Pregnancy and delivery after in vitro maturation of naked ICSI-GV oocytes with GH and transfer of a frozen thawed blastocyst: case report

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Submitted November 1, 2005; accepted November 15, 2005; published online January 31, 2006

Purpose: To determine if GV oocytes, collected at the time of ICSI, can be matured in vitro and rescued for therapeutic treatment. A patient for whom all the collected oocytes at the GV stage after a classical COH protocol were matured in vitro with GH.

Method: All the naked oocytes were matured in a culture medium (ISM2) containing 15% patient serum +1.6 units of GH (Saizen) per millilitre. Oocytes were incubated overnight at 37°C. The MII oocytes obtained were micro-injected. A fresh transfer was performed and a supernumerary blastocyst was frozen.

Results: The patient was pregnant and delivered a healthy girl after transfer of the frozen/thawed blastocyst. The baby girl is now 2 years old.

Conclusion: In vitro maturation with GH allows rescuing naked GV oocytes collected at the time of ICSI. GH action does not pass through the cumulus cells. According to the possible lack of synchrony between the embryo and the uterus, we recommend to freeze the embryos obtained and to replace them in a controlled cycle.

KEY WORDS: Growth hormone; GV oocytes; in vitro maturation; ICSI; blastocyst.

INTRODUCTION

In vitro maturation of cumulus enclosed oocytes has been introduced for patients with high risk of ovarian hyper stimulation syndrome, including polycystic ovaries (PCOs), as an alternative to conventional controlled ovarian hyper stimulation. It is now proposed as a therapy for diminished ovarian response. For all these patients IVM is carried out on cumulus enclosed oocytes; and no adequate treatment is proposed for patients submitted to COH for ICSI and for whom most of the oocytes, if not all, are at GV stage at the time of denudation. We recently found (1) that the GH receptor is present in the

oocytes and the early pre-implantation human embryos, and that GH stimulates in vitro maturation of naked oocytes. Growth hormone receptor has been described in mouse, bovine oocytes and early pre-implantation embryos (2,3). GH has in vitro, a positive effect through an increase in cytoplasmic competence (5) leading to a better developmental capacity of the embryos. In human, growth hormone has been added in controlled ovarian hyper stimulation (COH) protocols for more than 10 years and its efficiency is rather a matter of controversy. On these bases, we have performed in vitro maturation of oocytes in a patient scheduled for ICSI and for whom all the oocytes were at GV stage at the time of ovum pick up, in a “rescue” protocol.

CASE REPORT

CL a 32-year-old Caucasian woman entered our program on November 2002 to achieve an ICSI

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procedure. Her file showed a partial tube removal following a probable Chlamydia infection on the left side and a blocked tube on the right side. The patient had regular menstrual cycles (28 days) and she did not show any sign of endometriosis. Hormonal stimulation was performed according to classical protocols involving a short treatment with LHRH agonists (decapetyl, Serono, Geneva Switzerland) followed by ovarian stimulation with rFSH (Gonal F:120 units per day during 7 days followed by 75 units the two following days). Ovulation was triggered by 5000 units of hCG on day 10. Fifteen oocytes were collected. They were denuded using Hyaluronidase (Synvitro Hyadase, MediCult*, Jyllinge, Denmark), one hour after retrieval, in U-IVF (MediCult*). All the oocytes except one were at the GV stage, the remaining one was at MI. A 5–10% collection of immature oocytes is usually observed in our unit. The MI oocyte was incubated in ISM2 medium alone, waiting for the first polar body expulsion and micro-injection in the afternoon: it did not mature and was discarded in the afternoon. In order to mature the oocytes, a GH solution was prepared 40 milliunits of GH (Saizen SERONO) in 2.5 mL of ISM2 medium (MediCult*) containing 15% patient serum, collected 2 h after oocytes retrieval, after denudation has been achieved. The oocytes were incubated overnight at 37°C, under 5% CO₂ atmosphere in air. The following morning, 13 of the 14 GV oocytes had reached MII. They were all micro-injected and placed in ISM1 medium (MediCult*), according to our classical procedure. The sperm sample parameters were rather of poor quality: 10 million cells/mL, 2 mL, 60% living cells, 1/2 high-quality motility, 74% abnormal forms according to WHO criteria). After 20 h, nine of the oocytes had two PNs, one was lysed. We did not observe for the last two, any sign of fertilization. On day 3, four embryos were at eight-cell stage, four at the six-cell stage, and two showed late developments (one five-cell and one four-cell originating from the oocytes showing no sign of fertilization 20 h after micro-injection). Two eight-cell embryos with small fragments were transferred; the patient received 100 mG Utrogestan intravaginally for 15 days to support the luteal phase. No pregnancy resulted.

From the six supernumerary embryos, placed in ISM2 medium, one expanded blastocyst was obtained and frozen on day 6. Three months after the procedure, a frozen-thawed embryo replacement cycle was induced by daily stimulation with 50 IU of

rFSH (Gonal F) daily; after 7 days of stimulation, a sonographic control was performed daily in order to check endometrial receptivity. Six thousand International Units of hCG were injected on day 10 and the thawed blastocyst was transferred on day 17, 25 January 2003. Luteal support was performed with Utrogestan (100 mG per day intravaginally). A pregnancy resulted and the patient delivered a healthy girl, 24 October 2003. The baby is now 2 years old showing no health problems.

CONCLUSION

It is always a matter of frustrations and interrogations, when a high proportion or the totality of the oocytes is at the GV stage at the time of ICSI, especially as it is usually totally unexpected. Our observation confirms that GH can be used successfully to mature GV oocytes in a culture medium containing the patient serum, with no extra hormone addition. A role for GH is possible in vivo during human oocyte maturation (4). GH effects on the oocytes can be direct and not necessarily through the cumulus cells even if the GHR mRNA is abundant in cumulus cells (1). Our data observed here are in close agreement with our preliminary data (1) for IVM of GV oocytes with GH: 60/92 = 65% vs. 14/42 = 33% without GH, but they are in contradiction with a mandatory role exerted by the cumulus cells (3). A role in cytoplasmic maturation is obvious according to the improvement in developmental capacity of the embryos obtained after fertilization of in vitro GH matured bovine oocytes (5). Cytoplasmic maturation and regulation of mRNA polyadenylation process are linked and thus lead to a developmental competence. Growth hormone may interfere with this process. GH could also increase the DNA repair capacity in the embryonic cells as observed in the liver cells (6): pre-implantation embryos and liver cells have similar metabolic pathways. DNA repair is obviously an important process at the time of fertilization and early embryogenesis and could fit with the observations made in vitro for aged patients (5). Our procedure has been applied to two other patients: 8 out of the 13 oocytes have been successfully matured, 5 embryos were obtained. The two “fresh” transfers of 6–8 cells on day 3, lead in one clinical pregnancy resulting in a miscarriage, no spare embryo was frozen. However, it seems that the best solution is to freeze the embryo obtained, according to what we did for the successful pregnancy. The risk is

quite high to have a misfit between the embryo and the endometrium: the embryos are transferred with a 24-h late asynchrony, which is always deleterious, as observed in all animal species. Our protocol offers a new simple opportunity to rescue GV oocytes collected at the time of ICSI.

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