Clinical embryologists invest a major part of their working day in observing oocytes, zygotes and embryos in order to optimize the chance of selecting an embryo that has the greatest potential of leading to the birth of a healthy baby after transfer (Puissant et al., 1987). Through gradually evolving processes that are largely based upon experience, in vitro fertilization (IVF) laboratories invariably develop their own systems of assessment and scoring. In 2011, two major professional societies, Alpha (Scientists in Reproductive Medicine) and the European Society for Human Reproduction and Embryology (ESHRE) Special Interest Group for Embryology reached a consensus on gamete and embryo assessment, which was published as the Istanbul Consensus or I.C. (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). Practice guidelines that include assessment of embryo morphology have also been proposed by ASEBIR, the Spanish Embryologists’ Society (Torello et al., 2005), and by the UK’s Association of Clinical Embryologists (ACE) in collaboration with the British Fertility Society (Cutting et al., 2008). Although it is universally recognized that assessment and scoring of gametes and embryos is largely a matter of experience, which requires a relatively long learning period and is known to be subject to substantial intra-observer variation (Arce et al., 2006; Baxter Bendus et al., 2006; Paternot et al., 2009), dedicated teaching makes it possible to achieve excellent levels of agreement regarding embryo classification. This atlas has been compiled as a teaching tool, providing a full collection of oocytes and embryos at each stage of development, placed within the context of clinical and treatment parameters associated with each couple. The cases illustrated were gathered from routine patient treatment cycles at two centres: Kantonsspital Baden A.G., Switzerland (KSB) and Kinderwunsch Zentrum Landes-Frauen- und Kinderklinik (LFKK) in Linz, Austria. Because of restrictions imposed by legislation in Switzerland, blastocyst culture and transfer is not a routine procedure at KSB, and the additional cases from Austria that demonstrate extended culture to the blastocyst stage therefore add a significant and important dimension to the atlas. The inclusion of cases, images and descriptions from two entirely separate, independent IVF units also provide an excellent opportunity to compare and contrast all of the different parameters of clinical assessment and controlled ovarian hyperstimulation that are theoretically common to all assisted reproductive technology (ART) practices, and yet can be applied with differences that may be quite subtle.

The 111 cases are divided into two groups: those that ended in transfer of cleavage stage embryos (KSB) and those that were placed into extended culture, aiming for blastocyst transfer (LFKK). Each set of cases was categorized in the first instance on the basis of female indication for treatment. Because female age is accepted as the most important contributory factor in the prognosis for a successful outcome, the cases were sorted according to female age within each category. For easy reference, supplementary summary tables have been provided so that cases with specific parameters can be identified, for instance, semen parameters, stimulation protocol, total gonadotropin dose, oestradiol level, and number of oocytes retrieved.

Cause of infertility, indication for treatment

1. No female cause of infertility (COI) identified: male infertility, or ‘idiopathic’ infertility

   Semen assessment was carried out according to the fourth edition of the WHO guidelines (1999), with the following limits for parameters defined:
   
   - Normozoospermia: \( >20 \times 10^6/\text{mL} \)
   - >50% type A+B or >25% type A
   - >14% normal forms
   
   Oligospermia: \( <20 \times 10^6/\text{mL} \)
2. Introduction

Asthenospermia: < 50% type A+B or < 25% type A
Type A fast progressive motility
Type B non-linear motility
Type C non-progressive motility
Type D immotile
Teratozoospermia: < 14% normal forms
Cryptozoospermia: < 100 single spermatozoa in the entire ejaculate
OATS: oligoasthenoteratozoospermia

The male factor diagnosis is based upon two diagnostic semen assessments prior to the initiation of treatment; in some cases, the parameters found on the day of oocyte retrieval differed from those that were used to define the diagnosis.

2. Endometriosis: diagnosed and graded according to the revised ASRM guidelines for staging and classification (Practice Committee of the American Society for Reproductive Medicine, 2006).
3. Tubal damage: confirmed by hysterosalpingogram or laparoscopy
5. Poor response/ovulatory dysfunction/anoovulation/premature ovarian failure (POF)
6. Hyperprolactinaemia
7. Cervical factors
8. ‘Other’: fibroids, translocation

Clinical and laboratory protocols

Controlled ovarian hyperstimulation

Long gonadotropin releasing hormone agonist protocol (luteal downregulation protocol, LDP)

Pituitary downregulation was achieved with the use of either the gonadotropin releasing hormone (GnRH) agonist goserelin (Zoladex®, AstraZeneca, Zug, Switzerland), depot injection of 3.6 mg on day 21, or buserelin (Suprecur®, Sanofi-Aventis, Frankfurt am Main, Germany). Ovarian stimulation was carried out with either menotropin (Humegon®, Organon, Pfäffikon, Switzerland), human menopausal gonadotropin (HMG) (Menopur®, Ferring, Kiel, Germany) or recombinant follicular stimulating hormone (FSH) (Puregon®, Aesc Pharma, Vienna, Austria). Stimulation was initiated with a routine starting dose of not less than 150 IU human menopausal gonadotropin/FSH and adapted to the follicular response ascertained by vaginal ultrasound and the assessment of oestradiol levels.

Short gonadotropin releasing hormone agonist protocol (follicular phase downregulation, FDP)

The GnRH agonist was administered from day 1 or 2 of the cycle, and ovarian stimulation initiated on day 2.

Gonadotropin releasing hormone antagonist protocol

Human menopausal gonadotropin (Menopur®, Ferring Pharmaceuticals, Saint-Prex, Switzerland) or recombinant FSH (Puregon®, Aesc Pharma, Vienna, Austria) was started on day 3 of the cycle, and GnRH antagonist ganirelix (Orgalutran®; Organon, Vienna, Austria or Ganirelix®, Gentaur, Zürich, Switzerland) was administered after 5–6 days of stimulation, when a 12–13 mm follicle was detected by ultrasound monitoring.

Ovulation was induced with 10 000 IU human chorionic gonadotropin (hCG) (Pregnyl®, Aesc Pharma, Vienna, Austria or Organon, Pfäffikon, Switzerland), and vaginal oocyte retrieval was carried out under ultrasound guidance 36 hours after hCG administration.

KSB: all cases reported are intracytoplasmic sperm injection (ICSI) cycles, and cumulus cells were removed immediately after oocyte collection using SynVitro Hydase (Medicult Origio GmbH, Berlin, Germany).

After complete enzymatic and mechanical denudation, germinal vesicle (GV) oocytes were discarded, and metaphase I were separated from metaphase II oocytes. These were placed in 30 μL drops of universal IVF medium under a layer of liquid paraffin oil (Medicult Origio) in a 3001 Falcon dish, and cultured in an atmosphere of 5% O2, 5% CO2 and 90% N2 until the time of injection, 5–6 hours after retrieval. Metaphase I oocytes that had matured to metaphase II, on the basis of an extruded polar body, were also injected.

LFKK: cumulus–oocyte complexes (COC) were collected in BM1 medium (Eurobio, Les Ulis, France) and incubated for 2–3 hours prior to IVF or ICSI. Denudation for ICSI was performed after brief exposure to 80 IU hyaluronidase (Origio, Berlin, Germany) 2–3 hours after oocyte retrieval. ICSI was carried out on all oocytes with the first polar body (PB) extruded (metaphase II) immediately after enzymatic and mechanical denudation.
Sperm preparation

**KSB:** all semen samples were routinely prepared by centrifugation over a two-layer (40%/100%) density gradient at 500 g for 20 minutes. The 100% layer was washed twice with 5 mL of universal IVF medium (Medicult Origio GmbH, Berlin, Germany) and centrifuged for 10 minutes at 500 g. Cryptozoospermic samples were washed twice with universal IVF medium, similar to the density gradient washing steps. The injection dishes (BD Falcon 353655 Becton Dickinson, Alschwil, Switzerland) contained a central 20 μL droplet of SpermSlow™ (Medicult Origio GmbH, Berlin, Germany), surrounded by six 20 μL droplets of flushing medium (Medicult Origio GmbH, Berlin, Germany), overlayed with liquid paraffin. A small amount of the sperm preparation was added to a drop and incubated for 20 minutes prior to the injection. If available, hyaluronan-bound spermatozoa were selected and immobilized by tail crushing as described in detail by Van den Bergh et al. (1995, 1998). Testicular spermatozoa were preferentially prepared and cultured in universal IVF medium 24 hours before the oocyte collection, but were not treated further (Emiliani et al., 2000, 2001; Van den Bergh et al., 2009).

**LFKK:** normozoospermic ejaculates were incubated in a sperm selecting chamber (Zech-selector, AssiTIC Medizintechnik GmbH, Leutsch, Austria), a device that consists of two concentric wells overlaid by a U-ring and a cover glass. Progressively motile spermatozoa migrate from the ejaculate in the outer well to concentrate in the medium-filled inner well via a capillary bridge created by the overlying U-ring. After approximately 1–2 hours, a 500 μL sperm sample can be aspirated from the central chamber, analysed and used for insemination. This method results in the accumulation of an adequate number of motile sperm without exposure to centrifugation stress (Ebner et al., 2011a).

Samples to be used for ICSI were prepared by a mini swim-up technique, and then incubated on the injection dish (Falcon type 1006) in 10 μL droplets of fresh BM1-medium. Two small droplets of a polyvinylpyrrolidone (PVP) solution (MediCult, Copenhagen, Denmark) were also prepared in the same dish. This constellation of droplets under mineral oil avoids potential contamination of the PVP by debris carried over from the sperm suspension. The sperm samples were subjected to additional treatment where appropriate, for example, a ready-to-use ionophore in case of previous ICSI failure (GM508 Cult-active®, Gynemed Lensahn, Germany) or preincubation with theophylline (GM501 SpermMobil, Gynemed, Lensahn, Germany) in order to increase motility in samples obtained by testicular sperm extraction (TESE).

**Intracytoplasmic sperm injection**

The technique of ICSI has been described in detail (Ebner et al., 2001). Briefly, micromanipulation was performed on an inverted microscope (x200 magnification, Olympus, Vienna, Austria) with Hoffman modulation contrast (Modulation Optics Inc., Greenvale, NY, USA), an electronically controlled heated stage and hydraulic micromanipulators (Luigs and Neumann, Ratingen, Germany). Injection was performed exclusively with the PB at the six o’clock position, using Microtech pipettes (Gynemed, Lensahn, Germany). A similar micromanipulation set-up was used at KSB, consisting of a combination of electronically guided micromanipulators (Transferman NK and CellTram Vario microinjectors, Vaudaux-Eppendorf AG, Basel, Switzerland) installed on a Leica DMI 3000 B with modulation contrast and Leica MATS heating stage. Images were captured using the Leica DFC295 camera and processed with the Leica application suite (Leica Microsystems, Heerburg, Switzerland).

**Culture conditions**

**KSB:** oocytes were cultured individually in 20 μL universal IVF medium (Medicult Origio GmbH, Berlin, Germany) overlaid with liquid paraffin, from the time of retrieval until pronuclear observation 18 hours after injection.

Zygotes were transferred on day 1 into Medicult ISM1 medium for further culture. An optimal pH of 7.3 was obtained in Forma Scientific water-jacked Series II incubators in an atmosphere of 5% O2, 5% CO2 and 90% N2. Because of the restrictions imposed by the Swiss law on reproductive medicine, a maximum of three selected zygotes were kept in culture; in general, only two zygotes were cultured further, in order to keep the overall multiple pregnancy rate below 20% as defined in our ISO 9001:2008 certified quality management system. Surplus zygotes were cryopreserved.
4 Introduction

**LFKK:** oocytes and embryos were cultured individually in small drops (10 μL) of either sequential medium (EmbryoAssist® and BlastAssist®, MediCult, Copenhagen, Denmark) or global medium (GM501 Cult®; Gynemed, Lensahn, Germany), using Falcon type 351016 culture dishes and a K-MINCI-1000 benchtop incubator (Cook, Brisbane, Australia). The gas mixture consisted of 5% O₂, 6% CO₂ and the remainder N₂.

After evaluation of all prognostic markers assessed from day 0 to day 5, a maximum of two embryos/blastocysts were chosen for intrauterine transfer. Embryos were loaded into an Gynetics catheter (Gynemed, Lensahn, Germany) using <10 μL of BlastAssist medium 2 or GM501 Cult, inserted transcervically and then expelled approximately 1 cm from the fundus. The embryo transfer procedure was performed without sedation or anaesthesia.

All images were produced using the Octax EyeWare® (MTG, Altdorf, Germany) at a magnification of x400. In occasional cases where the expanding blastocyst was larger than the image section, Adobe Photoshop CS5 was used to automatically calculate an image from four to six raw images.

**Luteal support**

**KSB:** Utrogestan vaginal tablets (300 mg) and Aspirin Cardio 100 (Bayer, Zürich, Switzerland) were administered daily from the day after follicular puncture until the result of a pregnancy test was known.

**LFKK:** a total of 9000 IU hCG (Pregnyl®, Organon, Vienna, Austria) was injected over a period of three days: the day of ovum pick-up (3000 IU), the day of embryo transfer (3000 IU) and then three (1500 IU) and six (1500 IU) days post-transfer. The hCG was not administered to patients at risk of ovarian hyperstimulation syndrome (OHSS). Progesterone was also administered from the day after follicular puncture until the day of the pregnancy test, using either in-house prepared vaginal suppositories (400 mg) or vaginal tablets (300 mg) (Utrogestan®, Meda Pharma, Vienna, Austria).

Serum hCG levels were measured 17 days after intrauterine transfer. Clinical pregnancy was determined by visualization of at least one gestational sac with positive fetal heart activity 4 weeks after embryo transfer. Biochemical pregnancies were diagnosed if no fetal heartbeat was detected.

**Oocyte and embryo assessment/image captions**

Embryologists are presented daily with oocytes of varying qualities, the majority of which result from desynchronization between nuclear and cytoplasmic maturation. At one extreme, cytoplasmic fusion between two oogonia may lead to so-called giant oocytes, which are diploid or, before meiosis, tetraploid, and thus cannot be used for ICSI (Rosenbusch et al., 2002). In other cases, oocytes may be ‘over-mature’, having aged either in the follicle or in the incubator (Miao et al., 2009).

In order to facilitate discussion among scientists, oocyte anomalies may be classified into extra- and intracytoplasmic dysmorphisms (Alpha (Scientists in Reproductive Medicine) and ESHRE Special Interest Group of Embryology, 2011). Intracytoplasmic anomalies represent a heterogeneous group of dysmorphisms that include first PB morphology (Ciotti et al., 2004; Ebner et al., 2000, 2002), perivitelline space (PVS) size (Xia, 1997) and granularity (Farhi et al., 2002; Hassan-Ali et al., 1998), discoloration (Esfandiari et al., 2006), zona pellucida (ZP) defects (Esfandiari et al., 2005) and shape anomalies (Ebner et al., 2008b). The majority of these morphological phenomena seem to have a negligible effect on further outcome.

The same holds true for the vast majority of intracytoplasmic anomalies, for example, inclusions or refractile bodies (Otsuki et al., 2007), dense central granulation (Kahraman et al., 2000), vacuoles (Ebner et al., 2005) and aggregation of the smooth endoplasmic reticulum (sER) (Ebner et al., 2008a; Otsuki et al., 2004). Any impact of these dysmorphisms on further pre-implantation development is closely related to the size and the number of anomalies. The only exception to this is clustering of the sER, which, according to reported consequences, is the most detrimental of the observed intracytoplasmic features (Ebner et al., 2008a; Otsuki et al., 2004).

The KSB has a policy whereby no specific scoring system is used but, instead, a clear and simple description is provided, in order to allow the readers to attribute a score based upon their own preferred system. Readers of this atlas should also refer to the details provided in the guidelines for minimum gamete and embryo assessment standards suggested by the I.C. (Alpha (Scientists in Reproductive Medicine) and ESHRE Special Interest Group of Embryology, 2011).
The descriptive parameters provided by the KSB cases include:

1. Zona pellucida thickness
   Although the I.C. suggests that ZP thickness measurement adds no benefit to assessment, we have previously found that this parameter may be correlated to oestradiol levels at the time of ovulation induction, and a thick ZP is sometimes apparent in cases with reduced fertilization rates in the presence of normal semen parameters (Bertrand et al., 1996). Therefore, we systematically annotate a ZP of atypically thick appearance.

2. Perivitelline space
   The size and appearance of the PVS is mentioned, although we have been unable to use this as a prognostic feature.

3. First polar body
   The presence of the first PB is used to confirm that the oocyte has reached the metaphase II stage but, in our opinion, the morphology of the first PB is relevant only when its size is atypically large. Metaphase II oocytes with large first PBs were not used for therapy because of the potential aneuploidy risk.

4. Cytoplasmic dysmorphism
   The cytoplasm of the human oocyte often contains a variety of inclusions, among which discs or aggregates of sER and vacuoles are easy to recognize. Other inclusions appear in the form of refractile bodies, dense or degenerate areas of cytoplasm, and 'clustering', as described in the I.C. Clustering is easily recognized, even with a simple binocular light microscope, and is associated with poor oocyte quality. Inclusions are described as 'major' or 'minor' on the basis of their size and number.

   The presence of sER aggregates may be associated with an abnormal outcome (Ebner et al., 2008a; Otsuki et al., 2004) and must be reported; if the abnormalities persist, the oocytes should not be used for therapy. Vacuoles observed at the oocyte stage tend to persist and are also associated with a poor prognosis. However, vacuoles may appear on day 1 during the stage of pronuclear formation, and disappear at the cleavage stage. The presence of vacuoles impairs fertilization and interferes with cleavage planes (Istanbul Consensus, (Alpha Scientists in Reproductive Medicine) and ESHRE Special Interest Group of Embryology, 2011).

Pronuclear assessment
The detailed assessment of pronuclei 16–18 hours after sperm injection or oocyte insemination provides a considerable amount of useful information about the further potential of the embryo. In countries where ART is governed by restrictive legislation, pronuclear and oocyte morphology are the only tools at the disposal of the embryologist, especially in situations where a maximum of three zygotes can be kept in culture and embryo freezing is forbidden. Scott and Smith (1998) were the first to correlate zygote morphology with rates of implantation and pregnancy. The basis of their grading system was a combination of pronuclear size with number and distribution of nucleoli. Changes in cytoplasmic appearance (Ebner et al., 2003) and progression to first cleavage division were also considered as part of the scheme (Sakkas et al., 1998). However, this scoring system was quite complex and the requirement for multiple observations made it rather impractical. Consequently, these authors (Scott, 2003; Scott et al., 2000) reconsidered their scoring system and introduced a simple single-observation score (so-called Z-score). The revised system took into account nuclear size and alignment, together with number and distribution of nucleoli. The zygotes of best prognosis showed nucleoli that were aligned at the pronuclear junction, a finding that was in line with observations reported by others (Tesarik and Greco, 1999).

Irrespective of the type of scoring system applied on day 1, zygotes showing pronuclei that are either unequal in size or peripherally located, and those with non-abutted pronuclei are considered to be abnormal.

The KSB developed a system of classifying pronuclear morphology in three groups:

(i) Symmetric or synchronous configuration, with nucleoli or nucleolar precursor bodies (NPBs) aligned in an equatorial plane facing each other, corresponding to the Z1-score (see previous discussion).

(ii) Symmetric or synchronous configuration without alignment of the NPBs, corresponding to the Z2-score.
All other configurations are described as asynchronous, and as mentioned before, are associated with a lower developmental and implantation potential.

The terms synchronous, symmetric and aligned correspond with the Z-patterns O, 1 and 3 as described by Tesarik and Greco, 1999; however, the observation of fewer than three or four aligned NPBs was considered to be prognostic of lower competence compared with between three and seven aligned NPBs. To date, the factors that may influence pronuclear morphology remain unclear, and no correlation has been found with the spermatozoon selected for intracytoplasmic injection. From more than 4000 pronuclear (PN) scores collected in our database, we were able to observe a lower frequency of synchronous patterns in older women, and in cycles in which controlled ovarian hyperstimulation continued for more than 12 days.

Assessment of cleavage stage embryos

Assessment of cleavage stage embryos on days 2–3 post insemination is based mainly on blastomere number and symmetry, as well as degree of fragmentation (Johansson et al., 2003). A correlation has been found between blastomere size and irregularity and the presence of multinucleated cells (Hardarson et al., 2001).

In the timescale of normal in vitro development, a good quality embryo is expected to have four blastomeres of equal size at least 41 hours after injection or eight cells 65 hours after ICSI, with no or minimal fragmentation; blastomeres are geometrically aligned in a pyramidal fashion. The daily practice at KSB is to perform the ICSI procedure around 2 p.m., and a subsequent fertilization check is systematically carried out at 7.30 a.m., that is, 17.5 hours post-ICSI, a timescale that is within the suggested 17/C61 hour proposed by the I.C.

It is widely accepted that a significant degree of fragmentation is associated mainly on blastomere number and symmetry, as well as degree of fragmentation (Johansson et al., 2003). A correlation has been found between blastomere size and irregularity and the presence of multinucleated cells (Hardarson et al., 2001). In the timescale of normal in vitro development, a good quality embryo is expected to have four blastomeres of equal size at least 41 hours after injection or eight cells 65 hours after ICSI, with no or minimal fragmentation; blastomeres are geometrically aligned in a pyramidal fashion. The daily practice at KSB is to perform the ICSI procedure around 2 p.m., and a subsequent fertilization check is systematically carried out at 7.30 a.m., that is, 17.5 hours post-ICSI, a timescale that is within the suggested 17 ± 1 hour proposed by the I.C.

Blastocyst assessment

Viable blastocyst stage embryos were scored strictly according to the guidelines published by Gardner et al. (2000). In brief, the implantation potential of a given blastocyst is assessed on the basis of three parameters: blastocoel expansion, quality of the inner cell mass (ICM), and quality of the trophectoderm (TE), based upon cell number and cohesion. It should be noted that it is only possible to assess the quality of both cell lineages from the full blastocyst stage onwards, that is, Gardner scores III–VI (described as follows). A distinct ICM cannot be detected in early blastocysts (scores I–II). Exclusion of fragments and blastomeres (Kovacic et al., 2004) and other abnormalities (Ebner et al., 2011a) were also recorded as part of the assessment.

I Early blastocyst: the blastocoel occupies less than half the volume of the embryo.
II Blastocyst: the blastocoel occupies half the volume of the embryo or more.
III Full blastocyst: the blastocoel completely fills the embryo, but the zona pellucida has not thinned.
IV Expanded blastocyst: the volume of the blastocoel is larger than that of the embryo, and the zona pellucida is thinning.
V Hatching blastocyst: the TE has started to herniate through the zona pellucida.
VI Hatched blastocyst: the blastocyst has completely escaped from the zona pellucida.

The morphology of the ICM and TE are then assessed under an inverted microscope:

<table>
<thead>
<tr>
<th>Inner cell mass</th>
<th>Trophoderm</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Tightly packed, many cells</td>
<td>Many cells forming a cohesive epithelium</td>
</tr>
<tr>
<td>B Loosely grouped, several cells</td>
<td>Few cells forming a loose epithelium</td>
</tr>
<tr>
<td>C Very few cells</td>
<td>C Very few large cells</td>
</tr>
</tbody>
</table>
REFERENCES

Alpha (Scientists in Reproductive Medicine) and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Human Reproduction 2011; 26: 1270–1283; Reproductive Biomedicine Online 2011; 22(6): 632–646.


Johansson M, Hardarson T, Lundin K. There is a cutoff limit in diameter between a blastomere and a small anucleate.
8 Introduction


Part A

Cleavage stage transfer (KSB, Switzerland)
Case 1  

4 years 1\textdegree infertiltiy  Diagnosis: azoospermia

<table>
<thead>
<tr>
<th>Female partner</th>
<th>Male partner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 24, kitchen assistant</td>
<td>Age 39, statistician</td>
</tr>
<tr>
<td>Tubal status: unexplored</td>
<td>Congenital bilateral absence of vas deferens</td>
</tr>
<tr>
<td>MH: oligo-amenorrhoea</td>
<td>Karyotype normal 46 XY</td>
</tr>
<tr>
<td>Endocrine status: normal</td>
<td>CFTR-gene: no mutations found</td>
</tr>
<tr>
<td>Non-smoker, no alcohol abuse</td>
<td>No microdeletions</td>
</tr>
<tr>
<td>Non-smoker, no alcohol abuse</td>
<td>Non-smoker, no alcohol abuse</td>
</tr>
</tbody>
</table>

**Semen assessment**: fresh testicular spermatozoa used for ICSI  
Previous treatments: none  
Cycle: ICSI 2010

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation protocol</td>
<td>Long protocol, GnRH agonist, depot 3.6 mg</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>Carried out at external centre, not recorded</td>
</tr>
<tr>
<td>Daily dose</td>
<td>2 × 75 IU HMG</td>
</tr>
<tr>
<td>Total dose</td>
<td>Not known</td>
</tr>
<tr>
<td>Oestradiol at the time of hCG administration</td>
<td>Not recorded</td>
</tr>
<tr>
<td>Number of follicles ≥ 15 mm</td>
<td>Not recorded</td>
</tr>
<tr>
<td>Total number of retrieved oocytes</td>
<td>11</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>9</td>
</tr>
<tr>
<td>Metaphase I</td>
<td>0</td>
</tr>
<tr>
<td>Germinal vesicles</td>
<td>2</td>
</tr>
<tr>
<td>Atretic oocytes</td>
<td>0</td>
</tr>
<tr>
<td>Injected</td>
<td>9</td>
</tr>
<tr>
<td>Lysed</td>
<td>0</td>
</tr>
<tr>
<td>Diploid</td>
<td>2</td>
</tr>
<tr>
<td>Monoploid</td>
<td>4</td>
</tr>
<tr>
<td>Polyploid</td>
<td>0</td>
</tr>
<tr>
<td>Cryopreserved</td>
<td>0</td>
</tr>
<tr>
<td>Replaced</td>
<td>2-cell embryo and 6-cell embryo</td>
</tr>
</tbody>
</table>

**Outcome**: biochemical pregnancy