Quality Control Measures in an Embryo Research Program

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ABSTRACT

The need to establish quality control measures in handling mammalian embryos has been illustrated by a number of recent studies demonstrating a relationship between "toxic laboratory supplies" and embryo mortality. Routine, coordinated testing of biological preparations (e.g., hormones, media, serum, water) and the maintenance of sanitary practices are critical for routine embryo production, handling and effective use. A quality control program that incorporates the principle that "an ounce of prevention is better than a pound of attempted cure" can reduce or eliminate most of the problems that consume valuable time and resources. In a multidisciplinary research program, a systematic approach to quality control relies on careful organization, accurate records, communication, routine testing/verification and common sense. The purpose of this discussion is to highlight basic quality control guidelines that will generate accurate and reproducible research data.

Keywords: embryo, hygiene, laboratory, quality control, research

INTRODUCTION

Laboratory sanitation and quality control practices are essential to successful embryo research (AETS, 1986; IETS, 1987). Even the earliest laboratory studies of mouse embryos (e.g., Whittingham, 1971) recognized that a highly purified water source was necessary for successful embryo culture. Based on: 1) known health-care advantages to using high surgical standards during embryo transfer (ET) (Brand et al., 1976) and 2) the sensitivity of cell cultures to microbial contamination (Atkin, 1983; Hay, 1985; Freshney, 1987), certain aseptic practices have become routine for maximizing production of live offspring. As recently as the mid-1980's, however, one survey indicated that there was considerable variation in quality control practiced by suppliers of commonly used ET drugs and biological products (Wollen, 1985). Additionally, several controlled studies have demonstrated that commonly used methods for gas sterilization (Schiewe et al., 1984) and medium handling (Kruger et al., 1985; Takeda and Hasler, 1986) can have toxic effects on embryos *in vitro*. In light of these and other reports suggesting marked variations in the potency of exogenous gonadotropic preparations (Donaldson and Ward, 1986; Armstrong and Opavsky, 1988; Schmidt et al., 1989), concerns over water quality (Bavister and Andrews, 1988; Rinehart et al., 1988) and toxicity of disposable supplies (e.g., latex catheters: Critser et al., 1983; plastic syringes: Bondioli and Hill, 1986, Takeda and Hasler, 1986; bacteriological filters: Bavister and Andrews, 1988; Wells et al., 1988; gas sterilized porous materials: Schiewe et al., 1984, 1985a, 1988; Hagele et al., 1987; Takeda et al., 1987), embryo researchers and ET practitioners have become more keenly aware of the need for improved quality control measures.

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Collaboration between the National Zoological Park's Reproductive Physiology Program and the Embryo Cryopreservation Program at the National Institutes of Health focuses on both basic and applied embryo research. Embryo studies are multidisciplinary and cross several spectrums (i.e., oocyte maturation, *in vitro* fertilization, pleiomorphic sperm/ooctye interaction, embryo transfer, embryo cryopreservation) using a variety of laboratory (mouse, rabbit, rat) and domestic (cats, ferrets, goats, pigs, sheep) animal species (Camp et al., 1983; Diehl et al., 1986; Schiewe et al. 1985b, 1987; Schmidt et al. 1985, 1987; Goodrowe et al., 1988; Wildt et al., 1989). Some of these species also serve as models for applied and fundamental research on related wildlife taxa (Wildt et al., 1986; Wildt, 1989, 1990). The first level of basic quality control is achieved by providing excellent animal care through strict conformation to the provisions of the U.S. Public Health Service's Guide for the Care and Use of Laboratory Animals (PHS, 1985). The Guide provides recommendations on optimal animal husbandry, veterinary care and facilities needed to ensure research on healthy animals. A second source of general guidance is generated from published information for maintaining high quality tissue culture laboratories and cryobanking programs (AATB, 1985; Hay, 1985; Freshney, 1987). These publications give suggestions for sustaining a clean laboratory environment (suitable for embryo research) including proper maintenence of equipment.

Although many genetic and environmental factors influence the success of embryo research, basic laboratory sanitation and quality control measures are relatively simple, often overlooked procedures that have a real effect on research productivity. Others have discussed the impact of gonadotropic hormones (Murphy et al., 1984; Donaldson and Ward, 1986; Lindsdell et al., 1986; Torres et al., 1987; Armstrong and Opavsky, 1988; Schmidt et al., 1989), donor artificial insemination (Hawk, 1988) and nutrition (Aughtry, 1982, Seidel, 1988) on embryo collection, culture and transfer success. These topics will not be reviewed here, but do represent critical factors which must be considered in attempting to interpret research results. In this manuscript, we will assume that these factors are standardized and optimal. Rather, we will focus on suggested guidelines and methods which likely will maximize embryo hygiene *in vitro* and thereby eliminate microbial and chemical contamination as a research variable. Although the discussion is directed at techniques which will improve laboratory research, obviously, these suggestions are relevant for improving handling of embryos under farm or field conditions.

GUIDELINES FOR QUALITY CONTROL

Quality control measures are of two types, those that do not normally contact the embryo, referred to as "general laboratory hygiene", and those which directly influence embryo viability, termed "embryo hygiene". The goal of both is to ensure **clean habits** and **aseptic techniques**. In addition, new batch preparations of biological products (e.g. hormones, serum, medium) should be screened routinely to avoid potential setbacks caused by casual use of poor quality materials. Preventative measures presented below are derived from recommendations for cell and embryo culture work (Atkin, 1983; Hogan et al., 1986; Freshney, 1987; Hay, 1985, 1988).

Laboratory Hygiene

Every item in the laboratory should be perceived as a potential contamination threat. Eating, drinking or smoking in the laboratory should be strictly prohibited and distractions (e.g., conversations) while working with embryo or cell cultures discouraged. Ideally, a given laboratory should be designated only for embryo handling procedures, and within this room, the following should be considered:

Laboratory: Unauthorized personnel, other than regular ET unit members, should have limited access to the laboratory to reduce sources of outside contamination. Floor mats should be installed at all laboratory entrances to assist removing foreign debris from footware. Alternatively, disposable shoe covers or a change of shoes should be encouraged when entering the laboratory from an animal handling area. The laboratory ceiling and walls should be constructed of non-

porous materials, and surfaces should be sealed thoroughly to reduce dust or access to pests. A pest control program (e.g., poison, traps) will eliminate outside contamination introduced by rodents and insects. High efficiency particulate air (HEPA) filters should be used in the air ventilation system (Atkin, 1983; Coriell, 1984). If these are cost-prohibitive, bag-type filters are acceptable. Intake areas of the air conditioning and heating units should be disinfected weekly. Windows and doors should be kept closed and sealed, especially when embryo handling and preparation procedures are in progress. Because microorganism-containing dirt and dust settle on floors and horizontal surfaces, these are the primary places to attack contamination vectors by routine treatment with a disinfectant solution (e.g., Roccal®, O-Syl®; Atkin, 1983). Sink drains also should be decontaminated routinely with either a disinfectant (e.g., Amphyl®; Atkin, 1983) or sodium hypochlorite (e.g., bleach; Atkin, 1983) solutions. The immediate work surface on which embryos will be handled should be cleaned daily with a 70% ethanol solution. Under field/farm conditions, clean (preferably sterile) drapes should be used to cover the embryo handling surface. If at all possible, all animals (including laboratory mice) should be excluded from the embryo culturing room. The laboratory should be separate from animal holding and handling areas. If animals are brought into the laboratory for embryo collection or transfer, then all procedures should be performed within a biological safety cabinet (i.e., laminar flow hood).

Equipment: Unnecessary furniture, equipment and supplies should be banned from the embryo laboratory. These items only serve as additional places for dust and spores to collect and, in turn, require more time and labor to clean. The exterior surface of all equipment (especially cooling units and vents on refrigerated units) should be cleaned weekly. Small equipment items (e.g., microscopes, pipette pullers, microforges, manipulators, video accessories) should be protected by plastic covers and examined routinely for cleanliness. The stages of light and stereomicroscopes should be wiped with 70% ethanol on at least a daily basis before use.

Embryo incubators warrant special attention. The elevated temperature and humidity of incubators provide ideal conditions for microorganisms to thrive. Routine quality control here will conserve hours, days and even weeks of frustration associated with contamination. Cell culture incubators should be placed in the laboratory at locations with minimal air movement and personnel traffic. Other sources of incubator contamination include the gas and water supply and culture medium. A 200 nm filter should be installed in the lines of all incoming gas sources, if not already built-in, and should be replaced on an bi-annual basis. To maintain the proper and clean humid environment for *in vitro* embryo culture, the water reservoir should be cleaned weekly or bi-monthly and refilled with a solution of zephrin- or Roccal® II 10% (2 to 4.5 ml disinfectant/l distilled water; Atkin, 1983; Freshney, 1987). To safeguard against controller drift, incubator temperature and CO_2 levels should be monitored and adjusted weekly or even daily if necessary. The frequency of incubator disinfection depends on each laboratory environment and personal preferences. Many cell culture laboratories adhere strictly to monthly or even weekly cleaning, whereas the general consensus by others is that a properly working, uncontaminated incubator should be left alone. Our laboratories commonly disinfect embryo incubators semi-annually using procedures described elsewhere (Atkin, 1983; Freshney, 1987). In brief, the incubator should be thoroughly washed with a Roccal® II solution (4.5 ml/l water). The use of bleach or aerosols with phenol derivatives should be avoided since these products can corrode or pit stainless steel or leave toxic residues. After 15 minutes, the incubator should be rinsed twice with purified/distilled water or even tap water, but not resin deionized water that could contain bacteria (e.g., Pseudomonas). Caution must be taken to properly cleanse and disinfect delicate sensors and electronic components according to manufacturer recommendations. The incubator and its component parts should be allowed to dry completely before reuse. Additionally, the water jacket should be drained and disinfected (as described above), and then refilled with demineralized water containing 5 ml Roccal® II.

Incubator sterility can be maintained easily using an alternative approach whereby embryos are cultured in individual, closed desiccator units (e.g., modular incubator chambers) placed inside the larger, common incubator. These small chambers are particularly attractive if several

individuals share the same incubator or if several different gas mixtures are required simultaneously. The individual units can be cleaned easily and offer the advantage that if one chamber becomes contaminated, other embryos in other chambers are not necessarily compromised.

Personnel: The clothing, skin and hair of laboratory personnel represent significant sources of contamination. Any person handling embryos must practice acceptable personal hygiene and at all times wear a laboratory coat which should be washed at least weekly. Easily disinfected footwear (plastic boots) or disposable paper "booties" also are recommended for persons moving embryos from animal handling areas into the laboratory. Frequent discussions of embryo quality control should be made routinely at staff meetings and especially emphasized in the technical training of new personnel or visiting scientists.

Embryo Hygiene

The ability to promote and support embryo development *in vitro* depends on a controlled environment that offers nutrient support without competition from microorganisms. Many culture media are known to be effective for handling and culturing embryos isolated at several developmental stages from many mammalian species. However to sustain embryo viability, measures must be taken to maintain sterility and remove impurities from the culture medium. All disposable and reusable supplies coming into contact with the embryo, culture medium and culture atmosphere must be sterile and devoid of toxins and pyrogens. Toxic agents have been identified in association with materials commonly used for embryo recovery, culture and cryopreservation (see discussion below).

Culture Medium: The use of latex surgical or examination gloves in the preparation and handling of medium (and embryos) may be contraindicated because of the potential toxic effects of powder residues on the glove surface (Kruger et al., 1985). If used, the gloves should be pre-rinsed with purified water. Alternatively, bare hands should be washed (soap and water) and rinsed with a 70% alcohol solution.

In most research laboratories, tissue culture media either are purchased from commercial suppliers or support services organization (e.g., the National Institutes of Health Media Unit) or prepared directly in the laboratory. The latter is usually more cost-effective, but requires strict adherence to aseptic practices to avoid contamination. Generally, complex media should be used within 2 weeks when stored at 4°C (Kaighn, 1984), although some media (i.e., Minimal Essential Medium, Whitten's medium) may retain a longer, effective storage life (Herr and Wright, 1986). Changes in the pH of stored media are thought to be responsible for the deterioration of some nutrient components (Stewart-Savage and Bavister, 1988), although exposure to ultraviolet and visible light also can be detrimental (Taylor, 1984). Some medium components (e.g., L-glutamine, pyruvate) spontaneously degrade during storage and should be added immediately before use. In contrast, saline solutions and stock preparations devoid of proteins (e.g., serum) are less likely to become contaminated and can be stored (4°C or less) for 1 to 6 months. All media should be kept refrigerated (4°C) and only necessary aliquots used under ambient conditions. Many solutions can be frozen, especially basic salt solutions (e.g., PBS), although some ingredients (e.g., $CaCl_2$) will induce a precipitant upon freezing and, thus, should be omitted at initial preparation. Frozen media and supplemental nutrient components (e.g., serum, growth factors) should be stored in an ultralow freezer (-70°C), as opposed to a frost-free freezer (-10° to -20°C). This will avoid gradual separation and concentration of solutes on the container bottom which may lead to protein denaturation and insolubility (Kaighn, 1984).

Glass-distilled water (2 or 3X) or water purified by reverse osmosis-ion exchange filtration (e.g., RO/Milli-Q) are recommended as the base component (Whittingham, 1971; Freshney, 1987; Bavister and Andrews, 1988). Prolonged storage of purified water is not recommended because water will deteriorate in purity over time (Galber et al., 1983), and heavy metals will leach from some glass and plastic storage vessels (Abramczuk et al., 1977). Adding chelating agents, like ethylenediaminetetraacetic acid (EDTA, 100 μM), to culture medium may overcome heavy metal

ion contamination which normally inhibits *in vitro* embryo development (Abramczuk et al., 1977). Amino acid, albumin and serum components of media also may serve a similar purpose by nonspecifically binding heavy metal contaminants (Taylor, 1984).

All media and cryoprotective solution preparations should be checked for pH and osmolality to confirm desired levels as a final step before sterilization (Waymouth, 1981; Kaighn, 1984). Media then should be filter-sterilized in a biological safety cabinet or, at least, in a designated clean area with minimal air movement. The sterility of media is dependent on filtration (pore size, 0.22 μm) and storage in sterile containers (preferably silicone-coated borosilicate glass bottles or plastic culture tubes [Falcon®, No. 2001 or 2054], Bavister and Andrews, 1988). Protein-containing solutions should be sterilized by positive pressure filtration to avoid excessive bubbling and denaturing. The latter is achieved by using syringe-fitting bacteriological filter units (e.g., Millex-GV® Millipore filters, Bavister and Andrews, 1988). However, filters from some manufacturers may contain potentially toxic wetting agents on the membrane surface which can be diluted to negligible quantities or eliminated by discarding the first few milliliters of filtered medium (Bavister and Andrews, 1988; Wells et al., 1988). As an added precaution, the addition of phenol red to the medium provides a convenient, visual verification of pH.

Only reagent/analytical grade chemicals and tissue culture grade enzymes, antibodies and serum should be used in culture medium preparation. It may be necessary to evaluate several batches or lots of a particular component for effectiveness in the laboratory before purchase (Coriell, 1984). One common approach for comparing different product lots is to perform a comparative plating efficiency test using a cell culture system (Hogan et al., 1986). In brief, this involves using an established cell line, seeding tissue flasks with an equal number of fresh or frozen-thawed cells and then allowing them to grow under treatment conditions. After 2 to 5 days, the tissue cultures are redissociated into cell suspensions and comparative growth performance evaluated by counting cell populations/flask using a hemocytometer.

Fetal calf serum is a major source of contamination of tissue culture cell lines (Hook, 1984). The severity of potential microbial (especially viral or mycoplasmal) cross-contamination of embryos by serum (Atkin, 1983; Hare and Singh, 1984; Hay, 1985, 1988) warrants serious consideration and use of alternative growth-promoting supplements and/or bovine serum albumin which are less likely to be contaminated (Menezo et al., 1984; Leibo, 1985). Sera should be tested for the presence of microorganisms (e.g., mycoplasma, viruses, fungi, bacteria; Atkin, 1983; Coriell, 1984; Kaighn, 1984), heat-inactivated at 56°C (30 min), aliquoted into small sterile containers and then stored at -70°C. Generally, the use of antibiotics and antimycotics in embryo flushing and culture media is essential for controlling embryo pathogens. However, to test the sterility of media preparations the use of antibiotics is discouraged as they may mask the presence of underlying contaminants (Coriell, 1984).

All new media preparations should be tested and assayed for potential contamination and the ability to sustain and promote the development and growth of embryos (Coriell, 1984; Kaighn, 1984). The most convenient assay uses mouse embryos, however, embryos from different species have different culture requirements and toxicity sensitivities, especially at the 1- or 2-cell stage of development. Therefore, an alternative model like the rabbit (Kuehl et al., 1986) may be useful. Details for establishing mouse embryo cultures for quality control purposes appear elsewhere (Hogan et al., 1986; Monk, 1987; Gerrity, 1988). For human *in vitro* fertilization programs, 1- to 4-cell mouse embryos are used, and development is monitored to the hatched blastocyst stage over a 3- to 5-day period (Ackerman et al., 1984; Trouson and Conto, 1984; Fleming et al., 1987). Cryopreserved 2-cell mouse embryos can be purchased from commercial sources for quality control assessments (Leibo, 1990). The *in vitro* mouse embryo development system, however, does not detect certain water impurities (Silverman et al., 1987) that can adversely affect pregnancy rates (Quinn et al. 1985; Rinehart et al., 1988). As an alternative, a hamster sperm motility assay (HSMA; Bavister and Andrews, 1988; Rinehart et al., 1988) recently has been validated for assessing culture medium quality. This assay provides rapid (within 24 hours) information on the quality of water and media supplements and potential toxicity of media handling products at a much greater level of sensitivity than the mouse embryo bioassay. The

HSMA can be integrated easily into normal quality control practices and is recommended on a weekly or bi-monthly basis (Bavister and Andrews, 1988; Rinehart et al., 1988).

Sterilization: Disposable sterile plastic dishes, containers, syringes, needles and pipettes are recommended. Re-using these supplies is strongly discouraged. Before sterilization, all re-usable glassware, stainless steel accessories (i.e., surgical equipment, stylettes), filtering devices and catheters should be soaked and washed in a soap solution (Linbro® 7X detergent). These materials should be rinsed thoroughly with deionized water (5 to 7 changes) and distilled- or purified water (3 to 5 changes) to completely remove detergent residues and other impurities. Tap water should be avoided for all items used in medium- or embryo-handling systems, because contaminating heavy metals bind tenaciously to glass and can be removed only by acid washing (Kaighn, 1984). The appropriate method of sterilization depends on the properties of the material:

Heat sterilization: For glassware (e.g., watch glasses, volumetric flasks, media bottles) and stainless steel items (e.g., surgical equipment, cervical dilators, Cassou guns and stylettes), an autoclave (steam at 15 psi, ≥30 min exposure at 121°C) or a drying oven (dry heat, 1 hour exposure at 150°C) can be used effectively. Several packaging materials and systems are available to maintain sterility during storage, each of which dictates that items are enclosed in a thermally-sealed package.

Cold sterilization: This method can be used for unpackaged items that do not come into direct contact with embryos. Items are placed in a chemical disinfectant solution (e.g., Nolvasan®, Roccal® II) for at least 10 minutes at room temperature and thoroughly rinsed with sterile water or medium before use. This approach is used routinely in our laboratory for delicate surgical equipment (e.g., laparoscope and fiber-optic cable) and miscellaneous accessories (e.g., speculum, cervical dilator, stylette). The life-span of some materials (e.g., Foley catheter balloon cuffs) may be shortened after repeated or prolonged exposure to these chemical solutions.

Gas sterilization: Ethylene oxide (EtO) is the most commonly used method for sterilization of heat-labile plastic (e.g., straws, embryo filters, i.v./tom-cat catheters, Unopette tips) and rubber products (e.g., Foley catheters). It is a potent antimicrobial agent that can penetrate porous material, but in most cases residues continue to reside in pores following sterilization (Barbi and Butler, 1984; Schiewe et al., 1985a). Some latex products have been known to retain EtO residues for 1 to 3 months. An adequate aeration interval must be provided to avoid potential toxic effects caused by EtO residues in some medical materials (Jones, 1979; Barbi and Butler, 1984).

We have reported that EtO residues that are toxic to mouse embryos remain on plastic culture dishes after the 24 or 36 hour aeration period recommended by the manufacturer (Falcon®, No. 3001; Schiewe et al., 1984, 1985a). The toxic effect of EtO residues disappears after 1 week of aeration. This discrepancy with manufacturer recommendations results from the latter extrapolating data used to establish a minimum safety margin for human exposure. The manufacturer-recommended 24 hour aeration interval is based on *in vivo* dermatological skin reaction tests (Andersen, 1971) and not *in vitro* cell growth.

Other investigations (Hagele et al., 1987; Takeda et al., 1987; Schiewe et al., 1988) have demonstrated that embryos maintained for even short periods (30 min to 2 hours) in EtO-treated plastic straws following 0 to 7 days of aeration have reduced developmental capacity in culture. Differences in the composition of plastic straws also result in variations in EtO retention times. For example, Continental straws remain toxic to embryo development even after 30 days aeration, whereas 1 week aeration eliminates the toxicity of IMV straws (Takeda et al., 1987; Schiewe et al., 1988). We have held mouse embryo suspensions in manufacturer-sterilized or EtO-treated (0, 1 and 3 days aeration) straws from 30 min to 9 hours at 20°C before washing and *in vitro* culture (Schiewe et al., 1988). The extent of toxicity is related inversely to the duration of aeration and directly to the length of exposure (Table 1). Because EtO re-elutes as a gas from the product and packaging during aeration, these materials should remain isolated in a ventilated hood during the first 2 to 3 days of aeration to prevent reactions between eluting EtO and other miscellaneous supplies (i.e., straws, petri dishes, catheters) stored nearby. It also is important to emphasize that the rate of EtO elution is temperature dependent. Low temperature aeration requires more time for

the loss of toxic residues. Additionally, other factors (e.g., vacuum pressure, number of post-sterilization evacuations) can influence the rate of EtO residue dissipation (Barbi and Butler, 1984), suggesting that changes in the aeration procedure (in the sterilization chamber) can substantially reduce EtO residues faster than aeration at ambient conditions. Standardized EtO sterilization protocols should be validated using a variety of commercial devices including indicator tapes or a Steritest® dosimeter/bacterial spore preparation (Andersen Products, Inc., Oyster Bay, NY).

There should be a major concern about exposing personnel and animals to EtO gas during sterilization. All sterilization and aeration procedures must be performed in an isolated area within a ventilated hood. Pregnant women and animals should avoid inhaling EtO gas because of an increased risk of spontaneous abortion or abnormal fetal development (NIOSH, 1981). Gas sterilization is a very effective method for sustaining quality control in the embryo laboratory, but the use of EtO mandates detailed knowledge and the routine practice of safety precautions.

TABLE 1. *In vitro* development of mouse embryos stored in EtO-treated plastic straws following different post-sterilization aeration interval and duration of exposure treatments*.

Aeration period	Exposure period	N	DEV (%)	EDR (%)	Viability Grade†
Control					
	30 min	40	90.0^{ab}	10.0^{ab}	$3.7\pm.3^{a}$
	3 hours	40	92.5^{a}	7.5^{a}	$3.9\pm.3^{a}$
	6 hours	40	100.0^{a}	0.0^{a}	$3.8\pm.2^{a}$
	9 hours	40	100.0^{a}	0.0^{a}	$4.0\pm.2^{a}$
0 hours					
	30 min	40	87.5^{b}	12.5^{b}	$3.3\pm.3^{b}$
	3 hours	40	0.0^{e}	100.0^{e}	$1.0\pm.4^{d}$
	6 hours	40	0.0^{e}	100.0^{e}	$0.1\pm.1^{e}$
	9 hours	40	0.0^{e}	100.0^{e}	$0.1\pm.1^{e}$
24 hours					
	30 min	40	97.5^{a}	2.5^{a}	$3.9\pm.3^{a}$
	3 hours	40	65.0^{c}	35.0^{c}	$3.1\pm.4^{bc}$
	6 hours	40	5.0^{e}	95.0^{e}	$2.7\pm.3^{c}$
	9 hours	40	0.0^{e}	100.0^{e}	$1.9\pm.4^{c}$
72 hours					
	30 min	40	100.0^{a}	0.0^{a}	$4.2\pm.1^{a}$
	3 hours	40	100.0^{a}	0.0^{a}	$4.0\pm.2^{a}$
	6 hours	40	80.0^{b}	20.0^{b}	$3.7\pm.1^{a}$
	9 hours	40	25.0^{d}	75.0^{d}	$2.8\pm.2^{c}$

^{a,b,c,d,e} Mean (±SEM) column values with different superscripts differ ($p<0.05$).
*Data from Schiewe et al., 1988.
† Based on a fluorescein diacetate fluorescence intensity assay rated on a scale of 0 = no fluoresence to 5 = very intense, uniform fluoresence (Schiewe et al., 1985).
DEV = development of embryos to the blastocyst stage.
EDR = embryo degeneration rate.

Radiation: Gamma (^{60}Co, ^{137}Ce) and electron beam irradiation are used widely for sterilizing manufacturer-packaged, heat-sensitive medical materials (Dietz, 1986). Irradiation represents about 28% of all commercial sterilization service business compared to ~47% for EtO sterilization (V. Chamberlain, personal communication). Currently, there are fewer than 20 such companies nationwide (G. Dietz, personal communication). Irradiation is particularly attractive because it leaves no toxic residues, and the material is available for immediate use, requiring no aeration or quarantine period (V. Chamberlain, personal communication). The reliability of irradiation has been theorized to exceed all other sterilization methods (G. Dietz, personal communication).

The primary disadvantage of using radiation sterilization is that structural changes can occur in the material, especially polymers, leading to density fluctuations and increased brittleness (Skiens, 1980; Dietz, 1986). Because these changes are dose-dependent and cumulative, empirical data may be needed to determine (1) the total dosage of radiation leading to structural failure of the material and (2) the minimum dosage needed to safely and effectively sterilize the material. This information will yield the total number of practical irradiation cycles that a particular material will withstand. For many items including Foley catheters, syringes and cell culture dishes/flasks, this information already is available at the sterilization company. For large IVF and ET research programs, it may be worth considering the irradiation of certain single use items (e.g., straws, Unopettes®) purchased in bulk quantities. This could be an attractive alternative to the routine use of EtO. These items could be purchased and repackaged individually or in small lots in zip-lock or heat-sealed bags and then shipped to a sterilization service company for irradiation. Cost-effectiveness would depend on the volume of materials scheduled for sterilization, since most commercial companies have a minimum service charge. Detailed information on irradiation systems is available elsewhere (Dietz and Baker, 1988).

Miscellaneous:

Record keeping: Seidel (1988) recently discussed the essential role of record keeping in a quality control program. For example, all items should be properly dated upon initial use and embryo holding containers accurately identified. Accurate logs also should be maintained on gonadotropins and medium components (e.g., dates of purchase or reconstitution and lot numbers used), liquid nitrogen tanks (e.g., evaporation time, refilling schedule, inventory) and anesthetic products (e.g., inventory).

Ovulation Induction: Potency differences in gonadotropin preparations due to manufacturer or lot differences can cause considerable variability in ovarian response and embryo quality (cattle, Murphy et al., 1984; Donaldson and Ward, 1986; Lindsdell et al., 1986; sheep, Torres et al., 1987; rats, Armstrong and Opavsky, 1988; mouse, Schmidt et al., 1989). We test batches or sources of pregnant mares' serum gonadotropin and follicle stimulating hormone in a mouse and sheep model system, respectively, before purchasing large orders. It is particularly important to ensure that gonadotropins are effective before using in wildlife species as these animals can be stressed by multiple or daily hormone injections. Because the basic reproductive physiology of most nondomestic species is poorly understood, it is essential to minimize all possible sources of technical failure if embryo strategies are to be productive and successful.

Embryo collection: Nonsurgical embryo recoveries should be performed using a closed flushing system, especially under field or barn conditions. Most embryo filtering devices allow the flushing medium to pass directly from the Foley catheter through connector tubing to a closed filtering container. This approach protects the recovered embryos from possible environmental contamination. When embryos are destined for export, an aliquot of the flush fluid must be retained for official examination of potential pathogens (Hare and Singh, 1984). All embryos should be washed repeatedly after collection to control against disease transmission, especially if the possibility of importation or exportation exists. Detailed embryo washing protocols are available (Hare, 1985; Singh, 1987; IETS, 1987). In brief, this involves moving embryos through a series of 6 to 10 pools of medium (1 to 3 ml), using a new sterile pipette after each dilution. The

use of a trypsin-containing pool may be warranted, but can make embryo handling difficult since it requires the use of a serum/albumin-free medium. Alternatively, this step should be performed using sterile, siliconized glass (dishes and pipettes) to reduce potential damage or loss of the embryo during handling.

Light exposure: Long wavelength ultraviolet (320 to 400 nm) and visible light (400 to 750 nm) found in direct sunlight or cool-white fluorescent (CWF) light has been shown to cause chromosomal damage and mutagensis of tissue culture cells (Parshad et al., 1977). Furthermore, growth-promoting factors in medium can deteriorate after extended exposure to CWF (Taylor, 1984). Therefore, care should be taken to **minimize** direct light exposure to embryos or media contained in clear glass or plastic containers. Additional practical measures to protect embryos and media from harmful CWF exposure include: 1) enclosing all CWF light fixtures (e.g., laboratory, laminar flow hood, cold room) in gold or orange sleeves that absorb ultraviolet and low wavelength visible light (< 475 to 500 nm); 2) filtering media under yellow light and storing in the dark; and 3) using appropriate light filters (red, blue) in microscopic equipment (Taylor, 1984).

Syringes, catheters and filter units: Two independent studies have shown that certain brands and lots of disposable syringes adversely affect embryos (Bondioli and Hill, 1986; Takeda and Hasler, 1986), probably due to toxic leaching of benzothiazoles from the rubber plunger (Petersen et al., 1981; Korhonen et al., 1982). Both the concentration of the toxin and the interval that the embryos are exposed to the contaminated medium influence the extent of damage (Takeda and Hasler, 1986). Therefore, exposure of medium (especially small volumes) to the rubber plunger of some syringe types should be avoided. It may be possible to eliminate toxic residues found in syringes (Takeda and Hasler, 1986) as well as bacteriological filters (Bavister and Andrews, 1988; Wells et al., 1988) and latex catheters (Critser et al., 1983) by pre-use washing. An additional precaution can include maintaining an air space buffer (1 to 5 ml) between the medium and the plunger surface.

Oil: Paraffin or silicone oil, often used in embryo culture, should be washed using a sterile 0.9% NaCl saline solution which will reduce potential batch variability (Bavister and Andrews, 1988) and potential toxic effects on embryo development *in vitro* (Fleming et al., 1987). Pre-equilibration of the oil in the culture incubator also is recommended.

Pipetting: Solutions or medium should never be mouth-pipetted. When moving embryos using a mouth micropipette, a filtering device should be incorporated into the pipetting assembly.

Embryo cryopreservation: Several fundamental techniques associated with embryo cryopreservation can be viewed as quality control measures aimed at improving embryo survival. Areas of concern include: 1) moving embryos among cryoprotective solutions; 2) potential dilution effects which can alter the desired concentration of cryoprotective solutions; 3) proper sealing of embryo storage containers; and 4) adhering to optimum thawing procedures. When transferring embryos into cryoprotectant solutions or medium, the transfer pipette should be rinsed in holding medium. Alternatively, the pipette should be changed between steps and the embryos aspirated using a minimal volume of solution (2 to 5 µl). Each solution pool should contain a minimum volume of 1 ml to reduce possible concentration changes caused by evaporation or dilution with repeated use. After transfer, embryos should be mixed thoroughly in the new solution and allowed to equilibrate. All straw freezing containers can be prepared during the equilibration process. The proper amount of cryoprotective solutions can be added using pre-set levels indicated (i.e., with a sharpie marker) on the external surface of the 0.25 ml straw. In general, a 1 cm column containing the embryo-cryoprotectant solution is aspirated into the straw, followed by a 0.5 to 1 cm air space and then a 6.8 to 7.5 cm column of 1.0 M sucrose solution when using a one-step® straw approach (Leibo, 1982, 1985).

When straws are stored submerged in liquid N_2, the low temperature creates a negative pressure inside the straw capable of drawing liquid N_2 into the straw through a leaky heat seal or cotton plug. For this reason, a straw should be hermetically-sealed on both ends. The preferred method involves repeatedly heat-sealing on each side of the straw until it adheres to the electrode surface of the thermal sealer. The effectiveness of heat seals can be tested by dipping control straws into a beaker of water and observing for air bubbles when a positive pressure is placed on

the opposite end. This seal also allows an empty 0.5 ml straw to be slip-fitted onto the plugged-end to serve as a labelled handle (Leibo, 1982). The use of labelled handles greatly facilitates identifying and handling straws while the embryos remain safely submerged in liquid N_2. Recent evidence (Rall and Meyer, 1989) indicates that thawing straws in room temperature air (20°C) protects against damage to the zona pellucida, which may improve embryo survival and reduce import-export concerns.

For embryo cryobanking, various guidelines and standardized procedures (e.g., storage, distribution), established for cell and tissue storage, should be used (AATB, 1985; Hay, 1985). Of particular interest is the utility of biochemical and molecular genetic procedures (e.g., antigen-antibody reactions, 2-dimensional electrophoresis, isozyme analysis, DNA fingerprinting) for determining the identity of cryopreserved cell lines (O'Brien et al., 1977; Anderson, 1984; Peterson et al., 1984; Hay, 1984, 1985). Although presently impractical for direct application in the commercial ET industry, these strategies may assist managing the genetic diversity of laboratory animals and endangered wildlife species. It is likely that elaborate testing schemes, supplemental to karyotypic data (Hsu and Benirschke, 1975), will be essential for ensuring species and subspecies purity (O'Brien et al., 1984).

SUMMARY

The accuracy and reproducibility of embryo research data depend on integrated, high quality laboratory activities which provide consistent experimental results. Effective quality control has 3 essential components: 1) **prevention** which requires routine testing and planning before and during experiments to ensure that supplies are of exceptional quality and that equipment is functioning properly; 2) **internal controls** which allow checking the performance of standard protocols to determine the effectiveness and repeatability of component steps (examples are duplicate sampling, review of calculations and records by supervisors, and method/assay validation); and 3) **correction**, those actions taken to determine the causes of quality deficiencies or disruptions followed by restoration to expected efficiency levels. This will involve trouble-shooting to correct malfunctioning equipment, examining the need for additional controls, re-evaluating methods or even retraining personnel. Quality control guidelines, issued by several industry and public organizations, are available for standardizing many of the procedural steps involved in most embryo research programs.

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