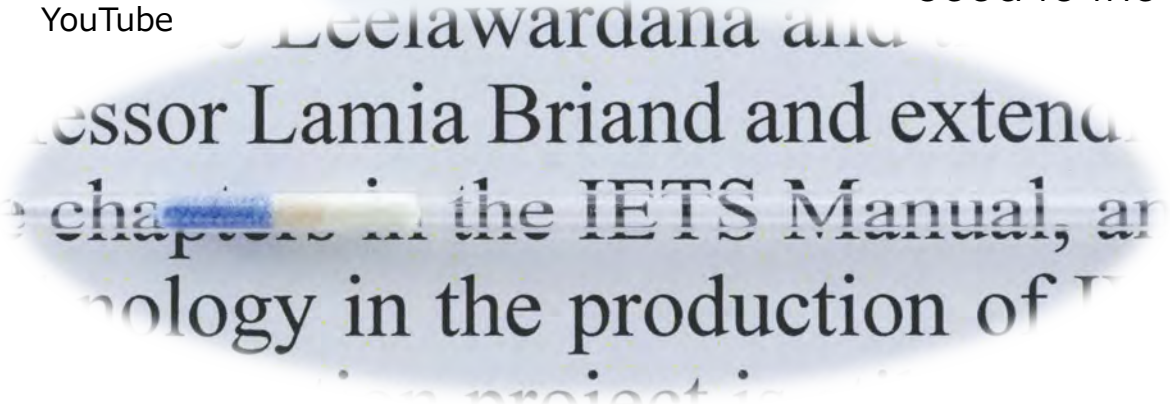
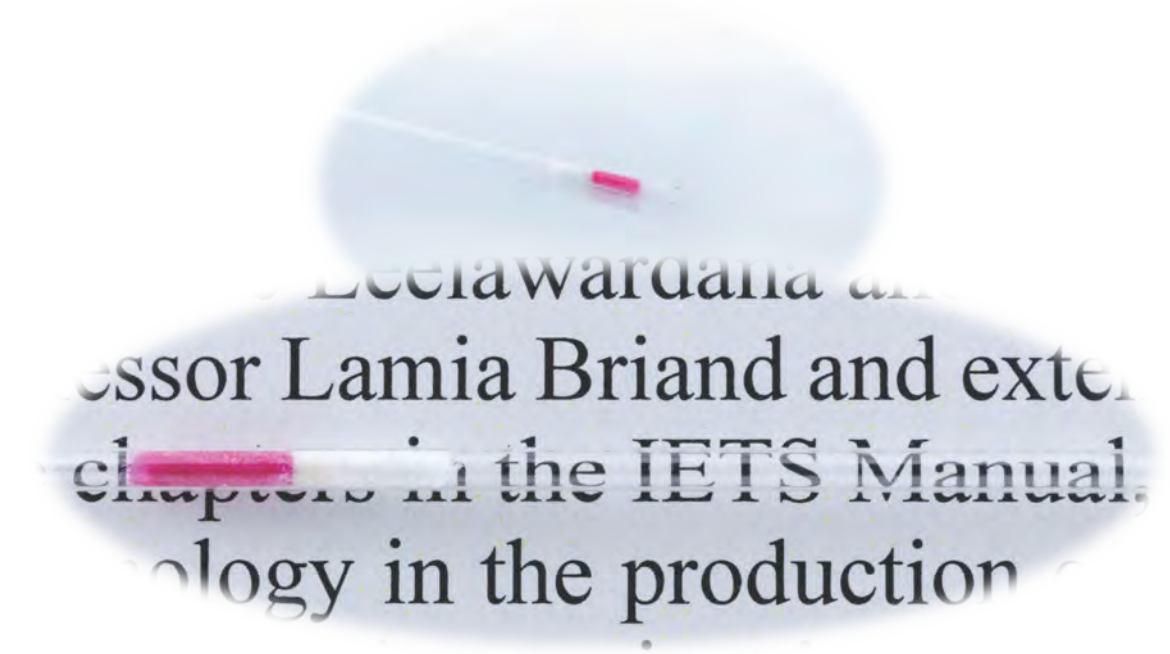
iSeeding Co., Ltd. 0.25ml Plastic Straw for Bovine Embryo Made in Japan

YouTube

Seed to the Future



iStraw®：with Function of automatic Ice Formation



Emvisible Straw®

The letters under the straw are clearly visible without distortion!

Manufacturer and distributor：iSeeding Co., Ltd.

2-1-18 Sakuradai, Ushiku, Ibaraki 300-1217, Japan TEL:

090-9546-1961 (President Toshiyuki Kojima, Ph.D.)

k1887523@kadai.jp; https://iseeding.co.jp
Official X: https://x.com/AnimoScience84
Manufacturer：Gunze Co., Ltd.

FAQ Frequently Asked Questions

① What makes iSeeding's straws different from other companies' straws?

The price of our straws is set higher than domestic and foreign non-sterile bulk straws (sold in units of 500~2000 straws). In addition, the straw is enclosed in a packaging material that does not break the seal for 3 years under proper storage conditions (avoid direct sunlight, ultraviolet rays of germicidal lamps, and high temperature and humidity), and the production cost is relatively high.
Since the start of sales, many users have praised our straws for being very convenient and highly evaluated for being able to use them immediately after purchase without worrying about the EO gas sterilization after washing and drying each bulk straw with sterile distilled water, and the subsequent degassing period.
In addition, since the start of sales, there have been no complaints that the cotton plug part is too hard to push out the straw contents, or conversely, the cotton plug falls out during thawing. This is because the pressing pressure is controlled by a digital control device during the manufacturing process. At the time of its release, there were complaints that liquid would leak from the outer cotton stopper, but in response to this, we took measures to increase the length of the outer cotton stopper and insert the core thread into the center of the braid. Next, we ask you to stop suction when the gelling agent (sodium alginate) is wet.

The straw itself is outsourced to a domestic plastic extrusion company, but the finished straw is much more transparent than the straws of foreign companies that use similar materials. Japan was able to reaffirm the high level of technical capabilities of the company, which has a higher raw material cost than PVC (polyvinyl chloride) used in bulk straws, but the benefits outweigh it.
Occasionally, we hear that the straw ruptures when melting a straw made by another company, but in most cases, this is due to liquid nitrogen entering the straw due to a defective seal/If you are using an identification plug, it may be due to air remaining between the plug and the liquid. All straws are carefully inspected visually.
In the case of iStraw® with a built-in automatic/automatic ice formation function, the inner cotton plug is impregnated with silver iodide, which is the ice crystal nucleus, and because the number of steps of the iStraw® is the other Emvisible Straw® the price is higher for more. For example, an ice crystal formation confirmation test (10% EG solution is aspirated and immersed in an alcohol bath at -7°C within 2 minutes to confirm that the straw with ice crystals is accepted as a pass within 2 minutes) is indispensable during the manufacturing process.
As mentioned above, we pay close attention to every corner of the manufacturing process, product inspection, and packaging so that users can use our straws with peace of mind. We will continue to make unremitting efforts to reduce costs at the manufacturing stage, including the automation of the manufacturing process, and we ask for your understanding.

The confirmation test to see if ice automaticly occurs within 2 minutes at -7℃ in the manufacturing process.

iStraw植氷試験

植氷可否(○=成功, ×=失敗)

2024.9.12

本数	植氷結果	本数	植氷結果
1	○	51	○
2	○	52	○
3	○	53	○
4	○	54	○
5	○	55	○
6	○	56	○
7	○	57	○
8	○	58	○
9	○	59	○
10	○	60	○
11	○	61	○
12	○	62	○
13	○	63	○
14	○	64	○
15	○	65	○
16	○	66	○
17	○	67	○
18	○	68	○
19	○	69	○
20	○	70	○
21	○	71	○
22	○	72	○
23	○	73	○
24	○	74	○
25	○	75	○
26	○	76	○
27	○	77	○
28	○	78	○
29	○	79	○
30	○	80	○
31	○	81	○
32	○	82	○
33	○	83	○
34	○	84	○
35	○	85	○
36	○	86	○
37	○	87	○
38	○	88	○
39	○	89	○
40	○	90	○
41	○	91	○
42	○	92	○
43	○	93	○
44	○	94	○
45	○	95	○
46	○	96	○
47	○	97	○
48	○	98	○
49	○	99	○
50	○	100	○

Carried out in Tsuyama Gunze.

② Advantages of automatic ice formation (probability of reducing human error). What does it mean?

In general, it is said that people will always make a mistake 3 out of 1,000 times, even if they work with a moderate amount of tension. There are 3 in 1,000 mistakes that are overlooked and leaked without noticing the mistake, and if you multiply this, you get 9 in 1,000,000. It is said that the probability that a human will make a mistake is 1 in 20 times when dialing a phone and 1 in 100 times when performing a simple repetitive task.
*"How to deal with human error" Hideki Kasahara (2007) Kajima Publishing

However, as shown on page 22 of this collection, “Factors that cause human error during manual ice transplantation," the actual ice planting error is more than 1 in 100 times because the technology is not standardized in manual ice transplantation. It is imagined that it will happen at a slightly higher rate.

With iStraw®, you can completely avoid the human error associated with manual ice planting, so you don't have to worry about ice planting. In other words, if the advent of iStraw® fosters understanding that ice transplantation is a very important step, it is expected that standardization will progress and errors during manual ice transplantation will naturally decrease.

③ By using iStraw®,does the conception rate increases?

The reasons for the increase in conception rate can be as follows.

A. By using electron beam sterilized straws, there is no concern about residual gas, unlike EO gas sterilized straws. 6

B. Due to automatic ice implantation, (1) the equilibrium time between the embryo and the cryopreserved solution is not unnecessarily long because the ice is implanted almost simultaneously even with a large number of ice plants, (2) the risk of excessive temperature stimulation on the embryo is reduced, and (3) the growth rate of ice crystals in the straw is constant and close to ideal.

C. Since the straw is highly transparent, it is possible to evaluate the quality of the embryo after thawing through the straw, which leads to the effective use of the embryo (transfer of poor-quality embryos can be avoided).

D. Although it is not possible to say for sure whether the above reasons alone will increase the conception rate, it is also considered that the straw that "gives a sense of security" to ET technicians is also an important factor.

* At present, there is no difference in conception rate in the control group (when manually icing using straws made by other companies or Emvisible straws® and using the automatic icing function by iStraw® is used (see the table on the next page).

In iStraw®, automatic ice crystal formation from the inner cotton plug was confirmed by immersion in a liquid layer at -7°C, and the liquid layer containing the embryo was completely frozen, and slow cooling was performed to -30°C (using YT programmable freezer made by Yamane Tech).

	移植数	受胎数	受胎率（%）
iStraw	86	43	50.0
通常のストロー	108	205	52.7

For ordinary straws, manual icing was performed 1 minute after immersion in a liquid layer at -7°C, and slow cooling was performed to -30°C after confirming that the liquid layer containing the embryo was completely frozen (using YT programmable freezer made by Yamane Tech). The composition of the cryopreserved solution is 1.5M (8.3%) ethylene glycol +

0 sucrose + 20% calf serum (composition of the base solution: Dulbecco PBS; Gibco14040-133 with 1mg/ml glucose, 36μg sodium pyruvate, 0.4% albumin + penicillin streptomycin)

- Currently (as of August 2023), we are continuing to conduct frozen embryo transfer trials in the test and control areas.

What our users are saying

[iStraw user Agricultural cooperative corporation in Tsu City, Mie Prefecture]:
With the advent of the dreamy iStraw®, this is it! I no longer have to worry about equilibrium time when freezing a large number of embryos!

[iStraw® user, practicing veterinarian in Iwate Prefecture]
I'm glad I used iStraw® because of the quality of it.

《Emvisible Straw Users: ET-related companies in Kagoshima Prefecture》
Until now, I have been using imported straws that have been sterilized by electron beams, but I liked the price and high transparency, so I replaced all the straws with Emvisible straws.

《Emvisible Straw User: Artificial Insemination Technician of the Fukuoka Prefecture Dairy Federation》：
When transferring embryos to the YT gun, in the case of imported straws that we had been using, the cotton stoppers were too hard to press, so we used a straw cutter to cut between the cotton stoppers, but with the Emvisible Straw, we have eliminated that trouble, so the work has become very smooth.

《Emvisible Straw user, practicing veterinarian in Aichi Prefecture》：
I always check the position of the embryo when I suck it into a straw, but with the Emvisible Straw, the time to find the embryo is reduced because of the high transparency, which is very helpful, especially when there are a lot of embryos.

《Emvisible straw user, practicing veterinarian in Ibaraki Prefecture》：
I think the Emvisible straw is a straw that gives a sense of security to ET technicians, especially lab technicians.

Here are some examples of technical questions related to ET

(1) To prevent degradation of survivability from egg recovery to the start of freezing, is there an acceptable amount of time?

It can be said that the time between egg recovery and the start of freezing (to be precise, the point at which straws are placed in a cooling vessel at -7 ° C and the iceing operation begins) is positively reflected in the conception rate (that is, the later it is, the higher the risk of a decrease in conception). It is necessary to transfer it into a suitable holding solution (isotonic, filtered sterile, containing the right amount of protein sources and nutrients) in a sterile test tube (and then apply it to the skin to prevent the temperature from dropping). It is necessary to bring a set of fertilized egg search tools, such as Pasteur pipettes, to the farm, and it is contraindicated to leave them in the recovery solution for several hours. Previously, a research paper was published in the American scientific journal Theriogenology that showed that the survival after freezing and thawing decreases with the passage of time. I remember that the limit was 2~3 hours with the collected solution, but please consider that the sooner the better. (1) To prevent degradation of survivability from egg recovery to the start of freezing.

(2) What is the suction style for the straw?

In the case of conventional manual ice transplantation, it is absolutely necessary to cool as far away from the embryo as possible (the meaning of "as far as possible" means the distance at which the embryo is not subjected to temperature shock by manual ice transplantation) to reliably produce ice crystals, and to confirm that the ice crystals grow in the straw. It is not clear how far away you need to keep the embryo, but empirically, icing is definitely possible by cooling a single straw in liquid nitrogen for 5 seconds and touching the outer wall of the liquid phase part of the straw tube for 3 seconds. Since it is almost impossible to see the position of the embryo with the naked eye, multiple columns are provided to prevent ice planting directly above the embryo, and the column to place the embryo is determined in advance. There is always a risk of excessive temperature shock to the embryo (the liquid temperature inside the straw sandwiched between tweezers cooled with liquid nitrogen will be approximately -80 ° C). If it is too large, it will take time for the ice crystals to spread over the entire straw. It is safer to use a maximum of 3 mm. If the cryopreservation solution is continuously sucked into one straw, the inner wall of the straw in the bubble part is wet, so there is no problem for the ice crystals to grow. Immediately after the bubble is sucked in, a measure may be taken to remove wetting of the inner wall of the straw by lightly touching the straw stump on the kimwipe, etc.

The total length of the straw is constant at 133mm, even if the manufacturer is different, I think it will vary slightly depending on the manufacturer, but the effective length is about 110mm, excluding the cotton plug part and the heat sealing part. If the air layer on the heat seal (seal) side is 10 mm, the length of the column will be about 31 mm each. Repeat the process of providing a 3 mm air layer (30 mm of liquid→ 3 mm of air→ 30 mm of liquid→ 3 mm of air→ 30 mm of liquid).

Currently, we are asking Professor Osamu Dochi of Rakuno Gakuen University to conduct a test on the effects of the appropriate liquid layer column arrangement and the position of the length and embryo on the survivability after thawing when using iStraw (as of August 8, 2024).

(3) Precautions for egg recovery and appropriate temperature in the embryo processing laboratory. How much is better to set?

When mammalian embryos are exposed to low temperatures (15°C or lower), the lipid bilayer of the cell membrane undergoes irreversible structural changes, making it difficult for the cell membrane to function properly (semi-permeability, etc.). It may be a good idea to switch to LED lamps that do not emit ultraviolet rays and compatible lighting fixtures in the lab.
However, even if you keep the temperature above 15°C, it is wise to avoid sudden fluctuations in temperature. For example, room temperature → 35°C→ room temperature is contraindicated.

(4) Tell me about the appropriate equilibrium time between the embryo and the cryopreservation solution.

The equilibrium time affects the viability of embryos after freezing and thawing. However, this does not mean that the shorter the better, but rather that “equilibrium” (the point at which the cryopreservation solution penetrates into the cells of the embryo and occupies almost the same volume as the embryo in the isotonic holding solution before being transferred to the cryopreserved solution) requires a certain amount of time. It is affected by the type of cell membrane permeable cryoprotectant such as ethylene glycol, propylene glycol (also known as 1,2propanediol), glycerol, DMSO (also known as Me2SO4), the species of the embryo, the stage of development of the embryo, and the presence and concentration of cell membrane impermeable freeze agents such as sucrose. The main reason why direct transfer has become possible is that a cryoprotective agent with a high cell membrane permeation rate for the embryo of the animal has been found. Therefore, it is theoretically okay to move the cow embryos into a petri dish containing 1.5 MEG, immediately suck them into a straw, and freeze them immediately.→ When using a cryoprotective agent other than EG or using it in combination with other antifreeze agents, it is recommended to immerse the embryos in the cryopreservation solution and measure the time from shrinkage to re-expansion to the original volume under a microscope. Propylene glycol also infiltrate into the cow embryo relatively quickly. In the case of glycerol, it takes 15< minutes to penerate into the cytoplasm of cow embryo at room temperature, so it necessary to avoid osmotic shock.

(**5) Rapid cooling from room temperature to ice-planting temperature, which is generally practiced, is a problem, isn't there?**

In the current slow freezing of cow embryos, straws that have been equilibrated at room temperature are immersed in and loaded directly into an alcohol vessel/cooling chamber at -7°C. Direct immersion at 7°C was thought to have no effect on survivability, and assuming a room temperature of 25°C, it was possible to save more than 30 minutes before the start of ice transplantation.

(6) I would like to know the proper ice planting temperature ot bovine embryos in slow freezing (so-called, slow freezing method).

First of all, let's review the significance of ice transplantation: by artificially inducing the formation of ice crystals in the extracellular fluid at a temperature slightly lower than the freezing point of the cryopreserved solution, it promotes "extracellular fluid concentration→", "osmotic pressure increase", →"excretion of fertilized egg free water from the cell". As the supercooling progresses, the entire cryopreserved solution,

usually sucked into a straw at around -12°C, freezes instantaneously, and the temperature rises sharply to near the freezing point of the solution (it rises almost vertically when monitored by a temperature sensor). This sudden increase in temperature itself is not harmful to the embryo, but rather the temperature inside the straw that rises due to the latent heat of coagulation is the temperature of the programmed freezer (in this case, -12° C), the cooling rate increases in an attempt to catch up, causing a fatal injury called intracellular freezing to embryos. Ice implantation has been performed since the beginning of development (1972) in the slow cooling method of embryos (mice). The first ice transplantation was performed in 1932 by Robert Chambers of the Low Temperature Research Station at the University of Cambridge, England and H. P. Hale (Proceedings of the Royal Society B).

I'm not sure why, it has been misunderstood that it is better to keep the latent heat of coagulation as low as possible in icing, because the increase in cooling rate due to coagulation latent heat generation at the time of supercooling progresses causes a fatal injury to cells called intracellular freezing. The most important thing in ice transplantation process is that the portion of the solution in which the embryo is located is covered by ice crystals during the holding time (generally set to 10 minutes) as ice crystals grow in the straw (it is debatable whether it is better for the ice crystals to reach the embryo earlier or later in time, or whether this has little to do with the survival after thawing). In general, in the slow freezing method of cow embryos, the cryoprotectant agent is often used at around 1.5 M, and the molar freezing point drop is as follows; The molar freezing point drop is determined by the type of solvent regardless of the type of solute, and the freezing point drop is
calculated × by the formula ΔTf = Kf (molar freezing point drop: specific to the solvent) × m (mass molar concentration). 1.5 = 2.775 ° C. However, since the base solution of the cryopreservation container is composed of an isotonic solution, if a molar concentration of 154 mmol / L of saline is used, 1.85 × 0.154 = 0.2849, and finally 2.775 + 0.2849 = 3.0599 ° C is the freezing point. If the latent heat of coagulation generated during that time catches up to -7°C within the retention time, the increase in the cooling rate at that time is negligible, so there is almost no risk of intracellular freezing in the fertilized egg after coagulation latent heat generation.

【Reference】

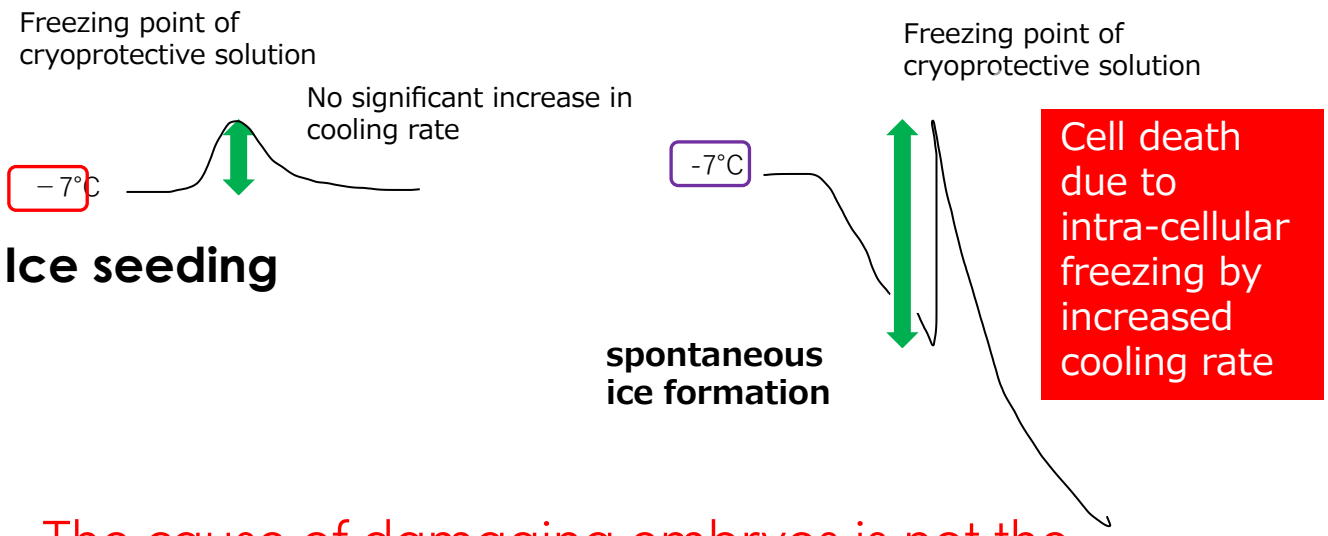
Keeping the height of the latent heat of solidification low (= Setting an ice seeding temperature close to the freezing point of the cryoprotective solution as much as possible) is necessary to improve the viability of the embryo after thawing.

This is misunderstanding!

Causes of misunderstanding

With ice seeding vs. Without ice seeding
(comparison of cooling curves)

latent heat of solidification



The cause of damaging embryos is not the magnitude of the latent heat of solidification, but the increase of the cooling rate when catching up with the temperature of the cooling tank from the highest point.

Therefore, the significance of the ice transplantation operation is to ensure the formation of ice crystals in the extracellular fluid, and for this purpose, -7°C is the temperature that ensures safe and reliable ice crystal formation.

(7) What is the significance of slow cooling after ice seeding?

Ice seeding is an opportunity to promote “extracellular fluid concentration→”, “osmotic pressure increase”, → “excretion of embryo’s free water from the cell” by artificially inducing ice crystal formation in the extracellular fluid at a temperature slightly lower than the freezing point of the cryopreservation solution. Normally, it is necessary to cool at a rate commensurate with the cell membrane permeability. Normally, it is set to -30°C at a speed of 0.3°C per minute.

If the temperature reached by the programmable freezer is set to -30℃, it may not reach -30℃ depending on the model, so it is suitable to set the temperature at -32℃, observe the temperature a little before the time when cooling ends, and immerse it to liquid nitrogen when it reaches to -30℃.

Many researchers have tested the temperature just before immersing to liquid nitrogen and the cooling rate just before immersing to liquid nitrogen. The cooling program may be different between bovine morula and bovine expanded blastocyst.

8) What are the precautions when immersing to liquid nitrogen?

It is basic to put it in quickly and reliably, but in order to do it quickly and reliably, liquid nitrogen is placed in a container with a suitable frontage made of Styrofoam, and sandwich the end of the straw with tweezer cooled (if you are using a liquid layer freezer, put it in an alcohol tank at -30 ° C for a few seconds) . Putting it directly into the canister of a liquid nitrogen tank is dangerous because it takes a long time to aim and is exposed to room temperature for a long time. When putting into liquid nitrogen, I think that the operation of putting into liquid nitrogen will be smoother if it is placed in a state close to vertical.

(9) Tell us the maintenance of alcohol tank type programmable freezer.

Mechanical maintenance by the manufacturer is required regularly, but here we will talk about the maintenance of alcohol (anhydrous ethanol or anhydrous methanol is used) after the cooling is completed. Alcohol absorbs moisture, which in turn reduces cooling performance, and you may see it left unattended, but it is contraindicated. This is one of the items to check the quality control and awareness of the laboratory.

(10) Is it necessary to calibrate the display temperature of the programmable freezer?

It is recommended to calibrate the displayed temperature of the programmable freezer by asking the manufacturer to make sure that the displayed temperature is correct about once a year, or it is necessary to check the accuracy of the displayed temperature of the programmable freezer by measuring with a thermocouple or platinum resistance thermometer sensor that has been calibrated with temperature. There is also a simple method of two-point calibration, that is, in pure water filled with ice, the temperature at which the ice remains without melting is 0℃, and the temperature of liquid nitrogen is -196℃.

(11) The color of the ice crystals that grow in the straw is sometimes different by the straw. Why is it different?

If we consider the mechanism by which the color of an object is visible, white light contains various colors of light, and when it hits an object, only a certain color of light is reflected. On the other hand, when a thin film of water is formed on the surface, and the irregularities are filled and smooth, the diffuse reflection of the surface is reduced, so the white light is reduced from the light that is reflected and reaches the eye, and it appears darker. It is not clear how this difference in color (the number of surface irregularities) affects the viability of fertilized eggs. Straws from each manufacturer are roughly made of three different materials; Polyvinyl Chloride resin (PVC), glycol-modified polyethylene terephthalate resin (G-PET; our straw). Polyamide resin (=nylon), etc. I have never compared whether the color of the ice crystals in the straw differs depending on the difference in these materials.

(12) At my laboratory, EO gas sterilization of straws and uterine perfusion tubes is performed in-house. In that case, please tell me what I need to be careful about.

EO gas sterilization is a very beneficial sterilization method that can be applied to almost all materials other than liquids. However, it is necessary to be careful about the degassing period (= aeration period). Please see page 21 and 22.

In conclusion, if the embryo straw is sterilized with EO gas, it will take 8 weeks at room temperature to vent the gas, after 50°C for 24 hours (which is programmed in the automatic EO gas sterilizer) . If it is consistently done at 50°C, a 2-week aeration period is required. The venting of the straw in which the embryo is trapped for a certain period of time needs to be more perfect than the uterine perfusion tubes, which the embryo passes through in an instant.

Title: Effects of heat- processing (50℃ 2wks) after EO gas sterilization on the viability of *in vitro* produced cow embryos

19 EOガス滅菌後の胚移植ストローに対する加温処理が胚の生存性に及ぼす影響

Effect of heat treatment for EO gas sterilized plastic straw on survival of *in vitro*-produced bovine embryos.

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金山佳奈子・米内美晴・小島敏之
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○ Makito TOHYAMA, Kei IMAI [1)], Syuji KOBAYASHI, Masayuki SHIN-NOH
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(National Livestock Breeding Center, [1)] National Institute of Animal Industry)

【目的】胚移植ストローなどの非耐熱性プラスチック製品の滅菌にはエチレンオキサイド（EO）ガス滅菌が用いられているが、滅菌物に浸透したEOガスが長期間に亘り残留し、胚の生存性に悪影響をもたらすことが漠然と危惧されている[1)]。しかし、このEOガス滅菌物を50～60℃前後に加温することでより早く残留EOガスを除去することができると言われている。本調査ではEOガス滅菌後の胚移植ストローに対する加温処理が胚の生存性に及ぼす影響について検討した。

【材料および方法】家畜改良センターの常法により牛体外受精胚を生産した。その牛胚を用いて以下の実験を行った。

実験①：供試胚は体外培養7日目および8日目（媒精日を0日目とする）の胚盤胞（Bl）および拡張胚盤胞（Exp.Bl）とした。この胚を0.1 mM β-メルカプトエタノール（β ME）を添加した20％牛胎子血清（FCS）加TCM199を用いて胚移植ストロー（IMV社製、0.25mlストロー、AAA:201）内またはマイクロドロップで38.5℃、5％ CO_2 in airの気相条件で72時間培養し、脱出胚盤胞（Hed.Bl）まで生育した胚を生存として Hed.Bl率を求め、その差を比較した。

胚移植ストローは、EOガス滅菌後室温で放置したもの（無処理区）とEOガス滅菌後50℃24時間加温した後に室温で放置したもの（加温処理区）を用い、それぞれの保存期間（0週、4週、8週）における胚の生存性を調査した。また、対照区としてEOガス滅菌後3～6ヶ月室温で放置したストローでの培養（ストロー対照区）およびマイクロドロップでの培養（マイクロドップ対照区）を行った。培養液量はストロー、マイクロドッロプ共に100 μl とし、胚は100 μl 当たり3～5個の培養密度とした。

実験②：供試胚は体外培養7日目および8日目のExp.Blで形態的品質がGoodのものとした。この胚を実験①と同様の方法により培養し、Hed.Blまで生育した胚を生存として Hed.Bl率を求め、その差を比較した。

胚移植ストローはEOガス滅菌後2週間室温で放置したもの（非加温区）とEOガス滅菌後2週間50℃で連続的に加温したもの（加温区）を用い、それぞれの胚の生存性を調査した。対照区および培養液量等の設定は、実験①と同様の方法を用いた。

実験①および②におけるEOガス滅菌は酸化エチレン濃度 10w/w%のボンベ（ノン

バクト-E）を接続した、Zクレーブ　E－3（三浦工業株式会社製）で行った。滅菌条件は加湿下で缶内圧力 1 kg/cm²、60 ℃、15 時間とし、洗浄は 500 mmHg の高真空条件で 15 回とした。また、加温処理は理化学用恒温器（三洋電機株式会社製　MIR-151）で行った。

【結果および考察】実験①の結果は表 1 のとおりであった。加温処理の有無に関わらず、滅菌後 8 週間で対照区とほぼ同レベルの濃度まで EO ガスが除去されることが明らかとなった。また、室温放置の状態よりも迅速に残留 EO ガスを除去するためには 50 ℃、24 時間の加温処理では時間的に不充分であり、より長い期間加温処理を続ける必要があると思われた。実験②の結果は表 2 のとおりであった。加温区は Hed.Bl 率が 90.0 %とマイクロドロップ対照区およびストロー対照区と比べ差はみられなかった。一方、非加温区はマイクロドロップ対照区、ストロー対照区および加温区に対して Hed.Bl 率が 42.3 %と低く、その差は有意であった（$P<0.01$）。以上の結果から、今回の滅菌条件において胚移植ストローから残留 EO ガスを除去するためには 50 ℃で 2 週間加温処理をすることが有効と思われた。今後は実験②の結果をふまえ、加温処理の期間が 2 週間よりも短縮できるかどうかについて検討する必要がある。ただし、EO ガス滅菌装置の機種や被滅菌物の材質により残留 EO ガス濃度が異なるため、それらの条件が異なる場合には今回の成果をそのまま適用するのは避けた方が良いかも知れない。

表 1．50 ℃ 24 時間の加温処理の有無と保存期間による胚の生存性．

		培養 72 時間後のステージ				
	胚数	Exp.Bl	Hing.Bl	Hed.Bl	Deg.	Hed.Bl 率
ﾏｲｸﾛﾄﾞﾛｯﾌﾟ対照区	58	13	3	41	1	70.7 a
ｽﾄﾛｰ対照区	83	10	3	65	5	78.3 a
無処理区（0 週）	15	0	0	0	15	0.0 c
加温処理区（0 週）	27	0	0	0	27	0.0 c
無処理区（4 週）	34	3	0	10	21	29.4 b
加温処理区（4 週）	44	5	3	18	18	40.9 b
無処理区（8 週）	25	1	3	20	1	80.0 a
加温処理区（8 週）	25	1	2	21	1	84.0 a

RT 8 wks

a>b>c：異符号間においてそれぞれ 2.5%水準で有意差有り．

表 2．50 ℃ 2 週間の加温処理の有無による胚の生存性．

		培養 72 時間後のステージ				
	胚数	Exp.Bl	Hing.Bl	Hed.Bl	Deg.	Hed.Bl 率
ﾏｲｸﾛﾄﾞﾛｯﾌﾟ対照区	54	2	0	52	0	96.3 a
ｽﾄﾛｰ対照区	54	3	0	51	0	94.4 a
加温区	50	1	1	45	3	90.0 a
非加温区	52	2	2	22	26	42.3 b

50℃ 2 wks

RT 2 wks

a>b：異符号間において 1%水準で有意差有り．

【参考文献】1) T.Takeda, L.J.Kennel and J.F.Hasler ;Theriogenology, 1987, 27: 286.

(13) Please tell us about the development history of iStraw®.

The representative director of our company used silver iodide (conceived as an ice crystal-forming organic agent because it has the property of forming ice crystals at -4°C in pure water), which is used as a seeding agent for artificial rainfall, as an ice crystal-forming inducer nucleus at the time of ice planting in the cryopreservation of domestic rabbit morula stage embryos (without manual icing), and cryopreserved and obtained baby rabbits from the thawed embryos (T. Kojima et al., Theriogenology 26:341-52, 1986). Subsequently, this method was used for cryopreservation of pig embryos, and the production of piglets from frozen and thawed pig embryos, which had a low probability of success at the time, was successful (Y.Fujino et al., Cryobiology 30:299-305, 1993). Subsequently, it was reported that it was used for cryopreservation tests of swine semen at livestock testing and research institutes in several prefectures in Japan, as well as cryopreservation tests of bovine semen at Cornell University in the United States.

Immediately after the current representative director retired from Kagoshima University, he established AnimoScience Co., Ltd. with the representative of COWOX Co., Ltd., and as one of his businesses, he launched developmental research on straw with automatic ice crystal formation. With the advice of Professor Hitoshi Iwahashi of the Faculty of Agriculture of Gifu University, and Dr. Yoichi Kadokami, Director of the Kadokami Research Institute (Hakodate City), we worked diligently on it, but we gave up on the use of ice nucleating bacteria due to the bottleneck of their ability to form ice crystals in a room temperature environment. Initially, due to the size of the market, we considered using it for cryopreservation of cow semen, and repeated the experiment with the cooperation of the Livestock Improvement Technology Research Institute of the Livestock Improvement Corporation, but the current cooling protocol could not find the significance of ice planting for the cryopreservation of cow semen, so the test was suspended. Then, I decided to concentrate on the development of automatic ice planting straws targeting cow embryos, which have a proven track record.

(14) What are the direct benefits of using iStraw®?

The direct advantages of using iStraw include: (1) Various human errors* during manual icing can be avoided (please refer to the table for a summary) (2) Saving the time and effort of manual icing (including the preparation of liquid nitrogen; Ice planting is possible even in an environment where there is no liquid nitrogen →any animal's embryo freezing experiment in the space shuttle) (3) It saves time; The time required for ice planting (especially when freezing a large number of straws) & the time required to hold at the ice planting temperature (because all straws are ice-planted at about the same time) (4) The equilibrium time (from immersion of embryos in a cryopreserved solution to ice crystals covering the entire column containing fertilized eggs) can be shortened. (5) The growth rate of ice crystals that occur naturally at -7°C (about 1 cm/min) is ideal speed according to the literature, which promotes effective dehydration from embryos (6) Since ice crystals are generated from the inner cotton plug, it is possible to set the ice planting temperature higher than -7°C by using a liquid that freezes at a higher temperature than the storage solution in the liquid in contact with the inner cotton plug.
In addition, it has been reported that the growth rate of ice crystals in straws of iStraw by Professor Osamu Dochi of Rakuno Gakuen University is close to the ideal value (see figure of next page).

***Factors that cause human error during manual ice transplantation**
4 Factors of Human Error & Possible Effects

要因	内容	結果
Lack of awareness	The person who made the mistake is not aware of it. Or they are not able to recognize the surroundings because they are correcting their mistakes by themselves.	Human error is not recognized and becomes latent.
Lack of standards	Since it is spot work, they continue to work without setting a "standard".	Human error is not defined and is left unattended.
Inadequacies in the standard	The purpose and instructions for complying with the standard are ambiguous, and the definition of NG is unclear.	Because it depends on the interpretation of the worker, human error becomes diverse.
Lack of education, training, and appropriateness	At the same time as there is a lack of education and training for workers, it is difficult to make appropriate judgments.	It becomes difficult to avoid human error.

※"Standard": A standard for how many seconds a metal (e.g., tweezers) immersed in liquid nitrogen should be touched to which part of the outer wall of the straw for how many seconds, and with what force.

㈱AnimoScience社製自動植氷ストロー「iStraw」の植氷発生確認試験結果

- 凍結媒液（FM）：1.5Mエチレングリコール ＋ 0.1Mショ糖 in ダルベッコPBS+20%CS
- 凍結器：アルコール液槽式（FHK-ET-1N）
- 氷晶発生確認

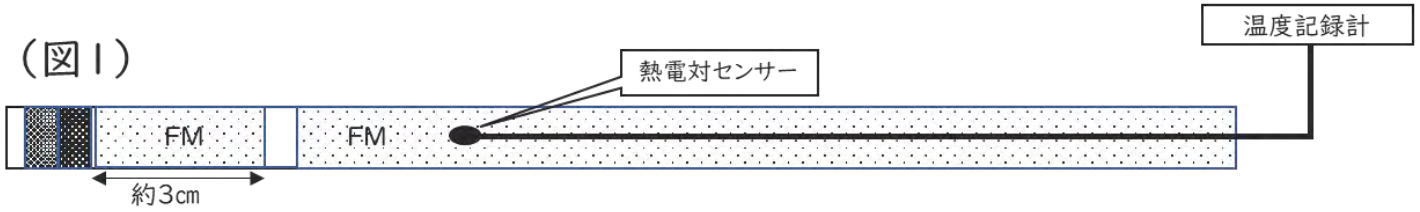
① 氷晶発生確認方法：凍結媒液をストローに吸引（図1）し、熱電対センサーをストロー内に挿入して、凍結器のチャンバー内のストローラックに装填した時間を0秒として、目視で氷晶発生を確認した時間

✓ 結果：1回目：54秒、2回目：28秒、3回目：38秒

所見：

- 3回の実験とも1分以内に氷晶発生を確認
- 氷晶発生時間に差があった理由として、ストローパウダーの重点密度の差が影響した可能性が考えられた（FMをストローに吸引する際にパウダーを超えてFMが吸引されるストローがあった）。
- また、ストローをチャンバーに浸漬した時のチャンバー内のアルコール液槽の状況（アルコール液の対流の強弱がチャンバー内で一定でないこと）が影響した可能性も考えられた。
- しかし、氷晶発生時間の差は、受精卵の生存率や受胎率に影響する可能性は皆無と判断された。

（図1）



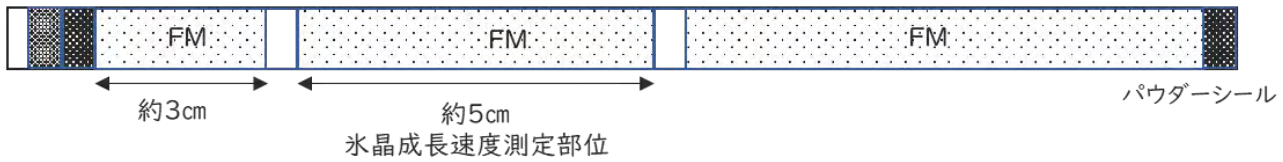
②氷晶成長速度の測定

ストロー内に5cmのFM層を作り（図2）、自動植氷後、氷晶が5cmのFM液層に侵入し、次の空気層（5cm層全体）に氷晶が完全に到達するまでに要した時間を測定

✓ 結果：1回目：1.31cm/分、2回目：1.22cm/分

所見：理想的な氷晶成長速度は毎分1cm程度とされていることから、ほぼ理想に近い値と判断される。氷晶成長速度は、凍結媒液（濃度）の種類によって異なることから、本実験で使用したFMに適した氷晶成長速度であるとも判断される。

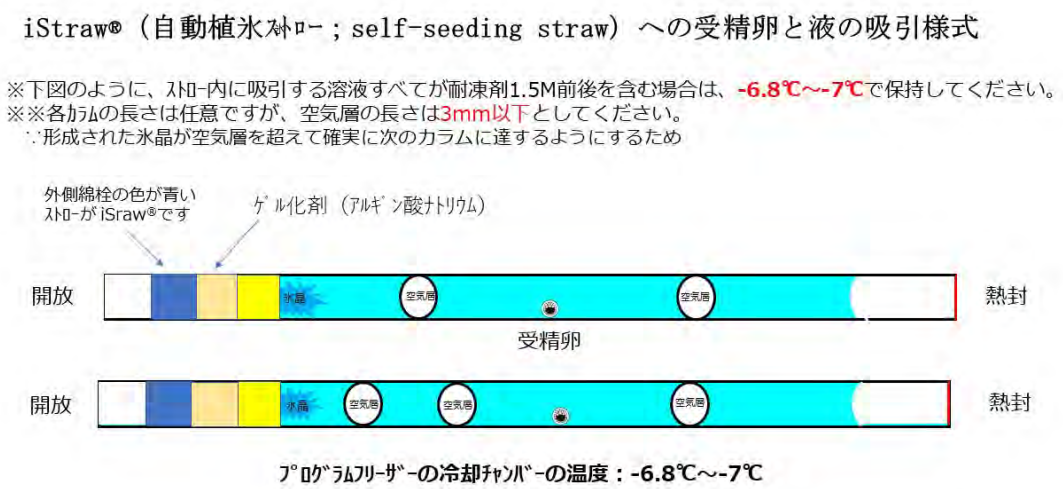
（図2）



Evaluation of iStraw by Professor Osamu Dochi, Rakuno Gakuen University

The growth rate of ice crystals in straws of iStraw by Professor Osamu Dochiof Rakuno Gakuen University is close to the ideal value

⑮Tell us about aspiration manner for cryo-preservation solution and embryo into iStraw®



The length of air bubble is ideally 3 mm.

In Japan, the alcohol bath type programmable freezer made by Fujidaira Industry and a YT freezer (dry type) made by Yamane Tech are commonly used. iStraw® is compatible with both freezers.
In addition, in foreign-made simple freezers straw has to be hold perpendicular, but iStraw can also be used.
If you would like to check how to use it, please contact to the following telephone or e-mail.
→ 090-9546-1961 or k1887523@kadai.jp

(16) Silver iodide is used for ice crystal nuclei in automatic icing for iStraw.Is there any adverse effect on embryos?

We asked Kazuko Ogata, a researcher at the Livestock Research Division of the National Agriculture and Food Research Organization, to co-culture bovine fertilized eggs with our iSeed (beads immobilized with silver iodide with calcium alginate) and compare the growth results with the control group (Please see pages 28, 29)

CONCLUSION

Silver iodide group was not significantly different from the control group in all indices in the co-culture performance with in vitro fertilized eggs, indicating that silver iodide can be used as an ice crystal formation inducer for cryopreservation of cow embryos without any concern of adverse effect of silver iodide. Silver iodide is still used in many countries including Japan as an ice crystal nucleus for artificial rainfall, and is also used in the veterinary pharmaceutical field and antibacterial filters due to its bactericidal properties.
The extractable test imposed on embryo straws stipulated by the Japan Veterinary Drug Association was also "compliant" in all items.

Effect of Silver iodide (AgI), which is used as an ice crystal nucleus, on the development of bovine IVF embryos (Co-culture test; Self-test)

iSeed is commercial name of silver iodide alginate gel droplet.
Co-culture means embryos are cultured with iSeed.

Table 1. Result-1

	cleavaged/cultured			
	Yes	No	%	total
Control	85	24	78.0	109
Co-culture	96	23	80.7	119
total	181	47		228

No significant difference (P=0.6159)

Table 2. Result-2

	Developed to embryo/cultured			
	Yes	No	%	total
Control	33	76	30.3	109
Co-culture	43	76	36.1	119
total	76	152		228

No significant difference (P=0.34851)

Table 3. Result-3

	Developed to embryo/cleavaged			
	Yes	No	%	total
Control	33	52	38.8	85
Co-culture	43	53	44.8	96
total	76	105		181

No siginificant difference (P=0.41683)

Effect of Silver iodide (AgI), which is used as an ice crystal nucleus, on the development of bovine IVF embryos (Co-culture test; Self-test)

iSeed is commercial name of silver iodide alginate gel droplet.
Co-culture means embryos are cultured with iSeed.

Table 4. Result-4

	Hatched B/Blastocyst			
	Yes	No	%	total
Control	14	19	42.4	33
Co-culture	27	16	62.8	43
total	41	35		76

No significant difference（P=0.34851）

Table 5. Result-5

	Hatched B/cultured			
	Yes	No	%	total
Control	33	76	30.3	109
Co-culture	43	76	36.1	119
total	76	152		228

No significant difference（P=0.07747）

The extractables test is based on the Veterinary Medical Device Standards (April 1, 17; This test is imposed on the "XVIII Straw Tube for embryos for Animals" of the Japan Veterinary Pharmaceutical Association.

Extractable Test Results:

(October 3, 2022: Tsukuba Center, Sumiju Atex Co., Ltd.)
Sample: iStraw®

a) Appearance and pH : **Conforming** Difference between sample → blank and sample: 1.00 (less than or equal to standard 2.0)

b) Heavy metals: **Conforming**
The color of the test solution was not darker than that of the comparison solution.

c) Potassium permanganate reducing substance**:** **Conforming** Difference between sample and blank sample consumption: 0.9 mL (standard 2.0 mL or less)

d) Evaporation residue: **Conforming** Amount of residue → sample: 0.4 mg (standard 1.0 mg or less)

Conforming for all items in the iStraw® sample!

(17) Tell us about Key points of quality control of iStraw and Emvisible Straws.

Only straws that have passed the visual inspection (visual inspection of all dirt, scratches, etc.) and dimensional inspection (extraction) are moved to the next process.
As a result of the uniformity inspection of the length of the solution suction part of the straw, if there is a defect, it is corrected and the next process is carried out
After aspirated 10% ethylene glycol solution (saline) into a straw, check whether ice crystal formation starts within 2 minutes after immersion at -7° C (alcohol bath in a program freezer) (sampling inspection method by our original test; Lot conformity judgment is operated according to the company's own management standards)
After the tests in (1), (2), and (3), check whether the force is extruded by a digital force gauge (ZTS-5N, IMADA) with a force of 4 to 5 Newtons.
Empty the extruded liquid into a 35mm dish and inspect for contaminants.

Straws that have passed the above inspections (4) and (5) are heat-sealed (20 mm wide; It is done by hand wearing gloves in a clean environment)
After visual inspection of the appearance of the straw enclosed in the packaging material, the position of the cotton stopper, etc., it is packed into a package and subjected to the electron beam sterilization (Sumiju Atex) process.

* For Emvisible straws, the dimensions and materials of the straws are the same as those of iStraw®, but they do not have the function of forming ice crystals, so the inspection in (3) is not performed.

Digital Forcegauge used to control pressing pressure of cotton plugs.

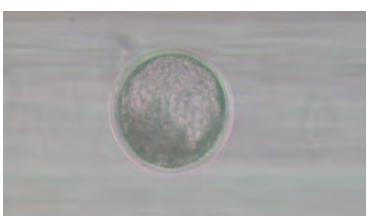
(18) The transparency of iStraw and Emvisible straw is very high. But what are the benefits?

One of the most important requirements for development was that the straws should be sterilized by electron beams. However, it was found that some plastic materials turn yellow when irradiated with electron beams, and as a result of searching for materials that do not cause this yellowing (≈ material deterioration), As a result of asking a company specializing in plastic extrusion in Japan to make straws using G-PET, we were able to produce very transparent and uniform straws. I found that the transparency is obviously different, which I think is proof of the high level of Japan technology.

Comparison of digital microphotographic images of the surface of G-PET straws

"Emvisible Straw® & iStraw®

Imported G-PET straw



With self-assurance, you can evaluate the developmental stage and the quality of the embryo.

If the transparency of the straw is high, (1) the embryo sucked into the straw can be found immediately (if there are a large number of eggs, the time accumulates, so it cannot be ignored), (2) in the case of direct transfer, the quality of the embryo can be evaluated more accurately through the straw than with a conventional straw, (3) this leads to the effective use of the embryo (transfer of a completely dead embryo is stopped, embryos of very high quality may be considered to be an advantage, such as transfer them into recipients in better condition.

(19) Why does iSeeding take 1, 3, or 5 individually wrapped straws?

Most of the sterilized embryo straws made in foreign countries are one type of individually wrapped 5 straws, but for example, if only 4 embryos can be collected, there will be a surplus of 1 straw. Until now, there was a difference in the unit price of straws, but now that the price difference has been eliminated, we hope that our commitment to sterile straws will be conveyed more strongly to users.

(20) The quality of iSeeding's sterile straws is understandable. There is a business that use the unsterile bulk straws. But tell me why iSeeding are so particular about straws so much so.

The textbook for the training course that must be taken to obtain the embryo transfer qualification states that the instruments and equipment used for embryo transfer should be sterilized in an appropriate manner (Livestock artificial insemination course textbook published by the Japan Association of Livestock Artificial Insemination; Pages 100~107, 116~120 of Livestock In Vivo Derived Embryo Transfer and Livestock In Vitro Produced Embryo Transfer), but the straw is not described in detail, but when the collected embryo is freshly transferred, and when the collected embryo is frozen and stored and filled into a deep injection implanter after thawing, straw can be considered as a container in which the embryo is stored for a relatively long time. Sterilization of straws and the use of straws with guaranteed sterilization are absolutely necessary to ensure the fertility of transferred embryos and to prevent unintentional intrauterine infection of recipient cows.

(21) Preceding electron beam sterilization of straws, validation test for sterilization was done. What is the validation test?

In the sterilization validation test, the commercial electron beam irradiation dose is determined (actually 25 kGy) through (1) the recovery rate is measured by putting the number of known bacteria in the straw, (2) the bioburden test (the number of bacteria in the straw before sterilization is calculated), (3) the electron beam dose is determined based on the above results, (4) the test dose is determined, and (5) the dose distribution test in the package where the electron beam is actually irradiated (actually 25 kGy).
In general, when sterilizing veterinary medical devices, it is necessary to perform under sterilization conditions set after scientifically verifying (validating) in order to ensure sterilization assurance level 10^{-6} (1 or less product with 1,000,000 products that can develop bacteria is attached). The iStraw® and Emvisible straws® we sell use an electron beam sterilization method that does not have any concerns about residual gas. In addition, Meiwa Pax Co., Ltd., which manufactures packaging materials for both straws, conducts accelerated weathering tests on the seal strength of the packaging materials to indicate the sterilization retention period.

(22) Is it possible to print on iSeeding straws with an inkjet printer?

Our straws, which were printed on the surface with alcohol-resistant MEK-free ink (inks not applicable to organic laws = consideration for the health and safety of workers), were soaked in anhydrous ethanol (-7°C) for 10 minutes and then taken out to check the printing condition. The printing condition was kept in good condition, and the print did not disappear even after rubbing.

(23) Straws manufactured and sold by iSeeding Co., Ltd. are said to be different from the products made by other manufacturers. Please tell me the characteristics of the straws of iSeeding.

First of all, we were the first in the world to develop iStraw®, a straw with a built-in automatic ice seeding function.

The second is that it is an electron beam sterilized product that determines the amount of electron beam irradiation after sterilization validation testing.

The third is that it is completely manufactured in Japan, so it is less susceptible to exchange rate fluctuations and international logistics.

The fourth is that we have a direct sales system from the manufacturer, so we can respond quickly to user feedback.

The fifth is that the straw has a high transparency because it uses G-PET (glycol-modified polyethylene terephthalate) as the material.

As a result, the unit price of straws is higher than that of conventional unsterilized bulk straws, but we are confident that users will have a sense of security beyond the price difference, and as a result, users and farmers will feel a high cost-effectiveness.
I would like to cherish the commitment of "just a straw, but a straw".

(24) Effects of the difference in the price of straws and the difference in conception rate on management. Please let me know if you have any thoughts.

Please note that the example is a hypothesis, and it is assumed that a farmer raising 100 recipient cows is targeted. iStraw® (price 385 yen including tax) and unsterile bulk straw (price 11 yen including tax) are used to freeze cow embryos, respectively, and assume that the conception rate of the former is 56% and the latter is 55% (this difference of 1% is considered to be a valid figure due to the advantages of automatic icing, sterilization effect, accuracy of quality assessment of embryos just before embryo transfer, etc.). The market price of Japanese Black calf at 10 months will be **624,158 yen** (Ibaraki Prefecture All Agricultural Statistical Data 2023).

Assuming that ET is repeated a total of 6 times until conception for the above 100 recipient cows, a total of 179 times (56/100 → 44×0.56/44 → 20×0.56/20 → 9×0.56/9 → 4×0.56/4 → 2×0.56/2; denominator total 179 times), and bulk straw → a total of 184 times (55/100 45×0.55/45 → 21×0.55/21 → 10×0.55/10 → 5×0.55/5 → 3 ×0.55/3; denominator total 184 times) embryo transfers. Therefore, the cost of each straw is **68,915 yen** (= 385×179) for iStraw® and **2,024 yen** (= 11×184) for unsterile bulk straws, and the difference in straw costs is 66,891 yen. In iStraw®, the number of cows conceived during up to 6 times of ET is 56 + 24 + 11 + 5 + 2 + 1 = 97, while the number of cows conceived by bulk straw is 55 + 24 + 11 + 5 + 2 + 1 = 96, and **the number of conceived cows is calculated to increase by 1 if iStraw is used.**

As a result, when using iStraw® , if all conceived calves are successfully born, one more calf will be born than bulk straw. If you take into account the straw cost difference mentioned earlier, the increase in income is 624,158 yen – 66,891 yen = 557,267 yen.

Also, if you use iStraw® , 184 times - 179 times = 5 ET expenses (equipment costs such as transplanters, labor costs, working hours, etc. plus α) can be saved. You will need to pay a separate feeding and management fee for 9 months until the calf is shipped to the market, so if the production cost per calf is 425,000 yen, if you use iStraw®, you will end up with 557,267 yen - 425,000 yen = 132, 267 yen plus α. Then the increase of income is expected.

In conclusion, if one more calf is born by 6 times of ET on a farm with 100 recipient cows using iStraw (unit price 385 yen including tax) compared to using inexpensive unsterilized bulk straws (assuming conception rates of 55% and 56%, respectively), you can expect to earn 132,267 yen more than using inexpensive unsterile bulk straws. This amount varies depending on feed prices, utility costs, labor costs, etc. In this case, assuming that the farmer bears the entire straw cost of 66,891 yen, if the veterinarian pays the full amount, the farmer's income will increase by 199,158 yen (624,158-425,000). You will be able to fully absorb the straw expenses.
The argument developed here is full of assumptions, and it may be a bit of a leap to say that replacing straws with sterile automatic ice seeding straws will improve conception rates compared to unsterile straws + manual ice seeding. However, using sterile instruments and ensuring successful ice seeding is one of the factors that improves conception rates. It is assumed that other factors that are essential for improving conception are met.

(25) Direct transfer straw breaks during thawing
What are the possible causes of break?

This is not something that usually happens often, and if it does, check the defective lot number and straw; Contact your supplier to see if similar cases have occurred elsewhere, and provide comments on this problem and possible solutions.

a) Ingress of liquid nitrogen into the straw, which is not properly sealed/closed (PVA polyvinyl alcohol) or the straw itself has a crack. 38

b) A small amount of air remains between the identification plug and the liquid phase, under positive pressure conditions, the air pocket at the tip of the straw will exert pressure on the plug. If the seal is defective, liquid nitrogen will enter the space (650 times expansion factor at 24℃ from liquid to gas).

c) Liquid nitrogen enters the straw during storage, and the rapid expansion during thawing will cause rupture.

d) PVA powder; Our straws are made of sodium alginate (not PVA) are filled between the outer and inner cotton stoppers, but you can check if they are separated by holding the direct transfer (DT) straw and exposing it to light. The separation of the cotton plugs can be identified by magnifying the straw package with a stereo microscope (in the photo below, the six straws on the right are defective, but they cannot be seen with the naked eye).

Reference：Top 10 Questions of the New Bovine Embryo Practitioner （Embryo Technology Newsletter Volume 41, Number 2, June 2023 IETS）.

Possible workarounds:

a) Remove the identification plug while most of the thawing straw is in liquid nitrogen or during thawing, taking care not to immerse the open end of the straw in warm water. 39

b) Remove the identification plug and hold the straw in the vial containing the preservation solution, if one straw ruptures, the other straws may also have the same effect.

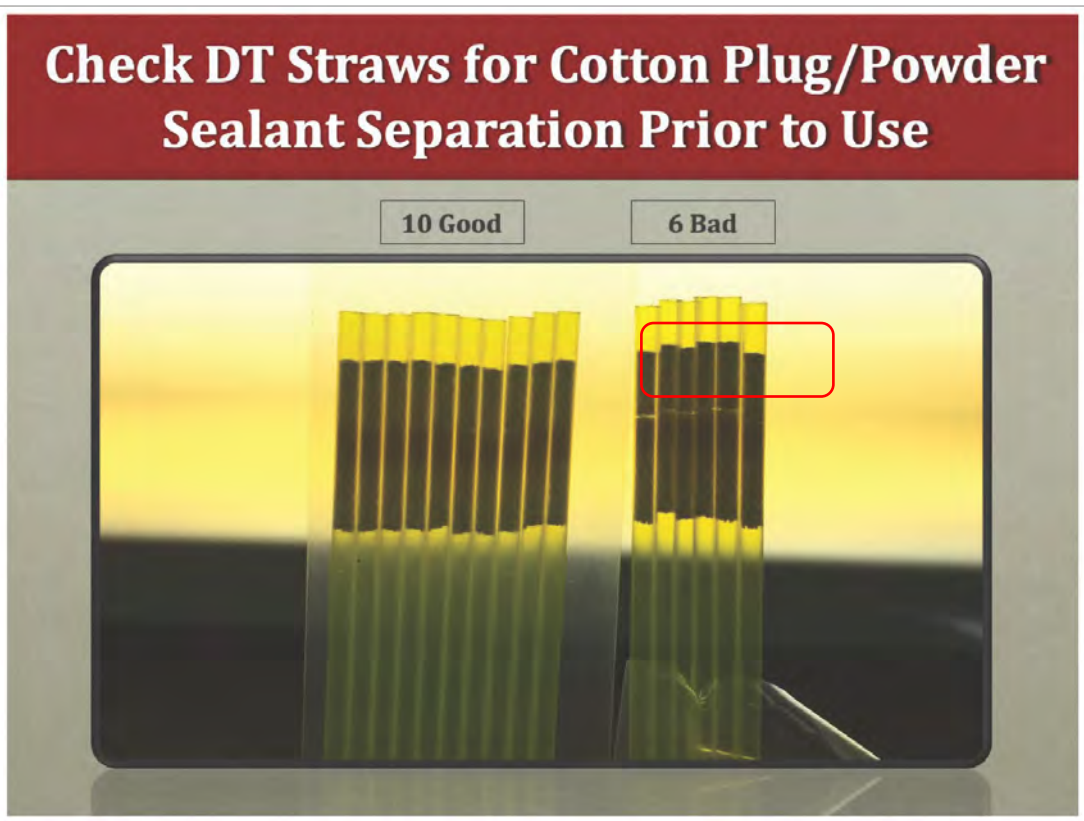
c) For glycerol: Unplugging the straw while holding it in air (20°C) for 10 seconds before immersing it in warm water at 20°C will help reduce straw rupture, which also helps prevent cracking of the zona pellucida (Rall and Meyer, 1989).

d) For direct transfer: Unplug the straw while holding it in air (20°C) for 5~10 seconds before immersing it in warm water at 30°C for 30 seconds.

e) Proper filling of the straw with embryos and cryopreservation solution and secure sealing can reduce the occurrence of straw rupture.

f) Store direct transfer straws in a place where they are not subject to violent vibration. If the straws are stored in a warm, rattling mobile unit for a long period of time, we have often seen the phenomenon of the gelling agent separating from the cotton plug (J. H. Pryor, personal communication）.

g) The straw is heat sealed and the sealing end is separated after melting.



(26) Teach what to pay attention to when transplanting embryos into recipient cows.

From the stage of collecting the embryos by uterine flushing to the completion of transfer to the recipient cow, it is important to use sterilized instruments and solutions that are suitable for each of them. In the case of EO gas sterilization, pay attention to the degassing (aeration) period, which requires 8 weeks at room temperature or 2 weeks in 50℃ incubator. In the case of electron beam sterilization, it can be used immediately after sterilization, but whether the dose has been determined through sterilization validation tests, the type of seal of the packaging material in which the instrument or equipment is enclosed, and the seal strength also affect the shelf life. You should definitely avoid situations where the embryo is in a straw that is unsterilized.

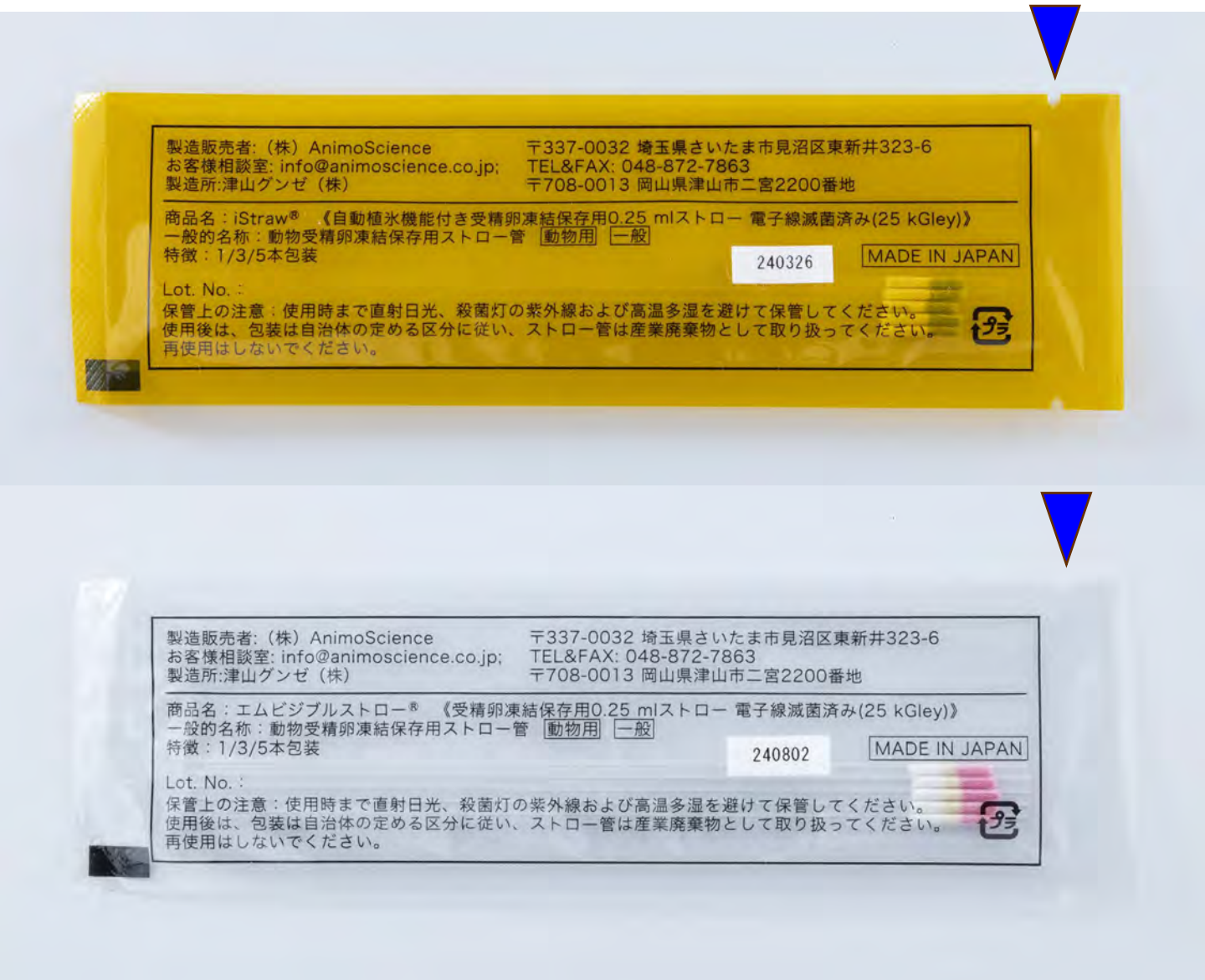
Since the recipient cow of embryo transfer is in the luteal phase, the resistance to bacterial infection is reduced in the uterus. Therefore, it is necessary to clean, dry, disinfect, and dry the vulva during the actual embryo transfer, and to cover the outer sheath of the implanter. It is strongly recommended to visually insert the tip of the injector into the opening of the uterus into vagina using a vaginal speculum at the time of embryo transfer. Although some skilled technicians do not like to use a vaginal speculum, it is 100% more likely to develop endometritis due to conception and unintentional intrauterine contamination. It is recommended to use a vaginal speculum to avoid it, and another benefit is that it is easy to spot cow with urovagina.

As to the criteria for selecting a recipient, basically, it is important that the signs of estrus are clear and that the estrus is expressed with a periodicity (21 days ± 3 days). If the above two points are satisfied, I think that there is no big problem by saying that if there is a corpus luteum, it will be transferred. If the process of forcibly advancing the injector into the deep part of the luteal side uterus leads to bleeding of the endometrium, the conception rate will be lowered, so in such cases, it is recommended to put it on the non-luteal side.

Feeding management, nutritional management (improvement of diet by conducting metabolic profile tests) and stress management of the recipient cow will lead to a high conception rate from before embryo transfer until conception confirmation is confirmed at least 60 days.

* In the case of freeze-thawed embryos (direct transfer is basic), it is necessary to pay attention to the time from thaw to transplantation (as short as possible), and in the case of fresh embryos, it is necessary to pay attention to temperature control from the lab to the transfer site, ultraviolet light shielding, etc.

弊社製ストローの包装



Description of the picture:
It will be delivered enclosed in the above packaging material. iStraw® is in the yellow transparent packaging material (upper part of the photo), Emvisible straws® are contained in a colorless and transparent packaging materia Both packaging materials retain electron beam sterilization.
(3 years for optimal storage, 1 year for iStraw)
All of them are individually wrapped in 5 bottles.
The white label indicates the date of electron beam sterilization. Each packaging material is opened from the cut on the right side of the photo and a straw cotton stopper
It is possible to grasp the part and take it out.

iStraw's® packaging material (the yellow bag that houses iStaw®) is made of PET*12μm/UV500*printing/LLDPE*40μm, which can protect the straw from ultraviolet and visible light when the embryo is aspirated before implantation.

After taking out the iStraw®, do not throw it away and use it for the above purposes.

* PET: Polyethylene terephthalate
* UV500: In addition to ultraviolet rays, it has the function of blocking more than 98% of visible light up to 550 nm.
* LLDPE: Linear low-density polyethylene

The strengths of iSeeding!

The straws we manufacture and sell are Made in Japan
No concern about supply shortages, less susceptible to foreign exchange fluctuations
Direct sales from the manufacturer and developer
User feedback can be quickly reflected in product improvements.
"It's just a straw, but it's a straw."
It is a container that contains a fertilized egg until just before implantation
Add new value as a container for cryopreservation
Electron beam sterilization with no risk of residual gas
Individually wrapped (1/3/5 straws) sterile straws that do not produce waste
Checking for cracks by visual inspection of straws
Provision of technical information related to cryopreservation straws
* Liquid suction is stopped when the inner cotton plug and gelling agent are wet.

(If the outer cotton stopper is wet, there is a high risk that the cotton stopper will come off during melting.)

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