The Role of a Human Chorionic Gonadotropin Burst in In Vitro Fertilization

ADRIAN SHULMAN,^{1,3} ISAAC BEN-NUN,² YEHUDIT GHETLER,² MICHAL YONISH,¹ and SHLOMO MASHIACH¹

Submitted: June 21, 1996

Accepted: September 18, 1996

Findings: No oocytes were found during four ovum pickups (OPU), despite a satisfactory ovarian response to controlled ovarian hyperstimulation. After the first attempt failed in the fourth case, five eggs were retrieved, fertilized, and cleaved after cycle rescue with hCG.

Conclusions: Whenever oocytes are not aspirated during OPU due to a lack of hCG administration, the cycle may be rescued if 10,000 IU of hCG is injected immediately and OPU planned for 33–36 hr later.

KEY WORDS: assisted reproduction; empty follicles; menotropins; ovum pickup.

INTRODUCTION

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 undergo a form of follicular stimulation that will result in the recruitment of a cohort of preovulatory follicles and hence supernumerary oocytes.

Empty or cystic follicles are very rare, at least in in vitro fertilization (IVF) programs with gonadotropin releasing hormone agonist (GnRH-a) and gonadotropins.

It has been suggested that an absence of oocytes at ovum pickup (OPU) reflects an underlying ovulatory disorder existing before entry into the IVF program (1). Recently, Asch *et al.* (2) reported on five women

with “empty follicles” at OPU, despite an appropriate ovarian response to controlled ovarian hyperstimulation with GnRH-a and menotropins, numerous follicular lavages, and aspiration of peritoneal fluid. The absence of a human chorionic gonadotropin (hCG) injection before OPU seems to be the cause of the lack of oocytes in these patients.

We report here an additional four cycles from four patients in our IVF program with no oocytes found at OPU, although they had several successful previous cycles with the same protocol of induction of ovulation and comparable hormonal responses. As in the report by Asch *et al.* (2), the only explanation for the absence of oocytes in the aspirate was the lack of hCG administration prior to OPU. We suggest a practical approach for such cases.

MATERIALS AND METHODS

Controlled ovarian hyperstimulation protocols in all cases included pituitary suppression by GnRh-a (*Decapeptyl*, 3.2 mg; CR, Ferring, Malmö, Sweden) and human menopausal gonadotropins (hMG; *Pergonal*; Teva Ltd., Kfar Saba, Israel), as described by Ben-Nun *et al.* (3). Human menopausal gonadotropin stimulation was started 2 weeks after GnRH-a administration, always after ensuring that 17- β -estradiol (E_2) and plasma progesterone (PP) levels were within the castration range ($E_2 < 20$ pg/ml, and PP < 1 ng/ml), and no ovarian cysts were found at ultrasound. Follicular development was monitored by transvaginal ultrasound assessment and daily estimation of E_2 and progesterone levels. An intramuscular injection of 10,000 IU of hCG (*Chorigon*; Teva Ltd.) was given when E_2 levels were at least 1000 pg/ml and at least three or four follicles of 17–18 mm were measured by ultrasound. Thirty-three to thirty-six hours after hCG administra-

¹ IVF Unit, Department of Obstetrics and Gynecology, Chaim Sheba Medical Center, Tel Hashomer (affiliated with the Sackler Faculty of Medicine, Tel Aviv University), Israel.

² Sapir Medical Center, Kfar Saba (affiliated with the Sackler Faculty of Medicine, Tel Aviv University), Israel.

³ To whom correspondence should be addressed.

tion, transvaginal ultrasound-guided oocyte retrieval was performed.

Laboratory Procedures. Oocytes were aspirated using a transvaginal ultrasound technique (*Elscint Ltd., Israel Sector*; vaginal probe, 6.5 MHz). During the OPU procedure, a direct and continual report on the number of retrieved oocytes was received from our laboratory assistants (Y.G., Y.M.). If three or four follicles were aspirated and no oocytes were found, repeated flushing of each follicle was started. Whenever oocytes were not found or only one or two immature eggs were retrieved, despite the high number of follicles aspirated, the patient was asked to show the pack of hCG ampoules, the number of diluent ampoules, and the number of ampoules with active powder, and these were compared with the original prescription. Furthermore, a blood test for qualitative and quantitative β -hCG assessment was performed immediately at the end of the procedure and was found to be negative in all cases.

In the fourth case, after verifying that all active ingredient hCG ampoules were intact, we decided immediately to inject 10,000 IU of hCG, and 34 hr later we retried OPU. By this time, we were able to retrieve five mature eggs, which fertilized and cleaved. Relevant data on the patient's medical history, as well as data on the previous and actual IVF cycles, are presented in Tables I and II.

DISCUSSION

In spontaneous cycles, the maturation of human oocytes in their expanding chambers (follicles) is controlled by the combined presence of hormones from the anterior pituitary gland (FSH and LH) and hormones secreted from the follicles themselves (estrogens). This process leads to the maturation of one single oocyte through a biologically determined time sequence.

The use of GnRH-a and hMG facilitates stimulation of multiple follicular development in women undergo-

ing IVF (4). By producing a hypogonadotropic state, it is possible to control follicular development more readily than when the hypothalamic pituitary system is functioning normally. In particular, an unexpected premature rise of LH can be avoided, and hence, oocyte recovery can be timed conveniently. hCG is used as a surrogate LH surge to trigger the final follicular maturation before oocyte retrieval. After the combination of GnRH-a and hMG, 85% of the oocytes retrieved 33–36 hr after hCG are in the metaphase II stage (5).

With the cases presented in this study, there was no difficulty in recovering oocytes from their follicles in former or subsequent cycles that used similar induction protocols and displayed similar ovarian responses. In these other cycles, oocyte recovery occurred in at least 80–90% of the follicles aspirated.

In spontaneous cycles, ovulation is the release of the oocyte with a surrounding layer of follicular cells (corona radiata) from the follicle chamber. The process is controlled by a burst of LH secretion that may dissolve the surface layer of the follicle. In IVF cycles, the oocytes are needle aspirated, thus the changes in the follicular surface layer induced by LH have no great functional impact.

It is generally accepted that LH induces morphological changes in cumulus cells by inducing dissociation between the cumulus cells and the oocyte by expanding the cumulus–corona complex. Thus, the most likely explanation for failure of oocyte retrieval during IVF is that cumulus expansion and detachment or loosening from the mural granulosa did not occur because neither an endogenous nor an exogenous stimulus was provided (6).

The few oocytes retrieved in one cycle, which did not receive hCG, did not fertilize. Several explanations for fertilization failure exist. Maturation of the oocyte is a prerequisite for fertilization; the LH surge acts as a trigger to resume meiosis (germinal vesicular breakdown, extrusion of the first polar body at the metaphase II stage). Lack of LH causes immature eggs. Further-

Table I. Cycle Characteristics During hCG Administration

	Patient No.		
	1	2	3
No. of hMG amp./cycle	60;84;50;65	80;98;98;101	30;27;24
No. of induction days	13;13;14;16	20;15;15;16	10;10;10
E ₂ levels on day of hCG (pg/ml)	1640;1970;751;2235	1861;2135;3225;3083	3600;4017;3663
No. of follicles aspirated	8;8;5;7	11;11;12;16	30;35;35
No. of eggs retrieved	6;6;4;6	10;9;12;14	27;33;35
No. of embryos/cycle	4;5;4;3	8;7;8;6	23;20;14

Table II. Clinical Information and Presentation of the Patients in Cycles of Failed Oocyte Retrieval^a

	Patient No.			
	1	2	3	4 ^b
Age (years)	27	34	22	31
Parity	G1Ab1P0	G2Ab1P0	G3Ab2Ect1P0	G2Ect1P1
Fertility etiology	Mechanical	Mechanical	Mechanical	Mechanical
No. of hMG amp./cycle	65	91	46	27
No. of induction days	14	14	8	9
E ₂ level on day of hCG (pg/ml)	2014	3400	3026	3770
No. of follicles aspirated	8	15	55	17
No. of eggs retrieved	0	2 immature	1 immature	0
No. of embryos/cycle	0	0	0	0
hCG injection	Only diluent	Only diluent	Only diluent	None

^a Ab, abortion; amp., ampoules; Ect, ectopic; G, gravida; P, para.

^b This patient received 10,000 IU hCG once no oocytes were retrieved at OPU; 34 hr later, five eggs were retrieved by transvaginal ultrasound-guided OPU.

more, spermatozoa cannot penetrate the cumulus oophorus before cumulus expansion. The expansion of the cumulus and production of mucus are LH dependent, and provide an environment in which spermatozoa can pass between cumulus cells to reach the zona pellucida. There is evidence that the cumulus could play a role in the capacitation of spermatozoa, increasing sperm motility, and protecting the zona from hardening. The cumulus oophorus has also been reported to induce the acrosome reaction. In the absence of a hCG burst, cumulus expansion failed to occur.

The fourth case, with rescue of five oocytes after delayed hCG administration, emphasizes the role of the LH burst in the process of oocyte maturation beyond the facilitation of oocyte outbreak. Although the eggs were present in the follicles during the first aspiration attempt, we failed to aspirate them at that time. Only after hCG administration later in the same cycle were they successfully aspirated, emphasizing the role of hCG in both the maturation process and the detachment of the eggs.

As a practical approach, despite the ultrasonographically cloudy appearance of the follicles on the second attempt, due to blood clots, follicular puncture should be performed again, accompanied with meticulous flushing.

The results of this study underscore that hCG administration is imperative to elicit the process of cumulus expansion, the ovulatory stimulus, and detachment from the mural granulosa. Whenever oocytes are not aspirated at OPU, despite a good follicular response

on induction of ovulation, assessment of hCG administration must be done immediately by comparing the contents of remaining ampoules to the initial prescription and by rapid assessment of the patient's blood serum for β -hCG. Both tests must be done to exclude a possible delay in hCG administration. Once the lack of hCG administration is proved, we suggest cycle rescue by immediate administration of 10,000 IU hCG and OPU planned for 33–36 hr later.

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