Media for Embryo Production

IVF, ICSI, ET

and

Cryo Preservation

Stroebech media

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Helping you succeed is our passion!

Dr. Lotte Stroebech and Dr. Birthe Avery

Bovine

Equine

Buffalo

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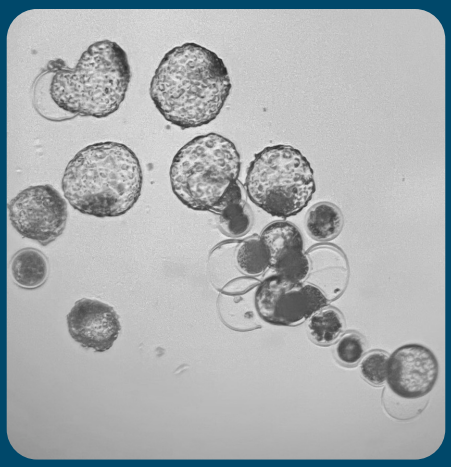


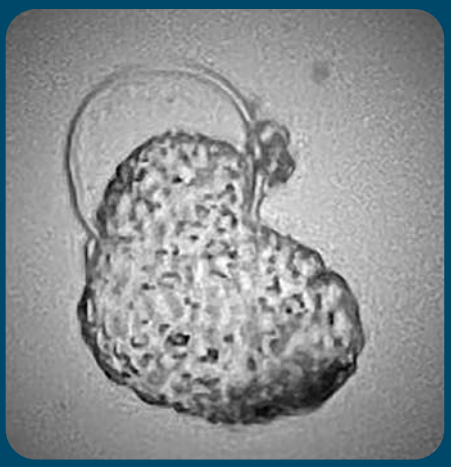


Photo: Poul Hyttel

IVF media for Assisted Reproductive Technologies in Animals

With more than 40 years of experience within media manufacturing and assisted reproductive technologies we have a new and optimized media product line for in vitro fertilization in cows, sheep, goat, camels and buffaloes, as well as assistance with IVF in exotic animals.

Furthermore, we have an entire media range for Equine ICSI with a detailed protocol.

We offer individual training and support as well as courses within our media and protocols as well as extensive distributor training.

Quality Control

The Bovine Embryo Assay (BEA) test is the most important!

There are huge differences in reproductive physiology between the mouse and cow, therefore a MEA test is not sufficient QC for a bovine medium.

The oocyte maturation is more complex in cows and fertilization and sperm capacitation are species specific. A MEA test mostly assesses blastocyst development from either one-cell or two-cell stage embryos. Should a batch variation occur it will not be detected in a MEA test, where release parameter is > 80 % blastocyst rate. In the BEA test, however, a difference in medium quality can be detected even though the medium passes the MEA test. For instance, 35 % vs 55 % blastocyst rates indicate two media are different, however both media will have passed the MEA.

- ✓ Each batch of media comes with a certificate for BEA test as well as sterility, fungal and endotoxin tests
- ✓ The production site is ISO9001 and ISO13485 certified
- ✓ All media are delivered in glass bottles

Our scientific background

Lotte Stroebech

DVM, PhD

As the CEO and CSO of Stroebech Media, founded in 2019, Dr. Stroebech has been a pioneer in the field of animal IVF, successfully producing over 500,000 embryos globally using the Stroebech Media's innovative protocols and media.

In 2012 she was the first in the world to create and launch a commercial serum-free, ready-to-use media range for animal IVF, sold to IVFBioscience in the UK.

Previously, Dr. Stroebech established and led the bovine IVF research laboratory at Origio, Cooper Surgical.

Academic work
Guest Professor of Animal Breeding and Biotechnology at the Estonian University of Life Sciences. Supervision of international PhD students and postdoctoral researchers. Dr. Stroebech collaborated on numerous international research projects, including EliteOva, Elitesemen, and GIFT Brazil.

Dr. Stroebech was also the Laboratory Director at the IVF Laboratory within the Faculty of Health and Medical Sciences, Department of Veterinary and Animal Sciences in Copenhagen, Denmark.

Previous board position
Chairman of the Danish Society of Reproduction and Fetal Development (DSRF) and a board member of the International Embryo Technology Society (IETS) and the Association of Embryo Transfer Society Europe (AETE).

IVF Experience:
With extensive IVF experience, Dr. Stroebech has consulted for over 300 IVF laboratories in animal research and production worldwide, providing insights to establish new facilities and enhance results in existing laboratories.

Birthe Avery

MD, PhD, DVSc

International recognized pioneer within IVF. Retired associate professor from the Veterinary Faculty, University of Copenhagen, Denmark.

Dr Avery is an Internationally recognized pioneer in IVF. and was for 27 years head of the IVF laboratory, and responsible for the research, which mainly included bovine, porcine and equine in vitro embryo technology. Dr Avery is a noteworthy embryologist, researcher and educator.

She became Doctor of Medicine, University Copenhagen, 1977 and PhD in Embryology, Royal Veterinary and Agricultural University, Copenhagen, 1991. And in 2007 Doctor in Veterinary Sciences, Royal Veterinary and Agricultural University, Copenhagen.

She has been a guest professor at Cornell University, New York, 1984, University Estate São Paulo, Jaboticabal, Brazil, 1993, University Wisconsin, Madison, 1996. Furthermore, she has been invited speaker to numerous national and international scientific conferences. She has worked from 2010 to currently, as Scientific Advisor and Consultant with Dr Stroebech optimizing and developing IVP media, and commercializing them into ready-to-use serum-free products. Thus, rendering a more accessible solution to IVP of bovine embryos and facilitated for more laboratories to implement the technique, without the trouble of media making and batch variation.

Martin Thomsen

CFO, COO

Martin Thomsen's ambition to collaborate with top professionals in the industry led him to join forces with Dr. Stroebech, forming a formidable team in the IVF market.

This partnership has been instrumental in delivering some of the most high-performing media globally, backed by rigorous quality standards.

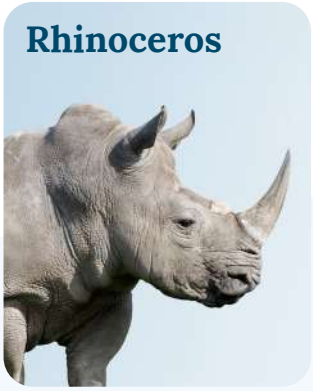
With 15 years of experience as CFO and COO for multinational corporations operating in various countries including China, USA, UK, Germany, Ukraine, and Scandinavia, Martin Thomsen brings a wealth of expertise to the table. Additionally, he has successfully spearheaded the transformation of three start-ups into thriving enterprises, demonstrating his proficiency in due diligence processes on both the selling and buying ends, including the successful divestment of a company to a private equity firm.

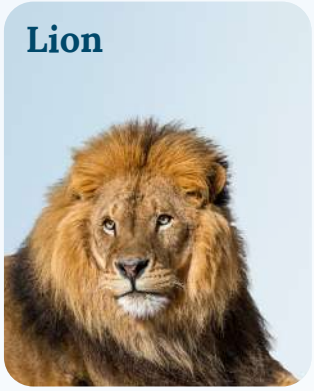
Notably, Martin Thomsen played a pivotal role in establishing a manufacturing facility in Hundested, Denmark, catering to Chinese investors for the production of infant formula intended for the Chinese market. His responsibilities included overseeing factory operations and production processes. Furthermore, he boasts extensive experience in engaging with financial institutions, suppliers, auditors, and government agencies, showcasing his adeptness in negotiations and relationship management across various stakeholders.

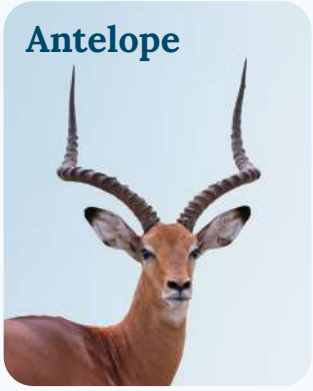
IVF Media Products

The media range applies to in vitro production of bovine embryos from both Ovum Pick Up and Slaughterhouse ovary collection. Additionally, the media can also be utilized for the production of endangered species, zoo animals, and wildlife.

All the media are ready-to-use and require no supplements, except for the equine range, which requires serum supplementation. As a customer you get a detailed Manual of Procedure and access to online support. Furthermore, we offer online training courses and individual troubleshooting sessions.







Quality Control Tests and Manufacturing

- Sterility tested (Fungal and Bacterial)
- Osmolality tested and pH tested
- Endotoxin tested
- Manufacturer is ISO9001 and ISO13485 certified
- Controlled environment and to human standards
- Bovine Embryo Assay (BEA)
- Batch analysis certificate available

Distribution

Stroebech Media is delivered within 1-3 days in Europe and within 7 days to most parts of the world. Get in touch to learn more about our distribution network.

Manufacturing

Our factory is ISO13485 certified

- Each batch of medium comes with a certificate for BEA test as well as sterility, fungal and endotoxin tests
- The production site is ISO9001 and ISO13485 certified
- All media are delivered in glass bottles

ISO standards are internationally agreed by experts and when a factory is certified it ensures state-of-the-art manufacturing in a controlled environment and is your assurance of quality.

ISO 13485, also known as the medical device regulatory system, is a quality system for the medical device industry. It also means that the factory is audited through the Medical Device Single Audit Program (MDSAP) .

Bovine IVF Media

Every medium batch is provided with a Certificate of Analysis with a high level of Quality Control (QC) release parameters.

As a customer you get a detailed Manual of Procedure and access to online support.
Furthermore, there will be online training courses and individual troubleshooting sessions.

All the media are serum-free and ready-to-use and require no supplements.

Key ingredients are listed at www.stroebech-media.com
The media range applies to in vitro production of bovine embryos from both Ovum Pick Up and Slaughterhouse ovary collection. The media can also be used to produce sheep, goat, buffaloes and camelid embryos. For endangered species, ZOO- and wildlife animals get in touch.

OPU Medium

500 ml. Prod. No. 2.01.500

For retrieval of oocytes from Ovum Pick Up and for Embryo Flushing (ET).

The medium is HEPES buffered, and does not require CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 37° C prior to use.

WASH Medium

50 ml. Prod. No. 1.02.050

For handling of oocytes and embryos outside of the incubator.

The medium is HEPES buffered, and does not need CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use.

IVM Medium

20 ml. Prod. No. 1.03.020

For in vitro maturation of oocytes in the laboratory.

The medium is Sodium Bicarbonate buffered and requires CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use.

H-IVM Medium

20 ml. Prod. No. 1.04.020.

For the in vitro maturation of oocytes outside the incubator.

The medium is HEPES buffered and does not require CO_2 equilibration, but it can also be used in a CO_2 Incubator (recommended without vial lid, if oocytes arrive to the laboratory before 20 hours of after IVM).

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use.

Semen Wash Medium

50 ml. Prod. No. 1.05.050

For washing of semen during centrifugation prior to in vitro fertilization.

The medium contains a very low concentration of Sodium Bicarbonate and is phosphate buffered and must therefore not be CO_2 equilibrated.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use with the lid on.

Sperm Gradient Upper Layer 45%

20 ml. Gradient Medium, Prod No. 2.10.020

For semen preparation for IVF ICSI and to be used in combination with Stroebech Semen Wash Medium.

The media are supplied sterile in 20 ml glass bottles for sperm separation by density gradient centrifugation.

The medium is Hepes buffered and does not require CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 35-37°C prior to use.

Sperm Gradient Lower Layer 90%

20 ml. Gradient Medium, Prod No. 2.11.020

For semen preparation for IVF ICSI and to be used in combination with Stroebech Semen Wash Medium.

The media are supplied sterile in 20 ml glass bottles for sperm separation by density gradient centrifugation.

The medium is Hepes buffered and does not require CO_2 equilibration.

The medium is ready-to-use and does not need any supplements

Medium should be preheated to 35-37°C prior to use.

IVF Medium

20 ml. Prod. No. 1.06.020

For in vitro fertilization of matured oocytes.

Suitable for culture in 4WP dishes with 500 µl/well or in 100 µl drops. An overlay of Stroebech Oil must be used to avoid evaporation.

The medium is Sodium Bicarbonate buffered and requires CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use.

IVC Medium

20 ml. Prod. No. 1.07.020

For in vitro culture of embryos.

The medium is a one-step medium that can be used without change of medium for the entire period from inseminated oocytes to the blastocyst stages. Suitable for culture in 4WP dishes with 500 µl/well or in 100 µl drops. Use Stroebech Oil as overlay to avoid evaporation. The medium is Sodium Bicarbonate buffered and requires CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use.

Holding Medium

20 ml. Prod. No. 1.09.020

For Embryo Holding/Transfer/Biopsy.

For Embryo transfer and holding, transportation and biopsy of in vitro and in vivo ET embryos. Keep embryos for as short time as possible in transfer straws. For longer transportation use vials and for transportation more than 24 hours use CO_2 equilibrated IVC medium. The medium is ready-to-use and does not need any supplements. Medium should be preheated to 38.8° C prior to use. The medium is HEPES buffered and does not require CO_2 equilibration, but it can also be used in a CO_2 Incubator.

Stroebech Heavy Oil

50 ml. Prod. No. 2.09.050

Peroxide tested Pre-washed Oil.

Stroebech Heavy Oil is a pharmaceutical grade light mineral oil*), pre-washed and sterile filtered light paraffin oil. Oil is often the key to poor results, use only BEA tested and Peroxide Value (POV) tested oil as minimum standard quality control.

*) Liquid paraffin oil is a mineral oil and is a by-product of crude oil distillation.

Vitrification Kit

3 vials each containing 2 ml. Prod. No. 2.20.006

3 Vials for Embryo Vitrification.

Obtain higher embryo survival and pregnancy rates with vitrification than after slow freeze.

Particularly in vitro and biopsied embryos benefit from this cryo-preservation method.

Media should not be pre-heated to more than 30° C prior to use – leave lid on in order to avoid evaporation.

Warming Kit

4 vials each containing 2 ml. Prod. No. 2.21.008

4 Vials for Embryo Warming.

Obtain higher embryo survival and pregnancy rates with vitrification than after slow freeze.

Particularly in vitro and biopsied embryos benefit from this cryo-preservation method.

Pre-heat media 38.8° C prior to use – leave lid on in order to avoid evaporation.

Oocyte Vitrification Kit

6 vials each containing 2 ml medium in glass bottles.
Prod. No. 2.22.010

6 Vials for Oocyte Vitrification.

Media should not be pre-heated to more than 30°C prior to use – leave lid on in order to avoid evaporation

Oocyte Warming Kit

6 vials each containing 2 ml medium in glass bottles.
Prod. No. 2.23.010

6 Vials for Oocyte Warming.

Pre-heat media 38.8°C prior to use -leave lid on in order to avoid evaporation

Small Ruminant IVF Media

Every medium batch is provided with a Certificate of Analysis with a high level of Quality Control (QC) release parameters.

The most important is the Bovine Embryo Assay BEA Test. There are huge differences in reproductive physiology between the mouse and cow, therefore a MEA test is not sufficient QC for a bovine medium.

OPU Medium

500 ml. Prod. No. 6.01.500

For retrieval of oocytes from Ovum Pick Up and for Embryo Flushing (ET).

The medium is HEPES buffered, and does not need CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 37° C prior to use.

WASH Medium

50 ml. Prod. No. 5.02.050

For handling of oocytes and embryos outside of the incubator.

The medium is HEPES buffered, and does not need CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use.

IVM Medium

20 ml.Prod. No. 5.03.020

For in vitro maturation of oocytes in the laboratory.

The medium is Sodium Bicarbonate buffered and requires CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use.

H-IVM Medium

20 ml. Prod. No. 5.04.020

For the in vitro maturation of oocytes outside the incubator.

The medium is HEPES buffered and does not require CO_2 equilibration, but it can also be used in a CO_2 Incubator (recommended without vial lid, if oocytes arrive to the laboratory before 20 hours of after IVM).

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use.

Semen Wash Medium

50 ml. Prod. No. 5.05.050

For washing of semen during centrifugation prior to in vitro fertilization.

The medium contains a very low concentration of Sodium Bicarbonate and is phosphate buffered and must therefore not be CO_2 equilibrated.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use with the lid on.

Sperm Gradient Upper Layer 45%

20 ml. Gradient Medium, Prod No. 2.10.020

For semen preparation for IVF ICSI and to be used in combination with Stroebech Semen Wash Medium.

The media are supplied sterile in 20 ml glass bottles for sperm separation by density gradient centrifugation.

The medium is HEPES buffered, and does not need CO_2 equilibration.

The medium is ready-to-use and does not need any supplements

Medium should be preheated to 35-37°C prior to use.

Sperm Gradient Lower Layer 90%

20 ml. Gradient Medium, Prod No. 2.11.020

For semen preparation for IVF ICSI and to be used in combination with Stroebech Semen Wash Medium.

The media are supplied sterile in 20 ml glass bottles for sperm separation by density gradient centrifugation.

The medium is HEPES buffered, and does not need CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 35-37°C prior to use.

IVF Medium

20 ml. Prod. No. 5.06.020

For in vitro fertilization of matured oocytes.

Suitable for culture in 4WP dishes with 500 µl/well or in 100 µl drops. An overlay of Stroebech Oil must be used to avoid evaporation. The medium is Sodium Bicarbonate buffered and requires CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use.

IVC Medium

20 ml. Prod. No. 5.07.020

For in vitro culture of embryos.

The medium is a one-step medium that can be used without change of medium for the entire period from inseminated oocytes to the blastocyst stages. Suitable for culture in 4WP dishes with 500 µl/well or in 100 µl drops. Use Stroebech Oil as overlay to avoid evaporation.The medium is Sodium Bicarbonate buffered and requires CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use.

Holding Medium

20 ml. Prod. No. 5.09.020

For Embryo Holding/Transfer/Biopsy.

For Embryo transfer and holding, transportation and biopsy of in vitro and in vivo ET embryos. Keep embryos for as short time as possible in transfer straws. For longer transportation use vials and for transportation more than 24 hours use CO_2 equilibrated IVC medium. The medium is ready-to-use and does not need any supplements. Medium should be preheated to 38.8° C prior to use. The medium is HEPES buffered and does not require CO_2 equilibration, but it can also be used in a CO_2 Incubator.

Stroebech Heavy Oil

50 ml. Prod. No. 6.09.050

Peroxide tested Pre-washed Oil.

Stroebech Heavy Oil is a pharmaceutical grade light mineral oil*), pre-washed and sterile filtered light paraffin oil. Oil is often the key to poor results, use only BEA tested and Peroxide Value (POV) tested oil as minimum standard quality control.

*) Liquid paraffin oil is a mineral oil and is a by-product of crude oil distillation.

Vitrification Kit

3 vials each containing 2 ml. Prod. No. 6.20.006

For vitrification of in vitro and in vivo embryos.

Obtain higher embryo survival and pregnancy rates with vitrification than after slow freeze. Particularly in vitro and biopsied embryos benefit from this cryopreservation method.

Media should not be pre-heated to more than 30° C prior to use – leave lid on in order to avoid evaporation.

Warming Kit

4 vials each containing 2 ml. Prod. No. 6.21.008

For warming of in vitro and in vivo embryos.

Obtain higher embryo survival and pregnancy rates with vitrification than after slow freeze. Particularly in vitro and biopsied embryos benefit from this cryopreservation method.

Pre-heat media 38.8° C prior to use – leave lid on in order to avoid evaporation.

Oocyte Vitrification Kit

6 vials each containing 2 ml medium in glass bottles.
Prod. No. 2.22.010

6 Vials for Oocyte Vitrification.

Media should not be pre-heated to more than 30°C prior to use – leave lid on in order to avoid evaporation.

Oocyte Warming Kit

6 vials each containing 2 ml medium in glass bottles.
Prod. No. 2.23.010

6 Vials for Oocyte Warming.

Pre-heat media 38.8°C prior to use -leave lid on in order to avoid evaporation

Equine IVF/ICSI Media

Every medium batch is provided with a Certificate of Analysis with a high level of Quality Control (QC) release parameters.

Equine OPU Medium

500 ml. Prod. No. 4.01.500

For retrieval of oocytes from Ovum Pick Up and for Embryo Flushing (ET).

The medium is HEPES buffered, and does not need CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.8° C prior to use.

Oocyte Holding Medium

20 ml. Prod. No. 3.12.020

For holding of oocytes prior to maturation.

For holding of oocytes to postpone maturation Oocyte Holding must be supplemented with 10% -20% serum.

Medium should be preheated to 22 °C prior to use.

The medium is HEPES buffered and does not require CO_2 equilibration.

Equine IVM Medium

20 ml. Prod. No. 3.03.020

For in vitro maturation of oocytes in the laboratory.

The medium is Sodium Bicarbonate buffered and requires CO_2 equilibration. The medium must be supplemented with 5 % serum.

Equine Hyaluronidase

5×1 ml. Prod. No. 4.02.005

For denudation of mature equine oocytes.

Equine Hyaluronidase medium is used in the oocyte denudation process. Hyaluronidase digests the hyaluronic acid between the cumulus cells, which makes it easier to denudate the oocytes.

Medium should be preheated to 37° C prior to use with the lid on.

NOTE: Do not incubate in a CO_2 incubator as it will lower the pH below 7.

Equine Semen Wash Medium

50 ml. Prod. No. 3.05.050

For washing of semen during centrifugation prior to ICSI.

The medium contains a low concentration of Sodium. Bicarbonate and is phosphate buffered and must therefore not be CO_2 equilibrated.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.2° C prior to use with the lid on. Advantages of using a non-gradient medium. Potential toxic gradients and excessive centrifugation is avoided and motility better preserved. Gentler for treating thawed re-frozen semen, Particularly suitable for when pieces of straw are used.

Sperm Gradient Upper Layer 45%

20 ml. Gradient Medium, Prod No. 2.10.020

For semen preparation for IVF ICSI and to be used in combination with Stroebech Semen Wash Medium.

The medium is HEPES buffered, and does not need CO_2 equilibration.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 35-37°C prior to use.

Sperm Gradient Lower Layer 90%

20 ml. Gradient Medium, Prod No. 2.11.020

For semen preparation for IVF ICSI and to be used in combination with Stroebech Semen Wash Medium.

The media are supplied sterile in 20 ml glass bottles for sperm separation by density gradient centrifugation.

The medium is HEPES buffered and does not require CO_2 equilibration.

The medium is ready-to-use and does not need any supplements Medium should be preheated to 35-37°C prior to use.

Equine PVP Medium

1 ml PVP medium in glass bottles. Prod. No. 4.10.001

For decreasing the motility of sperm for ICSI.

The PVP immobilizes spermatozoa due to the high viscosity.

Easier and more accurate selection of a single spermatozoa for ICSI.

Medium should be preheated prior to use.

Equine Swim Up Medium

50 ml. Prod. No. 3.11.050

For sperm swim up.

The medium is HEPES buffered, and does not need CO_2 equilibration, however, the medium can be placed inside the incubator during the swim up procedure.

Place tube at an angle to maximize surface to harvest more spermatozoa.

The medium is ready-to-use and does not need any supplements.

Medium should be preheated to 38.2° C prior to use.

Equine ICSI Medium

20 ml. Prod. No. 3.06.020

For intra cytoplasmic injection of matured oocytes.

The medium is Hepes buffered and does not require CO_2 equilibration.

The medium must be supplemented with 5 % serum.

Medium should be preheated to 38.2° C prior to use.

Equine One Step IVC Medium

20 ml IVC medium in glass bottle. Prod. No. 3.13.020

For in vitro culture of equine embryos from day 0 until blastocyst stage

The medium is sodium bicarbonate buffered and requires CO_2 equilibration.

The medium must be supplemented with 5 % serum.

Refresh medium at day 5-6.

Equine IVC Medium 1 - Cleavage

20 ml IVC medium in glass bottle. Prod. No. 3.07.020

For first step in vitro culture of embryos low glucose.

The medium is Sodium Bicarbonate buffered and requires CO_2 equilibration

The medium must be supplemented with 5 % serum.

Equine IVC Medium 2 - Blastocyst

20 ml IVC medium in glass bottle. Prod. No. 3.08.020

For in vitro culture of embryos from day 5 high glucose.

The medium is Sodium Bicarbonate buffered and requires CO_2 equilibration.

The medium must be supplemented with 5% Serum.

Equine Holding Medium

20 ml. Prod. No. 3.09.020

For transfer and handling of embryos outside the incubator.

For Transfer of equine in vitro or in vivo produced embryos. Keep embryos for as short time as possible in transfer straws. For longer transportation use or CO_2 equilibrated IVC-2 medium. Medium should be preheated to 38.2° C prior to use.

The medium is HEPES buffered and does not require CO_2 equilibration, but it can also be used inside a CO_2 Incubator. The medium can be used for in vivo derived equine embryos prior to transfer or vitrification.

Equine Stroebech Heavy Oil

50 ml. Prod. No. 4.09.050

Peroxide tested Pre-washed Oil.

Stroebech Heavy Oil is a pharmaceutical grade light mineral oil*), pre-washed and sterile filtered light paraffin oil. Oil is often the key to poor results, use only BEA tested and Peroxide Value (POV) tested oil as minimum standard quality control.

*) Liquid paraffin oil is a mineral oil and is a by-product of crude oil distillation.

Equine Vitrification Kit

3 vials each containing 2 ml. Prod. No. 4.20.006

3 Vials for Embryo Vitrification.

Obtain higher embryo survival and pregnancy rates with vitrification than after slow freeze Particularly in vitro and biopsied embryos benefit from this cryopreservation method.

Media should not be pre-heated to more than 30° C prior to use – leave lid on in order to avoid evaporation.

Equine Warming Kit

4 vials each containing 2 ml. Prod. No. 4.21.008

For warming of in vitro and in vivo embryos.

Obtain higher embryo survival and pregnancy rates with vitrification than after slow freeze. Particularly in vitro and biopsied embryos benefit from this cryopreservation method.

Pre-heat media 38.8° C prior to use – leave lid on in order to avoid evaporation.

Oocyte Vitrification Kit

6 vials each containing 2 ml medium in glass bottles.
Prod. No. 2.22.010

6 Vials for Oocyte Vitrification.

Media should not be pre-heated to more than 30°C prior to use – leave lid on in order to avoid evaporation.

Oocyte Warming Kit

6 vials each containing 2 ml medium in glass bottles.
Prod. No. 2.23.010

6 Vials for Oocyte Warming.

Pre-heat media 38.8°C prior to use -leave lid on in order to avoid evaporation.

Equine One Step IVC Medium

For in vitro culture of equine embryos from after ICSI until blastocyst stage

- ✓ Tailor-Made for continuous culture of Equine blastocysts: Equine embryos have unique requirements.
- ✓ Experience unparalleled success in equine embryo development with our revolutionary new IVC Medium.
- ✓ Say goodbye to the outdated belief that a 2-step IVC system with high glucose is the sole solution. Uncover the remarkable breakthroughs achieved through our highly efficient and effective Equine One Step Medium.
- ✓ There has been a widespread belief that equine embryos required sequential media containing high levels of glucose in the second step of culture. However, recent advancements have demonstrated that it is actually more efficient to continue the culture of equine embryos in a one-step medium without the inclusion of high glucose. This new approach has yielded more favorable outcomes in terms of embryo development and overall success rates.

Equine One Step Continuous Culture IVC Medium!

Our innovative formula is meticulously developed to provide an optimal environment for the growth, proliferation, and maintenance of equine cells in vitro for the entire culture period from after ICSI until blastocyst stage

- ✓ Enhanced Efficiency
- ✓ Stable Performance: Our advanced IVC Medium guarantees consistent and reliable results
- ✓ High-Quality Ingredients: We believe in providing only the best for your embryo production.
- ✓ Each component of our IVC Medium undergoes rigorous quality control measures to ensure purity, consistency, and performance, resulting in optimal cell growth and viability.
- ✓ The medium contains a synthetic serum replacement, but you still need to supplement with serum.

Key Ingredients

Insulin, Hyaluronic Acid, Calcium L-Lactate, Selen, L-Carnitine, Ethanolamine, Sodium Pyruvate, Transferrin, Albumin, Inorganic Salts, Essential and non-essential Amino Acids, Vitamins, Sodium Bicarbonate, L-Glutamine, Glucose, Phenol Red, Gentamycin.

Quality Control Tests and Manufacturing

- Sterility tested (Fungal and Bacterial)
- Osmolality tested and pH tested
- Endotoxin tested
- Manufacturer is ISO9001 and ISO13485 certified
- Controlled environment and to human standards
- Bovine Embryo Assay (BEA)
- Batch analysis certificate available

Storage

- Store in original unopened container refrigerated 3-9 °C and protected from light
- Do not expose to temperatures higher than 39 °C
- Do not freeze product it may precipitate < 2 °C
- The medium must be used within 10 days after opening, providing it has been handled aseptically and is kept refrigerated after opening
- Discard unused medium if it has been warmed

Bovine Embryo Development Rates are Affected when Oocytes are Matured in Different Vials Containing HEPES/Bicarbonate Buffered Medium

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Introduction

Lab ware for *in vitro* produced embryos is generally made from embryo tested plastic instead of glass. The quality of the plastic is crucial for the outcome, as plastic is often toxic to the gametes. In addition, gas molecules permeate through the plastic at a rate that depends on a variety of factors, such as diffusion coefficient and thickness of the plastic, thus influencing medium pH. Furthermore, medium volume in the vial and the number of cumulus-oocyte-complexes (COCs) are important factors contributing to pH alteration in the medium. Emission of CO_2 from cumulus cell metabolism contributes to a decrease in pH in low gas permeable vials, whereas a low medium volume increases pH in high gas permeable vials. HEPES buffer does maintain pH for a longer period of time, but not indefinitely when bicarbonate is also present.

Objectives

To choose the optimal system for oocyte transportation during maturation (IVM). Blastocyst rates were compared after maturing different numbers of oocytes, 5, 20 and 45, in glass vs plastic vials.

After screening several plastic vials, the least toxic was chosen. Two different maturation medium volumes, 50 % and 95 %, were assessed in the two different vials.

Results

In Experiment 1 the highest blastocyst rates, 40 % and 43 %, were obtained in glass vials with COC numbers between 5 and 20, in 50 % medium volume. The corresponding pH values in the maturation medium were 7.7 and 7.6 respectively, after 21 hours of IVM. The maturation medium pH was 7.3 at the start of IVM.

In Experiment 2 the highest blastocyst rates were also obtained in the glass vials, though not significantly different. In both experiments the lowest blastocyst rates, 28 % and 29 %, were obtained in glass vials with 45 COCs in both medium volume groups. The corresponding pH value in both groups was 7.0. Furthermore, there was a clearly visible pH gradient in the glass vials with 95 % medium volume. See Fig. 2.1.

Experiment 1
50 % medium volume/vial

Figure 1.1

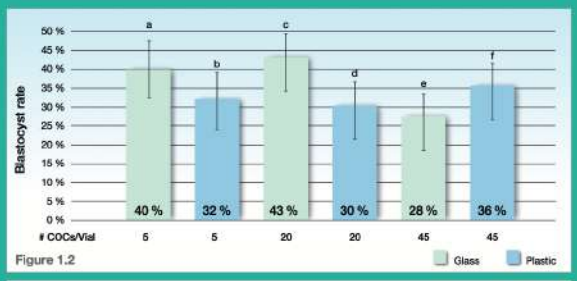

Figure 1.2

# COCs/Vial	COC	Blastocyst %	pH
Glass 5	167	40±7.4	7.7
Plastic 5	171	32±7.0	7.6
Glass 20	245	43±6.2	7.6
Plastic 20	223	30±6.0	7.6
Glass 45	220	28±5.9	7.0
Plastic 45	236	36±6.1	7.5

Table 1.1

Table 1.1, Figure 1.1 and Figure 1.2 Blastocyst rates expressed as percentage of inseminated oocytes (Mean ± SD). Four replicates with a total of 1488 COCs. Values in a column and within experiment with different superscripts were different
[a,b]: $P \leq 0.1$; [c,d]: $P \leq 0.05$; [e,f]: $\leq P\ 0.1$

Experiment 2
95 % medium volume/vial

Figure 2.1

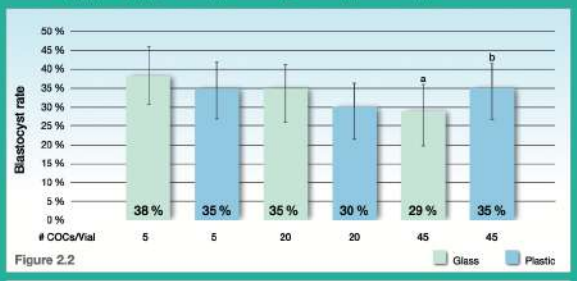

Figure 2.2

# COCs/Vial	COC	Blastocyst %	pH
Glass 5	178	38±7.1	7.3 - 7.5
Plastic 5	196	35±6.6	7.6
Glass 20	212	35±6.4	7.2 - 7.5
Plastic 20	164	30±7.0	7.5
Glass 45	301	29±5.1	7.0 - 7.2
Plastic 45	257	35±5.8	7.4

Table 2.1

Table 2.1, Figure 2.1 and Figure 2.2 Blastocyst rates expressed as percentage of inseminated oocytes (Mean ± SD). Four replicates with a total of 1375 COCs. Values in a column and within experiment with different superscripts were different
[a,b]: $P \leq 0.1$.

Materials and Methods

pH indicator: A pH indicator guide was created using color-matched photographs for glass (VWR DK, NSCAC 4015-96, 1 ml) and plastic vials (Sigma-Aldrich, CLS430661, 2 ml) containing media at various pH levels with measurements taken using a pH Meter (Fig. 3). The visual pH value estimated from vials was reconfirmed by measuring pH (Fig. 3).

Media: All media used were from IVF Bioscience, UK.

Experiment 1: Slaughterhouse oocytes were randomized into 6 maturation groups: 5, 20 and 45 COCs per vial in glass and plastic, respectively.

- **Matured** (IVM) 21 hours in BO-HEPES IVM, at 38.8 °C without CO_2. Vials contained 50 % medium volume/vial.
- **Fertilized** (IVF) overnight in BO-IVF under a 5.5 % CO_2 in a 38.8 °C humidified atmosphere.
- **Cultured** (IVC) in BO-IVC under a 5.5 % CO_2, 5.5 % O_2, 89% N_2 in a 38.8 °C humidified atmosphere.

Twenty-one hours post IVM the medium pH was assessed from Fig. 3 and presumptive zygotes were vortexed to remove cumulus cells and then cultured in BO-IVC for 7 days (8 days post-IVF) when blastocyst rates were assessed (Table 1.1., 1.2, Fig. 1.2. and 2.2).

Experiment 2: Same protocol as above, but with 95 % maturation medium volume/vial.

Statistical Analysis: Statistical analysis was performed with Chi Square and levels of significance at $P \leq 0.1$ and ≤ 0.05.

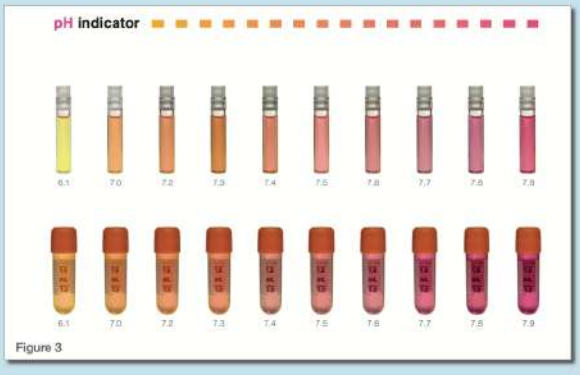

Figure 3

Conclusion

Oocytes can be successfully matured without CO_2 incubation during transport with the selection of the:

- Correct vial
- Correct medium volume
- Correct number of oocytes
- Correct maturation medium

Surprisingly, the acceptable pH range in the maturation medium is rather wide for subsequent good blastocyst rates. The highest rates, 40 %, 43 %, 38 %, and 35 % were obtained in pH 7.7, 7.6, 7.3 and 7.2, respectively.

The toxicity of plastic vials was demonstrated as the glass vial groups showed significantly higher blastocyst rates with more or less the same pH in both vial volume groups with the same number of oocytes, 5 and 20, during maturation.

The gas permeability in the plastic vials was clearly demonstrated as the pH was maintained in all plastic groups at 7.4 - 7.6 independently of number of oocytes and medium volume. In the glass vials with 45 oocytes in both medium volume groups the pH dropped to 7.0, impairing the blastocyst rates. Furthermore, when the medium volume was 95 % a pH gradient occurred (0.2 to 0.3 pH units), with the lowest pH in the medium surrounding the COCs at the bottom of the glass vial.

Final Conclusion:

The optimal blastocyst rates were obtained when oocytes were matured in glass vials in 50 % medium volume/vial and with 5 - 20 oocytes per vial.

Acknowledgements: The authors acknowledge and appreciate the financial support from the Danish Market Development Fund

In vitro production of bovine embryos: revisiting oocyte development and application of systems biology

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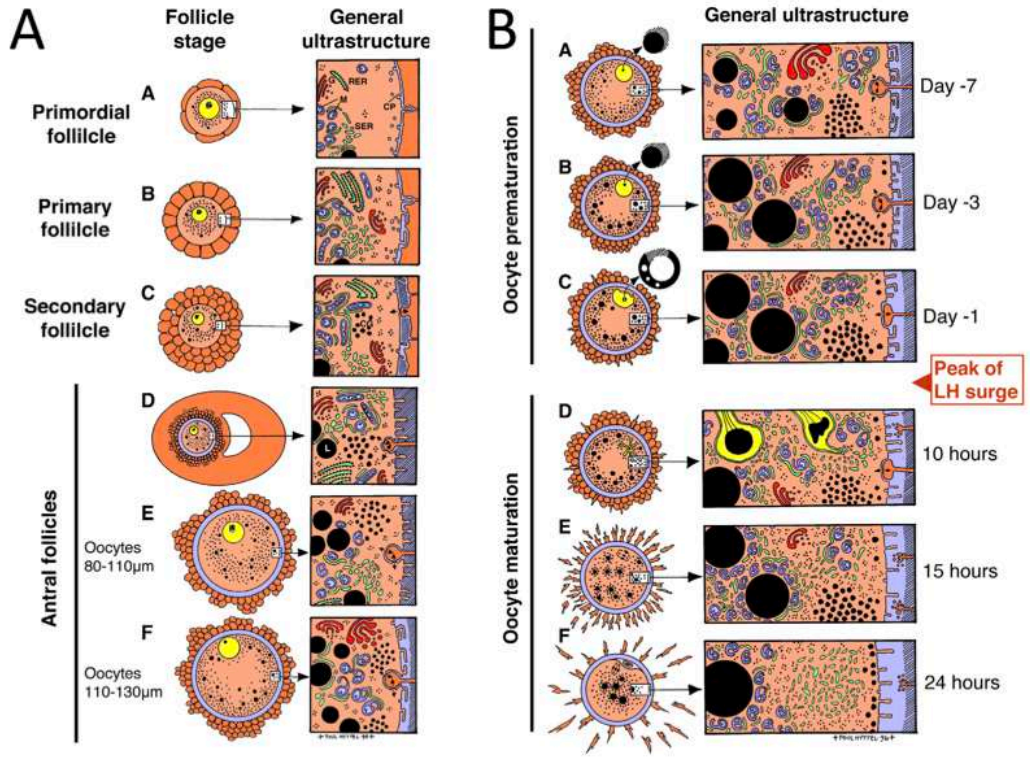


Figure 2. **(A)** Schematic drawing of bovine oocyte growth. **(A_A)** Primordial follicle with oocyte surrounded by a single layer of flattened granulosa cells. The central oocyte nucleus (yellow) is surrounded by round mitochondria (M), smooth (SER) and rough (RER) endoplasmic reticulum and small Golgi complexes (G). The oocyte cortex presents numerous coated pits (CP) and vesicles. The oocyte is transcriptionally quiescent. **(A_B)** Primary follicle with oocyte surrounded by a single layer of cuboidal granulosa cells. The eccentric oocyte nucleus is surrounded by round and elongated mitochondria. **(A_C)** Secondary follicle with oocyte surrounded by more than one layer of cuboidal granulosa cells. Small patches of zona pellucida material (hatched areas) have appeared and gap junctions (small arrows) developed between the oocyte and the granulosa cells. In the oocyte, the first small clusters of cortical granules (CG). The oocyte displays initial transcriptional activity. **(A_D)** Early tertiary follicle up to about 1mm. The follicular antrum has developed and the oocyte is surrounded by cumulus cells of which the innermost possess projections that penetrate the zona pellucida, invaginate the oolemma and make gap junctional contact to it. In the oocyte, the organelles have attained a more even distribution throughout the ooplasm, elongated mitochondria have become more numerous, lipid droplets (L) have become common, and the number and size of the cortical granule clusters have increased. Erect microvilli have become embedded within the zona pellucida. The oocyte is transcriptional active. **(A_E)** Tertiary follicle up to about 3 mm as represented by oocytes at 80 to 110 µm in diameter. The number of lipid droplets in the oocyte has increased. Oocytes less than 100 µm are transcriptionally active, whereas such at 100 to 110 µm transcription decreases in abundance. **(A_F)** Larger tertiary follicles as represented by oocytes at more than 110 µm in diameter. In the oocyte, the organelles have been dislocated to the periphery, the number of lipid droplets have increased as have the size of the Golgi complexes. The microvilli have been released from the zona pellucida and pile up in stacks in the perivitelline space. The peripheral oocyte nucleus presents has deceased its transcriptional activity to a minimum. **(B)** Schematic drawing of ultrastructural aspects of bovine oocyte prematuration in the dominant follicle up to the LH peak and maturation after the peak. **(B_A)** Oocyte from a dominant follicle 6 days before the LH peak. The general ultrastructure is identical with that obtained at the end of oocyte growth (A_F). **(B_B)** Oocyte from a dominant follicle 3 days before the LH peak. The number of microvilli stacks have decreased as have the size of the Golgi complexes, the amount of lipid droplets has increased, and the cortical granule clusters have dislocated to a more superficial location. **(B_C)** Oocyte from a dominant follicle on the day before the LH peak. Some individual corona cells display elongation and the corona cell projections have been retracted to a more superficial location, the perivitelline space has enlarged, the microvilli have become more erect, and the size of the Golgi complexes has been further reduced. Moreover, the envelope of the oocyte nucleus has become undulating and the nucleolar remnant has transformed into a ring-like structure. **(B_D)** Oocyte at "germinal vesicle breakdown" from an ovulatory follicle at 9-12 h after the LH peak. The perivitelline space develops further and in the oocyte the mitochondria tend to arrange around the lipid droplets and the nuclear envelope is dissolved into tubules of SER and microtubules appear adjacent to the condensing chromosomes. **(B_E)** Oocyte at MI from an ovulatory follicle at about 15 h after the LH peak. The number and size of the lipid droplets has increased and mitochondria have assembled around the droplets and these conglomerates have attained a more even distribution. Numerous ribosomes have appeared especially around the chromosomes and the size of the Golgi complexes has decreased further. **(B_F)** Oocyte at MII from an ovulatory follicle at about 24 h after the LH peak. The bulk of the cortical granules are distributed at solitary positions along the oolemma. The lipid droplets and mitochondria have attained a more central location in the ooplasm leaving a rather organelle free peripheral zone in which the most prominent features are large clusters of SER (adapted from Hyttel, 2011).

Stroebech Heavy Oil

Stroebech Heavy Oil

50 ml Peroxide tested Pre-washed Oil

Description

Stroebech Oil is a pharmaceutical grade light mineral oil*), pre-washed and sterile filtered light paraffin oil. Oil is often the key to poor results, use only BEA tested and Peroxide Value (POV) tested oil as minimum standard quality control.

*) Liquid paraffin oil is a mineral oil and is a by-product of crude oil distillation.

Oil viscosity influences temperature, pH and osmolarity. Oil with higher viscosity has demonstrated higher yields compared to regular oil, in terms of blastocyst development and quality in terms of blastocyst formation rates and cell counts.

Advantages: easier to handle, droplets are maintained better, and oil doesn't spill over as easy.

Temperature, pH is better maintained and increased media osmolarity is avoided both during handling and during culture. This has a positive impact on blastocyst rate formation – especially for the longer culture periods require in some species.

The unit of viscosity is newton-second per square metre, which is usually expressed as pascal-second in SI units, cP.

We have increased the viscosity from < 30 cP to 95-140 cP.

Quality Control: we still apply the strict quality control tests of each batch which besides a bovine embryo assay (BEA) test also includes *) POV, sterility, and endotoxin tests.

*) The peroxide value (POV) is defined as the reactive oxygen contents expressed in terms of milliequivalents (meq) of free iodine per kilogramme of fat. It is determined by titrating iodine liberated from potassium iodide with sodium thiosulphate solution. Oils with POV well below 10 meq/kg are considered fresh – The limit for our oil is <0.1meq/kg

Quality Control

All media batches are Bovine Embryo Assay, BEA tested in addition to MEA prior to release.

There are huge differences in reproductive physiology between the mouse and a woman, obviously also between a cow and a woman. The most obvious similarity in cow and woman is gestation time and that they do not give birth to litters like mice.

However, the MEA test is considered sufficient for quality control release of ART media. When media are tested in the BEA, that all have passed MEA, they often give significantly different blastocyst rates as well as considerable morphologic and kinetic differences in the BEA test.

Fertilization and capacitation are species specific and a MEA test mostly assesses blastocyst development from either one-cell or two-cell stage embryos. Should a batch variation occur it will not be detected in a MEA test, where release parameter is > 80 % blastocyst rate. In the BEA test, however, a difference in medium quality can be detected even though the medium has passed the MEA test. For instance, 35 % vs 55 % blastocyst rates indicate two media are different, however both media will have passed the MEA. Hence, the MEA is a mere toxicological bio-assay, whereas the BEA also provides functional and performance data.

Therefore, we have decided that every single batch manufactured will undergo the BEA test in addition to the required MEA Test.

How is the BEA test conducted?

Ovaries from slaughterhouse cows are collected and in the laboratory the oocytes are aspirated from all follicles size 10 mm-35 mm. Approximately 8 oocytes per ovary is on average obtained. They are subsequently matured, fertilized and cultured. Each new medium is tested in a control group with a minimum 150 oocytes per group. Blastocyst rates and kinetics and morphology are evaluated as well as hatching rates. The average blastocyst rates in the BEA test are between 40-50% and thus indicate performance differences, whereas 80 – 90 % blastocyst rates in the MEA test only indicate whether the medium is toxic or not.

Plastic vs Glass bottles

Only in glass bottles can you maintain stability of the active compounds in the media for up to 18-24 months. Plastic will decrease quality of media over time and even contribute to toxicity and will always have a limited shelf-life. The entire batch must be manufactured in one day. That means that no stock solutions should be stored for a longer period of time and that the entire batch should be manufactured and bottled within the same day and not stored overnight.

Batch Certificate

Every single batch should always come with a batch certificate stating that sterility, fungal and endotoxin tests have been performed and passed quality control specifications. For some products pH will be measured, but it doesn't make sense to have a set pH value release criterion for pH, if the buffer is CO_2 calibrated with bicarbonate.

Comparison of embryo development in human, cow and mouse.

	Human	Cow	Mouse
Oocyte diameter (µm)	110-120	120	70-90
Stage at zygotic genome activation	4-8 cell	8-16 cell	2 cell
Time to reach			
2-cell stage (hours)	30	36	18-20
Blastocyst (hours)	120	150	70
Hatching (hours)	150	200	100
Implantation (days)	9	21	4

Based on: Virtues and limitations of the preimplantation mouse embryo as a model system, Robert A Taft, Theriogenology 2007.

Read the full article here:
https://doi.org/10.1016/j.theriogenology.2007.09.032

Amino acid

Amino acid uptake and utilization also differ among species with mouse embryos not requiring amino acids to develop to the blastocyst stage, in contrast to bovine and human embryos.

pH

Mouse embryos are less sensitive to and recover more easily from changes in pH than either human or bovine embryos.

Glucose

The ability to utilize glucose varies among species. Human embryos do not utilize glucose due to limited availability of hexokinase. The situation is more complicated for the mouse, as embryos from some strains can metabolize glucose while others cannot. Cattle embryos are able to metabolize glucose, although this is affected by culture conditions.

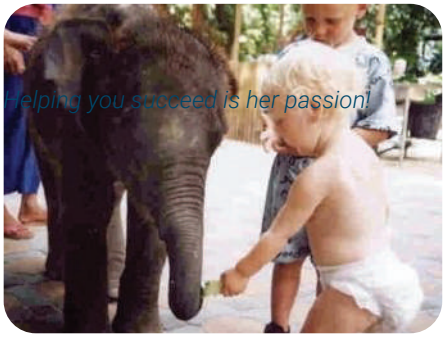
The strains of mice and developmental stage

Some of the controversy regarding the perceived value of the mouse embryo assay is likely the result of the conditions under which the assay was conducted. The strain of mouse used, stage of embryo used and the culture conditions employed may also affect the outcome. Thus, embryos from inbred strains may provide a better model, and the developmental stage of the embryo will also impact the result. For instance, one-cell embryos are more sensitive than two-cell, four-cell or eight-cell embryos. Finally, the type of media also influences the assay. For instance the absence of protein in the media improves the sensitivity of the assay.

Our Soul and Passion

Dr Stroebech is dedicated to improve IVF worldwide and contributed to the first frozen IVF calves born in India, the first buffalo pregnancy in Pakistan and counts many happy laboratory owners worldwide.

She has consulted with more than 300 IVF laboratories globally to help them get established or improve their results world-wide and has been involved in numerous animal reproduction research projects at Universities in many countries.



Helping you succeed is her passion!

Testimonials

"We have been using Stroebech media for bovine IVF for a short time but have seen an immediate improvement in blastocyst yield and quality. Having such off-the-shelf, ready-to-use, media has significantly simplified our laboratory workload and workflow."

Patrick Lonergan PhD, DSc, MRIA
Professor of Animal Reproduction
School of Agriculture and Food Science
University College Dublin, Ireland

"I want to acknowledge the efficiency of Stroebech Media for buffalo in vitro embryo production. Since, we included Stroebech Media for Buffalo IVF Program, embryo production and pregnancy has considerably improved."

Qaisar Shahzad
Technical Manager, Royal
Cell Biotechnology
Royal Cell Biotechnology
China/Pakistan

"Using Stroebech Media for bovine embryo production we achieved 10% better cleavage and blastocyst rate in group culture. And for single embryo culture the cleavage and blastocyst rate improved remarkably even up to 20%."

Marilin Ivask, PhD
Estonian University of Life Sciences, Tartu, Estonia
Institute of Veterinary Medicine and Animal Sciences
Chair of Animal Breeding and Biotechnology

"It was such a wonderful feeling when I had beautiful blastocysts in my first SH IVP program in a new lab which I was setting up. Stroebech Media is a really great IVF media with the best technical support, thank you very much Lotte for continuous support and encouragement!"

Zsófia Vigh,
Lab manager
Milkmen Ltd.-Embryo Ltd,
Hungary

"The Stroebech Media IVF suite is working extremely well in our bovine IVF laboratory. We have high blastocyst yield and excellent morphology. We appreciate the professional technical support and fast reply. I highly recommend Stroebech Media for research IVF."

Ylva Sjunnesson, DVM, PhD
Associate Professor
University of Agricultural Sciences, Sweden

"I am using Stroebech Media for equine embryo production and have very satisfactory results with high blastocyst and pregnancy rates, also after vitrification. I appreciate all the technical support and help we get"

Ruben Francisco Vazquez, PhD
IVF Laboratory, Xenetica Fontao, Galicia, Spain

"Stroebech Media comes with a detailed protocol and prompt support with lots of world class knowledge and professional consulting. The success of my embryo lab is based on the help from Dr Stroebech. Lotte is a wonderful person with endless passion in the field of IVF. Thank You!"

Elina Mark, DVM
Embryologist Scientist
Estonian University of Life Sciences, Tartu, Estonia

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