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ASSISTED REPRODUCTION TECHNOLOGIES

Prolonged gonadotropin stimulation for assisted reproductive technology cycles is associated with decreased pregnancy rates for all women except for women with polycystic ovary syndrome

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Abstract
Purpose To determine if etiology of infertility modifies the relationship between the duration of ovarian stimulation and success during assisted reproductive technology (ART) cycles.
Methods A prospectively collected database was analyzed in an academic infertility practice. Eight hundred and twelve infertile women undergoing their initial fresh embryo, non-donor in vitro fertilization (IVF) or Intracytoplasmic Sperm Injection ICSI) cycle between January 1999 and December 2010 were evaluated. Clinical pregnancy was the main outcome measured.
Results Out of 663 cycles resulting in oocyte retrieval, 299 produced a clinical pregnancy (45.1%). Women who achieved a clinical pregnancy had a significantly shorter stimulation length (11.9 vs. 12.1 days, p=0.047). Polycystic ovary syndrome (PCOS) was the only etiology of infertility that was significantly associated with a higher chance for clinical pregnancy and was a significant confounder for the association of duration and success of treatment. Women with 13 days or longer of stimulation had a 34 % lower chance of clinical pregnancy as compared to those who had a shorter cycle (OR 0.66, 95% CI:0.46-0.95) after adjustment for age, ovarian reserve, number of oocytes retrieved, embryos transferred and PCOS diagnosis.
Conclusion Prolonged duration of stimulation is associated with decreased ART success for all couples, except for women with PCOS.

Capsule In this study, prolonged duration of stimulation was a poor predictor of ART success for all couples, except for women with PCOS.

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Introduction

Utilization of assisted reproductive technology (ART) is increasingly common as almost 155,000 cycles were reported in 2010 in the United States [1]. Outcomes have been steadily improving over the last three decades; however, more than half of all initiated cycles do not result in a live birth even for women younger than 35 years old [2]. While female age and ovarian reserve have been recognized as the strongest determinants predicting success, these parameters cannot be optimized. Identification of modifiable predictors of ART outcomes is essential for cycle management and patient counseling [3]. Prolonged duration of controlled ovarian stimulation is associated with decreased chance of live birth after ART. Chuang et al. demonstrated that patients with shorter stimulation length were more likely to have day 5 embryo transfers and improved pregnancy rates, implying either more favorable ovarian reserve or a detrimental impact of prolonged stimulation on embryo quality [4]. On the other hand, several infertility diagnoses have been associated with significantly increased odds of pregnancy. In a large study of 225,889 patients, Baker et al. demonstrated that tubal and uterine factor infertility and ovulation disorders were associated with improved ART success [5]. Although diagnosis of infertility may influence the relationship between duration of stimulation and success rates, little data is currently available.

Significantly longer duration of stimulation was reported for patients with polycystic ovary syndrome (PCOS) in a retrospective study of 392 patients [6]. Although two smaller studies were not consistent with this trend [7, 8], a subsequent meta-analysis of data from 632 patients demonstrated that stimulation length in PCOS women was 1.2 days longer on

average as compared to general infertility population [9]. However, a critical clinical question remains: does a shorter or longer duration of stimulation influence success of ART based upon diagnosis of infertility. The objective of the current report was to determine if etiology of infertility modifies the relationship between the length of ovarian stimulation during ART and clinical pregnancy.

Methods

Patients

All IVF and ICSI cycles at the University of Colorado for the period between January 1999 and December 2010 were identified (Fig. 1). Only first fresh autologous oocyte cycles were considered eligible for inclusion. Repeat ART cycles were excluded to avoid confounding effect of dose adjustment after initial ART cycle thus potentially influencing duration of stimulation. Cycles canceled prior to oocytes retrieval (n=147), or data entry error (n=2) were excluded. Reasons for cycle cancellation included inadequate (n=112) or exaggerated (n=20) follicular response, personal reasons for withdrawal (n=13), or abnormal endometrium (n=2). The final analytic sample was restricted to 663 women undergoing their first cycle using fresh autologous oocytes. Patient characteristics were evaluated, including age, maximum historical baseline follicle-stimulating hormone (FSH) level, and prior reproductive history. Analyzed cycle characteristics included the total amount of gonadotropin used, stimulation protocol, number of oocytes retrieved, number of embryos transferred, use of intracytoplasmic sperm injection (ICSI), and day of embryo transfer. At the time of initial IVF consultation, the patient's physician designated etiology of infertility; infertility diagnoses were not mutually exclusive. In addition to analyzing the duration of stimulation as a continuous variable, it was also categorized as more than 13 days versus else. The study was approved by the Colorado Multiple Institutional Review Board.

Stimulation protocols

Prevention of premature LH surge was achieved with ovarian down regulation via luteal GnRH agonist protocol, GnRH agonist flare or with GnRH antagonist. Gonadotropin stimulation was initiated after down-regulation was established or after discontinuing oral contraceptives. Standard formulations of either recombinant FSH (rFSH) or human menopausal gonadotropins (HMG) were used for stimulation with initial dosing ranging from 150to 450 IU per day. Gonadotropin dose was determined per the discretion of the individual physician based on ovarian reserve and patient age. Ovarian response was monitored with serum 17β-estradiol (E2) assessments and by serial follicular ultrasound. Gonadotropin dose was assessed after 3–5 days and was adjusted as needed as per routine clinical practice. In patients on the antagonist protocol, suppression was introduced once the lead follicle reached 14 mm. In patients on the GnRH agonist flare protocol, suppression was started on cycle day 2 with gonadotropins started on the following day (day 3). Human chorionic gonadotropin (hCG) was used as a trigger when at least 3 follicles were greater than or equal to 18 mm and the majority of the cohort above 14 mm. Oocyte retrieval was then performed 36 h following the administration of hCG. Oocytes were inseminated with or without ICSI, as per our clinical practice. Evidence for fertilization was assessed approximately 18 h post-insemination. An embryo transfer was performed under ultrasound guidance 2–6 days post insemination. Indications for blastocyst transfer included the following: at least 6 embryos on day 2 that were at least 4 cells with ≤15 % fragmentation. The number of embryos transferred was based on the number and quality of embryos available, the age of the women and previous pregnancy history. Luteal support was provided by intramuscular or vaginal progesterone supplementation until the first pregnancy test and was continued in all cycles with a positive serum β-hCG until documentation of fetal cardiac activity.

Statistical analysis

The ART cycle outcome of interest was clinical pregnancy rate and defined as presence of an intrauterine gestational sac on ultrasound. For continuous variables, associations between demographic and clinical characteristics and the outcome were assessed using Student's t-test for normally distributed data and Mann–Whitney U test for skewed data as appropriate. Categorical variables were analyzed using Chi-square. A multivariable analysis was performed using a logistic regression model with clinical pregnancy as the dependent variable and stimulation length and etiology of infertility as the independent variables of interest. Logistic regression analysis was conducted using model building strategies suggested by Hosmer and Lemeshow (Hosmer DW, Lemeshow S. Applied Logistic Regression. New York: Wiley, 2000) [10]. Appropriate regression diagnostics were performed to examine whether the model fit was supported over the entire set of covariate patterns. Variables were considered for inclusion in the model if they exhibited a significant association with the outcome in the bivariate analysis or were acknowledged clinical predictors (ie age). Dosage of gonadotropin was not included in the final model because of concern for multicollinearity. The presence of each infertility diagnosis compared with absence of the

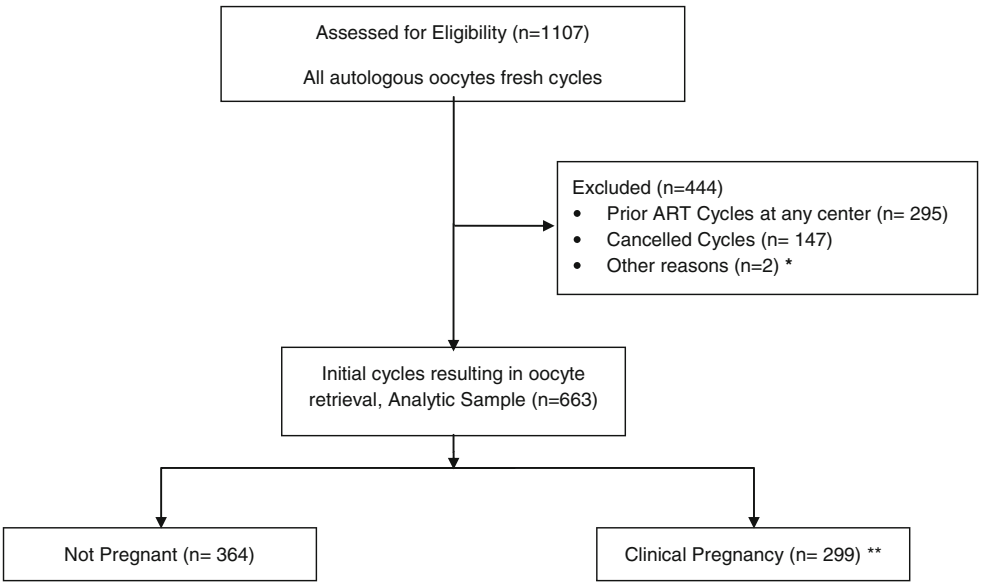

Fig. 1 CONSORT analytic sample diagram *apparent data entry error **defined as gestational sac on ultrasound

diagnosis, similar to the strategy that was previously employed in analyses of for the Society for Assisted Reproductive Technology reporting System [5]. The strength of association between the likelihood of clinical

Table 1 Mean (standard deviation) or prevalence (n) of demographic and cycle characteristics by ART outcome

	Not pregnant (*n*=364)	Clinical Pregnancy (*n*=299)	P-value
Female Age, years	33.6 (4.3)	32.5 (3.9)	<0.001[1]
Nulligravidity, %	58.0 (211)	57.5 (172)	0.909
History of livebirth, %	21.4 (78)	17.4 (52)	0.193
Maximum historical FSH, mIU/ml	7.6 (2.7)	7.2 (2.2)	0.093
Partner's sperm, %	54.8 (354)	97.7 (292)	0.742
Diagnosis of infertility, %,[2, 3]			
Male factor, *n*=296	55.7 (165)	44.3 (131)	0.696
Endometriosis, *n*=75	50.7 (38)	49.3 (37)	0.434
PCOS, *n*=58	41.4 (24)	58.6 (34)	0.030[a]
Diminished Ovarian Reserve, *n*=193	59.1 (114)	40.9 (79)	0.167
Tubal Factor, *n*=141	59.6 (84)	40.4 (57)	0.209
Uterine Factor, *n*=10	60.0 (6)	40.0 (4)	0.744
Unexplained, *n*=123	55.3 (68)	44.7 (55)	0.925
Total dose of gonadotropins, IU	2762 (1016)	2510 (819)	<0.001
Duration of gonadotropin stimulation, days	12.1 (1.4)	11.9 (1.1)	0.047[a]
Suppression, %			
GnRH agonist luteal	61.8 (225)	70.2 (210)	–
GnRH agonist flare	34.3 (125)	27.1 (81)	0.095
GnRH antagonist	2.2 (8)	2.3 (7)	–
Oocytes retrieved, n	16.5 (10.3)	17.9 (8.5)	0.054
ICSI, %	75.6 (275)	74.3 (222)	0.700
Blastocyst transfer, %	7.1 (26)	13.0 (39)	0.011[a]
Embryos transferred	2.8 (1.4)	2.9 (1.1)	0.278

[1] Statistically significant

[2] Distribution of couples by outcome within each etiology

[3] Categories may not add to 100 % due to multiple etiologies attributed to the same couple

pregnancy after ART and etiology of infertility as well as duration of stimulation is presented as an odds ratio (OR) with 95 % confidence interval (CI). All statistical tests used a two-tailed alpha of 0.05. Analyses were performed using STATA 10 (StataCorp LP, College Station, TX).

Results

Out of 663 cycles resulting in oocyte retrieval, 299 produced a clinical pregnancy (45.1 %). Patients with a clinical pregnancy following ART were younger, more likely to have a blastocyst transfer, required lower doses of gonadotropins, and had a significantly shorter stimulation length (Table 1). No difference was seen in the percentage of women who were nulligravid, had a previous live birth, or previously used injectable gonadotropins. Follicle stimulating hormone value was not different between groups. The use of partner's sperm was not different between the two groups. Women who achieved pregnancy had a higher number of oocytes retrieved; however, this was not statistically significant. A similar number of embryos were transferred regardless of the outcome. Notably, PCOS was the only cause of infertility that was significantly associated with a higher chance of clinical pregnancy.

Cycle outcome by stimulation length in our cohort is graphically depicted in Fig. 2. The clinical pregnancy rate was dramatically decreased when duration of gonadotropin stimulation took 13 days or longer ($p<0.001$). A logistic regression analysis was performed using clinical pregnancy as the dependent variable and stimulation length and etiology of infertility as the independent variables of interest (Table 2). When examining the impact of the cause of infertility on the outcome, PCOS was the only significant positive predictor of clinical pregnancy in our cohort. Increased duration of ovarian stimulation for the entire cohort was associated with a decreased chance of success in an unadjusted analysis (OR 0.90, CI 0.08–0.99, $p=0.048$). Next, we examined the impact of stimulation length on the outcome for each respective etiology. After addition of PCOS to the model, the association of stimulation length and the outcome was no longer significant ($p=0.071$). In addition to analyzing the duration of gonadotropin stimulation as a continuous variable, it was also categorized as greater than or equal to 13 days of gonadotropin stimulation versus else. This cut-off was chosen based on the inflection point in the clinical pregnancy rate (Fig. 2). The final multivariable logistic regression model suggested that 13 days or longer of stimulation decreased the chance of clinical pregnancy by 34 % as compared to shorter cycles (OR 0.66, 95 % CI: 0.46–0.95) after adjustment for female age, maximum historical FSH, number of oocyte retrieved, embryo transferred and PCOS diagnosis.

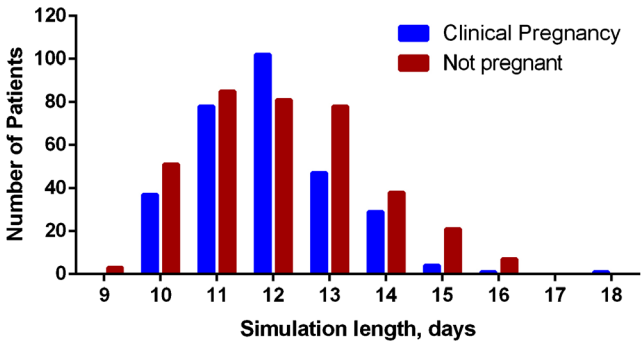

Fig. 2 Clinical pregnancy rate decreases with duration of gonadotropin stimulation of 13 days or longer

Discussion

In this multivariate analysis of 663 fresh autologous oocyte IVF cycles, we demonstrate that prolonged ovarian stimulation is associated with poor clinical pregnancy rates in all etiologies of infertility except for women with PCOS. To the best of our knowledge, this is the first study that specifically evaluated the relationship of the etiology of infertility in regards to duration. Multiple studies have indicated that a "low and slow" ovarian stimulation protocol with PCOS reduces ovarian hyperstimulation syndrome without compromising pregnancy outcomes. Homburg et al. showed that using a chronic low dose stimulation protocol vs. a conventional dose improved pregnancy rates for PCOS to 40 % from 24 % [11]. Andoh et al. used a low-dose step up protocol and compared it to conventional dosing. In this study, the low-dose step-up protocol had a lower risk of excessive ovarian enlargement 7 days after hCG trigger [12]. Our findings extend and add to the extant literature that suggests an overall positive prognostic outlook for women with PCOS undergoing ART regardless of duration of the stimulation phase.

ART outcomes in patients diagnosed with PCOS have been historically favorable. Clinical pregnancy rates have ranged from 33 to 45 % [13, 14]. Although fertilization rates and follicle maturity appear to be suboptimal in PCOS women, clinical pregnancy and live birth rates are similar compared to patients with tubal factor infertility. This implies a compensatory impact of overall higher than average values for ovarian reserve parameters [6, 9, 14, 15] . The major risk associated with ART in patients with PCOS is ovarian hyperstimulation syndrome (OHSS), which has been reported in up to 40 % of cases in some studies [14, 16, 17]. While patients with PCOS are thought to be at increased risk for early pregnancy loss due the inherent endocrine dysfunction associated with the syndrome, multiple studies have documented no elevated abortion risk in these patients undergoing IVF when compared to tubal factor infertility controls [14, 15]. This again implies a

Table 2 Likelihood of clinical pregnancy after ART by specified determinant[4]

Etiology	Estimates for couples within each etiology OR (95 % CI)	Estimates for length of stimulation in days		
		P value	OR (95 % CI)	p value
Entire cohort			0.90 (0.80–0.99) [b]	0.048[c]
Male factor	0.93 (0.68–1.26)	0.633	0.89 (0.80–1.00)	0.046
Endometriosis	1.24 (0.77–2.01)	0.381	0.89 (0.80–1.00)	0.044[c]
PCOS	1.74 (1.01–3.03)	0.046[c]	0.90 (0.81–1.00)	0.071
Diminished Ovarian reserve	0.85 (0.60–1.21)	0.371	0.91 (0.81–1.02)	0.094
Tubal Factor	0.79 (0.54–1.16)	0.230	0.90 (0.80–1.00)	0.053
Uterine Factor	0.81 (0.23–2.92)	0.753	0.90 (0.80–1.00)	0.049[c]
Unexplained	0.96 (0.64–1.43)	0.642	0.90 (0.80–1.00)	0.050

[a] Logistic regression models with clinical pregnancy as the outcome and days of stimulation and each respective etiology as the independent variables of interest

[b] Unadjusted

[c] Statistically significant

greater than average ovarian reserve capacity of PCOS women [18] and ability to achieve desired family size that is not smaller than that of unaffected women [19].

Prolonged stimulation has been associated with poor ART outcomes. Chuang et al. reported that prolonged duration of ovarian stimulation was a negative predictor of ART success, whereas a stimulation length of greater than or equal to 13 days decreased their likelihood of live birth by 53 % [4]. This study reported an increased stimulation length for women with PCOS and improved live birth rates; however, it was not designed to specifically look at infertility diagnosis in relation to stimulation length. Our study is novel in that it demonstrates that PCOS diagnosis per se appears to exempt women from the detrimental impact of prolonged gonadotropin stimulation on ART success. Additionally, our findings are consistent with this study performed in a different population, as pregnancy rates in the present cohort likewise declined after 13 days of stimulation (Fig. 2). Greater duration of stimulation as well as higher gonadotropin requirements underscore an underlying detrimental prognosis that is frequently associated with diminished ovarian reserve [20]. Our study highlights a fundamentally dissimilar etiology of infertility in patients with PCOS as prolonged stimulation length was not detrimental to pregnancy outcomes in this cohort and is in concert with findings by Chen et al. in Chinese women [21].

Limitations of this study include its retrospective nature and a moderate sample size for patients with PCOS. AMH levels and antral follicle counts were not captured in the database used for our analysis at the time of data collection; however, these values inherently represent determinants that cannot have been optimized prior to or during the ART cycle. Serum FSH was included in the analysis as a marker for ovarian reserve in lieu of AMH levels and antral follicle counts. Additionally, endometrium characteristics were not collected during the study period. Further, morbid obesity has been shown to affect ART outcomes and has been associated with higher gonadotropin requirements and lower chance of livebirth for all comers [22] and women with PCOS alike [23]. It remains to be determined if mild or moderate obesity plays a role, specifically in the relationship between the favorable impact of PCOS diagnosis on the outcome and duration of stimulation.

In summary, our study demonstrates that prolonged duration of ovarian stimulation is a negative predictor of ART success for all couples, except in women with PCOS. Poor outcomes associated with prolonged ovarian stimulation are thought to be due to the ovary's inability to effectively respond to exogenous gonadotropins, often indicating either testing-confirmed diminished reserve or "dynamic" diminished reserve [24, 25]. This is not thought to be the same mechanism contributing to subfertility in PCOS. Our results suggest that more days of ovarian stimulation during ART in PCOS women do not result in sacrificed chances of success. The take home point of our report is that going "low and slow" is fine for PCOS patients when it comes to progressing through ovarian stimulation.

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