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Failed oocyte retrieval after lack of human chorionic gonadotro administration in assisted reproductive technology

Ricardo H. Asch, M.D.*†
Huey-Po Li, M.D.*
John L. Yovich, M.D.‡
K. Paul Katayama, M.D., Ph.D.§
Jose P. Balmaceda, M.D.*
Francisco J. Rojas, Ph.D.*
Sergio C. Stone, M.D.*

University of California-Irvine, Orange, California; Pivet Medical Center, Perth, Australia; and University of Wisconsin Medical School, Milwaukee, Wisconsin

Objective: To document the absence of oocytes in follicular aspirates in women who, during controlled ovarian stimulation with gonadotropin-releasing hormone agonist (GnRH-a) and menotropins, fail to receive human chorionic gonadotropin (hCG) administration.

Design: Retrospective analysis of clinical laboratory data.

Setting: Multicentric.

Patients: Five women undergoing controlled ovarian hyperstimulation with GnRH-a and menotropins for programs of assisted reproductive technologies.

Results: The documented absence of an hCG injection produced "empty follicles" at transvaginal guided aspiration, despite numerous follicular lavages and aspiration of peritoneal fluid. The lack of oocytes and granulosa-cumulus complex in the follicular fluid was reverted in other cycles in the same patients when hCG was properly administered.

Conclusions: (1) This study emphasizes the importance of proper patients' and nurses' instructions for preparation of hCG injections and proper mixture of vehicle and powder before follicular aspiration. (2) In the absence of cumulus-corona-oocyte complex at aspiration, measure serum β-hCG to ascertain whether hCG injection was administered or not. (3) Routine preoperative β-hCG levels may be helpful to avoid unnecessary surgeries. Fertil Steril 1992;58:361–5

Key Words: Oocyte, follicular aspiration, human chorionic gonadotropin

It is now universally accepted that multiple follicular development is a prerequisite for a successful assisted reproductive technology (ART) program. It is therefore necessary for the patient to und form of follicular stimulation that will result recruitment of a cohort of preovulatory follicl hence supernumerary oocytes. The most co regimen used for controlled ovarian hypersti tion in ART currently is a combination of go tropin-releasing hormone agonist (GnRH-a menotropins (1). Gonadotropin-releasing ho induces pituitary suppression before meno stimulation and prevents premature lutei hormone (LH) surge, allowing better contro the process of follicular stimulation. Huma rionic gonadotropin (hCG) is used as a sur LH surge to trigger the final follicular matu before oocyte retrieval. Coulam et al. (2) we first to report in vitro fertilization (IVF) cy

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* Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, University of California-Irvine.

† Reprint requests: Ricardo H. Asch, M.D., University of California-Irvine, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, and Infertility, UCI Center for Reproductive Health, 101 The City Drive, Pavilion II, Orange, California 92668.

‡ Pivet Medical Center.

§ Department of Obstetrics and Gynecology, University of Wisconsin Medical School.

which no eggs were found in follicular aspiration, despite the acceptable serum estradiol (E_2) levels and follicular size. The term "empty follicle syndrome" was coined by the authors to describe the phenomenon. Since then, others have reported similar findings (3–7). However, the number of cases reported is small and therefore its incidence and etiology are unknown. In the past 2 years, we have witnessed five cases of failed oocyte retrieval from four ART centers. Four of the five patients had a prior or a subsequent cycle in which multiple oocytes were recovered successfully. The differences between our cases and the cases reported by Coulam et al. (2) are that GnRH-a was included in the controlled ovarian hyperstimulation regimens and failure of giving hCG before follicular aspiration was well documented. In this study, the clinical presentations of the index and additional ART cycles in the same woman are compared, and the possible cause of failed oocyte retrieval is discussed.

MATERIALS AND METHODS

Four ART centers participated in this study. The centers were asked to do a retrospective file research based on their ART registry to identify the cases of failed oocyte recovery upon the failure of the hCG trigger injection. Totally, there were five well-documented cases found that had occurred between 1989 and 1990. During that period of time, over 80% of treatment cycles in these four ART centers included GnRH-a suppression. The controlled ovarian hyperstimulation protocols that included pituitary suppression were a combination of one of the following: GnRH-a (Lupron, leuprolide acetate; TAP Pharmaceuticals, Chicago, IL; Suprefact; Hoechst Pharmaceuticals, Hounslow, United Kingdom; Lucrin; Abbott, Sydney, Australia); human menopausal gonadotropin (hMG, Pergonal; Serono Laboratories, Randolph, MA); and pure follicle-stimulating hormone (FSH, Metrodine; Serono Laboratories). Gonadotropin-releasing hormone started either in the midluteal phase of the preceding cycle (long protocol) or on day 1 or day 2 of the treatment cycle (short protocol). The daily doses of hMG and FSH were individualized according to the response of patients. The treatment cycles were monitored by transvaginal ultrasound (US) and serum E_2 levels. When two or more follicles had reached a diameter of 18 mm on vaginal US and the serum E_2 levels had reached ≥300 pg/mL for each main follicles (≥18 mm), patients were counseled to receive the hCG (Profasi; Serono Laboratories) 10,000 IU intramuscular (IM) injection. However, in one of the five cases th trigger injection was withheld intentionally the others were given improperly. Transvagi piration of follicles under US guidance wa formed 34 to 36 hours after hCG injection. graphic signs of ovulation (8) (decrease in fol size, fluid in the periovulatory area or cul- and appearance of intrafollicular echoes) checked to exclude ovulation before the transv aspiration. All of the visible follicles were asp If an oocyte was not found in the initial aspi the follicle was then flushed for at least five before attempting a subsequent follicular aspi During the same period of time, in patients re documented hCG administration, oocyte re rates were >90% per follicle aspirated in a centers. If no egg was recovered in follicle as and flushes, aspiration of the cul-de-sac was out to check for presence of any oocytes. St data collection forms were used to record the of patients' histories, the index cycles, and the with successful oocyte recovery.

RESULTS

There were five cycles of failed oocyte retri five patients because of documented failure administration before the transvaginal aspi All patients were given a GnRH-a for con ovarian hyperstimulation in the index cycle. 1 shows the summary of clinical informati the details of index cycle of the patients. Th age of the patients was 31.8 years (range 24 Patient 2 was the oocyte donor for a recipie premature ovarian failure. The decision of for the alleged hCG administration was ba follicular size by US scans and by serum E_2 Follicular aspirations occurred 13 to 14 day starting menotropins. Human chorionic gon pin was withheld deliberately in patient 1 w consent to prevent the occurrence of severe hyperstimulation syndrome because of a hi chance (9). All the remaining cases were ins to receive hCG at night, 34 to 36 hours bef programmed transvaginal oocyte aspiration. nighttime injections were not given at the AF ters but were given at a nearby emergency r by the patients themselves. In these index each follicle was flushed from 5 to 11 times, mature preovulatory oocytes were recovered patient. On completion of follicle aspirati flushes, follicles were re-entered and aspirate Only one atretic oocyte without cumulus wa

Table 1 Summary of Clinical Information and Presentation of the Five Patients in the Cycle of Failed Oocyte Retrieval

	Patient				
	1	2	3	4	5
Age (y)	26	24	29	42	3
Parity	G1P0	G1P0	G0P0	G0P0	G1
Cause	Tubal factor	Oocyte donor	Male factor	Unexplained	Male
Controlled ovarian hyperstimulation protocol	GnRH-a (FSH/hMG)	GnRH-a (FSH/hMG)	GnRH-a (hMG)	GnRH-a (FSH/hMG)	GnRH-a
No. of ampules*	12/27	4/20	34	40/40	5
Day of hCG†	11	12	12	11	1
E_2 level (pg/mL)‡	7,089	3,532	2,305	1,771	2,0
Follicles aspirated	40	17	10	8	
HCG injection	Withheld	Only diluent	Only diluent	Only diluent	Only d

* 75 IU per ampule.
† The day of hCG supposed to be given, day 1 = start of menotropins.
‡ Serum E_2 level on the day of hCG supposed to be g

ered with minimal granulosa cells (GCs) in patient 3. It had a dark pyknotic center and a thin incomplete corona, and it failed to be fertilized after insemination in vitro. In the rest of the patients, the follicle aspirates and flushes were entirely clear without presence of GCs or cumulus massed. Blood samples taken from the patients immediately after the failed retrievals showed β-hCG levels < mIU/mL in all cases. This finding confirmed that only the diluent was given to the patients. Patients questioned after the failed retrieval acknowledged that this mistake occurred and that the diluent was not mixed with the powder containing the active hCG.

Patients 1, 2, and 3 had a subsequent successful oocyte retrieval cycle in which the hCG trigger injection was effectively given, and patients 1 and 3 got pregnant with the embryos resulting fr retrieval. Patient 4 had one prior treatment c which 3 oocytes were collected but failed to tilized for unexplained reasons. Patient 3 al two prior successful cycles in which 9 and spectively, oocytes were retrieved, but both resulted in failed fertilization because of sev goasthenospermia. The controlled ovarian stimulation protocols used in the index an cessful cycles were almost identical except th was not given in the index cycles. Table 2 p the clinical and laboratory data of the prior sequent successful oocyte retrieval cycles of t patients. All oocytes were collected with eas 2 flushes per follicle). Patient 1 was at seve of ovarian hyperstimulation again in the subs

Table 2 Summary of the Prior or Subsequent Successful Oocyte Retrieval Cycle From Four of the Patients

	Patient			
	1	2	3	4
Controlled ovarian hyperstimulation protocol	GnRH-a (FSH/hMG)	GnRH-a (FSH/hMG)	GnRH-a (hMG)	GnRH-a (h
No. of ampules*	15/14	4/20	34	52
Day of hCG†	10	12	9	11
E_2 level (pg/mL)‡	7,299	3,883	3,090	1,305
Follicles aspirated	71	17	13	5
Oocytes retrieved	66	16	13	3
Oocytes fertilized	34	8	4/5 by donor 0/8 by husband	0
ART procedure	Frozen ZIFT	IVF/recipient	TEST§	
Outcome	Twin/ongoing		Singleton/ongoing	

* 75 IU per ampule.
† Day 1 = start of menotropins.
‡ Serum E_2 level on the day of hCG administration.
§ Tubal embryo stage transfer

successful cycle, and embryo transfer (ET) was withheld. A zygote intrafallopian transfer (ZIFT) procedure with four cryopreserved embryos was performed later in a hormone replacement cycle that resulted in a quadruplet gestation. After embryo reduction, a twin pregnancy is currently still ongoing. The recipient of patient 2 did not get pregnant from an IVF procedure. Patient 3 eventually got pregnant with the oocytes fertilized by donor sperm and transferred through an IVF-ET procedure.

DISCUSSION

Empty follicle syndrome was coined by Coulam et al. (2) in 1986 to describe the phenomenon of failed oocyte retrieval during IVF cycles. None of the five cycles reported by the authors included GnRH-a in the controlled ovarian hyperstimulation protocols, and four of the five cycles involved a spontaneous LH surge. Awadalla et al. (4) suggested that ovulation before attempts for oocyte recovery or technical difficulty with follicular aspiration might be the most likely explanation for the phenomenon observed by Coulam et al. (2). The five cases reported here are different because GnRH-a was used in the controlled ovarian hyperstimulation protocol and because failure of giving hCG trigger injection was well documented. Because we examined only cases of complete failure oocyte collection, it cannot be totally ruled out that in other cases of missed hCG administration, some oocytes might have been retrieved. The use of GnRH-a in ART cycles has become widespread because of its advantages of decreasing rates of premature luteinization, unplanned spontaneous ovulation, and cancellations (1).

Ovulation is a sequential process that consists of four major components: (1) reactivation of meiosis; (2) luteinization and morphological changes in the GCs; (3) cumulus expansion and mucification; and (4) follicular rupture. The trigger of all these four events is the LH surge. In cycles in which the LH surge is attenuated by superovulation with gonadotropins (10) or prevented by GnRH-a suppression, a surrogate LH surge is required. Human chorionic gonadotropin is similar to LH in molecular structure and biological activity; therefore, it has been used as a surrogate LH surge in ART cycles to trigger the final stage of follicular maturation before oocyte retrieval. The minimum preovulatory effective dose of hCG administration in the human and domestic mammals is unclear. In a study using different doses of hCG (5 to 100 IU) on rabbits, Bomsel-Helmreich et al. (11) demonstrated that at low doses (5 to 10 IU), resumption of meiosis occurre follicle rupture. Higher doses were associa a more advanced stage of nuclear matura teinization, and the incidence of follicular Thus, the administration of progressivel doses of hCG showed a dissociation of the nents leading to ovulation. There have b few IVF studies on the effects of varying hCG on human oocyte retrieval rates (12). et al. (12) reported a significantly lower s oocyte recovery in patients who received of hCG (77.3%) compared with patients ceived either 5,000 IU of hCG (95.5%) or 1 of hCG (98.1%; $P < 0.001$). Eight of the pa whom oocyte retrieval failed when they 2,000 IU HCG, were given 5,000 or 10,0 hCG in a subsequent cycle in which oocy recovered and fertilized successfully. The (12) also indicated that in the stimulated cy process of separation of oocyte-cumulus com GCs from the follicular wall to be availabl trusion at spontaneous ovulation or to be a is dependent on the hCG and its dose. The (12) also showed that serum levels of hCG a peak 17 hours after hCG 5,000 IU IM and did not fall to <10 IU/L until 6 days administration of hCG. Gonen et al. (13) i that a single dose of GnRH-a in the midc able to induce an endogenous LH and FS similar to that of a nature cycle and coul follicular maturation in IVF patients. In the series, GnRH-a was used as either long or sh tocols; therefore, it was not possible to i midcycle gonadotropins surge for follicula ration. On the contrary, the pituitary dow lation and desensitization prevented the L (14); therefore, the hCG trigger injection w nitely required. According to the serum β-h says performed immediately after the faile vaginal aspiration, it was demonstrated t hCG trigger injections had not been give patients, thus resulting in failed oocyte ret

In the cases presented in this study, ther difficulty in recovering oocytes from their in the prior or subsequent cycles that used t or lower doses of gonadotropins, and oocyte rates were virtually >90% of follicles aspirat hCG administration was documented. The ings excluded the problems of follicular as technique and doses of gonadotropins use could be suggested as the causes of failed oo covery in the index cycles. The most likel

nation for the failure to retrieve the oocytes is that cumulus expansion and detachment or loosening from the mural granulosa did not take place because neither the endogenous nor the exogenous stimulus was provided.

It has been suggested that FSH has a specific periovulatory action on cumulus and GCs. Follicle-stimulating hormone in vitro causes expansion of mouse cumulus cells, whereas LH has no effect (15). It is not known if this is the case in the human. The results of the present study indicate that appropriate hCG levels are sufficient to elicit the process of cumulus expansion and be the ovulatory stimulus. This cannot rule out that suprabasal surges of FSH may play a role in the ovulatory mechanism in the human.

Empty follicle syndrome has been reported as a sporadic (7) or a recurrent event (16). However, none of the previous reports has determined serum β-hCG levels after failed retrieval to document if it was because of the failure of hCG administration. In cases of very poor or failed oocyte recovery, the ART team should measure circulating levels of serum β-hCG to confirm whether hCG was administered or not. Furthermore, routine determination of β-hCG levels before oocyte recovery may be helpful in ART cycles. In patients on GnRH-a with undetectable β-hCG levels before oocyte retrieval, hCG could theoretically be given that same day with continuation of GnRH-a treatment, and oocyte retrieval could then be postponed to 36 hours later.

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