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ARTICLE

Cryopreserved embryo transfer in an artificial cycle: is GnRH agonist down-regulation necessary?

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Abstract The use of GnRH agonist downregulation in artificial endometrium priming cycles for cryopreserved embryo transfer was retrospectively investigated to establish whether higher live birth rates resulted. Six hundred and ninety-nine patients underwent 1129 artificial endometrium priming cycles for the transfer of cryopreserved embryos between 1 July 2009 and 1 June 2012. Hormonal supplementation with (group A, $n = 280$ cycles) or without (group B, $n = 849$ cycles) GnRH agonist co-treatment was given. Live birth rates were comparable between the two groups per started cycle (14.9% [41/275] in group A versus 15.1% [127/839] in group B) or per embryo transfer (17.5% [41/234] in group A versus 17.6% [127/723] in group B). After logistic regression analysis, the only variables that were significantly associated with live birth rates were day of embryo transfer (OR 0.69; 95% CI 0.48 to 0.98) for day 3 versus day 5 embryos, the number of embryos transferred (OR 2.13; 95% CI 1.58 to 2.86) for two embryos versus one embryo transferred and the endometrial thickness on the day of embryo transfer (OR 1.15; 95% CI 1.05 to 1.25). Live birth rates after cryopreserved embryo transfer in artificial cycles did not increase when a GnRH agonist was administered.

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KEYWORDS: artificially prepared cycle, cryopreserved embryo transfer, endometrium, GnRH agonist, live birth rate

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Introduction

Embryo cryopreservation has become a routine procedure in assisted reproductive technology ever since the transfer of a frozen-thawed human embryo resulted in pregnancy in 1983 (Trounson and Mohr, 1983). The increased use of single embryo transfer strategies in many assisted reproductive technology programmes worldwide has substantially enhanced the number of surplus embryos for cryopreservation, urging the development of successful frozen–thawed embryo transfer (FET) protocols (Tiitinen et al., 2001). This was further reinforced by investigators supporting the use of FET as an effort to eliminate the incidence of ovarian hyperstimulation syndrome (OHSS) through segmentation of the IVF treatment (Devroey et al., 2011). More recent data suggest that FET results in significantly higher ongoing pregnancy rates compared with fresh embryo transfer (Roque et al., 2013).

Although advances in embryo cryopreservation techniques, such as the introduction of embryo vitrification, have substantially improved embryo survival after warming (Loutradi et al., 2008), optimal synchronization between the endometrial development and the embryo remains essential (Edwards, 1988; Harper, 1992). When an FET is performed, endometrial preparation may be achieved in a natural or an artificial way. Several randomized controlled trials have been published examining the effects of different methods of endometrial preparation for cryopreserved embryo transfer, but evidence of a single best endometrium priming protocol is still lacking (Glujovsky et al., 2010; Groenewoud et al., 2012, 2013).

In patients with a regular menstrual cycle, the transfer of a cryopreserved embryo can be successfully conducted in a natural cycle based on detection of the LH surge that precedes ovulation (using serum or urine LH monitoring) or by triggering ovulation with HCG (Cohen et al., 1988). In a randomized controlled trial by Fatemi et al. (2010), a significantly lower pregnancy rate was observed after triggering with HCG compared with the detection of the spontaneous LH peak. Nevertheless, these findings have been questioned in a further small randomized controlled trial, which showed no difference between both approaches (Weissman et al., 2011). Impediments to the use of the natural cycle include oligomenorrhoea and the occurrence of early spontaneous ovulation resulting from cycle irregularities; this leads to cycle cancellation, despite cycle monitoring.

To minimize the risk of cycle cancellation, intensified cycle monitoring may be considered, which is time consuming and more expensive, despite the omittance of medication.

Another approach for endometrial preparation is the artificial hormonal cycle, originally developed for patients undergoing oocyte donation, which has also been used successfully in patients undergoing FET (Younis et al., 1996). Taking into account the minimal cycle monitoring related to such a practice (i.e. hormonal analyses and ultrasound scans of the endometrium), the protocol of exogenous oestrogen and progesterone administration is widely used for endometrial preparation (Younis et al., 1996). Disadvantages of this approach include the cost, inconvenience for the patient, side-effects of oestrogen supplementation (e.g. increased thrombotic risk) and prolonged treatment (especially in case of pregnancy).

In addition to the administration of oestrogen and progesterone, a GnRH agonist is often added to the artificial cycle protocol to prevent spontaneous ovulation. In a randomized controlled trial involving 234 patients undergoing FET, cycles without ovarian suppression using GnRH agonist were associated with reduced clinical pregnancy and live birth rates per cycle (El-Toukhy et al., 2004), mainly due to a higher cycle cancellation rate. Endocrine cycle monitoring, however, was not carried out in that study, and data on the incidence of premature ovulation were not available. The results of this trial strongly contradict the results of a Cochrane systematic review of four studies, including the study by El-Toukhy et al. (2004), (n = 725), which could not demonstrate any benefit of clinical pregnancy rate and cancellation rates when GnRH agonists were used (Ghobara and Vandekerckhove, 2008). The three other randomized trials included in this meta-analysis failed to find any significant difference in clinical pregnancy rates (Dal Prato et al., 2002; Loh et al., 2001; Simon et al., 1998) when the GnRH agonist was omitted. Similarly, another systematic review (Glujovsky et al., 2010) evaluating clinical pregnancy rates in women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes, had similar results, failing to reveal any significant benefit in clinical pregnancy rates. In view of the conflicting clinical pregnancy and live birth rates, we decided to analyse the data available from a large cohort of FET cycles in our centre, to evaluate whether addition of GnRH agonist may indeed increase live birth rates.

Materials and methods

Between 1 July 2009 and 1 May 2012, data from all artificial endometrium priming cycles for cryopreserved embryo transfer were analysed. Inclusion criteria were women's age 39 years or younger at the time of embryo cryopreservation and having undergone one or more cycles with the same protocol for all consecutive cycles (either with or without GnRH agonist). No cross-over was allowed across different cycles.

The FET cycles were carried out either after ovarian stimulation followed by conventional IVF or intracytoplasmic sperm injection (ICSI). The use of donor oocytes was excluded. The study protocol was approved by the Ethics Committee of our institution (B.U.N. 143201214922 on 20 September 2012).

Ovarian stimulation and IVF–ICSI treatment

Different protocols for ovarian stimulation were used (i.e. long and short GnRH agonist protocols and the GnRH antagonist protocol). Triggering with HCG was carried out as soon as three follicles 17 mm or wider were observed by ultrasound scan. Oocytes were retrieved 36 h after HCG injection. The collected oocytes were inseminated either via conventional IVF or via ICSI.

Cryopreservation and thawing–warming procedure

Embryos were cryopreserved on day 3 or on day 5 of embryo culture. Day 3 embryos were cryopreserved using either slow

freezing or vitrification. For slow-controlled freezing and thawing, the protocol was used with dimethylsulphoxide (DMSO) as the cryoprotectant as described previously (Van den Abbeel and Van Steirteghem, 2000). Embryos were vitrified by closed vitrification using closed blastocyst vitrification high security straws (Cryo Bio System) combined with with dimethylsulphoxide and ethylene glycol bis(succinimidyl succinate) as the cryoprotectants (Irvine ScientificR Freeze Kit) (Van Landuyt et al., 2011). Day 5 and day 6 blastocysts were vitrified using the same protocol, as described for day 3 embryo vitrification.

Preparation of the endometrium

In both groups, artificial preparation of the endometrium consisted of 7 days of oestradiol valerate (Progynova®; Bayer-Schering Pharma AG, Berlin, Germany) at a dose of 2 mg twice daily, followed by 6 days of oestradiol valerate at a dose of 2 mg three times daily. On day 13, endometrial thickness was measured by ultrasound scan, and serum oestradiol and progesterone levels were analysed. If serum progesterone levels were over 1.5 ng/mL, the cycle was cancelled. If the endometrial thickness was 7 mm or less, and a triple-line endometrium was present, progesterone supplementation was started, as described below. If the endometrium was less than 7 mm mm in thickness, patients continued to take oral oestradiol at a dose of 2 mg three times daily until the endometrium thickness was 7 mm or greater, at which point progesterone supplementation was started. If the endometrial thickness remained less than 7 mm in spite of prolonged oestradiol priming (with a maximum of 7 days additional oestrogen supplementation), the cycle was cancelled.

Patients were eligible only if the same endometrial preparation protocol was used in consecutive FET cycles (no crossover was allowed between GnRH downregulated and non-downregulated cycles).

In GnRH-downregulated cycles (group A) buserelin nasal spray (Suprefact®, Hoechst UK Ltd., Hounslow, Middlesex, UK) was initiated from day 21 of the cycle onwards at a dose of 0.2 mg three times daily. This regimen was continued for 14 days. If basal hormonal serum levels were observed after 2 weeks (oestradiol < 80 ng/l and progesterone < 1.5 ng/ml), oestradiol valerate was started. If not, buserelin nasal spray was continued until basal hormone levels were achieved. Daily buserelin nasal spray was continued until the first pregnancy test if patients still had cryopreserved embryos available, to be able to perform a next cryopreserved embryo transfer in case no pregnancy occurred after the current cryopreserved embryo transfer. If no embryos were left, buserelin was stopped on the day progesterone was started (El-Toukhy et al., 2004). The decision of whether or not to add GnRH agonist to the protocol depended on physicians' preference.

In the control group, cycles that did not use GnRH agonist (group B), oestradiol valerate priming was started on days 1–3 of the cycle, after hormonal serum analysis, to detect a premature oestradiol rise owing to early follicular recruitment or the presence of an ovarian cyst. If circulating oestradiol levels were more than 80 ng/l, the cycle was cancelled.

In both groups, micronized vaginal progesterone (Utrogestan®; Besins, France) 200 mg three times daily) was started as soon as endometrial thickness measured 7 mm or more.

Cryopreserved day 3 or day 5 embryos were transferred 5 days and 7 days after progesterone initiation, respectively. Cryopreserved embryo transfers were performed under ultrasound guidance using a Cook ET catheter (K-soft-5100; Cook, Bloomington, IN).

Assessment of pregnancy outcome

Serum beta-HCG levels were measured 12 days after embryo transfer. If the test was positive, daily oestradiol valerate and progesterone supplementation were continued until the 12th week of pregnancy (Remohi et al., 1995). An ultrasound scan was carried out 4–5 weeks after embryo transfer to determine fetal viability. Clinical pregnancy was defined as the presence of one or more gestational sacs on ultrasound at 7 weeks of gestational age. Spontaneous abortion was defined as the loss of a clinical pregnancy up to 20 gestational weeks. Live birth was defined as the delivery of at least one live born baby, irrespective of the duration of the pregnancy (Zegers-Hochschild et al., 2009).

Outcome measures

The primary end-point of the study was the live birth rate per cycle after cryopreserved embryo transfer. Secondary endpoints included clinical pregnancy rate, early pregnancy loss (spontaneous abortion and biochemical pregnancy), ectopic pregnancy rate and the occurrence of premature serum progesterone rise after administration of oestradiol valerate, which was defined as a serum progesterone level of over 1.5 ng/ml.

Statistical analysis

Continuous variables were analysed using the independent *t*-test or Mann–Whitney U test depending on the normality of the distribution. Normality was examined by the use of the Shapiro–Wilk test.

Categorical variables were analysed by Pearson's chi-squared test or Fisher's exact test. All values were two-tailed with a level of significance set at 0.05. All analyses were performed with the Statistical Package for Social Sciences (SPSS) version 10 SE (SPSS Inc., USA).

To identify variables that may be related to live birth rate, stepwise forward logistic regression analysis was conducted to identify independent variables associated with live birth rates. Live birth was set as the dependent variable in the model, with independent variables being the day of embryo transfer (day 3 or day 5), parity (nulliparous or multiparous), preparation of the endometrium (with or without GnRH agonist), indication for fertility treatment (**Table 1**), outcome of the fresh IVF–ICSI treatment, cryopreservation protocol (slow freezing or vitrification), age, number of embryos transferred, endometrial thickness and body mass. The significance level of the candidate predictive variables to enter the model was set to 0.05 and to stay in the model, it was set to

Table 1 Baseline characteristics of the patients included.

	Group A[a] *(n = 280)*	*Group B*[b] *(n = 849)*
Age (years) (SD)	32.5 (3.8)	31.3 (4.1)[c]
Height (cm) (SD)	166.3 (7.5)	165.7 (7.1)
Weight (kg) (SD)	65.1 (14.6)	66.7 (14.3)
BMI (kg/m^2) (SD)	23.6 (4.8)	24.3 (5.1)
Indication for fertility treatment		
Male (%)	72 (25.7)	204 (24.0)
Tubal (%)	15 (5.4)	57 (6.7)
Ovulation disorder (%)	71 (25.4)	268 (31.6)
Endometriosis (%)	27 (9.6)	52 (6.1)
Genetic (%)	37 (13.2)	136 (16.0)
Idiopathic (%)	52 (18.6)	120 (14.1)
Other (%)	6 (2.1)	12 (1.4)

[a]Oestrogen plus progesterone plus GnRH agonist.
[b]Oestrogen plus progesterone.
[c]$P < 0.01$.

0.10. After selection of the candidate variables, the final model included those prognostic variables with statistical significance according to the Wald statistic test at a threshold of 0.05. The goodness-of-fit of the normal regression models was assessed by the Hosmer–Lemeshow goodness-of-fit test.

Results

In total, 1129 cycles were included in the analysis. Two hundred and eighty cycles ($n = 185$) were carried out with hormonal supplementation with GnRH agonist co-treatment (group A), and in 849 cycles ($n = 514$) only hormonal supplementation without the use of GnRH agonist co-treatment was adopted (group B). Patients' baseline characteristics are presented in Table 1. As shown, no significant difference was found between the two groups in demographic characteristics such as weight, height and body mass index (Table 1). Mean age was significantly higher in group A (32.5 years, SD 3.8) compared with group B (31.3 years, SD 4.1) ($P < 0.01$). Indications for fertility treatment included male factor infertility, tubal infertility, ovulation disorders, endometriosis, genetic factors (i.e. pre-implantation genetic diagnosis) and idiopathic infertility, and were similar in both treatment arms (Table 1). The outcome of the fresh embryo transfer (in terms of clinical pregnancy) was not significantly different between the two groups (78 out of 185 fresh cycles [42.2%] in group A versus 212 out of 514 [41.2%]) in group B).

Endometrial thickness at the moment of progesterone administration was comparable between groups (8.3 mm [SD 2.0] in group A and 8.2 mm in group B [SD 2.0]). The number of thawed/warmed embryos (with a median of 2 in group A [quartile range: 1–2] and 2 in group B [quartile range 1–2]), as well as the number of transferred embryos per cycle (with a median of 1 in group A (quartile range 1–2) and 1 in group B [quartile range 1–2]) was similar (Table 2). No statistically significant difference were observed in the distribution of cleavage stage or blastocyst transfers in both groups: 38.1% (91/239) in group A versus 41.9% (307/733) in group B for cleavage stage embryos; 61.9% (148/239) in group A versus 58.1% (426/733) in group B for blastocyst transfers (Table 2), nor in the distribution of slow freezing versus vitrification in both groups: 15.6% (42/270) in group A versus 15.3% (123/802) in group B for slow freezing; 84.4% (228/270) in group A versus 84.7% (679/802) in group B for vitrification) (Table 2).

Out of the 280 started cryopreserved embryo transfer cycles in group A, 41 did not reach embryo transfer (14.6%); The number of cancelled cryopreserved embryo transfer cycles in group B was 116 out of 849 cycles (13.7%). The reason for cancellation was similar between the two study groups, except for premature progesterone rise. In group B, premature progesterone rise occurred in 1.9% (16/849), whereas there was not a single cycle with premature progesterone rise in group A ($P = 0.021$) (Table 3). The mean value of progesterone in case of a premature progesterone rise, was 8.4 ng/mL (SD 4.6). Cycle cancellation owing to insufficient embryo quality after thawing and warming occurred in 31 out fo 280 cycles in group A (11.1%), versus 69 out of 849 cycles in group B (8.1%). Other reasons for cycle cancellation (e.g. thin endometrium, fluid in the uterine cavity, decision of the patient to stop treatment) were not statistically significant between the two groups (group A 10/280 [3.6%] versus group B: 31/849 [3.7%]).

Live birth rate per started cycle (41/275 in group A [14.9%] versus 127/839 in group B [15.1%] and live birth rate per embryo transfer (41/234 in group A [17.5%] versus 127/723 in group B [17.6%]) were not statistically different. No information could be found on pregnancy outcome after 10 weeks of gestational age in five and 10 cycles in group A and B, respectively. They were considered lost to follow up.

Clinical pregnancy rates per started cycle did not differ between the two groups: 47 out of 280 in group A (16.8%) versus 137 out of 849 in group B (16.1%). Clinical pregnancy rates per embryo transfer were not statistically different either (47/239 in group A [19.7%] versus 137/733 in group B [18.7%]. Also, early pregnancy loss (spontaneous abortion and biochemical pregnancy) and ectopic pregnancy rates were similar in both arms (Table 3).

Multivariable logistic regression analysis showed that age, body mass index, parity, endometrial preparation protocol, cryopreservation protocol, outcome of the fresh IVF–ICSI cycle and indication for fertility treatment were not associated with live birth rates. On the contrary, number of embryos transferred, day of embryo transfer and endometrial thickness at day of planning were the only independent variables significantly associated with live birth rate (odds ratio [OR] 2.13; 95% confidence interval [CI] 1.58 to 2.86 for live birth for women having two embryos transferred compared with women having one embryo transferred; OR 0.69, 95% CI 0.48 to 0.98) for live birth for women having a cleavage stage embryo transfer compared with women having blastocyst transfer; and OR 1.15, 95% CI 1.05 to 1.25) for live birth for endometrial thickness on the day of progesterone administration.

Discussion

The results of this large cohort study in 1129 cryopreserved embryo transfer cycles show that cryopreserved embryos can successfully be transferred in an artificial endometrium priming cycle without the use of GnRH agonist before endometrial preparation. Live birth rates were unaffected, regardless of

Table 2 Embryological data.[a]

	Group A[b]	Group B[c]
Number of embryos thawed and warmed: median (quartile range)	2 (1–2)	2 (1–2)
Number of embryos transferred per cycle: median (quartile range)	1 (1–2)	1 (1–2)
Day of embryo transfer		
Day 3 (cleavage stage) (%)	91/239 (38.1)	307/733 (41.9)
Day 5 (blastocyst stage) %	148/239 (61.9)	426/733 (58.1)
Cryopreservation protocol		
Slow freezing (%)	42/270 (15.6)	123/802 (15.3)
Vitrification (%)	228/270 (84.4)	679/802 (84.7)

[a]No statistically significant differences between the two groups.
[b]oestrogen plus progesterone plus GnRHa.
[c]Oestrogen plus progesterone.

Table 3 Clinical outcome.

	Group A[a]	Group B[b]
Cycles without embryo transfer	41/280 (14.6)	116/849 (13.7)
Failed embryo thaw–warming (%)	31/280 (11.1)	69/849 (8.1)
Premature progesterone rise (%)	0/280 (0)	16/849 (1.9)[f]
Other (%)	10/280 (3.6)	31/849 (3.7)
Clinical pregnancy		
Per started cycle (%)	47/280 (16.8)	137/849 (16.1)
Per embryo transfer[c] (%)	47/239 (19.7)	137/733 (18.7)
Early pregnancy loss		
Per started cycle (%)	27/280 (9.6)	90/849 (10.6)
Per embryo transfer[c] (%)	27/239 (11.3)	90/733 (12.3)
Ectopic pregnancy		
Per started cycle (%)	3/280 (1.1)	10/849 (1.2)
Per embryo transfer[c] (%)	3/239 (1.3)	10/733 (1.4)
Live birth		
Per started cycle[d] (%)	41/275 (14.9)	127/839 (15.1)
Per embryo transfer[e] (%)	41/234 (17.5)	127/723 (17.6)

[a]Oestrogen plus progesterone plus GnRHa.
[b]Oestrogen plus progesterone.
[c]In group A, 41 patients did not undergo embryo transfer; in group B, 116 did not undergo embryo transfer.
[d]In group A, five patients pregnancy was unknown (i.e. lost to follow up); in group B, 10 pregnancy outcomes were unknown.
[e]In group A, 41 patients did not undergo embryo transfer and pregnancy outcome was unknown in five patients (i.e. lost to follow-up); in group B, 116 patients did not undergo embryo transfer and 10 patients were lost to follow-up.
[f]$P = 0.021$.

co-administration of a GnRH agonist. Because of the retrospective design of this trial, results need to be interpreted with caution.

As far as clinical pregnancy rates are concerned, our results are in line with previously published prospective randomized studies (Dal Prato et al., 2002; Loh et al., 2001; Simon et al., 1998). In those studies, the investigators concluded that endometrial preparation for frozen–thawed embryo transfer using steroid supplementation only is as effective as endometrial preparation involving preliminary pituitary desensitization (Dal Prato et al., 2002). Another randomized trial, however, showed significantly lower pregnancy and live birth rates without GnRH agonist co-treatment (El-Toukhy et al., 2004). In the latter trial, however, cycle monitoring was carried out by ultrasound scans only, without endocrine monitoring. In addition, the mean duration of the proliferative phase was nearly 3 weeks, which may have predisposed patients in the non-down-regulated group to a higher rate of ovulation (El-Toukhy et al., 2004).

An interesting observation in relation to our study dataset is that age was statistically significantly higher in the GnRH agonist downregulated group (32.5 versus 31.3 years, $P < 0.01$), although this difference is probably not clinically significant. This finding might be attributed to the treatment

policies obtained by individual physicians. It is a fact that women of advanced reproductive age may experience shorter menstrual cycles and therefore an increased incidence of early spontaneous ovulation, given that increasing age is associated with a shortening of the mean menstrual cycle length (Brodin et al., 2008; Treloar et al., 1967). Therefore, it may be hypothesized that the physician's decision of whether or not to administer GnRH agonist was based on their willingness to prevent cancellation owing to a shorter cycle in women of more advanced reproductive age.

In our study, cancellation rates caused by premature ovulation (defined as a premature progesterone rise) were only 1.9% in cryopreserved embryo transfer cycles when no GnRH agonist was administered. Cryopreserved embryo transfer cycles without GnRH agonist co-treatment are more patient-friendly because of the avoidance of potential side-effects caused by GnRH agonist, such as fatigue and other symptoms of oestrogen deprivation. Furthermore, omittance of GnRH agonist lowers the cost of a treatment cycle, without compromising pregnancy and live birth rates. Overall, cryopreserved embryo transfer cycle cancellation rate of 2% has been reported (Queenan et al., 1997), but the risk of cancellation owing to ovulation has been reported to be as high as 7.4% (Dal Prato et al., 2002; Pattinson et al., 1992), which has been ascribed to a delay in oestrogen initiation (Remohi et al., 1993) or to an insufficient oestrogen dose (Simon et al., 1998).

Given the low cancellation rates owing to progesterone rise in our study (1.9%), the results need to be interpreted with caution. Although this difference was statistically significant (which simply implies that measurement of progesterone should be performed), from a clinical and cost-effective point of view, it is questionable whether measurement of progesterone levels may indeed significantly improve patients' outcome, owing to the extremely low incidence of progesterone rise and escape ovulation in these patients.

An additional preventive measure to further reduce the incidence of premature progesterone rise, and thus totally avoid hormonal measurement, might be the use of higher oestrogen starting doses (e.g. 6 mg daily from day 1–3 of the cycle onwards), to further suppress gonadotrophin release and prevent the occurrence of follicular dominance and excessive LH secretion (Simon et al., 1998). Finally, starting oestrogen treatment from day 3 (or later) of the cycle onwards has been shown to be associated with a higher spontaneous LH secretion and postovulatory changes observed on day 15 compared with cycles in which oestrogen was given from day 1 onwards (de Ziegler et al., 1991; Remohi et al., 1993). It is questionable, however, whether such an approach may increase both the physical burden and the cost related to treatment.

In terms of patient friendliness and convenience, natural cycle cryopreserved embryo transfer is indeed more friendly and less expensive owing to the lack of exogenous medication administration and the lower treatment cost. Artificial endometrial preparation, however, can be of paramount importance for everyday clinical practice, as it is the only option to treat women with oligo- or amenorrhoea, whereas it may contribute towards avoiding embryo transfers during weekends or holidays. Therefore, identification of the optimal protocol for artificial endometrial preparation has a key role in patients' fertility care.

In conclusion, live birth rates are not impaired when GnRH agonist is omitted in artificial endometrium priming cycles when adequate endocrine monitoring is carried out. Although GnRH agonist co-treatment eliminates the risk of premature progesterone rise, its low incidence does not justify the routine use of a GnRH agonist for cryopreserved embryo transfer cycles in everyday clinical practice, whereas it is questionable whether meticulous hormonal level monitoring may indeed contribute towards an increase in live birth rates.

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