Outcome of frozen-thawed blastocysts derived from gonadotropin releasing hormone agonist or antagonist cycles

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Abstract *Purpose:* The aim of this retrospective study was to compare the outcome of frozen-thawed blastocysts derived from the cycles using controlled ovarian stimulation with GnRH agonists vs. GnRH antagonists.

Methods: Survival, pregnancy and cumulative live birth rates in 231 freeze-thaw cycles derived from the GnRH agonist cycles (GnRH agonist group), and in 175 freeze-thaw cycles derived from the GnRH antagonist cycles (GnRH antagonist group) were compared.

Results: In the GnRH agonist group significantly higher proportion of blastocysts survived the thawing procedure than in the GnRH antagonist group (86.1% versus 78.5%; $p < 0.01$). The differences in cumulative live birth rates did not differ significantly between the groups: in the GnRH agonist group the cumulative live birth rate was 16.5%, and in the GnRH antagonist group it was 14.2%.

Conclusions: Frozen-thawed blastocysts derived from the GnRH agonist cycles have better survival rates and similar cumulative live birth rates than those derived from the GnRH antagonist cycles.

Keywords ART · Frozen-thawed blastocysts · GnRH agonists · GnRH antagonists

Introduction

The long protocol with gonadotropin releasing hormone (GnRH) agonists has been the golden standard of ovulation induction in in vitro fertilization (IVF) cycles worldwide for the last two decades. New possibilities for the prevention of premature luteinizing hormone (LH) surge were open with the development of GnRH antagonists causing rapid pituitary desensitization leading to a decrease in gonadotropin serum levels without the initial flare-up effect. Additionally, they shorten the procedure with fewer ampoules of gonadotropins used [1, 2].

Controlled ovarian stimulation (COS) with GnRH antagonists proves to be equally efficacious as COS with GnRH agonists in terms of pregnancy rates [3–5]. So far, almost all the published clinical studies of GnRH antagonists have referred to the cycles involving fresh embryos. The outcome of freeze-thaw cycles with two pronuclear (2PN) oocytes or embryos obtained after COS with GnRH antagonists has not been studied sufficiently [6].

The aim of this retrospective study was to compare the outcome of frozen-thawed blastocysts derived from the cycles using COS with GnRH agonists versus GnRH antagonists in terms of survival, pregnancy and cumulative live birth rates.

Materials and methods

Patients

In the time period from 1 January 2002 to 31 December 2004, 931 freeze-thaw cycles were performed in infertile couples that had undergone either conventional IVF or intracytoplasmic sperm injection (ICSI) with fresh embryo transfer (ET)

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and surplus embryos cryopreservation at the Reproductive Unit, Department of Obstetrics and Gynecology, University Medical Center Ljubljana.

In this retrospective study only the cycles of frozen-thawed blastocysts derived from the cycles using COS with recombinant follicle stimulating hormone (rFSH) and GnRH agonist buserelin acetate (GnRH agonist group; $n = 231$) or GnRH antagonist cetrorelix (GnRH antagonist group; $n = 175$) were enrolled. Clinical data of blastocyst freeze-thaw cycles in both groups were collected, and the outcome of frozen-thawed blastocysts (blastocyst survival rate, whether ET of thawed blastocysts was performed or not, pregnancy and cumulative live birth rates) with regard to the COS (agonists vs. antagonists) used were analyzed.

The choice between an antagonist and an agonist in the fresh cycle was coincidental.

Procedures

GnRH agonist group

COS with rFSH and GnRH agonist buserelin acetate according to a standard long protocol was used. On day 22 of the menstrual cycle buserelin acetate administration in a daily dose 0.6 mg s.c. was started and continued for 14 days, after which the first ultrasound (US) examination (SonoAce 8000 EX, Medison, Seoul, South Korea) and estradiol measurement (IMMULITE® Estradiol, DPC, Los Angeles, USA) were performed. When the criteria for pituitary desensitization were reached (estradiol concentration <0.07 nmol/L, endometrial thickness <4 mm, absence of follicles >5 mm), COS with rFSH was started. If the signs of pituitary desensitization were not registered, buserelin acetate in a daily dose 0.9 mg was administered for further 7 days, after which COS with rFSH and buserelin acetate continued until the criteria for the administration of human chorionic gonadotrophin (HCG) were reached (diameter of the dominant follicle at least 18 mm, estradiol concentration at least 0.4 nmol/L per follicle).

GnRH antagonist group

COS with rFSH and GnRH antagonist cetrorelix, using modified single- or multiple-dose protocols, was applied, starting on day 2 of the menstrual cycle with daily rFSH administration. From day 6 onwards US examinations were performed daily. When the dominant follicle reached the diameter of 12–14 mm, a single dose of cetrorelix (3 mg) or multiple doses of cetrorelix (0.25 mg per day) were administered s.c. rFSH administration was continued until the same criteria as in GnRH agonist group for the administration of HCG were reached. If these criteria were not reached within 96 h from the cetrorelix administration (single-dose protocol

only), 0.25 mg cetrorelix was added daily until the criteria for HCG administration were achieved.

HCG was then administered at a dose of 10,000 IU in both groups. Transvaginal US guided aspiration of ovarian follicles was performed 31–32 h after HCG administration. IVF or ICSI were performed using conventional methods [7].

Embryos were cultured to the blastocyst stage in sequential media M1 and M2 (Blast Assist System, MediCult, Jyllinge, Denmark).

At most two embryos at the blastocyst stage were transferred on day 5. Surplus blastocysts of normal morphology (normally developed inner cell mass, trophectoderm and blastocoel cavity) were cryopreserved using the freeze-thawing procedures described previously [8].

Two different methods of endometrial preparation for ET of thawed blastocysts were used:

- spontaneous cycles without ovarian stimulation (patients with regular menstrual cycles) and
- cycles with minimal stimulation, using 75 IU of rFSH daily started on day 7 of the menstrual cycle (patients with irregular menstrual cycles) [8, 9].

Blastocyst survival rate was defined as the proportion of survived blastocysts per all thawed blastocysts in a group. The quality of thawed blastocysts was evaluated according to the percentage of non-damaged cells. Thawed blastocysts were considered as having survived, when $\geq 50\%$ of cells were non-damaged. Further, thawed blastocysts were classified as completely non-damaged (100% non-damaged cells) or partially damaged (50–99% non-damaged cells).

Statistical analysis

The Student *t* test was used to compare normally distributed variables between the groups. For variables not distributed normally Mann–Whitney *U* test was used. Chi-square test was used to compare attributive variables. Data were analyzed using SPSS 12.0.1 (SPSS Inc., Chicago, USA). Statistical significance was set at $p < 0.05$.

Results

There were no significant differences between the two groups in baseline characteristics as shown in Table 1.

There were no significant differences between the two groups in mean number of thawed blastocysts per woman, mean number of blastocysts transferred and in the percentage of cycles with ET of completely non-damaged blastocysts. In the GnRH agonist group, statistically significantly more blastocysts survived the freeze-thawing procedure. In the GnRH antagonist group, ET could not be performed in statistically

Table 1 Baseline characteristics of the two groups

	GnRH agonist group	GnRH antagonist group	<i>p</i>
Number of cycles	231	175	–
Number of women	194	134	–
Age (years) ^a	33.2 ± 4.3	33.6 ± 4.4	NS
Number of previous fresh IVF/ICSI trials ^a	2.3 ± 1.5	2.3 ± 2.1	NS
Cycle day 3 FSH (IU/L) ^a	5.6 ± 1.7	5.7 ± 1.8	NS
BMI ^a	22.7 ± 3.3	22.6 ± 3.2	NS
Number of COCs retrieved in fresh cycle ^a	13.4 ± 5.5	13.6 ± 7.1	NS
Number of embryos transferred in fresh cycle ^a	1.8 ± 0.5	1.9 ± 0.3	NS
Number of cryopreserved embryos from fresh cycle ^a	3.9 ± 2.5	4.1 ± 3.2	NS
Endometrial thickness on the day ET of thawed blastocysts was planned (mm)	9.5 ± 1.8	9.2 ± 2	NS
Indication for IVF			
Tubal factor	51.5%	52.6%	NS
Endometriosis	14.7%	14.9%	NS
Endocrinological factor	28.6%	36.6%	NS
Uterine factor	27.3%	28.6%	NS
Male factor	44.6%	37.7%	NS
Other	4.3%	6.9%	NS

Note. FSH, follicle stimulating hormone; BMI, body mass index; COCs, cumulus-oocyte complexes; ET, embryo transfer. NS, not significant.

^aValues are mean ± SD.

significantly more cycles due to no survived blastocysts. Data are shown in Table 2.

The method of endometrial preparation for ET of thawed blastocysts was distributed evenly between the groups. In the GnRH agonist group, endometrial preparation in spontaneous cycles without ovarian stimulation, and endometrial

Table 2 Outcome of frozen-thawed blastocysts

	GnRH agonist group	GnRH antagonist group	<i>p</i>
Total number of thawed blastocysts	409	317	–
Mean number of thawed blastocysts per woman ^a	1.77 ± 0.78	1.81 ± 0.88	NS
Blastocyst survival rate	352/409 (86.1)	249/317 (78.5)	<0.01
Cycles without ET due to no survived blastocysts	6/231 (2.6)	14/175 (8)	<0.01
Mean number of blastocysts transferred ^a	1.56 ± 0.51	1.55 ± 0.5	NS
Cycles with ET of completely non-damaged blastocysts	151/225 (67.1)	108/161 (67.1)	NS

Note. The value in parenthesis are in percentage; ET, embryo transfer. NS, not significant.

^aValues are mean ± SD.

Table 3 Pregnancy and cumulative live birth rates

	GnRH agonist group (%)	GnRH antagonist group (%)	<i>p</i>
Pregnancy rates per thawed cycle	41/231 (17.7)	24/175 (13.7)	NS
Pregnancy rates per ET	41/225 (18.2)	24/161 (14.9)	NS
Cumulative live birth rate	32/194 (16.5)	19/134 (14.2)	NS
Cumulative live birth rates regarding women's age			
Age ≤ 38 years	29/165 (17.6)	17/113 (15)	NS
Age > 38 years	3/29 (10.3)	2/21 (9.5)	NS

Note. ET, embryo transfer. NS, not significant.

preparation in cycles with minimal stimulation were performed in 121 (52.4%) and in 110 (47.6%) cycles, respectively, and in the GnRH antagonist group in 104 (59.4%) and in 71 (40.6%) cycles, respectively.

Of the 41 pregnancies in the GnRH agonist group 32 deliveries (two pairs of twins) resulted; of the 24 pregnancies in the GnRH antagonist group, 19 deliveries (once twins) resulted. No woman delivered twice. The differences between the groups were not statistically significant neither in pregnancy rates nor in cumulative live birth rates. Data are shown in Table 3. Additionally, there were no statistically significant differences in cumulative live birth rates regarding the women's age neither between the groups nor overall.

Discussion

GnRH antagonists are new medications for the prevention of premature LH surge, introduced into assisted reproductive techniques (ART) for COS only recently. Consequently, the experience with frozen-thawed embryos derived from GnRH antagonist cycles is scarce, and so is the literature concerning this topic.

At our institution GnRH antagonists were introduced in ART program in 2000, when a prospective randomized study was performed with the aim to compare the efficacy of GnRH antagonists vs. GnRH agonists in the standard IVF procedure. Similar pregnancy rates were registered in both groups. The main advantages of GnRH antagonists found were a shorter duration of ovulation induction and less gonadotropins used [10]. In the same year we started to culture embryos to the blastocyst stage. In our experience ET of blastocysts on day 5 improves pregnancy rates and lowers miscarriage rates. All surplus blastocysts of normal morphology are cryopreserved. Freezing and thawing of blastocysts cultured in sequential media are preformed using a modified method [8].

Although our study was retrospective, our results show that there were no significant differences between the two groups in baseline characteristics, in the method of

endometrial preparation for the transfer of thawed blastocysts, in the mean number of thawed blastocysts per woman, in the mean number of blastocysts transferred and in the percentage of cycles with embryo transfer of completely non-damaged blastocysts, which indicates the homogeneity and good comparability of the groups we observed. We should also stress that the same embryo culture in sequential media M1 and M2 (Blast Assist System, MediCult, Jyllinge, Denmark) to reach the blastocyst stage, and the same freeze-thawing procedures were used for all cycles in both groups, therefore we consider the results of the collected data to be reliable.

In most studies comparing the efficiency of GnRH antagonists versus GnRH agonists, the pregnancy rates were for some percentage, but not significantly, lower with GnRH antagonists [10–15]. However, in a Cochrane review, Al-Inany and Aboulghar [16] found significantly fewer clinical pregnancies in women treated with GnRH antagonists. With most recent and modified stimulation protocols with GnRH antagonists the results are improving. In the updated meta-analysis by Daya [3], the clinical pregnancy rate is marginally lower with the antagonists; he concludes that both agonists and antagonists are equally efficacious. The results of the German IVF registry also show that there is no significant difference in the pregnancy rates between the GnRH agonist long protocol and the GnRH antagonist protocol [4].

Hernandez [17] ascertains that GnRH receptors are ubiquitously localized in the reproductive tract, oocyte and embryo and that activation of the GnRH receptor by the GnRH antagonist may be possible. This binding could decrease the synthesis of growth factors involved in the control of the cell cycle, compromising the mitotic program of granulosa and endometrial cells, and fertilization mechanism and zygote development as well. On the basis of Hernandez's findings we may presume that at the transfer of thawed embryos there is no prolonged influence of GnRH antagonists on the reproductive tract, but the influence of GnRH antagonists on thawed embryo is very likely prolonged. That is why the studies on the outcome of frozen-thawed embryos derived from GnRH antagonist cycles are important.

Retrospective studies comparing the outcome of freeze-thaw cycles resulting from the GnRH antagonist- and GnRH agonist-stimulated cycles, used 2PN oocytes for cryopreservation [18–20]. The results of the three studies did not demonstrate any significant differences between GnRH antagonists and GnRH agonists regarding the viability of 2PN oocytes after thawing and the quality of embryos transferred. Moreover, the pregnancy rates in all studies were satisfactory indicating that the cryopreserved oocytes had unhampered implantation potential [6]. Kol et al. in their study [21] found that high doses of GnRH antagonist (1 or 2 mg once daily) are associated with a low implantation rate but do not adversely affect the potential of em-

bryos to establish clinical pregnancy in freeze-thaw cycles. They presume that high doses of GnRH antagonist do not have a direct negative effect on the quality of oocytes and embryos. The only paper dealing with frozen-thawed blastocysts in GnRH antagonist cycles, without comparison to GnRH agonist cycles [22], ascertains that GnRH antagonists probably do not exert an adverse effect on oocyte or embryo quality, and that the chances of blastocyst replacement and achievement of an ongoing pregnancy in a freeze-thaw ET cycle are not dependent on the duration of GnRH antagonist administration.

In our study we found that statistically significantly less blastocysts survived the freeze-thawing procedure and that there were statistically significantly more cycles without ET due to no survived blastocysts in the GnRH antagonist group. According to these results we may not exclude the possibility of GnRH antagonists to have a negative prolonged influence on thawed blastocysts. In the GnRH antagonist group pregnancy and cumulative live birth rates were found to be lower for some percentage, but not significantly, than in the GnRH agonist group.

Although there were statistically significantly more cycles (8%) without ET due to no survived blastocysts in the GnRH antagonist group, the comparison to 25.8% obtained by Zikopoulos et al. [22] shows that ours is a rather satisfactory outcome. However, the pregnancy rates in GnRH antagonist cycles in our study were lower than theirs; they reported 15.1% ongoing pregnancies per started replacement cycle, and 20.3% per ET of frozen-thawed blastocysts.

In conclusion, frozen-thawed blastocysts derived from the cycles stimulated with rFSH and GnRH agonist buserelin acetate have better survival rates and similar pregnancy and cumulative live birth rates than those derived from the cycles stimulated with rFSH and GnRH antagonist cetrorelix.

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