Follicular waves during the natural cycle: rationale for double ovarian stimulation

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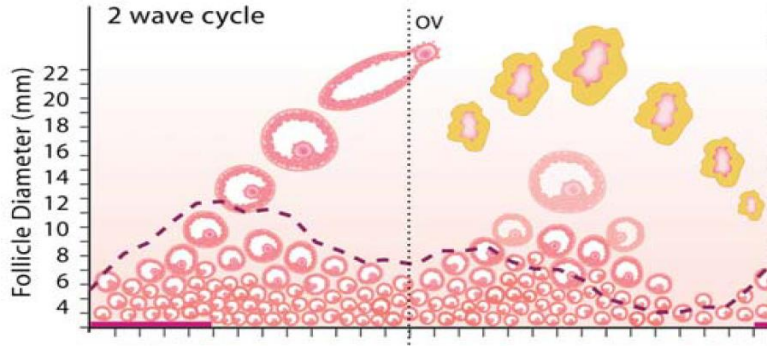
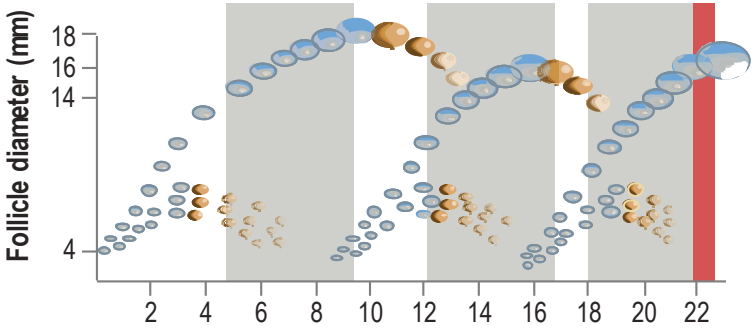
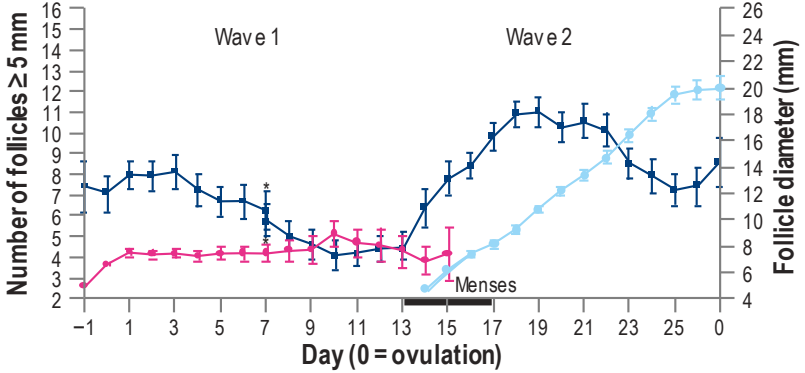
Educational objectives

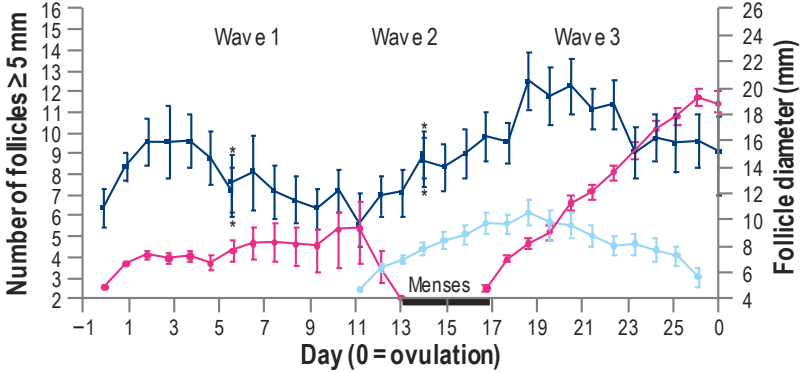
- Describe the physiology of the development of multiple follicular waves during the menstrual cycle
- Discuss the rationale for new ovarian stimulation protocols in increasing the number of available oocytes
- Assess the evidence and clinical considerations for double ovarian stimulation as a useful strategy for improving patient outcomes

Folliculogenesis in an ovarian cycle





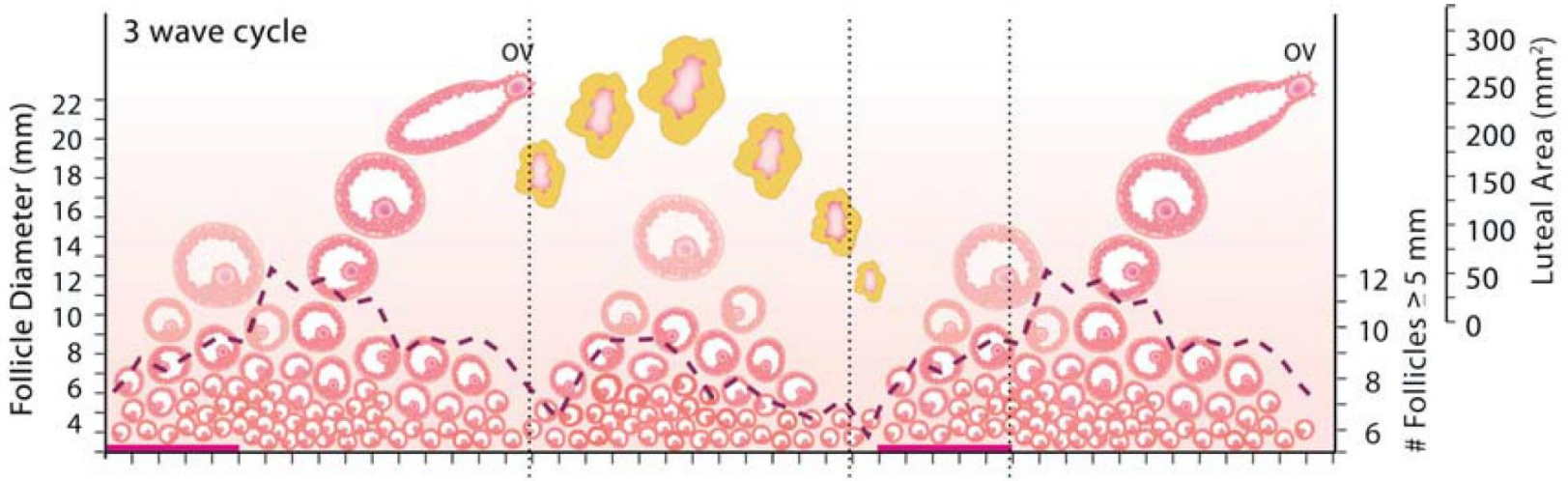




■ Number of follicles > 5 mm ■ Diameter of the largest follicle

Adams GP, et al. J Reprod Fertil. 1992J;94:177-88.
Baerwald AR, et al. Hum Reprod Update. 2012;18:73-91.
Baerwald AR, et al. Fertil Steril Fertil Steril. 2003;80:116-22.

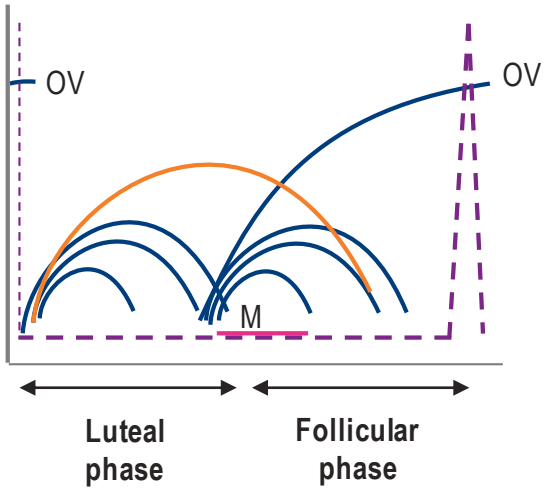
Folliculogenesis in an ovarian cycle



M, menses; OV, ovulation.

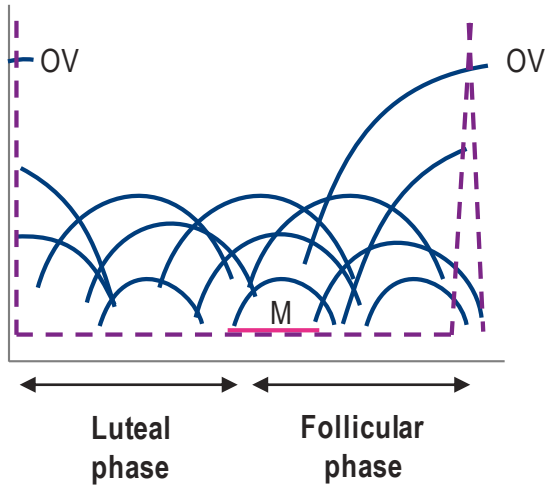
Baerwald AR, et al. Hum Reprod Update. 2012;18:73-91.

Theories on folliculogenesis in an ovarian cycle



"Waves theory"

Two or three cohorts of antral follicles are recruited per ovarian cycle



"Continuous recruitment theory"

Follicles start growing and regress continuously during the ovarian cycle

The mechanisms regulating each individual cohort of follicles are not yet fully understood

M, menses; OV, ovulation.

Baerwald AR, et al. Hum Reprod Update. 2012;18:73-91.

Scepticism has arisen...

More than one fertile ovulation per cycle?

To the Editor:

Baerwald et al. (1) proposed a new model for ovarian follicular development in the human menstrual cycle. They showed additional waves of follicular development in the follicular and luteal phase of normal ovulatory cycles. Although no additional ovulation was observed, the authors speculated that the anovulatory follicles from the additional waves of follicular development may be able to ovulate in the presence of an additional LH surge.

We point out that the bulk of evidence suggests that a second ovulation does not play any clinical role in natural cycles. Among more than 30,000 cycles in users of modern natural family planning methods studied at the research center in Duesseldorf, no pregnancy occurred when intercourse took place during the luteal phase, as diagnosed by elevated basal body temperature and evaluation of cervical mucus. This is consistent with findings from effectiveness studies of the symptothermal method of natural family planning showing that when intercourse occurs outside the fertile window (as determined by the above signs), the probability of pregnancy is far less than 0.1% per cycle (2, 3).

In an evaluation of 1,681 cycles with a standardized mucus-only system of natural family planning (the Creighton Model System), no evidence of conception was found outside a fertile window of 6 days before and 4 days after the mucus peak day, a clinical marker of ovulation (4). Each of these recent publications cites many older studies with similar findings.

For anecdotal evidence of unplanned "late" pregnancies during the use of the calendar method, delayed ovulation is a much more plausible explanation than an additional ovulation. Delayed ovulation is relatively frequent (5).

Although the study by Baerwald et al. (1) provides some interesting insights on ovarian function, the authors clearly failed to consider a large volume of existing evidence in the interpretation and presentation of their findings.

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To the Editor:

The article by Baerwald and colleagues (1) has been a lightning rod for controversy. A major component of the controversy has been the expectations generated by the media coverage and the difficulties in interpreting the waveform data.

The data from this dynamic, serial ultrasonographic study of follicular diameters and wave patterns in 63 patients does not seem to live up to the media hype. Of the 63 participants, 50 had normal biphasic cycles and could thus be included. Thirteen patients were excluded because of deviations in cycle duration that were considered to be outside the range of normal ovulation. Of the remaining 50 patients, 34 exhibited two waves of follicular development and 16 patients had three waves of follicular diameter. However, it was always the last wave that resulted in a dominant follicle and ovulation. It is not surprising that the women ovulated during the last wave of follicular growth. This wave is under the influence of LH, whereas the first wave is under the domination of P, which stops ovulation altogether until the end of the cycle.

This new research serves to confirm what we already knew: Follicular development during the luteal phase is regularly observed by women using the symptothermal method. During this time, they usually note 1 day of cervical fluid increase and a short decrease in basal body temperature.

Contrary to reports in the press of this article, women experience only one fertile ovulation per cycle. The still widely unknown safety and usefulness of the symptothermal method of birth regulation remains unchanged.

R. Hanri Wettstein, Ph.D., M.A., M.D.A.
Symptotherm Foundation
Morges, Switzerland
September 8, 2003

The dynamics of folliculogenesis introduced new stimulation regimens

Random start approach:

COH can be started at any time during the ovarian cycle (in a setting for urgent fertility preservation)

Von Wolff M, et al. 2009; Sonmezer M, et al. 2011; Nayak SR, et al. 2011; Ozkaya E, et al. 2012; Cakmak H, et al. 2013.

Luteal phase stimulation:

COH can be started between Day 17 and Day 21 of a spontaneous ovarian cycle

Bentov, et al. 2010; Buendgen, et al. 2013; Martínez, et al. 2014; Zhag, et al. 2015; Wang, et al. 2016; Li, et al. 2016; Qin, et al. 2016; Boots, et al. 2016; Wang N, et al. 2016; WY, et al. 2017.

DuoStim (double stimulation in 1 ovarian cycle):

Combination of FP stimulation and LP stimulation in poor prognosis patients (advanced maternal age, POR)

Kuang Y, et al.2014; Ubaldi, et al. 2016; Liu, et al. 2017; Vaiarelli A, et al. 2017, 2018; Cimadomo D, et al. 2018.

COH, controlled ovarian hyperstimulation; FP, follicular phase; LP, luteal phase; POR, poor ovarian reserve.

Luteal phase stimulation was proposed for urgent tasks, such as fertility preservation for oncology patients…

All Gynecologic Cancers

Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase

Michael von Wolff, M.D.,[a] Christian J. Thaler, M.D.,[b] Torsten Frambach, M.D.,[c] Cosima Zeeb, M.D.,[e] Barbara Lawrenz, M.D.,[d] Roxana M. Popovici, M.D.,[a] and Thomas Strowitzki, M.D.[a]

Fertility and Sterility® Vol. 92, No. 4, October 2009

Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles

Murat Sönmezer, M.D.,[a,b] Ilgın Türkçüoğlu, M.D.,[c] Uğur Coşkun, M.D.,[d] and Kutluk Oktay, M.D.[e]

Fertility and Sterility® Vol. 95, No. 6, May 2011

Random-start gonadotropin-releasing hormone (GnRH) antagonist–treated cycles with GnRH agonist trigger for fertility preservation

Shweta R. Nayak, M.D., and Anthony N. Wakim, M.D.

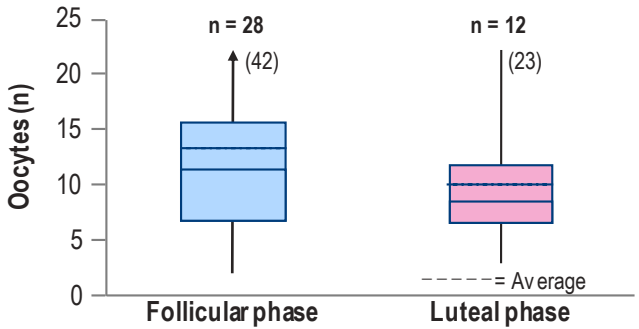
Fertility and Sterility® Vol. 96, No. 1, July 2011

Effective method for emergency fertility preservation: random-start controlled ovarian stimulation

Hakan Cakmak, M.D., Audra Katz, R.N., Marcelle I. Cedars, M.D., and Mitchell P. Rosen, M.D.

Fertility and Sterility® Vol. 100, No. 6, December 2013

Number of oocytes collected after initiation of OS in the follicular vs luteal phase



Comparison of outcomes of conventional and random start COS cycles

	Conventional start (n = 88; 103 cycles)	Random start (n = 35; 35 cycles)	p value	Late follicular phase start (n = 13; 13 cycles)	Luteal phase start (n = 22; 22 cycles)	p value
AFC	13.0	11.5	NS	10.5	12.1	NS
Days of OS	9.3	10.9	< 0.001	10.5	11.2	< 0.001
Total dose of gonadotropins (IU)	3,404	4,158	0.001	3,842	4,344	0.005
Gonadotropin daily dose (IU/d)	361	372	NS	371	373	NS
Follicles ≥ 13 mm	10.5	11.8	NS	10.9	12.3	NS
Oocytes retrieved	14.4	14.5	NS	13.0	15.5	NS
Mature oocytes (MII) retrieved	9.7	9.9	NS	9.1	10.3	NS
MII oocytes/total oocytes ratio	0.66	0.67	NS	0.68	0.67	NS
Oocytes/AFC ratio	1.09	1.26	NS	1.24	1.28	NS
Mature oocytes/AFC	0.73	0.85	NS	0.84	0.86	NS
Fertilization rate after ICSI (2PN/MII)	0.72	0.87	NS	0.85	0.88	NS

2PN, 2 pronuclei; AFC, antral follicle count; ICSI, intracytoplasmic sperm injection; MII, metaphase II; NS, not significant; OS, ovarian stimulation.

von Wolff M, et al. Fertil Steril. 2009;92:1360-5. Cakmak H, et al. Fertil Steril. 2013;100:1673-80.

And then, live births were reported from poor prognosis patients...

An ongoing pregnancy from two waves of follicles developing during a long follicular phase of the same cycle

Yaakov Bentov, M.D., M.Sc.,[a,b,c] *Navid Esfandiari, D.V.M., Ph.D., H.C.L.D.,*[a,b] *Asli Gokturk, M.Sc.,*[a] *Eliezer Burstein, M.D.,*[a,b,c] *Ofer Fainaru, M.D., Ph.D.,*[a,b,c] *and Robert F. Casper, M.D., F.R.C.S.C.*[a,b,c]

Fertility and Sterility® Vol. 94, No. 1, June 2010

Objective: To report an ongoing pregnancy after in vitro fertilization (IVF) with ovarian stimulation using a gonadotropin-releasing hormone (GnRH) antagonist that resulted in two waves of follicular growth.
Design: Case report.
Setting: University of Toronto affiliated infertility clinic.
Patient(s): A 33-year-old woman with a 3-year history of secondary infertility.
Intervention(s): In vitro fertilization and embryo transfer.
Main Outcome Measure(s): Ongoing pregnancy.
Result(s): This patient successfully conceived after the GnRH antagonist-induced demise of the first cohort of follicles and the emergence of a second wave of follicles followed by oocyte retrieval on cycle day 30 and fresh embryo transfer.
Conclusion(s): This case report is consistent with previous observations of multiple waves of follicle recruitment and growth per cycle. The window of implantation may not be adversely affected by prolonged or even variable estrogen exposure in the follicular phase of the cycle. (Fertil Steril® 2010;94:350.e8–e11. ©2010 by American Society for Reproductive Medicine.)
Key Words: In vitro fertilization, ovarian stimulation, follicular growth

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

Yanping Kuang, M.D.,[a] Qingqing Hong, M.D.,[a] Qiuju Chen, Ph.D.,[a] Qifeng Lyu, Ph.D.,[a] Ai Ai, M.D.,[a] Yonglun Fu, M.D.,[a] and Zeev Shoham, M.D.[b]

Fertility and Sterility® Vol. 101, No. 1, January 2014

Pregnancy outcomes from frozen-thawed embryos originating from OS during the luteal phase

	First FETs	Second and third FETs	Total FETs
Thawed cycles, n	173	56	229
Thawed embryos, n	2.0	1.9	1.9
Thawed survival rate, %	97.4	91.6	96.0
Thawed highest-quality embryos, n	1.8	1.7	1.8
Endometrial thickness, mm	12.4	12.1	12.3
Pregnancy outcome of FET			
Positive hCG rate per transfer, %	63.0	58.9	62.0
Clinical pregnancy rate per transfer, %	59.0	55.6	55.5
Implantation rate, %	43.9	28.7	40.4
First trimester miscarriage rate, %	4.9	25.0	7.9
Second trimester miscarriage, %	1.0	0.0	0.8
Multiple pregnancy rate, %	42.2	16.0	37.0
Ectopic pregnancy rate, %	3.9	0.0	3.2
Ongoing pregnancy rate per transfer, %	53.2	35.7	48.9
Cumulative pregnancy rate per cycle initiated, %			64.7

FET, frozen embryo transfer; hCG, human chorionic gonadotropin.

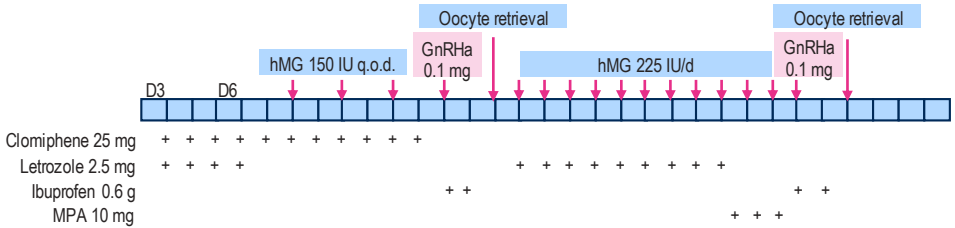
Bentov Y, et al. Fertil Steril. 2010;94:350.e8-11.
Kuang Y, et al. Fertil Steril. 2014;101:105-11.

Double stimulation in POR (Shanghai protocol)

Double stimulations during the follicular and luteal phases of poor responders in IVF/ICSI programmes (Shanghai protocol)

Yanping Kuang [a,b,*], Qiuju Chen [a,b], Qingqing Hong [a,b], Qifeng Lyu [a,b], Ai Ai [a,b], Yonglun Fu [a,b], Zeev Shoham [c]

The protocol of double stimulation during the follicular and luteal phases in patients with POR



Cryopreserved ET cycle outcomes using embryos derived from double stimulation in patients with POR

	Total	Embryos from first oocyte retrieval	Embryos from second oocyte retrieval	Two embryos from two oocyte retrievals
Patients, n	21	12	6	3
Cryopreserved embryo transfer cycles n	23	13	7	3
Embryos warmed, n	43	22	15	6
Embryo transferred, n	41	21	14	6
Embryo survival rate, n/N (%)	41/43 (95.3)	21/22 (95.5)	14/15 (93.3)	6/6 (100)
Clinical pregnancy rate, n/N (%)	13/23 (56.5)	8/13 (61.5)	5/7 (71.4)	0/3
Implantation rate, n/N (%)	15/41 (36.6)	10/21 (47.6)	5/14 (35.7)	0/6
Spontaneous abortion rate, n/N (%)	2/13 (15.4)	1/8 (12.5)	1/5 (20.0)	0
Ongoing pregnancy rate, n/N (%)	11/23 (47.8)	7/13 (53.8)	4/7 (57.1)	0/3

Basic characteristics of patients with POR (n = 38)

Parameter	Values
Mean age, years ± SD	36.4 ± 5.0
Mean BMI, kg/m^2 ± SD	22.6 ± 3.7
Mean infertility duration, years ± SD	4.4 ± 3.8
Mean basal FSH, IU/L ± SD	6.9 ± 2.3
Mean AFC in follicular phase ± SD	3.8 ± 1.8
Primary infertility, n/N (%)	24/38 (63.2)
Secondary infertility, n/N (%)	14/38 (36.8)
Previous IVF failure, n/N (%)	
0	12/38 (31.6)
1–2	15/38 (39.5)
≥ 3	11/38 (28.9)

BMI, body mass index; ET, embryo transfer; FSH, follicle-stimulating hormone; GnRHa, gonadotrophin-releasing hormone agonist; HMG, human menopausal gonadotrophin; IVF, in vitro fertilization; MPA, medroxyprogesterone acetate; q.o.d., every other day; SD, standard deviation.

Kuang Y, et al. Reprod Biomed Online. 2014;29:684-91.

No differences between clinical outcomes: FPS vs LPS in a donation programme…

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

Francisca Martínez, Ph.D., Elisabet Clua, B.Sc., Marta Devesa, M.D., Ignacio Rodríguez, B.Sc., Gemma Arroyo, B.Sc., Clara González, B.Sc., Miquel Solé, B.Sc., Rosa Tur, Ph.D., Buenaventura Coroleu, Ph.D., and Pedro N. Barri, Ph.D.

Characteristics of the oocyte recipients

Reception cycles, n	R-D15 (n = 12)	R-D2 (n = 8)
Recipient age, years	43.92	44.13
Donated oocytes, n	8.75	8.38
Inseminated oocytes, n	8.50	8.50
Fertilization rate, %	76.47	73.33
Transferred embryos, n	1.67	1.50
Quality of transferred embryos	8.50	8.50
Cryopreserved embryos, n	3.08	2.88
Pregnancies, n (pregnancy rate/transfer)	7 (58.3%)	5 (62.5%)

FPS, FP stimulation; LPS, LP stimulation;
R-D2, recipients of oocytes after Day-2 donor-stimulation start; R-D15, recipient of oocytes after Day-15 donor-stimulation start.

Martinez F, et al. Fertil Steril. 2014;102:1307-11.

Physicians started to be less sceptical…

Reproductive BioMedicine Online (2014) 29, 659–661

www.sciencedirect.com
www.rbmonline.com

ELSEVIER

COMMENTARY

Dual ovarian stimulation is a new viable option for enhancing the oocyte yield when the time for assisted reproductive technnology is limited

Rebecca Moffat [a], Paul Pirtea [a], Vanessa Gayet [a], Jean Philippe Wolf [b], Charles Chapron [a,c], Dominique de Ziegler [a,*]

Kuang's data (2014b) and our findings indicate that a dual back-to-back ovarian stimulation protocol is a viable option for coping with the insufficient ovarian responses that are sometimes encountered in ART. Moreover, the short overall duration of these approaches (<30 days) is valuable for cases of fertility preservation. Indeed, the DPX approach permits coping with the time constraints of fertility preservation and the desire to accumulate the largest number of oocytes possible.

DPX, Duplex protocol.

Moffat R, et al. Reprod Biomed Online. 2014;29:659-61.

DuoStim in patients with POR/poor prognosis

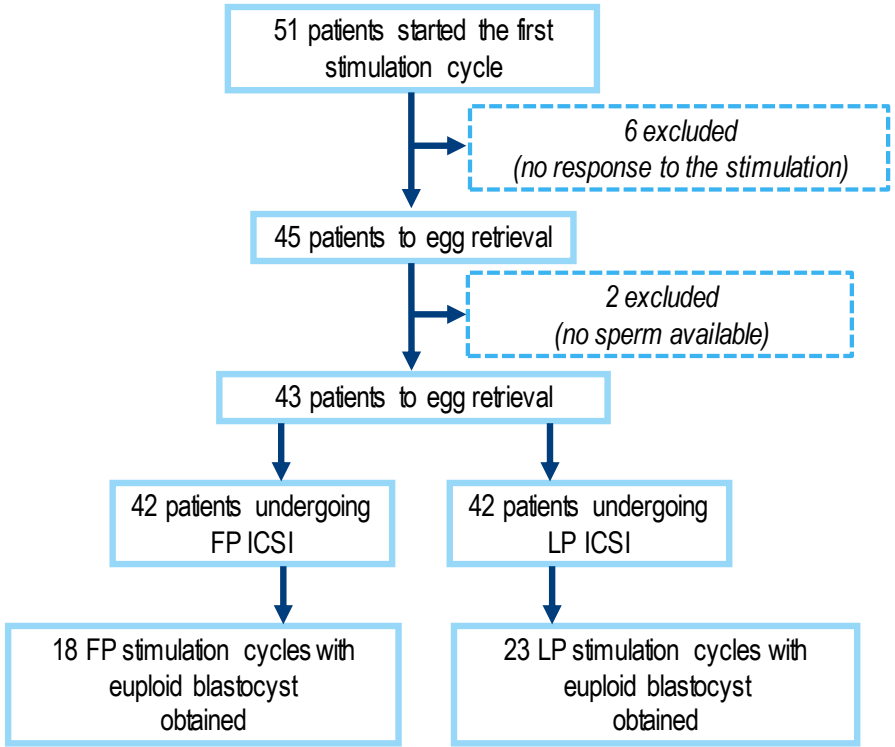
Fertility and Sterility® Vol. 105, No. 6, June 2016

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

Filippo Maria Ubaldi, M.D., M.Sc.,[a,b,c] Antonio Capalbo, Ph.D.,[a,b,c] Alberto Vaiarelli, M.D., Ph.D.,[a,b] Danilo Cimadomo, M.Sc.,[a,b,d] Silvia Colamaria, M.D.,[a,b] Carlo Alviggi, M.D., Ph.D.,[d,e] Elisabetta Trabucco, M.D.,[a,b] Roberta Venturella, M.D.,[a,b,f] Gábor Vajta, Ph.D.,[g,h] and Laura Rienzi, M.Sc.[a,b,c]

51 patients with POR (AMH ≤1,5 ng/mL, AFC ≤ 6 follicles and/or ≤ 5 oocyte retrieved in previous COH) undergoing ICSI treatment and PGT-A

Primary outcome measure
Euploid blastocyst rate
Secondary outcome measures
Number of retrieved COCs and MII oocytes



AMH, anti-Müllerian hormone; COC, cumulus-oocyte-complex; PGT-A, preimplantation genetic testing for aneuploidy.

Ubaldi FM, et al. Fertil Steril. 2016;105:1488-95.

DuoStim in patients with POR/poor prognosis

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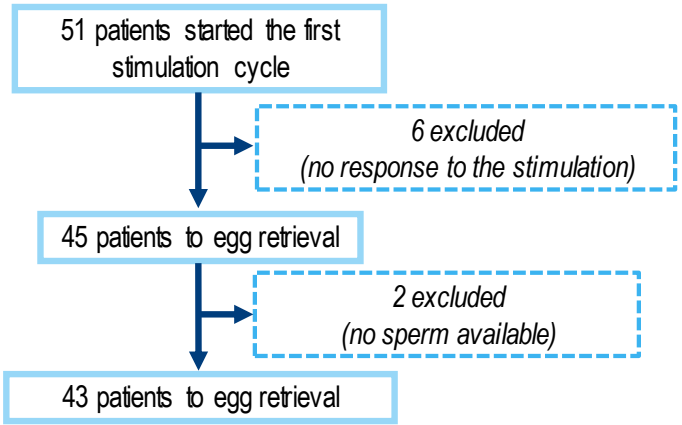
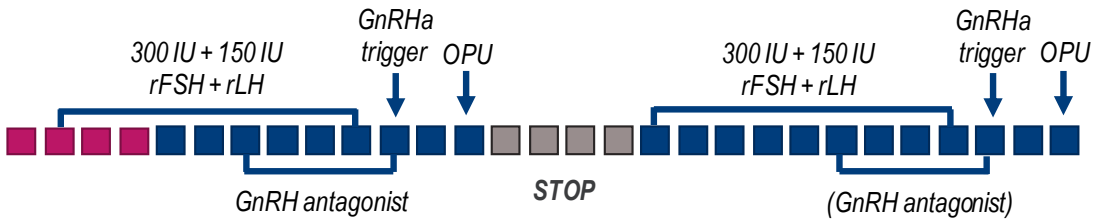
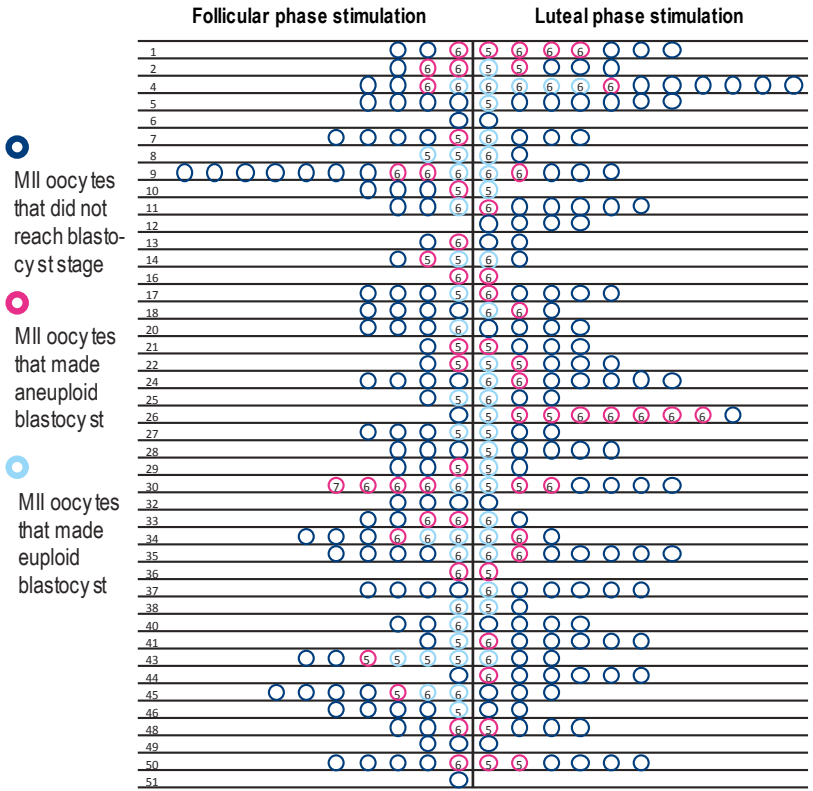




FSH, follicle-stimulating hormone; LH, luteinizing hormone; OPU, oocyte pick-up rate; rFSH, recombinant FSH; rLH, recombinant LH.

Ubaldi FM, et al. Fertil Steril. 2016;105:1488-95.

Large intraovarian follicle wave variability: similar laboratory results for FPS and LPS

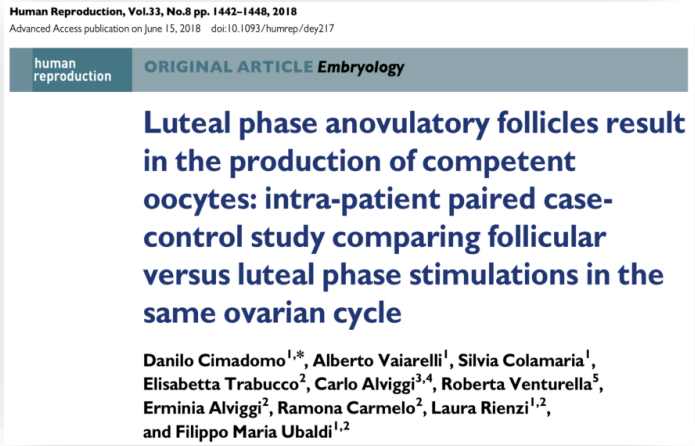


Data according to follicular and luteal phase stimulation

	Stimulation phase		
Data basis	**Follicular**	**Luteal**	**p value**
Per patient			
Days of stimulation	9.6	10.3	NS
COCs	5.1	5.7	NS
MII oocytes	3.4	4.1	NS
Fertilized oocytes	2.3	3.2	NS
Biopsied blastocysts	1.2	1.4	NS
Euploid blastocysts	0.6	0.7	NS
Per injected MII oocyte			
MII oocytes	142	173	
Fertilized oocytes	99	136	NS
Biopsied blastocysts	49	58	NS
Euploid blastocysts	23	26	NS
Per biopsied blastocyst			
Biopsied blastocysts	49	58	NS
Day of blastulation			
5	19	22	NS
6	29	36	NS
7	1	0	NS
Blastocyst quality			
Excellent	21	26	NS
Good	9	10	NS
Average	12	12	NS
Poor	7	10	NS
Aneuploidy			
Euploid	23	26	NS
Single/double aneuploid	18	25	NS
Complex aneuploid	8	7	NS

Ubaldi FM, et al. Fertil Steril. 2016;105:1488-95.

Similar oocyte competence after FPS and LPS



Human Reproduction, Vol.33, No.8 pp. 1442–1448, 2018
Advanced Access publication on June 15, 2018 doi:10.1093/humrep/dey217

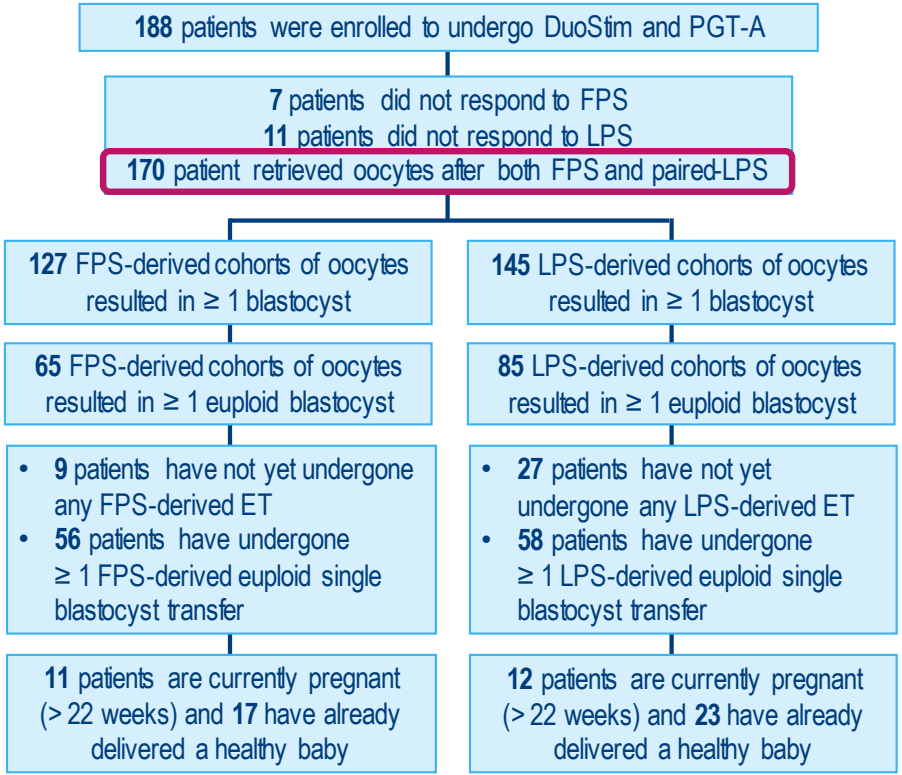
human reproduction ORIGINAL ARTICLE *Embryology*

Luteal phase anovulatory follicles result in the production of competent oocytes: intra-patient paired case-control study comparing follicular versus luteal phase stimulations in the same ovarian cycle

Danilo Cimadomo[1,*], Alberto Vaiarelli[1], Silvia Colamaria[1], Elisabetta Trabucco[2], Carlo Alviggi[3,4], Roberta Venturella[5], Erminia Alviggi[2], Ramona Carmelo[2], Laura Rienzi[1,2], and Filippo Maria Ubaldi[1,2]

Study question: Are the mean numbers of blastocysts obtained from sibling cohorts of oocytes recruited after FPS and LPS in the same ovarian cycle (DuoStim approach) similar?

Answer: The follicles recruited during the anovulatory phase of the ovarian cycle may be rescued through LPS, and originate larger cohorts of oocytes with comparable competence to paired-FPS-derived ones



Cimadomo D, et al. Hum Reprod. 2018;33:1442-48.

Similar oocyte competence after FPS and LPS

Embryological data after FPS and LPS conducted, from the 188 couples included in the study

	FPS mean ± SD	LPS mean ± SD	z value	p value	Correlation between LPS and FPS (R) p value
MII oocytes, n	n = 684 3.6 ± 2.1	n = 804 4.3 ± 2.8	−2.8	< 0.01	0.50 p < 0.01
Fertilized oocytes, n	n = 485 2.6 ± 1.9	n = 595 3.2 ± 2.4	−2.8	< 0.01	0.34 p < 0.01
Mean fertilization rate per oocyte retrieval	68.2% ± 33.0%	70.0% ± 30.8%	−0.5	NS	0.01 NS
Blastocysts, n	n = 227 1.2 ± 1.1	n = 308 1.6 ± 1.6	−2.7	< 0.01	0.09 NS
Mean blastocyst rate per oocyte retrieval	33.1% ± 30.3% (0–100%)	37.4% ± 30.8% (0–100%)	−1.2	NS	−0.03 NS
Euploid blastocysts, n	n = 93 0.5 ± 0.8	n = 133 0.7 ± 1.0	−2.4	0.02	0.17 p = 0.02
Mean euploidy rate per oocyte retrieval	13.6% ± 22.8%	16.3% ± 23.4%	−1.1	NS	0.08 NS

Preliminary clinical outcomes of euploid single blastocyst transfers of embryos obtained after FPS or LPS

	FPS (n = 52)	LPS (n = 57)
Euploid single blastocyst transfers, n	62	64
Clinical pregnancies, n (% of transfers)	31 (50)	39 (60.9)
Miscarriages, n (% of clinical pregnancies)	5 (16.1)	4 (10.3)
Ongoing pregnancies, n (> 22 weeks)/deliveries (% of transfers)	26 (41.9)	35 (54.7)

Cimadomo D, et al. Hum Reprod. 2018;33:1442-8.

LPS-derived cohort of oocytes larger than FPS: confirmed in a multicentre study

Double stimulation in the same ovarian cycle (DuoStim) to maximize the number of oocytes retrieved from poor prognosis patients: a multicentre experience and SWOT analysis.

Alberto Vaiarelli[1*], Danilo Cimadomo[1], Elisabetta Trabucco[2], Roberta Vallefuoco[2], Laura Buffo[3], Ludovica Dusi[3], Fabrizio Fiorini[4], Nicoletta Barnocchi[4], Francesco Maria Bulletti[5], Laura Rienzi[1, 2, 3, 4], Filippo Maria Ubaldi[1, 2, 3, 4]

frontiers

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DuoStim was proposed to **353** patients

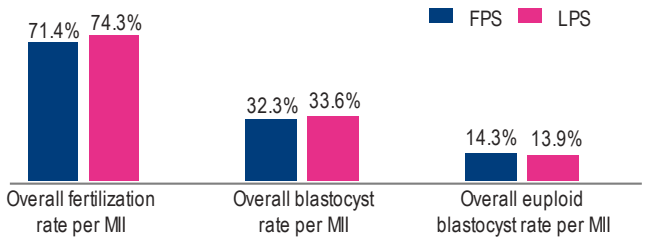
→ 17 patients did not respond to FPS

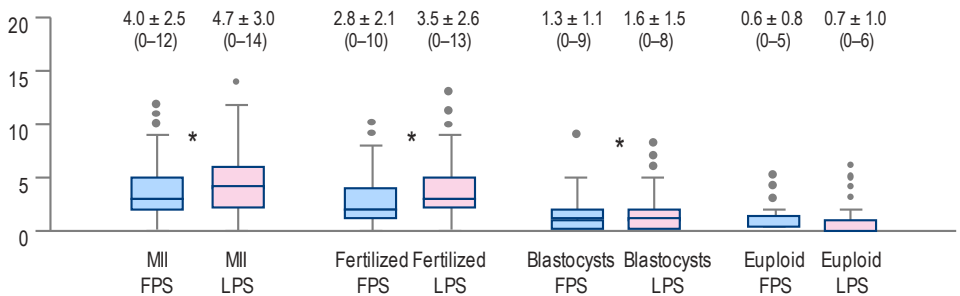
336 patients completed FPS and underwent the 1st oocyte retrieval

→ 26 patients did not respond to LPS

310 patients completed LPS and underwent the 2nd oocyte retrieval

	FPS	LPS
Mean fertilization rate per cycle	69.4%	69.8%
Mean blastocyst rate per cycle	34.9%	32.6%
Mean euploidy rate per cycle	15.7%	13.0%





* $p < 0.05$.

Vaiarelli A, et al. Front Endocrinol (Lausanne). 2018;9:317.

More patients obtaining competent blastocyst(s) per ovarian cycle

Double stimulation in the same ovarian cycle (DuoStim) to maximize the number of oocytes retrieved from poor prognosis patients: a multicentre experience and SWOT analysis.

Alberto Vaiarelli[1*], Danilo Cimadomo[1], Elisabetta Trabucco[2], Roberta Vallefuoco[2], Laura Buffo[3], Ludovica Dusi[3], Fabrizio Fiorini[4], Nicoletta Barnocchi[4], Francesco Maria Bulletti[5], Laura Rienzi[1, 2, 3, 4], Filippo Maria Ubaldi[1, 2, 3, 4]

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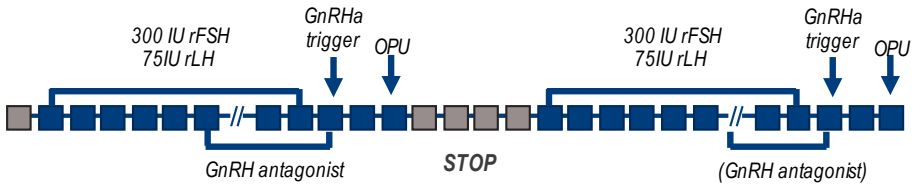
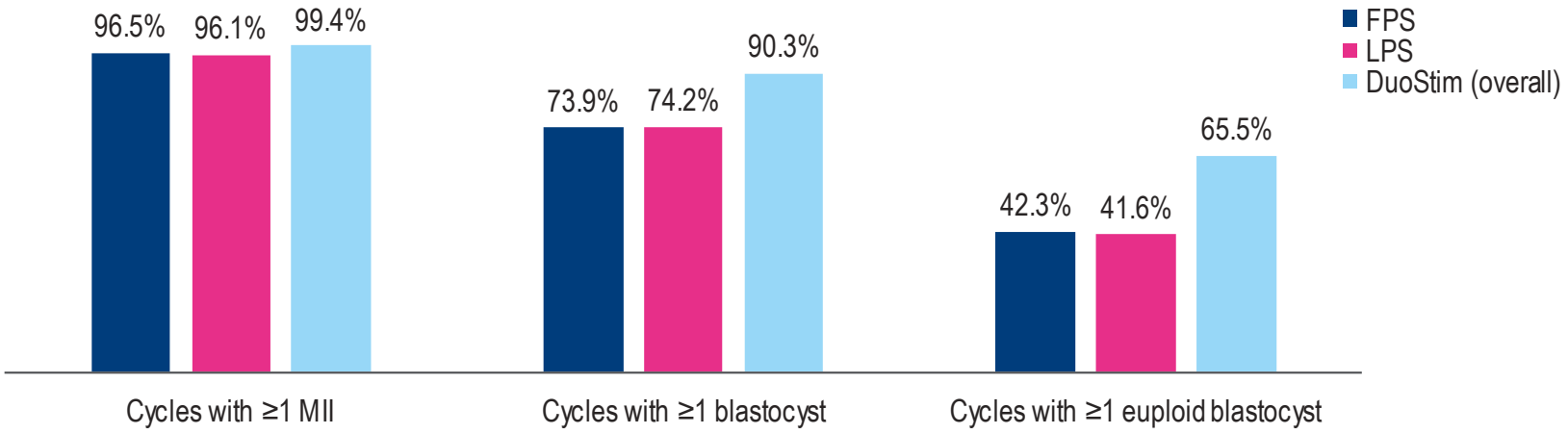




Vaiarelli A, et al. Front Endocrinol (Lausanne). 2018;9:317.

What about poor responders according to the Bologna criteria?

- Study design:
  - Paired observational study was performed in a private IVF clinic between January 2015 and January 2018
  - Primary outcome: LBR per ITT
  - Secondary outcome: embryological outcomes in FPS and LPS
- Results:
  - DuoStim resulted in a significantly higher number of MII oocytes and blastocysts vs FPS or LPS alone
  - Greater increase in LBR/ITT was observed in the DuoStim group compared to FPS-only
- In "Bologna poor responders", the contribution of LPS in the DuoStim protocol might increase the chance of obtaining a reproductively competent embryo to transfer

Vaiarelli A, et al. Accepted for publication.

What about poor responders according to the Bologna criteria?

Study design:
Paired observational study was performed in a private IVF clinic between January 2015 and January 2018

Inclusion criteria:
100 consecutive poor responder patients fulfilling the **Bologna criteria** undergoing PGT-A were enrolled

- AMH < 0.5–1.1 ng/mL, or AFC ≤ 5–7 follicles
- ≤ 3 oocytes retrieved in a previous cycle after conventional stimulation
- Advanced maternal age (≥ 40 years)

} ≥ 2 criteria

Exclusion criteria:
Severe male factors

- Azoospermia and severe OAT < 1 mil sperm count
- Abnormal karyotype
- PGT-M cycles

OAT, oligoasthenoteratozoospermia; PGT-M, preimplantation genetic diagnosis for monogenic disease.

Vaiarelli A, et al. Accepted for publication.

Outcome measures and results

Primary outcome:
LBR per ITT

Secondary outcome:
All embryological outcomes in FPS and LPS were monitored

100 patients fulfilling the Bologna criteria

5 patients did not respond to FPS

4 patients did not respond to LPS

91 patients: oocytes retrieved after both FPS and LPS

ITT, intention to treat; LBR, live birth rate.

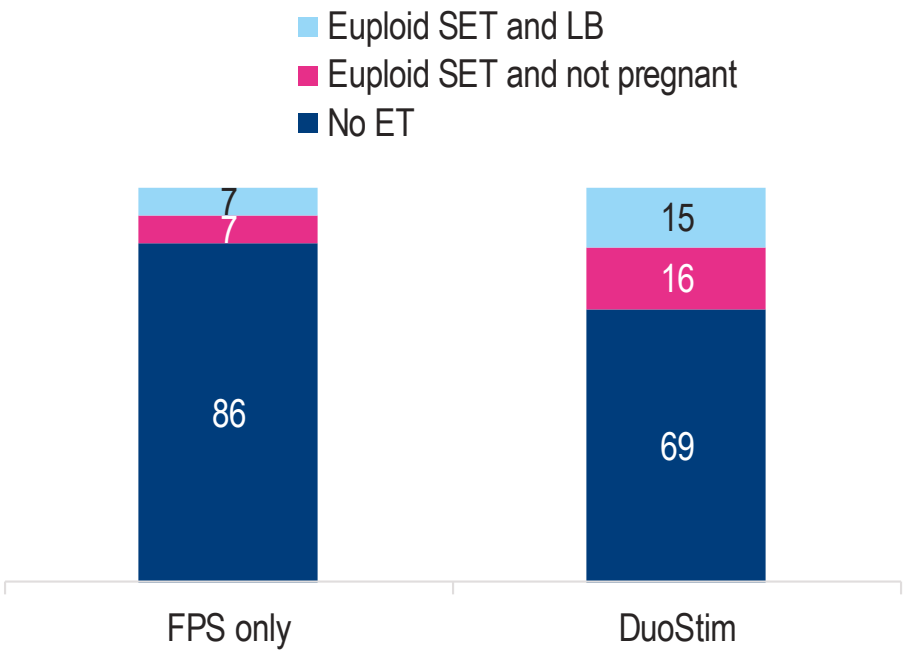
Vaiarelli A, et al. Accepted for publication.

DuoStim: laboratory results in “Bologna patients”

	FPS	LPS	p value	DuoStim
MII oocytes, n Mean ± SD (range)	237 2.4 ± 1.5 (0–6)	309 3.1 ± 2.2 (0–10)	< 0.01	546 5.5 ± 2.8 (0–12)
Blastocysts, n Mean ± SD (range)	70 0.7 ± 0.8 (0–4)	107 1.1 ± 1.1 (0–6)	< 0.01	177 1.8 ± 1.5 (0–7)
Mean blastulation rate per MII per cycle ± SD	30.7% ± 32.8%	36.2% ± 33.5%	NS	32.2% ± 24.4%
Euploid blastocysts, n Mean ± SD (range)	14 0.1 ± 0.4 (0–1)	21 0.2 ± 0.5 (0–2)	NS	35 0.4 ± 0.6 (0–2)
Mean euploid blastocyst rate per MII per cycle ± SD	4.8% ± 12.7%	6.6% ± 16.2%	NS	6.4% ± 13.6%

Vaiarelli A, et al. Accepted for publication.

DuoStim: clinical results in “Bologna patients”



- In *“Bologna poor responders”*, the contribution of LPS in the DuoStim protocol might increase the chance of obtaining a reproductively competent embryo to transfer
- This might also increase the LBR/ITT from 7% in the FPS-only to 15% in the DuoStim

LB, live birth; SET, single-embryo transfer.

Vaiarelli A, et al. Accepted for publication.

Clinical, obstetric, and perinatal outcomes after SET of euploid blastocysts from LPS and FPS: interim analysis

Study design:
Multicentre prospective study performed between October 2015 and July 2017 (interim analysis)

- **Primary outcome:** ongoing pregnancy rate (> 20 weeks)
- **Secondary outcome:** miscarriage rate and obstetric/perinatal outcome

Sample size analysis:
To achieve 80% power ($\alpha = 0.05$) to rule out a 15% difference in ongoing implantation rate between FPS- and LPS-derived euploid blastocysts, we require 174 first SETs per arm (**348 overall**)

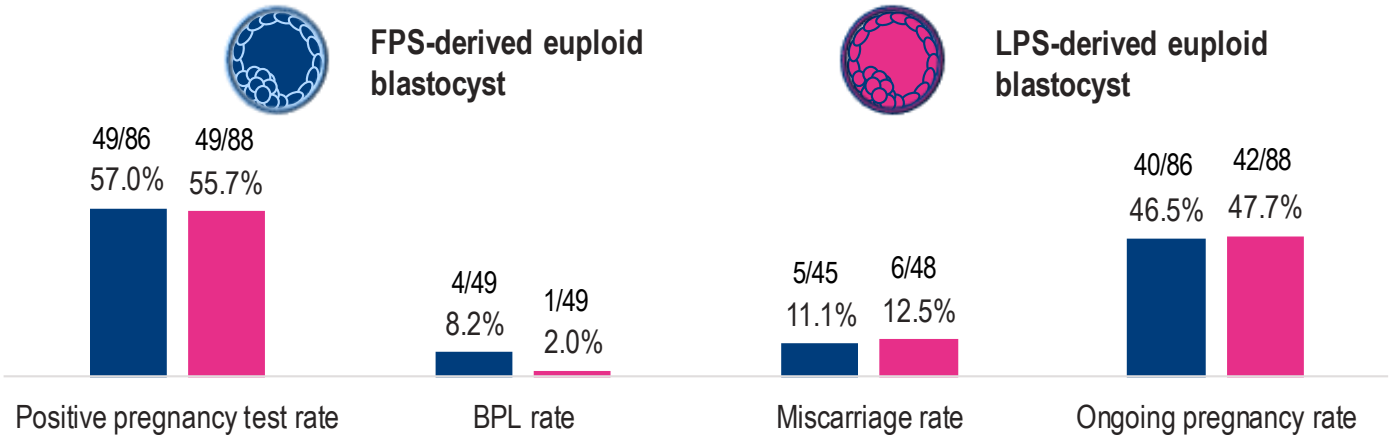
Only the first SET performed was included in this study

Results (interim analysis):

- **174** patients obtained and transferred at least 1 euploid blastocyst either from FPS and/or LPS

Vaiarelli A, et al. Oral presentation at ESHRE 2018.

Clinical, obstetric, and perinatal outcomes after SET of euploid blastocysts from LPS and FPS: interim analysis



1 embryo twinning
1 gestational diabetes
1 polyhydramnios

30 babies born to date

Gestational age: 38.1 ± 1.1 weeks
Birthweight: 3,308 ± 880 g
1 neonatal respiratory distress (7 days in NICU)

2 gestational diabetes

32 babies born to date

Gestational age: 38.0 ± 2.2 w
Birthweight: 3,217 ± 584 g
No complications or malformations

BPL, biochemical pregnancy loss; NICU, neonatal intensive care unit.

Vaiarelli A, et al. Oral presentation at ESHRE 2018.

DuoStim to maximize the number of oocytes for fertility preservation in oncology patients

HUMAN FERTILITY, 2017

ORIGINAL ARTICLE

Double ovarian stimulation (DuoStim) protocol for fertility preservation in female oncology patients

Nikolaos Tsampras, Della Gould and Cheryl T. Fitzgerald

St Mary's Hospital Reproductive Medicine Unit, Manchester, UK

… some cancer patients might have only a single cycle opportunity to collect oocytes before starting their oncology treatment

Patients' characteristics, stimulation details, and oocyte yields

No.	Age (years)	Diagnosis	US findings	AFC	AMH (pmol/L)	hMG dose (IU)	Days of stimulation	Oocytes retrieved (MII)	Interval between stimulations (days)	hMG dose (IU) second cycle	Days of stimulation	Oocytes retrieved (MII) second cycle	Oocytes retrieved in total (MII)	Comments
1	17	Aplastic anaemia	Normal	20	16.5	150	10	9 (9)	5	300	12	13 (12)	22 (21)	Elective platelet transfusion for oocyte retrieval
2	31	Bowel Ca	1 cm endometrioma	16	16.4	300	13	9 (6)	3	300	17	4 (1)	13 (7)	–
3	17	Myelodysplasia	Normal	19	18.0	150–300	14	1 (1)	7	300	12	19 (12)	20 (13)	–
4	34	Bowel Ca	Left oophorectomy, right ovarian cyst (5 cm)	4	2.1	450	14	6 (6)	1	450	15	1 (1)	7 (7)	–
5	34	ER^{-ve} breast Ca	Normal	23	47.1	300–262.5	9	13 (9)	0	300–375	9	20 (18)	33 (27)	–
6	37	ER^{+ve} breast Ca	Normal	4	2.3	450	8	3 (3)	0	450	11	3 (3)	6 (6)	Letrozole 0.5 mg daily
7	29	ER^{+ve} breast Ca	Right ovarian cyst (4 cm)	36	50.0	150	10	12 (8)	–	225	10	11 (10)	23 (18)	Letrozole 0.5 mg, metformin 1,000 mg daily
8	38	ER^{+ve} breast Ca	Right ovary not seen; left ovary normal	3	3.0	300–375	13	12 (5)	4	375	8	3 (2)	15 (7)	Letrozole 0.5 mg daily
9	37	ER^{+ve} breast Ca	Normal	9	7.1	300	11	12 (10)	0	300	10	1 (1)	13 (11)	Letrozole 0.5 mg daily
10	37	ER^{+ve} breast Ca	Normal	16	11.8	300	12	4 (4)	2	450	12	7 (7)	11 (11)	Letrozole 0.5 mg daily

After the 2nd OPU, patients continued GnRH ant for 1 week, and those with ER $^{+ve}$ were also continued on aromatase inhibitors

ER, estrogen receptor; US, ultrasound.

Tsampras N, et al. Hum Fertil (Camb). 2017;20:248-53.

SWOT analysis: putative advantages and disadvantages of DuoStim

Double stimulation in the same ovarian cycle (DuoStim) to maximize the number of oocytes retrieved from poor prognosis patients: a multicentre experience and SWOT analysis.

Alberto Vaiarelli[1*], Danilo Cimadomo[1], Elisabetta Trabucco[2], Roberta Vallefuoco[2], Laura Buffo[3], Ludovica Dusi[3], Fabrizio Fiorini[4], Nicoletta Barnocchi[4], Francesco Maria Bulletti[5], Laura Rienzi[1, 2, 3, 4], Filippo Maria Ubaldi[1, 2, 3, 4]

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Strengths
- Higher number of oocytes and embryo/ovarian cycle
- More patients obtaining competent blastocysts/ovarian cycles
- No differences in competence between oocytes after FPS and LPS

Weaknesses
- Higher number of stimulations cancelled in the LPS
- No RCT or cost-effectiveness analysis performed
- Freeze-all approach is mandatory
- Applied so far to poor prognosis patients only

SWOT analysis

Opportunities
- It may reduce time to obtain at least one competent embryo in a single ovarian cycle
- It may be emotionally better tolerated from the patients than two consecutive FPS cycles
- Theoretically, it may reduce the drop-out rate

Threats
- Cost-effectiveness?
- Increased total dose of gonadotropin administrated than in conventional COS
- Few biological, gynaecological, obstetric, and prenatal evidence of safety produced

COS, controlled ovarian stimulation; RCT, randomized controlled trial; SWOT, strengths–weaknesses–opportunities–threats.

Vaiarelli A, et al. Front Endocrinol (Lausanne). 2018;9:317.

SWOT analysis: putative advantages and disadvantages of DuoStim

Strengths

- Higher number of oocytes and embryos in the LPS and ovarian cycle
- More patients obtaining competent blastocysts/ovarian cycle
- No differences in competence between oocytes after FPS and LPS: same blastulation and euploidy rate

Opportunities

- It may reduce time to obtain at least 1 competent embryo in a single ovarian cycle
- It may be emotionally better tolerated by the patients than two consecutive FPS cycles
- It may reduce the drop-out rate
- It may increase the knowledge of the mechanisms of follicular recruitment and ovarian physiology

Vaiarelli A, et al. Front Endocrinol (Lausanne). 2018;9:317.

SWOT analysis: putative advantages and disadvantages of DuoStim

Weaknesses

- Higher number of stimulations cancelled in the LP
- No RCT comparing DuoStim vs two consecutive FPS or cost-effectiveness analysis performed
- Freeze-all approach is mandatory
- So far, applied to poor prognosis or cancer patients only

Threats

- Increased costs, cost-effectiveness (?)
- Increased total dose of gonadotropin administered compared with conventional COS
- Few biological, gynaecological, obstetric, and neonatal evidence of safety produced so far

Vaiarelli A, et al. Front Endocrinol (Lausanne). 2018;9:317.

And, although several papers have been published…

Study	Design	Patients, n
Xu and Li, 2013	Case report	1
Kuang et al., 2014	Pilot study	38
Moffat et al., 2014	Commentary paper	No information
Ubaldi et al., 2016	Prospective study	51
Wei Li-Hong et al., 2016	Retrospective study	23
Tsampras et al., 2017	Pilot study	10
Vaiarelli et al., 2017	Observational study	128
Cardoso et al., 2017	Retrospective	13
Liu et al., 2017	Retrospective case-control	
Cimadomo et al., 2018	Paired case-control study	
Zhang Wei et al., 2018	Retrospective study	
Rashtian and Zhang, 2018	Retrospective	
Madani et al., 2018	Prospective clinical study	
Bailing et al., 2018	Retrospective study	
Vaiarelli et al., 2018	Multicentre observational study	
Sighinolfi et al., 2018	Review opinion paper	
Alsbjerg B et al., 2019	Case series	

Guideline on Controlled Ovarian Stimulation in IVF/ICSI

Responsible Special Interest Group: SIG Reproductive Endocrinology

Recommendation	
Double stimulation in low responders should only be used in the context of clinical research	Research only
Double stimulation can be considered for urgent fertility preservation cycles.	GPP

Available from: https://www.eshre.eu/Specialty-groups/Special-Interest-Groups/Reproductive-Endocrinology. Accessed May 2019.

Several opinion leaders co-authored a reviewer comment on DuoStim

REVIEWER COMMENTS FORM

Guideline:	Controlled ovarian stimulation for IVF/ICSI
Review period:	12 February – 26 March 2019

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Page	Line	Comment
80-81	2145-2172	The growing knowledge of human ovarian follicular waves introduced new models to describe folliculogenesis. This concept has opened a new scenario in which non-conventional COS represents new and intriguing opportunity to fully exploit the waves of human follicular development and to maximize the utilization of the ovarian reserve via tailored protocols especially in very poor prognosis patients. In this scenario, Dual Stimulation (follicular and luteal stimulation) in the same ovarian cycle should be considered a clinical evolution of random start and luteal phase stimulation in order to collect a higher number of oocytes and obtain a adequate number of embryos in all situations where the time is limited and entail non-transfer cycle. We recognize that there are no prospective randomized trials (RCT) that compare dual stimulation with two conventional stimulations in terms of efficacy (cumulative live birth rates) or efficiency (reduced time to live birth) of the two strategies. We also recognize that mandatory freeze-all of oocytes or embryos may be a disadvantage of this protocol because of additional procedure and oocyte manipulation, which, may not be allowed by some national health care. Nevertheless, it must be noted that freeze-all is mandatory also in case of luteal phase stimulation-only, random start, oocyte/embryo accumulation through sequential conventional stimulations and blastocyst stage PGT-A cycles. In addition, we do not understand why Committee Members did not mention in the evidence section that, according to all the papers published on the topic, the mean number of oocytes retrieved in the luteal phase stimulation is significantly higher than follicular phase as are the mean number of blastocysts and of euploid blastocysts. Moreover, the chance to find an euploid embryo or a blastocyst to transfer is significantly higher per started ovarian cycle in the dual stimulation if compared to standard stimulation (Ubaldi et al., 2016, Cimadomo et al., 2018, Vaiarelli et al., 2018). Finally, dual stimulation is applied successfully by many centers in different countries. And the evidence published in favor of this procedure in increasing day by day (Xu and Li, 2013, Kuang et al., 2014, Moffat et al., 2014, Ubaldi et al., 2016, Wei et al., 2016, Tsampras et al., 2017, Vaiarelli et al., 2017, Cardoso et al., 2017, Liu et al., 2017, Rashtian and Zhang, 2018, Zhang et al., 2018, Madani et al., 2018, Jin et al., 2018, Vaiarelli et al., 2018, Alsbjerg et al., 2019, Sighinolfi et al., 2018, Vaiarelli et al., 2019). While we understand that this procedure cannot be suggested for standard patients, poor prognosis patients (e.g., with reduced ovarian reserve, AMA, Bologna POR) or women deserving fertility preservation (oncologic patients) might benefit from it. Hence, confining this technique "only for research" does not reflect the available evidence and could have serious consequences in case of reimbursement or clinical complication In conclusion, although there are no RCTs that show the superiority of dual stimulation vs conventional stimulation in terms of efficacy and efficiency, the author of this guideline could not ignore and/or underestimate the available evidence. We believe that there are enough clinical data to state that "dual stimulation can be considered in poor prognosis patients when freeze-all is mandatory. It is not clear why the Committee stated that: "Luteal phase stimulation could be used in the non transfer cycles" although it has far less clinical and laboratory evidence (some of which use data from dual stimulation) reported in the literature.

Conclusions

- Evidence has shown that mutliple follicular waves during a single ovarian cycle allows collection of the highest number of oocytes per ovarian cycle in poor prognosis or oncology patients
- Evidence shows that oocytes obtained from LPS seem to be developmentally, genetically, and reproductively competent
- LPS in the DuoStim protocol increases the chance of obtaining a reproductively competent embryo for transfer in poor prognosis patients
- A multicentre prospective RCT is needed to evaluate the efficacy, efficiency, and costs of DuoStim vs two consecutive FPS cycles

Grazie per la vostra attenzione

g.en.e.r.a.

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Thank you for your attention