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Draft – Not for Implementation

Mouse Embryo Assay for Assisted Reproduction Technology Devices

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Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

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For questions about this document, contact DHT3B: Division of Reproductive, Gynecology, and Urology Devices at (301) 796-7030.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health

Preface

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Mouse Embryo Assay for Assisted Reproduction Technology Devices

Draft Guidance for Industry and Food and Drug Administration Staff

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff or Office responsible for this guidance as listed on the title page.

I. Introduction

FDA is issuing this draft guidance to outline draft recommendations on conducting the mouse embryo assay (MEA) to support premarket submissions and lot release of assisted reproduction technology (ART) devices. The majority of ART devices directly or indirectly contact gametes (i.e., sperm and oocytes) and/or embryos during their intended use. MEA is used to assess the potential for embryotoxicity of devices that contact gametes and/or embryos. Several classification regulations under 21 CFR part 884 include special controls that require MEA testing or information. MEA may also be used to support premarket submissions for other devices that are intended to contact gametes and/or embryos during their use. However, there are no voluntary consensus standards that describe how to conduct the MEA. This draft guidance provides recommendations for conducting the MEA to support premarket submissions for devices that are intended to contact gametes and/or embryos and to comply with the special controls for those devices classified under 21 CFR 884 that require MEA testing or information.

For the current edition of the FDA-recognized standard(s) referenced in this document, see the FDA Recognized Consensus Standards Database.[1] For more information regarding use of consensus standards in regulatory submissions, please refer to FDA guidance titled "Appropriate Use of Voluntary Consensus Standards in Premarket Submissions for Medical Devices."[2]

FDA's guidance documents, including this draft guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are

[1] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm.

[2] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/appropriate-use-voluntary-consensus-standards-premarket-submissions-medical-devices.

cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

II. Scope

The scope of this document is limited to devices that are regulated under 21 CFR part 884 that have direct or indirect contact with gametes and/or embryos. This draft guidance provides recommendations for how to comply with the special controls for those devices classified under 21 CFR 884 that require the MEA testing or information.[3] This guidance also applies to devices for which MEA testing or information may be used to support a premarket submission. Devices that only contact sperm during use (e.g., sperm handling or storage media, separation devices, handling labware) and the human sperm survival assay (HSSA) are outside the scope of this guidance document.

III. Method for MEA Testing

FDA recommends that MEA testing be used to assess the embryotoxicity of ART devices that have direct and/or indirect contact with gametes and/or embryos. MEA assesses blastocyst development from either one-cell or two-cell staged embryos (i.e., one-cell system or two-cell system). FDA is aware of the one-cell and two-cell system for assessing embryotoxicity and considers both methods acceptable. FDA's specific recommendations for MEA testing are described below.

A. Test Article

MEA should be conducted on ART devices in their final finished form (e.g., aseptically-filtered media in final packaging). The number of devices evaluated for each MEA test should be sufficient to ensure robust evaluation. FDA recommends that a minimum of three individual devices be evaluated in each test to account for potential variability between devices. To support a premarket submission, sponsors should perform testing on devices from one lot at both time zero (i.e., newly manufactured devices) and the end of the proposed shelf-life.

Depending on the device type and intended use, the MEA test article should be prepared as described below:

1. Liquid-based devices for MEA should be tested as provided without extraction or dilution (i.e., neat).

2. Oil indicated for oocyte/embryo culture should be evaluated under the intended use conditions. Embryos should be cultured in a drop of embryo culture medium overlaid

[3] These classification regulations include 21 CFR 884.6100, 884.6110, 884.6130, 884.6160, 884.6165, 884.6170, 884.6180, and 884.6195.

with the culture oil. The type of medium to be used in this study is recommended in Section III.C below.

3. Plates and dishes that are indicated for embryo culture should be tested directly without extraction.

4. Solid devices that are not indicated for embryo culture (e.g., embryo transfer catheters, cryopreservation devices) should be extracted before use. Only the portions of devices that will come in direct and/or indirect contact with oocytes or embryos should be used to prepare test article extracts.

B. Preparation of Test Article Extract

When preparing test article extracts for solid devices that are not indicated for embryo culture, the devices should be extracted in a standard embryo culture medium at 37 °C for at least 30 minutes. FDA recommends that the volume of extraction media is consistent with the FDA guidance "Use of International Standard ISO 10993-1, 'Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process'"[4] and ISO 10993-12: *Biological evaluation of medical devices – Part 12: Sample preparation and reference materials*.

C. Test Procedure

(1) Culture media and labware

Culture media and labware used in the study, except for the test article(s), should be intended for use in ART procedures. FDA recommends that a medium indicated for embryo culture during ART procedures be used in MEA. Manufacturers can check the marketing status of culture media and labware using a publicly available FDA database[5] and provide this information to FDA in the premarket submission.

(2) Mouse embryos

One-cell or two-cell embryos should be obtained from hybrid mouse strains (e.g., CBA/B6 hybrid[6]) that are sufficiently sensitive to detect embryotoxicity.[7] FDA recommends that the

[4] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/use-international-standard-iso-10993-1-biological-evaluation-medical-devices-part-1-evaluation-and.

[5] The 510(k) database is available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm.

[6] For more information about embryo strains, see (1) Khan, Zaraq, et al. "Mouse strain and quality control testing: improved sensitivity of the mouse embryo assay with embryos from outbred mice." *Fertility and sterility* 99.3 (2013): 847-854; and (2) Punt–van der Zalm, J. P. E. M., et al. "Toxicity testing of human assisted reproduction devices using the mouse embryo assay." *Reproductive biomedicine online* 18.4 (2009): 529-535.

[7] FDA supports the principles of the "3Rs," to reduce, refine, and replace animal use in testing when feasible. We encourage sponsors to consult with us if it they wish to use a non-animal testing method they believe is suitable, adequate, validated, and feasible. We will consider if such an alternative method could be assessed for equivalency to an animal test method.

mouse strain(s) used in MEA are scientifically justified. A minimum of 21 embryos should be used in each MEA. FDA believes that this is a sufficient sample size to evaluate potential embryotoxicity and control for biological variability based on previous submissions for legally marketed devices.

(3) Control

Each MEA should include a control group that uses a medium indicated for embryo culture during ART procedures, and the same embryo sample size and test procedure as the test article group.

(4) Duration of exposure of mouse embryos to test articles

For MEA test exposure to represent intended use, FDA recommends:

- In a standard MEA, embryos should be incubated in the test article extract under normal culture conditions (i.e., 37 °C and 5% CO_2) for 96 hours if a one-cell system is used or 72 hours for a two-cell system.
- If the device is a plate or dish intended for embryo culture, the embryos should be directly incubated in the device in a standard embryo culture medium under normal culture conditions until the end of MEA culture time.
- If the device is a one-step medium (e.g., a single media used for culture of embryos until Day 5/6 of development), MEA should be conducted using the test articles until the end of intended incubation period under normal culture conditions. For example, MEA should be assessed after six days of incubation (120 hours) for a medium intended for use until Day 6 of development.
- If the device is a media product with a shorter intended contact duration than a standard MEA (e.g., less than 72 in a two-cell system or 96 hours in a one-cell system), MEA should be conducted by exposing the embryos to the device for the total intended use duration provided in the labeling of the device. The embryos should then be transferred to standard culture medium for the remaining culture time.
- If the device is a culture oil, embryos should be cultured in standard culture medium overlaid with the device under normal culture conditions for 96 hours if a one-cell system is used or 72 hours for a two-cell system.
- If the device includes a series of solutions used sequentially (e.g., vitrification and warming of oocytes or embryos), testing should be conducted using a step-in/step-out approach. This approach sequentially exposes the embryos to all vitrification and warming solutions. Exposure to each solution should match the maximum exposure conditions described in the labeling. After step-in/step-out exposure, embryos should then be cultured in a standard culture medium under normal culture conditions until the end of MEA culture time.

(5) Evaluation of embryo development

At the completion of embryo culture, embryos should be evaluated for blastocyst development using the acceptance criteria described in Section III.D.

D. Acceptance Criteria

Depending on the system used, FDA recommends using the following MEA acceptance criteria for test and control articles:

1. One-cell system: ≥80% embryos expanded to blastocyst at 96 hours; or
2. Two-cell system: ≥80% embryos expanded to blastocyst at 72 hours.

E. Test report

For information on the recommended content and format of test reports for the testing described in this section, refer to FDA's guidance, "Recommended Content and Format of Complete Test Reports for Non-Clinical Bench Performance Testing in Premarket Submissions."[8] To facilitate FDA's review, we recommend that your MEA complete test report also include the MEA test method used, including mouse strain, number of embryos for test and control groups, culture conditions (e.g., gas, temperature, size of medium drop, number of embryos in each drop), and procedures. The name of the embryo culture medium used in the control group and test article group (as applicable) should be provided.

IV. MEA Information in Device Labeling

When MEA testing is conducted, FDA recommends that the package and vial labels and instructions for use state the MEA acceptance criterion used.

V. Certificate of Analysis (COA)

When MEA testing is conducted, FDA recommends that any COA state the MEA acceptance criterion used and include the lot-specific test results.

[8] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/recommended-content-and-format-non-clinical-bench-performance-testing-information-premarket.