6 October 2021
EMA/CHMP/499319/2021
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: **Vitrolife IVF media**

Ancillary medicinal substance: recombinant human albumin solution

Procedure No.: EMEA/H/D/004693/0000

Applicant: DNV Product Assurance AS*

* The notified body changed name from DNV Nemko Presafe AS to DNV Product Assurance AS between the adoption of the opinion and the publication of this report.

Administrative information

Invented name of medical device:	Vitrolife IVF media
INN (or common name) of the ancillary medicinal substance:	recombinant human albumin solution (Recombumin® Prime)
Applicant for medical device CE certification:	Vitrolife Sweden AB
Notified body:	DNV Product Assurance AS Veritasveien 3 1363 Hovik Norway
Applied intended purpose of the device:	In Vitro Fertilisation solutions designed to provide a stable liquid environment for oocytes and embryos outside the human body.
Intended purpose of the ancillary medicinal substance in the device:	To facilitate gamete and embryo manipulation in vitro by acting as a surfactant, maintaining embryo physiology and metabolism and chelating potential toxins during this process
Pharmaceutical form(s) and strength(s) of the ancillary medicinal substance:	Extracorporeal solution 20 mg/ml

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List of abbreviations

Ag	Antigen
ART	Assisted Reproductive Technology
BSE	bovine spongiform encephalopathy
CARTR Plus	Canadian Assisted Reproductive Technologies Register Plus
cDNA	complementary DNA
CHMP	Committee for Medicinal Products for Human Use
EC	European Commission
ET	embryo transfer
FDA	U. S. Food and Drug Administration
HAV	Hepatitis A Virus
HBC	Hepatitis B Virus core Antigen
HbsAg	Hepatitis B Virus surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HED	Human Equivalent Dose
HIMS	Heat inactivated maternal serum
HIV-1	Human Immunodeficiency Virus 1
HPLC	High Performance Liquid Chromatography
HSA	Human Serum Albumin
HTF	Human tubal fluid
HTLV	Humanes T-lymphotropes Virus
ICSI	Intracytoplasmic sperm injection
IM	Intramuscular
IPC	In-process control
IUI	intra-uterine insemination
IVF	In-Vitro Fertilisation
MA	Marketing Authorization
MCB	Master Cell Bank
MDM	medical Device Manufacturer
MEA	Mouse Embryo Assay
NF	National Formulary

NOAEL	no-observed-adverse-effect-level
PGD	Preimplantation genetic diagnosis
Ph. Eur.	European Pharmacopoeia
PPV	Porcine Parvovirus
R&D	Research and development
rHA	recombinant human albumin
S(m)PC	Summary of Product Characteristics
SC	subcutaneous
TSE	Transmissible spongiform encephalopathy
USP	United States Pharmacopeia
WCB	Working Cell Bank

1. Background information on the procedure

1.1. Submission of the dossier

The notified body DNV) Nemko Presafe AS* submitted to the European Medicines Agency (EMA) on 21 December 2016 an application for consultation on recombinant human albumin solution incorporated as ancillary medicinal substance in the medical device Vitrolife IVF 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 and the evaluation teams were:

Rapporteur: Daniela Melchiorri Co-Rapporteur: Peter Kiely

- The application was received by the EMA on 21 December 2016.
- The procedure started on 19 January 2017.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 10 April 2017. The Co-Rapporteur's first assessment report was circulated to all CHMP members on 5 April 2017.
- During the meeting on 18 May 2017, the CHMP agreed on the consolidated List of Questions to be sent to the applicant.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 29 August 2017.
- The Rapporteurs circulated the joint assessment report on the applicant’s responses to the list of questions to all CHMP members on 13 October 2017.
- During the CHMP meeting on 09 November 2017, the CHMP agreed on a list of outstanding issues to be sent to the applicant.
- The applicant submitted the responses to the CHMP consolidated list of questions on 18 December 2017.
- The Rapporteurs circulated the joint assessment report on the applicant’s responses to the list of outstanding issues to all CHMP members on 10 January 2018.
- During the meeting on 25 January 2018 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 recombinant human albumin solution as ancillary medicinal substance used in Vitrolife IVF media.

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1.3. Manufacturers

Manufacturer of the active substance used as ancillary medicinal substance

Albumedix Ltd.
Mabel Street
The Meadows
Nottingham NG2 3ED
United Kingdom

Manufacturer of the finished product used as ancillary medicinal substance

Not applicable.

Manufacturer responsible for batch release

Albumedix Ltd.
Mabel Street
The Meadows
Nottingham NG2 3ED
United Kingdom

Manufacturer responsible for import and batch release in the European Economic Area

Not applicable.

Manufacturer of the medical device

Vitrolife Sweden AB
Gustaf Werners gata 2
SE-421 34 Västra Frölunda
Sweden

1.4. 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:

Area[1]	**Description**
Clinical safety	The Manufacturer is asked to continue monitoring the outcome of pregnancies conceived using rHA media, by monitoring the literature and where possible by using the registries (e.g. consortia from the European Society of Reproductive Medicine (ESHRE) to gather data on ART outcome) and through reporting systems at national level.
Clinical safety	The Manufacturer should monitor and collect hypersensitivity reactions as part of their obligations for safety reporting for the Vitrolife rHA media.

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

2. Scientific overview and discussion

2.1. General information

Following Vitrolife Sweden AB submission application to the Norwegian Notified Body Det Norske Veritas (DNV) Product Assurance AS, DNV has requested the CHMP a scientific opinion (consultation) in regarding of the quality, usefulness and safety of recombinant Human Albumin (rHA) contained as ancillary substance in Vitrolife In Vitro Fertilisation (IVF) solutions/media. The IVF solutions are considered medical devices under Directive 93/42/EEC as amended.

The role of albumin in facilitating gamete and embryo manipulation *in vitro* by acting as a surfactant, in maintaining embryo physiology and metabolism, in chelating potential toxins during this process, and in acting as a protein supplement and micronutrient, is known. Significantly, albumin is the most abundant protein in the female reproductive tract.

Vitrolife IVF media contain rHA, produced in *Saccharomyces cerevisiae*, with an ancillary role (non-therapeutic use). The principle intended action of the whole medical device is to provide a stable liquid environment for oocytes and embryos outside the human body. While rHA has the potential to reduce the risk of transfer of human plasma-derived ligands and impurities (viral or prion contamination) vs Human Serum Albumin (HSA), the product may be contaminated with residues from the host organism, yeast proteins or show neoepitopes resulting in potentially immunotoxic effects. For a medical device like IVF media the quality, non-clinical and clinical characterisation regarding the ancillary substance, is aimed to verify both safety/tolerability for the mother to be and for the gametes and embryo.

Vitrolife IVF media are used sequentially in different stages of IVF and Assisted Reproductive Technologies (ART) treatment (embryo transfer, to culture embryos, to preserve sperms and embryo during IVF, for handling and manipulation of oocytes and embryos). During the preimplantation period, a sequential series of media are often used to accommodate changes in physiology and metabolism of the embryo from a 1-cell zygote to the differentiated blastocyst stage.

G-MM contains, the highest amount of rHA (20% w/v) among the device solutions.

G-MM is used to supplement the following media: G-1, G-2, G-IVF, G-MOPS, G-PGD, G-ThawKit Blast, by the end user.

rHA pre-supplemented devices are: Embryoglue and ICSI.

The rHA in G-MM media is claimed to be > 99% pure and is structurally identical to the naturally occurring HSA. Its molecular weight is 66438 Daltons.

The G Series media also have the same base composition (ionic composition, osmolality, pH), thus reducing the potential stress that the embryo is exposed to when media is changed during culture.

The current G Series were previously marketed as, and is identical to, the G5 Series launched in 2007 which is an upgraded version of the former GIII Series launched in 2002. Some changes in composition (not related to rHA) between GIII Series and current G Series were performed.

G-MM has been on the market since 2002 and some of the products that are to be used after supplementation with rHA have been on the market since the late 1990s however no later than 2005 (ICSI when supplemented with rHA).

2.2. Quality documentation

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

Introduction

Vitrolife IVF media consist of 8 solutions and a kit (G-ThawKit Blast) of 4 physiologically balanced salt solutions, containing a variety of amino acids, vitamins, glucose and gentamicin. Vitrolife IVF media are used sequentially at various stages of IVF.

Recombumin Prime 20% (w/v) is used as a source of albumin for Vitrolife IVF media and acts as an ancillary substance. Recombumin Prime is formulated with water for injection (USP/Ph. Eur.), sodium chloride (USP/Ph. Eur.), octanoate and polysorbate 80 (NF/Ph. Eur.) to give a final target concentration of 200 mg/mL total protein (20% w/v). The human albumin is produced in Saccharomyces cerevisiae by Albumedix Limited, UK, which holds appropriate GMP certification. The primary packaging is a 50 mL moulded clear type II glass vial sealed with a siliconized chlorobutyl rubber stopper. The primary packaging is secured with aluminium overseals with flip-off plastic caps.

Active substance

General information

Recombumin Prime is a clear to amber solution containing the active ingredient yeast derived rHA at a concentration of 20% (w/v), which meets the relevant requirements of the HSA (Ph. Eur.) and all of the rHA National Formulary (NF) monograph as published by the USP.

rHA has a molecular weight of 66,438 Daltons. Comparison of amino acid composition and peptide maps of rHA with an in-house working standard prepared from commercially available HSA verified the structural identity of the polypeptide chains. Both the N-terminal and C-terminal sequences of rHA were shown to comply with the known sequence of HSA. Like HSA, rHA consists of 585 amino acids and contains a single tryptophan (residue 214), one free thiol (Cys at residue 34), and 17 disulfide (Cys-Cys) bridges.

Manufacture, process controls and characterisation

Description of manufacturing process and process controls

The manufacturing process of Recombumin Prime has been adequately described. Main steps are fermentation in *S. cerevisiae*, harvest, purification through a series of chromatographic and ultrafiltration steps, formulation and filling. The manufacturing process is stated to be continuous and intermediates are defined. In-process hold times are appropriately justified.

The manufacturing process has been sufficiently described and in-process controls (IPCs) are adequately set to control the process.

The container closure components comply with Ph. Eur. requirements. An overview of the acceptance criteria is provided for the vials, stoppers, and overseal. The specifications for the container closure components include visual appearance, dimensions and additionally penetrability, fragmentation and physicochemical characteristics of the stoppers (specific gravity, ash, and elasticity) and tests for the vials (powdered glass test, water attack at 121°C, and hydrolytic resistance).

Control of materials

Sufficient information on raw materials used in the active substance manufacturing process has been submitted. No human or animal derived raw materials are used in the active substance manufacturing process and acceptable documents have been provided for raw materials of biological origin used in the establishment of the cell substrate.

A list of chemical raw materials is provided. With a few justified exceptions, all materials comply with the Ph. Eur. Also, the chromatographic matrices, ultrafiltration cassettes, and filters used in the manufacturing process are listed. A two-tiered cell banking system is used, and sufficient information is provided regarding testing of Master Cell Bank (MCB) and Working Cell Bank (WCB) and release of future WCBs. Genetic stability has been demonstrated for cells at and beyond the limit of cell age.

WCBs are routinely produced from the MCB approximately biennially. Specifications are defined for the MCB and WCB and a stability monitoring program is in place.

Controls of critical steps and intermediates

A comprehensive overview of critical IPCs and critical in-process tests performed throughout the Recombumin Prime manufacturing process is given. Acceptable information has been provided on the control system in place to monitor and control the active substance manufacturing process with regard to critical, as well as non-critical operational parameters and in-process tests. Actions taken if limits are exceeded are specified.

Critical process controls are defined as those controls that have a high potential impact on product safety or quality. All process controls of the Recombumin Prime manufacturing process have been analysed to identify the critical process controls. The critical process controls that were identified are mainly process parameters and a limited number of IPCs. With the exception of a control for microbial purity at the end of fermentation, IPCs for bioburden control during the manufacturing process are not included as critical process controls.

Process validation

The active substance manufacturing process has been adequately validated. Consistency in production has been shown on full scale commercial batches. All acceptance criteria for the critical process parameters and likewise acceptance criteria for the in-process tests are fulfilled demonstrating that the purification process consistently produces the active substance of reproducible quality that complies with the predetermined specification.

The manufacturing process of Recombumin Prime was first validated in 1999. Between 2000 and 2003 three further process qualifications were performed to support changes to the manufacturing process, and between 2003 and 2010 three process qualifications were performed to introduce new contract manufacturers for the filling operations. The latest process performance qualification was performed to support changes in the fermentation process. A brief summary of all validation exercises is provided. The validation reports of the latest exercise are available and are considered satisfactory.

To demonstrate that the filling process is appropriately validated, summaries of media fill studies, mixing validation, filler qualification studies, and transport qualification are provided.

Manufacturing process development

Development of Recombumin Prime has taken place over a twenty-year period via two previous formulations. During development the yeast organism and the manufacturing process were modified to significantly reduce the level of yeast-derived impurities resulting in the current Recombumin Prime product.

The formulation of Recombumin Prime was developed to produce a liquid formulation of rHA that would have equivalent stability properties to HSA. As Recombumin Prime is used in the further manufacture of other pharmaceutical products, its rHA concentration is not a critical factor. Regarding stabiliser selection, firstly octanoate is used because investigations showed a superior effect when compared to a combination of N-acetyltryptophan with octanoate or N-acetyltryptophan alone. Secondly, Polysorbate 80 is used to prevent the formation of particles and was chosen as an additional stabilizer as it has a precedence of use in parenteral products.

Characterisation

The active substance has been sufficiently characterised by physicochemical methods, revealing that the rHA has the expected structure and equivalence with HSA was demonstrated. It is noted that the electrospray mass spectra mainly show unmodified monomer in rHA, whereas peaks of monomer lacking N-terminal Asp-Ala and monomer + blocked free thiol are more pronounced in HSA. Gel permeation HPLC showed a small polymer peak in HSA, but not in rHA. The small dimer peak was a bit more pronounced in rHA compared to HSA. The observed minor differences are not relevant in view of the intended use of the product.

Specification

The active substance specification includes appropriate tests and limits. The product- and process-related impurities are appropriately evaluated.

Analytical methods

The applicant has provided brief descriptions of the methods used for testing the active substance. No Ph. Eur. monograph for rHA exists. However, as the protein is equivalent to HSA, rHA complies with relevant provisions from Ph. Eur. 0255 human albumin solution. The Applicant has provided the requested information on the compendial methods used. Equivalence to Ph. Eur. methods is sufficiently demonstrated and validation reports are provided.

Batch analysis

Batch analysis data (n=23) of the active substance were provided. The results are within the specifications and confirm consistency of the manufacturing process.

Reference standard

Information is provided on rHA reference standards derived from released Recombumin Prime batches. The reference standards are stated to be used in the SDS-PAGE, HPLC, ConA, and mass spectrometry assays.

Stability

The stability results indicate that the active substance is sufficiently stable and justify the proposed shelf life in the proposed container.

Real time, real condition stability data on 3 Recombumin Prime production batches were utilized for the overall stability program. All batches were tested at 5 ± 3°C in horizontal position, at 25 ± 2°C in horizontal and upright positions and under accelerated condition at 40 ± 2°C in horizontal position.

In addition, the container closure system has proved to be integral at all test temperatures and orientations. The 40 ± 2°C study was an accelerated study designed to generate additional stability data and will not be considered as a recommended storage temperature.

A shelf life of 60 months at 5 ± 3°C, and shipment at 25 ± 2°C up to 7 days are claimed by the Applicant and supported by data.

In accordance with EU GMP guidelines[i], any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

Finished product

Recombumin Prime is an active pharmaceutical ingredient and not a formulated medicinal product and consequently all information on Recombumin Prime is provided. The absence of Finished Product section was found acceptable.

Adventitious agents' safety

No human- or animal-derived materials are used in the production of Recombumin Prime. Transmissible spongiform encephalopathy (TSE) statements from the manufacturer/vendor of the chemical raw materials have been provided. In addition, TSE statements for the chromatography matrices, and container closure system have been included in the documentation.

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

The media G-MM, ICSI and EmbryoGlue contain rHA when delivered to the end user. The other media are supplemented by the customer with rHA using G-MM.

Description of method of manufacture

G-MM, ICSI, EmbryoGlue: After approved testing, the Recombumin Prime bottles are transferred to the clean room, opened aseptically, and pipetted into a container to obtain the correct amount. After all other chemical components are added to the bulk solution, rHA is added by pouring the rHA directly from the container into the bulk solution. The bulk solution is then stirred before in-process sampling.

G-1, G-2, G-IVF, G-MOPS, G-PGD: Recombumin Prime is not added during manufacturing. Instead, the customer supplements the media with rHA (using the product G-MM) prior to use according to the instructions for use.

Control of starting materials

Control of Recombumin Prime mainly relies on the certificate of the manufacturer. A limited number of tests are performed: bacterial endotoxins, Mouse Embryo Assay, and ammonium ions. Detailed method descriptions are provided.

Control test carried out at intermediate stages of the manufacturing process of the medical device

No control tests for Recombumin Prime are performed at intermediate stages of the manufacturing process.

Final control tests of the ancillary medicinal substance or the ancillary human blood derivative in the medical device.

The specifications for the final IVF media do not contain specific control tests for Recombumin Prime. A final functional Assay is performed for each lot to verify that the media provide a relevant physiological environment to the embryo.

Stability

Statements on stability of the different IVF media are provided and considered acceptable. Stability data are provided for the different media stored at 2-8°C for various time periods (25 weeks to 36 months). The parameter linked to degradation of rHA, formation of ammonium, showed no, or only slight increase during storage. The functional test showed no detectable negative effects.

2.2.3. Discussion on chemical and pharmaceutical aspects

Information on development, manufacture and control of the ancillary medicinal substance and on the ancillary medicinal substance as incorporated in the medical device has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that Vitrolife IVF media should have a satisfactory and uniform performance in clinical use.

No human- or animal-derived materials are used in the production of Recombumin Prime. Since Recombumin prime is produced in *S. cerevisiae*, the information about viral safety is not relevant. TSE statements from the manufacturer/vendor of the chemical raw materials have been provided. In addition, TSE statements for the chromatography matrices and container closure system have been included in the documentation.

2.2.4. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of ancillary rHA itself and as incorporated in the medical device, is considered to be

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.

2.2.5. Recommendation for future quality development

Not applicable.

2.3. Non-clinical documentation

For rHA, no new pharmacodynamic (PD) studies were performed but reference to published pre-clinical studies on the use of rHA as supplement in different IVF media, including some of Vitrolife media, in animal embryos was made in non-clinical and clinical parts of the dossier. Results from two pharmacokinetic (PK) studies in rabbits (acute IV and repeated-dose SC) were provided with the primary objective to demonstrate equivalence in respect to absorption, plasma distribution and clearance of Recombumin Prime and HSA (Bayer Human-Albumin N 20%) when used as therapeutic agents. Similarly, toxicity studies (single IV, repeated-dose IV, SC, IM, immunotoxicity in guinea pig and rabbit) have been performed on rHA.

A battery of studies including acute IV toxicity in rat and mouse and in vitro and in vivo genotoxicity testing has been performed on the potential process contaminants of rHA.

As regards the Vitrolife finished media, biocompatibility studies (i.e., Iso Vaginal Irritation Study in the Rabbit, Sensitisation Study in the Guinea Pig, Cytotoxicity Study Using the Agarose Overlay Method) have been performed only on those Vitrolife media which are intended to be in contact with the mother to be.

Pharmacodynamics

Since the role of albumin in facilitating gamete and embryo manipulation in vitro by acting as a surfactant, in maintaining embryo physiology and metabolism and chelating potential toxins during this process, is known and Vitrolife media will be used as non-therapeutic agents, no non-clinical PD studies were performed. Moreover, albumin is the most abundant protein in the female reproductive tract.

Published studies on the use of rHA as supplement in IVF media in manipulation of animal gametes/embryos were provided.

Pharmacokinetics

No PK assessment was performed on the final Vitrolife media nor on rHA since the amount likely absorbed following intravaginal administration is minimal.

Toxicity

A number of in vivo toxicity studies comparing safety and tolerability of Recombumin Prime vs HSA were carried out for use of rHA in further manufacturing purposes (e.g. as a component in other therapeutic products) and thus poorly relevant for the intended use of Vitrolife media.

Rat **single IV doses** up to 4000 mg/kg, and **repeated doses** over 5 days up to 2000 mg/kg/day (IV), and of 100 mg/kg/day (SC, IM), showed no relevant toxicity signs or minimal evidence of toxicity

by rHA. Plasma levels of Recombumin Prime in repeated-dose studies showed no accumulation post-dose following IV administration, while a slightly lower exposure was observed following SC administration 5 days the last dose, reasonably due to a reduced absorption process. The higher plasma levels observed 5 days the last dose following IM administration is considered by the Applicant likely due to animal variability.

If we consider the SC/IM route of administration the most representative for the Vitrolife use (in which an absorption phase might occur), 100 mg/kg/day for rats for both SC/IM can be considered the lowest NOAEL value: this is a conservative approach since 100 mg/kg was used as single dose in repeated-dose toxicity studies where no dose-range was tested, thus there is the possibility that higher doses would be non-toxic as well (as shown in IV acute and dose-repeated toxicity studies). The correspondent Human Equivalent Dose (HED, assuming 60 Kg human) calculated by the CHMP is 16.1 mg/kg that is a high multiple (107-fold) of Recombumin Prime dose expected to be in contact (provided absorption) with mother to be. Moreover, the use of the Vitrolife device is not expected to be repetitive.

rHA showed a good **local tolerance** since no signs of local reactions at injection sites (IV, SC, IM) after single and repeated doses were observed in rats and in rabbits.

No **genotoxicity** characterisation was performed on rHA since it is expected that proteins would not interact directly with DNA/chromosomal material (ICH S6 Guideline).

Long-term **carcinogenicity** and **reproductive/developmental** toxicity studies were not performed since Recombumin Prime, like HSA, is a human protein and therefore is immunogenic in animal species. This is demonstrated by the fact that all the animals in both rHA and HSA treatment groups developed antibodies against human albumin in a repeated-dose toxicity study in rabbit in which SC doses of 80 mg/kg of either rHA or HSA were administered for 5 consecutive days. However, the immunological response of Recombumin Prime in humans is considered assessed since the same rHA is present in trace in 2 vaccines approved in Europe, one of two M-M RVAXPRO (measles, mumps, rubella) contains rHA since 2006.

Studies in **juvenile animals** are considered not relevant for the intended use of the media containing Recombumin Prime.

While rHA has the potential to reduce the risk of transfer of human plasma-derived ligands and impurities (viral or prion contamination) vs HSA, the product may be contaminated with residues from the host organism, Saccharomyces cerevisiae or showed neoepitopes resulting potentially immunotoxic. As regards the potential **immunogenicity/antigenicity** of Recombinum Prime against the yeast-antigen, no passive cutaneous anaphylaxis reaction was observed after IV challenge with Recombumin Prime in guinea pigs intracutaneously sensitized against yeast-antigen preparations derived from yeast mock strains of S. cerevisiae and only 1 of 8 rabbits SC administered with Recombumin Prime for 5 consecutive days developed IgG antibodies against human albumin compared with 3 of 8 rabbits administered with HSA in the same study. In addition, a neoantigenicity study in rabbits that was performed to determine whether the rHA contained epitopes not present on HSA showed no evidence of neoepitopes to be present in Recombumin Prime.

It is noted that there was a great variability in the Aluminium content in the 2 Recombumin Prime batches used in non-clinical studies although both below the specification limit. Since most of the studies (except IV repeated-dose study) were performed with the batch with the higher Aluminium content (worst case scenario), toxicity results can be considered adequate also in terms of any possible Aluminium related-toxicity.

As regards Recombumin Prime **process-related impurities** (four chromatography column leachates: L1, L2, L3 and L4 and antifoam agent used in the fermenter) their potential toxicity was assessed by **acute IV toxicity** studies in rat and mouse. No dose-repeated toxicity studies were carried out with Recombumin Prime process-related impurities. Of the four column leachates, three (L1, L2 and L4) were non-toxic in mice and rats at IV doses up to 50 mg/kg b.w. The fourth (L3) had some limited toxicity in mice and rats. Nevertheless, it was concluded that, given the extremely low or unmeasurable amounts of these agents in the Recombumin Prime final product, the leachates do not represent a systemic toxic risk for human subjects. The wide safety margins calculated by the Manufacturer are considered reassuring also in light of the conservative approach of NOAEL values. In conclusion, considering the very low amount of impurities found in rHA, the low amount of rHA transferred to mother to be, the null or minimal absorption of rHA by the mother to be, the use of the Vitrolife device (assumed not to be repetitive), the potential overall systemic toxicity associated with the presence of rHA process-related impurities is considered to be negligible.

As a potential process-related contaminant, the **antifoam** was also included in a range of toxicity and genotoxicity/mutagenicity tests. The antifoam did not show any evidence of either acute toxicity (IV rat study): given the very large removal of the antifoam agent by the downstream purification process and the extremely large safety margin that was demonstrated between maximum possible levels in Recombumin Prime and the levels shown to be safe in the toxicity studies, it was concluded that the antifoam does not represent a systemic toxic risk for human subjects.

The column leachates were also characterised for their potential **genotoxicity**. One column leachate (L1) was not mutagenic or clastogenic in the in vitro assays (Ames test and chromosome aberration test in Chinese hamster ovary cells). A second leachate (L3) was mutagenic in the Ames test and a third leachate was clastogenic (L2) in the chromosome aberration test in Chinese hamster ovary cells at the highest tested doses. However, all three of these leachates (L1, L2 and L3) were not clastogenic in the higher-ranked in vivo mouse micronucleus assay (bone marrow) at IV doses as high as 50 mg/kg administered on two consecutive days. The fourth column leachate (L4) resulted negative in the Ames test, chromosome aberration test in Chinese hamster ovary cells and in vivo mouse micronucleus assay.

The antifoam agent resulted negative at both Ames test and in vitro chromosome aberration test in Chinese hamster ovary cells, while it was not tested in the in vivo mouse micronucleus assay.

Although in vivo mouse micronucleus test is considered more valuable than in vitro assays since it allows detecting potential aneuploidy (reduced chromosome number), the main limitation is the lack of demonstration of in vivo exposure (no TK determination). Indeed, as per ICH S2 guideline "when adequate exposure cannot be achieved (e.g. with compounds showing very poor target tissue availability), conventional in vivo genotoxicity tests have little value.

Although, the clastogenic potential of one of the column leachates (L2) in mammalian cells (in vitro) and the potential ability of another of the column leachates (L3) to induce frameshift mutations (Ames test) cannot be considered superseded by the negative results obtained in the in vivo study, considering the low specification limits for these 2 impurities in Recombumin Prime and the low amount of the Recombinum Prime final product in contact with the mother to be, the Manufacturer conclusion that the 2 impurities (L2 and L3) do not represent a genotoxic risk to humans (mother to be), can be considered acceptable and the potential genotoxic effect on oocytes, sperms and embryo can be reasonably excluded. The same consideration also applies to antifoam agent. In addition, as outlined in ICH M7 guideline, the Threshold of toxicological concern (TTC)-concept can be used for estimating acceptable daily intake levels for DNA reactive (mutagenic at the Ames test) impurities. For a duration of exposure up to 1 month, a daily intake of 120 ug/day of a mutagenic impurity can be acceptable. Further, a daily intake of 20 ug/day of a mutagenic impurity is acceptable for up to 1 year. As worst-case scenario, this means that 1 year of daily exposure to 40000 ml (40 L) of media

containing G-MM at the highest concentration and with the mutagenic leachate (L3), is considered to pose negligible carcinogenic risk. Although the exact duration of exposure of gametes and embryos to these solutions is unclear, these estimations are sufficiently reassuring to conclude that the solution with highest rHA concentration can be accepted. The estimated levels of the clastogenic leachate (L2) in G-MM is $>10^5$-fold lower than non-clastogenic effects.

These data taken together are sufficiently reassuring to conclude that there is no cause for concern with rHA.

The Manufacturer provided full study reports of 3 ISO **biocompatibility** assays which are considered relevant for testing the safety of those Vitrolife media intended to be in contact with the mother to be (Embryoglue, G-1, G-2, G-MOPS). Results showed no cytotoxicity in vitro, absence to minimal irritancy to vagina mucosal tissue of the rabbit, and no delayed dermal contact sensitisation (erythema, oedema) in the guinea pig. The lack of "in vitro cytotoxicity study" for those media not intended to be in contact with mother to be is considered acceptable since all media underwent "one-cell mouse embryo assay" and "human sperm motility recovery assay" (for ICSI) as product specification.

It is noted that biocompatibility studies were performed before some compositional changes (not involving Recombumin Prime) were introduced. The impact of these changes has been assessed from a biocompatibility standpoint. For what concerns citrate and hyaluronan, biocompatibility testing has been performed on a Vitrolife IVF media, EmbryoGlue, containing the same concentration of citrate and a concentration of hyaluronic acid which was 3-fold higher than the concentration in G-2. In relation to Gentamicin, biocompatibility testing was done on ASP, an old Vitrolife product containing a concentration of gentamicin 5-fold higher than the concentration in G-2. Biocompatibility testing performed on a Vitrolife IVF media old product (ASP), containing a concentration of gentamicin 5-fold higher than the concentration in EmbryoGlue, is thus relevant.

According to Annex A of ISO 10993-1:2009, genotoxicity is a relevant hazard for consideration in a biological safety evaluation. While the risk for mother to be is clearly included, whether this applies to gametes and embryos exposed to potential genotoxic media is not directly evident, anyhow although the exact duration of exposure of gametes and embryos to ART solutions is unclear, this is expected to be limited. Moreover, genotoxicity assessment of the rHA impurities did not reveal particular safety concerns both to mother-to-be and gametes/embryos considering the very high safety margins in animal studies following systemic administration of levels of rHA. Finally, the composition of the Vitrolife media is mainly comprised of known salts, amino acids and antibiotic (gentamicin) that do not trigger any genotoxicity potential. Overall considered, the absence of genotoxicity assessment of the finished media is acceptable.

Local tolerance

Please refer to section Toxicity above.

2.3.1. Discussion and conclusion on the non-clinical documentation

Based on the documentation provided, it can be concluded that the media subject of this assessment show no unreasonable risk of harm and constitute no recognized risk for the gamete, embryo or mother to be when the intended use is followed.

Overall, the results of the extensive published literature support the safety and usefulness of HSA as a supplement of ART solutions for sperm preparation, fertilisation, culture of embryos and

cryopreservation.

There are no objections from a non-clinical point of view to acceptance of the currently proposed ancillary substance rHA used in the medical device Vitrolife IVF media.

2.4. Clinical evaluation

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

The role of albumin in facilitating gamete and embryo manipulation in vitro by acting as a surfactant, in maintaining embryo physiology and metabolism, in chelating potential toxins during this process, and in acting as a protein supplement and micronutrient, is well known. Significantly, albumin is the most abundant protein in the female reproductive tract that the gametes came in to contact with. Recombumin Prime is structurally identical to HSA. Supplementation of IVF media with HSA is well established (Laverge et al., 1997, Blake et al., 2002).

No new clinical performance studies were carried out either on Recombumin Prime and Vitrolife media. The Manufacturer provided published clinical data supporting the clinical performance and safety use of rHA in G-MM, pre-supplemented EmbryoGlue and ICSI, and G-1, G-2. No publications are available describing clinical performance data for rHA supplemented media: G-IVF, G-MOPS, G-PGD, G-Thawkit Blast.

Since the launch of G-MM in 2002, its use as source of rHA has been the object of articles showing its clinical success rates in pregnancy and implantation (Valojerdi et al., 2008, Valojerdi et al., 2009, Murakami et al., 2013, Calhaz-Jorge et al, 2016).

The positive effect of EmbryoGlue pre-supplemented with G-MM and available on the market since 2003 has been evaluated in 2 meta analyses published by Cochrane Bontekoe et al., 2010, Bontekoe et al., 2014 that concluded that there are good results obtained after EmbryoGlue which indicates that rHA contributes to improved clinical outcome.

In a retrospective study intended to compare vitrification vs slow freezing methods for cryopreservation of human cleavage stage embryos, G-1 (version 3; Vitrolife, Kungsbacka, Sweden), supplemented with 10% rHA, Vitrolife, was used for 2 to 3 days oocytes culturing and for thawing and warming procedures for standard IVF and ICSI (Valojerdi et al., 2009). Other media containing HSA were also used, thus a direct effect of G-1 on the survival rate and deleterious effects on post-warming embryo morphology cannot be extrapolated.

G-1 (version 3; Vitrolife, Kungsbacka, Sweden), supplemented with 10% rHA Vitrolife was also used for 2 day oocytes culturing together with EmbryoGlue and other media containing HSA (Valojerdi et al., 2008). This prospective randomized study demonstrated that laser-assisted hatching does not appear to be efficient in improving pregnancy and implantation rates in patients with advanced female age (R37 y) and recurrent implantation failure (for R2 cycles). However, the methodology appears to be an effective strategy for improving implantation of frozen–thawed embryos. A direct effect of G-1 on clinical pregnancy and implantation rates cannot be extrapolated.

G-1 and G-2 media (Vitrolife) containing either HSA or rHA were used in a prospective randomized study (Murakami et al., 2013) in which culture of human embryos in the defined media with rHA yielded good-quality blastocysts, resulting in a high pregnancy rate after single cryopreserved embryo transfer. The nature of the media used for the vitrified-warmed blastocysts, is not known.

Further published data refer to other media different from Vitrolife, supplemented with rHA/G-MM

indicating the efficacy and safety in terms of fertilisation rate and development of human embryos.

Two articles also report live birth using G-MM for supplementation of other than Vitrolife media (Endo et al., 2012, Murakami et al., 2014).

The Notified Body particularly went through the recent retrospective article by Murakami et al. 2014, comparing the use of rHA (G-MM) against HSA as a protein supplement of homemade IVF culture media. In this study, 1,496 patients received vitrified/warmed embryo transfer (ET). Patients were divided in to two groups (rHA vs HSA). In HSA group, 831 patients received 1,163 cycles of ET (IVF=487; ICSI=676), using embryo-stage blastocysts vitrified/warmed in the conventional solutions containing HSA. In rHA group, 665 patients received 898 cycles of ET (IVF=343; ICSI=555), using blastocysts vitrified/warmed in the defined solutions containing rHA. There was no difference between these two patient groups other than the age (average age in HSA group was 34.8±0.1 while in rHA group it was 35.4±0.2). Clinical outcome of this study showed that most blastocysts survived warming in both groups (embryo survival rates 98.7 % vs. 98.9 %, HSA and rHA respectively) and the number of embryos transferred (1.08±0.01 vs. 1.06±0.01, HSA and rHA respectively) were similar. Outcome of the ET in terms of percentages of live delivery, stillbirth, and miscarriage per pregnancy were also similar in both groups. The HSA and rHA groups had similar live delivery rates/pregnancy (72.2 % vs. 72.3 %, respectively), multiple pregnancies and perinatal outcomes, including birth weight (2,988±28 vs. 3,046±26 g, respectively).

Table 14. Patient characteristics and clinical outcomes for vitrified/warmed embryo transfers

	HSA	rHA	*P*-value
No. of patients	831	665	
Patient age (range)	34.8±0.1 (22–44)	35.4±0.2 (23–45)	.005
BMI (range)	20.6±0.1 (14.4–32.5)	20.8±0.1 (15.1–36.7)	.109
Patients undergoing their first or second IVF/ICSI treatment cycle (%)	735 (88.5)	604 (90.8)	.149
Cause of infertility			
Male factor	326 (39.2)	264 (39.7)	.873
Female factor	142 (17.1)	102 (15.3)	.398
Combined and other factor	363 (43.7)	299 (45.0)	.638
No. of warmed cycles	1,163	898	
No. of embryos warmed	1,274	963	
No. of surviving embryos (%)	1,258 (98.7)	952 (98.9)	.848
No. of ETs	1,159	897	
No. of embryos transferred	1,250	951	
Mean no. of embryos transferred	1.08±0.01	1.06±0.01	.108
No. of implantations (%)	621 (49.7)	521 (54.8)	.018
No. of clinical pregnancies (%/ET)	597 (51.5)	502 (56.0)	.045
No. of live deliveries (%/pregnancy)	431 (72.2)	363 (72.3)	>.999
No. of miscarriages (%/pregnancy)	158 (26.5)	131 (26.1)	.945
No. of stillbirths (%/pregnancy)	2 (0.3)	2 (0.4)	>.999
No. of patients lost to follow up (%/pregnancy)	6 (1.0)	6 (1.2)	.779

Values are expressed as mean±SEM. The vitrification/warming solutions contained either HSA (HSA group) or rHA (rHA group)

Birth defects occurred in 0.9 % and 1.6 % of neonates in the HSA and rHA groups, respectively without significant statistical difference between the groups. There were no reports of neonatal mortality in either group.

Table 15. Incidence of birth defects in neonates born after vitrified/warmed embryo transfers

	HSA	rHA
Birth defects	1 (9 trisomy and double outlet right ventricle) 1 (patent ductus arteriosus)	2 (ventricular septal defect) 1 (multicystic dysplastic kidney [left], hydronephrosis [right], and hypertrophic pyloric stenosis)
	1 (cleft lip and palate)	1 (imperforate anus)
	1 (sebaceous nevus)	1 (polydactyly)
		1 (polysyndactyly)
Total (%)	4 (0.9)	6 (1.6)

The vitrification/warming solutions contained either HSA (HSA group) or rHA (rHA group)

One difference between the rHA group and HSA group is the clinical pregnancy rates/ET, which were higher ($P<0.05$) in the rHA group (56.0 %) than in the HSA group (51.5 %).

In addition, fewer males were born in the HSA group than in the rHA group ($P<0.05$).

Based on these results, authors suggest that rHA can effectively replace HSA in the base solutions for vitrification/warming of human blastocysts, and might yield high rates of pregnancy and live births after ET. Although long-term follow-up studies with more participants are required to validate the safety of the procedure including rHA, according to the authors, this new approach will aid in the development of standardized and defined ART systems. In conclusion, there is no evidence of an increased risk of congenital abnormality with the use of rHA. Perinatal risks that may be associated with ART might be related to the underlying cause of infertility or the risks associated with multiple pregnancy. However, the Manufacturer is asked to continue to monitor the outcome of pregnancies conceived using rHA media, by monitoring the literature and where possible by using the registries (e.g. consortia from the European Society of Reproductive Medicine (ESHRE) to gather data on ART outcome) and through reporting systems at national level. (**Recommended measure**)

It should be noted that in Murakami et al., 2014 the final concentration of rHA is 20 times lower than the one of HSA and 2-fold lower than the final concentration of rHA in Vitrolife G-ThawKit Blast media. In addition, in Murakami et al., 2014 a different cryopreservation procedure was tested i.e. embryo-stage blastocysts "vitrification/warming" rather than "slow freezing/thawing" claimed for Vitrolife G-ThawKit Blast medium. Among the indirect evidence (coming from published articles and a poster) provided by the Applicant, the most relevant is paper by Gardner et al., 2003 comparing the efficacy (in terms of embryo metabolism) of two slow-freezing protocols using the same cryopreservation solutions but with different start temperatures and cooling rates. The medium used for blastocyst freezing was G-2, while for thawing the media were G-1 and G-2. Results show that slow-freeze protocol (particularly with lower start temperature and quicker cooling rate) using culture media containing rHA leads to successful cryopreservation of blastocysts. This would suggest that the rHA ancillary functions is not compromised in cryopreservation technique for embryo-stage blastocysts "slow freezing/thawing" in terms of morphometric and morphokinetic -time to mitosis resumption and time to compaction- features.

Finally, as regards the higher concentration of rHA in Vitrolife media respect media used in the cited articles, the concentration of albumin in most media used for culture, cryopreservation and vitrification is higher than that in the Vitrolife G-ThawKit Blast medium, and no albumin concentration finding studies have been performed or presented for Vitrolife or other ART albumin containing media; thus, there is no reason to think that higher albumin concentration in Vitrolife media could compromise its ancillary functions in terms of pregnancies outcome or when different cryopreservation techniques for embryo-stage blastocysts are used (e.g. "vitrification/warming" used in published articles vs "slow freezing/thawing" claimed for Vitrolife G-ThawKit Blast medium).

As for toxicity non clinical studies, the 2 Phase 1 clinical trials (IV or IM, see below) were carried out to support the use of rHA in further pharmaceutical manufacturing and thus are not fully relevant for the intended use of Vitrolife media in ART. Both of these studies involved multiple doses (up to 65 mg IM and up to 50 g IV) and used HSA N 20% (Bayer) as comparator. The amount of HA administered in these clinical studies was much higher than those that may get in contact with the mother to be with Vitrolife media.

Both studies had as primary endpoints the safety and tolerability of rHA and HSA: however, in the **IV** study a secondary objective was to compare the PD of Recombumin Prime and HSA. The PD profiles of colloid osmotic pressure (COP), blood volume expansion (BVE) and hematological parameters were examined during the 24 hours after receiving the final (50 g) dose (on Day 42). The observed increase in albumin levels combined with the maximum extent (<400 mL) of BVE after the 50 g dose, suggested a distribution of the infused albumin beyond the central plasma compartment.

It was concluded that the observed PD effects of Recombumin Prime rHA and HSA were in close agreement.

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

G-MM has been on the market since 2002 and some of the media to be supplemented by G-MM have been on the market since late 1990s and no later 2005. During these years the Manufacturer states that no safety reports have been notified in relation with rHA supplementation.

Although some authors (Drylund et al., 2014, Kleijkers et al., 2016) concluded that G-MM respect to HSA sources, is free of contaminating proteins that makes it a good source of rHA in terms of reproducibility of composition, low levels of ammonium and lack of potentially immunotoxic proteins, yeast and human proteins (neoepitopes) still represent a potential risk in case of hypersensitive mother to be.

Two Phase 1 clinical trials compared safety and tolerability of rHA and HSA given IV and IM administration in healthy volunteers at doses higher than expected to be in contact with mother to be (0.15 mg/transfer).

In the **IV** study 30 healthy volunteers received increasing doses (10, 20 and 50 g) of either rHA or HSA via IV infusions. Each subject received a total of 80 g via three separate administrations 3 weeks apart (on Days 0, 21 and 42).

As for the IM study, only subjects with no known history of allergic reactions to S. cerevisiae or any other yeast products and with no history of anaphylactic or severe systemic response to human plasma proteins were selected.

Altogether, 6 (40%) subjects of the rHA group and 8 (53%) of the HSA group experienced adverse events. The observed, generally mild, adverse events (headache, rhinitis) could be expected in healthy volunteers and occurred independently of dose or treatment group. There were no critical events observed in any of the subjects. Further safety assessments (hematology, clinical chemistry, urinalysis and vital signs) revealed no noteworthy differences between rHA and HSA treatment.

As for the IM study, the clinical assessment for this study focused on potentially allergic events, which were defined as "critical events". However, no critical events were observed in the IV clinical study.

Assays to detect IgG and IgE against rHA, HSA, mannosylated rHA (m-rHA; ConA binding material) and yeast (*S. cerevisiae*) production host strain; mock preparation) were performed on all subjects on Day 0 (pre-dose), Day 42 (final dose) and Day 49 (follow-up) using ELISA. In addition, the IgE status

of all the trial subjects was evaluated prior to any dose administration using commercially available Radio Allergo Sorbent Test (RAST) methods to give an indication of the atopic status of the study population, particularly any hypersensitivity to yeast. However, no subjects, including those RAST positive to yeast, were excluded from the study on the basis of their RAST results.

Table 16. Positive RAST Data prior to treatment exposure

Treatment group	rHA	HSA
Number of subjects	15	15
Positive findings in RAST allergy test:		
-Phadiatope test	20%	27%
-*Saccharomyces cerevisiae*	7%	-
-*Candida albicans*	7%	-

The antibody analysis showed no clinically or statistically relevant differences between the rHA and the HSA treatment groups. There was no evidence of post-dose increase in antibodies in either dose group even given that, prior to any treatment exposure, positive RAST findings to yeast (either *Saccharomyces cerevisiae* or *Candida albicans*) were found in 14% of subjects in the rHA treatment group versus none in the HSA treatment group.In the **IM** study 500 healthy male and female subjects were enrolled. Only those subjects with no known history of allergic reactions to *Saccharomyces cerevisiae* or any other yeast products, and with no history of anaphylactic or severe systemic response to human plasma proteins were selected. Initially 100 subjects received either rHA or HSA at the lowest dose level of 5 mg. Then a further 100 subjects received either rHA or HSA at the second dose level of 15 mg and finally a further 300 subjects received either rHA or HSA at the highest dose level of 65 mg. All subjects received 5 intramuscular administrations over 1 month (Day 1, 8, 15, 22, and 29).

Adverse events were observed in a total of 205 subjects (rHA: 99, HSA: 106), but the overall incidence of adverse events was comparable between dose levels and treatment groups. The most frequently reported adverse event was rhinitis (rHA: 41, HSA: 46), although none of the rhinitis cases was considered study drug related. The severity of all but 2 of the adverse events, a ruptured ovarian cyst (65 mg HSA) and rhinitis (15 mg rHA), was considered to be mild or moderate. Study drug administration was discontinued in 3 subjects (rHA: 2, HSA: 1) because of study drug related adverse events. These were "exanthema on the trunk" (5 mg rHA; 4 administrations received), "exanthema, symmetrical erythematous eruption with peripheral distribution" (65 mg rHA; 2 administrations received), and "moderate new occurring reddening/swelling and mild reddening at the injection site" (65 mg HSA; 1 administration received).

As for the IV study, the clinical assessment for this study focused on potentially allergic events, which were defined as "critical events". A total of 16 critical events (rHA: 8, HSA: 8) in 15 subjects were reported (rHA: 8, HSA: 7). Nine of these events (rHA: 4, HSA: 5) were considered study drug related by the investigator. All but 2 (nausea rHA; abdominal pain HSA) of the 16 critical events were skin-related findings. The most frequent critical event was reddening/swelling at sites other than the injection site. Few cases of skin reactions (i.e. exanthemas, reddening at and away from the injection site, urticaria) and other symptoms not defined as critical events (pruritus) could be clinically interpreted as an expression of allergic reactions. These cases were equally distributed between the two treatment groups. The incidence of adverse events (included critical events) by sex showed an overall higher occurrence in females (46.2%) compared to male subjects (36.3%). Assays to detect IgG and IgE against rHA, HSA, mannosylated rHA (m-rHA; ConA binding material) and yeast (*S. cerevisiae)* production host strain mock preparation) were performed on all subjects at baseline (Day 1 pre-dose) and at follow-up (Day 36), which was 7 days after the final dose using ELISA. In addition, the IgE status of all the trial subjects was evaluated prior to any dose administration using

commercially available RAST methods to give an indication of the atopic status of the study population, particularly any hypersensitivity to yeast. However, no subjects, including those RAST positive to yeast, were excluded from the study on the basis of their RAST results.

Table 17. Positive RAST Data prior to treatment exposure

Treatment group	5 mg dose		15 mg dose		65 mg dose	
	rHA	HSA	rHA	HSA	rHA	HSA
Number of subjects	50	50	50	50	150	150
Positive findings in RAST allergy test:						
-Phadiatope test	34%	38%	38%	36%	39%	30%
-*Saccharomyces cerevisiae*	2%	4%	-	2%	5%	3%
-*Candida albicans*	4%	4%	4%	4%	8%	3%

Subsequently, for subjects in whom critical events were reported, additional antibody measurements (IgG and IgE) were performed on samples taken 1 week after each dose (Day 8, 15, 22 and 29). The antibody analysis showed no clinically or statistically relevant differences between the rHA and the HSA treatment groups. There was no evidence of post-dose increase in antibodies in any dose group even given that, prior to any treatment exposure, positive RAST findings to yeast (either *Saccharomyces cerevisiae* or *Candida albicans*) were found in both rHA and HSA treatment groups.

There was no correlation between the observed clinical symptoms in critical event subjects and the measured antibody time courses.

Overall, the 2 Phase 1 clinical studies demonstrated comparable safety and tolerability of Recombumin Prime vs HSA, with respect to potential immunogenicity when administered IM in doses of up to 65 mg and IV in doses of up to 50 g. Critical (potentially allergic) events were only reported in the IM study and their occurrence was equivalent between the rHA and HSA treatment groups. There were no relevant pre- versus post-dose changes in the levels of specific IgG or IgE to any of the four antigens (rHA, HSA, mannosylated rHA and yeast-derived proteins).

As requested by the CHMP, the Manufacturer has provided a literature search looking for documented hypersensitivity reactions in women who had ART using media containing yeast. While no cases of hypersensitivity have been reported to the Manufacturer, anaphylactic reactions may have occurred but have not been reported or published.

The Manufacturer has provided relevant literature i.e. 6 papers relating to the safety and immunogenicity of rHA when administered mainly in vaccines.

The incidence of hypersensitivity to yeast is expected to be small given the levels of anti-yeast positive antibodies as indicated in the paper by DiMiceli et al., 2006; however anti-yeast IGE was present in about 1-2% of those tested. Therefore hypersensitivity to yeast is a possibility. It is noted that the vaccine ProQuad & others (for which Recombumin is used as a component in the bulk manufacturing process) are contraindicated in the case of hypersensitivity to the active or any of the excipients.

The Manufacturer should monitor and collect hypersensitivity reactions as part of their obligations for safety reporting for Vitrolife rHA media. **(Recommended measures)**

As requested by the CHMP, information on the possible trace presence of yeast in rHA pre-supplemented EmbryoGlue and G-MM media has been introduced in the IFU and a warning on not to use the media in case a patients has a known saccharomyces or any other components hypersensitivity, has been added.

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

Although only published studies addressing G-MM, the pre-supplemented Vitrolife media EmbryoGlue and ICSI, and G-1, G-2 are available, the function of rHA in terms of pharmacological action is considered the same in all products, even though they are used at various stages of the embryo development.

In a recent retrospective clinical study (Murakami et al., 2014) showing comparable implantation rate and clinical pregnancy rate in both HSA and G-MM supplemented handmade IVF media used with vitrification/warming technique, a higher number of birth defects occurred in the rHA group containing 20-fold lower amount of rHA vs HSA. Although the number of congenital abnormalities is small in both groups and within the expected rate of congenital abnormality for live births, in order to investigate the potential causal relationship between rHA and birth defects and to assess any deviation from historical data with HSA, the Manufacturer should continue to monitor the outcome of pregnancies conceived using rHA media, by monitoring the literature and where possible by using the registries (e.g. consortia from the European Society of Reproductive Medicine (ESHRE) to gather data on ART outcome) and through reporting systems at national level, to provide long term data on developmental outcomes. **(Recommended measure)**

No albumin-concentration finding studies have been performed or presented for Vitrolife or other ART albumin containing media; however, there is no reason to think that higher albumin concentration in IVF media could compromise its ancillary functions in terms of pregnancies outcome or of different cryopreservation techniques.

While rHA has the potential to reduce the risk of transfer of human plasma-derived ligands and impurities (viral or prion contamination) vs HSA, and the predictability in its composition and relative batch to batch consistency makes rHA an attractive candidate as a protein supplement in the IVF media, the product may be contaminated with residues from the host organism, *S. cerevisiae* or may show neoepitopes resulting in potential immunotoxicity for the hypersensitive mother to be.

To allow the user to determine whether the patient has a pre-existing known hypersensitivity to yeast or any other components and therefore to use an alternative media for these patients, the IFUs for media EmbryoGlue and G-MM have been amended and information regarding the origin of recombinant human albumin and a warning on not to use the medium in case a patients has a known hypersensitivity has been added.

Data from 2 Phase 1 clinical trials, comparing the safety and tolerability of rHA vs HSA in healthy volunteers, showed wide safety margins, based on high doses administered and route of administrations, as regards the systemic toxicity of rHA for the mother to be, in case of absorption trough the vaginal and endometrial tissues. Potentially allergic events were considered as critical adverse events in the study protocols: skin-related findings other than the injection site were retrieved only following IM administration and were balanced in subjects treated with rHA vs HSA. The Applicant will request that Vitrolife continues to monitor and collect hypersensitivity reactions as part of the safety reporting for the Vitrolife rHA media and that this is specifically evaluated annually in the clinical evaluation reports. **(Recommended measure)**

2.4.4. Discussion and conclusion on the clinical evaluation

The role of HSA for in vitro manipulation of gametes and embryos including embryo cryopreservation is well known and supplementation of IVF media with HSA is well established.

The replacement of HSA with rHA is an accepted practice, and several published data showed

comparable non-clinical and clinical efficacy in terms of gamete viability (e.g. sperm capacitation and fertilisation) embryo development, post-thaw number of survival blastocyst cells after cryopreservation, implantation rate and clinical pregnancy rate.

Although only published studies addressing G-MM, the pre-supplemented Vitrolife media EmbryoGlue and ICSI, and G-1, G-2 are available, function of rHA in terms of pharmacological action is considered the same in all products, even though, they are used at various stages of the embryo development.

In a recent retrospective clinical study (Murakami et al., 2014) showing comparable implantation rate and clinical pregnancy rate in both HSA and G-MM supplemented IVF media other than Vitrolife, a higher number of birth defects occurred in the rHA group: the claimed absence of statistical significance is poorly relevant from the safety perspective. Perinatal risks might be related to the underlying cause of infertility or the risks associated with multiple pregnancy. However, the Manufacturer is asked to continue to monitor the outcome of pregnancies conceived using rHA media. (**Recommended measure**)

There is no reason to think that higher albumin concentration in Vitrolife media could compromise its ancillary functions in terms of pregnancies outcome or when different cryopreservation techniques for embryo-stage blastocysts are used (e.g. "vitrification/warming" used in published articles vs "slow freezing/thawing" claimed for Vitrolife G-ThawKit Blast medium).

G-MM has been on the market since 2002 and some of the media to be supplemented by G-MM have been on the market since late 1990s and no later 2005. During these years, the Manufacturer states that no safety reports have been notified in relation with rHA supplementation.

While rHA has the potential to reduce the risk of transfer of human plasma-derived ligands and impurities (viral or prion contamination) vs HSA, and the predictability in its composition and relative batch to batch consistency makes rHA an attractive candidate as a protein supplement in the IVF media, the product may be contaminated with residues from the host organism, *Saccharomyces cerevisiae* or may show neoepitopes resulting in potential immunotoxicity for the hypersensitive mother to be. Data from 2 Phase 1 clinical trials, comparing the safety and tolerability of rHA vs HSA in healthy volunteers, showed wide safety margins, based on high doses administered and route of administrations, as regards the systemic toxicity of rHA for the mother to be, in case of absorption trough the vaginal and endometrial tissues. Potentially allergic events were considered as critical adverse events in the study protocols: skin-related findings other than the injection site were retrieved only following IM administration and were balanced in subjects treated with rHA vs HSA.

To allow the user to determine whether the patient has a pre-existing known hypersensitivity to yeast or any other components and therefore to use an alternative media for these patients the IFUs for media EmbryoGlue (Encl. 3) and G-MM (Encl. 4) have been amended and information regarding the origin of recombinant human albumin and a warning not to use the medium in case a patients has a known hypersensitivity has been added.

The Applicant will request that Vitrolife continues to monitor the outcome of the of pregnancies conceived using rHA and continues to monitor and collect hypersensitivity reactions as part of the safety reporting for the Vitrolife rHA media and that this is specifically evaluated annually in the clinical evaluation reports. The Applicant will request Vitrolife to explicitly describe how to actively collect and evaluate data related to outcome of the pregnancies and hypersensitive reactions. **(Recommended measure)**

Overall, no unacceptable clinical risk, when used for rHA intended purpose in IVF procedures, is expected.

The Notified Body conclusions that the use of the rHA in IVF cell culture media products is suitable and has a useful ancillary effect, on the intended purpose of the medical device, is shared by the CHMP.

The benefit of including rHA is considered to outweigh the risk.

2.5. Overall conclusions

Overall, quality, non-clinical and clinical data support the conclusion that recombinant human albumin Recombumin Prime is able to achieve its intended purpose as ancillary ingredient in Vitrolife IVF media (i.e., embryo development, implantation rate and clinical pregnancy rate), and that it is safe and well-tolerated for both the mother to be and gametes/embryos.

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 recombinant human albumin solution used as ancillary medicinal substance(s) in the Vitrolife IVF media was favourable and therefore granted a positive opinion in the consultation procedure.