CLINICAL ASSISTED REPRODUCTION

Effects of Growth Hormone on In Vitro Maturation of Germinal Vesicle of Human Oocytes Retrieved from Small Antral Follicles

HASSAN ALY HASSAN,^{1,2,3} HOSSAM AZAB,^{1,2} ASHRAF ABDEL RAHMAN,^{1,2} and TAMER M. NAFEE^{1,2}

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Purpose: Evaluation of the effect of GH, administered *in vivo*, on *in vitro* maturation of human GV oocytes obtained from small follicles.

Methods: The study included 88 ICSI women stimulated with long agonist protocol. They were randomly allocated to two groups (Group 1, 44 women with 86 GV oocytes), were cotreated with GH (Group 2, no GH: 44 women with 67 GV oocytes). GV oocytes from small follicles were matured *in vitro*, injected, and their development observed.

Results: Resumption of meiosis was significantly higher in Group 1 compared to Group 2 (60 and 37.3% respectively). Significantly more M2 oocytes were obtained in Group 1 than in Group 2 (45.5 and 23.9% respectively). Fertilization rates were also significantly higher (51.9 and 18.8% respectively). No embryos were obtained in Group 2, whereas 21.1% of fertilized oocytes in Group 1 cleaved into class A embryos.

Conclusion: *In vivo* administration of GH enhanced *in vitro* maturation and fertilization of human GV oocytes retrieved from small antral follicles.

KEY WORDS: Germinal vesicle oocytes; growth hormone; *in vitro* maturation.

INTRODUCTION

The stage of follicular development (size of the follicle at the time of retrieval) greatly affects the oocyte's competence to resume meiosis (1). Also the duration and amount of gonadotropic exposure to which the oocyte-cumulus complex is being exposed has an impact on the degree of follicular development, and oocyte maturation, which eventually influences its developmental potential (2).

Growth hormone (GH) has been added to ovarian stimulation protocols aiming to improve ovarian response in low responders (3–5), especially in those with endogenously low GH levels (6). It is known to affect the ovary indirectly through amplification of gonadotropic actions (7), an effect which is mediated through insulin-like growth factor-1 (IGF-1) (7–9).

GH receptor gene expression has been detected in granulosa cells, cumulus cells, and oocytes, indicating that the GH has a direct effect on the ovary (10). Recently, GH mRNA was demonstrated to be expressed in bovine oocytes and granulosa cells recovered from small follicles maturing *in vitro* indicating that GH is being synthesized at its site of action. Such an effect is not controlled by growth hormone releasing hormone (GHRH), implicating the presence of paracrine and autocrine beside the known endocrine effect of GH (11). In granulosa cells, GH was demonstrated to help differentiation and replication (12), in addition to the modulation of steroidogenic gene expression (13,14). Moreover GH induces significant

¹ Obstetrics & Gynecology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt.

² Alexandria IVF & ICSI Center, El Hedaya, Alexandria, Egypt.

³ To whom correspondence should be addressed at Alexandria IVF & ICSI Center, 4 El Mogahedin Street, El Hedaya, Alexandria, Egypt; e-mail: hassanali2000@hotmail.com

cumulus expansion via cyclic AMP signaling pathway (15).

In vitro maturation of bovine oocytes in the presence of GH accelerates nuclear and cytoplasmic maturation, an effect which is exerted through the cumulus (12,16). It is also demonstrated to positively affect cumulus expansion, which is mediated by the cAMP signal transduction pathway (15).

Most of the data available about the effect of GH on oocyte maturation and follicular development are based on animal studies. The present study evaluates the effect of GH, administered in vivo, on the in vitro maturation of human GV oocytes obtained from class V and class VI follicles.

MATERIALS AND METHODS

The study included 88 women undergoing ICSI for male infertility stimulated with long agonist protocols. These women were counseled about the experimental nature of the protocol, and a signed consent was obtained from every patient and her partner to be included in the study. Patients were randomly divided into two groups:

Group 1: (GH Group)

Group 1 included 44 women receiving adjuvant GH 4 IU daily im injections throughout stimulation, starting on the first day of gonadotrophin treatment. Eighty-six germinal vesicle (GV) oocytes were retrieved from small follicles (class V and VI).

Group 2: (Control Group)

Group 2 included 44 women who did not receive adjuvant GH. Sixty-seven GV oocytes were retrieved from small follicles.

M2 oocytes obtained from large follicles (class VIII follicles) were injected, and the development was observed, and only the resulting class A embryos were transferred.

GV oocytes obtained from small follicles (class V and VI follicles) were incubated in nonsupplemented IVF-50 medium. They were then left to mature in vitro, and maturation was observed (GV break down and the resulting M2 oocytes). Those oocytes which resumed meiosis up to the M2 stage were injected and development was observed (fertilization and cleavage rates).

RESULTS

The study included 88 women undergoing ICSI for male infertility, using long agonist protocols. They were randomly divided into two groups: those who received adjuvant GH (Group 1), which included 44 women, and those who did not receive adjuvant GH (Group 2), which also included 44 women. Both groups were homogenous as regards age distribution ($32 \text{ years} \pm 0.4$ in Group 1, and $31.7 \text{ years} \pm 0.6$ in Group 2). The mean number of oocytes retrieved were comparable (10.4 ± 0.5 in Group 1 and 10.2 ± 0.8 in Group 2), with Group 1 yielding slightly more M2 oocytes ($94.1\% \pm 1.3\%$) than Group 2 ($87\% \pm 1.2\%$). The rates of fertilization for these oocytes on injection were $78.6\% \pm 4.8\%$ in Group 1, and $69.4\% \pm 2.4\%$ in Group 2, with cleavage rates of $79.2\% \pm 2.4\%$ and $76.6\% \pm 4.8\%$ respectively. Mean serum estradiol values on the day of hCG were $1608 \pm 97 \text{ pg/mL}$ in Group 1 as compared to $1269.5 \pm 133 \text{ pg/mL}$ in Group 2.

A significantly higher number of GV oocytes obtained from small follicles (class V and VI follicles) resumed meiosis in Group 1 than in Group 2 (60 and 37.3% respectively). GH cotreatment resulted in GV oocytes maturing in vitro to M2 oocytes, which were significantly more in Group 1 (GH group) than in Group 2 (control group) (45.5 and 23.9% respectively). Fertilization rates were also significantly higher in Group 1 than in Group 2 (51.9 and 18.8% respectively). In Group 2 who did not receive adjuvant GH, fertilized oocytes did not cleave (0% cleavage rate), whereas in Group 1 who received adjuvant GH, 21.1% of fertilized oocytes did cleave into class A embryos (Table I).

The overall pregnancy rates, however, were comparable in the two groups (32.1% for Group 1 and 24.6% for Group 2).

Table I. Showing the Development (GVBD, Number of M2 Oocytes, Fertilization, and Cleavage Rates) of the Germinal Vesicle Oocytes Obtained from Small Follicles (Class V and VI Follicles) in Both Groups, Those Who Received Adjuvant GH (Group 1), and Those Who Did Not (Group 2) (Control Group)

Parameters	Group 1	Group 2
GVBD	60% ^a	37.7%
M2 oocytes	45.5% ^a	23.9%
Fertilization rate	51.9% ^a	18.8%
Cleavage rate	21.1% ^a	0%

Note. GVBD: (germinal vesicle break down); M2 oocytes: (metaphase 2 oocytes).

^a $P < .05$.

DISCUSSION

The GH-induced nuclear maturation of the oocyte is cumulus dependent, which could explain the in vivo effect observed in the present study (17). The promoting effect of the hormone on cytoplasmic maturation, however, appears to be cumulus-independent (12). It is exerted through a cAMP-dependent signal transduction mechanism (15), which is probably, but not definitely, IGF-1 independent (7,10). Higher fertilization and cleavage, in response to in vivo GH, could be related to better cytoplasmic maturation after hCG. The action of GH on granulosa cells is mediated through/synergized by IGF-1 (7,18), which, likewise probably demonstrates a developmentally-regulated response, depending on the size of the follicle and the phase of the menstrual cycle (19). Maturation of oocytes in small follicles, therefore, could be enhanced through such an effect. Growth hormone, IGF-1, and gonadotrophins seem to converge on similar cAMP-mediated second messenger cascade in order to modulate the transcriptions activity of their target genes (15,17). IGF-1 receptor stimulation augments FSH receptor transcription by a mechanism that is distal to cAMP generation, and stabilizes the transcript (20). In the work of Homburg and Jacobs, GH augmented gonadotropin action on the ovary particularly in poor responders, and the doses of HMG were significantly reduced. They reported that this effect could be related to the increased effect of IGF-1 action on the ovary especially in PCOS patients where it is controlled with serum insulin levels (5,21–24). In fact FSH-beta subunit gene itself is a possible candidate for the action of GH (25). Enhanced FSH action could result in both better nuclear and cytoplasmic maturation. GH supplementation probably improved in vitro nuclear maturation of the oocyte in the present work. Although the better fertilization and cleavage rates are highly suggestive of an improved cytoplasmic maturation, a definitive effect has yet to be demonstrated.

In conclusion, GH, in vivo, allows access to oocytes before administration of hCG. The effect, therefore could be mediated by granulosa–oocyte intact gap-junctions. Such an effect cannot be provided by in vitro addition of GH, as gap junctions will be disconnected as a function of hCG (26–28).

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