RBM ONLINE

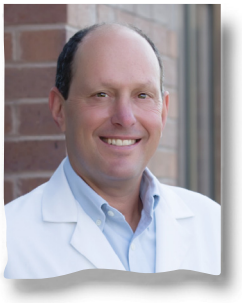
ELSEVIER

Article

GnRH agonist administration prior to embryo transfer in freeze-all cycles of patients with endometriosis or aberrant endometrial integrin expression

Eric S Surrey* *, Mandy Katz-Jaffe, Laxmi V Kondapalli, Robert L Gustofson, William B Schoolcraft

Colorado Center for Reproductive Medicine, Lone Tree, CO 80124, USA



Eric Surrey is Medical Director of the Colorado Center for Reproductive Medicine in Lone Tree, Colorado, USA. He is a past president of the Society for Assisted Reproductive Technologies, the Society of Reproductive Surgeons and the Pacific Coast Reproductive Society. He has written extensively on endometriosis, poor responders and elective single embryo transfer.

KEY MESSAGE

A prolonged course of GnRHa after freeze-all to patients with endometriosis or aberrant endometrial integrin expression results in high implantation and ongoing pregnancy rates after embryo transfer. This avoids excessive ovarian suppression associated with agonist administration prior to fresh embryo transfer and allows for elective vitrification of all embryos.

A B S T R A C T

Prolonged gonadotrophin-releasing hormone agonist (GnRHa) administration before IVF with fresh embryo transfer to patients with endometriosis or aberrant endometrial integrin expression (-integrin) improves outcomes but may suppress ovarian response and prevents elective cryopreservation of all embryos. This retrospective cohort pilot study evaluates freeze-all cycles with subsequent prolonged GnRHa before embryo transfer in these populations. Patients from 2010 to 2015 who met inclusion criteria and received a long-acting GnRHa every 28 days twice before FET were evaluated. A subset underwent comprehensive chromosomal screening (CCS) after trophectoderm biopsy. Three groups were identified: Group 1: + CCS, +endometriosis (20 patients, 20 transfers); Group 2: +CCS, –integrin (12 patients, 13 transfers); Group 3: no CCS, +endometriosis or –integrin (10 patients, 12 transfers); Group 4: all transfers after CCS for descriptive comparison only (n = 2809). Baseline characteristics were similar among Groups 1–3 except that the mean surgery to oocyte aspiration interval was longer for Group 1 than Group 3. Implantation and ongoing pregnancy rates were statistically similar among the three groups and compared favourably to Group 4. A non-significant trend towards improved outcomes was noted in Group 1. Prolonged GnRHa after freeze-all in these patients avoids excessive ovarian suppression and results in excellent outcomes.

* Corresponding author.
E-mail address: esurrey@colocrm.com (ES Surrey).

http://dx.doi.org/10.1016/j.rbmo.2017.05.004
1472-6483/

Please cite this article in press as: Eric S. Surrey, Mandy Katz-Jaffe, Laxmi V. Kondapalli, Robert L. Gustofson, William B. Schoolcraft, GnRH agonist administration prior to embryo transfer in freeze-all cycles of patients with endometriosis or aberrant endometrial integrin expression, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.05.004

Introduction

The impact of endometriosis in general on IVF outcomes is somewhat controversial, although the presence of more advanced disease (particularly in the presence of endometriomas) seems to have deleterious effects (Hamdan et al., 2015; Harb et al., 2013; Surrey, 2013). The benefit of a prolonged course of a gonadotrophin-releasing hormone agonist (GnRHa) in these patients prior to initiation of an IVF cycle with planned fresh embryo transfer has been demonstrated (Sallam et al., 2006; Surrey et al., 2002). Others have shown that this may also be beneficial in patients with recurrent implantation failure who were noted to have aberrant endometrial $\alpha v\beta_3$ integrin expression (Surrey et al., 2007).

One of the disadvantages of this approach is that prolonged pituitary down-regulation induced by the agonist may suppress ovarian response to gonadotrophin stimulation, particularly in patients with compromised ovarian reserve (Decleer et al., 2016). The duration of benefit derived from the administration of prolonged GnRHa has not been established, which may be a critical issue for patients requiring cryopreservation of all embryos, as in the case of planned comprehensive chromosomal screening (CCS) or who are at high risk of ovarian hyperstimulation syndrome. In addition, recent reports have suggested that transfer of blastocyst stage embryos after vitrification and warming may result in improved neonatal outcomes without affecting live birth rates in comparison to fresh transfers (Roy et al., 2014).

In an effort to avoid these issues, the current investigation is a pilot study that evaluates the impact of deferring administration of a prolonged course of GnRHa in these patient populations until after embryos have been vitrified and immediately prior to endometrial preparation for cryopreserved embryo transfer. To our knowledge, this is the first time that this issue has been reported upon.

Materials and methods

This is a retrospective cohort pilot trial performed in a single tertiary care assisted reproductive technology centre. All consecutive patients from 2010 to 2015 at the Colorado Center for Reproductive Medicine who underwent IVF with autologous oocytes, vitrification of all embryos and subsequent cryopreserved embryo transfer after prolonged administration of GnRHa were included. All patients had a prior surgical diagnosis of endometriosis and/or evidence of aberrant endometrial integrin expression. Patients considered to have endometriosis had received a surgical diagnosis within 10 years of initiation of their IVF cycle, although all but two patients had received a surgical diagnosis within 62 months of oocyte aspiration. Only patients with one or more of the following were offered prolonged GnRHa administration: (i) stage III or IV endometriosis (American Society for Reproductive Medicine, 1996); (ii) severe endometriosis-related symptoms; (iii) prior failed embryo transfers.

All patients underwent ovarian reserve testing including day 3 serum FSH, oestradiol and anti-Müllerian hormone (AMH) levels as well as antral follicle count determined at baseline transvaginal ultrasound examination performed in the early follicular phase. All patients were noted to have a normal uterine cavity documented at precycle office hysteroscopy. In an effort to eliminate a potential confounding variable, no patients were included who had received GnRH agonists or progestins for treatment of symptomatic endometriosis within 6 months of initiation of their IVF cycle. Only combination oral contraceptives and pain medications were permitted during this interval for those patients included in this investigation. No patients had endometriomas *in situ* that were >4.5 cm in mean diameter as measured at baseline ultrasound examination.

Endometrial biopsies to evaluate β_3 integrin expression were performed 9–11 days after documentation of an LH surge by urine ovulation induction kits. The tissue was evaluated for integrin expression using a commercial assay (Pathology Consultants, Greenville, SC) employing previously described techniques (Lessey et al., 1992). All biopsies were confirmed to be in phase ± 2 days by standard histological criteria before a decision was made to employ GnRHa if absent integrin expression was reported (Li et al., 1987). Typical indications for performing the biopsy included at least one of the following: clinically suspected endometriosis based on presenting symptoms but without prior laparoscopy and/or unexplained implantation failure as previously described (Surrey et al., 2007).

All patients underwent ovarian stimulation. The determination for the specific protocol to be used was based on age, the results of ovarian reserve testing, and prior response where appropriate. Transvaginal ultrasound-guided oocyte aspiration was performed 35 h after administration of HCG and/or GnRHa as trigger (Engmann et al., 2016; Katz-Jaffe et al., 2013). Embryos of patients who were to undergo CCS were cultured in sequential medium to the expanded blastocyst stage (days 5, 6 and/or 7 after oocyte aspiration) prior to trophectoderm biopsy as previously described (Schoolcraft et al., 2011). Blastocyst stage embryos were graded using a standardized system that has been used consistently in our centre (Gardner and Schoolcraft, 1999). Embryos from patients who were not undergoing CCS were vitrified at either the cleavage stage on day 3 or at the blastocyst stage on either days 5 or 6 after oocyte aspiration. All embryos were vitrified in Cryotops as described by Kuwayama (2007).

All patients subsequently were administered a long-lasting preparation of the GnRHa leuprolide acetate (Lupron Depot®; AbbVie, North Chicago, IL) 3.75 mg every 28 days for 56 days. The endometrium was then prepared for embryo transfer using exogenous oestradiol primarily administered by a transdermal route starting with two oestradiol 100 µg patches (Vivelle-Dot®; Novartis, Basel, Switzerland) changed every other day with doses increased at 4–6 day intervals as needed to a maximum dose of four patches to achieve adequate endometrial development (typically ≥8 mm and ≤15 mm with a triple pattern). If inadequate endometrial development was obtained, the transdermal oestradiol preparation was supplemented with orally and/or vaginally administered oestradiol 2 mg once or twice daily (Estrace®; Teva North America, North Wales, PA) and/or oestradiol valerate administered intramuscularly 25 mg twice weekly (Delestrogen®, Par Pharmaceuticals, Chestnut Ridge, NY) as needed. Once adequate endometrial development was documented, progesterone administration was initiated employing micronized progesterone 100 mg vaginally twice daily (Endometrin®, Fering Pharmaceuticals, Parsippany, NJ) and micronized progesterone in oil 25–50 mg intramuscularly every other day (West-Ward Pharmaceuticals, Eatontown, NJ). Blastocyst stage embryos were warmed and transferred on the sixth day of progesterone exposure. Cleavage stage embryos were warmed and transferred on the third day of progesterone exposure. Only embryos predicted to be euploid were transferred if CCS had been performed. Embryo transfers

Please cite this article in press as: Eric S. Surrey, Mandy Katz-Jaffe, Laxmi V. Kondapalli, Robert L. Gustofson, William B. Schoolcraft, GnRH agonist administration prior to embryo transfer in freeze-all cycles of patients with endometriosis or aberrant endometrial integrin expression, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.05.004

were performed under ultrasound guidance as previously described (Schoolcraft et al., 2001).

Serum pregnancy tests were performed 11 days after cleavage stage embryo transfer and 9 days after blastocyst stage embryo transfer. The vaginal progesterone dose was increased to 100 mg three times daily with a positive pregnancy test. Oestradiol and progesterone supplementation was slowly weaned in patients with evidence of ongoing pregnancy. In the absence of any pregnancy complications at 8.5 weeks of gestation, oestradiol preparations were decreased by one patch or tablet every 3 days along with initially discontinuing intramuscular progesterone followed by decreasing vaginal progesterone preparations by one tablet every 3 days with the goal of completing weaning by 10 weeks' gestation.

Three groups were identified for analysis. Group 1 consisted of patients with a prior surgical diagnosis of endometriosis whose blastocyst stage embryos had undergone CCS (20 patients, 20 cryopreserved embryo transfers). Group 2 consisted of patients without a surgical diagnosis of endometriosis but with aberrant endometrial integrin expression whose blastocyst stage embryos had also undergone CCS (12 patients, 13 cryopreserved embryo transfers). Group 3 consisted of patients who had either a history of endometriosis or aberrant integrin expression whose embryos were vitrified without CCS (10 patients, 12 cryopreserved embryo transfers). Patients with these two indications for prolonged GnRHa therapy were combined in Group 3 due to the small sample sizes. A fourth group (Group 4) was included for descriptive purposes only and consisted of outcomes from all patients at the Colorado Center for Reproductive Medicine who had undergone vitrified-warmed blastocyst stage embryo transfer cycles after CCS (2809 transfers). All embryos were transferred at the blastocyst stage with the exception of one cleavage stage embryo transfer performed in Group 3.

Ongoing pregnancy rate was defined as the presence of an intrauterine gestational sac with foetal cardiac activity documented by ultrasound evaluations performed both 4–5 and 6–8 weeks after a positive pregnancy test per embryo transfer procedure. Implantation rate was defined as the number of intrauterine gestational sacs with foetal cardiac activity as documented by ultrasound examination per number of embryos transferred.

Data were analysed using Student's group t-tests and chi-squared analysis where appropriate. *P*-values <0.05 were considered to be statistically significant. Results are expressed as mean ± standard deviation with 95% confidence intervals (CI) reported unless otherwise indicated.

Given the retrospective nature of the study design and de-identified data, institutional review board approval and informed consent were not sought for this study.

Results

Baseline characteristics of the three study groups are displayed in **Table 1**. There were no significant differences among the groups with regard to age, prior numbers of failed cycles, ovarian reserve testing or numbers of embryos transferred. It is interesting to note the relatively high incidence of prior failed embryo transfers in each of the groups (Group 1: 1.7 ± 1.76, 95% CI, 0.93–7.47; Group 2: 2.62 ± 1.64, 95% CI, 1.73–3.51; Group 3: 1.17 ± 1.34, 95% CI, 0.41–1.93). The distribution of severity of endometriosis was similar between patients in Group 1 and Group 3. Sixty per cent of Group 1 patients and 77% of Group 3 patients with endometriosis were diagnosed with stage III or IV disease. The mean interval from most recent surgical diagnosis of endometriosis to oocyte aspiration procedure in Group 1 was 30.35 ± 28.46 months (95% CI, 17.05–43.65; range 2–91 months) and in those Group 3 patients with endometriosis was 13.12 ± 7.34 months (95% CI, 7.0–19.24; range 2–26 months) ($P < 0.01$). As previously described, only two patients in Group 1 had more than a 62-month interval from initial surgical diagnosis to oocyte aspiration. Both of these patients not only were severely symptomatic in the 6 months prior to cycle initiation but also had ultrasound evidence of recurrent ovarian endometriomas suggestive of persistent or recurrent disease.

Cycle outcome data are presented in **Figure 1**. There were no significant differences in implantation or ongoing pregnancy rates among the groups. It is interesting to note that a trend towards higher implantation rates, which did not achieve statistical significance, was noted in Group 1 patients with a diagnosis of documented endometriosis who underwent transfer of euploid embryos after CCS. As an additional comparator, implantation and ongoing pregnancy rates of all 2809 cryopreserved blastocyst transfers of euploid embryos after trophectoderm biopsy and CCS at our centre are also displayed (Group 4). Implantation rates for all three study groups are slightly higher than those noted in Group 4, although the ongoing pregnancy rates were only higher in Group 1 patients. The percentage of 'top quality' embryos rated 3AB or better that were transferred was not significantly different among the three study groups (Group 1: 71%; Group 2: 82%; Group 3: 59%). In Group 4, the mean maternal age of patients was 36.5 years and a mean of 1.6 euploid blastocysts were transferred per embryo transfer procedure.

Discussion

In this retrospective cohort study, we have demonstrated that prolonged administration of GnRHa after vitrification of all embryos in

Table 1 – Baseline characteristics.

Group	Transfer cycles	Age (years)	Prior failed embryo transfers (range)	AMH (ng/ml)	Day 3 FSH (mIU/ml)	No. of embryos transferred
1	20	35 ± 3.7	1.70 ± 1.76 (0–6)	3.01 ± 2.59	8.17 ± 2.99	1.40 ± 0.49
		95% CI, 33.37–36.13	95% CI, 0.93–2.47	95% CI, 1.87–4.15	95% CI, 6.86–9.48	95% CI: 1.19–1.6
2	13	34.23 ± 5.24	2.62 ± 1.64 (0–5)	3.15 ± 1.92	6.12 ± 1.88	1.69 ± 0.46
		95% CI, 31.30–37.08	95% CI, 1.73–3.51	95% CI, 2.11–4.19	95% CI, 5.1–7.14	95% CI, 1.44–1.94
3	12	32 ± 4.07	1.17 ± 1.34 (0–4)	2.83 ± 2.9	6.58 ± 1.86	1.36 ± 0.48
		95% CI, 29.7–34.3	95% CI, 0.41–1.93	95% CI, 1.19–4.49	95% CI, 5.53–7.63	95% CI, 1.09–1.63

Results presented as mean ± standard deviation.
There were no statistically significant differences between the groups.
AMH, anti-Müllerian hormone; CI, confidence interval.

Please cite this article in press as: Eric S. Surrey, Mandy Katz-Jaffe, Laxmi V. Kondapalli, Robert L. Gustofson, William B. Schoolcraft, GnRH agonist administration prior to embryo transfer in freeze-all cycles of patients with endometriosis or aberrant endometrial integrin expression, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.05.004

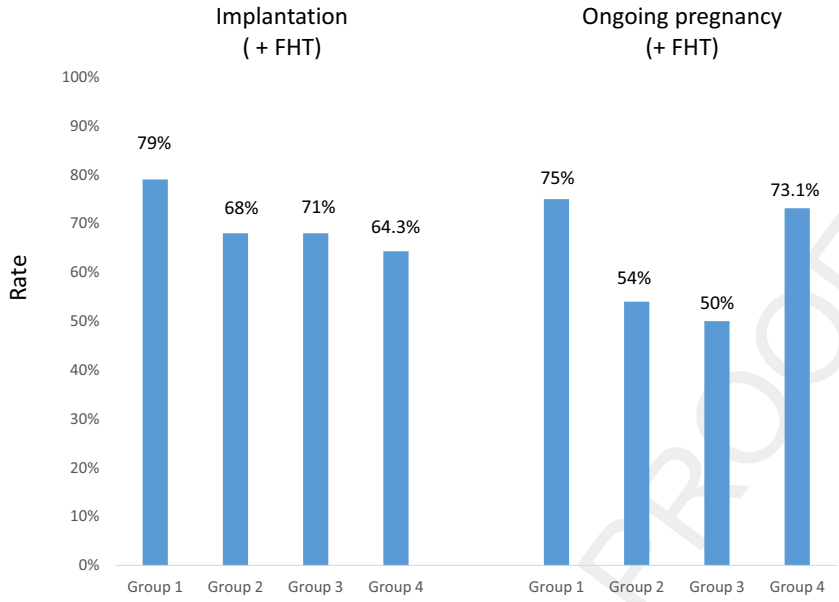


Figure 1 – Implantation and ongoing pregnancy rates after cryopreserved embryo transfer in the three study groups. Group 4, presented for descriptive comparison only, represents outcomes from all euploid blastocyst stage cryopreserved embryo transfers performed at the Colorado Center for Reproductive Medicine (*n* = 2809 transfers). There were no statistically significantly differences between the study groups (Groups 1–3). FHT = foetal cardiac activity noted on ultrasound examination.

IVF patients with endometriosis and/or aberrant endometrial integrin expression led to high implantation and ongoing pregnancy rates despite a high incidence of prior cycle failure. This approach avoids the potential for excessive ovarian suppression when prolonged GnRHa is administered prior to ovarian stimulation for IVF with fresh embryo transfer. To our knowledge, this is the first time that this novel approach has been reported.

The benefits of prolonged GnRHa administration prior to initiation of ovarian stimulation in patients with surgically confirmed endometriosis has been previously reported in a prospective randomized multicentre trial. Administration of a long-acting GnRHa for 3 months prior to cycle initiation in this patient population resulted in significantly higher ongoing pregnancy rates (80% versus 53.85%) with a non-significant trend towards higher implantation rates (42.7% versus 30.4%) in comparison to controls with endometriosis who underwent standard ovarian stimulation without prolonged GnRHa therapy (Surrey et al., 2002). Sallam et al. (2006) performed a meta-analysis of the outcomes of three previously published prospective randomized trials, which included 163 patients undergoing 3–6 months of pre-IVF cycle GnRHa therapy. Significantly enhanced live birth rates (odds ratio [OR] 9.1; 95% CI, 1.08–78.22) and clinical pregnancy rates (OR 4.28; 95% CI, 2.0–9.15) were noted. We have more recently demonstrated that a 2-month course of GnRHa therapy is also effective (Surrey et al., 2007).

The problematic aspect of this approach is the possibility that prolonged GnRHa therapy may suppress response to subsequent ovarian stimulation. Recently, Decleer et al. (2016) reported the results of a prospective randomized trial of 3 months of prolonged GnRHa in patients with peritoneal endometriosis planning IVF. They reported no differences between the two groups with regard to the primary endpoint: number of mature oocytes obtained per cycle or in pregnancy rates. However, patients in the prolonged agonist group required significantly higher doses of gonadotrophins and greater number of days of stimulation to achieve adequate follicular development. The use of a standard long protocol for the majority of patients in this study may have further suppressed response. No differences in pregnancy rates were noted although implantation rates were not reported. In light of the fact that endometriosis may have an impact on ovarian reserve, particularly after endometrioma resection (Seyhan et al., 2015), the idea of deferring prolonged agonist use until after embryos have been cryopreserved is highly appealing.

The mechanism of action of GnRHa on improving IVF outcomes in endometriosis patients has not been well established. Aside from suppressing the extent of disease, these agents may have a direct effect on the expression of inflammatory proteins and metalloproteinase tissue inhibitors within the peritoneal cavity (Bilotas et al., 2007; Ferrero et al., 2009; Sharpe-Timms et al., 1998). A direct endometrial effect on reducing nitric oxide synthase expression has also been described (Wang et al., 2006).

In addition, the impact of GnRHa on endometrial $\alpha v\beta_3$ vitronectin (integrin) expression has been evaluated. This protein may play a role in the initiation of trophoblast invasion and act as a site of interaction between the embryo and the endometrium (Creus et al., 2003; Lindhard et al., 2002). Lessey et al. (1994, 1995) had previously reported absent endometrial expression of the protein in women with both unexplained infertility and endometriosis. Others have demonstrated a high incidence of aberrant integrin expression in patients with prior implantation failure after IVF (Surrey et al., 2007; Thomas et al., 2003). These findings have not been confirmed by others (Hii and Rogers, 1998; Ordi et al., 2003).

Lessey (2000) demonstrated normalization of aberrant β_3 integrin expression in 64% of stage I/II endometriosis patients administered a long-acting GnRHa for 3 months. Surrey et al. (2007) reported that IVF outcomes after 2 months of agonist therapy in these patients were

Please cite this article in press as: Eric S. Surrey, Mandy Katz-Jaffe, Laxmi V. Kondapalli, Robert L. Gustofson, William B. Schoolcraft, GnRH agonist administration prior to embryo transfer in freeze-all cycles of patients with endometriosis or aberrant endometrial integrin expression, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.05.004

similar to untreated controls with positive expression. The beneficial impact of this approach has also been demonstrated in a murine model (Ruan et al., 2006).

There are additional indications for cryopreservation of all embryos aside from concerns regarding ovarian suppression from GnRHa. These include cases of incipient ovarian hyperstimulation syndrome (Greisinger et al., 2007) and premature elevation of progesterone levels (Shapiro et al., 2010). Cryopreservation at the blastocyst as opposed to earlier developmental stages has been demonstrated to result in enhanced outcomes in these circumstances (Anderson et al., 2003; Surrey et al., 2010a).

The successful application of CCS of blastocyst stage embryos after trophectoderm biopsy represents a means of significantly increasing implantation rates and live birth rates and decreasing miscarriage rates after IVF (Chen et al., 2015; Dahdouh et al., 2015; Scott et al., 2013). This approach is typically employed in conjunction with cryopreservation of all blastocyst stage embryos after biopsy (Schoolcraft et al., 2011).

The introduction of vitrification as the preferred means of cryopreservation has resulted in a significant increase in survival and pregnancy rates (Kuwayama, 2007; Zhu et al., 2011). Recent reports have suggested that clinical pregnancy rates are higher and perinatal outcomes are improved after transfer of vitrified-warmed blastocysts in comparison to fresh embryo transfer, suggesting an inherent benefit of this approach (Belva et al., 2016; Ishihara et al., 2014; Li et al., 2014; Nakashima et al., 2013; Özgür et al., 2015; Roque et al., 2015).

In the current investigation, implantation and ongoing pregnancy rates were not significantly different among the groups. A trend towards improved outcomes, which did not reach statistical significance, was noted in Group 1 patients pretreated with a prolonged course of GnRHa who had a previous diagnosis of endometriosis and underwent euploid embryo transfer after trophectoderm biopsy, CCS and blastocyst vitrification. The outcomes in all groups compared favourably with those in the cohort of all CCS cycles performed at our centre (Group 4). The similar implantation but lower live birth rates in Group 3 patients who did not undergo CCS can be explained by the likelihood of aneuploid embryo transfer leading to an increased rate of pregnancy loss.

The shorter time interval between surgical diagnosis of endometriosis and oocyte aspiration in Group 1 versus Group 3 patients with endometriosis could potentially be considered a confounding variable. As previously mentioned, the two patients in Group 1 who had extremely prolonged intervals beyond 62 months (91 and 97 months) experienced recurrent severe symptoms and also had ultrasound evidence of recurrent endometriomas. However, the clinical significance of these differences is not clear. Bedaiwy et al. (2008) demonstrated a lack of relationship between length of time from surgical diagnosis and treatment of endometriosis and subsequent IVF cycle outcomes. This study included patients who had a most recent surgery for endometriosis up to 10 years prior to the index IVF cycle. We had also demonstrated in a retrospective analysis of endometriosis patients undergoing IVF that there was no correlation between implantation rates and either surgery–oocyte interval or endometriosis score (Surrey and Schoolcraft, 2003). Nevertheless, patients in Group 3 with endometriosis who did not pursue CCS were more likely to have experienced severe symptoms and fewer failed cycles, which may have resulted in the shortened interval.

The aetiology of the more compromised responses in the Group 2 patients who were administered prolonged GnRHa due to aberrant endometrial integrin expression and underwent euploid embryo transfer after CCS is less clear. We had previously reported in a prospective randomized trial that in patients with diagnosed endometriosis, endometrial integrin expression did not predict which patients would benefit from extended GnRHa therapy prior to IVF in the absence of CCS (Surrey et al., 2010b). One potential problem with that study design was the fact that untreated control patients underwent ovarian stimulation in the cycle immediately after endometrial biopsy was performed. This may have introduced a confounding variable given data suggesting that endometrial injury *per se* may enhance implantation rates, particularly in patients with recurrent implantation failure (Potdar et al., 2012). In addition, other factors besides integrin expression that may not be responsive to GnRHa have been shown to have an impact on implantation in endometriosis patients, and may play a role (Burney et al., 2007; Kao et al., 2003; Matsuzaki et al., 2005; Wu et al., 2005). Other means of evaluating endometrial receptivity may allow for a more specific triage of implantation failure patients to alternate therapeutic interventions including changes in the duration of pre-transfer progesterone exposure (Garrido-Gómez et al., 2014; RoyChoudhury et al., 2016; Ruiz-Alonso et al., 2013).

There are several limitations to this investigation. The sample sizes of the three study groups are relatively small. However, this did represent the overall experience of a single large IVF centre, allowing for uniformity of treatment paradigms. Due to the small number of patients in Group 3 who were treated with prolonged GnRHa without CCS, those patients whose indications for agonist therapy included endometriosis or aberrant integrin expression were combined. This creates a confounding variable.

This is a retrospective cohort study and therefore, the ability to define an ideal control group is problematic. A more ideal study design would be to prospectively randomize appropriate patients to either prolonged GnRHa therapy prior to ovarian stimulation, IVF and fresh embryo transfer or to ovarian stimulation, IVF, vitrification of all embryos and subsequent prolonged GnRHa therapy prior to endometrial preparation and cryopreserved embryo transfer. This approach would, by necessity, eliminate any patients undergoing CCS in our centre because all embryos are vitrified after trophectoderm biopsy, as well as any patients who require vitrification of all embryos for other indications. It would not have been possible to evaluate retrospectively identified contemporaneously treated controls who received prolonged GnRHa and subsequent fresh transfer because this approach has not been offered to patients in our centre since 2009, after the introduction of successful vitrification techniques. This study also did not include as a control group endometriosis patients undergoing IVF whose embryos were vitrified but who were not offered prolonged GnRHa due to the fact that only those endometriosis patients with prior implantation failure, severe disease and/or incapacitating symptoms were offered prolonged GnRHa. Inclusion of other endometriosis patients as a control group presenting solely with less advanced disease, who were asymptomatic or who had not experienced prior failed transfers would have introduced a selection bias. In addition, the objective of this investigation was not to evaluate the efficacy of prolonged GnRHa in endometriosis patients *per se*, because we feel that this has already been demonstrated (Sallam et al., 2006; Surrey et al., 2002) but rather, to demonstrate efficacy of GnRHa in a subset of these patients after embryo vitrification. Therefore, overall data from CCS outcomes at our centre (Group 4) were provided solely for descriptive comparison without inclusion in statistical analysis.

Please cite this article in press as: Eric S. Surrey, Mandy Katz-Jaffe, Laxmi V. Kondapalli, Robert L. Gustofson, William B. Schoolcraft, GnRH agonist administration prior to embryo transfer in freeze-all cycles of patients with endometriosis or aberrant endometrial integrin expression, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.05.004

The strengths of this investigation include the fact that we believe this is the first publication on the subject of use of prolonged GnRHa in appropriately selected patients after vitrification of all embryos. The patient populations included a high incidence of those who had previously experienced failed embryo transfers. All patients were treated in a single centre, which allows for standardization of laboratory, biopsy and embryo transfer techniques. The transfer of only embryos predicted to be euploid in Groups 1 and 2 eliminates a further variable that could skew outcomes.

In conclusion, this study demonstrates that in a population of patients who are candidates for IVF and prolonged GnRHa therapy, administration of this agent after vitrification of all embryos results in high implantation and ongoing pregnancy rates despite a high incidence of prior cycle cancellation. A trend towards improved outcomes, which did not reach statistical significance, was noted in patients treated with prolonged GnRHa due to endometriosis who also underwent euploid embryo transfer after CCS. This 'freeze-all' paradigm avoids excessive ovarian suppression induced by prolonged GnRHa, particularly in patients with compromised ovarian reserve and allows for performance of CCS when indicated. The encouraging results from this pilot trial should lead to the development of an appropriately designed prospective randomized trial to more definitively address this issue.

ARTICLE INFO

Article history:
Received 19 December 2016
Received in revised form 1 May 2017
Accepted 3 May 2017
Declaration: The authors report no financial or commercial conflicts of interest.

Keywords:
Embryo transfer
Endometriosis
GnRH agonist
Implantation
Integrin
Vitrification

REFERENCES

American Society for Reproductive Medicine, 1996. Revised American society for reproductive medicine classification of endometriosis: 1996. Fertil. Steril. 67, 817–821.

Anderson, A.R., Weiker, W.L., Crain, J.L., 2003. Determining the most optimal stage for embryo cryopreservation. Reprod. Biomed. Online 8, 207–211.

Bedaiwy, M.A., Falcone, T., Katz, E., Goldberg, J.M., Assad, R., Thornton, J., 2008. Association between time from endometriosis surgery and outcome of in vitro fertilization. J. Reprod. Med. 53, 161–165.

Belva, F., Bonduello, M., Roelants, M., Verheyen, G., Van Landuyt, L., 2016. Neonatal health including congenital malformation risk of 1072 children born after vitrified embryo transfer. Hum. Reprod. 7, 1610–1620.

Bilotas, M., Barañao, R.I., Buquet, R., Sueldo, C., Tesone, M., Meresman, G., 2007. Effect of GnRH analogues on apoptosis and expression of Bcl-2, Bax, Fas, and FasL proteins in endometrial epithelial cell cultures from patients with endometriosis and controls. Hum. Reprod. 22, 644–653.

Burney, R., Talbi, S., Hamilton, A., Vo, K., Nyegaard, M., Nezhat, C., Lessey, B.A., Giudice, L.C., 2007. Gene expression analysis of endometrium reveals progesterone resistance and candidate genetic loci in women with endometriosis. Endocrinology 148, 3814–3826.

Chen, M., Wei, S., Hu, J., Quan, S., 2015. Can comprehensive chromosomal screening technology improve IVF/ICSI outcomes? A meta-analysis. PLoS ONE 10, e0140779.

Creus, M., Ordi, J., Fabergios, F., Casairijana, R., Carmona, F., Cardesa, A., Vanrell, J.A., Balasch, J., 2003. The effect of different hormone therapies on integrin expression and pinopode formation in the human endometrium: a controlled study. Hum. Reprod. 18, 683–693.

Dahdouh, E., Balayla, J., Garcia-Velasco, J., 2015. Impact of blastocyst biopsy and comprehensive chromosome screening technology on preimplantation genetic screening: a systematic review of randomized controlled trials. Reprod. Biomed. Online 30, 281–289.

Decleer, W., Osmanagaoglu, K., Verschueren, L., Comhaire, F., Devroey, P., 2016. RCT to evaluate the influence of adjuvant medical treatment of peritoneal endometriosis on the outcome of IVF. Hum. Reprod. 31, 2017–2023.

Engmann, L., Benadiva, C., Humaidan, P., 2016. GnRH agonist trigger for the induction of oocyte maturation in GnRH antagonist IVF cycles: a SWOT analysis. Reprod. Biomed. Online 32, 274–285.

Ferrero, S., Gillott, D.J., Remorgida, V., Anserini, P., Ragni, N., Grudzinkas, J.G., 2009. GnRH analogue remarkably down-regulates inflammatory proteins in peritoneal fluid proteome of women with endometriosis. J. Reprod. Med. 54, 223–231.

Gardner, D., Schoolcraft, W., 1999. Culture and transfer of human blastocysts. Curr. Opin. Obstet. Gynecol. 11, 307–311.

Garrido-Gómez, T., Quiñonero, A., Antunez, O., Diaz-Gimeno, P., Bellver, J., Simón, C., Francisco, D., 2014. Deciphering the proteomic signature of human endometrial receptivity. Hum. Reprod. 29, 1957–1967.

Greisinger, G., von Otto, S., Schroer, A., Ludwig, A., Diedrich, K., Al-Hasani, S., 2007. Elective cryopreservation of all pronuclear stage oocytes after GnRH agonist triggering of final oocyte maturation in patients at risk of developing OHSS: a prospective observational proof of concept study. Hum. Reprod. 22, 1348–1352.

Hamdan, M., Omar, S.Z., Dunselman, G., Chiong, Y., 2015. Influence of endometriosis on assisted reproductive technology outcomes: a systematic review and meta-analysis. Obstet. Gynecol. 125, 79–88.

Harb, H.M., Gallos, I.D., Chu, J., Harb, M., Coomarasamy, A., 2013. The effect of endometriosis on in vitro fertilization outcome: a systematic review and meta-analysis. B.J.O.G. 120, 1308–1320.

Hii, L., Rogers, P., 1998. Endometrial vascular and glandular expression of integrin $\alpha v\beta_3$, in women with and without endometriosis. Hum. Reprod. 13, 1030–1035.

Ishihara, O., Araki, R., Kuwahara, A., Itakura, A., Saito, H., Adamson, G.D., 2014. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. Fertil. Steril. 101, 128–133.

Kao, L., Germeyer, A., Tulac, S., Lobo, S., Yang, J., Taylor, R., Osteen, K., Lessey, B., Giudice, L., 2003. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease based implantation failure and infertility. Endocrinology 144, 2870–2878.

Katz-Jaffe, M., Surrey, E., Minjarez, D., Gustofson, R., Stevens, J., Schoolcraft, W., 2013. Association of abnormal ovarian reserve parameters with a higher incidence of aneuploid blastocysts. Obstet. Gynecol. 121, 71–77.

Kuwayama, M., 2007. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology 67, 73–80.

Lessey, B., 2000. Medical management of endometriosis and infertility. Fertil. Steril. 73, 1089–1096.

Please cite this article in press as: Eric S. Surrey, Mandy Katz-Jaffe, Laxmi V. Kondapalli, Robert L. Gustofson, William B. Schoolcraft, GnRH agonist administration prior to embryo transfer in freeze-all cycles of patients with endometriosis or aberrant endometrial integrin expression, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.05.004

Lessey, B., Damajanovich, L., Coutifaris, C., Castelbaum, A., Albelda, S., Buck, C., 1992. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. J. Clin. Invest. 90, 188–195.

Lessey, B., Castelbaum, A., Sawin, S., Buck, C., Schinner, R., Bilker, W., Strom, B., 1994. Aberrant integrin expression in the endometrium of women with endometriosis. J. Clin. Endocrinol. Metab. 79, 643–649.

Lessey, B., Castelbaum, A., Sawin, S., Sun, J., 1995. Integrins as markers of uterine receptivity in women with primary unexplained infertility. Fertil. Steril. 63, 535–547.

Li, T.-C., Rogers, A., Lenton, F., Dockery, P., Cooke, I., 1987. A comparison between two methods of chronological dating of human endometrial biopsies during the luteal phase and their correlation with histologic dating. Fertil. Steril. 48, 928–932.

Li, Z., Wang, Y.A., Ledger, W., Edgar, D.H., Sullivan, E.A., 2014. Clinical outcomes following cryopreservation of blastocysts by vitrification or slow freezing: a population based cohort study. Hum. Reprod. 29, 2794–2801.

Lindhard, A., Beutin-Ley, U., Raun, V., Islin, H., Hoiid, T., Rex, S., Bangsbøll, S., Sørensen, S., 2002. Biochemical evaluation of endometrial function at the time of implantation. Fertil. Steril. 78, 221–233.

Matsuzaki, S., Cams, M., Vaurs-Barriere, C., Beespflug-Tanguay, O., Dastugne, B., Mage, G., 2005. DNA microarray analysis of gene expression in ectopic endometrium from patients with deep endometriosis using laser capture microdissection. Fertil. Steril. 84, 1180–1190.

Nakashima, A., Araki, R., Tani, H., Ishihara, O., Kuwahara, A., Irahara, M., Yoshimura, Y., Kuramoto, T., Saito, H., Nakaza, A., Sakumoto, T., 2013. Implications of assisted reproductive technologies on term singleton birth weight: an analysis of 25,777 children in the national assisted reproduction registry of Japan. Fertil. Steril. 99, 450–455.

Ordi, J., Creus, M., Casamitjana, R., Cardesa, A., Vanrell, J., Balash, J., 2003. Endometrial pinopode and $\alpha v\beta_3$ integrin express is not impaired in women with endometriosis. J. Assist. Reprod. Genet. 20, 465–473.

Özgür, K., Berkkanoğlu, M., Bulut, H., Isikli, A., Coetzee, K., 2015. Higher clinical pregnancy rates from frozen-thawed blastocyst transfers compared to fresh blastocyst transfer: a retrospective matched-cohort study. J. Assist. Reprod. Genet. 32, 1483–1490.

Potdar, N., Gelbaya, T., Nardo, L.G., 2012. Endometrial injury to overcome recurrent embryo implantation failure: a systematic review and meta-analysis. Reprod. Biomed. Online 25, 561–571.

Roque, M., Valle, M., Guimarães, F., Sampaio, M., Geber, S., 2015. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. Fertil. Steril. 103, 1190–1193.

Roy, T., Bradley, G.K., Bowman, M.C., McArthur, S.J., 2014. Single-embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers. Fertil. Steril. 101, 1294–1301.

RoyChoudhury, S., Singh, A., Gupta, A., Gupta, N.J., Srivastava, S., Joshi, M.D., Chakravarty, B., Chaudhury, K., 2016. Repeated implantation failure versus repeated implantation success: discrimination at a metabolomic level. Hum. Reprod. 31, 1265–1274.

Ruan, H.-C., Zhu, X.-M., Luo, Q., Liu, A.-X., Qian, Y.-L., Zhou, C.-Y., Jin, F., Huang, H.-F., Sheng, J.-Z., 2006. Ovarian stimulation with GnRH agonist, but not GnRH antagonist, partially restores the expression of endometrial integrin $beta_3$ and leukaemia-inhibitory factor and improves uterine receptivity in mice. Hum. Reprod. 21, 2521–2529.

Ruiz-Alonso, M., Blesa, D., Diaz-Gimeno, P., Gómez, E., Fernandéz-Sánchez, M., Carranza, F., Carrera, J., Vilella, F., Pellicer, A., Simón, C., 2013. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil. Steril. 106, 818–824.

Sallam, H., Garcia-Velasco, J., Dias, S., Arici, A., 2006. Long term pituitary down-regulation before in vitro fertilization (IVF) for women with endometriosis. Cochrane Database Syst. Rev. (1), CD 004635.

Schoolcraft, W.B., Surrey, E.S., Gardner, D.K., 2001. Embryo transfer techniques and variables affecting success. Fertil. Steril. 76, 863–870.

Schoolcraft, W.B., Treff, N.R., Stevens, J.M., Ferry, K., Katz-Jaffe, M., Scott, R.T., Jr., 2011. Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray base comprehensive chromosomal screening in infertility patients. Fertil. Steril. 96, 638–640.

Scott, R.T., Jr., Upham, K.M., Forman, E.J., Hong, K.H., Scott, K.L., Taylor, D.T., Tao, X., Treff, N., 2013. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil. Steril. 100, 697–703.

Seyhan, A., Ata, B., Uncu, G., 2015. The impact of endometriosis and its treatment on ovarian reserve. Semin. Reprod. Med. 33, 422–428.

Shapiro, B.S., Daneshmand, S.T., Garner, F.L., Aguirre, M., Hudson, C., Thomas, S., 2010. Embryo cryopreservation rescues cycles with premature luteinization. Fertil. Steril. 89, 20–26.

Sharpe-Timms, K.L., Keisler, L.W., McIntush, E.W., Kusler, D.H., 1998. Tissue inhibitor of metalloproteinase-1 concentrations are attenuated in peritoneal fluid and sera of women with endometriosis and restored in sera by gonadotropin-releasing hormone agonist therapy. Fertil. Steril. 69, 1128–1134.

Surrey, E., Keller, J., Stevens, J., Gustofson, R., Minjarez, D., Schoolcraft, W., 2010a. Freeze-all: enhanced outcomes with cryopreservation at the blastocyst stage versus pronuclear stage using slow-freeze techniques. Reprod. Biomed. Online 21, 411–417.

Surrey, E.S., 2013. Endometriosis and assisted reproductive technologies: maximizing outcomes. Semin. Reprod. Med. 31, 154–163.

Surrey, E.S., Schoolcraft, W.B., 2003. Does surgical management of endometriosis within 6 months of an in vitro fertilization-embryo transfer cycle improve outcome? J. Assist. Reprod. Genet. 20, 365–370.

Surrey, E.S., Silverberg, K.M., Surrey, M.W., Schoolcraft, W.B., 2002. Effect of prolonged gonadotropin-releasing hormone agonist therapy on the outcome of in vitro fertilization-embryo transfer in patients with endometriosis. Fertil. Steril. 78, 699–704.

Surrey, E.S., Minjarez, D.A., Schoolcraft, W.B., 2007. The incidence of aberrant endometrial $\alpha v\beta_3$ vitronectin expression in a high-risk fertility population: could prolonged GnRH agonist therapy play a role? J. Assist. Reprod. Genet. 24, 553–556.

Surrey, E.S., Lietz, A.K., Gustofson, R.L., Minjarez, D.A., Schoolcraft, W.B., 2010b. Does endometrial integrin expression in endometriosis patients predict enhanced in vitro fertilization cycle outcomes after prolonged GnRH agonist therapy? Fertil. Steril. 93, 646–651.

Thomas, K., Thomson, A., Wood, S., Kingsland, C., Vince, G., Lewis-Jones, K., 2003. Endometrial integrin expression in women undergoing in vitro fertilization and the association with subsequent treatment outcome. Fertil. Steril. 80, 502–507.

Wang, J.H., Zhou, F.Z., Dong, M.Y., Wu, R.J., Qian, Y.L., 2006. Prolonged gonadotropin-releasing hormone agonist therapy reduced expression of nitric oxide synthase in the endometrium of women with endometriosis and infertility. Fertil. Steril. 85, 1037–1044.

Wu, Y., Halverson, G., Basir, Z., Strawn, E., Yan, P., Guo, S., 2005. Aberrant methylation at HOXA 10 may be responsible for its aberrant expression in the endometrium of patients with endometriosis. Am. J. Obstet. Gynecol. 193, 371–380.

Zhu, D., Zhang, J., Cao, S., Zhang, J., Heng, B.C., Huang, M., Ling, X., Duan, T., Tong, G., 2011. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles – time for a new embryo transfer strategy? Fertil. Steril. 95, 1691–1695.

Please cite this article in press as: Eric S. Surrey, Mandy Katz-Jaffe, Laxmi V. Kondapalli, Robert L. Gustofson, William B. Schoolcraft, GnRH agonist administration prior to embryo transfer in freeze-all cycles of patients with endometriosis or aberrant endometrial integrin expression, Reproductive BioMedicine Online (2017), doi: 10.1016/j.rbmo.2017.05.004