



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

20 July 2023
EMA/421977/2023
Committee for Medicinal Products for Human Use (CHMP)

Consultation procedure Public Assessment Report (CPAR)

Consultation on an ancillary medicinal substance incorporated in a medical device

Medical device: Origio A.R.T. Media

Ancillary medicinal substance: gentamicin sulfate / sargramostim (GM-CSF) / heparin sodium / insulin human

Procedure No.: EMEA/H/D/006090/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.



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List of abbreviations

| | |
|---------|---|
| A.R.T. | Assisted Reproductive Technology |
| AMA | Advanced Maternal Age |
| CER | Clinical Evaluation Report |
| COS | Controlled Ovarian Stimulation |
| EDTA | Ethylenediaminetetraacetic Acid |
| ERs | Essential Requirements |
| EPs | Essential Principles |
| ESHRE | European Society of Human Reproduction and Embryology |
| EU | European Union |
| FDA | Food and Drug Administration |
| FER | Frozen Embryo Replacement |
| GM -CSF | Granulocyte macrophage colony stimulating factor |
| HA | Hyaluronic Acid |
| hCG | Human Chorionic Gonadotropin |
| HLA | Human Leukocyte Antigen |
| HSA | Human Serum Albumin |
| I FU | Instructions for use |
| I CSI | Intra Cytoplasmic Sperm Injection |
| IVF | In vitro fertilization |
| KPI | Key Performance Indicator |
| MAUDE | Manufacturer and user facility device experience |
| MHRA | Medicines & Healthcare products Regulatory Agency |
| PESU | National Perinatal Epidemiology and Statistics Unit |
| PGT | Preimplantation Genetic Testing |
| PMCF | Post Market Clinical Follow-up |
| I UI | Intrauterine Insemination |
| PMS | Post Market Surveillance |
| ROS | Reactive Oxygen Species |
| GA | Therapeutic Goods Administration |
| TQE | Top quality embryo |
| WHO | World Health Organization |

1. Background information on the procedure

1.1. Submission of the dossier

The notified body BSI Group submitted to the European Medicines Agency (EMA) on 12 May 2022 an application for consultation on gentamicin sulfate / sargramostim / heparin sodium / insulin human incorporated as ancillary medicinal substance(s) in the medical device Origio A.R.T. Media, in accordance with the procedure falling within the scope of Directive 93/42/EEC, as amended.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Jayne Crowe

Co-Rapporteur: Larisa Gorobets

| | |
|--|-------------------|
| The application was received by the EMA on | 12 May 2022 |
| The procedure started on | 16 June 2022 |
| The Rapporteur's first Assessment Report was circulated to all CHMP members on | 5 September 2022 |
| The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on | 21 September 2022 |
| The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on | 13 October 2022 |
| The applicant submitted the responses to the CHMP consolidated List of Questions on | 20 December 2022 |
| The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on | 06 March 2023 |
| The CHMP agreed on a list of outstanding issues to be sent to the applicant on | 30 March 2023 |
| The applicant submitted the responses to the CHMP List of Outstanding Issues on | 20 June 2023 |
| The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on | 05 July 2023 |
| The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for quality and safety including the clinical benefit/risk profile of gentamicin sulfate / sargramostim / heparin sodium / insulin human as ancillary medicinal substance(s) used in Origio A.R.T. Media on | 20 July 2023 |

1.3. Manufacturers

Manufacturer(s) of the active substance used as ancillary medicinal substance

Gentamicin sulfate:
Fujian Fukang Pharmaceutical Co. Ltd
Jiangyin Industrial Estate
Fuqing
350309 Fuzhou
CHINA

Insulin:
Novo Nordisk A/S
Hallas Alle 1
4400 Kalundborg
DENMARK

rhu GM-CSF:
Partner Therapeutics, Inc.
2625 162nd Street SW
Lynnwood
Washington
98087
UNITED STATES

Heparin sodium:
Wexport Ltd. trading as LEO Pharma Cork
Wallingstown
Little Island
Cork
T45 RP82
IRELAND

Manufacturer(s) of the finished product used as ancillary medicinal substance

Gentamicin sulfate:
not applicable since the ancillary medicinal substance incorporated in the medical devices is the active substance.

GM-CSF (Leukine):
Pfizer Inc.
1776 North Centennial Drive
McPherson,
KS USA 67460-9301

Heparin solution, 5000 IU/mL: Heparin LEO :
Manufacturer responsible for batch release:
LEO PHARMA A/S
Industriparken 55DK-2750 Ballerup, Denmark
Mådevej 76, Esbjerg, 6705, Denmark

Human Insulin:
not applicable since the ancillary medicinal substance incorporated in the medical devices is the active substance.

Manufacturer(s) of the medical devices

CooperMedical SRL
Edificio N° B49
Zona Franca Coyol
La Garita, Alajuela
Costa Rica 20113

1.4. Remarks to the notified body

General remark

- The Medical device manufacture does not have access to the proprietary information regarding nitrosamines risk for the insulin and heparin sodium ancillary substances which is considered a significant limitation. It is emphasised that the overall risk of nitrosamine impurities is not linked only to ancillary substances and their incorporation and that changes to device processes in the future may modify the risk. It is assumed that the Notified Body has complete oversight on the entirety of the risks (including factors aside from the ancillary substances) and follows up on the evolution of risks during surveillance inspections.

- If the applicant would consider to use products from a different supplier or alternative products from the same supplier for any of the Ancillary Medicinal Substances, the notified body shall be informed of the changes and shall consult the Agency in order to confirm that the quality and safety of the ancillary substance is maintained (see 4.4 Post-consultation phase) in [EMA recommendation on ancillary consultation - EMA/CHMP/578661/2010 rev .1](#).

Labelling

Patients and physicians should be made aware of the addition of the medicinal product to the culture media used during their treatment. They should be informed about the reason for and usefulness of the addition of these products. Patients and physicians should be aware that to date there have been no safety concerns reported in children conceived using media containing the medicinal products.

As stated previously, there is currently no robust evidence of a beneficial effects of the addition of medicinal products on the clinical outcome of pregnancies conceived during A.R.T. treatment. There should be no claims in the IFU of their usefulness in improving implantation rate, pregnancy rate, live birth-rate or in achieving a pregnancy after previous implantation failure or recurrent pregnancy loss.

1.5. Recommended measures to the notified body

As discussed at CHMP, it would be recommended that the notified body request the following from the medical device manufacturer for device approval:

GM-CSF

| Area ¹ | Description |
|-------------------|--|
| Quality | The batch release CoAs provided for the medical devices EmbryoGen® and BlastGen™ (#1204 & #1205) correspond CooperSurgical Denmark (DK). The CoAs of batches manufactured at the CR site should be provided. Also, it is not clear where QC testing of the devices takes place; this point should be clarified and assurance provided that the relevant standards are in place at the sites. If relevant for medical devices, the results of method transfer for non-compendial methods should be presented. |

| Area ¹ | Description |
|-------------------|--|
| Quality | It is recommended that the single-use filters used for the sterilizing Filtration before aseptic filling be described in the dossier (table format), in the section on Description of Manufacturing Process and Process Controls. Unless otherwise justified, these filters have to correspond with the filters validated in the study performed by MediCult a/s and provided in "Sterile filtration validated processes for (Section 3_Appendix 3.P_8)". If the filters are not covered by that validation, the corresponding validation study results should be provided in the dossier. |
| Quality | For QC testing of ORIGIO media containing GM-CSF a cytotoxicity Mouse Embryo Assay (MEA) test is performed to verify absence of accumulation of any toxic impurities. For medicinal products QC testing methods should be validated and the relevant results have to be provided in the dossier. Particular attention is paid to validation of non-compendial biological methods. |

¹ Areas: quality, safety, including clinical benefit/risk profile.

Insulin

| Area ¹ | Description |
|-------------------|--|
| Quality | The batch release CoAs provided for the medical device BlastGen (#1205) correspond CooperSurgical Denmark (DK). The CoAs of batches manufactured at the CR site should be provided. Also, it is not clear where QC testing of the devices takes place; this point should be clarified and assurance provided that the relevant standards are in place at the sites. If relevant for medical devices, the results of method transfer for non-compendial methods should be presented. |
| Quality | It is recommended that the single-use filters used for the sterilizing Filtration before aseptic filling be described in the dossier (table format), in the section on Description of Manufacturing Process and Process Controls. Unless otherwise justified, these filters have to correspond with the filters validated in the study performed by MediCult a/s and provided in "Sterile filtration validated processes for (Section 3_Appendix 3.P_8)". If the filters are not covered by that validation, the corresponding validation study results should be provided in the dossier. |
| Quality | The Notified body is recommended to ensure that specifications for all media containing insulin are included in the dossier and contain a test for control of insulin content and numerical acceptance criteria (rather than "monitoring"). |
| Quality | The Notified Body is recommended to review the results of the stability study performed according to protocol Appendix 3.2.P_6_VAL-23-0128 to evaluate the stability of the insulin ancillary substance in the insulin stock solution as these results are not yet available. It should also be ensured that numerical acceptance criteria for insulin content are implemented during stability testing. |
| Quality | The Notified Body is recommended to review the results of the stability studies performed according to protocol Appendices 28 VAL-23-0122, 29 VAL-23-0120, 30 VAL-23-0119 and 31 VAL-23-0127 to evaluate the stability of the insulin ancillary substance during long-term storage of Origio media and in expired media as these results are not yet available. It should also be ensured that numerical acceptance criteria for insulin content are implemented during stability testing. |

¹ Areas: quality, safety, including clinical benefit/risk profile.

Heparin sodium

| Area ¹ | Description |
|-------------------|--|
| Quality | The batch release CoAs provided for the medical devices Flushing Medium with Heparin (#1076) and SynVitro® Flush with Heparin (#1576) correspond CooperSurgical Denmark (DK). The CoAs of batches manufactured at the CR site should be provided. Also, it is not clear where QC testing of the devices takes place; this point should be clarified and assurance provided that the relevant standards are in place at the sites. |
| Quality | It is recommended that the single-use filters used for the sterilizing Filtration before aseptic filling be described in the dossier (table format), in the section on Description of Manufacturing Process and Process Controls. Unless otherwise justified, these filters have to correspond with the filters validated in the study performed by MediCult a/s and provided in "Sterile filtration validated processes for (Section 3_Appendix 3.P_8)". If the filters are not covered by that validation, the corresponding validation study results should be provided in the dossier. |
| Quality | It is noted that the heparin sodium ancillary substance to be used for the medical device may contain the following substances: parahydroxybenzoate, propyl parahydroxybenzoate and benzyl alcohol (see Appendix 3.S_4 and table of Appendix 3.P_3 "In-Coming Control of Heparin (2141)"). It should be considered whether the levels or presence of these substances present in the final medical device should be declared in the product catalogue (Appendix 3.P_1) or other sections. |
| Quality | The Notified body is recommended to review the results of the formal stability study executed according to protocol Appendix 3.2.P 31 VAL-23-0119 Rev A to support the stability of heparin sodium in the final medical device as results are not yet available. |

¹ Areas: quality, safety, including clinical benefit/risk profile.

Gentamicin sulfate

| Area ¹ | Description |
|-------------------|--|
| Quality | A brief manufacturing flowchart is provided. It should be completed with: IPC, conditions, ranges, fields and amounts of ingredients pertaining to the incorporation of Gentamicin Sulfate in the medical device. |
| Quality | Regarding Purified Water quality used, please note that specifications should be provided. |
| Quality | Confirm that the commitment to update the dossier with batch analysis data for new incoming batches of Gentamicin Sulfate demonstrating compliance with the specification registered in 3.2.S.4.1 is fulfilled. |
| Quality | The results from stability studies performed in accordance with VAL-23-0122, VAL-23-0124, VAL-23-0120 and VAL-23-0119 should be reviewed to confirm they support the registered shelf life and in use periods of the Origio media products containing Gentamicin Sulfate. Results from VAL-23-0119 could also help in better understanding any pharmacological interaction between heparin and gentamicin. |
| Quality | The results for the interim study performed in accordance with VAL-23-0127 using expired Origio media products containing Gentamicin Sulfate should be reviewed to confirm they support the registered shelf life and in use periods of the Origio media products containing Gentamicin Sulfate. |

¹ Areas: quality, safety, including clinical benefit/risk profile.

General recommendations

| Area | Description |
|---|---|
| Labelling | <p>Should be updated as indicated to ensure that the patient and physician are aware of the indication for and usefulness of the addition of the medicinal products to the A.R.T. media.</p> <p>There is currently no robust evidence of a beneficial effects of the addition of medicinal products on the clinical outcome of pregnancies conceived during A.R.T. treatment. There should be no claims in the IFU of their usefulness in improving implantation rate, pregnancy rate, live birth-rate or in achieving a pregnancy after previous implantation failure or recurrent pregnancy loss.</p> |
| Follow-up of children conceived using the media | <p>Should ensure that the data source used for the clinical follow up study captures the required data which should include developmental follow-up. The quality of the data source should also be robust.</p> |

2. Scientific overview and discussion

2.1. General information

The notified body BSI Group submitted to the European Medicines Agency (EMA) on 12 May 2022 an application for consultation on gentamicin, heparin sodium, insulin and GM-CSF as ancillary medicinal substance(s) used in the medical device portfolio Origio Artificial Reproductive Techniques (A.R.T.)/In vitro Fertilisation (IVF) Media, in accordance with the procedure falling within the scope of Regulation (EU) 2017/745.

The Origio A.R.T. media portfolio is inclusive of media products covering all the steps in the A.R.T. processes and procedures.

The notified body has verified the usefulness of the ancillary medicinal substance(s)/ancillary blood derivative(s) as part of the medical device(s) and provided an assessment report on this verification. Data which are relevant to the quality and safety of the ancillary medicinal substances, including the clinical/benefit risk profile, have been supplied in the dossier.

These medical devices have been on the EU market for a long time; however, consultations under the Medical Device Directive had not been undertaken on the ancillary substances, (with the exception of Human Serum Albumin, HSA), as a result of a decision taken by the previous notified body and the Danish Competent Authority. This exemption was granted based on the low concentration of the agents used and on the fact that the full dose is not delivered to the mother but only comes into contact with the gamete. Following the publication of MEDDEV 2.2/4, and since the entry into application of Regulation (EU) 2017/745, a consultation on the ancillary medicinal substances gentamicin, heparin sodium, insulin and GM-CSF would be required as part of the re-certification of the medical device portfolio of Origio Artificial Reproductive Techniques (A.R.T.)/In vitro Fertilisation (IVF) Media.

The Origio A.R.T. media portfolio also contains Human Serum Albumin. However, for this ancillary medicinal substance a consultation has already been performed (EMEA/H/D000830); therefore, this substance is not the subject of this report.

Name of medical device(s):

Origio A.R.T. Media

Ancillary medicinal substance (s):

The devices contain one or more of the following:

Gentamicin Sulfate;

Sargramostim (GM-CSF);

Heparin sodium;

Insulin;

Human Serum Albumin (note, a re-consultation under Regulation (EU) 2017/745 has already been performed for this ancillary substance under the procedure EMEA/H/D000830)

Strength/concentration of ancillary medicinal substance(s)/ancillary human blood derivative(s):

Gentamicin Sulfate 10 ug/ml

GM-CSF; 2 ng/ml

Heparin sodium 10 IU/ml

Insulin 0.0054 to 0.5 ug/ml

Human Serum Albumin 1.0-12.5 mg/ml

Presentation(s) of ancillary medicinal substance(s)/ ancillary human blood derivative(s) as part of the medical device:

Solutions

Notified body:

BSI Group, The Netherlands

Medical device manufacturer:

Origio A/S

There are 32 products in the range:

| No. | Device Name | Ancillary medicinal substance(s)/human derivate(s) | Intended purpose per IFU |
|-----|--------------------------------------|---|--|
| 1. | Sperm Preparation Medium (1069/1070) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For washing of spermatozoa and isolation of motile viable spermatozoa by swim-up method. |
| 2. | UTM™ Transfer Medium (1052/1152) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For transfer of embryos and blastocysts. |
| 3. | PVP Medium (1089) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For slowing down the movement of the spermatozoa for ICSI. |
| 4. | PVP Clinical Grade (1090) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For slowing down the movement of the spermatozoa for ICSI. |
| 5. | SpermSlow™ (1094) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For slowing down the movement of the sperm to allow for the selection of the most mature, viable spermatozoa for ICSI. |
| 6. | Sperm Freezing Medium (1067) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For freezing of spermatozoa. |
| 7. | Embryo Freezing Pack (1026) | 1. Insulin 2. Human Serum Albumin | For freezing of human zygotes and cleavage stage embryos. |

| | | | |
|-----|---------------------------------------|--|---|
| 8. | BlastFreeze™ (1053) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For freezing of blastocysts. |
| 9. | BlastThaw™ (1054) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For thawing of blastocysts frozen using BlastFreeze™. |
| 10. | Flushing Medium (1084) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For retrieval, holding and washing of oocytes. |
| 11. | Flushing Medium with Heparin (1076) | 1. Gentamicin Sulfate 2. Heparin sodium 3. Insulin 4. Human Serum Albumin | For retrieval, holding and washing of oocytes. |
| 12. | Universal IVF Medium (1030/1031) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For fertilisation and culture until 2-8 cell stage. Can also be used for embryo transfer. |
| 13. | MediCult IVM® System (8221) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For pre-incubation and maturing of immature oocytes. |
| 14. | Biopsy Medium (1062) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For blastomere biopsy of cleavage stage embryos for Pre-implantation Genetic Diagnosis (PGD). |
| 15. | Embryo Thawing Pack (1098) | 1. Insulin 2. Human Serum Albumin | For thawing of human zygotes and cleavage stage embryos frozen using the Embryo Freezing Pack. |
| 16. | ICSI Cumulase® (1612) | 1. Human Serum Albumin | ICSI Cumulase® is for the removal of the cumulus complex and corona radiata surrounding the oocyte in preparation for ICSI. |
| 17. | SynVibro® Flush (1584) | 1. Insulin | For retrieval, holding and washing of oocytes. |
| 18. | SynVibro® Flush with Heparin (1576) | 1. Heparin sodium 2. Insulin | For retrieval, holding and washing of oocytes. |
| 19. | CryoSperm™ (1101) | 1. Gentamicin Sulfate | For freezing of human spermatozoa |
| 20. | MediCult Vitrification Cooling (1228) | 1. Gentamicin Sulfate 2. Human Serum Albumin | MediCult Vitrification Cooling is for ultra-rapid cooling of human oocytes, cleavage stage embryos and blastocysts. |
| 21. | MediCult Vitrification Warming (1229) | 1. Gentamicin Sulfate 2. Human Serum Albumin | MediCult Vitrification Warming is for warming of vitrified human oocytes, cleavage stage embryos and blastocysts |
| 22. | ORIGIO® Sequential Fert™ (8301/8302) | 1. Gentamicin Sulfate 2. Human Serum Albumin | For fertilisation of oocytes by conventional in vitro fertilisation (IVF) or Intracytoplasmic Sperm Injection (ICSI). |
| 23. | ORIGIO® Sequential Cleav™ (8303/8304) | 1. Gentamicin Sulfate 2. Human Serum Albumin | For culture until 2-8 cell stage. Can also be used for embryo transfer. |
| 24. | ORIGIO® Sequential Blast™ (8305/8306) | 1. Gentamicin Sulfate 2. Insulin 3. Human Serum Albumin | For culture from the 4-8 cell stage through to the blastocyst stage. Can also be used for embryo transfer. |

| | | | |
|-----|--|--|--|
| 25. | SAGE 1-Step™ (6701) | 1. Gentamicin Sulfate 2. Human Serum Albumin | For the in vitro culture of human embryos following fertilisation until Day 5/Day 6 of development. The medium can also be used for embryo transfer. |
| 26. | EmbryoGen® (1204) | 1. Gentamicin Sulfate 2. Leukine (GM-CSF) 3. Human Serum Albumin | EmbryoGen® is for the culture of human embryos until the 2-8 cell stage. EmbryoGen® can also be used for embryo transfer on Day 2 or Day 3. |
| 27. | BlastGen™ (1205) | 1. Gentamicin Sulfate 2. Leukine (GM-CSF) 4. Insulin 3. Human Serum Albumin | BlastGen™ is for the culture of 4-8 cell stage human embryos to the blastocyst stage. BlastGen™ may also be used for embryo transfer. |
| 28. | EmbryoGen®/BlastGen™ (1206) NB: 1206 are a system-pack and will not require a full submission | 1. Gentamicin Sulfate 2. Leukine (GM-CSF) 4. Insulin (only 1205) 3. Human Serum Albumin | EmbryoGen® is for the culture of human embryos until the 2-8 cell stage. BlastGen™ is for the culture of 4-8 cell stage human embryos to the blastocyst stage. BlastGen™ may also be used for embryo transfer. |
| 29. | ORIGIO® Sperm Wash (8405) | 1. Gentamicin Sulfate 2. Human Serum Albumin | ORIGIO® Sperm Wash is to be used for the washing of sperm, the isolation of motile viable sperm by swim-up method, the dilution of ORIGIO® Gradients, and as a holding medium for sperm prior to IUI. |
| 30. | ORIGIO® Gradient™ 90 (8401) | 1. Gentamicin Sulfate 2. Human Serum Albumin | for the efficient separation of motile sperm from the ejaculate by the density gradient method. |
| 31. | ORIGIO® Gradient™ 40/80 (8402) | 1. Gentamicin Sulfate 2. Human Serum Albumin | for the efficient separation of motile sperm from the ejaculate by the density gradient method. |
| 32. | ORIGIO Handling Medium (8310/8311) | 1. Gentamicin Sulfate 2. Human Serum Albumin | ORIGIO® Handling™ is intended for handling of oocytes outside the CO2 incubator. including oocyte retrieval and washing, as well as holding of oocytes prior to and during fertilization by Intracytoplasmic Sperm Injection (ICSI). |

2.2. Quality documentation

Origio ART media are a range of media products designed for use in Assisted Reproduction Technologies (ART). They have direct physical contact with human gametes or embryos for the purposes of preparation, maintenance or transfer or storage. The media consist of various compositions of physiological salts, nutritional and energy substances, buffer systems and synthetic serum replacement compounds. The media contain gentamicin sulphate, GM-CSF (leukine), insulin, and/or heparin sodium as ancillary medicinal substance(s) incorporated in the medical device. Therefore, they are considered Class III medical devices according to Rule 14 of the European Medical Device Regulation 2017/745. The current procedure is a consultation procedure for ancillary medicinal substances incorporated in medical devices.

Many of the media also contain human serum albumin as ancillary human blood derivative, for which consultation procedure EMEA/H/D/000830 was performed, assessment of which is not repeated in the current report.

All media are used in specialised hospital laboratories by laboratory technicians applying ART.

The Origio ART media are manufactured by CooperMedical SRL, Edificio no B49, Zona Franca Coyol, La Garita, Alajuela, Costa Rica 20113.

2.2.1. For the ancillary medicinal substance or the ancillary human blood derivative itself

2.2.1.1. *Gentamicin sulfate*

2.2.1.1.1. Active substance

As there is a monograph of gentamicin sulfate in the European Pharmacopoeia, the manufacturer of the active substance, Fujian Fukang Pharmaceuticals Co. Ltd., has been granted a Certificate of Suitability of the European Pharmacopoeia (CEP, i.e. R1-CEP 1998-155-Rev 10) for gentamicin sulfate which has been provided within the current application. The gentamicin sulfate is controlled by the supplier in line with the Ph. Eur. and additional tests as per the CEP.

The specification is identical to the specification registered by the CEP holder and includes additional tests performed on receipt of the material. A commitment is provided to update the dossier with batch analysis data and a recommendation is made for the Notified Body to follow up. As stability of gentamicin sulfate is not covered by the CEP, stability data generated by the manufacturer of the ancillary substance are provided supporting a re-test period of 4 years with no temperature restrictions.

The gentamicin sulfate active substance is incorporated into the medical device media.

A major objection (MO) was raised to request a nitrosamines risk evaluation which would consider any relevant factors such as the final concentration in the medical device. A risk assessment report on nitrosamine impurities in gentamicin sulfate was submitted based on which it was concluded that nitrosamine impurities are unlikely to be formed, and that there is no need to control nitrosamine impurities. The major objection was resolved.

2.2.1.1.2. Finished medicinal product

Not applicable since the ancillary substance is incorporated in the medical device media as active substance.

2.2.1.1.3. Adventitious agents' safety

Gentamicin sulfate is derived from fermentation of the bacteria *Micromonospora purpurea* and one of the nutrients included in the fermentation medium is fish peptone. A viral safety risk assessment is provided by the supplier of gentamicin sulfate addressing both the manufacturing process of the fish peptone and the extraction process for the gentamicin sulfate. The risk assessment supports the conclusion of very low risk of viral contamination. A transmissible spongiform encephalopathies (TSE) statement from the supplier of gentamicin sulfate was also provided. The information was considered sufficient.

2.2.1.2. GM-CSF (Leukine)

The GM-CSF ancillary substance is the Leukine finished product (250 µg/ml lyophilised powder for injection in a vial) which has been authorised in the US since 1991. This ancillary substance is not authorised in the EU.

The GM-CSF source used in the Origio IVF media is supplied from Partner Therapeutics Inc.

2.2.1.2.1. Active substance

A module 3.2.S was provided for the active substance relating to the GM-CSF ancillary substance. The information was considered sufficient to support the quality of the ancillary substance.

Sargramostim (recombinant human Granulocyte Macrophage-Colony Stimulating Factor; rhu GM-CSF) is a 127 amino acid glycoprotein that differs from native human GM-CSF by substitution of leucine (Leu) for arginine (Arg) at position 23. The primary amino acid sequence was provided. Three glycosylated forms of sargramostin are present in the drug substance.

Manufacturing process and controls

The active substance is manufactured and QC tested at Partner Therapeutics (Northpointe site), United States.

The fermentation process generates rhu GM-CSF for harvest and recovery is initiated from the working cell bank (WCB) and has 3 stages that include shake flask, seed fermentation and production fermentation. The production fermentation is a fed-batch process using a glucose solution feed. When the glucose feed is stopped, the promoter derepresses within the yeast resulting in rhu GM-CSF formation and secretion.

The harvest is performed via tangential flow filtration that separates the yeast cells from the secreted rhu GM-CSF, and the rhu GM-CSF is concentrated during the ultrafiltration (UF) unit operation. The purification process of rhu GM-CSF uses multiple chromatographic steps. The bulk active substance is cooled at 2 – 8 °C prior to transfer to – 70 °C freezers. No reprocessing of active substance is performed.

Critical process parameters (CPPs), process parameters (PPs) and in-process controls (IPCs) are defined for the process and are generally appropriate. CPPs are defined for the production fermentor and the capture and purification steps. CPPs are mainly defined on the basis of potential impact to glycoform ratio, N-terminal heterogeneity and process-related impurity clearance. Otherwise, process parameters are set to ensure consistent performance of unit operations.

Control of materials

The generation of the expression construct and cell banks is well described and generally in line with ICH Q5B/Q5D. The MCB was characterised with respect to identification (phenotypic properties) and the absence of contaminating microorganisms was confirmed. The genetic integrity of the plasmid and the expected sequence of the rhuGM-CSF was confirmed for the MCB. The procedure for characterisation of WCB is described and includes appropriate testing both at the level of the WCB vial and testing performed on fermentation lots generated from the WCB. The testing panel is sufficient to ensure identity, absence of contamination, viability, production of rhuGM-CSF, plasmid retention and the correct sequence for the rhuGM-CSF gene. Genetic stability for a production run is not specifically addressed but is supported by the results of the pilot fermentation runs and the presented characterisation data (where the primary amino acid sequence is confirmed for a number of production

runs). The shelf life of the WCB (3 years) is acceptable taking into account the cell bank storage conditions and considering the process controls to ensure consistent culture performance for the upstream process.

The specifications for non-compendial raw materials are provided or materials are accepted on the basis of supplier certificates of analysis. This is acceptable.

Process validation

Five successful process validation batches were manufactured at the Northpointe facility to validate the active substance manufacturing process. All results for CPPs, PPs and IPCs met their respective process validation acceptance criteria with the exception of a limited number of deviations which are adequately justified.

For the purification steps, removal of process-related solvents was demonstrated to acceptable residual levels. All reported profiles for endotoxin, protein impurities and DNA showed acceptable reductions across unit operations. Column lifetime qualification studies adequately verified the maximum column lifetimes for unit operations.

It was concluded that the process is in a validated state and capable of producing consistent batches which meet the required quality standard.

Manufacturing process development

The data presented focuses on a discussion of the optimisation of the process at the Northpointe (NP) facility and a comparability exercise to confirm comparability of the resultant bulk active substance with material manufactured at the previous commercial site (51U), approved for the US market. 6 engineering runs were performed at the new NP site to verify robustness of the manufacturing process. Further to this, 3 GMP batches were manufactured at the site and compared against 3 GMP batches from the 51U site (using release tests and extended characterisation). The processes were also compared using IPC test results. The results support the conclusion of comparability. The information provided is sufficient to support a well-developed process with understanding of relevant critical quality attributes and the function of unit operations in clearance of process-related and product-related impurities.

No justification was provided for the control strategy with respect to determination of process parameter criticality or associated acceptance ranges. Many of the process parameters are standard and in line with general expectations for this type of process. The extensive historical manufacturing experience along with the totality of evidence presented on historical batches and batches included in the comparability exercise, supports a consistent process capable of meeting the defined CPPs/PPs and producing bulk active substance which meets specification. Furthermore, the IPC testing applied throughout the upstream and downstream manufacturing process is sufficient to ensure that the purification chromatography steps operate as intended to produce material with the correct glycoform ratio.

Process-related impurity clearance is supported by the process validation and process development data presented, where clearance in the order of 4 – 5 logs has been demonstrated for HCP.

Characterisation

The structural and physicochemical characterisation of rhu GM-CSF is comprehensive and includes primary, secondary and tertiary structure. Primary structure was confirmed by peptide mapping and amino acid composition. Post-translational modifications (Methionine oxidation, aspartate isomerisation, deamidation) and disulfide bond characterisation were also evaluated by peptide mapping. Secondary and tertiary structure were evaluated by intrinsic fluorescence spectroscopy and

far UV circular dichroism (CD). Glycosylation site occupancy and N- and O- linked glycans were also characterised. The molecular masses of the different glycoforms were determined (MALDI-TOF-MS) and additional bioactivity analysis performed on the isolated peaks. The specific activities of the different glycoforms were comparable.

Process-related impurities that could be introduced into the rhu GM-CSF may originate from raw materials used in fermentation, fermentation by-products (e.g., yeast-cell secretion products, cell wall proteins, nucleic acids, cell membranes, etc.), and residual solvents from the purification process. The removal of host cell and media-related proteins and residual DNA was demonstrated during process characterisation and process validation. Residual solvents have been demonstrated to be removed as part of process validation or are detected at acceptably low levels in the drug substance. The absence of routine control for the aforementioned impurities is acceptable. The related substances of rhu GM-CSF are removed by the purification steps as demonstrated during process development, and related substances are routinely controlled by the RP-HPLC method.

Active substance specifications

The active substance specifications were presented. The testing panel is generally appropriate and addresses identification, quantity, biological activity, purity, monosaccharide composition and microbial purity.

The active substance specifications are based on analytical results from development batches of active substance and batches manufactured at Northpointe for the following tests: physical appearance, identification (IEF), SDS-PAGE (purity by reduced and non-reduced), and SE-HPLC (related substances) The acceptance criteria for endotoxin were set to ensure compliance with USP <85> (aligned to Ph. Eur.) at the maximum medicinal product dose. The limit for TAMC and TYMC is appropriate for an active substance used in manufacture of a sterile finished product. For all other test parameters, the proposed acceptance criteria are aligned with the presented batch data. Overall, the acceptance criteria are aligned to batch data and are sufficiently justified in the context of an ancillary substance.

Analytical methods

The analytical methods have been described and the methods are considered appropriate for testing of the active substance. Sufficient assurance was provided on the validation status of the methods given that the rhu GM-CSF active substance has been approved since 1991 in the US-authorized Leukine finished product.

Reference standards

The procedure for qualification and retesting of reference standard is outlined and is acceptable.

Container closure system

The bulk active substance container closure is a bottle with a screw cap closure. While it has not been confirmed that the container closure complies with relevant Ph. Eur. monographs, the material has been tested in accordance with the applicable USP standards. In addition, the container closure is confirmed to be suitable for food contact. Although not described in detail, it is noted that extractable and leachable studies have been performed and did not identify any concern. The information is sufficient.

Stability

The proposed shelf life of 60 months at – 70 °C is supported by the long-term stability data presented from 3 batches. No trends were observed. The stability protocol includes the relevant stability indicating parameters. The stability acceptance criteria are aligned to the release specifications.

Depending on the attribute tested, accelerated data is available for 6 – 9 months at 5 °C, 6 months at 15 °C, and 3 - 4 months at 25 °C and 40 °C respectively. The results from the accelerated/stressed studies support that the methods are sufficiently stability indicating.

2.2.1.2.2. Finished medicinal product

The medical device manufacturer uses vials of the lyophilised leukine medicinal product as the ancillary substance in manufacture of the IVF media. According to MEDDEV 2.1/3 rev 3 (Guideline on Borderline products, drug-delivery products and medical devices incorporating, Module 3.2.P (describing manufacture and control of the final lyophilised medicinal product) should be provided for the GM-CSF ancillary substance. The absence of any module 3.2.P for the GM-CSF ancillary substance was initially raised as a major objection. The requested information was provided (described hereafter) and is considered sufficient. As such, the major objection was resolved.

The finished product Leukine (GM-CSF) for injection is provided as a sterile, lyophilised white cake in a stoppered Type I, 5 ml glass vial. The vial contains an overfill of 14 µg of active ingredient to allow the labelled content of 240 µg to be withdrawn.

The medicinal finished product is manufactured at Pfizer Inc., Kansas, USA and QC tested by Partner Therapeutics (Northpointe Site), USA. The GMP status of the finished product manufacturing and testing sites has been verified.

The manufacturing process and control strategy are standard and acceptable. A schematic of the manufacturing process, including critical process parameters (CPPs), was provided. Appropriate in-process controls and acceptance criteria have been registered to the dossier. CPPs are defined for both the sterilisation and lyophilisation steps and are acceptable. In particular, acceptable controls are in place for bioburden prior to sterilisation, formulated bulk hold time, filter integrity testing and fill weight. Furthermore, the lyophilisation cycle CPPs are well detailed and include the relevant parameters for the freezing, primary drying, secondary drying and hold time steps.

The MAH maintains a lifecycle approach to manufacture of Leukine for Injection, a legacy product, through continuous process verification. The validated state is maintained through process monitoring, investigations as needed, updates based on regulatory expectations, and continuous process improvements, as demonstrated by manufacture since 1999. Finished product process validation results demonstrate that the manufacturing process successfully produces Leukine for Injection in a controlled and consistent manner.

The excipients (mannitol, sucrose and tromethamine) comply with pharmacopoeial requirements and there are no novel excipients.

The finished product specifications were provided.

Pharmacopoeial methods are used for some parameters. Non-compendial methods which are also used to control the bulk active substance were provided. Non-compendial methods specific to the finished product include reconstitution time and quantity. Method validation was performed to demonstrate the suitability of analytical procedure used for release and stability testing of the finished product. Analytical method validations, as appropriate, included accuracy, precision, specificity, detection limit, quantitation limit, linearity and range. Method suitability was confirmed for the pharmacopoeial methods. Batch analysis data was presented for 5 commercial lots. All batches meet the acceptance criteria. The specifications for finished product are based on manufacturing and development experience, ICH guidance, and the capabilities of the analytical methods.

A major objection was raised requesting a nitrosamines risk evaluation which should take into account any relevant factors such as the final concentration of the ancillary substance in the medical device. A nitrosamines risk assessment in line with the principles of the EMA Q&A for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products (EMA/409815/2020 Rev. 14) was provided for the GM-CSF when administered as medicinal product, considering a maximum daily dosage of 500 µg. Since Leukine, as an ancillary medicinal substance, is present at a concentration of only 2 ng/ml in the relevant Origio ART devices, the major objection was resolved.

It is proposed to include a general remark to the Notified Body (applicable to all four ancillary substances) reminding them that the overall risk of nitrosamine impurities is not linked only to ancillary substances and their incorporation and that changes to device processes in the future may modify the risk. It is assumed that the Notified Body has complete oversight on the entirety of the risks (including factors aside from the ancillary medicinal substances) and follows up on the evolution of risks during surveillance inspections.

The primary container closure system for lyophilised sterile finished product Leukine for Injection consists of a Type I glass vial (nominal fill volume of 5 mL) closed with a halo-butyl isoprene rubber stopper fastened by an aluminum crimp seal with a plastic flip-off cap. The glass vials and rubber stoppers meet appropriate USP standards.

Real-time long term stability data is available to support the 48 month shelf life for the medicinal finished product when stored at 2 – 8 °C. All stability data meet applicable specifications under long-term storage conditions within the batch expiry period.

2.2.1.2.3. Adventitious agents' safety

The rhu GM-CSF fermentation process utilises animal derived raw materials. Sufficient information on the materials was provided and the use of the animal derived materials in fermentation is considered acceptable. There are no animal derived raw materials in either the downstream process or the medicinal finished product fill-finish process. Thus, the risk of TSE transmission is agreed to be acceptably low. The production system in yeast will not support replication of mammalian viruses and the absence of any specific viral validation studies is acceptable.

2.2.1.3. Heparin sodium

Heparin sodium is present as ancillary medicinal substance in two of the media (Flushing and SynVitro media). Since 2008 the heparin is supplied from LEO Pharma A/S and it has been an authorised medicinal product in Denmark since 1955 (authorisation 00495/01613). The EU SmPC of this product has been provided.

2.2.1.3.1. Active substance

The active substance is covered by an EDQM Certificate of suitability (R1-CEP 2001-446- Rev 03, issued 21/02/2020) and is manufactured under GMP. Evidence of GMP has been provided for the manufacturing sites defined on the CEP. The starting material in the manufacturing process of heparin sodium is porcine intestinal mucosa. The process is split into three stages: (a) production of resin with heparin from mucosa, (b) production of heparin concentrated eluate from the resin and (c) production of heparin sodium from heparin concentrated eluate.

The heparin sodium is certified for compliance with Ph. Eur. monograph for Heparin sodium (0333). The animals from which heparin sodium is derived must be fit for human consumption and the identity of the source species (and absence of contaminating material from other species) is verified in accordance with the requirements of the aforementioned monograph.

While it is acknowledged that biological substances are excluded from the scope of the certificate of suitability procedure, for historical reasons there are a number of valid CEPs for such substances. In general, a CEP for a biological substance should not be used to replace the relevant data in the corresponding sections of module 3. However, taking into account that the heparin ancillary medicinal substance used as part of this product is an authorised medicine within the EU since 1955, the provided information is considered sufficient to support its use as an ancillary medicinal substance at low concentration in the medical device.

2.2.1.3.2. Finished medicinal product

The medical device manufacturer uses vials of the heparin medicinal finished product (5000 IU/vial) as the ancillary substance in manufacture of the Origio IVF media. Module 3.2.P (describing manufacture and control of the final lyophilised finished medicinal product) was requested for the Heparin finished medicinal product (5000 IU/vial) but could not be provided for reasons of confidentiality. Limited information has been provided for the heparin sodium medicinal finished product along with a risk

assessment regarding the absence of a full module 3.2.P. Given that the heparin sodium is an EU approved product and taking into account that appropriate QC controls are in place (i) on receipt of the heparin sodium medicinal finished product (raw material) by CooperSurgical and (ii) during further downstream processing, the absence of the requested data is not considered a major issue. The information provided is sufficient for an EU approved medicinal product.

A major objection was initially raised requesting a nitrosamines risk evaluation which should take into account any relevant factors such as the final concentration of the ancillary medicinal substance in the medical device. However, the requested information was not made available to the Applicant by the supplier of the heparin sodium (Leo Pharma) because it is claimed to be confidential information. Taking into account a number of risk mitigating factors, the major objection was downgraded to an other concern requesting the applicant to present a nitrosamines risk assessment addressing the concentration of the ancillary substance in the device and the level and duration of exposure to heparin sodium in the flushing media (for both patient and oocyte). An adequate risk assessment has now been presented. Patients are exposed to a concentration of heparin sodium at 3/50th the therapeutic dose for a limited exposure period (< 24 hr). The likely absence of metabolic activation of nitrosamines (if these were present) to DNA reactive intermediates during any oocyte incubation has also been considered. It is agreed that it is unlikely for oocytes in contact with the subject devices during the IVF procedures to have the capability to metabolize any possible nitrosamines present. Taking into account that the heparin sodium is an EU licensed product with no manufacturing process steps including chemical synthesis and no identified solvents of concern, it is agreed that the risk of nitrosamines due to the incorporation of heparin sodium into the device can be considered negligible.

2.2.1.3.3. Adventitious agents' safety

A risk assessment is presented with respect to TSE. In accordance with EMA 410/01 rev 3, pigs are not a TSE susceptible species. A TSE/BSE statement has been provided from the heparin manufacturer confirming compliance with the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents. The information provided was considered sufficient for an EU approved medicinal product.

2.2.1.4. *Insulin*

The insulin ancillary substance used in the Origio ART medical device range is the active substance for ActRapid, a centrally authorised medicinal product from Novo Nordisk, authorised under the procedure EMEA/H/C/000424 since 2002.

The insulin is obtained as crystalline biosynthetic recombinant insulin, complies with the European monograph for insulin and is manufactured under GMP. A letter of access is provided from Novo Nordisk authorising the EMA to refer to the relevant parts of the Novo Nordisk MAA's for information on the insulin active substance. Novo Nordisk also commits to ensure that the product (ancillary substance) purchased by the medical device manufacturer is the same as authorised in the Novo Nordisk MAs.

2.2.1.4.1. Active substance

The insulin active substance is manufactured at Novo Nordisk A/S, DK-4400 Kalundborg and the cell banks are prepared and stored at Novo Nordisk A/S, DK-2880 Bagsvaerd. Both sites hold valid GMP certificates. Brief information on the manufacturing process has been provided. The insulin is manufactured according to GMP by a standard process involving upstream fermentation (initiated from

WCB), recovery and downstream purification steps. The encoded product of secretion during fermentation is a single chain insulin precursor consisting of the first 29 amino acid residues of the insulin B chain linked with three amino acids to the insulin A chain. This single chain precursor is converted enzymatically to an insulin methyl ester, which is subsequently hydrolysed to yield human insulin, consisting of two chains (A and B) linked together with disulphide bridges. The purification process employs several chromatography and precipitation steps for isolation of the precursor, the intermediates, and the active substance respectively. This process is well established and recombinant human insulin has been manufactured by Novo Nordisk over a period of many years during which a number of improvements have been made. The fermentation, recovery and purification processes have been validated and critical parameters identified.

Human insulin is produced using a genetically modified strain of *Saccharomyces cerevisiae*. The strain carries a plasmid which codes for the expression of a single amino acid chain insulin precursor attached to a pre-pro leader region of the yeast mating factor (MFa1) gene. The plasmid is constructed based on the yeast 2 μ plasmid. The gene has been fully characterised from isolated plasmids from long-term production scale fermentation and cell bank (Original Mother Culture (OMC)). Constructional stability has been investigated in production strain, prolonged and very long-term fermentation and cell bank (OMC).

The insulin active substance complies with the Ph. Eur. monograph for insulin (0838). The active substance is stable for 60 months when stored at the recommended storage temperature.

Limited data has been provided in relation to the manufacturing process, control and stability of the active substance. However, taking into account that the insulin ancillary substance is the active substance in an authorised medicine within the EU, the provided information is considered sufficient to support its use as an ancillary substance at low concentration in the medical device. The quality of the active substance is considered sufficiently supported, also taking into account the commitment from Novo Nordisk to ensure that the ancillary substance purchased by the medical device manufacturer is the same as the active substance authorised for the approved Novo Nordisk MAs.

A major objection was raised requesting a nitrosamines risk evaluation which should take into account any relevant factors such as the final concentration in the medical device. The Applicant has not provided the requested nitrosamines risk assessment for insulin and, instead, re-confirmed that the insulin ancillary substance is the active substance approved in the centrally authorised ActRapid product range (EMA/H/C/000424). It is understood that the requested information is not made available to the Applicant (by Novo Nordisk) as it is claimed to be commercial confidential information. Nonetheless, on the basis of the information available for the ActRapid range and in the context of a recent article 58 procedure (EMA/H/W/005779/0000) which concluded that there is no risk of nitrosamines for the product in question, and considering the concentration of insulin in the Origio ART range (≤ 0.5 $\mu\text{g/ml}$ which is > 1000 fold lower than therapeutic levels), the major objection is resolved.

2.2.1.4.2. Finished medicinal product

Not relevant as the ancillary substance is the active substance (insulin crystals) for the centrally authorised ActRapid product range.

2.2.1.4.3. Adventitious agents' safety

A number of animal derived raw materials are used in the production of human insulin, (rDNA). These are peptone, beef extract and pepticase which are used in the preparation and storage of cell banks,

and L-threonine and trypsin used in the purification process to convert human insulin precursor to human insulin methyl ester. The TSE risk relating to these materials has been adequately addressed, in accordance with CPMP/CVMP Note for Guidance for minimising the risk of transmitting animal spongiform encephalopathy via medicinal products (EMA/410/01) and is considered to be low. According to the information provided, compliance with the relevant guidelines is ensured for viral safety issues. Although the information regarding viral risk is limited, it is accepted that the risk of mammalian virus propagation in a yeast-based production system is negligible. The information is considered sufficient for an ancillary substance that is approved in the EU as medicinal product.

2.2.2. For the ancillary medicinal substance or the ancillary human blood derivative as incorporated in the medical device

2.2.2.1. *Gentamicin sulfate*

Gentamicin sulfate has been included in selected ORIGIO ART media since 2010 at a concentration of 10 µg/ml to prevent microbial contamination during use of the media. The ancillary medicinal substance is added at the start of the manufacturing process where the drug substance is weighed and formulated into the media prior to sterilisation by filtration. There are no IPC controls specific to gentamicin sulfate included in the manufacturing process description, and process validation data reports do not include information regarding the fate of gentamicin sulfate. However, the medical device manufacturer has updated the device specifications to include a test for gentamicin sulphate concentration for all the Origio media products containing the ancillary substance.

Supplementary stability studies will be performed to support the shelf life and in-use periods registered for the Origio ART media products containing gentamicin sulfate. The relevant protocols were submitted, based on which a recommendation is made to the Notified Body to follow up on the results.

2.2.2.2. *GM-CSF (Leukine)*

GM-CSF has been included at a concentration of 2 ng/ml in the Origio ART range for over 10 years in the following media: EmbryoGen and BlastGen. The information presented regarding the manufacturing process (incorporation of the ancillary substance into the Origio IVF media) is generally sufficient. A detailed flow diagram of the process, the batch formula and the batch size were provided. In general, adequate information was provided in relation to preparation, quality control and stability of the GM-CSF stock solution.

There are no in-process controls around the step for incorporation of the ancillary substance into the device, but this is acceptable given that the final specifications for the relevant Origio IVF media contains tests for control of GM-CSF concentration and bioavailability. The methods for control of GM-CSF concentration (ELISA) and bioavailability (TF-1 assay) have been appropriately validated.

Data has been presented to support the stability of the GM-CSF ancillary substance in the Origio IVF media EmbryoGen and BlastGen for the proposed shelf life (25 weeks + 1 week after opening) at the intended storage condition, and stability of in the device under the normal conditions of use (37 °C), which are supported.

2.2.2.3. *Heparin sodium*

Heparin sodium is used in the two Origio IVF medical devices since 2008 at a concentration of 10 IU/ml: Flushing medium with heparin and SynVitro Flush with heparin. The information presented

regarding the manufacturing process (incorporation of the ancillary substance into the Origio IVF media) is generally sufficient. A detailed flow diagram of the process, the batch formula and the batch size has been provided. There is no stock solution for the heparin sodium.

There are no in-process controls around the step for incorporation of the ancillary substance into the device. However, this is acceptable given that it is intended to test the concentration of heparin sodium in the final device. The release specifications for the relevant Origio media have been updated to include the test for control of heparin sodium concentration using the compendial method for anti-factor IIa as per Ph. Eur. 2.7.5

Data has been presented to support the stability of the heparin ancillary substance in the final Origio IVF media by testing of two lots of Flushing medium with heparin near expiry. The study included in-use stability of the medical devices upon repeat opening of the bottles. A protocol has also been registered to perform a formal long-term stability study with representative media to specifically address the stability of the heparin sodium ancillary substance in the medical device. The notified body is recommended to review the results of the formal stability study, when available.

Presented data are sufficient to support the stability of the ancillary substance in the medical device until the formal stability study is executed, i.e. for Flushing Medium with heparin, the proposed shelf-life is 52 weeks in closed bottles, and 7 days after opening, and for SynVibro® Flush with Heparin, 27 weeks in closed bottles and 7 days after opening.

2.2.2.4. *Insulin*

Insulin is used in selected products of the Origio IVF medical device range at a concentration of 0.0054 to 0.5 µg/ml. Origio IVF media containing insulin have been available on the market for 16 – 30+ years. The information presented regarding the manufacturing process and in process controls for incorporation of the ancillary substance into the Origio IVF media is generally sufficient. A detailed flow diagram of the process, the batch formula and the batch size have been provided.

In general, adequate information has been provided in relation to preparation and quality control of the insulin stock solution. However, the Notified body is recommended to follow up on the results, not yet available, for stability of the insulin stock solution. There are no in-process controls described for the process step where the insulin is incorporated into the medical device. However, this is acceptable given that an insulin concentration test will be performed in the relevant Origio IVF media. The specifications for the insulin containing media have been updated to include the new test for concentration of insulin, and details of the analytical method and validation are presented in the dossier. However, the proposed acceptance criteria for insulin content is “monitoring”. The Notified Body is recommended to ensure that specifications for all media containing insulin are included in the dossier and contain a test for control of insulin content with numerical acceptance criteria (rather than “monitoring”).

No data has been presented to support the stability of the insulin ancillary substance in the final Origio IVF media. It is proposed to perform additional stability studies with representative media to specifically address the stability of the insulin ancillary substance in the medical devices. It is also proposed to test expired lots of representative Origio IVF media to support the stability (study date July 2023). The Notified Body is recommended to review the results of the stability studies performed according to the registered protocols and to ensure that numerical acceptance criteria are implemented for insulin content during stability testing.

2.2.3. Discussion and conclusion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the ancillary medicinal substance (active substance and/or finished product), and on the ancillary medicinal substance as incorporated in the medical devices has been presented in a satisfactory manner. The two MOs raised (request for nitrosamines risk assessment for each of the four ancillary substances, and for the drug product section for the GM-CSF medicinal product) were adequately resolved during the procedure. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in use.

At the time of the CHMP opinion, there were a number of remarks and recommendations to the notified body having no impact on the Benefit/Risk ratio of the product.

Gentamicin

The quality of the gentamicin sulfate ancillary substance is supported by a CEP; R1-CEP 1998-155-Rev 10. Incoming specification registered in the dossier for the ancillary substance complies with the Ph. Eur. and CEP and the manufacturer of gentamicin sulphate has confirmed that there is no risk for nitrosamines impurities to be present. A test to control the concentration of gentamicin sulphate in the relevant Origio IVF media has been included on the relevant media specifications. The applicant has submitted stability protocols to determine the concentration of gentamicin sulphate in the relevant media over the registered shelf life and in-use periods. A recommendation is made to the NB to follow up on the results of these studies.

GM-CSF (Leukine)

The information presented in relation the GM-CSF active substance and finished medicinal product is generally sufficient. Otherwise, the dossier sections relating to the medical device are sufficiently detailed regarding incorporation and control of the ancillary substance into the devices.

Heparin

The ancillary substance is an EU approved medicine. The EU SmPC for the ancillary substance has been provided. The active substance is covered by an EDQM Certificate of suitability (R1-CEP 2001-446-Rev 03, issued 21/02/2020). Sufficient detail is provided regarding the incorporation of the ancillary substance into the devices. The Notified body is recommended to review the results of the formal stability study to support the stability of heparin sodium in the final medical devices, as results are not yet available.

Insulin

It is noted that the insulin ancillary substance is the active substance of a centrally authorised medicinal product in the EU. Sufficient detail is provided regarding the incorporation of the ancillary substance into the device. While it has been agreed to control insulin content in the final media, the Notified body is recommended to ensure that specifications for all media containing insulin are included in the dossier and contain numerical acceptance criteria for insulin content (rather than "monitoring"). Stability protocols have been registered for the insulin stock solution and representative Origio IVF

media. The Notified body is also recommended to review the results of the stability studies performed to verify the stability of the insulin ancillary substance in both the stock solution and representative medical device (results not yet available).

Conclusion

The quality of this product is considered acceptable. Physicochemical and biological aspects relevant to the uniform performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.3. Non-clinical documentation

Pharmacodynamics and pharmacokinetics

According to MEDDEV 2.1/3 rev 3 on the non-clinical documentation, the lack of new pharmacodynamics studies is acceptable, since the role of the 4 ancillary medicinal substances in media for in vitro fertilisation is known, as well as the lack of new pharmacokinetic studies since their absorption through the vaginal and endometrial tissue is considered negligible.

Toxicity and local tolerance

Reference to the known toxicological profile of the ancillary medicinal substance was provided as scientific literature.

In addition, biological evaluation of the 4 ancillary substances incorporated as an integral part in the ORIGIO device, was carried on. The following ORIGIO A.R.T. media were considered the most representative of the 4 active substances and were selected by manufacturer for testing.

| | | |
|-------------------------------------|---|--|
| BlastGen™ (1205) | BlastGen™ is for the culture of 4-8 cell stage human embryos to the blastocyst stage. BlastGen™ may also be used for embryo transfer. | Gentamicin Sulfate Sargramostim Insulin Human Human Serum Albumin |
| Flushing Medium with Heparin (1076) | For retrieval, holding and washing of oocytes. | Gentamicin Sulfate Heparin sodium Insulin Human Human Serum Albumin |

However, in some cases other media were tested which are not representative for the 4 active substances:

- BlastAssist, which is similar in composition to BlastGen but without sargramostim, was tested
- Flushing Medium with heparin containing penicillin and streptomycin instead of gentamicin was tested (only in 2010 the Flushing Medium with heparin composition was changed to replace penicillin, streptomycin with gentamicin),
- SynVITRO Flush containing insulin and heparin
- MediCult Medium Mix (MMM) containing porcine heparin and insulin, but not gentamicin

The assays performed comprised:

- mouse embryo assay
- cytotoxicity in V79 Chinese hamster or L929 mouse cells
- sensitisation: mouse local lymph node assay or guinea pig maximisation test
- rabbit intravaginal irritation test
- sperm survival test
- Ames test (mutagenicity) (performed on BlastAssist on MediCult Medium Mix)

Gentamicin

Gentamicin is a broad-spectrum aminoglycoside antibiotic, which inhibits the growth of both gram-positive and gram-negative bacteria, as well as several strains of mycoplasma. The role of gentamicin sulfate in Origio A.R.T. media is to avoid the bacterial infection of (sterile) cell cultures during normal handling under strict hygienic conditions. Although the seminal fluid and the vagina are non-sterile environments, it is important that the culture systems for embryo culture and transfer are sterile since the presence of microorganisms can lead to lower fertilisation rates and poor embryo development. The final concentration of gentamicin in all ORIGIO A.R.T. media containing gentamicin sulfate is 10 µg/ml. This concentration is considered efficient in inhibiting growth of bacteria usually found in IVF procedures, without affecting the development of embryos. Gentamicin sulfate is present in almost all 32 ORIGIO media.

In 2009-10, the medical device manufacturer ORIGIO a/s changed the antibiotic agent from penicillin/streptomycin to the current gentamicin sulfate in the current concentration. Based on the comparison of toxicological profiles, both streptomycin and gentamicin are nephrotoxic and ototoxic. The cytotoxic effects of streptomycin and gentamicin seem to be related to induction of apoptosis and gentamicin seems to be more potent than streptomycin. The only cytotoxic effect of penicillin reported concerns red blood cells and neutrophils. The toxic effects on reproduction reported from studies of the three antibiotics indicate that both male and female reproductive parameters can be affected by gentamicin and streptomycin, whereas this is not reported for penicillin. This is also reflected in the FDA Pregnancy Categories, where penicillin is in the safest category, whereas the categorisation of the two aminoglycosides is less clearly defined.

The effect of antibiotics on development of in vitro hamster oocytes was investigated by Zhou et al., and they compared the use of Penicillin (100 IU/ml), Streptomycin (50 µg/ml) and Gentamicin (10 µg/ml) in culture media. Their investigation concluded that there was no difference in embryo development between the three antibiotics when penicillin and streptomycin were used alone, however penicillin and streptomycin in combination did produce lower percentages of 8-cell embryos and blastocysts. The authors conclude that gentamicin appears to be the safest antibiotic.

Hypersensitivity reactions are rarely reported from use of gentamicin, while streptomycin causes hypersensitivity reactions ranging from skin rashes (fairly common) to exfoliative dermatitis and anaphylactic shock. The major adverse effects of penicillin are hypersensitivity reactions (American Medical Association. American Medical Association, Council on Drugs. Hypersensitivity reactions from Gentamicin. Chicago: 1994.). It has been estimated that up to 10% of patients treated with penicillin experience hypersensitivity reactions to this drug. The irritative potential of the three antibiotics is not described in the literature but is judged not to be of any concern.

One of the major concerns to use antibiotics in media culture is given by the development of resistant bacteria. In particular, gentamicin is characterised by mechanism of resistance due to a failure of permeation, low affinity for the bacterial ribosome or inactivation of gentamicin by microbial enzymes. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has set limit values for gentamicin (clinical breakpoints), which provide information on the pre-determined range that classifies an organism as susceptible or not to a given antibiotic in clinical practice. These limits mean that in some types of infections the utility of gentamicin is questionable although development of resistant bacterial strains seems to be more pronounced following use of streptomycin/penicillin, compared to the use of gentamicin sulfate.

Moreover, in vitro studies have shown that aminoglycosides can catalyse the formation of reactive oxygen species (ROS) in the presence of iron and copper such as superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (HO•). Both iron and copper-containing compounds are present in IVF media, indicating that the formation of reactive oxygen species may take place. (Lesniak W, et al., 2005). Origio BlastAssist (9529 medium B) +1216 (red phenole) containing 10 µg/L and 16 µg/L gentamicin showed not to generate Reactive Oxygen Species (ROS) in a level which is harmful to spermatozoa (which are known to be extremely sensitive to ROS).

Further, in vivo data from rabbits indicate that gentamicin may interfere with metabolism of vitamin B6, i.e. injections of gentamicin (5 mg/kg bw) led to a 47% decrease in the plasma level of vitamin B6 (pyridoxal-5' -phosphate) (Weir MR, et al., Depression of vitamin B6 levels due to gentamicin. Vet Hum Toxicol. 1990). The implications of this aspect for vitamin B6 and gentamicin in A.R.T. media is unknown. However, the presence of vitamin B6 in culture media would seem important as it has been shown to reduce gentamicin-induced nephrotoxicity in rats (Jana S, et al. Molecular understanding of aminoglycoside action and resistance. Appl Microbiol Biotechnol. 2006).

Based on all bibliographic and laboratory data provided with Origio BlastAssist (9529 medium B) or BlastGen, which contain the same amount of gentamicin sulfate, it can be concluded that the change of antibiotics does not affect safety and/or performance of the IVF media and the advantages of using gentamicin instead of using streptomycin/penicillin G can be summarised as follows:

- At 10 µg/ml gentamicin, no adverse effects were observed on hamster embryo development, whereas a combination of 100 IU/mL penicillin and 50 µg/mL streptomycin adversely affected embryo development. The same amounts of penicillin and streptomycin also adversely affected development of human embryos.
- Many streptomycin and/or penicillin resistant bacterial strains are sensitive to gentamicin.
- Gentamicin results in less, if any, allergic reactions at systemic, clinical use in contrast to penicillin and streptomycin.
- The activity of gentamicin is not affected when serum proteins are present in contrast to penicillin and streptomycin.
- Gentamicin is heat stable and is not destroyed at autoclavation whereas streptomycin and penicillin cannot be autoclaved.
- Gentamicin is stable at pH 2-10 and more stable than streptomycin and penicillin. Gentamicin is effective on certain types of virus, (from 5 µg/ml), whereas penicillin has no effect on virus (White LA, et al., Effect of gentamicin on growth of viral, chlamydial, and rickettsial agents in mice and embryonated eggs. 1976).

To evaluate the safety of gentamicin as ancillary active substance integral part of the Origio A.R.T. media, ORIGIO BlastAssist media, the former commercially available CooperSurgical-ORIGIO similar in composition (including the same amount of gentamicin 10 µg/ml) to BlastGen but not containing sargramostim, was tested. BlastGen was also tested.

Since BlastAssist/Gen are surface device for cultivation of human embryos from 4-8 cell stage to the blastocyst stage (>24 hrs) and are also used for embryo transfer, they come into direct contact with human embryos as well as women (mucosal membrane (<24 hrs). Thus, these media were appropriately selected for toxicity screening for embryo and mother to be.

BlastAssist/Gen showed biocompatibility in the in vitro cytotoxicity test on V79 cells and on mouse embryo assay. This latter assay was performed over the media's shelf life for up to 36 weeks, thus excluding any potential degradation products harmful to embryos. Moreover, they resulted not be sensitiser, nor irritating at local lymph node and vaginal in mouse and rabbit, respectively.

The justifications provided for the waiver of genotoxicity, carcinogenicity and reproductive toxicity test with embryos at the blastocyst stage and patients are considered acceptable. In addition, BlastAssist resulted negative at bacterial mutagenicity assay (Ames test).

It is worth to mention that there are certain observations on gentamicin and heparin interaction at PK level, where the inhibitory effect of heparin on gentamicin in serum were observed at doses above 62.5 IU heparin/ml, but doses below 31.2 IU/ml resulted in a recovery of gentamicin above 90% (Nilsson L. Factors affecting gentamicin assay. Antimicrob Agents Chemother. 1980; 17(6):918-21). In another study, it was confirmed that aminoglycosides by an agar disk diffusion assay are inhibited by heparin in a dose-dependent way (Nilsson L. et al. Inhibition of Aminoglycoside Activity by Heparin. Antimicrob Agents Chemother. 1981; 20(2):155-158). Authors hypothesise that masking of the aminoglycoside activity by heparin is probably an inhibition by ionic interaction, rather than an

inactivation since the effect may be reversed by dilution.

The applicant, based on these data, theoretically concludes that a concentration of gentamicin sulfate of 10 µg/ml medium and 10 IU heparin/ml medium is not expected to result in lower activity of gentamicin.

In scientific literature, there are controversial opinions concerning gentamicin and heparin interaction, e.g. some authors confirm interaction between the two substances (Lavern M. Vercaigne L.M. et al. Antibiotic-Heparin Lock: In Vitro Antibiotic Stability Combined with Heparin in a Central Venous Catheter. *Pharmacotherapy* 2000; 20(4): 394–399), while others deny it (Bastani B. The myth of incompatibility of gentamicin and heparin revisited. *J Nephrol.* 2011 Mar-Apr; 24(2): 192-5. Doi: 10.5301/jn.2010.3115. PMID: 20623465).

Considering that “Flushing Medium with heparin” is the only Origio A.R.T. solution containing both gentamicin and heparin and that, according to the applicant, the “Flushing Medium with heparin” tested in the present application does not contain gentamicin but penicillin+streptomycin (formulation was tested before 200, but in 2010 the composition was changed to include gentamicin instead of penicillin+streptomycin), results from the biocompatibility tests are not representative of potential interactions between gentamicin and heparin. According to results from the VAL-23-0127 study, no interaction (specifically inactivation from heparin action on gentamicin as incorporated in the medical device) is expected. Overall, the interaction between gentamicin and heparin is not considered an issue. Nonetheless, the notified body is recommended to follow-up on the results of the long-term stability study.

Additionally, the applicant provided the IFU and labels of all ORIGIO A.R.T. medical devices containing Gentamicin Sulfate in accordance with Annex I, Chapter III, Section 23 of Regulation (EU) 2017/745 except for ORIGIO Handling Medium (8310/8311). The presence of Gentamicin Sulfate in Origio A.R.T. media is mentioned in the IFU for the individual Origio A.R.T. media containing Gentamicin Sulfate together with the concentration of Gentamicin Sulfate used in the final A.R.T. media, together with warnings to the Reproductive Medicine Specialist (i.e. embryologist) to check if the mother has allergy to Gentamicin Sulfate. The following warning is included: “This product contains gentamicin and should not be used on patients that have a known allergy to gentamicin or similar antibiotics”.

BlastAssist/Gen do not contain any unknown chemicals or drugs that are not already present in other commercial IVF blastocyst-stage culture media with the same intended use, and none of the chemical components in BlastAssist/Gen are considered to promote cytotoxicity, genotoxicity, sensitisation or irritation in the patient. No new risks or higher level of existing risks on the final product, including any raw material substitutions (e.g., currently sodium hyaluronate) during the product lifecycle, were identified. In conclusion, BlastGen is toxicologically safe and biocompatible with embryos and patients according to its intended use.

Since BlastGen also contains insulin and sargramostim, results described for gentamicin are also applicable to the other ancillary substances. Regarding the mutagenicity results carried out with BlastAssist, these cannot be extrapolated to sargramostim (GM-CSF), as BlastAssist does not contain this ancillary substance.

Sargramostim (GM-CSF)

Sargramostim is a GM-CSF, a multi-functional cytokine synthesised in the epithelial cells of the female reproductive tract, which is essential for modulating stress response genes, heat shock proteins and apoptosis. Sargramostim 2 ng/mL is present in 3 ORIGIO A.R.T. media with the ancillary role to provide an in vitro environment to better simulate the conditions in vivo for the embryo before the transfer into the woman’s uterus.

BlastGen is the Origio A.R.T. media tested for assessing the biocompatibility of sargramostim. Results from BlastAssist (similar to BlastGen, containing gentamicin and insulin but no sargramostim) and Flushing medium (containing gentamicin, heparin, insulin) are not representative for sargramostim since this active substance is not present in these media.

BlastGen resulted to be not cytotoxic in vitro on V79 cells and Mouse Embryo Assay, it did not induce sensitisation in mouse local lymph node assay, nor vaginal irritation in rabbits.

BlastGen resulted to be non-mutagenic to *S. typhimurium* tester strains TA98, TA100, TA1535, and TA1537, and to *E. coli* WP2uvrA tester strain, in presence and absence of metabolic activator. Thus, any potential mutagenicity from sargramostim as incorporated in the medical device can be excluded.

No studies assessing carcinogenicity and reproductive toxicity of sargramostim as incorporated in the medical device were carried out despite the fact that, according to the embryo contact, BlastGen is considered categorised as a surface device with contact for a prolonged period (> 24 hrs).

In the usefulness report, the manufacturer stated that colony-stimulating factors are known to be genotoxic in animal studies. The role of GM-CSF in breast cancer development (doi: 10.3892/mmr.2020.11017) was also reported. GM-CSF is known as a growth factor and, according to ICH S6 (R1) guidelines, "when there is a concern about the carcinogenic potential, a variety of approaches may be considered to evaluate the carcinogenicity risk". The manufacturer also stated that colony-stimulating factors are known to be fetotoxic in animal studies, but no further details were provided.

Finally, the manufacturer stated that the lower complexity of recombinant sargramostim compared to native GM-CSF is associated with less immunogenicity. Indeed, in the product information of Leukine (sargramostim), hypersensitivity reactions and induction of neutralising anti-drug antibodies are noted. However, in the product information of the medicinal product Leukine (sargramostim), it is reported that genotoxicity studies have not been conducted, at 200 mcg/kg/day LEUKINE had no effect on fertility of female rabbits, and no findings suggesting male or female reproductive toxicity were observed in cynomolgus monkeys. Overall, clinical data from the use of BlastGen since 2014, are reassuring confirming that concentration of sargramostim as in BlastGen has not shown to be harmful.

No remarks on the IFU are noted.

Insulin

Insulin is contained in almost all ORIGIO A.R.T. media at concentration ranging 0.0054 – 0.5 µg/mL. Similarly to sargramostim, its role in ORIGIO A.R.T. media as growth factor for improving embryo development is known, promoting glucose uptake as well as other mechanisms to protect against cell apoptosis/death and promote cell proliferation/growth.

The pharmacological profile of insulin as medicinal product for the treatment of type II diabetes is well known.

Insulin is present in BlastGen, Flushing medium with heparin, and also in BlastAssist (the previous version of BlastGen without sargramostim), therefore the results from biological evaluation obtained with the 3 media are also relevant for insulin.

Biocompatibility of BlastGen, in which the two substances sargramostim and insulin coexist, has been assessed. With regards to the potential pharmacodynamic interaction between sargramostim and insulin, and how this interaction could affect the performance of the Origio A.R.T. media, the Manufacturer clarified that insulin-stimulating glucose uptake occurs in specialized cells within the cumulus-oocyte complex (Scott H. Purcell et. al, 2012). p85 (alpha) is not the only possible factor involved in the mechanism of insulin resistance. Evidence has shown that GM-CSF driven myeloid cells lead to reduced adipose tissue 2-oxoglutarate dehydrogenase complex (DHTKD1) levels and subsequently increase in plasma 2-aminoadipate (2-AA) levels, both of which are reported to correlate

with insulin resistance (Deanna L Plubell et. al, 2018). However, the Manufacturer states that p85 (alpha), as one of the possible factors important in the mechanism of insulin resistance, is not present at the time of oocyte retrieval or during up to 5 days of culture in vitro. Therefore, it can be assumed that GM-CSF is not able to cause insulin resistance in the culture media for up to 5 days duration.

No remarks on the IFU are noted.

Heparin

Heparin belongs to a family of naturally occurring acid mucopolysaccharides called glycosaminoglycans. It is a well-known anticoagulant and produces its major anticoagulant effect by inactivating thrombin and activated factor X (factor Xa) through an antithrombin (AT)-dependent mechanism.

Flushing Medium and SynVITRO Flush are the 2 ORIGIO A.R.T. media containing heparin. Flushing Medium and SynVITRO Flush are well-established products and have been on the market since 1988 and 2000 respectively. The ancillary role that heparin performs to the device is also known, as it prevents the formation of clots blood that could interfere with the isolation of oocytes at the retrieval. Heparin in these media comes from porcine intestinal mucosa, and the risk of transmission of spongiform encephalopathy (TSE) infection of the users must be considered even if as stated by the manufacturer, porcine intestinal mucosa carries no detectable infectivity. As a mitigation measure due to its animal origin, the relevant ORIGIO A.R.T. media IFU and labels contain a warning regarding the heparin contained in the devices.

Since the media are used for follicle flushing, they come in direct contact with oocytes and the mother to be, therefore a biological evaluation was performed. Both Flushing Medium and SynVITRO Flush resulted biocompatibility at in vitro cytotoxicity test on L929 cells and on mouse embryo assay.

This latter assay was performed over shelf life for up to 36 weeks, thus excluding any potential degradation products harmful to embryos. While both were not sensitiser at rabbit vagina test, divergent results were observed at guinea pig maximisation test i.e. only Flushing Medium showed evidence of delayed contact hypersensitivity in 3 out of 20 animals 48 hours after treatment. In the Flushing Medium with Heparin tested (formulation referred to year 2009), besides insulin and human serum albumin, there are penicillin+streptomycin (instead of gentamicin in the marketed ORIGIO A.R.T. media) as ancillary substances, while in SynVITRO Flush with heparin there is also insulin. If the results from the 2 media containing the same amount of porcine heparin 10 IU/ml and human insulin 14.25 UI/ml and the same salt composition are compared, the delayed contact hypersensitivity observed in guinea pig with Flushing Medium with heparin, could be potentially attributed to the presence of penicillin G sodium, streptomycin or human serum albumin. It is known that the major adverse effects of penicillin are hypersensitivity reactions. The marketed Flushing Medium with heparin contains gentamicin instead of penicillin+streptomycin. BlastAssist medium (containing Gentamicin Sulfate sulfate 10 ug/mL, Insulin Human, Human Serum Albumin) tested in mice in local lymph node assay was not considered a skin sensitizer. Thus, the overall data indicate that heparin does not carry any potential toxicity for oocytes and mother to be.

According to results from the VAL-23-0127, no interaction (specifically between heparin and gentamicin, as incorporated in the medical device) is expected. Nonetheless, the notified body is recommended to follow-up on the results of the long-term stability study.

The justifications provided for the waiver of carcinogenicity and reproductive toxicity test (with oocytes and patients) are considered acceptable. In addition, MediCult Medium Mix (MMM), with similar composition of Flushing Medium and SynVITRO Flush, resulted negative at bacterial mutagenicity assay (Ames test).

Lastly, the justification provided for waiver of acute systemic toxicity and hemocompatibility is acceptable, since the interaction of heparin with blood is the basis of its intended mode of action/role in the 2 devices (i.e., prevent the formation of clots blood that could interfere with the isolation of oocytes at the retrieval).

2.3.1. Discussion and conclusion on the non-clinical documentation

This assessment refers to an initial consultation on known ancillary medicinal substances incorporated as an integral part, in a class III medical device, i.e. ORIGIO in vitro 32 fertilisation solutions. These media solutions cover all the steps in the A.R.T. process and procedures e.g. egg or sperm collection, storage, selection, in vitro fertilisation, embryo transfer, cryopreservation and oocyte implantation.

The ancillary substances are gentamicin sulfate, human recombinant sargramostim, human recombinant insulin, porcine heparin.

The 4 ancillary substances are well known active substances/medicinal products; their final concentration in the devices is significantly lower than the therapeutic dose administered to patients. Although present in the composition of Origio A.R.T. media for many years, none of the 4 ancillary substances have been previously assessed by any EU national medicines competent authority. Since these substances, if used separately, can be considered medicinal products, in line with the new Regulation 2017/745, the Notified Body is consulting the competent authority (EMA) for the assessment of their safety, taking account of the intended purpose in the device.

The lack of new pharmacodynamics studies is acceptable, since their role in buffers and media for in vitro fertilisation is known, as well as the lack of new pharmacokinetic studies since their absorption through the vaginal and endometrial tissue is considered negligible. Reference to the known toxicological profile of the ancillary medicinal substance was provided as scientific literature.

In addition, toxicity studies were performed on 5 Origio solutions already on the market for several years and 3 of these solutions can be considered as representative of the 4 ancillary substances:

1. BlastGen including gentamicin, sargramostim, insulin
2. BlastAssist (the previous version of BlastGen without sargramostin).
3. Flushing medium with heparin, insulin, penicillin and streptomycin (only in 2009-10 gentamicin replaced penicillin and streptomycin).

Not all the 3 media underwent the same assays, but all studies presented were carried out in line with the relevant guidelines for non-clinical assessment of class III/IVF medical devices (EN 10993 series). BlastGen and BlastAssist resulted non-mutagenic at Ames test. Moreover, MediCult Medium Mix (MMM) with similar composition of Flushing Medium and SynVitro Flush, resulted negative at Ames test. Justification for waiver of carcinogenicity, reprotoxicity and hemocompatibility studies were considered acceptable on the basis of available knowledge of the safety profile of ancillary substances and the intended use of the media.

Local tolerance is of particular relevance since the route of exposure to the ancillary medicinal substances is different from their conventional use as medicinal products. In vitro cytotoxicity test on V79 or L929 cells and on mouse embryo, were performed; the media containing the 4 ancillary substances resulted biocompatible. Mouse embryo assay was performed over shelf life for up to 36 weeks, thus excluding any potential degradation products harmful to embryos.

Local lymph node assay in mouse and guinea pig maximisation test were carried out to assess sensitisation potential; vaginal irritation test in rabbit was carried out to test potential irritation. The results for both tests were negative i.e. no sensitisation or vaginal irritation were observed.

From non-clinical perspective, no new/additional risk for gametes, embryo and mother to be coming from the presence of the 4 ancillary active substance in Origio A.R.T. media:

- gentamicin sulfate 10 µg/ml,
- human recombinant sargramostim 2 ng/mL
- human recombinant insulin 0.0054 – 0.5 µg/mL
- porcine heparin 10 IU/ml

has been identified.

With regards to the IFU, the inclusion of the below warnings as mitigation risk measures is agreed:

- presence of heparin of animal origin, to inform about potential transmissible infections
- presence of gentamicin, to inform about potential hypersensitivity reactions of the mother to be

To conclude, from a non-clinical point of view, the use of gentamicin, heparin sodium, insulin and GM-CSF in the Origio A.R.T. media is considered acceptable.

2.4. Clinical evaluation

2.4.1. Usefulness of the ancillary medicinal substance incorporated in the medical device as verified by notified body

The usefulness report provided by the applicant describes a range of ancillary substances used, for the same intended purpose, in a range of media for A.R.T./ IVF procedures. The ancillary medicinal substances are:

Gentamicin Sulfate, Insulin, Heparin sodium, Sargramostim (GM-CSF)

The Origio A.R.T. media portfolio also contains Human Serum Albumin. However, for this ancillary medicinal substance a consultation has already been performed (EMA/H/D000830); therefore, this substance is not the subject of this report.

Throughout the A.R.T./ IVF process, there is the need for different solutions and therefore the composition, including of the ancillary substance, is different depending on the use. The devices (A.R.T. solutions) that are in scope of this procedure have been on the market for many years already.

Gentamicin

Gentamicin is an aminoglycoside antibacterial used, often with other anti-bacterial agents, to treat severe systemic infections due to sensitive Gram-negative and other organisms. Gentamicin is also applied topically for localised infections. Aminoglycosides are taken up into sensitive bacterial cells by an active transport process which is inhibited in anaerobic, acidic, or hyperosmolar environments. Within the cell they bind to the 30S, and to some extent to the 50S, which are subunits of the bacterial ribosome, inhibiting protein synthesis and generating errors in the transcription of the genetic code. The manner in which cell death is brought about is not fully understood and other mechanisms may contribute, including effects on membrane permeability.

Gentamicin included as a sulfate, but doses are expressed in terms of gentamicin only. For many of the infections mentioned, above it is given intramuscularly every 8 hours to provide a total daily dose of 3 to 5 mg/kg. For urinary-tract infections, if renal function is not impaired, 160 mg once daily may be used.

The aminoglycosides can produce irreversible, cumulative ototoxicity. This affects both the cochlea (manifest as hearing loss, initially of higher tones and which, because speech recognition relies greatly on lower frequencies, may not be at first apparent) and the vestibular system (manifest as dizziness or vertigo). The incidence and relative toxicity with different aminoglycosides are a matter of some dispute, but netilmicin is probably less cochleotoxic than gentamicin or tobramycin, and amikacin, more so. Vestibular damage is more common than hearing loss in patients receiving gentamicin. The nephrotoxicity of gentamicin is reported to be largely due to the gentamicin C2 component.

Hypersensitivity reactions have occurred, especially after local use, and cross-sensitivity between aminoglycosides may occur. Very rarely, anaphylactic reactions to gentamicin have occurred.

Reversible nephrotoxicity may occur, and acute renal failure has been reported, often in association with the use of other nephrotoxic drugs.

Insulin

Insulin is a hormone that plays a key role in regulating carbohydrate, protein, and fat metabolism.

In these devices, the manufacturer claims Human insulin recombinant acts as a growth factor that improves embryo development.

The insulin receptor has been confirmed in oocytes, cumulus cells, and in embryos from zygote to blastocyst stage, and binding of insulin promotes glucose uptake, as well as other mechanisms to protect against cell apoptosis/death and promote cell proliferation/growth. Pre-clinical experiments are generally showing beneficial effects of 0.0017 ng/mL - 20 µg/mL insulin in various A.R.T. culture media, with increased oocyte maturation, embryo development to morula/blastocyst stage and increased cell numbers.

The Origio A.R.T. media portfolio is including products that contain 0.005 – 0.5 µg/mL insulin and, generally, it combined with glucose in concentrations of 1 – 5 mM. The products are used for a few minutes up to a maximum of 4 days (when used for culturing of embryos from the 4-8 cell stage until blastocyst stage). Finally, the Origio A.R.T. media products with insulin have been available on the market for 16 – 30+ years, with the product specific CERs documenting state-of-the-art clinical performance and safety without any negative trends.

Heparin

Heparin is added to the media in order to prevent coagulation of blood in the aspirated follicle fluid. Clotted blood in the needle may disturb the isolation of oocytes at the retrieval. Heparin inhibits clotting of blood in vitro and in vivo by enhancing the action of antithrombin III. Antithrombin III, which is present in plasma, inhibits the activity of activated clotting factors including thrombin (factor IIa) and activated factor X (factor Xa). Heparin increases the rate of this inhibition, but in a manner that is dependent on its dose. With normal therapeutic doses, heparin has an inhibitory effect on both thrombin and factor Xa. The inhibition of thrombin blocks the conversion of fibrinogen to fibrin, and the inhibition of factor Xa blocks the conversion of prothrombin to thrombin. The low doses that are given subcutaneously for the prophylaxis of thromboembolism have a selective effect on inhibition of factor Xa. Very high doses are reported to reduce the activity of antithrombin III. Heparin also has some effect on platelet function, inhibits the formation of a stable fibrin clot, and has an antilipidaemic effect.

Heparin is well established as an anticoagulant and antithrombotic and acts mainly by binding to, and enhancing the activity of, antithrombin III. However, it has other actions, and the physiological role of endogenous heparin has not been clearly defined, despite its presence in mast cells, its ability to interact with many proteins and its close structural similarity to heparan sulfate (suleparoid), the ubiquitous cell-surface glycosaminoglycan (GAG). GAGs are ubiquitous compounds naturally found in reproductive tract secretions, including uterine, tubal and follicular fluid and the cumulus oophorous complex, where they may play a role in sperm capacitation and initiation of the acrosome reaction.

Heparin has numerous medical applications, for instance it is used as an injectable anticoagulant during surgery or for treatment of several coagulation disorders. Furthermore, various medical devices such as catheters are coated with heparin to form an inner anticoagulant surface.

Sargramostim (GM-CSF)

GM-CSF (Leukine) or sargramostim is a granulocyte-macrophage colony-stimulating factor (GM-CSF), a haematopoietic growth factor that stimulates the development of white blood cells, particularly granulocytes, macrophages, and monocytes. It is used to treat or prevent neutropenia in patients

receiving myelosuppressive cancer chemotherapy and to reduce the period of neutropenia in patients undergoing bone marrow transplantation. It is also used after bone marrow transplantation when engraftment is delayed or has failed. Sargramostim may be used to mobilise peripheral blood progenitor cells for collection and subsequent use in autologous peripheral blood stem cell transplantation, as well as after transplantation to improve engraftment. It is typically given in a dose of 250 micrograms/m² daily by intravenous infusion over 2 hours.

GM-CSF in relation to human embryo culture to day 3 has been studied thoroughly and a tendency towards a higher number of top-quality embryos (TQE) were observed in the embryos cultured with GM-CSF-supplemented culture media as compared to the control when used in non-therapeutic concentration (2 ng/ml) as ancillary medicinal substance.

The human data indicate that GM-CSF have a physiological role in promoting the development of the human embryo/blastocyst and regulate cell viability. So far, one study based on spare discarded frozen/thawed human cleavage stage embryos has demonstrated improved developmental rates and quality of blastocysts when cultured in GM-CSF-supplemented culture medium (Sjöblom et al. 1999). The proportion of embryos that developed to the blastocyst stage was found higher in the presence of GM-CSF, and in addition the developmental competence of these blastocysts (hatching and attachment to extracellular matrix-coated culture dishes) was also improved by GM-CSF. No negative effect of 2 ng/ml GM-CSF in culture media to day 3 or day 5 has been identified.

2.4.2. Clinical safety of the ancillary medicinal substance incorporated in the medical device

The substances in the Origio A.R.T. media have been on the market for many years and there is no evidence, either from post-marketing report or literature, of harm from these substances to the mother to be or the pregnancy. The main issue with A.R.T. is multiple pregnancy, which is the most important risk factor, and it is unlikely to be related to the A.R.T. media composition. A.R.T. is also associated with long-term perinatal outcomes, including cerebral palsy, autism, neurodevelopmental imprinting disorders, and cancer. A.R.T. media composition and association with cerebral palsy, autism, neurodevelopmental imprinting disorders, and cancer should be further explored, and a literature review should be conducted.

2.4.3. Clinical benefit/risk profile of the ancillary medicinal substance incorporated in the medical device

The risks related to the ancillary substances are generally known from clinical use in human beings with significantly higher doses than used in Origio A.R.T. media. In addition, A.R.T. media are complex mixtures of substances: ancillary medicinal substances, physiological salts, buffers, energy substrates, amino acids and other substances all designed to mimic conditions in vivo or protect from artificially introduced risks and lead to improved outcomes in A.R.T. procedures. Whilst the benefits (live, healthy births) may be a relatively easy measure, the complexity of the solutions and the procedures make the individual benefits and risk harder to discern. A.R.T. media, such as those described in this report, have been on the market for 20-33 years with clinical evaluation playing a key part of the medical device certification process to ensure state-of-the-art.

Gentamicin

With respect to gentamicin, In Vitro Fertilisation followed by embryo transfer bypasses the natural defences in the female genital tract, including antimicrobial secretion of lysozyme and lactoferrin.

Therefore, the presence of microbes may be of greater significance than in vivo and control measures need to be taken to minimize the risk of bacterial contamination.

One approach to reduce microbial contamination of culture media during IVF procedures is the enrichment of culture media with antibiotics. Antibiotics are added to swim-up, preparation, washing or incubation media. Culture media with antibiotics were originally developed by Singh in 1985.

Supplementation of media with antibiotics is routinely carried out using concentrations determined in pioneering studies which evaluated their toxicity in cell culture systems. The recommended concentration for gentamicin is up to 50 mg/litre. These standard concentrations have been adopted in culture media.

Bacteria in culture media have been shown to have a possible detrimental effect on gametes and embryos, resulting in A.R.T. outcomes below the average. Therefore, the benefits of adding antimicrobial agents are clear. Gentamicin has been chosen for its spectrum of activity and stability during processing and throughout the shelf life. The doses used in vitro are significantly lower than clinical doses and so risks of ototoxicity are very low for any residual gentamicin introduced into the mother. There remains an allergic risk, and the label and IFU contain warnings to counteract this. Evidence of (probably reversible) damage to immature nephrons of the foetal kidney associated with use, and eighth cranial nerve damage associated with aminoglycoside exposure in utero, have been reported. There are no controlled data in human pregnancy; however, these effects are not associated with the dose ranges used in IVF. This concentration of gentamicin has been validated for use, in terms of MIC testing and is used in many different A.R.T. media.

Insulin

Side effects of insulin are well known and are related mainly to the parenteral administration in diabetes mellitus. There are no reports of negative effects when using insulin supplemented A.R.T. media for women suffering from Polycystic Ovary Syndrome, hyperinsulinemia, and elevated levels of AMH. A clinical study with parallel culture of sibling oocytes from day 0 until day 5/6 using these media with and without 0.05 µg/mL insulin supplementation, found a statistically significant higher amount of good quality embryos already from day 2 (82% versus 71%; $P < 0.0001$) and through to blastocysts stage (50% versus 27%; $P < 0.0001$), as well as a statistically significant higher overall blastocyst rate in the intervention group (62% versus 46%; $P < 0.0001$). A multivariate logistic regression analysis identified no correlation between cycle characteristics, patient demographics, and clinical variables and the clinical pregnancy rate, except for Body Mass Index (BMI) and insulin supplementation; with a statistically significant higher clinical pregnancy rate in the intervention group (adjusted OR 1.60, 95% CI 1.03–2.49; $P = 0.037$).

Heparin

Similarly to insulin, adverse events for heparin are well documented when given parenterally, but in this case the route of delivery is different. When used to flush intravenous devices, the low levels of heparin that reach the systemic circulation are unlikely to cause adverse effects; however, immune-mediated thrombocytopenia and thrombosis have been reported rarely.

Addition of heparin to human follicular aspiration/flushing/pick-up medium is common to prevent the formation of blood clots in the tubing during the aspiration and/or flushing of oocytes during A.R.T.. In fact, blood clots might block the aspiration needle and hinder the identification and isolation of oocytes for use in A.R.T.. The use of media for oocyte pick-up with or without the inclusion of heparin is the choice of the physician performing the procedure.

Sargramostim (GM-CSF)

GM-CSF may cause transient hypotension and flushing, bone pain and musculoskeletal pain, fever and chills, dyspnoea, rash, fatigue, and gastrointestinal effects when given parenterally. When administered parentally, neutralising anti-drug antibodies have been detected. Anaphylactic reactions, pleural and pericardial effusion, and cardiac arrhythmias have been reported rarely, following parenteral administration to adults. CooperSurgical media only contain GM-CSF in non-therapeutic concentration (2ng/ml) as ancillary medicinal substance.

Therapeutic doses of colony-stimulating factors are known to be fetotoxic in animal studies. GM-CSF in relation to human embryo culture to day 3 has been studied thoroughly. It has been demonstrated that no significant difference in chromosomal constitution (chromosomes 13, 16, 21, 22, X and Y) between embryos cultured in culture media with or without GM-CSF was observed. Moreover, although not significant, a tendency towards a higher number of top-quality embryos (TQE) were observed in the embryos cultured with GM-CSF-supplemented culture media as compared to the control.

The human data indicate that GM-CSF has a physiological role in promoting the development of the human embryo/blastocyst and regulate cell viability. No negative effect of 2 ng/ml GM-CSF in culture media to day 3 or day 5 has been identified.

Overall, the clinical benefit/risk profile of the ancillary medicinal substances incorporated in the medical device may be considered to be positive.

2.4.4. Discussion and conclusion on the clinical evaluation

Gentamicin

The usefulness of gentamicin to prevent contamination of gametes and embryos in culture is agreed. The rationale for the dose used in the media is acceptable. The impact on the safety of clinical outcomes of the addition of Gentamicin to Origio A.R.T. media is difficult to study due to the number of confounding variables that could impact clinical outcome in couples undergoing A.R.T. procedures. The antibiotic has been added to media for over 20 years with no safety signal detected. The in vitro safety and well-established use of Gentamicin in A.R.T. culture media is reassuring. The applicant has included a warning in the labelling concerning the risk of hypersensitivity in subjects with an allergy to gentamicin. In addition, the applicant also confirmed that the ongoing effectiveness of gentamicin contained in the media is monitored through user feedback, PSURs and post-market complaints.

The benefit-risk of the addition of the gentamicin to Origio A.R.T. media can be considered as positive.

Heparin

Heparin is added to the media in order to prevent coagulation of blood in the aspirated follicle fluid. Clotted blood in the needle may disturb the isolation of oocytes at the retrieval. The usefulness of Heparin in the media for this indication is acceptable. Heparin is added to flushing media only and is not added to embryo culture or to embryo transfer media. There is contact of the gametes retrieved with heparin. Biocompatibility, genotoxicity and cytotoxicity tests have been conducted. Heparin has been added to A.R.T. media since 2008 with no safety adverse events associated with its use reported. The dose of heparin is small and less than a therapeutic dose of heparin. There is little or no risk of systemic absorption in the patient treated. The estimated systemic exposure in women is not considered clinically relevant. Moreover, there have been no adverse events or reactions related to heparin use in the media reported.

The benefit-risk of the addition of Heparin to Origio A.R.T. media can be considered as positive.

Insulin

Insulin is added to the media as recombinant Human insulin acts as a growth factor that improves embryo development. The data presented to support the usefulness of insulin in Origio A.R.T. media includes preclinical data, clinical data, live birth data and literature. Interpretation of the clinical data is limited by the number of confounding factors including patient, in vitro, laboratory and medical factors.

The insulin receptor has been confirmed in oocytes, cumulus cells and in embryos from zygote to blastocyst stage and binding of insulin promotes glucose uptake, as well as mechanisms to protect against cell apoptosis/death and promote cell proliferation/growth. Pre-clinical experiments are generally showing beneficial effects of 0.0017 ng/mL - 20 µg/mL insulin in various A.R.T. culture media, with increased oocyte maturation, embryo development to morula/blastocyst stage and increased cell numbers. The usefulness of insulin for this purpose, i.e. to promote embryo development, could be accepted.

The studies of Fawzy, Liu et al and the meta-analysis of Sha et al, are reassuring about the laboratory and clinical outcome in pregnancies conceived using media containing insulin. Whilst the addition of insulin may be considered useful in improving embryo growth, some concerns relating to safety have also been reported, as outlined in Chronopolou and Harper - Human Reproduction update vol 21 39-55 2015. For instance, even though development rate is used as an end-point to evaluate culture conditions, faster is not necessarily better. The faster developing mouse embryos presented loss of genomic imprinting raising safety issues for media that promote fast growth (Market Velker et al., 2012). Also, growth factors (GFs), if not well regulated, can have adverse effects on development and have been associated with large offspring syndrome (LOS) (Young et al., 2001). Furthermore, embryos produce GFs creating a closed loop of autocrine signaling (O'Neill, 2008) and the addition of a single GF could disturb the balance (Menezo et al., 2013). Finally, after embryo culture with LIF, IGF-1 and HB-EGF, distinct deviations were noticed in the expression of various cell fate genes (Kimber et al., 2008).

However, in more than 34-years of experience of human embryo culture using A.R.T. media with insulin in concentrations of 0.005 – 0.5 µg/mL, there are no reports of insulin pathways that induce epigenetic changes or alter gene expression in the developing embryo which could impact the birth weight of offspring conceived in the media containing insulin. There is also no clinical evidence of a detrimental effect on developmental or cognitive outcomes on children conceived with embryos or gametes using the media. Therefore, the concerns are not considered relevant in the context this application.

In addition, the dose of insulin to woman undergoing A.R.T. treatment cycles due to exposure to the culture media is extremely small and unlikely to have a clinical impact in women with diabetes or impaired insulin metabolism. The estimated systemic exposure in women is not considered clinically relevant. Moreover, there have been no adverse events or reactions related to insulin use in the media reported.

The benefit-risk of the addition of insulin to Origio A.R.T. media can be considered as positive.

Sargramostim (GM-CSF)

GM-CSF is added to Origio A.R.T. media to promote the development of the human embryo/blastocyst and regulate cell viability. No negative effect of 2 ng/ml GM-CSF in culture media to day 3 or day 5 has been identified *in vitro*.

The safety and effectiveness of human embryo culture media supplemented with GM-CSF versus culture media not supplemented with GM-CSF in women or couples undergoing assisted reproduction was subject to a Cochrane review in 2020. The review included RCTs comparing GM-CSF (including granulocyte colony-stimulating factor)-supplemented embryo culture media versus any other non-GM-CSF-supplemented embryo culture media (control) in women undergoing assisted reproduction.

The authors concluded that, due to very low to low quality of evidence, it is uncertain whether GM-CSF is any more or less effective than culture media not supplemented with GM-CSF for clinical outcomes that reflect effectiveness and safety. It is important that independent information on the available evidence is made accessible to those considering using GM-CSF-supplemented culture media.

The usefulness of GM-CSF in promoting embryo development could be acceptable but the evidence that this leads to a positive effect on pregnancy rates or outcome is not robust.

The Cochrane review did not conclude that addition of GM-CSF led to any safety concerns, but the review concluded that the data was not of sufficient quality to rule out any harmful effects. Although more data would be required conclude on this aspect, the clinical data provided by the applicant from their study and vigilance data is reassuring.

According to the data provided from literature and published studies looking at the in vitro and in vivo use of GM-CSF and the effects at a cellular level, there is no evidence of an epigenetic effect on embryos cultured in GM-CSF containing media. However, it is to be noted that the clinical data presented relates to birthweight, gestational age, congenital abnormality and ploidy, and developmental and cognitive outcomes are not reported.

Moreover, in more than 11-years of experience of human embryo culture using A.R.T. media with 2 ng/mL GM-CSF, there are no reports of GM-CSF pathways that induce epigenetic changes or alter gene expression in the developing embryo which could impact the birth weight of offspring conceived in the media containing GM-CSF. There is also no clinical evidence of a detrimental effect on developmental or cognitive outcomes on children conceived with embryos or gametes using the media. Thus, the concerns are not considered relevant in the context this application. In addition, the notified body has requested continuous proactive post-market clinical follow-up. The planned proactive clinical follow-up and ongoing vigilance should provide further clinical data on these outcomes.

The claims from marketing information that GM-CSF has a positive effect on pregnancy rates are not supported by the available evidence presented here; further well designed, properly powered RCTs are needed to lend certainty to the evidence. In this regard, the applicant clarified that a clinical benefit is not claimed, and that physicians and embryologists are only informed about the in-vivo effects. They are informed about the potential benefit to poor prognosis patient, considering that a clinical outcome benefit to poor prognosis patients has not been demonstrated.

Additional clinical data to determine the long-term outcome of children conceived using the enriched media is collected directly from their users. In addition, there are ongoing PMCF literature and vigilance checks to monitor for signals of adverse clinical outcomes using the media, which is supported.

The benefit-risk of the addition of GM-CSF to Origio A.R.T. media can be considered as positive.

Conclusion

Overall, the benefit-risk for the usefulness and safety of the addition of the medicinal products into Origio A.R.T. media can be considered positive.

2.5. Overall conclusions

The known risks of these ancillary medicinal substances are related to their administration in adult medicine, where the route of administration is different and the doses significantly higher, compared to their administration as part of A.R.T. media. The evidence for the safety and effectiveness for the ancillary substances are a combination of in vitro data, animal studies and clinical studies. Measures such as fertilisation, cleavage, embryo quality, implantation, and pregnancy have been evaluated and shown to be positive for the media containing these substances. The substances have been on the

market for many years and there is no evidence from post-market reports or literature of harm from these substances to the mother to be, or the pregnancy. The usefulness of these substances in A.R.T. media is therefore confirmed.

The benefit risk can be considered positive from a quality, non-clinical and clinical perspective.

2.6. Recommendation

Based on the CHMP review of data submitted, the CHMP considered by consensus that the quality and safety including the benefit risk profile of gentamicin sulfate / sargramostim (GM-CSF)/ heparin sodium / insulin human used as ancillary medicinal substance(s) in the Origio A.R.T. Media was favourable and therefore granted a positive opinion in the consultation procedure.