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Original Article

Luteal phase progesterone supplementation following induced natural cycle frozen embryo transfer: A retrospective cohort study

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ABSTRACT

Introduction. – The objective of this study was to assess the impact on the clinical pregnancy rate of luteal phase progesterone treatment in patients being prepared for natural cycle frozen embryo transfer (FET) with induced ovulation.
Material and methods. – This retrospective cohort study collect all the FET protocols over a 6-month period at Strasbourg University Hospital fertility unit between December 2016 and May 2017. In total 293 consecutive patients with regular menstrual cycles were prepared for natural cycle FET during this period. All patients had an embryo cryopreservation secondary to in vitro fertilisation (IVF) or by intracytoplasmic sperm injection (ICSI). There were 2 protocols during this period and patients either received or did not received progesterone. Ovulation was routinely triggered in all patients by injection of choriogonadotrophin alfa. Patients in the treated group received vaginal natural micronized progesterone treatment of 400 mg daily, starting on the day of ovulation. The principal assessment criterion was the occurrence of pregnancy.
Results. – In total, 231 patients were analysed: 108 in the group not receiving progesterone and 123 in the group receiving progesterone. Patient characteristics were comparable between groups. A higher clinical pregnancy rate (39% vs. 24.1%, $p = 0.02$; 95CI [1.10; 3.74]) was recorded in the treated group.
Conclusions. – Our results suggest that luteal phase support with vaginal progesterone statistically increases the clinical pregnancy rate following hCG-triggered natural cycle FET and that it should be used more widely.

Introduction

The first live birth after frozen embryo transfer (FET) took place in 1983 [1]. Since then, embryonic cryopreservation and FET procedures have greatly advanced and become essential tools in the treatment of infertility [2–5]. Today FET procedures account for 25% of births in the field of assisted reproductive techniques (ART) [6]. They allow storage of supernumerary embryos and have enabled the indications for single embryo transfer to be greatly extended, thus limiting multiple pregnancies [2,4,7–11]. They also strive to minimize iatrogenic risks by storage of all embryos in cases of ovarian hyperstimulation syndrome [2,7,10,12]. And they allow preimplantation diagnoses [13,14].

In spite of the ubiquity of FET procedures, the most effective endometrial preparation protocol is still a topic of debate [2,4,8,11–13,15–17]. There are in fact a number of methods for synchronising the endometrium and the stage of embryonic development: natural or spontaneous cycles, cycles stimulated by exogenous gonadotrophins or even a replacement hormone therapy [2,4,8,13,15–18]. Current research has yet to provide a definitive decision on the matter but the natural cycle, since it approximates most closely to the physiological situation, appears to provide the best endometrial response [2,11,13,17]. Its further utility lies in its being less restrictive for patients and less costly than a stimulated cycle [7,8,10,12,17,18]. It should be preferred in patients with regular cycles [8,10,13,14,17,18]. Moreover, some authors raise the possibility of impaired endometrial receptivity secondary to ovarian stimulation with gonadotrophins [2,8,9,17].

There may be differences in treatment protocol even within natural cycle frozen-thawed embryo transfers [9,10]. Various authors have studied utility of routine triggering of ovulation by

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administration of exogenous hCG which is called modified natural cycle [10,16,18]. This approach appears to enable better planning of FET as well as an increased pregnancy rate [2,18].

Administration of luteal phase progesterone can also be considered [15]. In point of fact, endometrial receptivity can be compromised by several factors including luteal phase hormonal insufficiency, which is thought to occur in 8.1% of natural cycles [3,6,8,9]. An inadequate serum level of progesterone at the time of implantation is implicated in miscarriages [6,8,9,12,17]. The most appropriate method of administration appears to be vaginal even though no statistical difference has been shown to exist in the pregnancy rate between different delivery systems [6,9,16,19,20]. Vaginal administration ensures a stable progesterone concentration in the endometrium even when serum levels are low, thus reducing the risk of systemic effects [5].

The aim of this study was to determine the utility of luteal phase treatment with vaginal natural micronized progesterone following natural cycle FET with induced ovulation, by comparing the difference in pregnancy rate between a treated group and a control group.

Material and methods

This was a retrospective cohort study extending over a 6-month period from 1 December 2016 to 31 May 2017 at the Strasbourg University Hospital fertility unit.

In total 293 consecutive patients with regular menstrual cycles were prepared for natural cycle FET during this period. All patients had an embryo cryopreservation secondary to in vitro fertilisation (IVF) or by intracytoplasmic sperm injection (ICSI).

Patients provided their written informed consent and the study was validated by a local ethics committee before data analysed.

All patients underwent ovulation monitoring, with an initial check on the 9th day of their cycle. Endovaginal ultrasound was performed, along with assay of the serum oestradiol, progesterone and LH levels. Sonographic factors in favour of triggering were the presence of one or two follicles measuring 16–18 mm together with an adequate endometrial mucosa of more than 7 mm and less than 15 mm. Concerning the lab test values, serum oestradiol was required to be above 100 pg/ml with a rising LH and low serum progesterone of less than 1 ng/ml. When these conditions were all met, an injection of choriogonadotrophin alfa (ovitrelle©) was administered in order to trigger ovulation. Where necessary, a repeat ultrasound and lab tests were scheduled 2–3 days later in the hope that both the sonographic situation and lab test results would be such as to allow ovulation to be triggered.

There were 2 protocols during this period and patients either received or did not receive progesterone during luteal phase. We gathered patients in 2 groups: treated group received vaginal natural micronized progesterone in the dose of 200 mg twice daily. Treatment was started on the day of ovulation, i.e. 2 days after its induction. It was continued until first ultrasound confirming pregnancy (6 weeks following embryo transfer, i.e. 8 weeks' gestation) and then gradually tailed off over a week. Control group did not receive any treatment following embryo transfer.

The principal assessment criterion was the occurrence of pregnancy. We defined absence of pregnancy as a negative beta hCG assay 14 days following embryo transfer. Clinical pregnancies corresponded to an intrauterine pregnancy with cardiac activity visualised on ultrasound. Patients who had a miscarriage following a positive beta HCG assay on day 14 post-transfer or after visualisation of an intrauterine pregnancy on ultrasound were assigned to the spontaneous miscarriage group.

Results are shown in terms of actual numbers and weighted percentages. Interferential analysis for qualitative variables was performed either using a χ^2 test or a Fisher's exact test based on theoretical numbers in the cross-tabulation tables. The *t*-test was applied to continuous variables exhibiting normal distribution, and the Mann–Whitney test to variable not exhibiting normal distribution. Analyses were carried out using ShinyStat™ software as well as the various software packages required for complete analysis. A p value of less than or equal to 0.05 was considered as being significant.

Results

Of the 293 patients receiving ART with a view to FET, only 231 patients were enrolled in our study. A total of 52 were unable to undergo embryo transfer and were therefore excluded from the analysis. The principal reasons for non-embryo transfer are displayed in Table 1. These were mainly related to premature ovulation (15 patients, i.e. 28.8%), an inappropriate follicular response which was either excessive or inadequate (13 patients, i.e. 25%) and in some cases to administrative issues (11 patients, i.e. 21.1%). Ten patients refused to be in the study, and were therefore also excluded. Ultimately, the group without progesterone comprised 108 patients and the group treated with progesterone 123 patients (q.v. flowchart).

In total, 231 patients were analysed, 108 in the group not receiving progesterone and 123 in the group treated with progesterone. Biometric parameters were comparable between groups in terms of age, body mass index (BMI) and anti-Mullerian hormone (AMH). The percentage of patients who were active smokers, or who had undergone ICSI was also similar. Lastly, causes of infertility and embryo storage techniques were also equally distributed in both groups. Table 2 summarises these data.

Transfer characteristics were also comparable between groups. In fact, all patients were induced by hCG. In 95% of cases, a single embryo had been transferred in both groups. Blastocyte rate was similar in both groups. These data are displayed in Table 3.

There was a statistically significant difference between groups with regard to the outcome of transfer ($p = 0.05$). Clinical pregnancy rate was higher in the group treated with progesterone ($n = 48$, 39%) than in the control group not receiving progesterone ($n = 26$, 24.1%) ($p = 0.02$; 95CI [1.10;3.74]). Number of patients presenting a negative beta hCG level was considerably higher in the group not receiving progesterone (71.3% vs 56.1%; $p = 0.02$; 95CI [0.29;0.92]). Lastly, miscarriage rate was identical in both groups: 5 patients in the group not receiving treatment (4.6%) and 6 patients in the treated group (4.9%) ($p = 0.93$). Table 4 sets out these results.

Discussion

Our study demonstrates an increase in the clinical pregnancy rate of patients being prepared for natural cycle FET with triggered ovulation when receiving supplemental luteal phase vaginal progesterone. But this study is a retrospective cohort, a prospective

Table 1
Reasons for absence of embryo transfer.

	Reasons for non-transfer *n* (%)
n	52
Premature ovulation	15 (28.8)
Inappropriate follicular response	13 (25)
Administrative issue	11 (21.1)
Inappropriate endometrium	6 (11.6)
Inappropriate serum progesterone level	6 (11.6)
Intercurrent disease	1 (1.9)

Table 2
Patient characteristics.

Patient characteristics	Not receiving progesterone	Receiving progesterone	*p*
n	108	123	
Age (years)	35.3 [24;44]	35.2 [25;43]	0.91
BMI (kg/m^2)	22.7 [17;37]	22.9 [17;35]	0.68
Duration of infertility (years)	7.5 [2;17]	7.6 [2;17]	0.84
AMH (ng/mL)	3.4 [0.51;12.4]	3.04 [0.32;13.6]	0.25
Active smoker *n* (%)	20 (18.5)	28 (22)	0.57
ICSI *n* (%)	68 (63)	77 (62.6)	0.7
Causes of infertility *n* (%)			0.89
Preimplantation diagnosis (PID)	12 (11.1)	9 (7.3)	0.51
Endometriosis	6 (5.6)	7 (5.7)	
Tubal	9 (8.3)	12 (9.7)	
Male factor	29 (26.9)	36 (29.3)	
Idiopathic	52 (48.1)	59 (48)	
Vitrification technique for embryo freezing *n* (%)	107 (99)	121 (98.3)	0.9

Table 3
Embryo transfer characteristics.

Characteristics of transfer	Not receiving progesterone	Receiving progesterone	*p*
n	108	123	
Induction	108	123	
Embryo transfer *n* (%)	103 (95.4)	118 (95.9)	1
Blastocyst *n* (%)	68 (62.9)	70 (56.9)	0.35

Table 4
Embryo transfer outcomes.

Transfer outcome	Not receiving progesterone	Receiving progesterone	*p*
n	108	123	0.05
No pregnancy *n* (%)	77 (71.3)	69 (56.1)	0.02 95CI[0.29;0.92]
Clinical pregnancies *n* (%)	26 (24.1)	48 (39)	0.02 95CI[1.10;3.74]
Spontaneous miscarriages *n* (%)	5 (4.6)	6 (4.9)	0.93

randomized study double blind would be necessary to confirm our results

With the aim of improving pregnancy rates after hCG-induced ovulation [2,18], we decided to make this treatment routine in our fertility unit. In fact, hCG has a central role in endometrial preparation. It appears to induce modifications in the endometrium necessary for embryo implantation (decidualisation, optimisation of endometrial thickness) [6,10]. It enables a more exact estimation: the transfer day can be closely time-matched with the stage of embryo development. This degree of precision in synchronising between the day of embryo transfer and the date of ovulation may explain the increase in pregnancy rates compared to the natural cycle where the exact day of ovulation is difficult to determine.

A cautious approach must be maintained however in the administration of hCG. Some authors suggest that it can have a detrimental effect in patients in whom ovulation has already started. In fact, it appears that hCG leads to early closure of the implantation window [10,18,21]. This effect is however still under discussion [12]. In our study, routine hCG administration gave rise to an acceptable pregnancy rate, rendering this hypothesis rather unlikely.

Administering hCG to trigger ovulation is responsible for a hormone deficit in the luteal phase; an increase in the clinical pregnancy rate when patients receive hormone support during this period is consistent with this hypothesis [3,13,17]. It would seem that hCG induces a reduction in pituitary LH production via a negative feedback mechanism [3,8,16,17]. This action is probably related to the fact that the hCG molecule is similar to that of LH [3,16]. LH insufficiency is then thought to cause luteolysis, the corpus luteum being deprived of the continuous section of LH required for it to exert it role [3,16].

It should be noted that some teams use hCG also as a luteal phase support, but this carries a high risk of ovarian hyperstimulation syndrome [15].

Implantation of the embryo requires an adequate endometrium [9]. In order to allow correct embryonic implantation, the endometrium undergoes major biochemical and conformational changes from the proliferative phase to the secretory phase [6,8]. There is a short-lived implantation window during which it acquires its receptivity [6,8]. This window appears in response to a rise in the serum progesterone level but requires prior oestrogenic stimulation [8,9,17].

Administration of progesterone would therefore appear necessary in all patients being managed by hCG-triggered natural cycle FET. Progesterone enables the transformation of the endometrial mucosa and also promotes embryonic survival through an anti-inflammatory effect secondary to the production of Th2 cytokines as well as a local vasodilatory effect mediated by nitric oxide synthesis [8]. Initiating a supplemental progesterone treatment too early could have a detrimental effect on endometrial maturation and lead, like hCG, to early closure of the implantation window. Treatment must therefore be started more than 24 h following hCG injection or more than 24 h after the LH level has peaked [2,22]. Accordingly, we chose to administer progesterone on the evening of ovulation, i.e. 2 days following hCG. The vaginal form of progesterone produces a blood peak in approximately 8 h which then steadily declines in the next 8 h [9]. A twice-daily administration regimen would therefore seem best suited to ensure that the serum progesterone level remains stable throughout the day.

Theoretically, during the natural cycle in a fertile woman, luteal phase support is redundant since it would be provided by the fully functional corpus luteum. By contrast, women undergoing ART are either subfertile or even infertile, and are likely to produce insufficient amounts of progesterone. There is therefore a rationale for supplemental luteal phase progesterone even in the absence of hCG triggering [17].

Conclusion

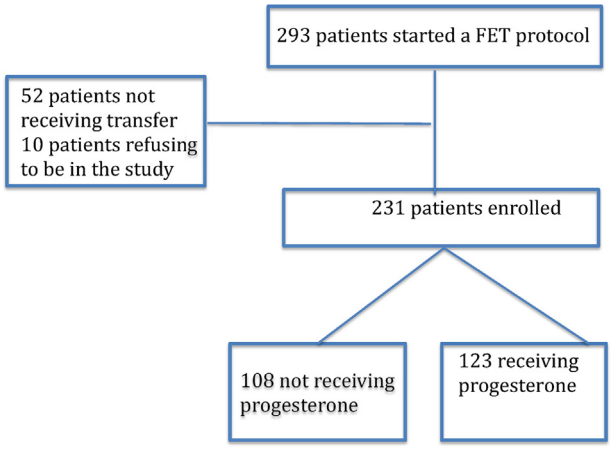
In conclusion, administration of vaginal progesterone in the luteal phase appears to increase significantly the clinical pregnan-

cy rate. With regard to the literature supplemental progesterone after hCG triggering seems to be necessary. Anyway, a prospective randomized study double blind would be necessary to confirm our results. Given its lack of adverse effects and its beneficial effect on the outcome of embryo transfer, this treatment ought to become more widespread [5,6,9,14,17,19].

Disclosure of interest

The authors state that they have no conflict of interests.

Annexes. Flowchart



References

[1] Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature 1983;305(October (5936)):707–9.
[2] Montagut M, Santos-Ribeiro S, De Vos M, Polyzos NP, Drakopoulos P, Mackens S, et al. Frozen-thawed embryo transfers in natural cycles with spontaneous or induced ovulation: the search for the best protocol continues. Hum Reprod Oxf Engl 2016;31(December (12)):2803–10.
[3] Ortega I, García Velasco JA. Progesterone supplementation in the frozen embryo transfer cycle. Curr Opin Obstet Gynecol 2015;27(August (4)):253–7.
[4] Chang EM, Han JE, Kim YS, Lyu SW, Lee WS, Yoon TK. Use of the natural cycle and vitrification thawed blastocyst transfer results in better in-vitro fertilization outcomes: cycle regimens of vitrification thawed blastocyst transfer. J Assist Reprod Genet 2011;28(April (4)):369–74.
[5] Wang Y, He Y, Zhao X, Ji X, Hong Y, Wang Y, et al. Crinone gel for Luteal phase support in frozen-thawed embryo transfer cycles: a prospective randomized clinical trial in the Chinese population. PLoS ONE 2015;10(7):e0133027.
[6] Zarei A, Sohail P, Parsanezhad ME, Alborzi S, Samsami A, Azizi M. Comparison of four protocols for luteal phase support in frozen-thawed Embryo transfer cycles: a randomized clinical trial. Arch Gynecol Obstet 2017;295(January (1)):239–46.
[7] Melnick AP, Setton R, Stone LD, Pereira N, Xu K, Rosenwaks Z, et al. Replacing single frozen-thawed euploid embryos in a natural cycle in ovulatory women may increase live birth rates compared to medicated cycles in anovulatory women. J Assist Reprod Genet 2017.
[8] Eftekhar M, Rahsepar M, Rahmani E. Effect of progesterone supplementation on natural frozen-thawed embryo transfer cycles: a randomized controlled trial. Int J Fertil Steril 2013;7(April (1)):13–20.
[9] Bjuresten K, Landgren B-M, Hovatta O, Stavreus-Evers A. Luteal phase progesterone increases live birth rate after frozen embryo transfer. Fertil Steril 2011;95(February (2)):534–7.
[10] Kyrou D, Fatemi HM, Blockeel C, Stoop D, Albuarki H, Verheyen G, et al. Transfer of cryopreserved – thawed embryos in hCG induced natural or clomiphene citrate cycles yields similar live birth rates in normo-ovulatory women. J Assist Reprod Genet 2010;27(December (12)):683–9.
[11] Haas J, Lantsberg D, Feldman N, Manela D, Machtinger R, Dar S, et al. Modifying the luteal phase support in natural cycle frozen-thawed embryo transfer improves cycle outcome. Gynecol Endocrinol Off J Int Soc Gynecol Endocrinol 2015;31(11):891–3.
[12] Le QV, Abhari S, Abuzeid OM, DeAnna J, Satti MA, Abozaid T, et al. Modified natural cycle for embryo transfer using frozen-thawed blastocysts: a satisfactory option. Eur J Obstet Gynecol Reprod Biol 2017;213(June):58–63.
[13] Yarali H, Polat M, Mumusoglu S, Yarali I, Bozdag G. Preparation of endometrium for frozen embryo replacement cycles: a systematic review and meta-analysis. J Assist Reprod Genet 2016;33(October (10)):1287–304.
[14] Orvieto R, Feldman N, Lantsberg D, Manela D, Zilberberg E, Haas J. Natural cycle frozen-thawed embryo transfer-can we improve cycle outcome? J Assist Reprod Genet 2016;33(May (5)):611–5.
[15] Ghobara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. Cochrane Database Syst Rev 2017;7:CD003414.
[16] Kyrou D, Fatemi HM, Popovic-Todorovic B, Van den Abbeel E, Camus M, Devroey P. Vaginal progesterone supplementation has no effect on ongoing pregnancy rate in hCG-induced natural frozen-thawed embryo transfer cycles. Eur J Obstet Gynecol Reprod Biol 2010;150(June (2)):175–9.
[17] Kim C-H, Lee Y-J, Lee K-H, Kwon S-K, Kim S-H, Chae H-D, et al. The effect of luteal phase progesterone supplementation on natural frozen-thawed embryo transfer cycles. Obstet Gynecol Sci 2014;57(July (4)):291–6.
[18] Weissman A, Horowitz E, Ravhon A, Steinfeld Z, Mutzafi R, Golan A, et al. Spontaneous ovulation versus HCG triggering for timing natural-cycle frozen-thawed embryo transfer: a randomized study. Reprod Biomed Online 2011;23(October (4)):484–9.
[19] Guo W, Chen X, Ye D, He Y, Li P, Niu J, et al. Effects of oral dydrogesterone on clinical outcomes of frozen-thawed embryo transfer cycles. Nan Fang Yi Ke Da Xue Xue Bao 2013;33(June (6)):861–5.
[20] Mamouni N, Derkaoui A, Bougern H, Bouchikhi C, Chaara H, Banani A, et al. Subcapsular hematoma of the liver complicating preeclampsia: about 6 cases. Pan Afr Med J 2011;9:47.
[21] Groenewoud ER, Macklon NS, Cohlen BJ, ANTARCTICA Study Group. The effect of elevated progesterone levels before HCG triggering in modified natural cycle frozen-thawed embryo transfer cycles. Reprod Biomed Online 2017;34(May (5)):546–54.
[22] Lee VCY, Li RHW, Ng EHY, Yeung WSB, Ho PC. Luteal phase support does not improve the clinical pregnancy rate of natural cycle frozen-thawed embryo transfer: a retrospective analysis. Eur J Obstet Gynecol Reprod Biol 2013;169(July (1)):50–3.