

DEBATE

Quality control and quality assurance in IVF

Twins of mixed races: consequences for Dutch IVF laboratories

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In June 1995, people working in the field of in-vitro fertilization (IVF) were startled by newspaper reports mentioning the birth of an IVF twin of mixed races. What has been thought of as the 'nightmare of the embryologist' had actually happened in a busy IVF setting of the Academic Hospital in Utrecht, The Netherlands. The unfortunate event gave rise to a whole series of questions: how are the parents of the twins going to live with it, how are the children going to cope with it and also, of course, what went wrong in the IVF laboratory and what measures have to be taken to further minimize such kind of risks. In the Dutch Society for Clinical Embryologists a discussion on quality assurance and safety has been opened and will lead to practical consequences for Dutch IVF laboratories. Apart from letting it be a national debate, we felt it would be appropriate to inform IVF laboratories more widely. An inquiry into the scientific literature on the subject of 'gamete exchange in IVF' did not provide us with any publication although in the lay press misuse of gametes in other occasions was reported. In a review on donor insemination (Linden *et al.*, 1995), however, mislabelling or mishandling of gametes is mentioned as a potential risk factor and a few examples are discussed. The recommendations of the safety and standards committee of the European Society of Human Reproduction and Embryology (ESHRE) stresses the importance of good labelling for the identification of specimens, according to good laboratory practice, and labels should bear the names of the patient (ESHRE, 1991). However, no further details or specific requirements are given. It is the purpose of this communication to report the incident in Utrecht to our international colleagues and open a discussion about safeguarding in our work, assuming that similar incidents can occur elsewhere or even have occurred elsewhere, maybe even going without notice.

Inquiry of the incident itself

Long before newspaper publicity started, the twins and their parents underwent extensive blood group typing. This established that one of the boys was the natural child of both

parents but that the other boy had a different genetic father. Furthermore, an internal inquiry commission of the hospital, completed with an external member with embryological expertise, reviewed the IVF procedures in the hospital in an effort to clarify what exactly went wrong. Several possibilities were investigated: insemination of at least one of the Petri dishes with oocytes with two different types of spermatozoa or contamination of one of the pipettes used during sperm preparation. The possibility of criminal intent was considered not to be applicable. The inquiry commission concluded that it was impossible to exactly pinpoint the cause of the event. The commission further concluded: 'although the commission understands that the IVF procedures practised are according to normal standards in Dutch IVF laboratories, it endorses improvement of the current IVF practice'. The report of the commission further states: 'Because a unique living product comes into being in the IVF laboratory, the procedures in such a laboratory should follow the strict guidelines of Good Manufacturing Practice (GMP), as used by the Pharmaceutical Industry'. The commission also advised to bring this part of their opinion to the attention of the Dutch Health Inspection Authority. The board of directors of the hospital agreed with the conclusions of the inquiry commission and instructed the IVF laboratory to start implementation of GMP regulations into its day-to-day practice.

Can we apply GMP in IVF?

The above mentioned inquiry commission considers the embryo to be transferred after IVF [or *mutatis mutandis* the sperm suspension to be inseminated for intrauterine insemination (IUI), etc] as a 'product' of the IVF laboratory. Although such a concept is debatable, it is of interest to investigate how far GMP rules can be applied to IVF laboratories considering the embryo or the sperm suspension as its products. It is not the purpose of this contribution to elaborate on GMP regulations, only to summarize some important GMP rules which might and which might not apply to our 'products' (summarized from ref 3, code of regulations FDA).

The following GMP rules might apply: (i) written procedures should be available designed to assure the quality of the product; (ii) production and process control procedures shall be followed and documented at the time of performance; (iii) critical steps in the procedures need to be validated; (iv) components removed from a container to a new container shall be adequately labelled and identified by a second person to assure proper identification; (v) each component shall be added to a batch by one person and verified by a second person; (vi) use of major equipment shall be identified by a distinctive code to show the specific equipment used in the manufacture of each batch or product; (vii) appropriate written procedures

designed to prevent microbiological contamination of products purporting to be sterile shall be established and followed, including validation of any sterilization process; and (viii) written procedures shall be available to assure that products are correctly labelled and packaged, this includes inspection immediately before packaging. Results of this inspection shall be documented.

GMP regulations that, at first sight, probably do not apply to IVF-laboratories are: (i) regulations on automatic labelling and packaging of products; (ii) taking of representative samples of finished products; (iii) maintaining of reserve samples; and (iv) regulations on returned products and recall procedures.

Furthermore, GMP contains a whole set of regulations on personnel responsibilities and qualifications, buildings and facilities, availability of proper equipment and purity assessment of components that contribute to the endproduct. As some important rules might apply [see for instance rules (iv) and (v) on double-checks] and some not, special GMP rules have to be set-up for IVF and in general for assisted reproduction techniques (ART) involving not only laboratory aspects but also clinical aspects.

Examples of GMP procedures in IVF

In most IVF laboratory manuals it reads: 'Each semen sample should be handled individually and each stage of the procedure should be completed before moving to the next sample'. In daily practice this often means that semen samples are located next to each other in one tray. The embryologist or technician indeed moves from sample to sample, for instance while pipetting the Percoll pellet from a tube into a fresh tube with wash medium. In between he or she discards the pipette or pipettes that came into contact with a particular specimen. In this way 'each stage of the procedure is completed before moving to the next sample'. All tubes are placed in one centrifuge and after centrifugation a similar procedure is performed before final preparation of the samples for insemination. Samples destined for IUI or IVF follow roughly the same procedure and there are many laboratories who receive and treat these kind of samples together on one bench in one run, sometimes also in combination with semen samples to be analysed for diagnostic purposes only. Although in Utrecht IVF samples were strictly separated from IUI samples in space and time, several tubes with IVF sperm samples could indeed be present in one tray handled one after the other by one technician or embryologist. Of course, a well-trained technician, able to concentrate on his or her work (no telephone in between and no other people addressing him or her during the work, one of the elements in good laboratory practice) will handle the specimens safely, but when he or she is processing several samples alone on one bench it cannot be guaranteed, let alone validated [see for instance GMP rules (ii), (iii) and (iv)], that no cross-contamination has occurred. The same can be argued for the insemination of the oocytes in IVF. When dishes and tubes with oocytes are properly labelled it is hardly likely that a dish will be incorrectly inseminated. However, because in most laboratories, the inseminations will not be controlled by a second person [GMP rule (v)] the correct

action cannot be guaranteed. Validation by a double-check in this example means that the two persons involved in the act of the insemination sign at the appropriate place on the written process control sheet. In the written process control procedure on insemination it must not only be indicated exactly how the insemination is performed but also which elements during the procedure have to be double-checked and signed for. Also, it should be validated that both persons are aware of the written procedure and understand it. Only in this way can it be investigated (even after many years) how the procedure was at a certain moment (we all know that procedures tend to change in IVF) and whether the proper execution of the action with the particular gametes could be guaranteed.

What has already been changed?

Since we supposed that somewhere during the process of pipetting or by the insemination itself the fatal error was made we set out to carefully identify all 'GMP sensitive' steps in our procedure. That is to say all elements where a change of tube or vessel seemed necessary for a sample or suspension (e.g. taking up embryos into the straw for freezing), all moments where products of different origin came near to each other (e.g. several men handing in their semen specimens around the same time) and all procedures regulating transfer of products to and from patients (for instance the embryo transfer or the ovum retrieval). All procedures were further detailed [GMP rule (i)] and written procedures were incorporated [GMP rules (iv), (v) and (viii)] in order to be able to validate that the particular procedure had taken place correctly. Although the process of identification of GMP-sensitive steps and translating this in detailed written procedures proved to be an enormous amount of work, the implementation of many of the practical consequences in daily IVF work went smoothly and did not lead to a substantial enhancement of the daily workload.

Role of the Dutch Society of Clinical Embryologists

The Dutch Society of Clinical Embryologists was officially founded in 1991. Membership is restricted to those who are graduates working in one of the 12 licensed IVF centres in The Netherlands with at least 1 year's experience in one of these laboratories. In the Netherlands the Society is the addressing point for the government and the Public Health Inspection concerning clinical embryological expertise. In 1995 the Society embarked on a process in which all members were invited to start in their laboratory quality control and validation systems. The society became a member of the Dutch 'Coordinating Committee for the Promotion of Quality Control of Laboratory research and testing pertaining to the Health Care sector' (the so-called CCKL) in The Netherlands, the general authority in relation to quality care and quality system development in medical laboratories. In cooperation with CCKL the Society started to develop a 'Model Quality Handbook Clinical Embryology' (Van Inzen *et al.*, 1996). As a starting point the guidelines of CCKL were used (Loeber *et al.*, 1995). In these

guidelines the conditions to fulfill quality systems are described in general terms, according to international standards.

The guidelines were worked out and interpreted for the field of IVF laboratory practice. The actual quality system has to be described in every laboratory's own local Handbook, according to the guidelines of the Model Quality Handbook Clinical Embryology. This Model Handbook does not contain protocols, only guidelines to set up these protocols in a standardized manner. Once set-up, the quality system must be put to internal and external testing. The Society's goal is to achieve quality judgement of the Dutch IVF laboratories leading to certified IVF centres.

This certification ensures the patients of optimal quality control. Furthermore, it should ensure future investigators who want to relate (for instance) the health of IVF children to certain factors during the IVF process, that all relevant data are stored in an orderly manner, accessible even after many years. Finally, certification and thorough inspection of IVF units in The Netherlands and Europe will contribute to the acceptance of IVF and related techniques by political authorities and the general public.

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Quality control and medically assisted procreation in French laboratories

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As underlined by van Kooij *et al.* (1997) neither the spermatozoa, the oocyte nor the embryo could be considered as

'products'. This is not only because of the ethical value of such cells but mostly because the medical specificity of a medically assisted procreation (MAP) laboratory: embryologists and technicians involved in artificial insemination or in-vitro fertilization (IVF) and embryo culture are not asked to analyse biological samples but to resolve infertility problems. In fact they have a medical rather than an analytical function and the usual guidelines for good practice are not easily adapted from those used in pharmaceutical industry or clinical chemistry (European Communities Confederation of Clinical Chemistry, 1997).

French embryologists and MAP laboratories receive specific accreditation for sperm preparation, oocyte culture, IVF or intracytoplasmic sperm injection (ICSI), gamete donation or embryo freezing. The Ministry of Health give these accreditations according to scientific and technical skill and to laboratory facilities. However, the criteria for quality system were poorly described in official directives (i.e. the 1994 law on MAP) and embryologists do not find responses to their specific need in international (ISO 9001, EN 45001, ISO guide 25) or in European (EC4) (European Communities Confederation of Clinical Chemistry, 1997) standards. National standards are those from the French Guide de Bonne Execution des Analyses de Biologie Médicale (GBEA) and were continuously improved and adapted to MAP specially with an official 'Guide des bonnes pratiques' actually in preparation. Certain specific standards for artificial insemination and sperm donation were already available whereas standards for IVF and embryo culture and freezing are proposed by the French Federation des Biologistes des Laboratoires d'Etude et de la Conservation de l'Oeuf (BLEFCO).

Most of the rules cited by van Kooij *et al.* (1997) are obvious and, theoretically, they are observed in the MAP French laboratories. We particularly agree with rules 1–3 and 6–8 and we feel are important to avoid disturbance during technical manipulations. However, we do not really understand how a second person may verify the component removed or added to a batch (rules 4 and 5): as the manipulations occurred under microscopic control, the second person could only see the gesture but not the cell. May be the most important procedures are the constant identification of each batch and the separation in time between manipulations concerning different couples by the same technician. But we must also define quantitative criteria with a maximum number of MAP procedures according to the number of people working in the laboratory. Finally the opinion of the majority of the French IVF biologists (members of BLEFCO) is that, more than industrial standards, our patients need constant great care only ensured by conscientious approach to our work.

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A simplified in-vitro fertilization (IVF) using disposable materials

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There are more than 300 hospitals and clinics registered to perform in-vitro fertilization (IVF) in Japan. Most of the hospitals and clinics have embryologists to carry out good laboratory practice according to their own guidelines. However, in some clinics like ours, gynaecologists perform IVF treatment without embryologists. In order to perform IVF under such circumstances it is essential to use a simple and easy method. Surprisingly, the birth of an IVF twin of mixed races in Utrecht, the Netherlands was reported in June 1995. In this issue, as van Kooij *et al.* (1997) ask, what went wrong in the IVF laboratory and what measures have to be taken to further minimize such risks in the future? We do not know whether our simplified IVF method is able to assist to minimize such risks in the future and to prevent such an unfortunate event. However, here we as gynaecologists would like to report our simplified IVF procedure using disposable materials.

Oocyte retrieval

Before beginning IVF at our clinic in 1988, a preliminary trial was carried out to evaluate whether anaesthesia is needed for the retrieval of a single oocyte during a natural cycle. One author (M.F., gynaecologist) attempted puncture of the dominant follicle of the other author (K.F., anaesthetist) without any kind of anaesthesia. When M.F. tried to perform the follicle puncture, K.F. felt extreme and intolerable visceral pain. Since then oocyte retrieval has always been done with anaesthesia. We usually use diazepam and ketamin for i.v. anaesthesia at first. If a patient shows a painful induration at the injection site or the relevant venous vasculature afterwards, thiamylal is substituted. We believe that anaesthesia for oocyte retrieval is of great importance because a patient who has experienced intolerable pain is never likely to undergo IVF treatment again. The pain may also interfere with the chance of conceiving afterwards.

We usually carry out natural cycle IVF for infertile couples with regular menstrual cycles and clomiphene citrate (CC) cycle IVF for couples with an ovulation disorder. The protocols for natural cycle IVF or CC cycle IVF have been described previously (Fukuda *et al.*, 1995, 1996b). During natural cycle IVF, it is sometimes difficult to aspirate the oocyte, especially when the dominant follicle is sited far from the tip of vaginal probe (i.e. the ovarian cortex is situated between the dominant follicle and vaginal probe). In order to optimize chances of obtaining an oocyte we use a corset on the lower abdomen and put two hard baseballs under the corset to fix the ovaries firmly in place. This corset, and the two balls, may also reduce

the amount of bleeding from the ovaries and vagina during ovarian stimulation cycles.

Prior to oocyte retrieval, the vagina is cleaned with 1000 ml of physiological saline (500×2 ml) after which it is rinsed with 10 ml of culture medium (Ménézo B2; CCD, Paris, France). The B2 medium is enclosed by a glass ampoule and hence the gas milieu (pH, pCO₂ and pO₂) is more stable than for media enclosed by a plastic material. Serono GPM (Serono, Tokyo, Japan) can also be used as the culture medium (Gadd *et al.*, 1990); this medium is also enclosed by a glass ampoule.

Needles of 17 gauge (CCD, Paris, France) are used for transvaginal ultrasound-guided follicle puncture. These needles are γ -irradiated and non-toxic. Connected to 5 or 10 ml syringes (γ -irradiated; Terumo, Tokyo, Japan) the follicle is aspirated using manual suction. Before the procedure, several 5 ml syringes are filled with culture medium (2-2.5 ml) and kept on the wrap covering the 37°C hot water bath. We always place these syringes vertically in a cup with the needle of syringe pointing down after manual suction because the cumulus-enclosed oocyte is heavier than the culture medium and so the enclosed oocyte sinks to the bottom of the syringe and, therefore, allows swift recovery of the oocytes (Tatsumi, 1995).

Semen preparation

Semen liquefaction is performed using a 5 or 10 ml disposable syringe connected to an 18 gauge needle (γ -irradiated; Terumo). We use disposable tubes (γ -irradiated: Falcon 2099; Becton Dickinson, NJ, USA) for semen preparation. Semen preparation is performed using the two-layered Percoll method (55 and 80%) described by Ziebe and Yding Andersen (1993). We use a 1 ml Tuberculin disposable syringe connected with 18 gauge needle to measure the amount of culture medium or Percoll medium instead of a glass or plastic pipette. Immediately after cutting open the ampoule of the culture medium we aspirate the medium using a 10 ml syringe with a needle. There should be as little contact as possible between the culture medium and the air because of the extremely low pCO₂ (~0.3 mm Hg) of air (Fukuda *et al.*, 1996a). The medium in the syringe may retain its pH, pCO₂ and pO₂ for several hours but not for 1 day because of leakage of CO₂ from the medium into the air through the joints and/or plastic material. Opened disposable materials such as syringes or tubes should be firmly labelled, otherwise they should be discarded at once without any hesitation. In order to minimize possible risks, we carry out semen preparation one by one instead of preparing the semen of several patients at the same time.

Conventional IVF

In order to find oocytes in the follicular fluid and/or flushing media we use culture dishes of Falcon 3037 (γ -irradiated; Becton Dickinson). For the manipulation of oocytes we use 18 or 20 gauge angiocutter's outer plastic thin tube (electrically irradiated; Vitaflon Plus, BOC Ohmeda, Heisingborg, Sweden) connected with 1 ml Tuberculin syringe. We find this useful for manipulation of oocytes. For the embryo culture we use

4-well multidish (γ -irradiated; Nunclon, Nunc, Kamstrup, Denmark). We write each patient's name clearly on the cover of culture dish with magic ink.

We use 1×10^5 motile spermatozoa for insemination in one well and on the next day we observe the presence of two pronuclei and change the medium, easily moving an oocyte(s) to another well filled with new culture medium. We never use the patient's serum. We only use Ménéz B2 which is already supplemented with amino acids (or GPM which is available supplemented with human serum albumin).

Intravaginal culture (IVC)

Intravaginal culture (IVC) was developed by Ranoux *et al.* in 1988. Up to 10 oocytes are placed into a 3 ml cryotube (γ -irradiated: Nunc) completely filled with culture medium (Ménéz B2), $3\text{--}6 \times 10^4$ motile spermatozoa are added and the tube is hermetically sealed without air. This cryotube is then wrapped tightly in a cryoflex envelope to prevent vaginal contamination. The sealed cryotube is placed into the vagina and held in place with a diaphragm. Approximately 44–50 h later the cryotube is removed and the presence of embryos is determined (Sterzik *et al.*, 1989; Hewitt, 1991). When this IVC technique is combined with natural cycle, this method is called 'natural oocyte retrieval with intravaginal fertilization' (NORIF) (Taymor *et al.*, 1992). NORIF is a simplified IVF procedure because of elimination of medium change and control of CO₂ incubator and only one oocyte retrieval.

The mechanism of IVC was recently demonstrated (Fukuda *et al.*, 1996a). The pO₂ and pCO₂ of the culture medium in cryotube are able to equilibrate with the local environmental gas milieu owing to the permeability of O₂ and CO₂ through the plastic material. IVC keeps the pH and pCO₂ of the medium constant by equilibration with the vaginal epithelium gas milieu, but the pO₂ of the medium is reduced because of the lower pO₂ in vaginal epithelium. Thus, in this system the vagina works as a CO₂ generator and an O₂ reducer.

Intravaginal transport and partial zona dissection in relation to this system have been reported (Sterzik *et al.*, 1993). It might also be possible to do assisted hatching using transport IVC 2 days after the oocyte retrieval.

Embryo transfer

We use a Frydman catheter (γ -irradiated; CCD) for embryo transfer. We also use a Faicon tube of soft silicone (not γ -irradiated but sterilized with EOG; Fuji systems, Tokyo, Japan). Flushing with culture medium is necessary when we use this Faicon tube in our IVF procedure. In a questionable case whether the embryo is truly from the infertile couple owing to mishandling of sperm preparation or mislabelling or no confirmation of labelling, embryo transfer should not be done after obtaining the couple's informed consent.

We use transvaginal ultrasound in order to improve the quality of embryo transfer. The endometrial cavity to-and-fro movement in the late follicular phase (Fukuda and Fukuda, 1994) can be slightly reproduced at embryo transfer (Fukuda and Fukuda, 1997). We have experienced a case showing a

very strong distinct vertical to-and-fro endometrial cavity movement at embryo transfer (Fukuda and Fukuda, 1997). This movement has also been reported by Woolcott and Stanger (1997). The strong vertical to-and-fro endometrial cavity movement of embryo-containing medium may cause delayed expulsion of transfer fluid after embryo transfer as described by Schulman (1986) and Mansour *et al.* (1994). Very recently Kunz and Leyendecker (1997) have demonstrated that uterine peristalsis is controlled by oestradiol and enhanced by oxytocin. Further studies are obviously needed to seek a method to prevent strong endometrial cavity movement at embryo transfer, perhaps using such hormonal control.

Higher ectopic pregnancy rates were associated with embryo transfer <15 mm from the fundus (Chase *et al.*, 1993). If the medium containing embryo is placed too close to the fundus, it may be influenced by the endometrial cavity horizontal to-and-fro movement at the fundus (Fukuda and Fukuda, 1994) and thus might cause ectopic pregnancy. We always assess the site of the medium containing the embryo disposed in the uterine endometrium by transvaginal ultrasound at embryo transfer.

Conclusions

It is thus possible to use disposable materials during the whole IVF procedure which is essential for a simplified IVF method. In order to make IVF even more simplified each procedure from the oocyte retrieval to embryo transfer should be easier and simpler as described above. We believe that our simplified method is able to assist to minimize unfortunate risks and maximize the quality control and quality assurance of IVF. We must pay the maximum attention to the minimum requirements and procedures of a simplified IVF.

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Quality control and quality assurance in IVF laboratories in the UK

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The incident in The Netherlands that produced twins of mixed race shocked the world of assisted conception and brought into question the scientific practice of the laboratory. However, despite the fact that protocols were in place and apparently adhered to, a mistake occurred with disastrous consequences. Embryology and andrology by their very nature require meticulous attention to detail since any error will have far reaching effects. As a result of this incident further safety measures and checks have been put into place by the Dutch Authorities, however, there are lessons to be learned here by everyone.

In this country, a state regulatory authority, the Human Fertilisation and Embryology Authority (HFEA), was established in 1991 by Act of Parliament. Its function is to licence and regulate treatment and research activities involving human gametes and embryos. Amongst the many requirements of the first Code of Practice of the HFEA (1991), one is that the laboratory staff should be adequately trained, protocols in place and equipment and laboratory to be of an acceptable standard. Assessment is made by inspection on an annual basis and a licence to practice is not issued until the HFEA is

satisfied that all criteria have been met. A team of professionals and lay people carry out the inspection and examine the working practice and procedures of the clinic.

In the UK we also have the Association of Clinical Embryologists (ACE), a legally constituted professional association, which was registered in 1993. Membership of the ACE is currently restricted to graduate embryologists working in appropriately licensed units.

The immediate priorities of the ACE upon its foundation were two-fold. Firstly to set up a training programme for practising embryologists and secondly to provide guidelines on good practice for IVF Laboratories. The Diploma in Clinical Embryology (Dawson *et al.*, 1996) was launched in April 1996 and is a postgraduate diploma that is designed to be completed in the candidate's own workplace and during the course of their working day to minimize disruption to normal practice. It is modular in design incorporating both a practical continuous assessment and a theoretical component, which enables it to be completed at the candidate's own pace. Since the practical component is performed under supervision, it provides a means of assessing and validating new trainee embryologists as well as allowing more experienced embryologists who choose to take it, the opportunity for discussion on laboratory procedures.

The second objective of ACE was to set up guidelines for good laboratory practice. A working party was established in 1994 which looked at various existing models of laboratory accreditation to see if IVF laboratories could be incorporated. Eventually guidelines published for pathology laboratories by Clinical Pathology Accreditation (UK) Ltd (CPA) were used and adapted to be meaningful for embryology laboratories. A draft document of the guidelines was distributed to all UK senior embryologists early in 1996 (McDermott *et al.*, 1996). Use of guidelines is voluntary at the moment. The ACE committee are still working on the details of how to implement the accreditation procedure, who will conduct the inspections and how the scheme will be financed.

The guidelines are not restricted to points of laboratory protocol, but cover the wider aspects of organization, staffing, facilities, equipment and safety, quality control, and staff development and training, all of which are involved in constituting good laboratory practice. In this way a safer working environment that benefits and protects both staff and patients alike is hopefully created. The document outlines the importance of written procedures relating to specimen collection, processing of gametes and embryo handling. It stresses the need for signed and dated protocols for the performance of every procedure. In addition it states that there should be written procedures relating to the secure labelling of material from individual patients which ensures unique identification of patient material and records at all stages of treatment. It also recommends that there should be written procedures for dealing with incorrect or unsatisfactory identification of specimens or documentation.

Specifically with respect to laboratory procedures, guidance is given on quality control, use of protein media supplements, general principles of gamete and embryo handling, record maintenance, patient identification and labelling of all tubes and dishes containing gametes and embryos. The document

also incorporates guidance from other publications relevant to working practice in the UK, e.g. from the Health and Safety Commission and HFEA's own code of practice.

Nevertheless, however good the protocols and practice in place, they will never make up for negligence or human error. Validation of procedures will always be a problem in small laboratories where only one embryologist may be employed. Therefore the greatest onus is on the individual carrying out the procedures, it is their responsibility to ensure that they are performing the work to the best of their ability. Adequate staffing levels are also essential if individuals are not to make mistakes due to stress and overwork.

The responsibility of scientists working in assisted conception laboratories cannot be underestimated. The ultimate product of our work, if we are successful, is to help a couple have a child. The HFEA code of practice concerns the welfare of the child. However, scientists have a duty to ensure that no problems should arise with a child created in our laboratories as a result of poor practice or human error. It is a fear that lives with the most able and thorough of professionals that a mistake or cross-contamination could occur whatever safeguards are in place. Most embryologists and andrologists can identify with the incident in The Netherlands in terms of 'there but for the grace of God go I'. However, this sobering thought in itself serves to strengthen the resolve and feelings of responsibility that people working in the field of assisted conception must take.

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Practices contributing to quality performance in the embryo laboratory and the status of laboratory regulation in the US

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Clinical embryology laboratories operate in the US without federally mandated laboratory regulations. In contrast, virtually all other types of clinical laboratories in the US, including chemistry, pathology, microbiology, haematology, andrology and endocrinology laboratories, are regulated under the Clinical Laboratory Improvement Amendment of 1988 (CLIA'88), a federal statute that mandates stringent patient test management procedures, personnel requirements for laboratory personnel, a quality assurance programme and quality control programmes, both internal and external (proficiency testing). Additionally, hospital-based laboratories, sometimes including embryo laboratories, are reviewed and inspected through the general hospital accreditation process conducted by the Joint Commission on Accreditation of Health Care Organizations (JCAHO).

Although certification of embryology laboratories is not required by law, a comprehensive joint programme designed specifically for reproductive laboratories has been accrediting assisted reproductive technology (ART) laboratories in the US since 1992. Accreditation, however, is sought by embryo laboratories solely on a voluntary basis. This programme, the Reproductive Laboratory Accreditation Program (RLAP), offered jointly by the College of American Pathologists (CAP) and the American Society for Reproductive Medicine (ASRM), was fashioned intentionally in accordance with both the language and the provisions of CLIA'88 so that the programme would be eligible for 'deemed status' through the Department of Health and Human Services at a later date (Visscher, 1991), should embryo laboratories eventually fall under this regulatory umbrella. The term 'deemed status' in this case means simply that RLAP contains enough of the essential elements of CLIA'88 that accreditation with CAP, through the application and on-site inspection processes of the RLAP programme, could also serve simultaneously to certify embryology laboratories under CLIA'88 in lieu of a separate, governmental inspection. Finally, another avenue for voluntary inspection and certification exists for embryo laboratories that qualify as physician office laboratories through the Committee on Laboratory Accreditation (COLA).

The voluntary process, however, is an expensive one and, in the absence of a legal mandate, has left many laboratories unregistered, uninspected and not accredited. Currently, 116 (35.7%) laboratories out of the ~325 embryology laboratories in the US are accredited through the RLAP programme of CAP/ASRM (Dr William Byrd, Commissioner, RLAP Program, CAP, personal communication). Therefore, although a specific embryology accreditation programme has been available in the US for 5 years, approximately two-thirds of the laboratories have opted to not seek accreditation under this voluntary system. In response to growing concerns in the US over the lack of required certification for embryo laboratories, the Society for Assisted Reproductive Technology (SART) has recently required the ART laboratories of all SART member programmes to undergo certification. SART membership, however, is not required for ART centres in the US.

Thus, the current situation, whereby a majority of embryo laboratories in the US remain unaccredited, does not stem from a lack of effort or availability of accreditation within the profession, but from the lack of legislative requirement. It is

clear that the ASRM and its affiliated society, SART, have not only been instrumental in developing a thorough laboratory inspection program (RLAP), but have instituted all measures available to a professional organization to ensure that laboratories gain accreditation. What is much less clear, however, is how voluntary oversight of embryo laboratories will be tolerated in the future by both consumers and the courts in light of irregularities such as those revealed at the University of California (Irvine) in the US and the Academic Hospital in Utrecht, The Netherlands. In spite of this uncertainty, the Executive Committee of SART this year has voiced formal opposition to the inclusion of embryology under the mandatory federal regulatory purview of CLIA'88, a perplexing move since RLAP is very much a clone of CLIA'88, enough so for RLAP to now have gained formal 'deemed status'.

Work practices that protect sample identification and integrity

Procedures commonly employed by embryo laboratories in the US

Mandated oversight aside, the vast majority of embryo laboratories in the US engage in methodical work practices that ensure the maintenance of sample identification, sample integrity, patient confidentiality and high quality testing throughout the ART procedure. CAP-accredited laboratories are required to perform in this manner, but this uniformity has two roots in unaccredited laboratories: (i) laboratory-based organizations such as the Reproductive Biology Professional Group (RBPG) and the Reproductive Laboratory Technology Professional Group (RLTPG) of the ASRM, and the College of Reproductive Biology, a special interest group of the American Association of Bioanalysts (AAB), conduct numerous educational programs in laboratory management aimed specifically at producing laboratory results of high quality and (ii) as indicated previously, andrology laboratories are covered by the statutes of CLIA'88. With the unusual situation that andrology is regulated while embryology is not, it is most efficient to utilize the same operational procedures for embryology that are required for andrology since these functions most often are housed within the same or in contiguous laboratories in an IVF centre.

The following, whether prescribed by CLIA'88, CAP, or JCAHO, are used to produce quality results in the embryo laboratory with particular reference to proper specimen identification throughout the pre-analytical, analytical and post-analytical phases of testing. These include: systematic patient test management, active quality control and quality assurance programs and regular tests of personnel competence in the laboratory. A concise review of these elements, as required by CLIA'88, can be found in Keel (1997).

Patient test management

Under proper patient test management, laboratories perform tests or procedures only at the written or electronic request of an authorized person. This ensures the laboratory can make appropriate preparations in supplies, staffing and time that are requisites for maintaining specimen integrity. Further, a written

requisition system documents to laboratory personnel the identification of patients prior to their arrival at the centre. The laboratory then maintains a written system that provides for proper specimen collection, identification, preservation, transportation, processing and accurate test reporting. Specifically, laboratories have written policies and procedures for each of these with a documented system for maintaining the identity of the specimen from its initial receipt through logging the sample into the laboratory, all steps of sample preparation, final testing or utilization of the sample and the reporting of results or procedural outcome. This is done with patient name and a unique identifying number that label the sample throughout the laboratory process, from receipt until final report. Both name and identifier are written on all tubes, dishes (top and bottom), incubator shelving, interim paperwork and final reports. In addition to redundant identification, this system provides two search queries to recall archived laboratory data.

A second and equally crucial component of patient test management is termed the 'chain of custody'. Although this term has a strict legal definition, in its applied sense it means that all laboratory documents associated with a given specimen indicate clearly the identity of the person who handled the sample at each point of manipulation, from receipt through final disposition. It is also standard procedure to mark each of these events with the date and time of handling as well. This allows the laboratory director or other responsible party to track custody of the sample throughout its duration in the laboratory should any irregularity emerge. Since laboratory records are retained for a minimum of 2 years after final disposition of the sample, an investigation into sample integrity can occur at a later date under this system.

Quality control

There are rigorous standards set forth in CLIA'88 and in RLAP for quality control (QC). Under both, the laboratory is required to establish and follow written quality control procedures that are designed to implement, monitor and evaluate quality laboratory tests and procedures. An effective QC programme prescribes adequate facilities, instrumentation, materials, supplies, test methods, calibration procedures, equipment maintenance and function checks. The hallmark of the program, however, is the procedure manual. A written procedure manual, available in the laboratory to all personnel, details all aspects of laboratory procedures including specimen requirements, supplies needed and their location within the laboratory, calibration procedures, control procedures and a step-by-step guide to the performance of each test. The procedures are approved, dated and signed by the laboratory director initially and when any changes are made. It is an informed laboratory, through written requisition, that can staff appropriately for upcoming laboratory procedures but it is also strict adherence to the performance of laboratory methods, as outlined in the procedure manual, by laboratory personnel that prevents the unintentional intermixing of samples in the embryology laboratory. Patient test management and QC are inseparable in the protection of specimen integrity in the embryo laboratory.

An additional safeguard has been instituted in many laborat-

ories to ensure that specimens, either semen samples for insemination of oocytes or embryos for transfer to patients, are not intermixed. This involves at least two individuals confirming and documenting, in writing, the identity of specimens prior to insemination or embryo transfer. In our laboratory, two individuals must read both the patient name and unique identifying number from each dish or tube, confirm in the case of insemination that both spermatozoa and oocyte vessels are identified in the same way and confirm, in the case of embryo transfer, that all culture vessels are labelled correctly. The identity of patients for embryo transfer is, likewise, confirmed and documented by two individuals prior to transfer. We have now extended this practice to our cryobiology programme and have two individuals concur on and document specimen identification prior to thawing.

Quality assurance

While rigorous QC is aimed at ensuring quality during a laboratory testing or procedural event, quality assurance (QA) examines overall laboratory quality, including both the pre-analytical (i.e. requisitions, sample labelling, specimen collection) and post-analytical (i.e. data management and reporting) components as well as the analytical event. Furthermore, a suitable QA programme examines the effectiveness of communication between the laboratory and other components of the centre and stipulates that remedial actions be taken, documented and monitored should evidence of miscommunication surface. Perhaps this aspect of QA is the most useful to the IVF laboratory in ensuring that appropriate laboratory procedures are performed for a given patient and that laboratory activities are closely coordinated with those of the clinic. QA programmes are written, QA meetings are convened in prescribed and regular intervals, the findings are documented and both the QA programme and any actions taken are reviewed for effectiveness by the laboratory director on an ongoing basis.

Competency testing

Most embryo laboratories in the US conduct some sort of personnel competency testing at regular intervals to ensure not only aptitude, but also uniformity in analytical laboratory procedures. This, in fact, is required under CLIA'88 and the test should span responsibilities in the pre-analytical and post-analytical phases also. The exact nature and extent of the test is left to the discretion of each laboratory but the results are reviewed by the laboratory director and are archived. One convenient and popular method in the US is to enrol in proficiency testing whereby uniform samples, such as semen specimens and culture media, are sent to laboratories by an independent source. For example, >400 US laboratories performing semen analysis have enrolled in the andrology proficiency testing module offered through the American Association of Bioanalysts. This allows for both intra- and inter-laboratory comparison of results and can serve as a source of reference samples for competency testing, as well as a basis for remedial action should performance be below par.

Future prospects for uniform quality performance in embryo laboratories in the US

The laboratory strategies enumerated above are intended to ensure quality performance with particular attention to specimen identification and integrity. Fortunately, these practices or even more stringent versions of them, are used widely in embryo laboratories throughout the US on a voluntary basis. Without mandatory regulation, however, it is both possible and legal in the US for patients to attend ART programmes where practices ensuring specimen integrity are not followed, where instrument function is not verified, where competence of personnel is not established and where communication between the clinic and laboratory, through faulty or lacking patient test management, is ineffective. There was some hope that the Fertility Clinic Success Rate and Certification Act of 1992 (HR 4773), also known as the Wyden Bill, would provide for appropriate embryo laboratory oversight (Gerrity, 1993). The Division of Laboratory Systems, Centers for Disease Control, of the federal government is currently developing a model inspection and certification program for embryo laboratories in accordance with the Wyden Bill. The law stipulates that this model, once developed and published, must be offered to the states for potential adoption on a voluntary basis. If a state adopts the inspection model, it must also be offered to laboratories on a voluntary basis. Therefore, despite the time, expertise and efforts of the model designers, the voluntary aspect of the Wyden bill prevents assurance to patients that all ART laboratories in the US meet minimum standards of QC and QA.

The dilemma that exists without mandatory standards is even broader. Given the litigious nature of life in the US, concern exists not only for patient welfare, but also for the liability exposure of embryo laboratory workers in the absence of mandatory uniform standards of performance. This leaves neither the possibility nor the probability, but the certainty that in the absence of regulation, a jury in the US will create the standards against which embryo laboratory workers will be measured.

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