Blastocyst formation—good indicator of clinical results after ICSI with testicular spermatozoa

I.Virant-Klun¹, T.Tomažević, B.Zorn, L.Bačer-Kermavner, J.Mivšek and H.Meden-Vrtovec

Reproductive Unit, Department of Obstetrics and Gynaecology, University Medical Centre Ljubljana, Šlajmerjeva 3, 1000-Ljubljana, Slovenia

¹To whom correspondence should be addressed. E-mail: irma.virant@kclj.si

BACKGROUND: The aim of this study was to evaluate the role of blastocyst culture in patients with azoospermia.

METHODS: In 98 cycles embryos were cultured for 2 days and in 128 cycles for 5 days to reach the blastocyst stage; a maximum of two of the most developed embryos were transferred in each group. RESULTS: There was a negative correlation between a high (≥20 IU/l) male serum FSH and embryo development, manifested as embryos not reaching the morula stage on day 5 (r = 0.387; P < 0.05). After prolonged culture, 23% of embryos reached the blastocyst stage. The pregnancy rates per transfer, and the abortion rates were approximately the same in the day 2 group and the day 5 group (20 versus 20% and 19 versus 18% respectively). After blastocyst transfer, a high clinical pregnancy rate (55%) and a low abortion rate (6%) were achieved, whereas the transfer of arrested embryos provided a low pregnancy rate (2%) and a high abortion rate (100%). If only blastocysts had been transferred on day 5, the clinical pregnancy rate per started cycle would have been approximately the same in both groups (13 versus 16%). CONCLUSIONS: Blastocyst formation is a good indicator of clinical results after ICSI with testicular sperm.

Key words: blastocysts/ICSI/spermatozoa/testis

Introduction

A number of studies have reported the successful culture of human embryos to the blastocyst stage in new sequential media without cell co-cultures (Gardner et al., 1998; Behr et al., 1999; Ménézo et al., 1999; Plachot et al., 2000; Langley et al., 2001; Karaki et al., 2002; Wilson et al., 2002). The transfer of blastocysts offers several advantages, the most important being synchronization of the embryo with the uterine endometrium, and selection of the best quality embryos with a high implantation potential (Gardner et al., 1998; Plachot et al., 2000). Blastocyst transfer is highly recommended for the indications related to maternal, paternal and cytogenetic aspects on the embryonic side to avoid repeated failure of implantation (Ménézo et al., 1999). It has been proposed to use blastocyst culture to avoid the negative paternal effects on embryo development after ICSI (Sakkas, 1999).

Abnormal sperm in terms of concentration, morphology and motility may manifest a negative effect on preimplantation embryo development to the blastocyst stage (Janny and Ménézo, 1994; Shoukri et al., 1998; Griffiths et al., 2000; Dumoulin et al., 2001; Miller and Smith, 2001). Additionally, the ICSI procedure in itself contributes to a reduced capacity of blastocyst formation in comparison with classical IVF (Griffiths et al., 2000; Dumoulin et al., 2001).

The source and maturity of sperm, most likely to be indicative of the severity of spermatogenic disorders, affect the rate of blastocyst formation and blastocyst implantation. It has been reported that sperm from non-obstructive azoospermic subjects, when utilized for ICSI, result in embryos that progress to the blastocyst stage at a lower rate than from obstructive azoospermic subjects and from ejaculated sperm (Balaban et al., 2001). Round spermatid injection is associated with a significantly lower embryo development rate and a significantly higher developmental arrest rate, when compared with the injection of testicular sperm (Balaban et al., 2000; Vidian et al., 2001). It has been reported that pronuclear morphology is correlated with chromosomal complement, and impacted upon by the sperm source (Kahraman et al., 2002).

Silber et al. (2002) found a higher rate of chaotic mosaicism in embryos derived from TESE–ICSI than in those derived from ICSI with ejaculated sperm. The difference was highly significant. It has been found (Obasaju et al., 1999) that paternal factors, which are likely to derive from the centromere, can contribute to embryonic numerical chromosomal abnormalities, which in turn may predispose to implantation failure. Sperm quality may adversely affect the chromosome constitution of embryos that result from ICSI (Obasaju et al., 1999). These findings indicate the importance of, and the need for, good embryo selection after TESE–ICSI.

Little is known about the factors affecting embryo development to the blastocyst stage after ICSI with testicular sperm. The question of whether the use of blastocyst culture selects the
best embryos after ICSI with testicular sperm and leads to a better implantation rate still remains to be answered.

The aim of this study was to find the factors affecting the developmental potential of embryos after ICSI with testicular sperm, and to evaluate the clinical role of blastocyst culture in patients with azoospermia in comparison with conventional cleaving stage embryos.

Materials and methods

Experimental design

This prospective, randomized study was performed at the Department of Obstetrics and Gynaecology, University Medical Centre Ljubljana, from January, 2000 to April, 2002.

The study was randomized so that each day of TESE–ICSI cycles with embryos cultured for 2 days (Group 1) was followed by the day of TESE–ICSI cycles with embryos cultured for 5 days (Group 2). In this manner 226 cycles of ICSI with frozen–thawed testicular sperm were included in the study. In 98 cycles (Group 1) ICSI-derived embryos were cultured conventionally for 2 days in the Universal IVF Medium (Medi-Cult, Denmark) and a maximum of two cleavage-stage embryos were transferred into the uterus on day 2. In 128 cycles (Group 2) embryos were cultured for 5 days in the Medium 1 and Medium 2 of Blast Assist System (Medi-Cult) and a maximum of two most developed embryos—blastocysts, delayed embryos—morulae, or arrested embryos—were transferred into the uterus on day 5. Supernumerary embryos were cryopreserved. We compared the clinical results between the groups. All men gave informed consent to participate in the study. The study had been approved by the National Medical Ethics Committee.

Studied population

In this study 226 ICSI cycles with testicular sperm were performed in 154 infertile men (74 men in Group 1 and 80 in Group 2) suffering from azoospermia: 29 obstructive azoospermia (16 in Group 1 and 13 in Group 2) and 125 non-obstructive azoospermia (58 in Group 1 and 67 in Group 2). Data on the age and testicular volume were collected for all the patients. Histological diagnosis of spermatogenesis was made in 62 patients (28 in Group 1 and 34 in Group 2), evaluation of serum FSH concentration in 77 patients (48 in Group 1 and 29 in Group 2). Serum FSH concentration was defined as increased at >10 IU/l and high at ≥20 IU/l.

Testicular biopsy and tissue cryopreservation

In all couples ICSI was performed using frozen–thawed testicular sperm. In our centre the diagnostic testicular biopsy has been followed by testicular tissue freezing to avoid possible negative effects of subsequent multiple biopsies since 1997. Moreover, with sperm cryopreservation we avoid ovarian stimulation and oocyte retrieval if no testicular sperm are retrieved.

Testicular tissue was extracted by open biopsy or aspiration. Each testicular specimen was analysed for the presence of sperm. A part of the sample was immediately fixed in the Bouin’s solution for histological analysis of spermatogenesis using modified Johnsen’s score (from score 1: Sertoli cells-only and thin epithelium, to score 10: normal spermatogenesis and thick epithelium) (Holstein et al., 1994). The remaining testicular specimen was frozen to be used in subsequent ICSI treatments. Testicular tissue was dissected, mixed with cryoprotectant solution—10% glycerol in Flushing Medium (Medi-Cult), put into ampoules, and frozen automatically (MiniCool 40, Air Liquide, France) to −150°C. Then it was plunged into liquid nitrogen (−196°C) and stored until thawing for ICSI. It was thawed at room temperature.

IVF

Female partners were stimulated using first a desensitizing protocol of GnRH agonist (s.c. buserelin acetate 0.6 mg once daily; Suprefact; Hoechst AG, Germany), started on day 22 of the menstrual cycle. If after 14 days serum estradiol concentrations were <40 pg/ml and no ovarian cystic structures were observed on ultrasound (US) examination, stimulation was started with 225 IU/day of hMG (Pergonal; Serono, Switzerland) or FSH (Metrodin, HP 75 IU; Serono). hCG (Pregnyl; Organon, The Netherlands) 5000 IU was administered when a leading follicle measuring >18 mm in diameter was obtained. Transvaginal ultrasound-guided aspiration of ovarian follicles was performed 36 h after hCG administration using a single lumen needle.

Oocyte and sperm preparation before ICSI

Cumulus–corona cell complexes were isolated from the follicular fluid and put into 5 ml Falcon tubes with 1 ml of Universal IVF medium (Medi-Cult); the tubes were gassed in 5% O2, 5% CO2 and 90% N2. The removal of the cells of the cumulus and the corona radiata by hyaluronidase has been described extensively elsewhere (Palermo et al., 1992; Van Steirteghem et al., 1993). Oocytes were placed in 80 IU hyaluronidase (Type VIII; Sigma, USA) and the cells of the cumulus and the corona radiata were removed with a denudation pipette (Swemed Lab, Sweden) and washed with Flushing Medium (Medi-Cult).

After testicular tissue preparation on PureSperm (Nidacon, Sweden) discontinuous concentration gradient and washing, 5 μl droplets of sperm suspension were prepared under liquid paraffin (Medi-Cult).

ICSI procedure

Metaphase-II oocytes were placed in 5 μl droplets of Universal IVF medium under liquid paraffin (Medi-Cult). ICSI was performed by the conventional method (Van Steirteghem et al., 1993) without using polyvinylpyrrolidone (PVP). A metaphase-II oocyte was aspirated with a slight negative pressure by the holding pipette (Swemed Lab) and a single spermatozoon was injected into the ooplasm by the micro-injection pipette (Swemed Lab) under an inverted microscope (Diaphot-TMD, Nikon, Japan) equipped with a hydraulic micro-manipulator (Narishige, Japan). Before micro-injection, no special selection of sperm was performed.

Embryo culture to the cleaving stage and transfer on day 2

In each patient, all embryos were cultured in a group in 0.5 ml of Universal IVF Medium (Medi-Cult) in a double-well dish (Falcon); a maximum of two embryos were transferred into the uterus on day 2 using the TDT embryo transfer set (Prodimed, Neuilly-en-Thelle, France). Supernumerary embryos of good quality (<15 % fragmentation) were cryopreserved.

Embryo culture to the blastocyst stage and transfer on day 5

Embryos were cultured to the blastocyst stage in sequential media, Medium 1 and Medium 2 of Blast Assist System (Medi-Cult). All embryos of each patient were cultured in a group. On day 1, fertilized oocytes were transferred into 0.5 ml of fresh Medium 1 in a double-well dish (Falcon). On day 2 they were transferred into fresh Medium 2. Then embryos were transferred each day into a fresh Medium 2, and on day 5 a maximum of 2 multi-cell embryos were transferred into the uterus. On day 5 we classified embryos as most developed embryos—blastocysts, delayed embryos—morulae, and arrested embryos—lower than morula stage. We transferred two blastocysts into the uterus.
uterus; if there were no blastocysts, we transferred the two most
developed embryos that did not reach the stage of blastocyst—
morulae, or arrested embryos. Supernumerary blastocysts were
cryopreserved.

Luteal support and follow-up of pregnancy
On the day of embryo transfer, the luteal phase support was started
using oral dydrogesterone, 300 mg/day (Dabroston: BELUPA,
Croatia). Biochemical pregnancy was confirmed by serum beta-hCG
determination 15 days after embryo transfer. Clinical pregnancy was
confirmed by US demonstration of at least one gestational sac at 6 to 7
weeks of pregnancy.

Statistical analysis
For statistical analysis the SPSS program (SPSS Inc., Chicago,
Illinois, USA) was used. Differences between the groups were
evaluated by Mann–Whitney U-test. Correlations between the clinical
parameters (female age, female indications of infertility, male age,
serum FSH, testicular volume, modified Johnsen’s score), and the
developmental potential of embryos after ICSI with testicular sperm
were evaluated by Spearman’s correlation coefficient. Statistical
significance was set at $P < 0.05$.

Results

General clinical results
In the studied population of men, the mean serum FSH
concentration was $12.1 \pm 10.3$ IU/l (range: 1.9–38.3), modified
Johnsen’s score $6.8 \pm 2.9$ (range: 1–9), and testicular volume
$29.9 \pm 13.3$ ml (range: 4.0–50.0). Serum FSH was elevated
($\geq 10$ IU/l) in 47 of the 77 (61%) men, and high ($\geq 20$ IU/l) in
22 (29%) men. The highest serum FSH concentration at which
testicular sperm were retrieved was 38.3 IU/l. There was a
strong negative correlation between the FSH level and
modified Johnsen’s score ($r = -0.649$, $P < 0.0001$). With
FSH serum concentrations 10 IU/l and 20 IU/l, the mean
Johnsen’s score was 5 and 2, respectively.

Clinical results concerning ICSI with testicular sperm are
presented in Table I. The group receiving embryos on day 2 did
not differ from the group receiving embryos on day 5 in the
male and female age, and proportion of female indications of
infertility.

After 226 ICSI cycles, embryos were transferred in 189
(83%) cycles resulting in 38 clinical pregnancies, i.e. in a 20% pregnancy rate per embryo transfer. Seven pregnancies (18%) ended in a spontaneous abortion. Clinical results of the group receiving embryos on day 2, and of the group receiving embryos after prolonged culture on day 5, arrested embryos included, were approximately the same. In both groups the twin pregnancy rates were approximately the same; there was no triplet pregnancy in either group.

Clinical results after day 2 transfer versus day 5 transfer
Blastocyst transfer provided a high clinical pregnancy rate per
embryo transfer (55%). After the transfer of blastocysts on day
5, pregnancy and implantation rates were significantly higher
than after the transfer of embryos on day 2; the abortion rate
was significantly lower (Table I).

| Table I. Clinical results after ICSI with testicular sperm according to the day of embryo transfer (day 2 versus day 5) |
|--------------------------|--------------------------|
| Embryo transfer on day 2 (cleavage stage embryos) | Embryo transfer on day 5 (blastocysts) |
| Started cycles | 98 | 128 |
| Couples | 74 | 80 |
| Male age (years) (range) | $34.05 \pm 6.85$ | $34.74 \pm 7.09$ |
| Female age (years) (range) | $32.93 \pm 5.01$ | $32.78 \pm 5.15$ |
| Female indications of infertility (%) | 22 (30) | 29 (36) |
| Oocytes | 749 | 850 |
| Oocytes per woman | $7.6 \pm 5.3$ | $6.6 \pm 4.9$ |
| Fertilized oocytes (%) | 240 (32) | 305 (36) |
| Embryos | 215 | 86 |
| Transferred (%) | 147 (68) | 62 (72) |
| Frozen (%) | 56 (26) | 24 (28) |
| Excluded (%) | 12 (6) | 0 (0) |
| Embryo transfers (%) | 81 (83) | 31 (24) |
| Clinical pregnancies | 16 | 17 |
| Clinical pregnancy rate per embryo transfer | 20 % | 55 % |
| Clinical pregnancy rate per started cycle | 16 % | 13 % |
| Twin pregnancies (%) | 1 (6) | 2 (13) |
| Triplet pregnancies (%) | 0 (0) | 0 (0) |
| Implantation rate | 125% | 31.7% |
| Spontaneous abortions (%) | 3 (19) | 1 (6) |
| Extrauterine pregnancies (%) | 0 (0) | 0 (0) |

$^a/b/c/d/e/f/g/h$Statistically significant differences as detected by Mann–Whitney $U$-test ($P < 0.05$).

Clinical results after embryo transfer on day 5
After prolonged culture, at least one blastocyst was transferred
in 31 of the 108 (24%) ICSI cycles. In the remaining transfer
cycles, embryos that did not reach the blastocyst stage were
transferred: morulae in 30 (28%) cycles and arrested embryos at
the lower than the morula stage in 47 (44%) cycles (Table II).

After the transfer of blastocysts clinical pregnancy and
implantation rates were significantly higher than after the
transfer of morulae and arrested embryos (Table II). Blastocyst
transfers resulted in a significantly higher twin-pregnancy rate,
and in a significantly lower abortion rate than transfers with
morulae and arrested embryos (6 versus 50% and 6 versus
100%; $P < 0.05$). With arrested embryos that did not reach the
morula stage, the pregnancy rate was very low (2%). After 47
transfers only one pregnancy was achieved, which ended in a
spontaneous abortion (Table II).

If only blastocysts had been transferred on day 5, the clinical
pregnancy rate per started cycle would have been approxi-
mately the same as in the day 2 transfer group: 13% (17
pregnancies in 128 cycles) versus 16% (16 pregnancies in 98
cycles) (Table I).

Developmental stage of embryos on day 5
After prolonged culture, 68 of the 290 (23%) embryos reached
the blastocyst stage and 54 (19%) embryos reached the morula
stage, whereas 35 (12%) embryos arrested before the morula
stage having more than 6 cells, and 134 (46%) embryos arrested at the 2–6-cell stage.

Clinical factors affecting developmental potential of embryos

There was no correlation between the female age ($r = -0.083$, $P = 0.390$), female indications of infertility ($r = 0.039$, $P = 0.687$), and the number of blastocysts in the couple. In the male partner, there was no correlation between the number of blastocysts and age ($r = 0.167$, $P = 0.197$), serum FSH ($r = 0.249$, $P = 0.143$), modified Johnsen’s score ($r = 0.098$, $P = 0.504$) and testicular volume ($r = 0.039$, $P = 0.687$).

The only correlation we observed was a positive correlation between the serum FSH concentration in the male partner and the number of arrested embryos that did not reach the morula stage on day 5 ($r = 0.387$; $P < 0.05$). In men with high serum FSH concentrations ($> 20$ IU/l) a significantly lower proportion had at least one morula and a significantly higher proportion of men had only arrested embryos in their ICSI cycles, compared with the men with normal serum FSH concentrations (Table IV).

There were no statistically significant differences in the developmental potential of embryos between the men with obstructive and those with non-obstructive azoospermia (Table V).

Discussion

In this study we provide evidence that prolonged culture of embryos after ICSI with testicular sperm does not decrease clinical results in infertile men with azoospermia. Blastocysts have a very good prognosis for pregnancy, whereas after the transfer of embryos that do not reach the blastocyst stage, clinical results are inferior: low pregnancy and implantation rates, most pregnancies ending in a spontaneous abortion.

In this study, 23% of embryos developed to the blastocyst stage after ICSI with testicular sperm. From our own experience, a significantly lower proportion of embryos develop to the blastocyst stage after ICSI with testicular sperm than after ICSI with ejaculate sperm (45%) or classical IVF (60%) thus confirming the negative paternal effects on embryo development.

Some recent studies show that blastocysts may have chromosomal abnormalities. It has been shown that some chromosomally abnormal human embryos develop to the

<table>
<thead>
<tr>
<th>Table II. Effect of the developmental potential of embryos transferred on day 5 on clinical results</th>
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<tbody>
<tr>
<td>Development potential of embryos on day 5</td>
</tr>
<tr>
<td>Blastocysts</td>
</tr>
<tr>
<td>Number of cycles with embryo transfer</td>
</tr>
<tr>
<td>Patients</td>
</tr>
<tr>
<td>Female age (years)</td>
</tr>
<tr>
<td>Male serum FSH concentration (IU/l; range)</td>
</tr>
<tr>
<td>Transferred embryos</td>
</tr>
<tr>
<td>Transferred embryos per woman</td>
</tr>
<tr>
<td>Clinical pregnancies</td>
</tr>
<tr>
<td>Clinical pregnancies per embryo transfer</td>
</tr>
<tr>
<td>Twin pregnancies (%)</td>
</tr>
<tr>
<td>Triplet pregnancies (%)</td>
</tr>
<tr>
<td>Implantation rate</td>
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<tr>
<td>Spontaneous abortions (%)</td>
</tr>
<tr>
<td>Extrauterine pregnancies (%)</td>
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</table>

Statistically significant differences as detected by Mann–Whitney U-test ($P < 0.05$).

<table>
<thead>
<tr>
<th>Table III. Developmental stage of embryos according to the male serum FSH concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum FSH concentration</td>
</tr>
<tr>
<td>≥20 IU/l (7 men/11 cycles)</td>
</tr>
<tr>
<td>&gt;10 IU/l (15 men/22 cycles)</td>
</tr>
<tr>
<td>&lt;10 IU/l (18 men/27 cycles)</td>
</tr>
</tbody>
</table>

Statistically significant differences as detected by Mann–Whitney U-test ($P < 0.05$).
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Table IV. Men and developmental stage of embryos after ICSI with testicular sperm according to the serum FSH concentration

<table>
<thead>
<tr>
<th>Serum FSH concentration</th>
<th>All men</th>
<th>Men with at least one blastocyst in all cycles (%)</th>
<th>Men with at least one morula in all cycles (%)</th>
<th>Men with arrested embryos only in all cycles (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥20 IU/l</td>
<td>7 (11 cycles)</td>
<td>2 (29)</td>
<td>0 (0)</td>
<td>5 (71)</td>
</tr>
<tr>
<td>&gt;10 IU/l</td>
<td>15 (22 cycles)</td>
<td>4 (27)</td>
<td>4 (27)</td>
<td>7 (46)</td>
</tr>
<tr>
<td>&lt;10 IU/l</td>
<td>18 (27 cycles)</td>
<td>7 (39)</td>
<td>6 (33)</td>
<td>5 (28)</td>
</tr>
</tbody>
</table>

ab, Statistically significant differences as detected by Mann–Whitney U-test (P < 0.05).

table V. Type of azoospermia and developmental potential of embryos

<table>
<thead>
<tr>
<th>Azoospermia</th>
<th>All embryos</th>
<th>Blastocysts (%)</th>
<th>Morulae (%)</th>
<th>Arrested embryos (%)</th>
<th>Serum FSH (IU/l; range)</th>
<th>Johnsen’s score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obstructive (13 men/25 cycles)</td>
<td>47</td>
<td>7 (15%)</td>
<td>16 (34%)</td>
<td>24 (51%)</td>
<td>4.4 ± 1.9 (2.5–10.3)</td>
<td>8.8 ± 0.4</td>
</tr>
<tr>
<td>Non-obstructive (67 men/82 cycles)</td>
<td>197</td>
<td>52 (26%)</td>
<td>30 (15%)</td>
<td>116 (59%)</td>
<td>15.7 ± 10.7 (1.9–38.3)</td>
<td>5.9 ± 3.1</td>
</tr>
</tbody>
</table>

The only correlation we found was a positive correlation between the serum FSH concentration in the male partner and the number of arrested embryos that did not reach the morula stage on day 5. In men with high serum FSH concentrations (≥20 IU/l) there was a significantly lower proportion of morulae and a higher proportion of embryos arrested prior to reaching the morula stage. We also found a higher proportion of men with only arrested embryos after ICSI with testicular sperm than in patients with normal serum FSH concentrations. Negative paternal effects were expressed at lower cell stages, whereas the proportion of blastocysts was approximately the same as in patients with normal serum FSH. We consider it very likely that an increased number of arrested embryos in men with high FSH is related to the switch from maternal to embryonic genome at the 4–8-cell stage (Bolton et al., 1989).

Serum FSH concentration is not a highly predictive factor for successful testicular sperm recovery in azoospermic patients (Tournaye et al., 1997). No strong predictors for successful testicular sperm recovery are available except histopathology (Tournaye et al., 1997). FSH concentrations correlate with spermatogonial activity. However, the prediction of successful sperm retrieval is not absolutely reliable. Testicular sperm extraction can also be successful when FSH hormone concentrations are outside the threshold levels (Bohling et al., 2002). It is possible to obtain sperm from patients with markedly elevated serum FSH (38.7 IU/l) as reported by Gil-Salom (Gil-Salom et al., 1995). In our study the highest serum FSH concentration at which testicular sperm were retrieved was 38.3 IU/l. Besides, 61% of men included in our study had increased serum FSH levels (≥10 IU/l), and 28% of men had high serum FSH concentration (≥20 IU/l). Therefore, the negative effect of high FSH should not be overlooked.

blastocyst stage (Sandalinas et al., 2001). However, in our study a high pregnancy rate per embryo transfer (55%) and a very low spontaneous abortion rate (6%) by blastocysts were obtained. Clinical pregnancies (13%) were also achieved by the transfer of morulae, although half of these pregnancies ended in a spontaneous abortion. Morulae are delayed embryos and a transfer of morulae, although half of these pregnancies ended in a spontaneous abortion. Our findings fit with the observation of a lower implantation rate of the embryos reaching the blastocyst stage on day 6. After 47 transfers of embryos arrested at a lower cell stage than the morula only one pregnancy was achieved, which ended in a spontaneous abortion.

By using blastocyst culture the genetic risk is not avoided, but is much decreased. A significantly higher proportion of genetically abnormal embryos is found among arrested embryos; aneuploidy mosaicism was found in almost 37% of arrested embryos (Ménézo and Ben Khalifa, 1995). In yet another study the percentage of abnormal cells was 54% in arrested embryos, and 17.1% in blastocysts (Veiga et al., 1999). Our study confirms the finding that after prolonged culture, arrested embryos lead to a spontaneous abortion, therefore the transfer of arrested embryos is not recommended. Moreover, if on day 5 only blastocysts had been transferred, the clinical pregnancy rate per started cycle would have been approximately the same as with the transfer on day 2. In this case, however, many women would have been saved from having a transfer and pregnancy that would end in an abortion. Also, prolonged culture of embryos improves the selection of embryos for cryopreservation thus avoiding the freezing of arrested embryos.

We did not observe any correlation between the female age, female indications of infertility, and the number of blastocysts in the couple. Most cycles were performed for the male factor of infertility—azoospermia.
FSH concentrations might reflect the testicular sperm quality. Egozcue showed that meiotic disorders in infertile men are frequent, and increase with high FSH concentrations (Egozcue et al., 2000). These patients produce more sperm with autosomal and sex chromosomal disomies and diploid sperm, originating either from meiotic mutations or from a compromised testicular environment. It has been stated that sperm concentration <1 × 10⁹/ml and/or serum FSH concentration >10 IU/l are the only predictors of male germ cell meiotic abnormalities (Vendrell et al., 1999).

There is a link between gonadal failure (high serum FSH levels) and the occurrence of sperm chromosome aneuploidies (Levron et al., 2001). Testicular sperm have higher rates of sperm aneuploidy and diploidy than ejaculated sperm (Bernardini et al., 2000; Mateizel et al., 2002). Paternal factors negatively affecting the embryo development are related to anomalies of male germ cell DNA condensation (Francavilla et al., 2001) and fragmentation (Tesarik et al., 2001; Evenson et al., 2002), as well as to centrosome anomalies (Hewitson et al., 2002). The factors leading to azoospermia may affect testicular sperm morphology (Yavetz et al., 2001).

In our study there was a strong negative correlation between FSH concentrations and Johnsen’s score. The serum FSH concentrations of 10 IU/l provided a mean Johnsen’s score of 5, and the FSH concentration of 20 IU/l, a Johnsen’s score of 2. These elevated FSH concentrations mainly correspond to immature germ cells or absence of germ cells. In most patients with high serum FSH concentration (≥20 IU/l) we extracted sperm from the sites of focal spermatogenesis; immature germ cells prevailed. FSH level >20 IU/l might represent the threshold level of testicular sperm maturity and explain the arrest of embryo development.

We found no statistical difference in the developmental potential of embryos in patients with obstructive versus non-obstructive azoospermia. Some men with non-obstructive azoospermia had a normal or only slightly elevated serum FSH concentration (i.e. azoospermia with maturation arrest), similar to that in men with obstructive azoospermia. Male serum FSH seems to be one of the most important factors for testicular sperm quality and embryo development.

Blastocyst formation is a good indicator of clinical results after ICSI with testicular sperm in terms of high implantation rate and low abortion rate. High male serum FSH concentrations negatively affect the developmental potential of embryos, these negative paternal effects being manifested before the morula stage.

Acknowledgements
We would like to thank the andrologist Sašo Drobnič and gynaecologists Martina Ribič-Pucelj, Andrej Vogler, Eda Bokal-Vrtačnik, and Bojana Pinter for collaborating in the IVF programme, Ms Mojca Pirc for revising the manuscript, and Ms Bibi Fissbechus for all her support.

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Submitted on November 5, 2002; accepted on January 1, 2003