Fertilization outcome, embryo development and birth after unstimulated IVM

Adriana Bos-Mikich · Marcelo Ferreira ·
Marcos Höher · Gerta Frantz · Norma Oliveira ·
Caroline G. Dutra · Nilo Frantz

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Abstract

Background/Purposes A common observation in oocyte in vitro maturation (IVM) cycles is poor embryo quality. However, no study was dedicated to assess zygote and early cleavage embryo quality in IVM cycles. The objective of this study is to analyze fertilization outcome, embryo development and the resulting pregnancy and births in unstimulated IVM cycles.

Methods IVM oocytes were collected 36 h post hCG and matured in vitro for 28–30 h. All oocytes were inseminated by ICSI. Resulting zygotes and embryos were assessed on day-1, 2 and 3, when transfers were made.

Results The overall oocyte maturation and fertilization rates were 63% and 62%, respectively. Abnormal fertilization rate was 1.7%. Ninety five and 14.6% of the 2Pn zygotes reached the 2-cell and 8 –cell stage at day-2 and day-3, respectively. Embryo quality assessment on day-3 at transfer revealed that only 9% of the embryos were of very good quality. Most embryos showed developmental delay. An average of 3.29 embryos were transferred per patient resulting in implantation and clinical gestation rates of 16% and 32%, respectively. Overall 14 healthy babies were born and there is one ongoing pregnancy.

Capsule In vitro maturation of human oocytes results in reasonably high rates of pregnancy and birth being particularly indicated for PCO or PCOS patients.

A. Bos-Mikich (✉)
Department of Morphological Sciences, ICBS,
Universidade Federal do Rio Grande do Sul,
Porto Alegre, RS, Brazil, CEP: 90.050-170
e-mail: adriana.bosmikich@gmail.com

M. Ferreira · M. Höher · G. Frantz · N. Oliveira · C. G. Dutra ·
N. Frantz
Nilo Frantz Research and Human Reproduction Centre,
Porto Alegre, RS, Brazil, CEP: 90.480-003

Conclusion Results show a significant rate of abnormal fertilization and poor embryo quality after IVM, which is reflected in a higher than average number of embryos being transferred. However, pregnancy, implantation and birth rates are reasonably high and allow us to consider IVM a valuable approach for the treatment of infertility in PCO or PCOS patients.

Keywords IVM · Embryo quality · Implantation · Pregnancy · Births

Introduction

The development of new biotechnologies for assisted reproduction (AR) is always desired. The introduction of in vitro maturation in human reproduction represented a valuable choice of protocol, when dealing with patients at risk of ovarian hyperstimulation using exogenous gonadotrophins for classical AR technologies. Since the first report of human oocyte IVM [6], the methodology has been refined and considerable progress has been achieved in terms of oocyte maturation and pregnancy rates.

IVM reported success rates vary considerably among services [1, 2, 4, 8, 11, 17, 18, 19, 21]. It is acknowledged that the methodology for IVM of oocytes collected from unstimulated ovaries and embryo development is still not ideal [12]. A common observation in IVM cycles is the poor embryo quality and the high number of cleavage stage embryos being transferred leading to lower implantation rates, when compared with classic stimulated cycles. We reported on the first IVM pregnancy and birth in Brazil [7]. Since our first attempts with the methodology, we noticed that IVM zygotes have a higher incidence of abnormal fertilization and embryos have a poorer in vitro perfor-

Table 1 Fertilization and embryo development outcome of 34 IVM cycles

Variable	
No. of patients	34
Age (y)(mean $\pm$ SD)	28.88 $\pm$ 4.67
No. Immature oocytes cultured (mean)	556 (16.3)
No. of MII oocytes (maturation rate)	350 (63%)
No. oocytes fertilized	218 (62%)
No. abnormal fertilization (1Pn/ $\geq$ 3PN)(mean)	58 (1.7)
No. cleaving 2Pn embryos	208 (95%)
No. of 8C-embryos at Day-3 (mean)	32 (0.94)
No. embryos transferred Day-3 (mean)	3.29
Pregnancy rate per transfer (%)	11/34(32)
Multiple pregnancies (%)	5/11 (45)
Implantation rate (%)	18/112 (16)
Births	10/11
Sex ratio (male/female)	6/8

mance, compared with stimulated cycles. Also, there are suggestions that birth rates are lower in IVM cycles, which would undermine its application as a valuable infertility treatment approach.

Here we report on the fertilization outcome, embryo quality and development, implantation, pregnancy and birth rates after in vitro maturation of oocytes in unstimulated cycles.

Material & methods

Patient selection

This is a retrospective analysis of 34 IVM cycles that occurred between January 2007 and December 2009. We analyzed data from 27 cycles of patients with polycystic ovarian syndrome (PCOS) and seven cycles of isolated polycystic ovaries (PCO) [14].

IVM patients had a baseline scan performed between days 2 and 4 of either a natural menstrual cycle or, if amenorrhoeic, after withdraw bleed induced by progesterone. This assessment recorded the number of follicles, endometrial thickness, ovarian volume and the presence of cysts. The same assessment was repeated between days 6 and 8 and timing of hCG was based on endometrial thickness (≥ 7 mm).

Oocyte retrieval, fertilization, embryo culture and transfer

Immature oocyte collection was performed 36 h post-hCG injection using a 17- gauge aspiration needle with a reduced aspiration pressure of 80 mmHg. Cumulus-oocyte-complexes were matured in IVM Medium (Medicult)

supplemented with 75 mIU/ml of FSH (Gonal-F, Serono) and 0.1 IU/ml of hCG (Chorimon, Meizler) for 28 to 30 h. After maturation, oocytes were denuded of cumulus cells by exposure to hyaluronidase and pipetting.

Insemination of MII oocytes was by ICSI using the partner's spermatozoa. Injected oocytes were cultured in ECM medium (Early Cleavage Medium, Irvine Scientific) in 5.5% CO₂ in air, at 37°C under oil. Fertilization check was performed 18 h post-ICSI for both groups of patients. Zygotes were further cultured in ECM medium till embryo transfer on day-3, when embryo quality was assessed [20], approximately 72 h post-insemination. All very good and good quality embryos (grades 1 & 2) that were not transferred were cryopreserved.

Patients received the best scoring embryos for transfer. All women received 6 mg of oestradiol valerate (Estrofem, Medley) for endometrial preparation, starting on the day of oocyte collection. Luteal support was provided by 800 mg of natural micronized progesterone (Evocanil, Zodiac) starting on day 1 and continued until 12 weeks of gestation together with the oestradiol valerate. Clinical pregnancy was diagnosed by the presence of a gestational sac, 4 weeks after oocyte pick up.

Results

Table 1 shows the age of the patients, maturation and fertilization rates, embryo development, pregnancy and implantation rates for the 34 IVM cycles here analyzed.

In vitro maturation rate and fertilization rates were 63% and 62% respectively. The frequency of abnormally fertilized oocytes (1Pn, $\geq$ 3Pn) was 1.7%, mainly due to second polar body retention. Nearly all normally fertilized zygotes reached the 2-cell stage on day-2, but only 14.6% of these embryos were at the 8-cell stage at transfer on day-3. In average, more than 3 embryos were transferred for each patient. Following embryo transfer, clinical pregnancy and implantation rates were 32 and 16%, respectively. There were five multiplets gestations, out of which four were twins (two became singletons) and one a quadruplet. There was one late abortion, nine healthy babies delivered (eight girls and six boys) and one ongoing gestation of a baby girl.

Table 2 Quality of IVM embryos at transfer on Day-3

Embryo grade	IVM
G 1	09 (08%)
G 2	27 (24%)
G 3	64 (57%)
G4	12 (11%)
Total D3 (TE)	112

Table 2 details the quality of day-3 embryos at transfer. Only 9% of all day-3 embryos were of very good quality and they were transferred to five different patients, all of which got pregnant. The majority of the embryos (68%) was of poor (G3) or very poor (G4) quality particularly due to retarded development.

Discussion

The data here presented show that embryo quality and development to day-3 are impaired in IVM cycles with oocytes from PCO or PCOS patients. However, implantation and pregnancy rates are reasonable and close to those usually attained in classical stimulated ICSI cycles. Birth and sex ratio of the babies delivered after IVM do not present any significant difference from babies born after other assisted reproduction technologies or natural conception.

There is a limited number of reports describing the embryological aspects of IVM [4, 8, 17, 18] particularly their developmental competence. This is a critical point in IVM procedures, as there are concerns that this approach leads to relatively high rates of developmental incompetent oocytes, which may affect gestation and subsequent birth rates.

Son et al. [18] reported that better pregnancy rate is obtained in hCG—primed IVM cycles, in which in vivo matured oocytes were collected compared with IVM cycles without any in vivo matured oocytes. However, the authors have a rather low implantation rate for both groups of patients and that may suggest that embryo quality was similar between the two groups. The lower embryo quality in that study may also be inferred from the elevated mean number of embryos transferred for both groups of patients.

Earlier studies comparing IVF and IVM claimed that implantation and pregnancy rates were lower after IVM in PCO and PCOS patients [5, 10]. However, these data have been questioned after a meta-analysis showed that there are no randomized controlled trials upon which one can base a conclusion on the use of IVM or conventional IVF for patients with PCOS [16]. On the other hand, a recent report [21] and our own experience show that implantation and pregnancy rates around 16 and 32–36%, respectively, after IVM are much superior to the first reports and close to results attained after classical IVF/ICSI cycles. This observation, together with the fact that we had five multiple gestations suggests that, although day-3 embryo quality seems to be impaired after IVM, their developmental potential is not affected and an IVM-own embryo scoring system should be proposed to compensate for their developmental delay. In doing so, a lower number of embryos should be transferred to avoid the occurrence of multiple gestations and its unwanted outcomes.

We found that after IVM, 1.7% of the fertilized oocytes present abnormal pronuclear formation, particularly 3Pn due to second polar body retention. This frequency is lower than that reported (2.5%) after insemination by IVF or ICSI of more than 5000 human oocytes in conventional IVF/ICSI cycles [13]. However, it is higher than the observed rate of polyploidy after conventional ICSI in our service. The reason for these discrepancies is not clear, however, it can be assumed that failure of second polar body extrusion after ICSI in IVM cycles is related to cytoskeletal abnormalities, possibly caused by manipulation or incomplete cytoplasmic maturation.

One main difference between IVM and classical IVF cycles is that IVM oocytes spend a more prolonged period in vitro before insemination. There are evidences that prolonged in vitro culture is related to an increased rate of monozygotic twinning in classical IVF/ICSI cycles [15]. However, we did not observe any monozygotic twinning among the multiplet gestations here described. These data agree with previous reports [3, 9] and suggests that IVM itself does not increase the rate of monozygotic twinning.

Except for the quadruplet pregnancy, all others were uneventful and 14 healthy babies were delivered, which demonstrates that IVM is a reliable alternative approach for PCO and PCOS patients that need assisted reproduction technologies for achieving a pregnancy.

In conclusion, despite the occurrence of abnormal fertilization and lower embryo quality, progresses in terms of implantation and pregnancy rates and the birth of healthy babies, together with its lower costs and avoidance of ovarian hyperstimulation lead us to believe that IVM is a clinically valuable technology in human AR, with clear application for young PCO and PCOS patients.

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