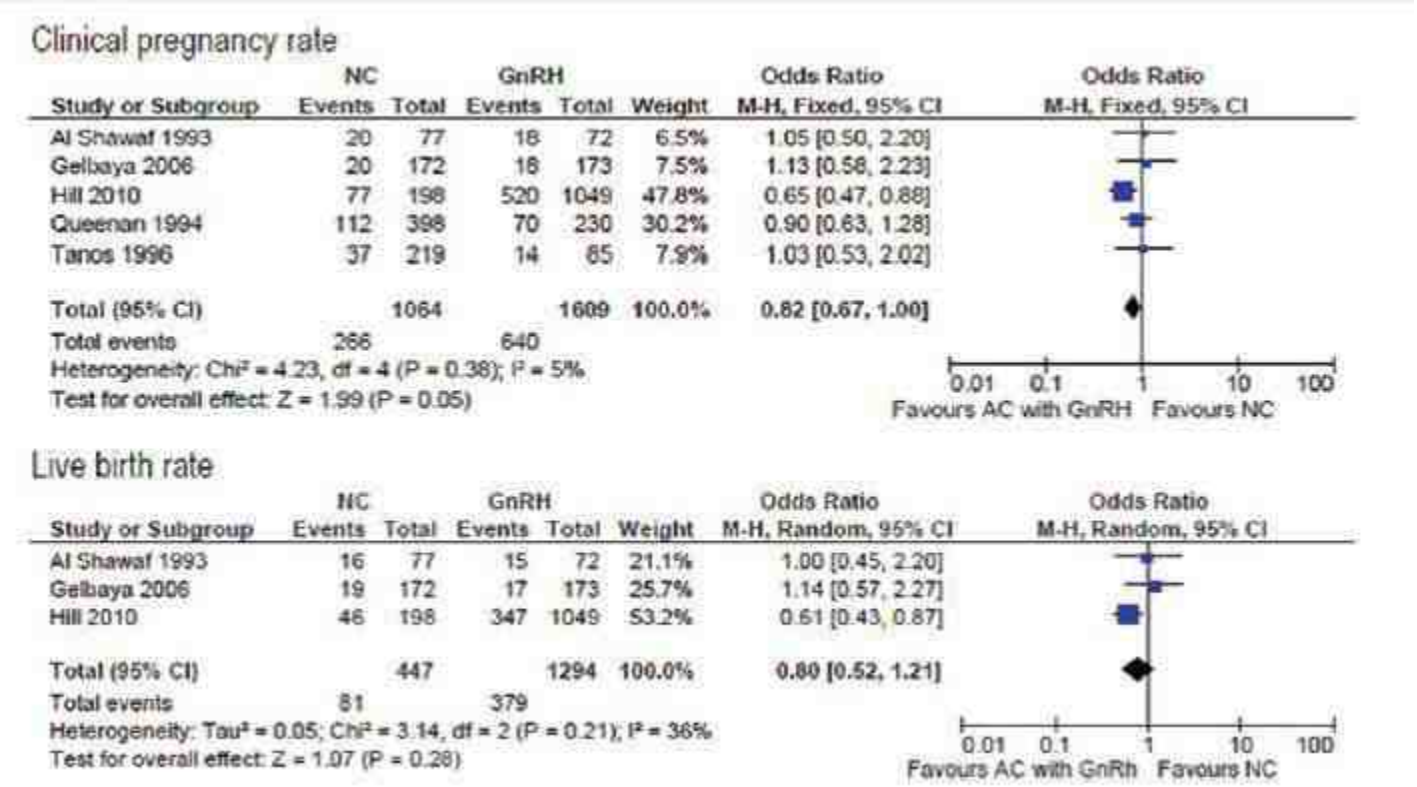
Heterogeneity: $Tau^2 = 0.15$; $Chi^2 = 5.63$, df = 2 (P = 0.06); $I^2 = 64\%$

Test for overall effect: Z = 0.90 (P = 0.37)



Groenewoud et al 2013

Clinical aspects of NC v AC



Groenewoud et al 2013

Artificial Cycle / P - support

Pregnancy outcomes in oocyte donation recipients: vaginal gel versus intramuscular injection progesterone replacement

Brian M. Berger · James A. Phillips

Pregnancy outcome	Descriptive statistic[a]	Vaginal progesterone gel (n=105)	Intramuscular progesterone (n=120)
Positive serum hCG rate	n (%)	65 (61.9)	71 (59.2)
	p value	0.685	
	Difference	2.7	
	95% CI	−10.9, 16.4	
Implantation rate	n (%)	89/203 (43.8)	91/245 (37.1)
	p value	0.175	
	Difference	6.7	
	95% CI	−2.9, 16.3	
Clinical pregnancy rate	n (%)	61 (58.1)	64 (53.3)
	p value	0.503	
	Difference	4.8	
	95% CI	−9.1, 18.6	
Delivery rate	n (%)	54 (51.4)	58 (48.3)
	p value	0.689	
	Difference	3.1	
	95% CI	−10.9, 17.1	
Total pregnancy loss rate	n (%)	11/65 (16.9)	13/71 (18.3)
	p value	1.000	
	Difference	−1.4	
	95% CI	−15.7, 12.9	

Berger et al 2011

Artificial Cycle / P - support

Intramuscular progesterone versus 8% Crinone vaginal gel for luteal phase support for day 3 cryopreserved embryo transfer

Daniel J. Kaser, M.D.,[a] Elizabeth S. Ginsburg, M.D.,[a] Stacey A. Missmer, Sc.D.,[a,b,c] Katharine F. Correia, M.A.,[a] and Catherine Racowsky, Ph.D.[a]

Clinical outcomes from day 3 cryopreserved embryo transfer cycles supported with intramuscular progesterone (IMP) versus Crinone.

Clinical outcome	IMP (n = 440)	8% Crinone (n = 298)	Effect estimate (95% CI)	*P* value[a]
Implantation rate[b]	30.4 ± 36.8	19.6 ± 32.2	0.82 (0.52–1.30)	.39
Biochemical pregnancy	51 (11.6)	39 (13.1)	1.08 (0.69–1.71)	.73
Clinical pregnancy	225 (51.1)	110 (36.9)	0.56 (0.41–0.76)	<.001
Spontaneous abortion	44 (10.2)	34 (11.5)	1.13 (0.71–1.80)	.61
Live birth[c]	169 (39.1)	72 (24.4)	0.51 (0.37–0.70)	<.0001

Kaser et al 2012

Artificial Cycle / P - support

Progesterone replacement with vaginal gel versus i.m. injection: cycle and pregnancy outcomes in IVF patients receiving vitrified blastocysts

Daniel B. Shapiro[1,*], Jennifer A. Pappadakis[2], Nancy M. Ellsworth[1], Howard I. Hait[3], and Zsolt Peter Nagy[1]

	IMP (n = 682)	Crinone 8% (n = 238)	Odds ratio (95% CI)	P-value
Implantation rate	46.4 ± 42.0	45.6 ± 42.5		0.81[a]
Positive serum hCG	496 (72.7)	168 (70.6)	0.90 (0.64–1.27)	0.58
Clinical pregnancy	421 (61.7)	144 (60.5)	0.95 (0.69–1.30)	0.80
Spontaneous abortion	91 (13.3)	28 (11.8)	0.87 (0.53–1.38)	0.62
Live birth[b]	332 (49.1)	116 (48.9)	0.99 (0.73–1.35)	>0.99

Shapiro et al 2014

Artificial Cycle / P - support

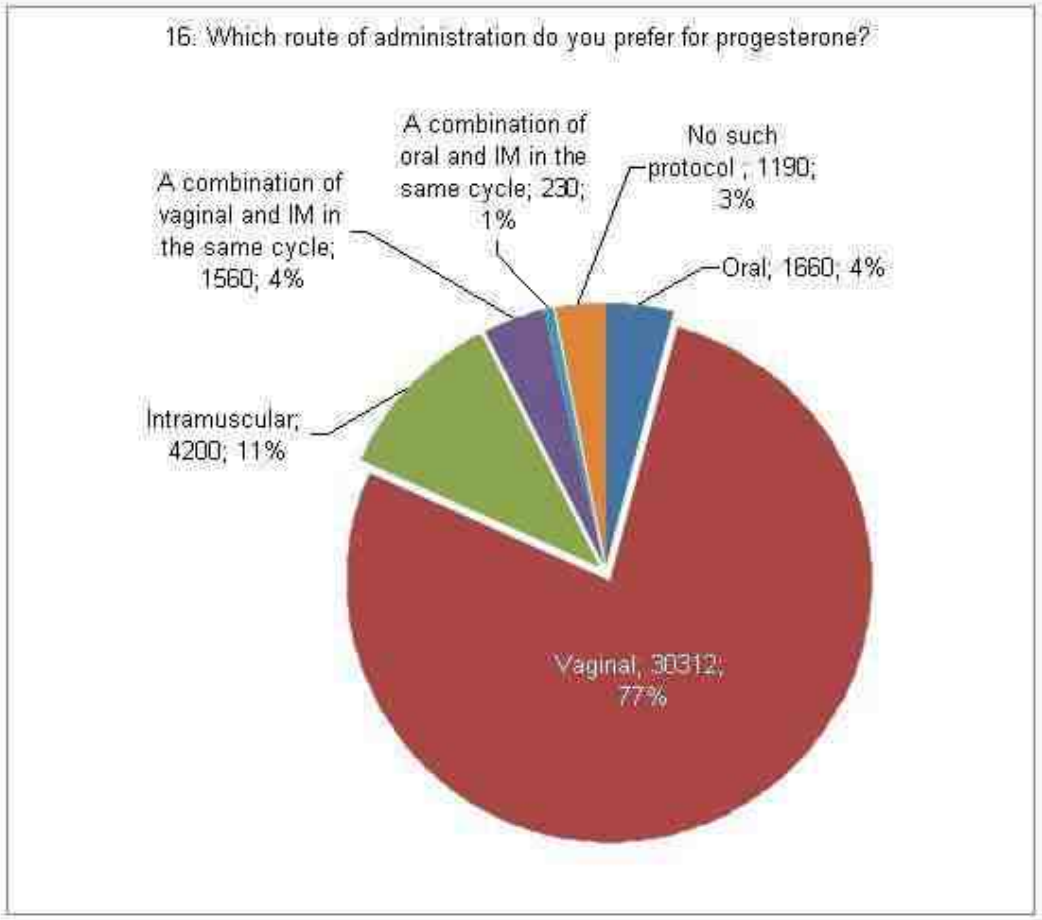
Luteal phase support for frozen embryo transfer cycles: intramuscular or vaginal progesterone?

Casper et al 2014 Discussion Forum in Fertil Steril

Estrogen increases the uterine and the subendometrial contractility in Artificial cycles,
P compansates this effectc of E,

IM Progesterone has better affect on contractility, and so causes decreased EP rates and increased PR. ?!

Artificial Cycle / P - support



IVF Worldwide Survey 2012

Artificial Cycle / P - support

Examining the evidence: progesterone supplementation during fresh and frozen embryo transfer

Daniel Shapiro [a,*], Robert Boostanfar [b], Kaylen Silverberg [c], Elena Hesina Yanushpolsky [d]

Shapiro et al 2014 Consensus Meeting

Table 5 Categories of evidence to support Summit consensus statements.

• Category I	Evidence obtained from at least 1 well-designed randomized, controlled clinical trial
• Category II	Evidence obtained from well-designed cohort or case-controlled studies
• Category III	Evidence obtained from case series, case reports, or flawed clinical trials
• Category IV	Evidence obtained from opinions of respected authorities based on clinical experience, descriptive studies, or reports of expert committees
• Category V	Evidence is insufficient to form an opinion

Table 6 Categories for Summit faculty level of agreement with consensus statements.

• Level 1	Accept completely
• Level 2	Accept with some reservations
• Level 3	Accept with major reservations
• Level 4	Reject with reservations
• Level 5	Reject completely

Artificial Cycle / P - support

Role of progesterone supplementation

- Progesterone administration is important for successful implantation.

  Evidence Category: II Agreement Level: 1

- The need for progesterone replacement in programmed cycles of frozen embryo transfer cycles is clearly established.

  Evidence Category: I Agreement Level: 1

- The value of progesterone supplementation in natural frozen embryo transfer cycles remains unclear.

  Evidence Category: V Agreement Level: 1

Timing and dosing of progesterone supplementation

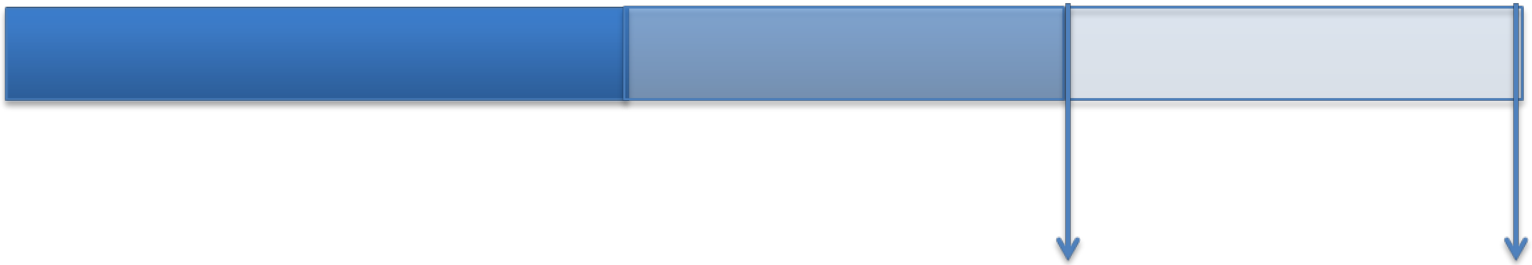
- Properly timed and appropriate dosage regimens of vaginal progesterone supplementation during stimulated IVF-embryo transfer cycles achieve birth rates at least equivalent to intramuscular progesterone supplementation.

  Evidence Category: I Agreement Level: 1

- With respect to frozen embryo transfer cycles, the data are conflicting.

  Evidence Category: III Agreement Level: 1

End. Prep. /Ovulation Induction



- D3-7 Letrozole 2.5 mg/ day
- D3-7 CC 50-100 mg / day

- Dom. Fol.> 17 mm
- Echo > 7 mm
- P < 1.5 ng/mL

D3/ D5 - ET

End. Prep. /Ovulation Induction

- Letrozole - 359 cycle / AC - 354 cycle / NC - 517 cycle

- IR ; Letrozol v AC 30,4% v 22,8%
- CP ; Letrozol v AC 53.2% vs 44.4%
- CP ; Letrozol v NC NS

Li SJ et al. Arch Gynecol Obstet 2014

End. Prep. /Ovulation Induction

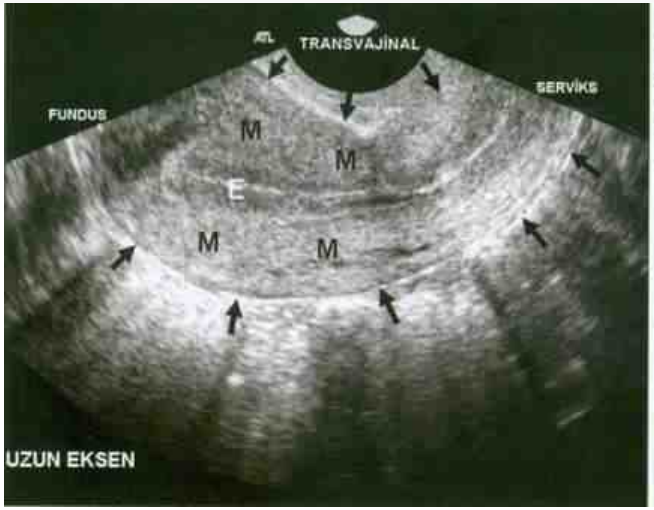
Transfer of cryopreserved – thawed embryos in hCG induced natural or clomiphene citrate cycles yields similar live birth rates in normo-ovulatory women

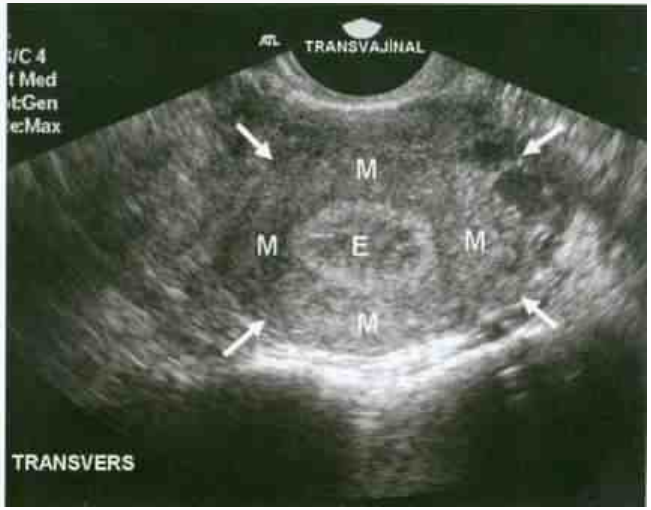
Dimitra Kyrou · Human M. Fatemi · Christophe Blockeel · Dominic Stoop · H. Albuarki · Greta Verheyen · Paul Devroey

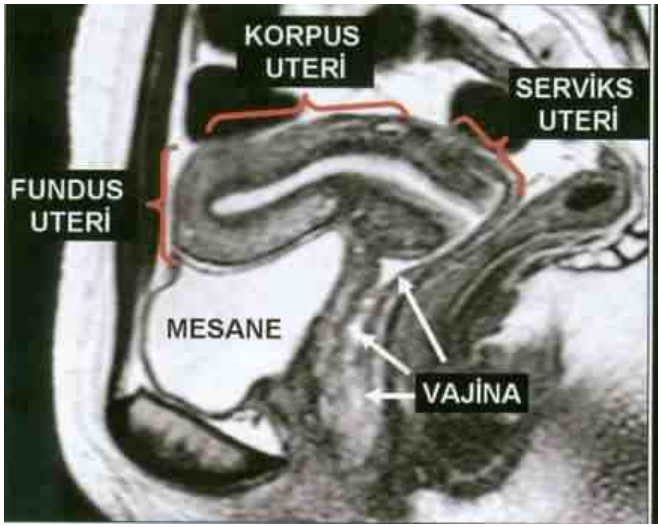
	Natural group (n=261)	CC group (n=167)	P value
Ongoing pregnancy rate (per cycle)	60 (23.0)	39 (23.4)	1.000[a]
Implantation rate (per ET)	76 (17.9)	57 (19.8)	0.557[a]
Number of pregnancies			0.892[b]
Singletons	50 (19.2)	31 (18.6)	
Twins	10 (3.8)	8 (4.8)	
Delivery outcome			0.708[a]
Live births	59 (22.6)	37 (22.2)	
Stillborn	1 (0.4)	1 (0.6)	
Elective termination	0 (0.0)	1 (0.6)	

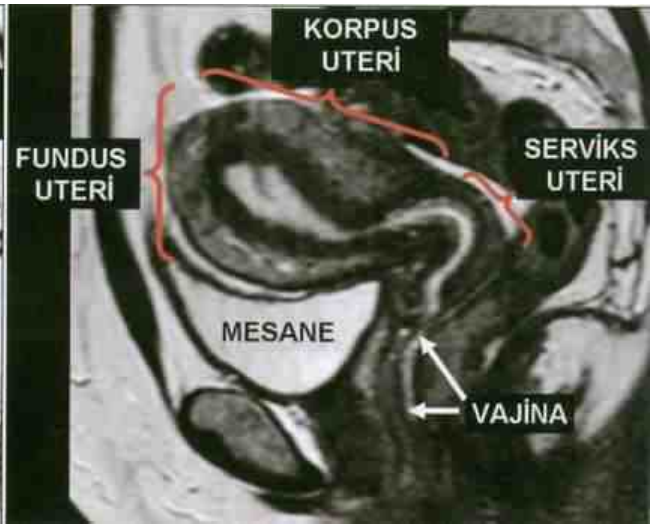
Kyrou et al 2010

End. Prep. / USG


TRANSVAJİNAL
FUNDUS
SERVİKS
M
M
E
M
M
UZUN EKSEN


TRANSVAJİNAL
M
M
E
M
M
TRANSVERS


KORPUS UTERİ
SERVİKS UTERİ
FUNDUS UTERİ
MESANE
VAJİNA


KORPUS UTERİ
FUNDUS UTERİ
SERVİKS UTERİ
MESANE
VAJİNA

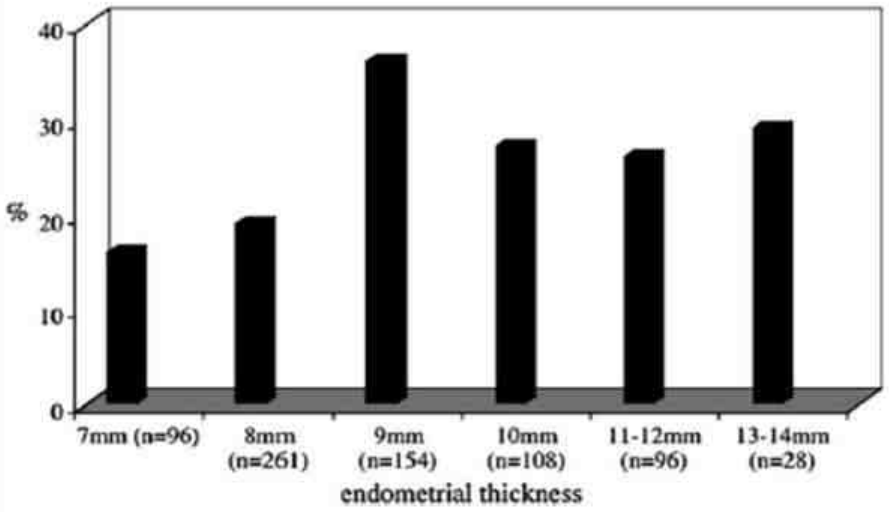
End. Prep. / USG

The relationship between endometrial thickness and outcome of medicated frozen embryo replacement cycles

Tarek El-Toukhy, M.R.C.O.G.,[a] Arri Coomarasamy, M.R.C.O.G.,[a] Mohammed Khairy, M.R.C.O.G.,[a] Kamal Sunkara, M.R.C.O.G.,[a] Paul Seed, M.Sc., C.Stat.,[b] Yacoub Khalaf, M.R.C.O.G.,[a] and Peter Braude, F.R.C.O.G.[a,b]

FIGURE 1

Clinical pregnancy rate according to endometrial thickness on day of P supplementation.



El-Toukhy. Endometrial thickness and frozen cycles. Fertil Steril 2008.

Fertil Steril 2008

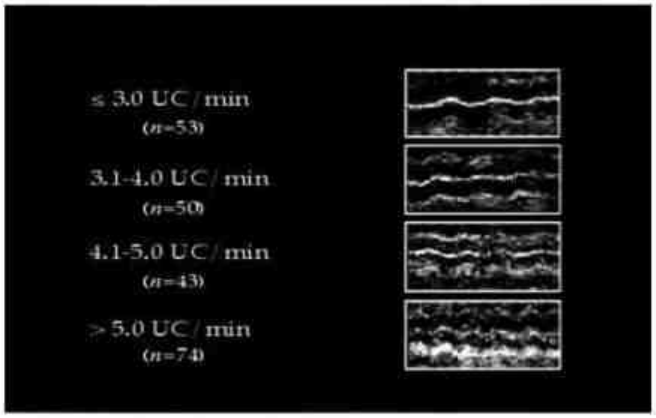
End. Prep. / USG

- **Uterine contractions at the time of embryo transfer alter pregnancy rates after in-vitro fertilization.**
- Fanchin R[1], Righini C, Olivennes F, Taylor S, de Ziegler D, Frydman R. / Hum Reprod. 1998 Jul;13(7): 1968-74

- **Abstract**
- To investigate the possible consequences of uterine contractions (UC) as visualized by ultrasound (US) on in-vitro fertilization (IVF)-embryo transferoutcome, we studied prospectively 209 infertile women undergoing 220 cycles of controlled ovarian stimulation. Inclusion criteria were age < or = 38 years, a morphologically normal uterus, and at least three good quality embryos transferred. Just before embryo transfer, women underwent 5 min digital recordings of the uterus using US image analysis software for UC assessment. Plasma progesterone and oestradiol concentrations were measured. Four groups were defined according to UC frequency: < or = 3.0 (n = 53), 3.1-4.0 (n = 50), 4.1-5.0 (n = 43), and > 5.0 (n = 74) UC/min respectively. Patients, controlled ovarian hyperstimulation and embryology characteristics were comparable in all groups. A stepwise decrease in clinical and ongoing pregnancy rates as well as in implantation rates occurred from the lowest to the highest UC frequency groups (53, 36, 21; 46, 32, 20; 23, 19, 10; and 14, 11, 4%; P < 0.001). Plasma progesterone and UC frequency were negatively correlated (r = -0.34, P < 0.001). Direction of UC did not affect embryo transfer outcome. As this study was controlled strictly for confounding variables and UC were assessed objectively by a computerized system, its results indicate that high frequency UC on the day of embryo transfer hinder IVF-embryo transfer outcome, possibly by expelling embryos out of the uterine cavity. The negative correlation between UC frequency and progesterone concentrations supports the uterinerelaxing properties of progesterone.

End. Prep. / USG

R.Fanchin *et al.*



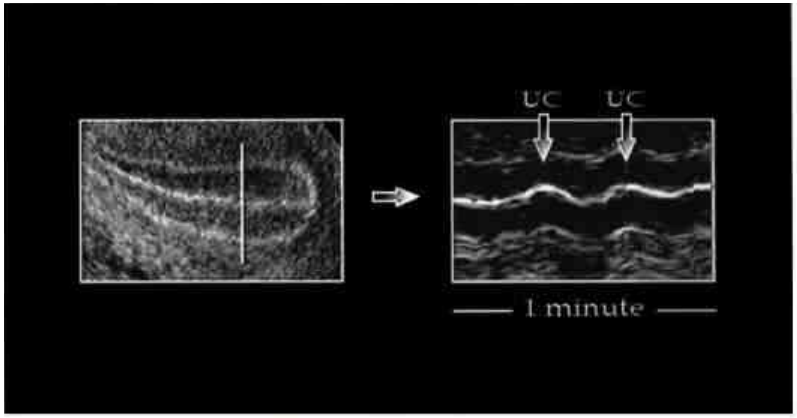


Figure 2. Definition of groups according to uterine contraction (UC) frequency.

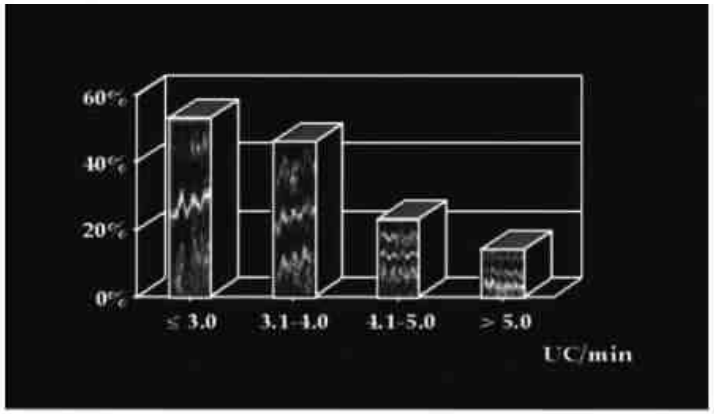


Figure 3. Stepwise decrease in clinical pregnancy rates from the lowest to the highest uterine contraction (UC) frequency groups ($P < 0.001$; ANOVA).

End. Prep. / ERA Test

- Profiling the gene signature of endometrial receptivity: clinical results

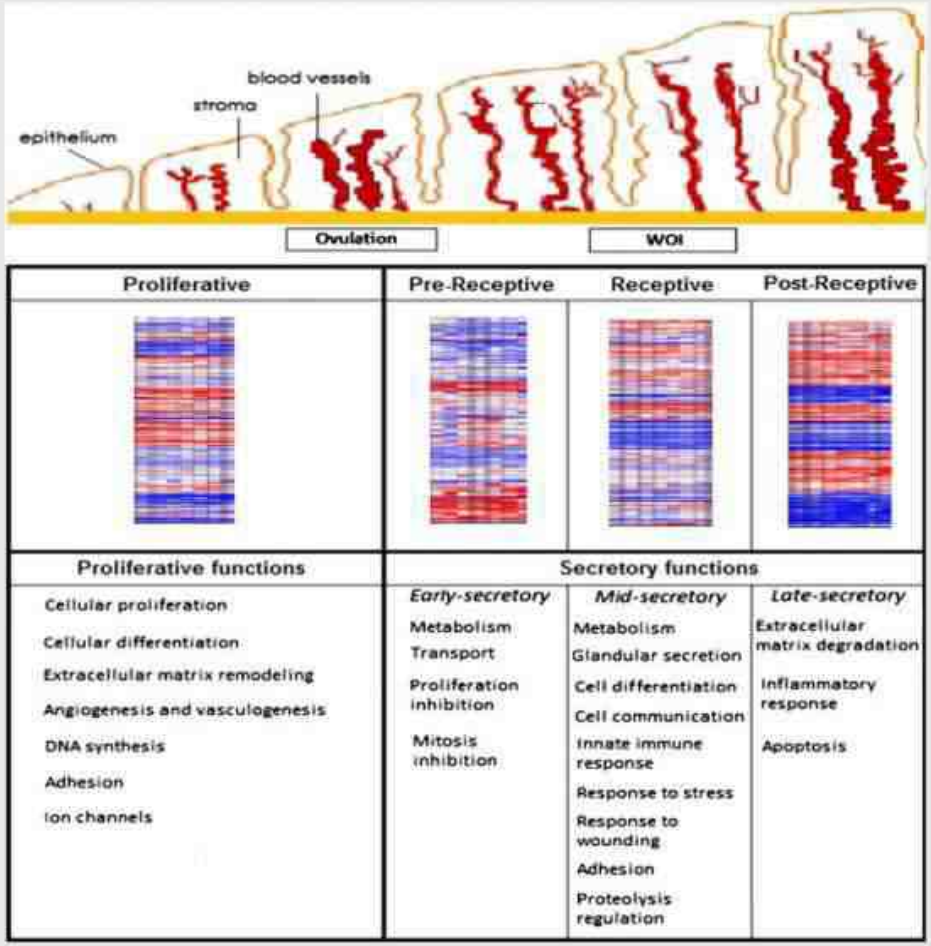
Tamara Garrido-Gomez, Ph.D., a María Ruiz-Alonso,b David Blesa, Ph.D.,a,b Patricia Diaz-Gimeno, Ph.D.,a,c Felipe Vilella, Ph.D.,a and Carlos Simon, M.D., Ph.D. a,b
a Fundacion Instituto Valenciano de Infertilidad (IVI) and Instituto Universitario IVI/INCLIVA (Investigaci on Clínico de Valencia), Valencia University; b Iviomics SL, Paterna; and c Computational Genomics Institute, Centro de Investigacion Príncipe Felipe, Valencia, Spain

- This article highlights the need for methods to objectively diagnose endometrial receptivity as a factor contributing to infertility in female patients. The correct identification of the appropriate window of implantation in a given patient, by using endometrial receptivity biomarkers, can help to prevent reproductive failure resulting from misplaced timing of the endometrial window of implantation (WOI). Although to date no single, clinically relevant morphologic, molecular, or histologic marker capable of indicating endometrial receptivity status has been identified, global transcriptomic analysis of human endometria performed in the last decade has given us insights into a genomic signature that is capable of identifying endometrial receptivity. As a consequence, a genomic tool named the Endometrial Receptivity Array (ERA), based on a customized microarray, was developed, and along with it a specially trained bioinformatic prediction computer algorithm was created to identify WOI timing in the endometrium. This tool has proven more accurate and consistent than histologic (Noyes) dating at identifying the personalized WOI day, thus leading to the new clinical concept of **personalized ET** on the optimum day of endometrial receptivity, identified individually case by case.

- FertilSteril 2013;99:1078–85. 2013 by American Society for Reproductive Medicine.

End. Prep. / ERA Test

FIGURE 1

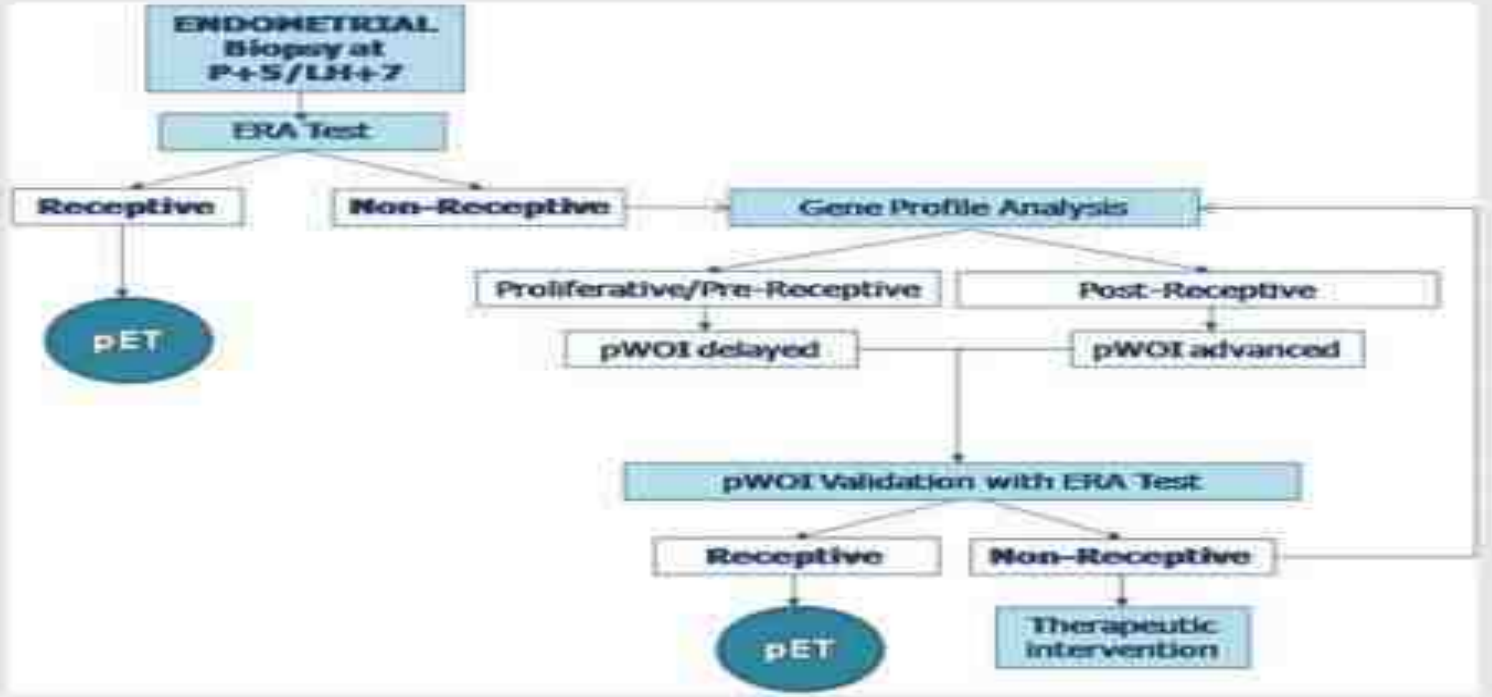


Evolution of endometrial tissue over time and the gene expression profile at each given stage. Heat map showing ERA gene expr
each endometrial cycle stage (proliferative, prereceptive, receptive, and postreceptive) and the major biological functions regulate
phases

Garrido-Gómez. Genomics of endometrial receptivity. Fertil Steril 2013.

End. Prep. / ERA Test

FIGURE 2



Clinical algorithm for ET personalization. This consists of a decision tree approach to health care treatment.

Garrido-Gómez. Genomics of endometrial receptivity. Fertil Steril 2013.

End. Prep. / ERA Test

	<=37				>37			
	Receptive		pET		Receptive		pET	
	aCGH-	aCGH+	aCGH-	aCGH+	aCGH-	aCGH+	aCGH-	aCGH+
ET cycles	30	38	7	5	25	11	7	9
b-hCG+	18	25	5	3	7	8	1	7
Sac+	17	23	4	3	7	7	0	7
FHR+	23	27	6	4	10	7	0	7
TNET	53	45	13	5	47	12	14	11
BPR	60,0%	65,8%	71,4%	60,0%	28,0%	72,7%	14,3%	77,8%
CPR	56,7%	60,5%	57,1%	60,0%	28,0%	63,6%	0,0%	77,8%
IR	43,4%	60,0%	46,2%	80,0%	21,3%	58,3%	0,0%	63,6%

(Findikli et al. unpublished)

Conclusion

- Although current literature favors freeze all approach, we still need strong evidence (Grade A) such as improved live birth rates from properly planned RCTs or large observational studies.

- The main hurdle which limits the wider application of this strategy is the existance of considerable differences in cryopreservation and FET strategies in clinics and labs.

- Once the standards are established, it will soon be an integral part of an IVF clinic.

Teşekkürler / Thank you