SUMMARY OF SAFETY AND CLINICAL PERFORMANCE

Gx-IVF™

This document is intended to provide an updated summary of clinical data and other information about the safety and clinical performance of the medical device Gx-IVF.

This document includes information in accordance with the requirements in Article 32 of the Medical Devices Regulation (EU) 2017/745 (MDR) and recommendations in Medical Device Coordination Group (MDCG) 2019-9 guidance document.

Document History:

Version	Description	Date	Sign
v.1.0	Initial version of SSCP for Gx-IVF (REP-2361)	2020-10-13	SM
v.2.0	Update of SSCP based on comments from DNV (REP-2361)	2021-06-04	SM
v.3.0	Update of SSCP due to a correction in section 5.2 and update of data in section 5.3. (REP-2361)	See publish date	SM

List of abbreviations:

ALA	Alpha Lipoic Acid
ALC	Acetyl-L-Carnitine
ART	Assisted Reproductive Technology
FET	Frozen Embryo Transfer
HSA	Human Serum Albumin
ICSI	Intracytoplasmic Sperm Injection
IFU	Instruction for Use
IVF	In Vitro Fertilization
IUI	Intrauterine Insemination
MDCG	Medical Device Coordination Group
MDR	Medical Devices Regulation 2017/745
NAC	N-Acetyl-L-Cysteine
PGT	Preimplantation Genetic Testing
PMCF	Post-Market Clinical Follow-up
SSCP	Summary of Safety and Clinical Performance
TESA	Testicular Sperm Aspiration
TESE	Testicular Sperm Extraction

SUMMARY OF SAFETY AND CLINICAL PERFORMANCE

This Summary of Safety and Clinical Performance (SSCP) is intended to provide public access to an updated summary of the main aspects of the safety and clinical performance of the device.

The SSCP is not intended to replace the Instructions For Use as the main document to ensure the safe use of the device, nor is it intended to provide diagnostic or therapeutic suggestions to intended users or patients.

The following information is intended for users/healthcare professionals.

1. Device Identification and general information

1.1 Device trade name: Gx-IVF™
1.2 Manufacturer's name and address: Vitrolife Sweden AB, Gustaf Werners gata 2, 421 32 Västra Frölunda, Sweden
1.3 Manufacturer's single registration number (SRN): SE-MF-000002389
1.4 Basic UDI-DI: 735002591AAPE8
1.5 Medical device nomenclature description/text: Not available
1.6 Class of device: Class III
1.7 Year when the first certificate (CE) was issued covering the device: Not yet approved
1.8 Authorized representative if applicable; name and SRN: Not applicable
1.9 NB's name (the NB that will validate the SSCP) and the NB's single identification number: Det Norske Veritas (DNV) Product Assurance AS and 2460

2. Intended use of the device

2.1 Intended purpose

Gx-IVF is a medical device intended for use in Assisted Reproductive Technology (ART) as a medium for preparation and handling of gametes, for *in vitro* fertilization and intrauterine insemination.

2.2 Indication (s) and target population (s)

The Indication for use of Gx-IVF is "medium for preparation and handling of gametes, for *in vitro* fertilization and intrauterine insemination". The intended target group is an adult or reproductive-age population that undergoes fertility treatment.

2.3 Contraindications and/or limitations

Gx-IVF contains gentamicin. Do not use in patients with known hypersensitivity/allergy to the component.

3. Device description

3.1 Description of the device

Gx-IVF is a bicarbonate buffered medium containing human serum albumin, gentamicin and a combination of three antioxidants (Acetyl-L-carnitine (ALC), N-acetyl-L-cysteine (NAC), α-lipoic acid (ALA)). Gx-IVF is designed to provide suitable physiological conditions during preparation and handling of gametes followed by in vitro fertilization (IVF) or intrauterine insemination as well as protection against oxidative damage. Gx-IVF is ready to use after equilibration at +37°C and 6% CO_2 atmosphere. Gx-IVF will have contact with patient when used for intrauterine insemination. The medium is sterile filtered using aseptic technique and is available in 60 ml bottles (see figure 1) that can be used for up to two weeks after first opening. Based on regulatory guidelines, the medicinal components present in Gx-IVF include N-acetyl-L-cysteine, gentamicin, human serum albumin (HSA).

Figure 1. Picture of Gx-IVF in a 60 mL bottle

3.2 A reference to previous generation (s) or variants if such exist, and a description of the differences

Not applicable.

3.3 Description of any accessories which are intended to be used in combination with the device

Not applicable

3.4 Description of any other devices and products which are intended to be used in combination with the device

Gx-IVF may be used in preparation of density gradient solution. Other devices that may be used in combination with Gx-IVF are sterile labware.

4. Risks and warnings

4.1 Residual risks and undesirable effects

For Gx-IVF, there are three residual risks that remain unacceptable after risk control measures. These risks have the hazardous situations ‘viral infection of the patient’ or ‘viral infection of user’ and are related to HSA present in the device. HSA is derived from human blood and could theoretically be a vector for various diseases such as hepatitis B (HBs-Ag), hepatitis C (Anti-HSV) and HIV 1/2 (Anti-HIV 1/2). The probability of patient or user being virally infected during IVF treatment is extremely small, yet the risk is considered unacceptable. Systematic literature search conducted during clinical evaluation has not identified any negative effects or infection associated with the use of HSA in IVF media. No undesirable effect of adverse event has been reported for any of the Vitrolife’s media containing HSA. The benefit-risk evaluation performed during risk analysis has concluded that the benefits of using HSA in IVF media are greater than the risks associated with blood-borne contamination as Vitrolife applies relevant safety measures. The raw material source of HSA used in Vitrolife’s media have been tested for blood-borne diseases by accredited laboratories.

The additional potential clinical risks that could occur during the use of Gx-IVF are the following:

- Chemical constituent has a negative effect on patient
- Allergic reaction or hypersensitivity in patient or user
- Patient suffers from cytotoxic reaction following use of the product
- Patient suffers from sensitization reaction following use of the product
- Patient suffers from irritation reaction following use of the product
- Patient infected by contaminated media

All these risks were acceptable after risk control measures. To control risks related to the use of Gx-IVF, all the raw materials are quality tested and each LOT of the final product is also tested for sterility,

bacterial endotoxin, sperm survival and embryo toxicity prior to its release. Biological evaluation conclude that all components are nutrients that are either naturally present in mammal tissues, or they have been used in the handling or treatment of human cells for an extended period. No materials or substances are listed as harmful. All chemicals, bottles and final products are MEA tested to confirm non-toxicity. Stability studies confirm the product is non-toxic for the entire lifetime. Additionally, the end user is informed about the device components, contraindication, precautions and the risk of using blood derived products by providing information on labels and Instruction for Use.

4.2 Warnings and precautions

Contraindications

Gx-IVF contains gentamicin. Do not use in patients with known hypersensitivity/allergy to the component.

Precautions

- Discard product if bottle integrity is compromised. Do not use Gx-IVF if it appears cloudy.
- Gx-IVF contains human serum albumin and acetylcysteine.
- To avoid contamination Vitrolife strongly recommends that media should be opened and used only with aseptic technique.
- The risk of reproductive and developmental toxicity for IVF media, including Vitrolife´s IVF media, has not been determined and is uncertain.
- Any serious incident that has occurred in relation to the device should be reported to the manufacturer.
- Not for injection.
- Discard the product according to standard clinical practice for medical hazardous waste when the procedure is finished.
- Caution: All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested for antibodies to HIV, HBc, HCV, and HTLV I/II and non-reactive for HbsAg, HCV RNA and HIV-1 RNA and syphilis. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.

Additional precautions related to the use of the device can be found in the package insert.

4.3 Other relevant aspects of safety, including a summary of any field safety corrective action (FSCA including FSN) if applicable

Not applicable.

5. Summary of clinical evaluation and post-market clinical follow-up

5.1 Summary of clinical data related to equivalent device, if applicable

Not applicable.

5.2 Summary of clinical data from conducted investigations of the device before the CE-marking, if applicable

a. Comparisons of human embryonic development in culture medium with and without antioxidant supplementation

Identity of the study	Interim analysis of the study performed in Melbourne IVF centers in Australia and registered with Australian New Zealand Clinical Trials Registry (ANZCTR) (ACTRN12618001479291).
Identity of the device	Gx-IVF manufactured by Vitrolife
Intended use of the device in the study	Gx-IVF was used for sperm selection which is part of the sperm preparation procedure.
Objective of the study	Investigate whether the combination of antioxidants favour embryo development in human IVF.
Study design	Prospective, randomized controlled superiority study
Primary and secondary endpoint (s)	Primary endpoint is percentage implantation rate following embryo transfer. Secondary endpoints are embryo development day 2 to day 6, embryo quality day 3 to day 6, total blastocyst formation (day 5 and 6), utilization rate, clinical pregnancy rate, neonatal outcome.
Inclusion/exclusion criteria for subject selection	Inclusion criteria: Couples with unexplained infertility intending to undergo IVF or ICSI and where there are no medical contraindications to perform the treatment. The couple should have received verbal and written information/consent about the study. Blastocyst culture and fresh transfer of a single blastocyst. Concomitant medications (e.g.; use of any oral antioxidants) will be recorded Exclusion criteria: Previous participation in the study. Use of PGT where all embryos will be frozen Testicular biopsy patients (TESA/TESE) Total freeze and no transfer possible.
Number of enrolled subjects	The target sample size is 1480. At the interim analysis 275 and 276 patients had received an embryo transfer in the control and antioxidants group, respectively.
Study population	Study is conducted in couples undergoing fertility treatment
Summary of study methods	A traditional IVF treatment including ovarian stimulation and oocyte pick-up was performed using the standard methods at the clinic. Sperm cells were selected according to standard laboratory procedures using Gx-IVF™ or control medium (G-IVF PLUS™, Vitrolife). Embryos were cultured from day 1 to day 5 or day6 in time-lapse systems manufactured by Vitrolife. Single embryo transfers were performed, and clinical pregnancy was determined by monitoring foetal heartbeat after 42 days of gestation.
Summary of results	The overall clinical pregnancy rate in the control group and study group (includes Gx-IVF) were 35% and 36%, respectively. The clinical pregnancy rate in female patients ≥35 years old was 27% and 33% in the control (205 transfers) and antioxidant (203 transfers) groups, respectively. The study is ongoing and the results will be updated after the completion of the study.

Limitations of the study, if any:	-
Device deficiency or replacements related to safety and/or performance, if any	-

b. Randomized controlled non-inferiority sibling oocyte study comparing blastocyst development in media with and without antioxidants

Identity of the study	Interim analysis of the study was performed in a single center in Japan and registered with UMIN Clinical Trials Registry (UMIN-CTR) (UMIN000034482)
Identity of the device	Gx-IVF manufactured by Vitrolife
Intended use of the device in the study	Gx-IVF was used for sperm preparation, oocyte incubation and *in vitro* fertilization
Objective of the study	Investigate the efficiency of culture system with antioxidants
Study design	Prospective, randomized controlled non-inferiority sibling oocyte study
Primary and secondary endpoint (s)	The primary endpoint of the study is *in vitro* embryo development. Secondary endpoints are fertilization rate, biochemical pregnancy rate, clinical pregnancy rate
Inclusion/exclusion criteria for subject selection	Inclusion criteria: Patients undergoing ART treatment with more than 8 oocytes and patients aimed for blastocyst culture until day 5/6 followed by cryopreservation and transfer of a single embryo in a frozen embryo transfer (FET) cycle. Patients should have received verbal and written information and provided informed consent to participation Exclusion criteria: Previous participation in the study Patients with surgically retrieved sperm, requiring split IVF/ICSI or presenting with less than 8 oocytes or more than 33 oocytes at pick-up
Number of enrolled subjects	A total of 143 patients were enrolled and oocytes from each patient were randomly allocated in a 1:1 ratio into either the media with antioxidants (study media group) or media without antioxidants (control group)
Study population	Study is conducted in patients undergoing ART treatment.
Summary of study methods	All the steps were performed using control (standard media) or the study media system (includes Gx-IVF). Washed oocytes were kept in Gx-IVF or control media until insemination or denudation prior to

	ICSI. Following fertilization, embryos were cultured to the blastocyst stage in the time-lapse system using low oxygen. Embryo development and quality was assessed according to Alpha/ESHRE consensus criteria on day 3 and using Gardner Score for blastocysts on day 5 / 6. Blastocysts of acceptable morphological quality were cryopreserved by vitrification and a single blastocyst is warmed and cultured prior to FET. Clinical outcome parameters (positive beta-human chorionic gonadotropin rate, implantation rate by gestational sac and by fetal heartbeat and live birth) will be monitored when all patients have received at least one embryo transfer.
Summary of results	Results showed no significant differences between control and study media in terms of fertilization rates (66% versus 71 %), embryo development on day 3 (46 % versus 50 %), good quality blastocysts (30% versus 31%), blastocyst formation day 5+6 (44% versus 45%) and embryo utilization rate (34 % versus 36 %). However, numerically higher rates of fertilization, embryo development were observed, and more embryos were available for transfer to the patient in the study media group (media containing antioxidants). This study has been terminated and the results will be updated when all patients have received at least one embryo transfer.
Limitations of the study, if any:	-
Device deficiency or replacements related to safety and/or performance, if any	-

5.3 Summary of clinical data from other sources, if applicable

Literature search has identified two peer reviewed articles and two meeting abstracts reporting data including the use of Gx-IVF for preparation and handling of oocytes or sperm (Gardner *et al*., 2020; Nakadate *et al*., 2020; Komure *et al*., 2020; Ueno *et al*., 2021). The publication by Gardner et al. (2020) evaluated the effect of antioxidant supplementation in IVF media and the outcomes after the use of Gx-IVF were compared with standard medium without antioxidants (G-IVF PLUS). Gx-IVF or G-IVF PLUS was used for handling/incubating oocytes until insemination or denudation. The results showed similar rates of fertilization in both groups but the number of good quality embryos on day 3 was significantly higher in the Gx-IVF group. Overall blastocyst development (day 5 and day 6), day 5 embryo quality and embryo utilization, implantation and ongoing pregnancy were numerically higher in the Gx-IVF group but the difference was not statistically significant. Ueno et al (2021) compared the outcomes between antioxidant supplemented media and standard media and Gx-IVF was used for oocyte incubation until denudation. The study also analyzed the results in relation to the type of incubator used (time-lapse or non-time lapse incubator). Results on fertilization, embryo development, blastocyst quality showed no significant difference between the antioxidant group (Gx-IVF) and the standard media group. However, results on clinical pregnancy rate and ongoing pregnancy rate showed significantly higher rates in the Gx-IVF group that used non-time-lapse incubator. In the study by Komure *et al* (2020), outcomes after the use of Gx-IVF for sperm preparation showed numerically higher rates of fertilization and good quality blastocyst and significantly higher blastocyst development after conventional IVF as compared to the control group which used media from another manufacturer. The overall performance of embryos in the Gx-IVF group was better than the control group but the difference was not statistically significant. In

addition, the sperm motility analysis following sperm preparation showed higher linear velocity for samples prepared using Gx-IVF. Nakadate *et al*. (2020) aimed to study the efficacy of Gx-IVF for *in vitro* oocyte maturation. Results from the study showed improved rates of oocyte maturation, fertilization, blastocyst formation and embryo utilization in the group that used Gx-IVF group as compared to standard media group.

Additionally, clinical experience data from 15 patients who underwent fertility treatment in a hospital in India showed fertilization and embryo development after the use of Gx-IVF for handling and storage of cumulus oocyte complexes. The clinical experience data from Japan after the use of Gx-IVF for sperm preparation and intrauterine insemination showed no significant difference in clinical pregnancy rates between the group using Gx-IVF (2903 cycles during March 2020 to February 2021) or the standard method without antioxidant media (2408 cycles during July 2019 to February 2020). The outcomes confirmed safe use of the device for intrauterine insemination.

In summary, the results observed after the use of Gx-IVF by Gardner *et al*., 2020; Ueno *et al*., 2021, Komure *et al*., 2020, Nakadate *et al*., 2020 and clinical experience data add support to the safety and performance of Gx-IVF.

5.4 An overall summary of the clinical performance and safety

Based on the Indication for Use, Gx-IVF is intended to provide clinical benefit by supporting preparation and handling of gametes without losing their function followed by *in vitro* fertilization or intrauterine insemination. For Gx-IVF, clinical investigations and published articles reporting rates of fertilization after the use of the device for preparation and handling of sperm and oocyte as well as in vitro fertilization confirm the performance of Gx-IVF. Results on embryo development, embryo utilization, implantation and pregnancy rate also demonstrate the safety and performance of Gx-IVF. The clinical pregnancy rates reported after the use of Gx-IVF align with the yearly European results published by ESHRE (Wyns *et al.*, 2021). Additionally, clinical experience feedback on the use of Gx-IVF in fertility procedures confirmed that the device performed according to its Indication for Use.

Since Gx-IVF is also intended for intrauterine insemination, the biological evaluation of the device has been performed. Results from biological evaluation confirms safe use of Gx-IVF in patients (internal data, Vitrolife). Results on sperm motility or survival obtained from testing Gx-IVF showed a beneficial effect of this device on that aspect (Meintjes *et al*., 2018). Further, data from clinical studies involving the use of Gx-IVF for sperm preparation also confirmed safe use of the device. Given that the device act as a carrier of sperm suspension during its transfer to the uterine cavity of the patient, the data showing safety of the device during sperm preparation and handling together with data from biological evaluation confirmed its safe use for intrauterine insemination. Additionally, clinical experience data obtained from the use of Gx-IVF for sperm preparation and intrauterine insemination in 2903 treatment cycles confirms its safe use for intrauterine insemination.

Data from the studies by Gardner *et al*., 2020; Ueno, *et al*., 2020 and Komure *et al*., 2020 comparing the outcomes between Gx-IVF (antioxidant supplemented medium) and standard medium reported improved outcomes in the Gx-IVF group. The study by Komure *et al*. (2020) showed numerically higher rates of fertilization, good quality blastocyst and significantly higher blastocyst development. In addition, the sperm motility analysis following sperm preparation showed higher linear velocity for samples prepared using Gx-IVF. Results from Gardner *et al*., 2020 demonstrated a significant difference on day 3 embryo quality and a trend to numerically higher rates on day 5 embryo quality, embryo utilization, implantation and pregnancy in the Gx-IVF group as compared to the standard medium group. A significant difference in clinical pregnancy rate and ongoing pregnancy rate was also observed in the Gx-IVF group that used non-time lapse incubator for culture (Ueno *et al.,* 2021). In addition, G-IVF PLUS supplemented with antioxidants (identical to Gx-IVF) showed protection against oxidative stress during its use for handling

of gametes and fertilization (Truong and Gardner, 2017). Overall, this evidence supports that the presence of antioxidants provides the chance of improved outcomes. No undesirable side-effects have been reported for the device during its use. The risks associated with the use of the device are considered acceptable when weighed against the benefits.

Together, these data confirm safety and performance of Gx-IVF for its Indication for Use and all clinical claims.

5.5 Ongoing or planned post-market clinical follow-up

There are no unanswered questions regarding the intended purpose or the performance and safety of the device. No further risks have been recognized during the clinical evaluation and the benefit-risk profile is acceptable. However, Gx-IVF being a new device, PMCF activity will be conducted during its post-market phase and that include collecting clinical experience data from a multi-center evaluation and feedback from the use of the device.

6. Possible diagnostic or therapeutic alternatives

ART is a treatment option for patients failing to conceive naturally as well as patients who have tried other treatments such as medications and surgical procedures without success. Hence, there are no therapeutic alternatives for patients at this stage.

Gx-IVF is a bicarbonate-buffered medium intended for use in ART for preparation and handling of gametes, *in vitro* fertilization and intrauterine insemination. G-IVF PLUS (Vitrolife) and ORIGIO Sequential Fert (CooperSurgical) are the similar devices for Gx-IVF.

Gx-IVF contains a combination of three antioxidants, and this makes the device unique for its intended purpose. Studies comparing the outcomes of Gx-IVF and G-IVF PLUS have shown improved results (e.g., fertilization rate) with the use of Gx-IVF but failed to reach significance. Hence, more studies are required to confirm the benefit of Gx-IVF over other commercial devices.

Several media are available in the markets for fertilization and culture of human preimplantation embryos. There is no clear evidence confirming the benefit of any media over the other. It should be determined within individual laboratories as to which medium best suits the procedure.

7. Suggested profile and training for users

The end user (IVF professional) is expected to be trained and qualified within ART field to understand the Indication for Use of Gx-IVF. Since no special design feature or safety concerns were identified for Gx-IVF, there is no specific training required for the end-users.

8. Reference to any harmonised standards and common specifications applied

Medical Devices Regulation (EU) 2017/745 (MDR)
MEDDEV 2.7.1 Rev.4

EN ISO 14971:2019	(risk management)
EN ISO 14155:2011	(clinical investigation; Good clinical practice, GCP)
EN ISO 15223-1:2016	(symbols to be used with medical device labelling)
EN 1041: 2008	(information supplied by the manufacturer of medical devices)
MEDDEV 2.7/4	(clinical investigation)
MEDDEV 2.12-2 Rev.2	(post-market clinical follow-up)

9. Revision history

SSCP revision number	Date issued	Change description	Revision validated by the Notified Body
			☐ Yes Validation language:

References

Gardner, D. K., Kuramoto, T., Tanaka, M., Mitzumoto, S., Montag, M., & Yoshida, A. (2020). Prospective randomized multicentre comparison on sibling oocytes comparing G-Series media system with antioxidants versus standard G-Series media system. *Reprod Biomed Online, 40*, 637-644.

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