Follicular versus luteal phase ovarian stimulation during the same menstrual cycle (DuoStim) in a reduced ovarian reserve population results in a similar euploid blastocyst formation rate: new insight in ovarian reserve exploitation

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Objective: To compare the euploid blastocyst formation rates obtained after follicular phase (FP) versus luteal phase (LP) stimulation performed in the same menstrual cycle in a preimplantation genetic diagnosis for aneuploidy testing (PGD-A) program in patients with reduced ovarian reserve.
Design: Prospective paired noninferiority observational study.
Setting: Private infertility program.
Patient(s): Forty-three reduced ovarian reserve patients undergoing a PGD-A.
Intervention(s): Both FP and LP stimulations using follicle-stimulating hormone and luteinizing hormone in combination with gonadotropin-releasing hormone (GnRH) antagonist starting on day 2 of the cycle and 5 days after the first oocyte retrieval, respectively, where GnRH agonist was used for both FP and LP ovulation triggering; a trophectoderm biopsy quantitative polymerase chain reaction–based PGD-A strategy; and single euploid blastocyst transfers during a subsequent natural cycle.
Main Outcome Measure(s): Primary outcome measure: euploid blastocyst rate per injected metaphase 2 (MII) oocyte; secondary outcome measures: number of cumulus-oocyte complexes (COCs), MII oocytes, and blastocysts.
Result(s): Patients with an antimüllerian hormone level of <1.5 ng/mL, antral follicle count of <6 follicles, and/or <5 oocytes retrieved in a previous cycle were included. No statistically significant differences were found in the number of retrieved COCs (5.1 $\pm$ 3.4 vs. 5.7 $\pm$ 3.3), MII oocytes (3.4 $\pm$ 1.9 vs. 4.1 $\pm$ 2.5), or biopsied blastocysts per stimulated cycle (1.2 $\pm$ 1.2 vs. 1.4 $\pm$ 1.7) from FP versus LP stimulation, respectively. No differences were observed in the euploid blastocyst rate calculated either per biopsied blastocyst (46.9% vs. 44.8%) or injected MII oocyte (16.2% vs. 15.0%).

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Conclusion(s): Stimulation with an identical protocol in the FP and LP of the same menstrual cycle resulted in a similar number of blastocysts in patients with reduced ovarian response. The LP stimulation statistically significantly contributed to the final transferable blastocyst yield, thus increasing the number of patients undergoing transfer per menstrual cycle. (Fertil Steril® 2016;105:1488–95.

Key Words: Euploid blastocyst yield, luteal phase stimulation, oocyte collection, ovarian stimulation, PGD-A

Multiple major and minor waves in follicular development have been described in wild ungulates and large domestic species, including cattle, horse, sheep, and goats, and also in primates (1–8) and in most animals where high-resolution ultrasonography has been used for detailed follow-up studies (9). During major waves, a dominant follicle develops; during minor waves, no selection occurs. In contrast, the traditional propitious moment theory suggested that in humans only a single major wave of follicle development occurs during the intraovulatory period (10–12), although even early investigations detected a limited number of nonatretic follicles with steroidogenic activity in the luteal phase (LP) (13).

In 2003, based on ultrasonographic studies, Baerwald et al. (14, 15) reported two or three follicular waves during the intraovulatory period of healthy women. They suggested that follicles developing during the LP may have the potential to ovulate in the presence of an luteinizing hormone (LH) surge, offering new possibilities for ovary stimulation. However, their theory was received with scepticism (16–19), and the suggestion has been disregarded for years.

Application of efficient vitrification techniques for oocyte and embryo cryopreservation (20, 21), successes in oocyte maturation (22), and the need for rapid interventions to preserve fertility in young cancer patients before the onset of therapy have led to attempts to obtain oocytes during the LP without (23, 24) and with stimulation (25–30). Although in vitro development has been observed after in vitro or in vivo maturation, no data are available regarding the developmental competence of these oocytes in vivo (31).

Sporadic indirect information has supported the possibility that oocytes from follicles developing outside the frames of the propitious moment scheme may be developmentally fully competent. In 1997, Hwang et al. (32) reported the birth of a healthy baby by caesarean delivery after oocyte retrieval, in vitro maturation, and intracytoplasmic sperm injection (ICSI) in a donation program. High conception rates after forced intercourse and heteropaternal superfecundation or superfetation could be explained by a stress-induced LH surge that may occur at any point of the menstrual cycle and even during periods of amenorrhea related to pregnancy and lactation (33–35).

Direct evidence for competent oocytes from a second follicular wave has also been published. In 2010, Bentov et al. (36) reported on a pregnancy that occurred after extended continuous stimulation, from oocytes derived from the second follicular wave. Similarly, Kuang et al. (37) reported the birth of healthy twins from a second wave of follicles after continuous stimulation and oocyte collection on day 20. The high progesterone level had no negative consequence on follicle maturation and oocyte retrieval. A subsequent study of 227 patients stimulated after spontaneous ovulation reported 68 live births and 44 ongoing pregnancies without any stimulation-related complications, proving the feasibility of this approach (38). A double-stimulation strategy applied to poor responders was also successful: mild stimulation in the follicular phase (FP) and a second stimulation after oocyte retrieval resulted in 13 pregnancies out of the 38 patients initially enrolled to the study (39).

Based on the limited available data, LP stimulation does not cause an elevated rate of abnormalities at birth (40). In a comparative study performed on a limited number of oocyte donation programs, Martínez et al. (31) found no differences between stimulation during the FP and LP in terms of outcomes, including fertilization, pregnancy, and implantation rates. A preliminary note of Moffat et al. (41) outlined a double-stimulation strategy with identical first and second stimulation protocols, resulting in a similar number of oocytes and blastocysts, thus doubling the final blastocyst yield.

Our study applied the double-stimulation approach within a single menstrual cycle (DuoStim) in a cohort of patients with a reduced ovarian reserve in a preimplantation genetic diagnosis for aneuploidy testing (PGD-A) infertility program. An identical protocol was used for both the FP and LP stimulations, and the euploid blastocyst formation rates per metaphase 2 (MII) injected oocyte were compared as the primary outcome.

MATERIALS AND METHODS

This prospective paired noninferiority observational study was performed in a routine infertility program of private IVF clinics between January and September 2015. Written informed consent was obtained from all patients who were enrolled. The study was approved by the Institutional Review Board of the clinic.

To exploit and compare the developmental quality of retrieved oocytes after FP and LP stimulation, the euploid blastocyst formation rate per MII injected oocyte by ICSI was set as the primary outcome measure. The secondary outcome

variables were the number of retrieved cumulus-oocyte complexes, MII-phase oocytes, and obtained/biopsied blastocysts.

During the study period, consecutive patients with reduced ovarian reserve—that is, with an antimüllerian hormone (AMH) level of $\leq$1.5 ng/mL, antral follicular count of $\leq$6 follicles, and/or $\leq$5 oocytes retrieved in a previous cycle—undergoing a preimplantation genetic diagnosis for aneuploidy testing (PGD-A) cycle were enrolled in the study before the first stimulation cycle. Both FP and LP stimulations were performed with recombinant follicle-stimulating hormone (FSH) and gonadotropin-releasing hormone (GnRH) antagonist treatment. In particular, after the ultrasonographic basal status of the ovaries had been assessed, the follicular stimulation was started on day 2 of the menstrual cycle with a fixed dose of 300 IU of recombinant FSH and 75 IU of recombinant LH (Gonal-F, Merck-Serono Italy; Puregon, MSD-Merck; Luveris, Merck-Serono Italy) for 4 days. The follicular growth was monitored with ultrasound scans and estradiol and LH assessment first on day 5, and then every 2 days. Daily administration of a GnRH antagonist (Cetrotide 0.25 mg, Merck-Serono Italy; Orgalutran 0.25 mg, MSD-Italy/Merck) was started when the leading follicle was 13–14 mm in diameter and continued until the day of the trigger of the ovulation.

When at least two follicles had reached 17–18 mm in diameter, ovulation was triggered with a single subcutaneous bolus of buserelin at the dose of 0.5 mL (Suprefact 5.5 mL; Hoechst Marion Roussel), and oocyte retrieval was performed after 35 hours. Five days after the first oocyte retrieval a second gonadotropin stimulation was started with a GnRH antagonist protocol identical to the first one. When at least two follicles reached 17–18 mm in diameter, the ovulation was triggered with a single subcutaneous bolus buserelin at the dose of 0.5 mL and the second oocyte retrieval was performed 35 hours after (Fig. 1A).

Oocyte retrieval, ICSI, and embryo culture procedures were described in detail elsewhere (42). Briefly, collection of cumulus-oocyte complexes was performed via transvaginal ultrasound-guided aspiration at 35 hours after the trigger. After 2 to 4 hours of incubation, the in vitro cumulus and corona radiata cells were removed by hyaluronidase treatment and pipetting. For insemination, all MII oocytes were subjected to ICSI using the technique described elsewhere (42). Presumptive embryos were cultured individually in 25 μL of Irvine continuous single culture medium (CSCM; Irvine Scientific Australia). Culture was performed at 37°C in 6% carbon dioxide and 5% oxygen tension with maximum humidity. Fertilization, cleavage, and development to the blastocyst stage were evaluated up to day 7. The blastocyst morphology was scored as described by Gardner and Schoolcraft (43). All embryos reaching the expanded stage on day 5, 6, or 7 were vitrified with the Cryotop method (44) after biopsy.

The blastocyst biopsy and aneuploidy screening were performed as described in detail elsewhere (45). Briefly, a 10–20 μm hole was opened on the zona pellucida by a diode laser, and 5 to 10 trophectodermal cells were removed. The samples were processed and analyzed at Genetyx SRL Laboratories (Marostica, VI, Italy) by using quantitative real-time polymerase chain reaction (46). Karyotype prediction was made for each embryo by a certified geneticist.

Embryo transfer was planned in the subsequent natural cycle after the genetic results on the blastocyst chromosomal status had been obtained. In all patients, single-embryo transfer (SET) was performed. Clinical pregnancy was determined by ultrasound demonstration of a gestational sac at 7 weeks. The miscarriage rate was assessed as the number of pregnancy losses per clinical pregnancy achieved. Ongoing pregnancy was defined as the number of pregnancies beyond the 20th week of gestation.

Statistical Evaluation

Continuous data are presented as absolute mean with standard deviation (SD). Categorical variables are presented as absolute percentage frequency with 95% confidence interval (CI). Fisher's exact test and paired *t* test were used to assess differences between categorical and continuous variables, respectively.

A power analysis on the primary outcome (euploid blastocyst formation rate) was conducted to evaluate the sample size to be included in this study. With a paired noninferiority design and an expected euploidy blastocyst formation rate of 17% per utilized mature egg in the FP stimulation (47), 320 oocytes were required to exclude a difference of 5% with a power of 80%. Considering a mean number of four MII-phase oocytes retrieved from each follicular and luteal oocyte pickup procedure, and a dropout rate of 10%, 50 patients (400 eggs) were thus planned to complete this study.

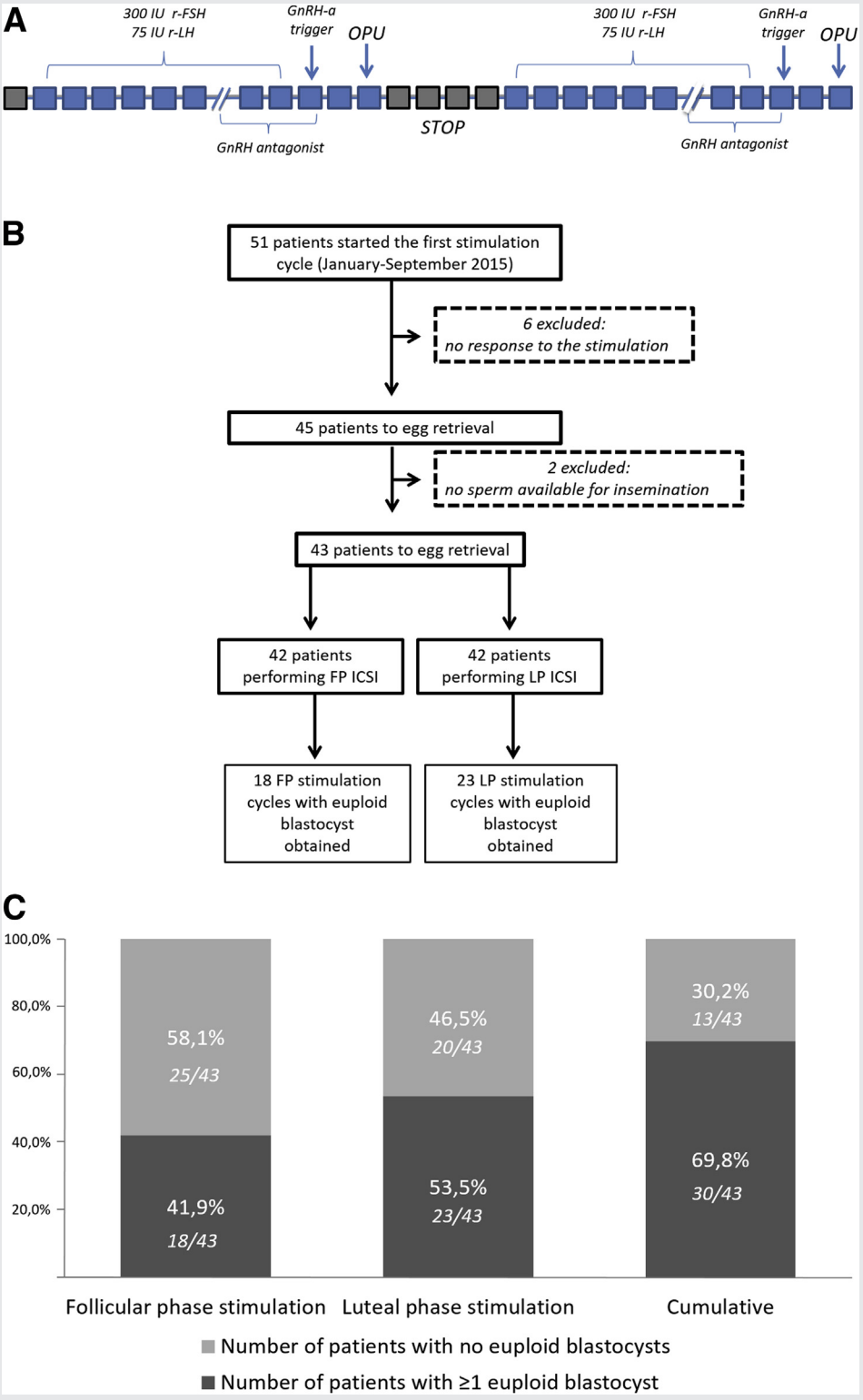
RESULTS

The flowchart of the study is shown in Figure 1B. Out of the 51 patients enrolled for this study, six were excluded because of no response to the stimulation. The nonresponding patients in the FP stimulation did not undergo LP stimulation. Forty-five patients underwent egg retrieval, but two were excluded after the first stimulation cycle because no sperm was available for ICSI (Fig. 1B). We performed 43 oocyte retrievals (OPU) in both FP and LP stimulation, respectively. We obtained MII oocytes in 84 stimulation cycles, 42 for each stimulation approach (Supplemental Fig. 1, available online).

The average age of the 43 patients was 39.2 $\pm$ 3.4 years (range: 32.0–44.0 years), the average duration of their infertility period was 2.9 $\pm$ 1.8 years (range: 1–9 years) and they already had a mean number of 0.55 $\pm$ 1.1 failed IVF cycles performed (range: 0–4 years) and 0.56 $\pm$ 1.0 previous miscarriages (range: 0–4 years). The mean FSH, LH, and AMH levels were 12.3 $\pm$ 7.5 IU (5.0–28.2), 5.1 $\pm$ 2.1 IU (3.0–9.0), and 0.7 $\pm$ 0.8 ng/mL (0.1–1.8), respectively. The mean antral follicle count registered was 5.2 $\pm$ 2.3 (2–9). These parameters are consistent with a patient population of poor prognosis.

Apart from reduced ovarian reserve, advanced maternal age was the most important infertility factor (23 patients, 53.5%). In 23.2% (n = 10) of the couples included in the study, a severe oligoasthenoteratozoospermia was also diagnosed after sperm analysis. Patients with endometriosis (n = 5), multiple miscarriages (n = 2), tubal factor infertility (n = 1), and endocrine-ovulatory dysfunctions (n = 2)

FIGURE 1



(**A**) Dual stimulation protocol. Five days after the first oocyte retrieval, luteal phase stimulation was performed with an identical protocol (as described in Materials and Methods). Each *square* represents a day of the cycle. (**B**) Patient flowchart. (**C**) Number of patients with at least one euploid blastocyst according to the relative contribution of each stimulation phase. The number of patients who could cumulatively obtain a euploid blastocyst increased from 18 (41.9%) of 43 to 30 (69.8%) of 43 when including the luteal phase-derived blastocysts. Twelve patients (27.9%) had euploid blastocysts exclusively after the luteal phase stimulation.

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accounted for 11.6%, 4.7%, 2.3%, and 4.7% of the population, respectively.

Two attempts of MII oocyte collections were unsuccessful, including one in the first and one in the second stimulation cycle. Fertilization was unsuccessful in three patients after the first stimulation cycle and in two after the second stimulation cycle. No blastocysts were obtained in 11 and 9 patients after the first and second stimulation cycles, respectively, including three patients who did not produce any blastocysts after both cycles. Eventually, one or more blastocysts were obtained in 31 and 33 patients from the first and second stimulation cycles, respectively (Supplemental Fig. 1).

Detailed comparative analysis of the first and second stimulation cycles (Table 1) did not reveal any difference in the length of stimulation or the mean number of cumulus-oocyte complexes, MII-phase oocytes retrieved, or fertilized eggs, as shown by paired *t* test analysis. Blastulation and euploid blastocyst rates per number of biopsied blastocysts or per MII injected oocytes were similar among FP and LP stimulations (Table 1). Additionally, no differences were found in the rates of excellent, good, average, or poor quality blastocysts, or in the percentage of blastocysts reaching full expansion on days 5, 6, and 7 in the two groups, respectively (Table 1).

Aneuploidy data were also consistent between blastocysts obtained after FP and LP stimulation, with also similar rates of single/double and complex aneuploidies obtained in the two different stimulation phases (Table 1). Eighteen and 23 patients produced at least one euploid blastocyst after the FP and LP stimulations, respectively (Fig. 1C; Supplemental Fig. 1). In 12 patients, euploid blastocysts were obtained only in the LP (Fig. 1C, Supplemental Fig. 1). Accordingly, the second stimulation in the LP increased the rate of patients with at least one possible euploid SET from 18 (41.9%) of 43 (a rate generally observed in matched poor-prognosis patients at our center) to 30 (69.8%) of 43 (Fig. 1C, Supplemental Fig. 1). In 12 (27.9%) of 43 patients more than a single euploid blastocyst was obtained after FP and/or LP stimulation cycles (Supplemental Fig. 1).

TABLE 1

Data according to follicular and luteal phase stimulation.

	Stimulation phase		
Data basis	**Follicular**	**Luteal**	***P* value**
Per patient[a]			
Days of stimulation	9.6 ± 2.4 (6–14)	10.3 ± 2.5 (8–15)	NS
COCs	5.1 ± 3.4 (1–22)	5.7 ± 3.3 (1–17)	NS
MII oocytes	3.4 ± 1.9 (0–10)	4.1 ± 2.5 (0–11)	NS
Fertilized oocytes	2.3 ± 1.7 (0–6)	3.2 ± 2.3 (0–10)	NS
Biopsied blastocysts	1.2 ± 1.2 (0–5)	1.4 ± 1.7 (0–9)	NS
Euploid blastocysts	0.6 ± 0.8 (0–3)	0.7 ± 0.8 (0–4)	NS
Per injected MII oocyte			
MII oocytes	142	173	
Fertilized oocytes	99 (69.7)	136 (78.6)	NS
Biopsied blastocysts	49 (34.5)	58 (33.5)	NS
Euploid blastocysts	23 (16.2)	26 (15.0)	NS
Per biopsied blastocyst			
Biopsied blastocysts	49	58	NS
Day of blastulation			
5	19 (38.8)	22 (37.9)	NS
6	29 (59.2)	36 (62.0)	NS
7	1 (2.0)	0	NS
Blastocyst quality			
Excellent	21 (42.8)	26 (44.8)	NS
Good	9 (18.4)	10 (17.2)	NS
Average	12 (24.5)	12 (20.7)	NS
Poor	7 (14.3)	10 (17.2)	NS
Aneuploidy			
Euploid	23 (46.9)	26 (44.8)	NS
Single/double aneuploid	18 (37.1)	25 (43.2)	NS
Complex aneuploid	8 (16.0)	7 (12.0)	NS

Note: Data are presented as number (%) unless otherwise indicated. Paired Student's *t* test was used to compare continuous variables, and Fisher's exact test for categorical ones. COC = cumulus-oocyte complex; MII = metaphase 2; NS = not statistically significant.
[a] Data are presented as mean ± standard deviation (range).

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Preliminary Clinical Outcomes

At the time of writing, 15 euploid blastocysts had been individually transferred during a single cryopreserved replacement cycle. Seven and eight embryos were derived from FP and LP stimulation, respectively. Five ongoing pregnancies (71.4%) were obtained with the transfer of blastocysts from FP stimulation and five (62.5%) with embryos obtained after LP stimulation (Table 2). In the latter only LP euploid blastocysts were available, thus confirming the significant contribution of LP stimulation to the pregnancy rate on a per menstrual cycle basis.

DISCUSSION

All infertility treatments aim to obtain a healthy baby—or establish the chance to have a healthy baby in the future—with the least possible physical and psychological distress, including shortening the length of required treatments. Time is important factor for all patients, but it is crucial for those with a foreseeable rapid loss/decrease of fertility, including a malignancy that requires gonadotoxic treatment or removal of gonads or in poor-prognosis patients. Although in the latter group the time frame is less constrained, reducing

TABLE 2

Preliminary clinical outcomes according to follicular or luteal phase stimulation.

	Stimulation phase		
Outcome	**Follicular**	**Luteal**	**Total**
No. of SET	7	8	15
No. of clinical pregnancies (%)	6 (85.7)	6 (75.0)	12 (80.0)
No. of miscarriages (%)	1 (16.7)	1 (16.7)	2 (16.7)
No. of ongoing pregnancies (%)	5 (71.4)	5 (62.5)	10 (66.7)

Note: SET, single-embryo transfers.

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the number of required stimulation cycles (preferably to one) to obtain an appropriate number of oocytes and subsequently transfer-quality embryos is a most desirable but very demanding goal. The incidence of poor responders in a routine infertility program is estimated to be between 9% and 24%, with a slight increase in the past decade (48).

Although various stimulation protocols and alternative approaches have been suggested, a consensus seems to form only for a few, including the application of GnRH antagonists and long-acting gonadotropins (48); however, an individual determination of the gonadotropin dose is strongly advised (49). In general, to obtain success in poor-responding patient is one of the most challenging tasks in assisted reproduction.

Our present approach to achieve advancement in this field was based on the following principles. The correlation between the number of obtained MII oocytes and the number of blastocysts developing from them may not be linear, and considerable individual variations may occur. Nevertheless, with the currently used stimulation treatments more oocytes mean a better chance to obtain transfer-quality blastocysts; and the higher the blastocyst number is, the better the chance to find euploid, developmentally fully competent ones (48, 49). The intrinsic handicap of poor responders restricts the number of retrievable oocytes. Accordingly, any new approach resulting in a significant increase in the number of retrieved oocytes during a specific time frame (a single menstrual cycle) would be a considerable achievement. Based on some recent findings about the feasibility of LP stimulation (24–29, 31), we tested double (i.e., FP and LP) stimulation in the same menstrual cycle for poor responders in a routine infertility program.

Our results proved that double stimulation (DuoStim) is successful for this cohort of patients, resulting in a similar number of MII phase oocytes after both FP and LP stimulation. The in vitro developmental competence to blastocyst stage was also similar, and with PGD-A no statistically significant difference in the proportion of euploid blastocysts derived from the two stimulation phases was found. These results confirm the feasibility of double-stimulation in a single menstrual cycle for poor responders.

Thus far, only one study has been published about DuoStim also for poor responder patients. Kuang et al. (39) reported a higher number of MII-phase oocytes and fertilized and cleaved embryos after LP versus FP stimulation, but no difference was found between the high-quality embryo and cryopreserved embryo numbers. Pregnancies were achieved from both FP and LP stimulation, but the limited numbers did not allow a statistical comparison. A remarkable difference between the work of Kuang et al. and our approach was that we used identical protocols for both the FP and LP stimulations. Our results regarding the efficiency of oocyte retrieval, in vitro embryo development, and the aneuploidy rate justify this simplified approach, although for any final conclusions, the pregnancy, birth and take-home baby rates, as well as detection of perinatal complications and postpartum anomalies will be indispensable (this work is in progress). For the latter, as already mentioned, promising data were published by Chen et al. (40), who did not find an elevated rate of birth defects after LP stimulation.

DuoStim obviously does not allow fresh embryo transfer. Cryopreservation of either oocytes or embryos (preferably blastocysts) is indispensable. With the introduction of a highly effective cryopreservation protocol for both human oocytes and blastocysts, the problem with logistics has been eliminated (50–53). Blastocyst biopsy is also the most efficient approach to select euploid embryos for transfer; by application of the proper embryo culture, biopsy, and vitrification techniques, the overall efficiency is not compromised (47, 54).

Possible application areas of the DuoStim approach include [1] all patients in whom obtaining oocytes is urgent, including those with malignant diseases or other medical indications, and [2] patients of advanced maternal age and/or reduced ovarian reserve. Additionally, if further studies prove that combined FP and LP stimulation increases the cumulative live birth rate, DuoStim together with a freeze-all strategy may be applied more extensively in the future. This approach clearly helps to save precious time for these difficult patient populations, and it also may be more cost-effective, depending on the center's policy. In our center, for example, the second oocyte retrieval was free of charge, and a significant reduction in the PGD-A cost was possible by cumulating embryos for a single genetic analysis.

It should also be noted that the discovery of similarities between humans and certain mammalian species may allow new models to be developed to study folliculogenesis and find improved models for controlled stimulation. By coincidence, cattle and horses, in which the most extensive knowledge about folliculogenesis and ovulation induction has been obtained, seem to be appropriate for the purpose. These species are monovular and polycyclic, with anatomic/pathologic conditions and ovary size similar to humans, allowing transrectal and transvaginal ultrasound investigations and serial blood collection (9, 55). Of the two species, horses seem to be the better model. In contrast to cattle where one or two major anovulatory waves occur during the menstrual cycle, in mares and humans only one major wave develops consistently (56, 57).

In conclusion, a similar number of euploid blastocysts per MII injected oocyte was obtained after FP and LP stimulation performed in the same menstrual cycle of patients with reduced ovarian response, respectively. The addition of LP stimulation significantly increased the final transferable blastocyst yield compared with FP stimulation alone. This novel stimulation strategy increased the number of patients with available euploid blastocysts within a single menstrual cycle and, in turn, the final clinical outcomes. This strategy may be applied in other situations where obtaining competent oocytes is an urgent task.

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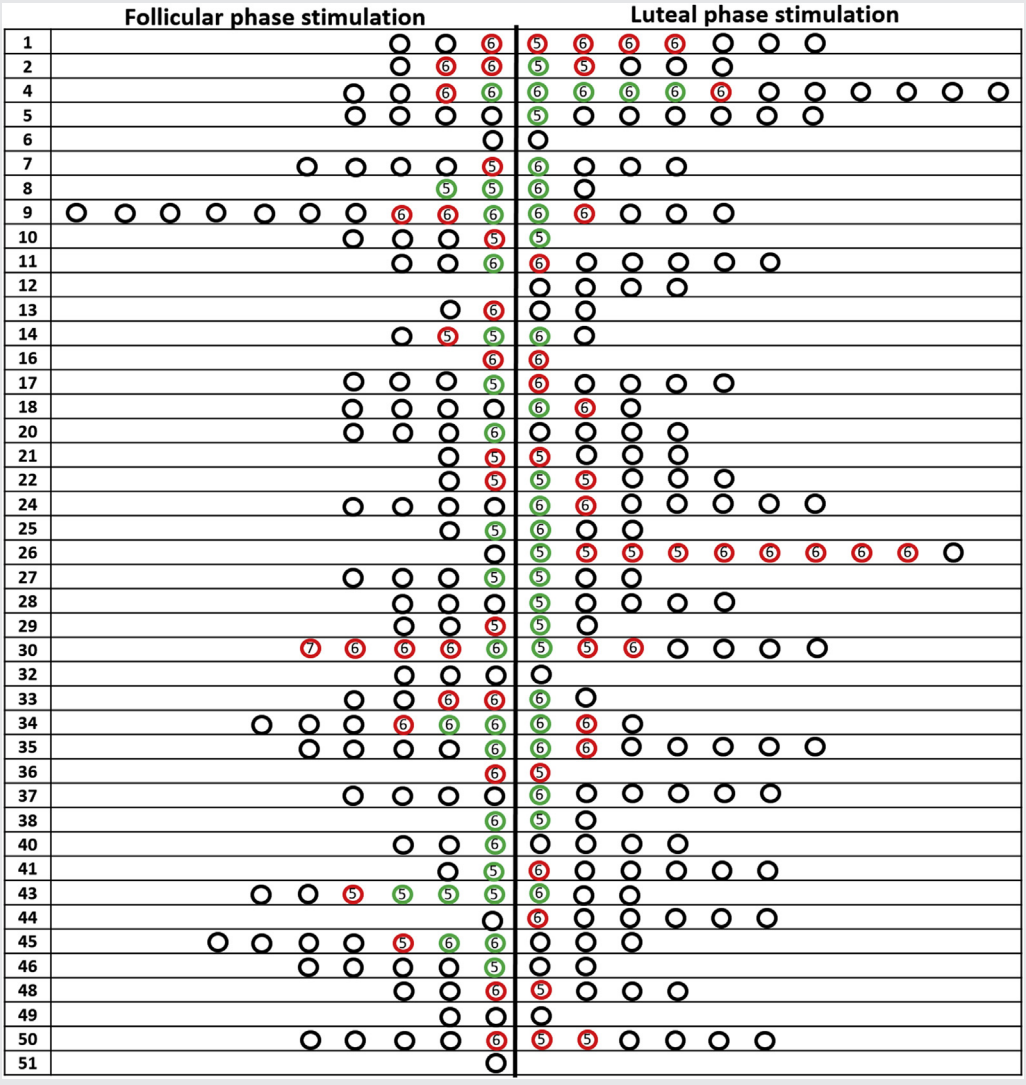
REFERENCES

1. Evans AC. Characteristics of ovarian follicle development in domestic animals. Reprod Domest Anim 2003;38:240–6.
2. Adamson GD. Global cultural and socioeconomic factors that influence access to assisted reproductive technologies. Womens Health (Lond Engl) 2009;5:351–8.
3. Adams GP. Comparative patterns of follicle development and selection in ruminants. J Reprod Fertil Suppl 1999;54:17–32.

4. McCorkell RB, Woodbury MR, Adams GP. Serial ovarian ultrasonography in wild-caught wood bison (*Bison bison athabascae*). Theriogenology 2013; 80:552–6.
5. McCorkell R, Woodbury M, Adams GP. Ovarian follicular and luteal dynamics in wapiti during the estrous cycle. Theriogenology 2006;65:540–56.
6. Coutinho LN, de Brito MB, Monteiro FO, de Andrade RS, da Conceição ME, Feliciano MR, et al. Analysis of follicular events in owl monkeys (*Aotus azarai infulatus*) using B-mode and Doppler ultrasound. Theriogenology 2013;80: 99–103.
7. Skidmore JA, Adams GP, Billah M. Synchronisation of ovarian follicular waves in the dromedary camel (*Camelus dromedarius*). Anim Reprod Sci 2009;114:249–55.
8. Adams GP, Sumar J, Ginther OJ. Effects of lactational and reproductive status on ovarian follicular waves in llamas (*Lama glama*). J Reprod Fertil 1990; 90:535–45.
9. Adams GP, Singh J, Baerwald AR. Large animal models for the study of ovarian follicular dynamics in women. Theriogenology 2012;78:1733–48.
10. Pache TD, Wladimiroff JW, de Jong FH, Hop WC, Fauser BC. Growth patterns of nondominant ovarian follicles during the normal menstrual cycle. Fertil Steril 1990;54:638–42.
11. Gougeon A. Dynamics of follicular growth in the human: a model from preliminary results. Hum Reprod 1986;1:81–7.
12. de Mello Bianchi PH, Serafini P, Monteiro da Rocha A, Assad Hassun P, Alves da Motta EL, Sampaio Baruselli P, et al. Review: follicular waves in the human ovary: a new physiological paradigm for novel ovarian stimulation protocols. Reprod Sci 2010;17:1067–76.
13. McNatty KP, Hillier SG, van den Boogaard AM, Trimbos-Kemper TC, Reichert LE, van Hall EV. Follicular development during the luteal phase of the human menstrual cycle. J Clin Endocrinol Metab 1983;56:1022–31.
14. Baerwald AR, Adams GP, Pierson RA. Characterization of ovarian follicular wave dynamics in women. Biol Reprod 2003;69:1023–31.
15. Baerwald AR, Adams GP, Pierson RA. A new model for ovarian follicular development during the human menstrual cycle. Fertil Steril 2003;80: 116–22.
16. Mikolajczyk RT, Frank-Herrmann P, Freundl G, Stanford JB. More than one fertile ovulation per cycle? Fertil Steril 2004;81:728–9.
17. Wettstein RH. More than one fertile ovulation per cycle? Fertil Steril 2004; 81:729.
18. Baird DD. More than one fertile ovulation per cycle? Fertil Steril 2004; 81:729.
19. McDonough PG. More than one fertile ovulation per cycle? Fertil Steril 2004; 81:729–30.
20. Kuleshova L, Gianaroli L, Magli C, Ferraretti A, Trounson A. Birth following vitrification of a small number of human oocytes: case report. Hum Reprod 1999;14:3077–9.
21. Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod Biomed Online 2005;11:300–8.
22. Son WY, Park SJ, Hyun CS, Lee WD, Yoon SH, Lim JH. Successful birth after transfer of blastocysts derived from oocytes of unstimulated woman with regular menstrual cycle after IVM approach. J Assist Reprod Genet 2002; 19:541–3.
23. Demirtas E, Elizur SE, Holzer H, Gidoni Y, Son WY, Chian RC, et al. Immature oocyte retrieval in the luteal phase to preserve fertility in cancer patients. Reprod Biomed Online 2008;17:520–3.
24. Maman E, Meirow D, Brengauz M, Raanani H, Dor J, Hourvitz A. Luteal phase oocyte retrieval and in vitro maturation is an optional procedure for urgent fertility preservation. Fertil Steril 2011;95:64–7.
25. von Wolff M, Thaler CJ, Frambach T, Zeeb C, Lawrenz B, Popovici RM, et al. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. Fertil Steril 2009;92:1360–5.
26. Bedoschi GM, de Albuquerque FO, Ferriani RA, Navarro PA. Ovarian stimulation during the luteal phase for fertility preservation of cancer patients: case reports and review of the literature. J Assist Reprod Genet 2010;27:491–4.
27. Sönmezer M, Türkçüoğlu I, Coşkun U, Oktay K. Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles. Fertil Steril 2011;95:2125.e9–11.
28. Nayak SR, Wakim AN. Random-start gonadotropin-releasing hormone (GnRH) antagonist-treated cycles with GnRH agonist trigger for fertility preservation. Fertil Steril 2011;96:e51–4.
29. Ozkaya E, San Roman G, Oktay K. Luteal phase GnRHa trigger in random start fertility preservation cycles. J Assist Reprod Genet 2012;29:503–5.
30. Cakmak H, Katz A, Cedars MI, Rosen MP. Effective method for emergency fertility preservation: random-start controlled ovarian stimulation. Fertil Steril 2013;100:1673–80.
31. Martínez F, Clua E, Devesa M, Rodríguez I, Arroyo G, González C, et al. Comparison of starting ovarian stimulation on day 2 versus day 15 of the menstrual cycle in the same oocyte donor and pregnancy rates among the corresponding recipients of vitrified oocytes. Fertil Steril 2014;102: 1307–11.
32. Hwang JL, Lin YH, Tsai YL. Pregnancy after immature oocyte donation and intracytoplasmic sperm injection. Fertil Steril 1997;68:1139–40.
33. Pape O, Winer N, Paumier A, Philippe H-J, Flatrès B, Boog G. [Superfetation: case report and review of the literature]. J Gynecol Obstet Biol Reprod 2008; 37:791–5.
34. Girela E, Lorente JA, Alvarez JC, Rodrigo MD, Lorente M, Villanueva E. Indisputable double paternity in dizygous twins. Fertil Steril 1997;67:1159–61.
35. Tarín JJ, Hamatani T, Cano A. Acute stress may induce ovulation in women. Reprod Biol Endocrinol 2010;8:53.
36. Bentov Y, Esfandiari N, Gokturk A, Burstein E, Fainaru O, Casper RF. An ongoing pregnancy from two waves of follicles developing during a long follicular phase of the same cycle. Fertil Steril 2010;94:350.e8–11.
37. Kuang Y, Quiuju C, Qinquing H, Qifeng L, Yonglun F, Ai A, et al. Luteal-phase ovarian stimulation case report: three-year follow-up of a twin birth. J IVF Reprod Med Genet 2013;1:1–3.
38. Kuang Y, Hong Q, Chen Q, Lyu Q, Ai A, Fu Y, et al. Luteal-phase ovarian stimulation is feasible for producing competent oocytes in women undergoing in vitro fertilization/intracytoplasmic sperm injection treatment, with optimal pregnancy outcomes in frozen-thawed embryo transfer cycles. Fertil Steril 2014;101:105–11.
39. Kuang Y, Chen Q, Hong Q, Lyu Q, Ai A, Fu Y, et al. Double stimulations during the follicular and luteal phases of poor responders in IVF/ICSI programmes (Shanghai protocol). Reprod Biomed Online 2014;29: 684–91.
40. Chen H, Wang Y, Lyu Q, Ai A, Fu Y, Tian H, et al. Comparison of live-birth defects after luteal-phase ovarian stimulation vs. conventional ovarian stimulation for in vitro fertilization and vitrified embryo transfer cycles. Fertil Steril 2015;103:1194–201.e2.
41. Moffat R, Pirtea P, Gayet V, Wolf JP, Chapron C, de Ziegler D. Dual ovarian stimulation is a new viable option for enhancing the oocyte yield when the time for assisted reproductive technology is limited. Reprod Biomed Online 2014;29:659–61.
42. Rienzi L, Ubaldi FM, Iacobelli M, Minasi MG, Romano S, Ferrero S, et al. Significance of metaphase II human oocyte morphology on ICSI outcome. Fertil Steril 2008;90:1692–700.
43. Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. Toward reproductive certainty: fertility and genetics beyond. Carnforth, United Kingdom: Parthenon; 1999:378–88.
44. Cobo A, Bellver J, Domingo J, Pérez S, Crespo J, Pellicer A, et al. New options in assisted reproduction technology: the Cryotop method of oocyte vitrification. Reprod Biomed Online 2008;17:68–72.
45. Capalbo A, Rienzi L, Cimadomo D, Maggiulli R, Elliott T, Wright G, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. Hum Reprod 2014;29:1173–81.
46. Capalbo A, Treff NR, Cimadomo D, Tao X, Upham K, Ubaldi FM, et al. Comparison of array comparative genomic hybridization and quantitative real-time PCR-based aneuploidy screening of blastocyst biopsies. Eur J Hum Genet 2015;23:901–6.
47. Ubaldi FM, Capalbo A, Colamaria S, Ferrero S, Maggiulli R, Vajta G, et al. Reduction of multiple pregnancies in the advanced maternal age population after implementation of an elective single embryo transfer policy coupled with enhanced embryo selection: pre- and post-intervention study. Hum Reprod 2015;30:2097–106.

48. Ubaldi F, Vaiarelli A, D'Anna R, Rienzi L. Management of poor responders in IVF: is there anything new? Biomed Res Int 2014;2014:352098.
49. La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: From theory to practice. Hum Reprod Update 2014;20:124–40.
50. Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, et al. Embryo development of fresh 'versus' vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod 2010; 25:66–73.
51. Ubaldi F, Anniballo R, Romano S, Baroni E, Albricci L, Colamaria S, et al. Cumulative ongoing pregnancy rate achieved with oocyte vitrification and cleavage stage transfer without embryo selection in a standard infertility program. Hum Reprod 2010;25:1199–205.
52. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, et al. Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence. Hum Reprod Update 2014;20: 808–21.
53. Kalampokas T, Sofoudis C, Boutas I, Aravantinos L, Kalampokas E, Deligeoroglou E. IVF/ICSI frozen replacement cycles; every cycle? Opinion expressed after a systematic review of the literature. Clin Exp Obstet Gynecol 2015;42:173–5.
54. Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril 2013; 100:624–30.
55. Ginther OJ. The mare: a 1000-pound guinea pig for study of the ovulatory follicular wave in women. Theriogenology 2012;77:818–28.
56. Jacob JC, Gastal EL, Gastal MO, Carvalho GR, Beg MA, Ginther OJ. Temporal relationships and repeatability of follicle diameters and hormone concentrations within individuals in mares. Reprod Domest Anim 2009; 44:92–9.
57. Ginther OJ, Knopf L, Kastelic JP. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. J Reprod Fertil 1989;87:223–30.

SUPPLEMENTAL FIGURE 1



Graphic representation of the 43 patients included in both follicular and luteal phase stimulation. Each *row* represents a single patient. Each *circle* represents a metaphase 2 (MII) oocyte. The *black circles* represent MII oocytes that did not reach the blastocyst stage after intracytoplasmic sperm injection (ICSI). The *red circles* represent MII oocytes that developed as aneuploid blastocysts after ICSI. The *green circles* represent MII oocytes that developed as euploid blastocysts after ICSI. The *numbers within circles* represent the day of blastocyst development.

Ubaldi. DuoStim for reduced ovarian reserve. Fertil Steril 2016.