Glycoprotein 130 promotes human blastocyst development in vitro

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Objective: To investigate the efficacy of leukemia inhibitory factor (LIF) and/or glycoprotein 130 (gp130) on in vitro growth of human embryos.
Design: Laboratory study.
Setting: University hospital-based IVF clinic.
Patient(s): A total of 164 frozen embryos that survived thawing were cultured in media supplemented with LIF and/or gp130 or control media.
Intervention(s): Morphological development was evaluated by light microscopy. Protein expression profiles of single blastocysts were evaluated using matrix-assisted laser desorption/ionization time of flight-based intact cell mass spectrometry.
Main Outcome Measure(s): Embryo development and protein content.
Result(s): Addition of gp130 to culture media improved blastocyst formation (73% vs. 43%). Addition of LIF to the culture media did not improve embryo development. Protein fingerprint spectra were obtained that revealed significant intensity changes for multiple molecular species including thymosin beta-10, thymosin beta-4, histone H2A, histone H2B, histone H4, ubiquitin, ubiquitin-T, and acyl-CoA binding protein.
Conclusion(s): Glycoprotein 130, but not LIF, seems to be beneficial for preimplantation embryo development, implicating a physiological role in regulating preimplantation development in humans and thus ought to be included in culture media designed for embryo culture to the blastocyst stage. Furthermore, these findings highlight the great potential of matrix-assisted laser desorption/ionization time of flight mass spectrometry and intact cell mass spectrometry as a versatile tool in reproductive medicine research. (Fertil Steril® 2013;99:1592–9. ©2013 by American Society for Reproductive Medicine.)
Key Words: Blastocyst, LIF, gp130, MALDI-TOF MS, ICMS

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Despite years of experience and many technical innovations in assisted reproduction techniques (ART), low implantation rate is still one of the major obstacles resulting in a large proportion of embryonic loss in the ART programs. One reason might be that a number of embryos fail to develop to the blastocyst stage probably due to suboptimal in vitro culture conditions with current culture media systems. A better understanding of basic needs and metabolic requirements of the early embryo at different developmental stages is needed for formulation of new sequential culture media, increased number of embryos reaching the blastocyst stage, and improvement of implantation rates.

Communication between the developing embryo and the maternal tract is of importance during the transport of the early preimplantation embryo through the fallopian tube. It is likely that the leukemia inhibitory factor (LIF) system contributes to this as it has been shown that LIF and its receptors are present in the fallopian tube and in the human preimplantation embryo (1, 2). Interestingly, glycoprotein 130 (gp130) is present in human embryos throughout embryo development; however, only

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in the inner cell mass (ICM) at the blastocyst stage (2), suggesting a need for soluble gp130 communication between the trophectodermal cells of the blastocyst to the maternal tract at later stages of embryo development, but not at earlier stages during transport and development in the fallopian tube. It has previously been shown that gp130 is needed for embryo development in mice (3), but the need for gp130 for human embryo development is not known.

Leukemia inhibitory factor is a cytokine, associated with reproductive processes such as embryo development and implantation (4). The LIF is an absolute requirement for implantation of murine blastocysts (5, 6). The action of LIF is mediated through a receptor constellation consisting of LIF receptor and gp130 subunits (7). The LIF receptor exists in both soluble and membrane-bound forms with opposite effects; the soluble form often antagonizes the actions of its ligands (8–10). gp130 can also combine with other cytokines, such as interleukin-6 (IL-6), IL-11, and ciliary neutrophic factor, to form a high affinity membrane-bound receptor complex (11, 12).

It has been shown that LIF, its receptors, and gp130, increase in the endometrium at the time of implantation (4, 7). Women with unexplained infertility have a lower LIF concentration in uterine fluid and lower protein expression of LIF receptor and gp130 in the endometrium compared with fertile women (1, 13–15), suggesting that LIF and its receptors are important for normal implantation. Soluble gp130 endometrial secretion is normally increased during the implantation window, whereas women with unexplained infertility have decreased secretion of gp130 (15).

In the present study we evaluated the influence of LIF and its receptor gp130 on embryo development using traditional morphological assessment using light microscopy. In addition to morphological assessment, protein analysis by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI TOF MS) was used for direct protein profiling of intact blastocysts followed by protein identification by using bottom-up proteomics and immunofluorescence staining.

The aim of the present study was to evaluate the effect of supplementation with LIF and/or gp130 to the embryo culture media. Furthermore, protein profiling of intact blastocysts by means of MALDI TOF MS was performed to identify potential markers of embryo development.

MATERIALS AND METHODS

Ethical Considerations

All embryos used in this study were donated by couples undergoing IVF treatment at the Centre for Reproduction, Uppsala University Hospital, Uppsala, Sweden. The embryos were thawed after 5 years, which according to the Swedish law is the limit for storage of embryos. After this time it is not possible to use the embryos for fertility treatment. All couples gave their written informed consent for the use of their frozen embryos. The study was approved by the regional research ethics review board in Uppsala.

Chemicals and Reagents

Ammonium bicarbonate, acetonitrile, 1,4-dithiothreitol (Cleland's reagent) (DTT), iodoacetamide, urea, and trifluoroacetic acid were purchased from Sigma Aldrich. Sequence grade trypsin from bovine pancreas was obtained from Roche. Sinapinic acid and protein calibration standard was purchased from Bruker Daltonics.

Embryo Scoring before Cryopreservation

On the morning of day 2, embryo quality was assessed morphologically on the basis of the number of blastomeres (cells), the rate of fragmentation (the proportion of the embryo with anucleate fragments), and the degree of multinucleation of blastomeres, as described elsewhere (16, 17). Each embryo received a score of 0 (top quality), 1 (good quality), 2 (fair quality), or 3 (poor quality). Embryos with a score of 0–2 were used for direct ET, and supernumerary embryos with scores of 0–1 were cryopreserved for later use. Three embryologists graded the embryos and together decided on which embryo(s) to transfer or cryopreserve. The embryo scoring system is validated twice a year, both internally and externally according to the International Organization for Standardization standard.

Freezing of Embryos

Before cryopreservation, embryos were cultured in microdroplets (G-1 v5 PLUS, Vitrolife AB) overlaid with mineral oil (OVOIL) at 37°C and 6% CO_2 in a humidified incubator. Supernumerary embryos were cryopreserved on day 2 (48 hours after ovum pick-up) according to a controlled rate freezing protocol involving the use of 1, 2 propanediol and sucrose solution in phosphate-buffered saline (PBS) as cryoprotectants (Vitrolife AB). This method has been described elsewhere (18, 19). All embryos used in the study had been cryostored for at least 5 years.

Thawing of Embryos

The thawing protocol was performed using a commercial kit according to the instructions from the manufacturer (Thawing Kit; Cook Medical). The embryos were then transferred into equilibrated culture medium (CCM; Vitrolife AB). Thawed embryos were then carefully assessed for blastomere survival, and each embryo received a score of A (100% survival rate), B (50%≤ survival rate <100%), or C (<50% survival rate). Only embryos with a score of A or B were used for the study. The survived embryos were then randomly allocated, with regard to embryo morphology/survival rate, into four different treatment regimens and cultured up to blastocyst stage. The four groups were control, LIF, gp130, or LIF+gp130. The control embryos were cultured in the standard medium that is used in the clinic (CCM; Vitrolife AB). For treatment, LIF (Human Recombinant, 100 ng/mL; Sigma) and/or gp130 (Human Recombinant, 300 ng/mL; Sigma) was dissolved in standard medium (CCM; Vitrolife AB).

Embryo Culture

The embryos were cultured individually in 20-μL droplets and assessed daily for developmental stage. Culture was