Frozen-warmed blastocyst transfer after 6 or 7 days of progesterone administration: impact on live birth rate in hormone replacement therapy cycles

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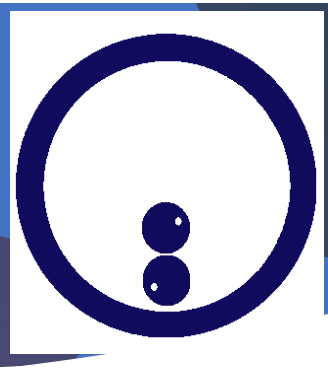
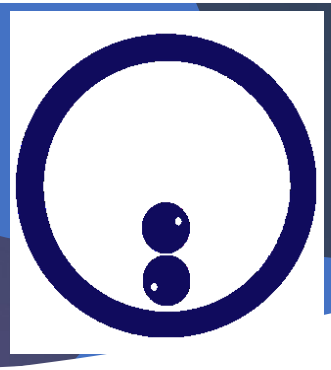
Objective:



- Synchronization between the embryonic stage and the endometrial window of implantation (WOI) is known to be crucial for the success rate of FET cycles.

- Progesterone is a crucial determinant of the WOI.

Progesterone

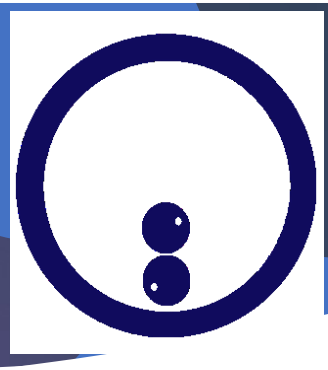


- Cochrane : Lower pregnancy rates after frozen cleavage stage embryo transfer when progesterone supplementation is **started** before in comparison to the day and the day after virtual oocyte retrieval.

- **Duration:** RCT: Progesterone administration before FET, did not show any difference in clinical pregnancy rates between protocol with 5 or 7 days before blastocyst transfer.

- Previous studies: endometrial receptivity could be achieved after very short progesterone exposure but: showed a higher risk of pregnancy loss if the endometrium was insufficiently **decidualized**.

Material & methods:



- Retrospective cohort study
- 619 patients
- December 2015-December 2017
- Standard protocol performed on the 7th and 6th day of progesterone supplementation.
- ***Group A*** =all patients underwent blastocyst transfer on the 6th day(n=346)
- ***Group B*** =all patients with transfer on the 7th day(n=273)
- Only one IVF/ICSI cycle with corresponding first FET cycle per patient, to avoid bias.

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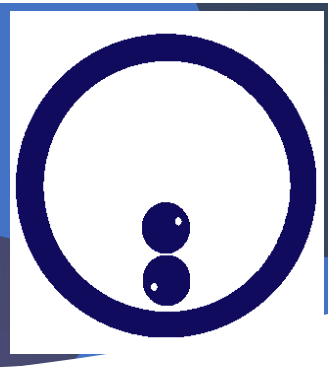
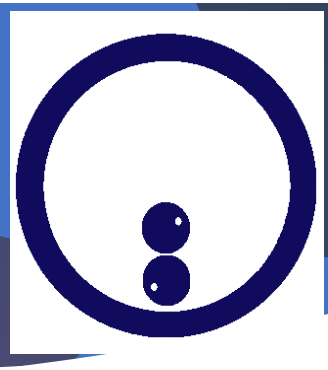


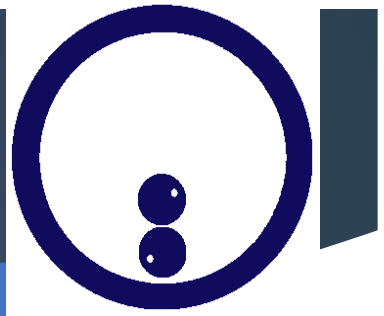
- The **outcome of preceding fresh cycle** and the application of a **freeze all** strategy were included in the study but were taken into consideration as confounding factors in the **multivariable regression analysis**.
- Age=18-43 yr
- Underwent blastocyst transfer
- Underwent HRT cycle
- Autologous and/or donor oocyte cycles
- Subgroup analysis: comparing clinical outcomes of the progesterone supplementation at 5 days versus 6 days of in vitro **development**.

Exclusion criteria:



1. blastocysts derived from in vitro maturation or PGT cycles
2. missing data regarding BMI and ET on the day of FET planning
3. FET of a cleavage stage embryo
4. Had a natural or managed natural cycle protocol before FET
5. escape ovulation despite HRT(serum progesterone >1.5 ng/ml)

Ovarian stimulation, cryopreservation and warming procedure of blastocysts:



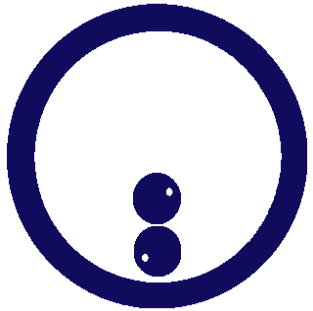
- GnRH agonist or antagonist protocol
- Oocytes insemination with sperm by IVF or ICSI, followed by development to the blastocyst stage(day 5 or 6)
- Either Fresh ET or freeze all strategy(risk for OHSS and/or serum progesterone levels on the day of ovulation triggering)
- Closed vitrification on day 5 or 6 of embryo culture.
- Only if they had reached to full blastocyst stage with a good quality ICM* and TE**.

*: Inner Cell Mass
**: Tropho Ectoderm

- Blastocysts were warmed on the morning of embryo transfer.
- Transferred only if **at least> 50%** of cells survived.

Endometrium preparation before FET:

- E2 (Oral or 0.06% gel) 2mg B.D. * 7days >>>> T.D.S. * 5 days
- U/s assessment of endometrium was performed at day 13.



- Endometrium adequacy: triple line appearance and at least 6.5 mm thickness.
  - Thickness <6.5mm on day 13: three possible options:
  1) vaginal estradiol valerate (2mg,TDS) was add
  2) the cycle was cancelled
  3) the transfer was planned anyway
- Finally vaginal progesterone 200 mg, TDS to induce secretory changes of endometrium.

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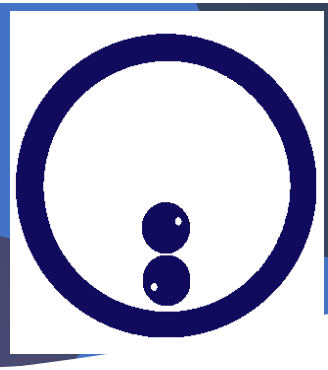
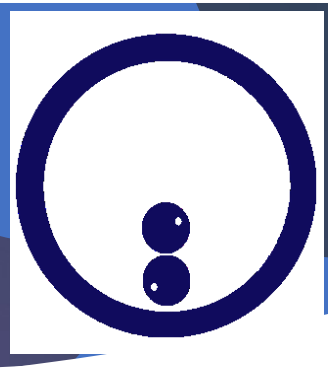


- Where ovarian suppression was used → GnRH agonist was started on day 21 of menstural cycle and continued until the start of progesterone supplementation.

- Estrogen administration was initiated **after 2weeks of suppression**

Outcome measures:



- The primary outcome → Live birth rate (live infant born after 24 weeks of gestation)
- Secondary outcomes →
  - clinical pregnancy (U/S visualization)(at 7 weeks gestational age)
  - biochemical pregnancy (detection of hCG 12 day after e.t.)
  - miscarriage(<24 week)

- Estrogen and progesterone supplementation was continued until 8 week gestation followed by a stepdown protocol.

Statistical analysis:

- Potential confounders: female age at cryopreservation , BMI , indication for treatment, parity, smoking, outcome of the preceding fresh IVF/ICSI cycle, day 6 of blastocyst transfer, source of oocytes, warmed embryo quality, Endometrial Thickness, duration of E2 supplementation before FET and single or double embryo transfer.

- Interaction analysis: with duration of progesterone administration and blastocyst developmental stage.

Result:

TABLE 1

Baseline and cycle characteristics for groups A and B, respectively.

Characteristic	Group A (n = 346)	Group B (n = 273)	*P* value
Age (y) at freezing, mean (SD)	31.7 (4.8)	31.9 (4.6)	.60
BMI (kg/m^2), mean (SD)	24.4 (4.7)	25.0 (5.6)	.12
Smoking, n (%)	67 (19.6)	43 (16.3)	.31
Parity, n (%)	136 (39.3)	102 (37.4)	.62
Indication for treatment, n (%)			
Male factor	116 (33.5)	97 (35.5)	.60
Tubal factor	24 (6.9)	28 (10.3)	.14
Ovulation disorder and PCOS	91 (26.3)	64 (23.4)	.42
Endometriosis	18 (5.2)	18 (6.6)	.46
Idiopathic	74 (21.4)	49 (17.9)	.29
Other	78 (22.5)	56 (20.5)	.54
Outcome fresh cycle, n (%)			
Clinical pregnancy	87 (25.1)	62 (22.7)	.48
Freeze all	171 (49.4)	117 (42.9)	.10
ICSI	319 (92.2)	247 (90.5)	.45
Oocyte acceptor	28 (8.1)	21 (7.7)	.85
Endometrium thickness at FET planning, mean (SD)	8.8 (1.9)	8.9 (2.2)	.59
Duration of estrogen supplementation until FET, mean (SD)	20.0 (3.7)	21.2 (5.1)	< .01
Suppression therapy, n (%)	9 (2.6)	9 (3.3)	.61
DET for FET, n (%)	41 (11.8)	55 (20.1)	.01
Top and good embryo quality for FET, n (%)	311 (89.9)	249 (91.2)	.58

Note: Statistical significance is defined as $P < .05$. BMI = body mass index; DET = double embryo transfer; FET = frozen embryo transfer; ICSI = intracytoplasmic sperm injection; PCOS = polycystic ovary syndrome; SD = standard deviation.

Roelens. *Progesterone administration in FET. Fertil Steril 2020.*

TABLE 2

Univariable analysis. Outcome measurements for groups A and B, respectively.

Variable	Group A (n = 346)	Group B (n = 273)	*P* value
Positive hCG per FET	205/346 (59.2)	147/273 (53.8)	.18
Biochemical loss per FET	16/346 (4.6)	16/273 (5.9)	.49
Biochemical loss per positive hCG	16/205 (7.8)	16/147 (10.9)	.32
Clinical pregnancy per FET	189/346 (54.6)	131/273 (48.0)	.10
Clinical miscarriage per FET	54/346 (15.6)	28/273 (10.3)	.05
Clinical miscarriage per positive hCG (excluding biochemical losses)	54/189 (28.6)	28/131 (21.4)	.15
Live birth (13 + 5 lost to follow-up)	122/333 (36.6)	98/268 (36.6)	.99

Note: Values presented at n (%), unless stated otherwise. Statistical significance is defined as $P < .05$. FET = frozen-warmed blastocyst transfer; hCG = human chorionic gonadotrophin.

Roelens. Progesterone administration in FET. Fertil Steril 2020.

TABLE 3

Multivariable regression analysis for live birth rate with adjusted odds ratios and 95% confidence interval according to the confounding factor.

LBR	aOR	95% CI		*P* value
FET on the 7th day of progesterone	1.073	0.740	1.556	.710
Age at freezing	0.980	0.939	1.022	.338
BMI	1.010	0.975	1.045	.588
Smoking	1.191	0.759	1.869	.448
Parity	0.709	0.480	1.046	.083
Indication for treatment				
Male factor	0.645	0.370	1.124	.122
Tubal factor	0.432	0.198	0.942	.035
Ovulation disorder and PCOS	0.654	0.376	1.137	.132
Endometriosis	0.775	0.330	1.821	.558
Idiopathic	0.501	0.248	1.015	.055
Other	0.532	0.274	1.035	.063
Outcome fresh cycle				
Clinical pregnancy	1.134	0.680	1.891	.630
Freeze-all	0.968	0.631	1.486	.883
Day 6 embryos	0.580	0.378	0.888	.012
ICSI	1.433	0.727	2.828	.299
Oocyte acceptor	1.199	0.547	2.632	.650
Top and good embryo quality for FET	1.369	0.746	2.514	.311
Endometrium thickness at FET planning	1.019	0.929	1.118	.690
Duration of E2 supplementation until Fret	0.990	0.949	1.033	.643
DET for FET	0.941	0.559	1.583	.818

Note: Statistical significance is defined as *P*< .05. aOR — adjusted odds ratio; BMI — body mass index; CI — confidence interval; DET — double embryo transfer; E2 — estradiol; FET — frozen-warmed blastocyst transfer; ICSI — intracytoplasmic sperm injection; LBR — live birth rate; PCOS — polycystic ovary syndrome.

Roelens. Progesterone administration in FET. Fertil Steril 2020.

TABLE 4

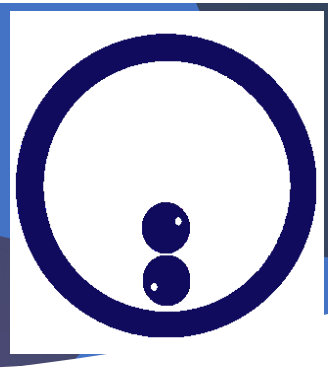
Univariable analysis. Outcome measurements of the subgroup analysis comparing day 5 and day 6 embryos for respectively group A and B.

	Day 5 embryos			Day 6 embryos		
Variable	**Group A (n = 252)**	**Group B (n = 211)**	***P* value**	**Group A (n = 94)**	**Group B (n = 62)**	***P* value**
Positive hCG per FET	161/252 (63.9)	116/211 (55.0)	.05	44/94 (46.8)	31/62 (50.0)	.70
Biochemical loss per FET	14/252 (5.6)	13/211 (6.2)	.78	2/94 (2.1)	3/62 (4.8)	.35
Biochemical loss per positive hCG	14/161 (8.7)	13/116 (11.2)	.49	2/44 (4.5)	3/31 (9.7)	.38
Clinical pregnancy per FET	147/252 (58.3)	103/211 (48.8)	.04	42/94 (44.7)	28/62 (45.2)	.95
Clinical miscarriage per FET	33/252 (13.1)	22/211 (10.4)	.38	21/94 (22.3)	6/62 (9.7)	.04
Clinical miscarriage per positive hCG (excluding biochemical losses)	33/147 (22.4)	22/103 (21.4)	.23	21/42 (50.0)	6/28 (21.4)	.02
Live birth (13 + 5 lost to follow-up)	102/240 (42.5)	76/206 (36.9)	.23	20/93 (21.5)	22/62 (35.5)	.06

Note: Values presented as n (%), unless stated otherwise. Statistical significance is defined as $P < .05$. FET = frozen-warmed blastocyst transfer; hCG = human chorionic gonadotrophin.

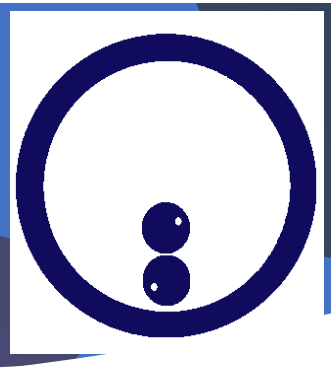
Roelens. Progesterone administration in FET. Fertil Steril 2020.

Result:



- The LBRs were same(36.6% in group A and group B)
- Multivariable analysis confirmed the higher clinical miscarriage rates and lower LBR when **day 6 blastocysts** were transferred.
- Significant results for the interaction between the **duration** of progesterone supplementation and the **developmental stage** of the blastocyst (day 5 versus day 6).
- The sample was not large enough to show a real difference between the subgroups according to the treatment group after adjustment for confounding factors.

Discussion:



- FET on the 6th day of progesterone administration resulted in LBRs similar to those of embryo transfer on the 7th day.
- Previous studies : lower implantation and clinical pregnancy rates with blastocysts expanded on day 6 compared day 5.
- These difference might be caused by a higher incidence of poor-quality embryos in day 6 blastocysts (because morphological high quality day 5 and 6 blastocysts conferred similar pregnancy outcomes)
- Haas et al found persistently higher clinical pregnancy rates for day 5 blastocysts regardless of embryo quality.
- Ferreux et.al showed significantly higher LBR with day 5 and day 6 blastocysts transferred on the 5th day of progesterone administration.

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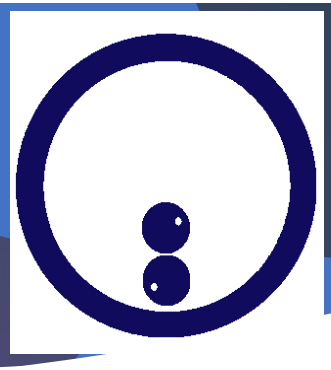

- Haas et al. performed thawing of day 5 blastocysts on the 5th day of progesterone supplementation followed by transfer 20-24 hr later, whereas day 6 blastocyst were thawed on day 6 and transferred 2-4 hr later.

- Exceeding 28 days of estrogen administration before blastocyst transfer has a deleterious effect on LBRs.
- Optimal duration of progesterone supplementation remains controversial.
- WOI starts approximately 48 hr after the start of progesterone and lasts for at least 4days.
- A tighter timeframe could potentially be more efficient.
- Enhanced flexibility is possible when planning a vitrified warmed day 5 blastocyst transfer after progesterone supplementation.(**no difference** between FET on the 6th or 7th day of progesterone on LBR and clinical miscarriage rates)

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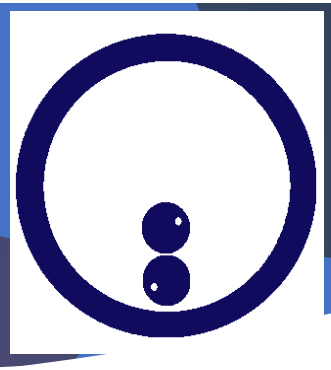

- Transfer of a vitrified warmed day 6 blastocyst deserves further investigation because these ""delayed"" blastocyst may seem to encounter a different and possibly more narrow WOI compared with day 5 embryos.

- This flexibility allow us to prolong progesterone supplementation if the recently defined threshold of 9.2ng/ml.
- This study was unpowered to difference in term of LBR below 11.5%.
- Progesterone supplementation protocols with FET on the 6^{th} day compared with 7^{th} day in similar LBRs.
- The transfer of day 5 embryos can be approached with a degree of flexibility.
- Optimal duration of progesterone exposure needs to be elucidated.

Limitations of the study

- Retrospective
- Underpowered for LBR <11.5%