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ASSISTED REPRODUCTION TECHNOLOGIES

Empty follicle syndrome after GnRHa triggering versus hCG triggering in COS

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Abstract
Purpose This study aimed to explore the incidence of empty follicle syndrome (EFS) in oocyte donors who had final oocyte maturation triggered with GnRHa and to compare the incidence of EFS in this group of patients with IVF patients who had final oocyte maturation with hCG.
Methods Data including 2034 oocyte donation cycles and 1433 IVF cycles performed between years 2009 and 2010 was retrospectively analyzed to identify cases of EFS in each group.
Results The incidence of EFS in the two groups did not differ significantly, 3.5% versus 3.1%, (n.s.).
Conclusions This large retrospective analysis indicates that the incidence of EFS is not increased after GnRHa triggering as compared to hCG triggering.

Keywords GnRH agonist · GnRH antagonist · GnRHa triggering · hCG · Empty follicle syndrome

Capsule Empty follicle syndrome is a condition in which no oocytes are retrieved after ovarian stimulation for IVF. The incidence seems to be similar after GnRHa triggering in oocyte donors as compared to hCG triggering in IVF patients.

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Introduction

During recent years, after the introduction of the GnRH antagonist protocol for clinical use, a single bolus of GnRH agonist (GnRHa) for final oocyte maturation has been proposed as an effective strategy to prevent OHSS and as a first line protocol for treatment of oocyte donors [1]. Recently, however, two case-reports described empty follicle syndrome (EFS) after GnRHa triggering in OHSS risk patients, despite the presence of a large number of apparently mature follicles at the oocyte pick-up (OPU) [2, 3].

EFS is a condition of uncertain etiology in which no oocytes are retrieved from apparently normally growing ovarian follicles with normal estradiol levels after ovarian stimulation for assisted reproductive techniques (ART). The incidence of EFS after hCG triggering in IVF patients has been estimated to be about 2–7% [4]. The available literature describes two subtypes of EFS; the so called genuine EFS - presumably related to intrinsic ovarian factors - and false EFS, mainly related to pharmacological problems or human administration error. Several hypotheses as to the background of EFS after hCG triggering of final oocyte maturation in gonadotropin stimulated cycles have been proposed: early oocyte atresia due to a dysfunctional folliculogenesis in the presence of an apparently normal hormonal response [4], a biological abnormality in the supply of mature oocytes to be retrieved despite normal bioavailability of hCG [5], genetic factors such as LH/hCG receptor mutations [6, REF 22 from Yariz 2011], abnormalities in the in vivo biological activity of some batches of commercially available hCG or GnRHa [7], rapid clearance of hCG by the liver [7], pharmacological problems [5, 8, 9] and human error [5, 7, 10] - in particular inappropriate timing of the triggering bolus of hCG [8]. Moreover, advanced ovarian ageing is considered a risk factor for EFS recurrence, probably due to altered folliculogenesis [6].

Although previous studies have shown that GnRHa effectively stimulates ovulation and final oocyte maturation [11, 12], there are significant differences between the GnRHa induced LH surge, the LH surge of the natural cycle and the continuous LH-like drive from a bolus of hCG. Thus, the LH surge of the natural cycle is characterized by three phases with a total duration of 48 h [13] as compared to the GnRHa induced LH surge, consisting of only two phases with a duration of 28–32 h [12, 13]. In contrast, a bolus of 10.000 IU hCG induces a LH-like activity still measurable in serum for 9–10 days due to the long biological half-life of hCG [14, 15]. Bearing in mind these differences, the background of EFS after hCG triggering may not be the same as after GnRHa triggering.

Until now, no study explored the incidence of EFS after GnRHa triggering. The objective of the present study was to retrospectively analyze from a large database the incidence of EFS depending on mode of triggering: GnRHa or hCG.

Materials and methods

Data including 2034 oocyte donation cycles and 1433 IVF cycles performed between years 2009 and 2010 was retrospectively analyzed. All oocyte donors were triggered with GnRHa for final oocyte maturation during this period of time. Only IVF patients under 35 years of age were included and EFS was defined as the complete absence of oocytes after retrieval.

Stimulation of donors

Donors were pre-treated with OCP (Microgynon30®, Organon) from day 1–2 of the menses of the previous cycle for 12 to 16 days. After a wash out period of 5 days after the last pill, stimulation commenced with a starting dose of recombinant FSH (Puregon®, Organon; Gonal F®, Serono) ranging from 150 IU to a maximum of 225 IU and 0.25 mg daily of the GnRH antagonist ganirelix (Orgalutran®, Organon) was introduced on day 5 or 6 of stimulation. As soon as two leading follicles reached ≥17 mm mean diameter, two ampoules of triptorelin 0.1 mg (Decapeptyl®, Ipsen Pharma) were administered and OPU was performed 36 h later.

Stimulation of IVF patients

IVF Patients were treated according to the same protocol as oocyte donors, i.e OCP pretreatment and stimulation with recombinant FSH followed by GnRH antagonist co-treatment. The only difference between IVF patients and donors was that IVF patients received rec hCG 250 microgr (Ovitrelle, MerckSerono, Spain) s.c. for final oocyte maturation as soon as two leading follicles reached ≥17 mm in mean diameter; OPU was performed 36 h later.

Statistical analysis

Categorical data were expressed as number and percentage, and numeric data as mean ± SEM. Statistical analyses were performed with the chi-square test and double-sided *t* test. Significance was set at .05. All statistical calculations were performed using Sigmastat for Windows 2.0 (Jandel Scientific, San Rafael, CA).

Results

A total of 2034 oocyte donors and 1433 IVF patients were included in this analysis. Even though all donors and patients were under 35 years of age, donors were significantly younger than patients (25.1±3.8 vs 31.9±2.3 years, $p<0.01$). Similarly, donors had a higher peak serum estradiol level (1892±1200 vs 1702±1050 pg/mL, $p<0.01$) and a higher number of MII oocytes retrieved (9.8±4.3 vs 7.2±4.6, $p<0.01$). Importantly, the incidence of EFS in the two groups, did not differ significantly (3.5% vs 3.1%, n.s.) (Table 1).

Table 2 shows cycle characteristics in cases of EFS versus non-EFS in donors and IVF patients. As shown, no differences could be found between age or peak estradiol levels. However, differences were found in the total dose of FSH administered: EFS donors received a lower dose of FSH (1660±402 vs 1791±477 ($p=0.02$)), whereas EFS IVF patients received a higher total FSH dose (1780±574 vs 1573±543 ($p=0.02$)).

In IVF patients when the cause of infertility was evaluated regarding the occurrence of EFS vs non-EFS, a statistical difference was notice in normoresponder patients 37.8% (17) vs 54.7% (785) $p=0.006$ and low responder patients 24.4% (11) vs 12.6% (175), $p=0.01$; whereas no differences were noticed for polycystic ovary 17.8% (8) vs 10.6% (147), $p=0.07$; endometriosis 11.1% (5) vs 10.7% (149), p=n.s; genetic 4.4% (2) vs 1.6% (22), p=n.s; tubal

Table 1 Cycle characteristics after triggering with GnRH agonist versus hCG

	Donors $n=2034$	Patients $n=1433$	p value
Age (years)	25.1±3.8	31.9±2.3	0.01
Peak serum E2 levels (pg/mL)	1892±1200	1702±1050	0.01
MII	9.8±4.3	7.2±4.6	0.01
% EFS (n)	3.5 (73)	3.1 (45)	n.s.

Mean ± SD and percentage where appropriate

Table 2 Characteristics of EFS vs non-EFS cycles after triggering with GnRH agonist versus hCG

	Donors			Patients		
	EFS	Non-EFS	*p* value	EFS	Non-EFS	*p* value
Age	24.4±4.1	25.1±3.9	0.13	32.1±2.4	31.9±2.3	0.56
Peak E2	1917±1272	1891±1198	0.86	1351±1298	1521±1041	0.38
Total FSH	1660±402	1791±477	0.02	1780±574	1573±543	0.02

4.4% (2) vs 6.2% (86), p=n.s, or recurrent miscarriage 0 vs 1.2% (24), p=n.s.

When analyzing basal characteristics of donors, no differences where found regarding BMI 21.5±2.3 vs 21.9±2.8 p=n.s. or AFC 18.1±4.9 vs 18.9±5.6 p=n.s. in EFS vs non EFS cycles, respectively.

Discussion

This is the largest analysis until now exploring the incidence of EFS after GnRHa triggering versus hCG triggering in more than three thousand patients. The analysis shows that the incidence of EFS seems to be similar regardless of whether GnRHa or hCG triggering is used for final oocyte maturation

EFS is a rare and frustrating complication of IVF, leading to cycle cancellation. As described previously, the exact etiology of EFS after hCG triggering is not fully explained. A systematic review reported that 67% of cases were so called 'false forms' of the syndrome mainly related to human error or pharmacological problems [16]. Regarding pharmacological abnormalities in the in-vivo biological activity of some batches of commercially available GnRHa, these have been described for hCG [7]; however, no data is available for GnRHa.

In genuine EFS, dysfunction of the folliculogenesis seems to be the most plausible etiology [23, 24]. In fact, when we analyzed the etiology of infertility in the IVF patients group, we found a higher proportion of PCO and low response and a lower percentage of unexplained infertility, which supports the concept of dysfunctional folliculogenesis. However, the donor population who had GnRHa trigger in our study challenges this concept. Donors are young healthy women in their early twenties with normal ovarian reserve; thus, it is logical to assume that dysfunctional problems would be lower in this population, although as observed, not negligible.

As previously mentioned, in contrast to hCG triggering, the action of a bolus of GnRHa is indirect via the endogenous release of LH and FSH from the pituitary after binding to and activation of the GnRH receptor [11, 12]. Thus, EFS after GnRHa triggering may represent a different pathology as compared to EFS after hCG triggering.

As the pituitary is the target organ for GnRHa, one might assume that under temporary or permanent dysfunctions of the pituitary, a sufficient flare up effect will not be achieved, resulting in a deficient final follicular maturation and EFS. An example of this is the hypogonadotropic/hypogonadal patient (WHO type I) who is characterized by endogenous levels of LH and FSH below 1.2 IU/l. GnRHa triggering in this type of patient will invariably result in EFS due to the induction of an insufficient surge of gonadotropins. In line with this concept, we could hypothesize that a "borderline" patient with low circulating levels of LH and FSH, however still above the hypo/hypo level would also run the risk of EFS after GnRHa trigger.

Other examples of patients who could be hypothesized to develop EFS after GnRHa triggering are patients with a GnRH receptor polymorphism [25], necessitating a higher dose of GnRHa to activate the receptor in line with the FSH receptor polymorphism (Ser/680 FSH-R) [26]. The same would account for patients with a LH receptor polymorphism [27]. Finally, patients with a variant LH β gene polymorphism specifically in the homozygous form, resulting in a less bioactive LH molecule [28, 29] might be at risk to have a blunted response after GnRHa trigger.

One drawback of the present study is its retrospective design. In fact, when evaluating a large population for any clinical parameter, statistically significant differences may arise, however their clinical relevance needs to be interpreted with caution. In our study, as expected, the donor population was younger and had higher estradiol levels as well as a higher number of eggs retrieved. On the other hand, these discordances between groups, albeit significant, do not defeat the purpose of the study because the physiological action of a bolus of GnRHa on the pituitary to induce final follicular maturation in GnRH antagonist cycles is independent of age, type of COS and even ovarian response, as described in ovulation induction cycles [17, 18] normoresponder IVF patients [19] hyperresponder patients [20] as well as donor population [21]. Hence, there is no reason to presume that GnRHa for triggering may act differently in donors and IVF patients. Alternative study designs to address the incidence of EFS could be the comparison of donor cycles triggered with hCG vs GnRHa, however, in view of the vast evidence in terms of security and comfort of GnRHa triggering in donors [1] it would be unethical to proceed with such a study, only for "scientific" purposes. Another valid (and feasible) approach would be the inclusion of IVF patients triggered with hCG vs GnRHa, and

ongoing studies are following this line. Moreover, one must take into account that some patients could be potential donors (e.g. IVF patients under 35 years old with male factor) and some donors may end up as patients in the future, so if both groups are young, it seems legitimate to present the current data. Finally, it seems that no specific protocol is related to the occurrence or recurrence of EFS [6].

In a further effort to identify specific characteristics of EFS cycles, we evaluated cycle features in EFS vs non-EFS. Interestingly, EFS cases received a lower dose of FSH in donors whereas the opposite occurred in IVF patients. As mentioned before, these differences, when looking at the absolute numbers, in fact are meaningless from a clinical point of view. Regarding the etiology of infertility in IVF patients, it seems that EFS cycles were more likely to occur in low responder patients compared to normal responder patients; this finding supports the hypothesis of an altered folliculogenesis as a plausible underlying cause of EFS; in donors, basal characteristics such as BMI or AFC were similar between EFS vs non-EFS cycles. Unfortunately, we were not able to retrieve further data from donors/patients such as basal day 3 hormones or the total number of mature follicles in order to understand these differences observed.

In conclusion, this large analysis shows for the first time that the incidence of EFS seems to be similar after GnRHa and hCG triggering. As in EFS cases seen after hCG, the exact reason for failure after GnRHa triggering remains uncertain. Most cases of EFS after either hCG or GnRHa triggering are related to human error, and, thus, a meticulous counseling and instruction of the patient prior to oocyte retrieval is of outmost importance.

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The authors disclose any potential conflict of interest.

References

1. Humaidan P, Kol S, Papanikolaou E, The Copenhagen GnRH Agonist Triggering Workshop Group. GnRH agonist for triggering of final oocyte maturation: time for a change of practice? Hum Reprod Update. 2011;17:510–4.
2. Honnma H, Hashiba Y, Asada Y, Endo T. Failure of triggering oocyte maturation with a GnRH agonist in polycystic ovary syndrome: two case reports. Eur J Obstet Gynecol Reprod Biol In Press. doi:10.1016/j.ejogrb.2011.03.002.
3. Griesinger G, Schultz L, Bauer T, Broessner A, Frambach T, Kissler S. Ovarian hyperstimulation syndrome prevention by gonadotropin-releasing hormone agonist triggering of final oocyte maturation in a gonadotropin-releasing hormone antagonist protocol in combination with a "freeze-all" strategy: a prospective multicentric study. Fertil Steril. 2011;95:2029–33.
4. Ben-Shlomo I, Schiff E, Levran D, Ben-Rafael Z. Failure of oocyte retrieval during invitro fertilization: a sporadic event rather than a syndrome. Fertil Steril. 1991;55:324–7.
5. Awonuga A, Govindbhai J, Zierke S, Schnauffer K. Continuing the debate on empty follicle syndrome: can it be associated with normal bioavailability of b-human chorionic gonadotrophin on the day of oocyte recovery? Hum Reprod. 1998;13:1281–4.
6. Zreik TG, Garcia-Velasco JA, Vergara TM, Arici A, Olive D, Jones EE. Empty follicle syndrome: evidence for recurrence. Hum Reprod. 2000;15:999–1002.
7. Zegers-Hochschild F, Fernandez E, Machenna A, Fabres C, Alteiri E, Lopez T. The empty follicle syndrome: a pharmaceutical industry syndrome. Hum Reprod. 1995;10:2262–5.
8. Ndukwe G, Thornton S, Fishel S, Dowell K, Aloum M, Green S. 'Curing' empty follicle syndrome. Hum Reprod. 1997;12:21–3.
9. Ubaldi F, Nagy Z, Janssenwillen C, Smitz J, Van Steirteghem A, Devroey P. Ovulation by repeated human chorionic gonadotrophin in 'empty follicle syndrome' yields a twin clinical pregnancy. Hum Reprod. 1997;12:454–6.
10. Quintans CJ, Donaldson MJ, Blanco LA, Pasqualini RS. Empty follicle syndrome due to human errors: its occurrence in an in-vitro fertilization programme. Hum Reprod. 1998;13:2703–5.
11. Gonen Y, Balakier H, Powell W, Casper RF. Use of gonadotropin-releasing hormone agonist to trigger follicular maturation for in vitro fertilization. J Clin Endocrinol Metab. 1990;71:918–22.
12. Itskovitz J, Boldes R, Levron J, Erlik Y, Kahana L, Brandes JM. Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist. Fertil Steril. 1991;56:213–20.
13. Hoff JD, Quigley ME, Yen SS. Hormonal dynamics at midcycle: a reevaluation. J Clin Endocrinol Metab. 1983;57:792–6.
14. Damewood MD, Shen W, Zacur HA, Schlaff WD, Rock JA, Wallach EE. Disappearance of exogenously administered human chorionic gonadotropin. Fertil Steril. 1989;52:398–400.
15. Weissman A, Lurie S, Zalel Y, Goldchmit R, Shoham Z. Human chorionic gonadotropin: pharmacokinetics of subcutaneous administration. Gynecol Endocrinol. 1996;10:273–6.
16. Stevenson TL, Lashen H. Empty follicle syndrome: the reality of a controversial syndrome, a systematic review. Fertil Steril. 2008;90:691–8.
17. Parneix I, Emperaire JC, Ruffie A, Parneix P. Comparison of different protocols of ovulation induction, by GnRH agonists and chorionic gonadotropin. Gynecol Obstet Fertil. 2001;29:100–5.
18. Díaz I, Guillén A, Pacheco A, Requena A, Garcia-Velasco JA. Endocrine modifications associated with final oocyte maturation with gonadotropin-releasing hormone agonists vs. human chorionic gonadotropin in women undergoing intrauterine insemination. J Reprod Med. 2008;53:33–9.
19. Fauser BC, de Jong D, Olivennes F, Wramsby H, Tay C, Itskovitz-Eldor J, van Hooren HG. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for in vitro fertilization. J Clin Endocrinol Metab. 2002;87:709–15.
20. Engmann L, Siano L, Schmidt D, Nulsen J, Maier D, Benadiva C. GnRH agonist to induce oocyte maturation during IVF in patients at high risk of OHSS. Reprod Biomed Online. 2006;13:639–44.
21. Bodri D, Guillén JJ, Trullenque M, Schwenn K, Esteve C, Coll O. Early ovarian hyperstimulation syndrome is completely prevented by gonadotropin releasing-hormone agonist triggering in high-risk oocyte donor cycles: a prospective, luteal-phase follow-up study. Fertil Steril. 2010;93:2418–20.
22. Yariz KO, Walsh T, Uzak A, Spiliopoulos M, Duman D, Onalan G, King MC, Tekin M. Inherited mutation of the luteinizing hormone/choriogonadotropin receptor (LHCGR) in empty follicle syndrome. Fertil Steril. 2011 (in press).
23. Khalaf Y, Braude P. 'Curing' empty follicle syndrome. Hum Reprod. 1997;12:1601.

24. Tsuiki A, Rose BI, Hung TT. Steroid profiles of follicular fluids from a patient with the empty follicle syndrome. Fertil Steril. 1988;49:104–7.
25. Beranova M, Oliveira LM, Bédécarrats GY, Schipani E, Vallejo M, Ammini AC, Quintos JB, Hall JE, Martin KA, Hayes FJ, Pitteloud N, Kaiser UB, Crowley Jr WF, Seminara SB. Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor mutations in idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2001;86:1580–8.
26. Perez Mayorga M, Gromoll J, Behre HM, Gassner C, Nieschlag E, Simoni M. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. J Clin Endocrinol Metab. 2000;85:3365–9.
27. Piersma D, Verhoef-Post M, Berns EM, Themmen AP. LH receptor gene mutations and polymorphisms: an overview. Mol Cell Endocrinol. 2007;260–262:282–6.
28. Alviggi C, Clarizia R, Pettersson K, Mollo A, Humaidan P, Strina I, Coppola M, Ranieri A, D'Uva M, De Placido G. Suboptimal response to GnRHa long protocol is associated with a common LH polymorphism. Reprod Biomed Online. 2009;18:9–14.
29. Pettersson KS, Söderholm JR. Individual differences in lutropin immunoreactivity revealed by monoclonal antibodies. Clin Chem. 1991;37:333–40.