Microdose follicular flare: a viable alternative for normal responding patients undergoing in vitro fertilization?

Eric D. Levens[a,b,c], **Brian W. Whitcomb**[d], **Jonathan D. Kort**[c], **Donna Materia-Hoover**[e], and **Frederick W. Larsen**[a,b]

a *Walter Reed Army Medical Center ART Program, Washington, DC*

b *Combined Federal Fellowship in Reproductive Endocrinology and Infertility at the National Institutes of Health, Walter Reed Army Medical Center, National Naval Medical Center, and Uniformed Services University of the Health Sciences*

c *Reproductive Biology and Medicine Branch, National Institute of Child Health and Human Development, NIH, Bethesda, MD*

d *Epidemiology Branch, National Institute of Child Health and Human Development, NIH*

e *A.R.T. Institute of Washington, Inc., Washington, DC*

Abstract

Objective—To compare cycle outcomes among normal responding patients ≤30 years receiving microdose follicular flare (MDF) and long-luteal agonist (LL).

Design—Retrospective cohort study.

Setting—Military-based ART center.

Patients—First, autologous ART cycles among 499 women ≤30 years old from 01/1999 to 12/2005.

Interventions—Following OCP administration prior to cycle start, patients were non-randomly assigned to either LL or MDF for LH surge suppression. LL received 1 mg/d leuprolide acetate (LA) on cycle day 21, which was reduced to 0.25 mg/day 10–14 days later. MDF received LA (40 µg BID) beginning 3 days after discontinuing OCPs. Both groups received a combination of hMG and rFSH.

Main Outcome Measures—Primary outcomes were implantation, clinical pregnancy and live birth rates; in cycle variables included peak E_2, oocytes retrieved, oocyte maturity, and fertilization rate.

Results—Multivariable models controlling for confounding by treatment indication found no significant differences between groups in implantation (MDF:36%; LL:38%), clinical pregnancy

(MDF:53%; LL:56%), and live birth rates (MDF:47%; LL:50%). No differences were observed in peak E$_2$, oocytes retrieved, oocyte maturity, fertilization rate, or embryos transferred.

Conclusions—MDF use among normal responding ART patients produced no differences in cycle outcome when compared to LL. Resultantly, MDF may be a viable alternative for normal responding patients.

Keywords

Introduction

Gonadotropin releasing hormone agonists (GnRH-a) produce an initial hypersecretion of pituitary derived gonadotropins followed by sustained pituitary desensitization and cessation of gonadotroph function by yet incompletely understood mechanisms. These properties have been exploited by altering the dosage and the timing of administration during stimulation to produce differing results among various target populations. The utility of GnRH-a in assisted reproductive technologies (ART) has been to prevent premature luteinization, to reduce cycle cancellation, and to improve oocyte recovery rates (1–4). Numerous GnRH-a pituitary desensitization regimens have been developed including long luteal (LL), ultra short, short/ flare-up and microdose flare (MDF) protocols (5). Oral contraceptive pills (OCPs) have been incorporated into stimulation protocols to provide for ease of cycle scheduling (6). The use of combined OCPs and GnRH-a yielded improved pregnancy outcomes when compared to GnRH-a use alone and there was no evidence of premature luteinization (7).

MDF stimulation protocols have traditionally been employed in an attempt to increase ovarian response during ART by taking advantage of the initial surge in gonadotropin secretion to increase peak estradiol (E$_2$), to reduce gonadotropin requirement, to initiate multi-follicular development, and to produce more mature ovarian follicles when compared to LL among patients with a prior poor ovarian response (8). Despite using approximately one fiftieth of the typical leuprolide acetate (LA) dose, no increased risk of a premature luteinization and no evidence of ovulatory events occurred. Subsequent studies have found improved outcomes among those receiving MDF, but evaluations have been limited primarily to those with a suspected poor response to gonadotropin stimulation (9–12).

Data comparing MDF and LL among normal responders remains limited as the majority of centers use LL protocols for pituitary down-regulation in this population. Our group previously reported that among women <40 years, MDF use resulted in similar clinical pregnancy rate per retrieval. However its use was associated with a significantly higher cycle cancellation rate when compared to LL (22.5% vs. 8.2%; p=0.032) (13). This study was confounded by an assignment bias favoring MDF use among women suspected of being at increased risk of cycle cancellation. Additionally, the age distribution preferentially included older women in the MDF group. Notably, as with previous experience with MDF, the study cycle cancellations were not found to be the result of premature luteinizing events suggesting that cancellation, in part, occurred due to ovarian reserve and age-related effects.

The suggestion of comparable clinical pregnancy outcome and cost-containment considerations (MDF uses a fraction of the total LA required for LL cycles) have led us to use MDF in a broader population. Consideration of this study population provides a unique opportunity to add to the limited outcome data regarding MDF use in the general ART population. Therefore this investigation was aimed at comparing cycle outcomes among good prognosis ART patients receiving either MDF or LL.

Materials and Methods

Study population

Cycle data for the Walter Reed Army Medical Center ART program from January, 1999 to December, 2005 were evaluated. To reduce the impact of age, oocyte quality, and multiple cycles on outcome, women ≤30 years undergoing their first, autologous ART cycle were selected for analysis. Patients were excluded if age >30 years or if not the first ART cycle. Oocyte donation cycles were not included in this cohort as no donation cycles are performed at our institution.

Simulation protocol

For purposes of scheduling, both groups were pre-treated with OCPs (Lo-ovral; Wyeth Pharmaceuticals, Collegeville, PA) for the cycle preceding ovulation induction. Stimulation protocols and initial gonadotropin starting doses were selected by the treating physician prior to beginning OCPs based on pre-cycle screening. Factors considered in the determination of the treatment protocol and starting dosages included diagnosis, day 3 FSH, antral follicle count, and ovarian volumes.

Gonadotropins administration included a combination of recombinant follicular stimulating hormone (rFSH) (Gonal-F; EMD Serono, Rockland, MA) and human Menopausal Gonadotropins (hMG) (Repronex; Ferring Pharmaceuticals, Suffern, NY) given twice daily. A baseline serum E_2 and ultrasonographic assessment were performed to verify ovarian quiescence for both groups. The LL group was treated with leuprolide acetate (LA; TAP Pharmaceuticals, Deerfield, IL) that started with subcutaneous (SC) LA 1 mg/day on cycle day (CD) 21. OCPs were continued for 5 days after the initiation of LA. After receiving LA for 10–14 days, these patients underwent a screening ultrasound and E_2 assessment. Provided that ovarian function was adequately suppressed, the LA dose was reduced to 0.25 mg/day and gonadotropin administration commenced 3 days later. The day of gonadotropin start was defined as stimulation day 1. The MDF group was treated as described by Leondires *et al.* (13). Briefly, pre-treatment OCPs were discontinued after the baseline ultrasound and E_2. Two days following the discontinuation of OCPs, patients began LA 40 µg SC every 12 hours. Gonadotropin administration began the following day which was defined as stimulation day 1.

As per our clinic's standard, throughout stimulation the response to gonadotropins was monitored using serial E_2 measurements and TV ultrasonography for follicular number and size. Regardless of pituitary down-regulation protocol, once at least 2 follicles reached ≥16 mm, 10,000 IU human chorionic gonadotropin (hCG; Novarel; Ferring Pharmaceuticals, Suffern, NY) was administered. Oocyte retrieval was performed 34–36 hours later and embryo transfer occurred at 72 or 120 hours post-retrieval depending upon the quality of the embryo cohort. The embryo transfer was performed using the Edward-Wallace Catheter (SIMS Portex Ltd, Kent, UK) under direct ultrasound guidance. Luteal phase support was with 50 mg progesterone in oil intramuscularly daily.

Data collection and definitions

Institutional review board approval was obtained prior to data collection. Data was collected by a retrospective review of electronic and hard copy patient records. Primary variables included implantation, spontaneous abortion, biochemical, ongoing pregnancy and live birth rates. Biochemical pregnancies were defined as a serum quantitative βhCG > 10mIU/ml on post retrieval day 14 in which an ultrasonographically visible pregnancy was never visualized and were not considered as implantations or as pregnancies. A TV ultrasound was performed at 6–7 weeks gestation to determine sac number and viability. For purposes of the analysis,

clinical pregnancies were determined by the presence of a gestational sac with a fetal pole having cardiac activity on transvaginal ultrasound and excluded spontaneous abortions. Live birth data was available for this cohort. Implantation rate was the number of gestational sacs per number of embryos transferred. Secondary variables included both pre-cycle and in-cycle assessments. Pre-cycle data including age, day 3 FSH, total antral follicle count, and SART diagnosis were assessed. In-cycle measurements evaluated included stimulation day 6 E_2, peak E_2, total gonadotropins received, total follicles developed, total oocytes retrieved, total mature oocytes, and cycle cancellation.

Statistical analysis

The sample size was determined by the study interval. Cycle characteristics and outcomes were evaluated using t-test and chi-square as appropriate. Linear mixed models were used for multivariable analysis of continuous outcome data; generalized estimating equations were used for modeling binary outcomes (SAS 9.1; SAS Institute, Cary, NC). These models utilized robust standard errors to account for the potential effects of non-independent, or correlated, data such as having multiple embryos transferred per cycle. As treatment was non-randomly assigned, multivariable models were specified to address potential confounding by indication; important predictors of treatment were included as covariates in multivariable models to address this concern. In-cycle measurements were not considered as potential outcome confounders as these observations followed treatment assignment and, as such, are likely on the causal pathway from treatment to outcomes.

Results

Of the 499 patients who met inclusion criteria, 287 received LL and 212 were treated with MDF. Although the cohort was limited to those ≤30 years, there was an age discrepancy of 1.3 years between groups (p<0.0001) (Table 1). Moreover day 3 FSH concentrations also differed between LL and MDF groups (6.0 and 6.9, respectively; p<0.0001). Total antral follicle count was lower among those in the MDF group (MDF: 15.7, LL: 17.7; p=0.04). The incidence of the etiology of infertility differed between groups as the LL group had more ovulatory dysfunction (19.9% vs. 5.2%) while MDF had more unexplained infertility (15.7% vs. 5.6%; p<0.0001), reflecting a bias towards assigning these diagnoses to the respective treatment regimens. However, patients in both groups received similar amounts of gonadotropin at cycle start (LL: 3.8 amps/day, MDF: 4.0 amps/day; p=0.10) with a similar starting dose range (LL: 1.5–6 amps/day, MDF: 2–8 amps/day).

In-cycle data is presented in Table 1. There were no differences between groups with regard to days of stimulation, stimulation day 6 E_2, peak E_2, total oocytes retrieved, total mature oocytes, and moderate to severe ovarian hyperstimulation syndrome (OHSS) risk. A total of 50 (10.0%) cycle cancellations occurred in this cohort and no cancellations were due to premature luteinization. Overall, however, there were no differences in cycle cancellations observed between groups (LL: 9.2%, MDF: 10.3%; p=0.60). Unadjusted results revealed that the MDF group used 6 more ampules of gonadotropins than were required by those in the LL group (MDF: 39.9 ampules, LL: 33.9 ampules; p<0.0001). Models adjusting for potential confounders (age, day 3 FSH, antral follicle count and diagnosis) suggested less of a difference in gonadotropin requirement; yet the remaining difference did approach statistical significance (LL: 35.5 ampules, MDF: 37.9 ampules; p=0.052).

In models of treatment effect on outcome unadjusted for potential confounders, the implantation rate was 39.2% for LL and 34.5% for the MDF group (p=0.23) (Table 2). There were no significant differences between groups with regard to cycle outcomes including biochemical pregnancy, spontaneous abortion and clinical pregnancy rates. There was a 50% live birth rate in the LL cohort and 46.6% live birth rate among those undergoing MDF down-

regulation (p=0.49). The unadjusted clinical pregnancy rate was higher among those undergoing LL down-regulation than those receiving MDF, although estimates were statistically non-significant (56.0% vs. 53.6%, respectively; p=0.60)

Factors identified as predictors of treatment (age, day 3 FSH, antral follicle count and diagnosis) were included in multivariable models to control for confounding by indication for treatment. In these models, differences between the groups tended to be smaller, and remained statistically non-significant. After adjustment, the cycle cancellation rate was lower in the MDF group (7.2%) compared to the LL group (9.3%), although this difference remained statistically non-significant (p=0.36). The implantation rate after accounting for confounding factors was 37.7% for the LL group and 36.0% among those undergoing MDF (p=0.75). Clinical pregnancy rates were not substantially altered by adjustment, and the adjusted group difference was not significantly different between the groups (LL: 56.0%, MDF: 53.2%; p=0.60). Similarly, adjusted rates for SAB and for live births were not greatly affected; the adjusted rate of SAB was 4.8% in the MDF group and 3.8% among those receiving LL (p=0.37), while live birth rate was 50.3% in the LL group and 46.6% in the MDF group (p=0.47).

Discussion

The objective of this study was to evaluate the use of the MDF protocol among young, presumably normal responding patients as there has been a paucity of data available in this study population. Previously, our group had reported results suggesting that MDF could be effectively utilized among more than just the standard poor responding patients. (13) With the cost advantage of down-regulating as many as 30 patients with the amount of LA required to down-regulate each LL patient, MDF has been routinely used in our program including among expected normal responders. This present study was undertaken to evaluate longer term data on a larger cohort of patients and to ensure that our current practice was sound.

Our group's previous report of women under age 40 utilizing MDF down-regulation was underpowered to detect a statistically significant difference in pregnancy outcome between treatment arms. The cancellation rates were significantly different between groups (MDF: 22.5%, LL: 8.2%; p=0.032). A notable limitation of previous work was the propensity to include older women with suspected poorer responses in the MDF group, creating selection bias which may have explained the higher cancellation rates.

In order to address the potential bias on estimates due to age-related discrepancies between the LL and MDF groups, we restricted our cohort to those with the most favorable predicted response (e.g. ≤ 30 years of age). Furthermore, the influence of multiple cycles was reduced by including only first cycle data, thus limiting the impact of prior cycle information. In the current investigation we found no differences between patients assigned to LL and those assigned to MDF among most in-cycle characteristics, including serum E_2 levels, number of oocytes retrieved, cycle cancellation rates and OHSS risk. This was significant as one might have expected E_2 levels or number of oocytes retrieved to have been increased in the MDF group given the "flare" effect. Moreover while we observed small non-significant group differences in terms of implantation and clinical pregnancy rates, our data confirms that previously reported differences in the cycle cancellation rates were likely due to patient selection and not due to intrinsic properties of the MDF down-regulation protocol. Ultimately, our findings suggest that among normal responding patients, MDF may result in similar cycle outcomes when compared to those undergoing the LL protocol.

Despite the advantage of excluding women older than 30 in our cohort, there were limitations related to the retrospective study design. Treatment assignment was non-random and there were statistically significant differences between groups regarding age at cycle start, day 3

FSH, total antral follicle count, days of stimulation and total ampules of gonadotropins administered. These differences were diminutive and of limited clinical importance. For example, there is no evidence that women 29 year-old would have poorer ART outcomes than a 27 year-old woman. Likewise, a day 3 FSH of 7 would be no more predictive of success than a FSH of 6. Moreover, in our cohort the mean total antral follicle count of those assigned to LL was statistically significantly different from that of the MDF (LL: 17.7, MDF: 15.7; p=0.04). Yet data from previous studies would suggest that these differences observed were immaterial and would not impact ART cycle outcome (14,15). Nevertheless, we accounted for the treatment group discrepancies through the use of multivariate modelling and, as expected, noted little impact of these variables on adjusted cycle outcomes.

As reflected by the proportion of patients receiving a starting dose of ≤ 3 ampules/day of gonadotropin (LL: 26%, MDF: 24%), there was a significant proportion of patients in each group expected to have an adequate response. Nevertheless, there was the propensity to include those with idiopathic infertility and to exclude those with ovulatory dysfunction in the MDF group. In total, this reflects a tendency in treatment assignment toward including those with a suspected lower response to the MDF group. It was not altogether surprising to find, then, that more ampules of gonadotropins were used in the MDF group. Patients with expected poorer response were simultaneously allotted to MDF and received higher doses of gonadotropins at the cycle outset. As these factors might have influenced cycle outcomes, these imbalances represent potential confounding by indication. We addressed this by the inclusion of these factors as terms in multivariable regression models. To whatever extent the adjustments were inadequate in accounting for the imbalance, observed differences in outcomes due to treatment were overestimates for which the factors leading to treatment assignment were in part responsible.

One criticism of the use of flare protocols has been that significantly increased serum progesterone levels may occur during the early follicular phase which may exert a negative impact on cycle outcome (2). Yet it appears that microdose GnRH-a administration may have little effect on serum progesterone levels, even following OCP pre-treatment (16). In our program, neither LH nor progesterone levels are routinely drawn. However if the clinical presentation suggests premature luteinization, progesterone assessment was performed. In this study there were no cycles cancelled for a premature LH surge and there was no biochemical evidence that such event occurred. Moreover if there were detrimental premature luteinization effects related to the use of MDF, a lower implantation rate would be expected. In our data, we observed a slight decrement in implantation rate among MDF-treated patients that was not statistically significant, suggesting that if any such effect were occurring, the impact would be small.

In conclusion, the use of MDF in patients under the age of 30 who were pre-treated with oral contraceptive pills produced similar cycle characteristics and pregnancy outcomes when compared to LL. Further consideration should be given to MDF use in good prognosis patients.

Acknowledgements

References

1. Janssens RM, Lambalk CB, Vermeiden JP, Schats R, Bernards JM, Rekers-Mombarg LT, et al. Dose-finding study of triptorelin acetate for prevention of a premature LH surge in IVF: a prospective, randomized, double-blind, placebo-controlled study. Hum Reprod 2000;15:2333–40. [PubMed: 11056128]

2. San Roman GA, Surrey ES, Judd HL, Kerin JF. A prospective randomized comparison of luteal phase versus concurrent follicular phase initiation of gonadotropin-releasing hormone agonist for in vitro fertilization. Fertil Steril 1992;58:744–9. [PubMed: 1426320]

3. Hughes EG, Fedorkow DM, Daya S, Sagle MA, Van de Koppel P, Collins JA. The routine use of gonadotropin-releasing hormone agonists prior to in vitro fertilization and gamete intrafallopian transfer: a meta-analysis of randomized controlled trials. Fertil Steril 1992;58:888–96. [PubMed: 1426372]

4. Porter RN, Smith W, Craft IL, Abdulwahid NA, Jacobs HS. Induction of ovulation for in-vitro fertilisation using buserelin and gonadotropins. Lancet 1984;2:1284–5. [PubMed: 6150318]

5. van Loenen AC, Huirne JA, Schats R, Hompes PG, Lambalk CB. GnRH agonists, antagonists, and assisted conception. Semin Reprod Med 2002;20:349–64. [PubMed: 12536358]

6. Gonen Y, Jacobson W, Casper RF. Gonadotropin suppression with oral contraceptives before in vitro fertilization. Fertil Steril 1990;53:282–7. [PubMed: 2105244]

7. Damario MA, Barmat L, Liu HC, Davis OK, Rosenwaks Z. Dual suppression with oral contraceptives and gonadotrophin releasing-hormone agonists improves in-vitro fertilization outcome in high responder patients. Hum Reprod 1997;12:2359–65. [PubMed: 9436663]

8. Scott RT, Navot D. Enhancement of ovarian responsiveness with microdoses of gonadotropin-releasing hormone agonist during ovulation induction for in vitro fertilization. Fertil Steril 1994;61:880–5. [PubMed: 8174725]

9. Schoolcraft W, Schlenker T, Gee M, Stevens J, Wagley L. Improved controlled ovarian hyperstimulation in poor responder in vitro fertilization patients with a microdose follicle-stimulating hormone flare, growth hormone protocol. Fertil Steril 1997;67:93–7. [PubMed: 8986690]

10. Detti L, Williams DB, Robins JC, Maxwell RA, Thomas MA. A comparison of three downregulation approaches for poor responders undergoing in vitro fertilization. Fertil Steril 2005;84:1401–5. [PubMed: 16275235]

11. Surrey ES, Bower J, Hill DM, Ramsey J, Surrey MW. Clinical and endocrine effects of a microdose GnRH agonist flare regimen administered to poor responders who are undergoing in vitro fertilization. Fertil Steril 1998;69:419–24. [PubMed: 9531870]

12. Sharara FI, McClamrock HD. Use of microdose GnRH agonist protocol in women with low ovarian volumes undergoing IVF. Hum Reprod 2001;16:500–3. [PubMed: 11228219]

13. Leondires MP, Escalpes M, Segars JH, Scott RT Jr, Miller BT. Microdose follicular phase gonadotropin-releasing hormone agonist (GnRH-a) compared with luteal phase GnRH-a for ovarian stimulation at in vitro fertilization. Fertil Steril 1999;72:1018–23. [PubMed: 10593374]

14. Frattarelli JL, Levi AJ, Miller BT, Segars JH. A prospective assessment of the predictive value of basal antral follicles in in vitro fertilization cycles. Fertil Steril 2003;80:350–5. [PubMed: 12909498]

15. Frattarelli JL, Lauria-Costab DF, Miller BT, Bergh PA, Scott RT. Basal antral follicle number and mean ovarian diameter predict cycle cancellation and ovarian responsiveness in assisted reproductive technology cycles. Fertil Steril 2000;74:512–7. [PubMed: 10973648]

16. Park WI, Kim SS, Chung HR, Shin SY, Lee JY. Early and late hormonal responses to the microdose gonadotropin-releasing hormone agonist in normal menstruating women. Fertil Steril 2004;81:1067–72. [PubMed: 15066465]

Table 1

Cycle patient characteristics of 499 women ≤ 30 years by down-regulation protocol

	LL (n=287)	MDF (n=212)	*p* -value
Pre-cycle	mean ± SD		
Age (years)	27.3 ± 2.0	28.6 ± 1.6	<0.0001
Gravidity	0.7 ± 1.3	0.8 ± 1.2	0.40
Body mass index (kg/m2)	25.2 ± 5.4	24.8 ± 5.2	0.63
Day 3 FSH (mIU/mL)	6.0 ± 1.8	6.9 ± 2.1	<0.0001
Day 3 estradiol (pg/mL)	43.9 ± 33.4	44.2 ± 25.2	0.90
Total antral follicle count (n)	17.7 ± 10.5	15.7 ± 9.3	0.04
Gonadotropin starting dose (amps/day)	3.8 ± 0.8	4.0 ± 2.0	0.10
In-cycle	mean ± SD		
Duration of stimulation (days)[*]	9.3	9.8	0.0003
Day 6 estradiol (pg/mL)	623 ± 556	604 ± 647	0.70
Peak estradiol (pg/mL)	3797 ± 1934	3891 ± 1963	0.80
Total gonadotropins (amps)	33.9 ± 11.8	39.9 ± 16.3	<0.0001
Total oocytes retrieved (n)	18.3 ± 8.6	17.8 ± 8.3	0.60
Total oocytes mature (n)	13.6 ± 7.6	13.9 ± 6.7	0.70
Moderate to severe OHSS (%)	1.7	2.8	0.41

[*] calculated from the first day of gonadotropin administration in each group

Table 2

Stimulation results by pituitary desensitization protocol

	Unadjusted			Adjusted *		
	LL	MDF	*p* -value	LL	MDF	*p* -value
	%			%		
Final cycle outcome results						
Cycles cancellation	9.2	10.3	0.60	9.3	7.2	0.36
Implantation rate	39.2	34.5	0.23	37.7	36.0	0.75
Biochemical pregnancy rate	4.6	7.2	0.20	4.6	7.2	0.20
Spontaneous abortion rate	4.7	4.8	0.98	3.8	4.8	0.37
Clinical pregnancy rate	51.2	48.6	0.50	56.0	53.2	0.60
Live birth rate	50.0	46.6	0.49	50.3	46.6	0.47

*
adjusted for potential confounders including age, day 3 FSH, antral follicle count and diagnosis