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Research Article

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Shorter Length of Gonadotropin Stimulation is Associated with Adverse IVF outcomes: A Retrospective Analysis

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Abstract

Background: *The impact of the length of gonadotropin stimulation (LOS) on IVF outcome has been studied by several groups. Results so far have been conflicting. The primary aim was to evaluate the impact of LOS on pregnancy rates and oocyte yield. Secondary outcomes included comparison of outcome based on LOS separately in gonadotropin-releasing hormone (GnRH) agonist (GnRH-a) and antagonist (GnRH-ant) cycles.*

Methods: *Retrospective review of IVF cycles managed by a single provider in a private clinic. Data was collected for demographic, stimulation, embryology and clinical outcome parameters. Oocyte yield (the ability to obtain an oocyte from a proper sized follicle) was calculated for each cycle (number of oocytes retrieved/ follicles >14 mm at last scan). LOS was divided into short (≤8 days), normal (9-12 days), and prolonged (≥13 days). Student's t-test, ANOVA, and Chi-Square tests were used.*

Results: *Outcome based on 295 IVF cycles (GnRH-a: 94 and GnRH-ant: 201) were analyzed. Overall pregnancy rate (PR) was 36.3%. Age, ovarian reserve, number of available and transferred embryos didn't differ in the three groups. Shorter cycles compared unfavorably to normal and prolonged stimulations regarding oocyte yield (1.2 vs 1.5 vs 1.9, P<0.05) and PR (17.6% vs 40.9% vs 28.7%, P<0.05). Oocyte yield was significantly lower in cycles ≤8 days in both the GnRH-a and GnRH-ant groups when compared to longer stimulation.*

Conclusions: *Quicker response to gonadotropin stimulation may suggest diminished ovarian reserve but could indicate inadequate time for oocyte/ endometrial maturation to occur. LOS should be considered prior to hCG trigger administration.*

Keywords: Length of Stimulation, In-Vitro Fertilization, Pregnancy Rates, Oocyte Yield, GnRH agonist, GnRH antagonist.

Introduction

The success of in vitro fertilization and embryo transfer (IVF-ET) cycles is primarily dependent upon successful recruitment of multiple follicles to controlled ovarian hyperstimulation (COH) which develop into multiple high quality embryos for selection for embryo transfer [1]. Identifying those factors that affect IVF outcome has been the Holy Grail of IVF. Non-modifiable factors include age (most predictive for outcomes), duration of infertility, ovarian reserve and previous reproductive history [2,3]. Some modifiable factors include life-style habits, comorbidities, body mass index, stimulation protocol and starting gonadotropin (Gn) dose. Other modifiable considerations include the day of ovulation trigger, length of stimulation (LOS), the laboratory methods, day of transfer and transfer technique [4-6].

In a typical IVF cycle, the final oocyte maturation is triggered using human chorionic gonadotropin (hCG) once the leading follicle(s) achieve a diameter of ≥17 mm. In the majority of cycles, this requires between 9 to 12 days of stimulation [7]. Concomitantly, the endometrium undergoes proliferative and subsequent secretory changes to allow successful implantation to occur. Too short or too prolonged gonadotropin stimulation may negatively influence both oocyte maturation and endometrial preparation [8-10].

The impact of LOS has been evaluated by several investigators. Reports have been conflicting regarding the impact of prolonged (≥10, ≥11, ≥12 or ≥13 days) Gn stimulation on implantation and pregnancy rates [8,11-14]. The conflicting results may be due to the different definitions of prolonged Gn stimulation, different stimulation protocols, total amount of Gn used, patient characteristics, laboratory protocols or other confounding factors. Thus, the primary aim of this study was to further evaluate the impact

of LOS (both prolonged and shortened) on IVF pregnancy rates and oocyte yield expressed as number of oocytes retrieved/ mature follicles. Secondary outcomes included a comparison of pregnant to non-pregnant cycles and outcomes based on LOS separately in GnRH-a and GnRH-ant cycles.

Materials and methods

Since this was a retrospective analysis, consent forms were not used and no IRB approval was needed as per local regulations. Data from all fresh IVF cycles (n=295) using autologous oocytes from January to December 2015 managed by a single provider at the Kaali IVF Institute in Budapest, Hungary were retrospectively reviewed and considered for analysis. Cycles using donated oocytes and those undergoing preimplantation genetic diagnosis or elective cryopreservation as well as cycles using other than GnRH-a/-ant protocols were excluded.

Ovarian stimulation using daily injections of 150-450 IU/day of recombinant follicle stimulating hormone (FSH) (Gonal-F, Merck Serono) or urinary human menopausal gonadotropin (hMG) (Menopur, Ferring) following either mid-luteal phase long GnRH-a (Suprefact, Sanofi Aventis) or GnRH-ant (Cetrorelix 0.25 mg; Merck Serono) flexible protocols were used. The stimulation protocol and the starting Gn dose were determined based on age, ovarian reserve markers, weight and/ or response to previous stimulation. All cycles were monitored starting on stimulation day 6, and dosing was adjusted as needed by serum hormone levels and transvaginal sonography. When the lead follicle reached ≥17 mm diameter in size, recombinant human chorionic gonadotropin (hCG; 250 mcg Ovitrelle, Merck Serono) was administered to induce final follicle maturation. Thirty-five to thirty-six hours later, the transvaginal oocyte retrieval was scheduled.

Retrieved eggs were fertilized by IVF or ICSI depending on the sperm parameters and reproductive history. Fertilization was checked 16-18 hours later. Embryos were cultured in groups up to cleavage or blastocyst stage. One to three embryos were transferred transcervically three to five days post-retrieval, based on cleavage rate and morphology. An embryo with at least 6 cells and less than 20% fragmentation on day 3 was considered good quality. Cycles with >3 good quality day 3 embryos were considered for blastocyst culture. On day 5, embryos that reached the blastocyst stage and had a tight, regular inner cell mass and outer cell layer were considered good quality.

Embryo transfers (ET) were performed using soft catheters (Wallace, Smith Medical International Ltd., UK) using the afterload technique under ultrasound guidance. Surplus embryos in both groups were cryopreserved using vitrification. Pregnancy was confirmed by serum β-hCG 12-14 days following ET; clinical (CP) and on-going (OG) pregnancies were defined as presence of gestational sac at 6 and 8 weeks of gestation, respectively.

Data collected were patient's age, ovarian reserve markers, including baseline FSH, estradiol (E2), anti-Müllerian hormone (AMH), and cycle stimulation characteristics. These characteristics included GnRH-a or GnRH-ant use, total Gn dose, LOS, number of follicles >14 mm at the last ultrasound (up to 3 days prior to hCG trigger), endometrial thickness, number of oocytes retrieved, oocyte yield (calculated by the number of oocytes retrieved/ follicles >14 mm at last scan), number of fertilized oocytes, number of available embryos, number of good quality embryos, day of ET, number of embryos transferred, cryopreservation, and pregnancy outcome. Oocyte yield could involve values > 1 since the number of oocytes collected could be higher than the number of mature follicles counted on last ultrasound a few days before the retrieval. LOS was divided into short (≤8 days), normal (9-12 days), and prolonged (≥13 days) based on previously published cut-offs.

Continuous variables were analyzed for normality allowing for parametric analysis. Student's t-test was used to detect differences in baseline and stimulation characteristics between pregnant and non-pregnant groups. ANOVA was used to analyze baseline characteristics and IVF cycle outcomes when comparing short, normal, and prolonged stimulation groups. Pearson product correlation coefficient was used to analyze the correlation between oocyte yield and duration of gonadotropin stimulation. Chi-Square tests were used to assess PR and oocyte yield in all cycles and individually in GnRH-a vs. GnRH-ant groups. Post-hoc analysis was used to compare each group individually. A p-value of <0.05 was considered significant. Statistical analysis was performed using the SPSS statistical package 18.0 (SPSS Inc., Chicago, IL).

Results

In total, 295 IVF cycles were included (GnRH-a [n=94]; GnRH-ant [n=201]). 55 (15.7 %) cycles meeting exclusion criteria were excluded. The overall PR was 36.3% (n=107). No differences were noted in pregnant versus non-pregnant cycles with respect to basal FSH [±SD] (8.1 ± 3.0 mIU/ml vs. 8.7 ± 3.6 mIU/ml), endometrial thickness (10.0 ± 1.6 mm vs. 9.8 ± 1.5mm), LOS (10.6 ± 1.4 days vs. 10.7 ±1.8 days), and number of retrieved oocytes (9.2 ± 3.8 vs. 7.3 ± 4.7, p=0.07) or oocyte yield (1.6 ± 1.0 vs. 1.56 ± 0.8) (Table 1).

In cycles resulting in pregnancy, female age was lower (36.8 ± 3.7 years vs. 37.1 ± 3.9 years, p<0.0001), baseline AMH was higher (3.0 ± 3.3 ng/ml vs. 2.2 ± 2.0 ng/ml, p<0.05), less Gn were used (1988 ± 769 IU vs. 2292 ± 809 IU, p<0.05), more follicles > 14mm were seen (5.9 ± 2.9 vs. 4.9 ± 2.5, p<0.001), and the number of embryos (5.5 ± 2.8 vs. 4.3 ± 3.2, p<0.001), good quality embryos (3.1 ± 2.0 vs. 2.4 ± 2.1, p<0.01), and number of embryos transferred (1.9 ± 0.4 vs. 1.6 ± 0.8, p<0.01) were higher (Table 1).

Table 1: Baseline and Stimulation Characteristics in Pregnant and Non-Pregnant Cycles ¶

	Pregnant (n=107)	Not pregnant (n=188)	P-value
Age (years)	36.8 ± 3.7	37.1 ± 3.9	<0.001
FSH (IU/l)	8.1 ± 3.0	8.7 ± 3.6	NS
AMH (ng/ml)	3.0 ± 3.3	2.2 ± 2.0	<0.05
Total gonadotropins (IU)	1988 ± 769	2292 ± 809	<0.01
Days of stimulation	10.6 ± 1.4	10.7 ± 1.8	NS

Follicles >14 mm	5.9 ± 2.9	4.9 ± 2.5	<0.01
Oocytes	9.2 ± 3.8	7.3 ± 4.7	NS
Endometrial thickness (mm)	10.0 ± 1.6	9.8 ± 1.5	NS
Embryos	5.5 ± 2.8	4.3 ± 3.2	<0.01
Good quality embryos	3.1 ± 2.0	2.4 ± 2.1	<0.001
Embryos transferred	1.9 ± 0.4	1.6 ± 0.8	<0.001

¶ t-test

When cycles were evaluated based on cycle length, age, basal FSH, and AMH were similar in groups based on LOS (≤8 days, 9-12 days, and ≥13 days). However, when comparing cycles with LOS ≤8 days, to 9-12 days and, ≥13 days, except for implantation rates, there were significant decreases in the number of dominant follicles ≥14mm (4.7 ± 1.9 vs. 5.6 ± 1.9 vs. 4.7 ± 2.7, p=0.01), number of MII oocytes (5.5 ± 3.2 vs. 8.0 ± 4.6 vs. 7.2 ± 4.4, p<0.05), oocyte yield (1.2 ± 0.5 vs. 1.5 ± 0.8 vs. 1.9 ± 1.3, p<0.05), and pregnancy rate (17.6% vs. 40.9% vs. 28.7%, p<0.05) (Table 2).

Table 2: Outcomes based on Length of Stimulation §

	≤8 days (n=18)	9-12 days (n=243)	≥13 days (n=34)	p value
Age (years)	36.8 ± 2.8	36.6 ± 3.9	36.8 ± 4.2	NS
FSH (IU/l)	9.6 ± 2	8.7 ± 3.2	8.7 ± 4.3	NS
AMH (ng/ml)	1.9 ± 1.4	2.5 ± 2.7	2.6 ± 2.5	NS
Total gonadotropins (IU)	1616 ± 538	1998 ± 652	2759 ± 905	<0.001
Follicles >14 mm	4.7 ± 1.9	5.6 ± 1.9	4.7 ± 2.7	0.01
M2 Oocytes	5.5 ± 3.3	8.0 ± 4.6	7.2 ± 4.4	<0.05
2pn	3.2 ± 1.9	4.4 ± 3.1	3.7 ± 2.6	NS
Embryos	3.6 ± 1.6	5.1 ± 3.2	4.4 ± 3.0	NS
Good quality embryos	2.2 ± 1.6	2.8 ± 2.1	2.7 ± 2.0	NS
Embryos transferred	1.9 ± 0.6	1.7 ± 0.7	1.8 ± 0.7	NS
Oocyte/follicle	1.2 ± 0.5	1.5 ± 0.8	1.9 ±1.3	<0.05
Implantation Rate	8.3 ± 19.1	24.3 ± 34.8	25.9 ± 37.2	NS
Pregnancy Rate	17.6%	40.9%	28.7%	<0.05

§ ANOVA

When cycles were analyzed based on GnRH-a or GnRH-ant use, no differences in ovarian reserve and other cycle stimulation characteristics, PR and IR including parameters based on LOS were noted. Similarly, oocyte yield correlated with duration of gonadotropin stimulation (r=0.19, p=0.001), and was significantly lower in cycles ≤8 days in both the GnRH-a (1.0 ± 0.5) and GnRH-ant groups (1.2 ± 0.5) compared to cycles 9-12 days (1.5 ± 0.9 and 1.6 ± 0.8) and ≥13 days (1.9 ± 1.3 and 1.6 ± 0.7, p<0.05). IR and PR were lower in cycles ≤8 days, but failed to achieve significance (Table 3).

Table 3: Outcomes based on Length of Stimulation in GnRH-a and GnRH-ant cycles ◊

	≤8 days*	9-12 days**	≥13 days***	P-value
GnRH-a Implantation Rate	8.3±20.4	20.3±31.5	32.2±44.7	NS
GnRH-a Pregnancy Rate	16.6%	34.7%	30.8%	NS
GnRH-a oocyte yield	1.0±0.5	1.5±0.9	1.9±1.3	<0.05
GnRH-ant Implantation Rate	8.3±19.4	26.0±35.9	21.0±30.3	NS
GnRH-ant Pregnancy Rate	18.2%	42.9%	26.8%	NS
GnRH-ant oocyte yield	1.2±0.5	1.6±0.8	1.6±0.7	<0.05

◊ Chi-square test
*(GnRH-a, n=8 and GnRH-ant, n=12); **(GnRH-a, n=73 and GnRH-ant, n=170); ***(GnRH-a, n=15 and GnRH-ant, n=19)

Comment

The objective of this study was to evaluate the effects of shortened LOS on IVF pregnancy outcomes and oocyte yield expressed as number of oocytes retrieved/ mature follicles. This study demonstrates that LOS ≤8 days was associated with lower PR and reduced oocyte yield from mature follicles compared to normal and prolonged LOS. While a decreased ovarian reserve in women undergoing shorter LOS may explain these findings, there were no differences in age or AMH levels among the three groups. This simply could reflect decreased oocyte maturation or insufficient endometrial maturation preventing proper implantation. No differences in pregnancy outcome based on LOS were seen when specifically comparing cycles using GnRH-a or GnRH-ant protocols, but this finding may represent an inadequate sample size.

COH is an important part of IVF treatment. COH is individualized for each patient with the aim to ideally retrieve 10-15 mature oocytes [1]. Follicular growth is a dual event where the granulosa-theca cell compartment increases its activity producing steroid hormones. These promote nuclear-cytoplasmic maturation of the oocyte thus enabling fertilization following retrieval and endometrial preparation for implantation.

While endometrial development is monitored by measuring endometrial thickness, the oocyte cannot be directly evaluated during stimulation, though follicle size and E2 levels are used to obtain information on follicular maturation. Follicle growth is not standard. In most women undergoing COH, the follicle grows approximately 1.7 mm/day and optimal length of COH that yields the most oocytes is 11 days [7,15]. However, in some cases follicular growth is faster, while in others it is prolonged.

There are potential disadvantages of prolonged and shortened COH. Prolonged LOS requires increased amounts of gonadotropins, which may adversely affect the oocyte, through increasing aneuploidy rates, as well as endometrial effects where prolonged E2 exposure may negatively influence endometrial gene expression and adversely impact implantation [16-18]. Conversely, too short COH may not allow proper oocyte nuclear-cytoplasmic maturation and may result in the collection of fewer mature oocytes, lower fertilization, suboptimal embryo development, and perhaps inadequate endometrial preparation. Pre-ovulatory progesterone levels ≥1.5 ng/mL, often associated with prolonged stimulation and greater numbers of recruited follicles are known to reduce endometrial receptivity and pregnancy outcomes [19]. While this study did not measure peri-ovulatory progesterone levels, a premature progesterone rise could have an adverse impact. Subsequent studies should evaluate the incidence of premature progesterone rise with shorter and longer follicular phases.

Most studies have focused on the potential negative effects of extended LOS on IVF outcomes, though with conflicting results. Bar-Hava et al. reported that women with a median of 9.8 days of COH had similar PR than women with two standard deviations above this (16.9 days) [11]. Martin et al. reported similar IR and PR regardless of LOS including 6-9, 10-12 and ≥12 days of stimulation [8]. Alport et al. reported that while there was a reduction in the number of follicles and oocytes if stimulation duration deviated from the optimal 11 days, IR and PR were not adversely affected by shorter or longer stimulation [13]. This contrasts with others who have reported up to a 2-fold reduction in pregnancy outcomes with LOS over 13 days [12,20,21].

However, there is a paucity of literature describing the impact of shortened LOS. Literature in natural cycles suggests that shorter menstrual cycle lengths are associated with poor pregnancy outcomes. In woman >40 years, the average menstrual cycle is on average 4 days shorter than in women in their 20s, and the FSH peak is 3 days earlier than in younger cohorts. When adjusted for age, menstrual cycle length prior to IVF treatment appears to be an independent predictor of IVF outcome, where women with cycles >34 days are almost twice more likely to have a live birth than women with cycles <26 days [22]. Whether this is the result of greater rates of aneuploidy or altered endometrial synchrony remains unclear [23]. COH for IVF where follicular maturity is achieved faster than normal may suggest diminished oocyte-embryo quality resulting in reduced pregnancy outcomes. Martin et al. reported no impact of shortened LOS on IVF outcomes when categorized 6-9 days, 10-11 day and ≥12 days [8].

This study contrasts these findings, where LOS <9 days results in both lower numbers of retrieved oocytes and pregnancy outcomes. Unlike Martin et al., this study included both agonist long and antagonist cycles, which may have affected the study's results. Our study differs from the study by Pereira et al, who included only first IVF cycles into their analysis, since we included repeat cycles too [21]. This may be an additional confounding variable; though Chuang et al. in a sensitivity analysis of their data failed to show an impact on the association between cycle length and IVF outcome when the order of the treatment cycle was considered [12]. The limitations of this study stem from its retrospective nature, which does not allow control for all confounding variables. The trend in lower IR and PR but lack of significance in GnRH-a and GnRH-ant groups separately probably represents the small sample in both groups. Despite their methodological weaknesses retrospective studies are often used to generate and test a hypothesis and serve the basis of other prospective study designs. This retrospective review might generate a cohort trial in the future. While a retrospective study may suffer from methodological issues it is not realistic to perform any other study model as the industry standard suggests follicle size warrant a trigger at 17mm.

Like the other studies we referred to in the manuscript, our analysis is also based on data obtained from a single center. One could argue that a multicenter design adds to the strength of a study and this is mostly true for prospective randomized studies when confounding variables are controlled for by the randomization. In the case of a retrospective study a single center design allows much stricter control of confounding variables (e.g.: sonography, lab conditions, and embryo transfer technique) that may influence the outcome.

The strength of this study is the attempt to provide a biological explanation for the potential adverse impact of short LOS. In addition to the reduced clinical outcome when compared to LOS 9 days or longer, when the stimulation was short (≤8 days), oocyte yield was reduced (lower oocyte/ mature follicle ratio) suggesting dysfunctional follicle development where fewer mature follicles provide oocytes for the IVF process. A potential explanation is that patients with LOS <9 days may have reduced ovarian reserve and faster follicular growth which may not provide adequate time for proper oocyte maturation that is not reflective in basal FSH and AMH levels, though the latter is significantly underpowered and may represent a type II error. Follicular development and oocyte competence during COH are critically important for fertilization

and embryogenesis. The success of an IVF cycle depends on the size and quality of the oocyte cohort.

This study provides more insight on total Gn use and pregnancy outcomes and more importantly, the impact of the length of Gn stimulation in women undergoing autologous antagonist and agonist protocols. Knowledge of particular characteristics of each patient, including ovarian reserve, length of menstrual cycles prior to IVF cycles, and length and dosing of gonadotropin stimulation are imperative in selecting the best protocol to optimize IVF outcome. While these results should be interpreted with caution, due to the retrospective nature of the study, this information could be used during the management of the stimulation phase of the cycle. Based on these results, women with shortened length of stimulation during IVF have fewer oocytes retrieved and lower PR. Waiting for the right time to administer hCG and potentially extending the stimulation by 1-2 days, even if the follicles reach criteria to trigger, may further improve oocyte number and quality and possibly IVF outcomes.

Conflict of Interest

Authors declare no conflicts of interest.

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