ORIGINAL ARTICLE: ASSISTED REPRODUCTION

# Integrating insulin into single-step culture medium regulates human embryo development in vitro

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**Objective:** To evaluate the effect of supplementing single-step embryo culture medium with insulin on human embryo development.
**Design:** Comparative study.
**Setting:** Two private centers.
**Patient(s):** The study involved a sibling oocyte split of 5,142 retrieved oocytes from 360 patients.
**Intervention(s):** Sibling oocytes split after intracytoplasmic sperm injection for culture from day 0 through day 5 or 6 in insulin-supplemented or control medium. Women were split to receive their embryos from insulin-supplemented or control medium.
**Main Outcome Measure(s):** Clinical pregnancy rate.
**Result(s):** There were significantly higher rates of clinical, ongoing, and twin pregnancies in the insulin-supplemented arm than in the control arm. On day 3, embryo quality and compaction were higher in insulin-supplemented medium. On day 5, insulin supplementation showed higher rates of blastocyst formation, quality, and cryopreservation.
**Conclusion(s):** Insulin supplementation of single-step embryo culture medium from day 0 through day 5 or 6 improved clinical pregnancy rate and human embryo development. However, these findings need further confirmation through a multicenter randomized controlled trial that may include other patient populations and different culture media. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
**Key Words:** Embryo culture media, insulin embryonic effect, blastocyst

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Despite significant strides over the past four decades, success of in vitro fertilization (IVF) remains relatively elusive, and the benchmark varies among clinics and around the world. An optimistic analysis reported cumulative live birth rates of up to 86% after six cycles of IVF in young good-prognosis women (1). In another study, women younger than 40 years achieved a cumulative live birth rate of 68% after six cycles, but only 32% for the first cycle (2). However, patient retention through six cycles to achieve this high live birth rate is challenging. Suboptimal embryo culture condition contributes to this relative inefficiency with myriad factors. These include, but are not limited to, pH, temperature, incubator $O_2$ level, volatile organic compounds, incubator type, and in particular, culture medium (3–7).

Culture medium is a complex solution that comprises a range of elements to provide the embryo with hydration, ions, and nutrients while maintaining a homeostatic and relatively nonstressful environment. Over the past decades, endeavors to determine the optimal medium have followed two main approaches: the "sequential media" paradigm which provides the developing embryo with stage-specific nutrients, and "single-step" medium formulation that provides all of the required nutrients continuously (8). However, to date, neither approach has produced a clearly superior medium (9, 10). Using unconditioned culture media, which contain embryotrophic factors, such as cytokines, steroid hormones, growth factors, and insulin, results in better preimplantation embryo development in vitro (11–14). Thus, the supplementation of culture media with specific embryotropic factors may provide better support for embryo development in vitro.

Of particular interest is the hormone insulin. Insulin tends to increase

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cell proliferation and differentiation and decreases apoptosis of in vitro cultured embryos (15). In a mouse model, insulin supplementation of culture medium yielded a 25% increase in the inner cell mass (ICM) and increased compaction and blastulation rate, and conversely the lack of insulin resulted in decreased embryo development and cell proliferation (16–19). This suggests that embryonic growth may be sensitive to insulin levels. Although the exact mechanism by which insulin influences embryo development remains unclear, oocytes and preimplantation embryos express insulin receptor genes in several species, including humans (20, 21). In human embryonic stem cell culture, insulin supplementation yields to increase epiblast cell numbers but not ICM, suggesting a particular role in differentiating ICM toward more pluripotent cells rather than a general mutagenic activity (22). Furthermore, a recent study that used a high concentration of insulin in the culture medium successfully cultured human embryos in vitro to day 13 independently from any maternal tissue contact (23).

With this background in mind, the aim of the present comparative study was to determine whether insulin supplementation of a single-step culture medium would have an influence on and embryo development in vitro and pregnancy rate.

## MATERIALS AND METHODS

From January 2015 to December 2015, two private IVF centers conducted this prospective comparative study. The research ethics committees of the two centers "IbnSina Center (Sohag, Egypt) and Banoon Center (Assiut, Egypt)" approved the protocol (Ethical Clearance no. 005-2014).

Stage 1 ("allocation I") involved a sibling oocyte split between insulin-supplemented and control media; stage 2 ("allocation II") of the trial involved a patient split between transferred embryos from insulin-supplemented and control media.

Women included in this study were 18–37 years of age with body mass index (BMI) $\leq 31$ kg/m$^2$ and normal response to stimulation with ten or more follicles (seven or more mature oocytes expected) as well as normal endometrial thickness (8–12) and echographic pattern at the time of the hCG trigger. They were undergoing their first treatment cycle or had had a previous successful treatment cycle. The study only included fresh semen samples with a total concentration of $10 \times 10^6$/mL and 5% progressive motility, excluded globozoospermia and pinhead samples, and did not use any other morphologic criteria.

In stage 1, an embryologist that not involved in the study design split each woman's inseminated oocytes (all subjects underwent intracytoplasmic sperm injection [ICSI]) evenly between each of two dishes that were unlabeled regarding the containing medium, which was either insulin-supplemented or control medium. For odd numbers of injected oocytes, the last oocyte was assigned randomly to one of the treatments. Color codes were used to label the culture dishes to differentiate between insulin-supplemented and control medium. Oocyte quality was not a factor in assigning oocytes. According to the Istanbul consensus, embryologists graded and recorded the oocyte and embryo quality (24).

For stage 2, 360 women were enrolled to receive embryos on day 5 from either the treatment or the control group according to a list generated with the use of a computer program (Excel). On the day of oocyte retrieval, a research counselor sent a sealed opaque envelope that contained the results of assignment to the laboratory. On the day of embryo transfer, a research counselor opened the sealed opaque envelope and passed the result to the laboratory director. The two highest-quality blastocysts from the insulin-supplemented medium and the control medium were each placed in labeled droplets (coded). The decision regarding transfer of embryos from either the insulin-supplemented arm or the control arm was received from the laboratory director, based on the result of the sealed envelope.

### Ovarian Stimulation Protocol and Luteal Phase Support

A midluteal pituitary down-regulation protocol was used for all women. GnRH agonist (Decapeptyl, 0.1 mg; Ferring) was started on day 21 of the cycle preceding treatment and continued throughout the next cycle. On cycle day 2, quiescent ovaries were confirmed by laboratory and ultrasound examinations. Gonadotropin injection (recombinant FSH [Puregon; MSD] and hMG [Menogon; Ferring]) for multifollicular ovarian stimulation was begun at a dose of 150–300 IU per day and was continued throughout the cycle. An ultrasound scan was done every other day, starting on day 5 of stimulation. When 3 follicles $\geq 18$ mm were seen on ultrasound, a 10,000 IU hCG trigger injection (Choriomon; IBSA) was given for final oocyte maturation. For luteal phase support, intramuscular P (100 mg/mL, Prontogest; IBSA) was started on the day after retrieval ("day 1") and continued up to 8 weeks of gestation.

### Sperm Preparation, Oocyte Retrieval, Denudation, and ICSI

Semen samples were prepared through a density gradient, according to the World Health Organization manual (25) (except for diluting gradient with buffer) with the use of Puresperm (Nidacon). The pellet was washed twice and then maintained at room temperature in HEPES-buffered medium (Allgrade Wash, Life Global). Ultrasound-guided oocyte retrieval was performed 36 hours after hCG administration, and each follicle was aspirated into 1 mL HEPES-buffered medium (Global/HEPES; Life Global) with aspirates handled at 37°C with the use of tube warmers. Oocyte denuding was performed 1 hour after collection with the use of hyaluronidase (Life Global) and mechanical aid (denudation pipettes; Vitrolife). Only metaphase II (MII) oocytes were injected 4 hours after denudation in Global/HEPES medium according to Palermo et al. (26).

### Culture Protocol and Embryo Scoring

The insulin-supplemented medium was freshly prepared the day before oocyte retrieval and ICSI. Insulin (10 mg/mL,