Journal of Gynecology Obstetrics and Human Reproduction 48 (2019) 91–94

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Original Article

Luteal phase stimulation, the future of fertility preservation? Retrospective cohort study of luteal phase versus follicular phase stimulation☆,☆☆

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ARTICLE INFO

Article history:
Received 19 August 2018
Received in revised form 7 November 2018
Accepted 9 November 2018
Available online 12 November 2018

Keywords:
Fertility preservation
Luteal phase
Random start

ABSTRACT

Research Question: Is luteal phase stimulation capable of improving fertility preservation?
Materials and methods: We performed a retrospective cohort study of consecutive ovarian stimulations, during July 2012 and September 2018 at Strasbourg University Teaching Hospital in France. Enrollment criteria were patients aged below 40 who had been referred to our center following a diagnosis of cancer or requiring gonadotoxic treatment. All patients enrolled had regular menstrual cycles and normal ovulation. Non-enrollment criteria were an expected low ovarian response (defined by an anti-Müllerian hormone (AMH) level <0.75 μg/L and/or an antral follicle count inferior (AFC) inferior than 5), polycystic ovarian syndrome, amenorrhea, prior history of infertility or gonadotoxic treatment. The primary endpoint is the number of mature oocytes (metaphase II) obtained. Secondary outcomes were oocyte yields obtained, stimulation duration, initial gonadotropin dose and total gonadotropin dose.
Results: A total of 100 patients were included: 20 in luteal phase and 80 in follicular phase. A larger number of mature oocytes was obtained in luteal phase versus follicular phase (13.1+/8.0 versus 9.2 +/−5.8 with p = 0.01). A greater amount of total (mature and immature) oocytes was obtained in luteal phase versus follicular phase with a significant difference (16.8+/−9.3 versus 11.8+/−7.3 with p = 0.01). No difference was found for the initial and total doses of gonadotropin.
Conclusions: Luteal phase stimulation has the advantage of a better flexibility with positives results as to the number of oocytes obtained in fertility preservation.

Introduction

Ovarian stimulation with oocyte/embryo cryopreservation is currently the most commonly used technique for preserving fertility in women. Ovarian stimulation is usually initiated at the beginning of the follicular phase (on D2-3 of the menstrual cycle). However this method requires, depending on the day of patient's menstrual cycle at the time of consultation, between 2 and 6 weeks for full implementation, which has the effect of delaying the onset of cancer therapy. In some cases, chemotherapy nevertheless has to be started urgently so as not to reduce the chances of survival. In this context, luteal phase stimulation seems to provide a solution. It appears to reduce this implementation time and thus enables a cancer therapy or other gonadotoxic treatment to be rapidly started. Several studies have examined the efficacy of luteal phase stimulation in the field of fertility preservation [1–5], but owing to the ethical difficulty of conducting a level 1 study in this setting their results are wildly divergent, especially with regard to the stimulation duration, total gonadotropin dose and estradiol level [6]. The objective of this study was to compare the efficacy of luteal phase versus

☆ **Where the work was done:** Centre Médico-Chirurgical d'Obstétrique (CMCO), 19 rue Louis Pasteur, 67,300 Schiltigheim, France.
☆ **Presented at a meeting:** ESHRE 2018 in Barcelona (1–4 July 2018): poster, 20ème congrès national de la Société de la Médecine de la Reproduction (SMR) à Montpellier (16–18 mai 2018) : communication orale (**awarded first prize**).
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http://dx.doi.org/10.1016/j.jogoh.2018.11.003
2468-7847/

follicular phase stimulation in patients requiring fertility preservation and thus to provide additional results for the possible uptake of luteal phase stimulation in fertility preservation.

Materials and methods

This was a retrospective cohort study of consecutive ovarian stimulations conducted between July 2012 and September 2018 at Strasbourg University Teaching Hospital in France. This study was approved by the Strasbourg University Teaching Hospital, local ethics review board and the French Data Protection Authority. Enrollment criteria were patients aged below 40 who had been referred to our center following a diagnosis of cancer or requiring gonadotoxic treatment. All patients enrolled had regular menstrual cycles and normal ovulation. Non-enrollment criteria were an expected low ovarian response (defined by an anti-Müllerian hormone (AMH) level <0.75 μg/L and/or an antral follicle count inferior (AFC) inferior than 5), polycystic ovarian syndrome, amenorrhea, prior history of infertility or gonadotoxic treatment.

The stimulation technique was selected based on the day of the patient's menstrual cycle at the time of consultation and how urgent chemotherapy was. All patients consented to receive treatment and undergo processing of their data. A written consent form was archived in each patient file.

Patients in the follicular phase group received conventional ovarian stimulation based on the antagonist protocol. Generally, hormone testing (oestradiol, LH, FSH, AMH, progesterone) was performed on D2-3 of the menstrual cycle, along with a basal antral follicle count by means of endovaginal ultrasound. Daily gonadotropin injections were initiated between D1 and D3 of the cycle. Ovarian response was assessed on D6 of stimulation, and every 2–3 days thereafter. Whenever oocytes were present >12–14 mm or estradiol level >300 ng/mL, GnRH antagonists (0.25 mg cetrorelix acetate or 0.25 mg ganirelix) were administered in order to avoid premature LH peaking. Ovulation was triggered by means of a subcutaneous HCG injection (250 μg choriogonadotropin alfa). Oocyte puncture was scheduled 36 h later. Any mature oocytes (metaphase II) retrieved were subsequently either cryopreserved by vitrification, or fertilized and preserved as frozen embyros. As in the follicular phase, an initial assessment with hormone testing (oestradiol, LH, AMH and progesterone) and endovaginal ultrasound was performed at the start of luteal phase stimulation. Stimulation is initiated at any time during the luteal phase, with the exception of the time of the LH peak. Ultrasound monitoring of the stimulation is performed without taking into account the endometrial measurement. GnRH antagonists (0.25 mg cetrorelix acetate or 0.25 mg ganirelix) were administered if follicules >12–14 mm or estradiol level >300 ng/mL were detected. Ovulation was triggered by an injection of HCG (250 μg choriogonadotropin alfa) and oocyte puncture scheduled 36 h later. The triggering criteria are identical to those of the follicular phase stimulation.

The luteal phase was determined by the day of the menstrual cycle (>D14) and/or by a progesterone level > 2 ng/L. In both groups, the initial gonadotropin dose was calculated on the basis of the AMH level, FSH level and initial antral follicle count, and was modified in the course of stimulation depending on the ovarian response (follicle size and estradiol level).

The primary outcome was the mature oocyte yield (metaphase II) obtained. Secondary outcomes were oocyte yields obtained, stimulation duration, initial gonadotropin dose and total gonadotropin dose.

Medifirst© AMP software enabled extraction of study data from medical dossiers at Strasbourg University Teaching Hospital. Data analysis was performed using R software (R Foundation for Statistical Computing, Vienna, Austria), version 3.4.4. Data are expressed as mean +/− standard deviation for normal numerical variables, median (quartile 1 – quartile 3) for numerical variables without a normal distribution, and number and percentage (%) for categorial variables. The Kolmogorov-Smirnov test was used to determine whether the data were normally distributed or not. For normally distributed variables, a Student *t*-test was used. For not normally distributed variables, a Mann-Whitney U test was realised. A Fisher exact test was used for categorial variables. $p < 0.05$ was considered significant.

Results

In all, 100 patients were enrolled in this study: 20 in the luteal and 80 in the follicular phase. Indications for fertility preservation were breast cancer, ovarian cancer, sarcoma, lymphoma, gastrointestinal cancer, cervical cancer, cerebral tumor, myelodystrophy, endometriosis, BRCA 1 mutation and auto-immune disease. There was a difference in the mean age between groups: the mean age was 31.7 +/− 4.0 for the luteal phase and 28.1 +/− 5.6 years for the follicular phase group with $p = 0.008$ (Table 1). No significant difference was detected between groups with regard to follicle stimulating hormone (FSH), anti-Müllerian hormone (AMH) levels and antral follicular count (AFC). To be noted that FSH was available for 12 out of 20 patients in the luteal phase group. This information was provided in the event of recent follicular phase stimulation or, in most cases, in the event of early prescription by the oncologist in anticipation for fertility preservation. Body mass index (BMI) was also comparable between groups.

No significant difference was observed in respect of the estrogen peak on the day of triggering with 1045 [678–1812] ng/ml in the follicular phase versus 1264 [821–2323] ng/ml in luteal phase. The progesterone level was significantly superior in the luteal phase than in the follicular phase (1.47 [0.81–2.54] vs 0.71 [0.48–1.10] ng/ml; $p = 0.005$). GnRH antagonist was administered earlier in luteal phase compared to follicular phase stimulation (6 [2–6] vs 7 [6–8] respectively; $p = 0.002$).

With regard to the principal assessment criterion, a higher number of mature oocytes was retrieved in luteal phase compared to follicular phase stimulation (13.1 +/- 8.0 vs 9.2 +/− 5.8]; $p = 0.01$) (Table 2). A higher total oocyte yield (mature and immature) was also obtained in luteal phase compared to follicular phase stimulation, the difference being significant (16.8 +/− 9.3 vs 11.8 +/− 7.3]; $p = 0.01$). No difference was detected in respect of the initial or total gonadotropin dose. Patients stimulated in the luteal phase received a median dose of 2650 [2250–2700] IU gonadotropin versus 2475 [2212–3243] IU for patients stimulated in the follicular phase. The duration of stimulation was also identical with 9 [9–11] days in the luteal phase and 10 [9–11] days in the follicular phase. Oocytes were vitrified or fertilized according to the wishes of the patient and partner. In the follicular phase group, 8 patients underwent ICSI and a total of 35 embryos were frozen compared to 2 patients in the luteal phase group where 4 embryos were frozen. It should be noted that 25.0% of the follicular phase cycles were programmed using estrogen.

Discussion

The results of our study suggest that luteal stimulation is as effective and even better than follicular phase stimulation regarding the number of mature oocytes retrieved. In fact, the mature and total follicle yield obtained on completion of stimulation was significantly higher in the luteal compared to the follicular phase. This result deviates from those of most fertility preservation studies in which no significant different has been observed [1,2]. A 2016 meta-analysis pooling eight studies on luteal phase stimulation in cases of infertility or fertility preservation, concluded however that there was a slight increase in the mature

Table 1
Initial patient characteristics.

	Follicular phase (n = 80) Median [Q1-Q3]	Luteal phase (n = 20) Median [Q1-Q3]	p value
Age (years)	28.1 +/− 5.6[a]	31.7 +/− 4.0[a]	0.008
Previous pregnancy	24 (30.0%)	5 (25.0%)	0.79
AMH (ng/ml)	2.6 [1.8–3.4]	3.5 [2.7–4.6]	0.08
Baseline FSH	6.0 [4.8–8.6]	5.8 [4.6–6.9]	0.70
AFC	13 [9–19]	15 [9–21]	0.60
BMI (kg/m^2)	22.9 +/− 4.3[a]	23.7 +/− 4.5[a]	0.46
Disorder			
Breast cancer	15	8	
Ovarian cancer	20	5	
Sarcoma	7	1	
Lymphoma/Leukemia	12	3	
Cerebral tumor	1	1	
Cervical cancer	1	1	
Gastrointestinal cancer	4	1	
Myelodystrophy	2	0	
Other cancer	2	0	
Endometriosis	12	0	
BRCA 1 mutation	2	0	
Auto-immune disease	2	0	

AMH = Anti-Müllerian hormone/FSH = Follicle-stimulating hormone / BMI = Body mass index.
[a] Mean +/− SD.

Table 2
Comparison between conventional and luteal start cycles.

	Follicular phase (n = 80) Median [Q1-Q3]	Luteal phase (n = 20) Median [Q1-Q3]	p value
Initial gonadotropin dose (IU)	250 [225–300]	300 [219–300]	0.32
Total gonadotropin dose (IU)	2475 [2212–3243]	2650 [2250–2700]	0.96
Duration of stimulation (days)	10 [9–11]	9 [9–11]	0.28
Estrogen programming	20 (25.0%)	0 (0%)	0.01
Pg on day of triggering (ng/mL)	0.71 [0.48–1.10]	1.47 [0.81–2.54]	0.005
E2 peak value (ng/mL)	1045 [678–1812]	1264 [821–2323]	0.17
Day of antagonist initiation	7 [6–8]	6 [2–6]	0.002
Pg on day of antagonist initiation (ng/mL)	0.5 [0.20–0.73]	4.05 [1.06–7.07]	0.002
Number of oocytes retrieved	11.8 +/− 7.3[a]	16.8 +/− 9.3[a]	0.01
Number of mature oocytes	9.2 +/− 5.8[a]	13.1 +/− 8.0[a]	0.01

Pg = progesterone/E2 = estradiol.
[a] Mean +/− SD.

oocyte yield obtained with luteal phase stimulation, without an increase in the total oocyte yield [6]. In view of the retrospective nature of our study, the existence of various biases cannot be excluded.

Moreover, although many studies suggest that a higher dose of gonadotropin for a longer duration of time is necessary in luteal phase stimulation [3,5,6], our study did not show any significant difference between the two protocols either in respect of the stimulation duration or total gonadotropin dose used. The case-controlled prospective study by Buendgen et al. in 2013 indicated the utility of using a mean dose of 3495 IU gonadotropin over 11.7 days in the luteal phase versus 2040 IU over 9.10 days in the follicular phase. The initial FSH dose was 300 IU in the luteal phase, whereas only 150 to 225 IU was used in the follicular phase. A more recent study in 2014 [4], comparing pregnancy rates in a donated oocyte program from donors stimulated at D2 versus D15, proved to be more optimistic. No significant difference was observed for pregnancy rates obtained in the recipients (62.5% for luteal phase versus 58.3% for follicular phase) or for mature oocyte yields in donors (14.0 versus 16.9), and on this occasion the duration and total dose of gonadotropin administered were identical for both protocols.

One of the problematic aspects of our study was in determining the progesterone level in order to define the luteal phase onset. A progesterone level of more than 2 ng/mL was arbitrarily set in order to define the luteal phase. Many other studies have used a higher level, notably that of Buendgen and al. in 2013 who defined the beginning of the second part of the cycle based on a progesterone level of more than 7 ng/mL and Qin and al. in 2016 who adopted a progesterone level of more than 6.5 ng/mL. For their part, Cakmak and al. in 2013 used a level closer to ours at 3 ng/mL.

Another point to be noted in our study was the routine and early use of GnRH antagonist in the luteal phase protocol. GnRH antagonist was indeed initiated several times at the start of stimulation out of concern that the LH level would peak prematurely. We know now from several studies [7–9] that endogenous progesterone alone in the luteal phase would have been sufficient to block a premature LH peak by inhibiting GnRH pulsatility, thus preventing positive estrogen “feedback”.

Furthermore, in our study, 25.0% of follicular cycles received estrogen priming. The impact of estrogen programming on oocyte yields obtained or pregnancy rates currently remains highly controversial. A Cochrane meta-analysis in 2017 [10] concluded that there was insufficient evidence to determine whether there was a difference between the two groups, with or without estrogen, on the pregnancy or live birth rates. We therefore take the view that these programs had little impact on our results.

Luteal phase stimulation would appear to be a promising alternative to conventional protocols in view of the number of mature oocytes retrieved [11,12]. The principal utility of developing

luteal phase stimulation is the advent of new protocols such as the "random start". Until recently the freezing technique used was slow freezing, with outcomes that were often less than optimal (embryo survival rate of about 52%) [13]. Vitrification, trialed for the first time in 1999, was a real advance since it offered a survival rate of about 90–95% [14–16]. Combined with advances in luteal phase stimulation, it enabled the development of a new approach to medically assisted procreation (MAP) and especially to fertility preservation. And given that luteal phase stimulation appeared to be as effective as follicular phase stimulation, why should "random start" stimulation not be possible, the objective being to create a new flexibility and rapidity in fertility preservation and MAP techniques? This is what Qin et al. sought to demonstrate in his 2016 study in Shanghai's Ninth Peoples Hospital [17]. Three groups of 50 patients were identified from the day of their menstrual cycle at the beginning of stimulation: early follicular phase stimulation (conventional group), late follicular phase stimulation and luteal phase stimulation. This retrospective study failed to find any significant difference between these three groups in terms of the mature oocyte yield (metaphase II) obtained or number of frozen embryos. The duration of ovarian stimulation was however shorter by about two days in the conventional phase group. The study by Cakmak et al. in 2013 reached the same conclusions [3]. The new flexible approach to ovarian stimulation, which enables it to be initiated both in the follicular phase (early or late) and in the luteal phase, therefore appears not just to be feasible, but effective. Its wider uptake could enable implementation times in fertility preservation to be reduced and thereby allow rapid oncological management. In our study, only six patients were in the late follicular phase at the beginning of stimulation. They were therefore included among the patients in early follicular phase, thus constituting one single follicular group.

Conclusion

Luteal phase stimulation offers the advantage of improved flexibility with satisfactory outcomes in terms of the number of oocytes retrieved. It can readily be imagined that this new protocol will have a future especially in the field of fertility preservation where reducing the time needed to implement stimulation is of capital importance.

Financial support

None.

Conflict of interest

All authors do not declare any conflict of interest.

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