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Datasheet for the decision of 23 March 2010

Case Number: T 0506/07 - 3.3.01

Application Number: 99951907.7

Publication Number: 1121015

IPC: A01N 1/02

Language of the proceedings: EN

Title of invention:
Method for vitrification of a biological specimen

Patentees:
Forest, Katrina T., et al

Opponents:
Greenpeace e.V.
Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V.

Headword:
Vitrification of cells/K. FOREST

Relevant legal provisions:
EPC Art. 100(a)(b), 123(2)

Relevant legal provisions (EPC 1973):
-

Keyword:
"Sufficiency of disclosure (yes)"
"Main request and first auxiliary request: inventive step (no) - the embryologist would consult documents in neighbouring fields when solving a mechanical problem"
"Second auxiliary request: inventive step (yes)"

Decisions cited:
T 0270/90

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0506/07 - 3.3.01

D E C I S I O N
of the Technical Board of Appeal 3.3.01
of 23 March 2010

Appellants: (Patent Proprietors)
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and

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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 30 January 2007 revoking European patent No. 1121015 pursuant to Article 102(1) EPC 1973.**

Composition of the Board:

Chairman: P. Ranguis
Members: C. M. Radke
D. S. Rogers

Summary of Facts and Submissions

I. The patent proprietors appealed against the decision of the opposition division to revoke European patent no. 1 121 015.

II. The oppositions were based on the grounds under Article 100(a) (exceptions to patentability under Article 53(a) EPC, lack of novelty and of inventive step) and Article 100(b) EPC. The opponents requested that the patent be revoked in its entirety.

III. The following documents were cited during the opposition proceedings:

(E1) M. Lane et al., Theriogenology, vol. 51, issue 1 (1 January 1999), 167
(E2) A. Martino et al., Biology of Reproduction, vol. 54 (1996), 1059-1069
(E3) D. W. Rodgers, Methods in Enzymology, vol. 276 (1997), 183-203
(E4) S. Parkin and H. Hope, Journal of Applied Crystallography, vol. 31 (1998), 945-953
(E5) H. Hope, Acta Crystallographica, vol. B44 (1988) 22-26
(E6) German utility model DE-U-G 91 09 683.9
(E7) DE-A-39 12 723.

IV. The claims of the proprietors' main request before the opposition division were claims 1 to 12 filed as auxiliary request 4 with the letter dated 20 November 2006.

Its only independent claim reads as follows:

"1. A method of vitrification of a biological specimen, selected from the group consisting of oocytes, embryos, blastocysts and morulas, comprising:
a) placing the biological specimen on a transfer instrument chosen from the group consisting of a loop and a paddle, and wherein the biological specimen is treated a cryoprotectant; and
b) placing the transfer instrument directly into a freezing material, wherein the biological specimen is directly exposed to the freezing material, thereby undergoing vitrification and further wherein the biological specimen will be viable after the biological specimen is thawed."

V. The opposition division decided that the subject-matter of the claims did not contravene the requirements of Article 53(a) EPC and that the priority was validly claimed so that document (E1) did not form part of the prior art. It concluded that the subject-matter of the claims was novel.

Document (E2) was considered to represent the closest prior art for the consideration of inventive step. The problem to be solved was to provide a further method for the vitrification of biological specimens. The use of loops as transfer instruments was obvious in view of any of the documents (E3) to (E7).

Hence, the subject-matter of the claims of the main request and of the auxiliary request then on file was not deemed to involve an inventive step.

VI. The following documents were *inter alia* additionally cited during the appeal proceedings:

(E13) Affidavit of Josiane Van der Elst dated 25 May 2007, 19 pages including cv

(E14) Affidavit of Michelle T. Lane dated 25 May 2007, 25 pages including cv.

VII. The claims presently on file are

- claims 1 to 11 of the Main Request,
- claims 1 to 11 of the First Auxiliary Request, or
- claims 1 to 11 of the Second Auxiliary Request,

all submitted during the oral proceedings before the Board.

(a) The only independent claim of the Main Request reads as follows:

"1. A method of vitrification of a biological specimen, selected from the group consisting of oocytes, embryos, blastocysts and morulas, comprising:

a) placing the biological specimen on a transfer instrument chosen from the group consisting of a loop and a paddle **wherein the transfer instrument is not an electron microscopy grid by either:**

(i) placing the biological specimen in a base medium and using the transfer instrument to scoop the biological specimen from the base medium; or

(ii) placing the biological specimen in a base medium and pipetting the biological specimen onto the transfer instrument;

wherein the base medium comprises a cryoprotectant; and

b) placing the transfer instrument directly into a freezing material, wherein the biological specimen is directly exposed to the freezing material, thereby undergoing vitrification and further wherein the biological specimen will be viable after the biological specimen is thawed."

(Note from the Board: The features in bold are those differing from the ones of the version of the claim cited under point IV above).

(b) The claims on the First Auxiliary Request are identical with the claims of the Main Request except for claim 1 which reads as follows:

"1. A method of vitrification of a biological specimen, selected from the group consisting of oocytes, embryos, blastocysts and morulas, comprising:

a) placing the biological specimen on a transfer instrument which is a loop wherein the transfer instrument is not an electron microscopy grid by either:

(i) placing the biological specimen in a base medium and using the loop to scoop the biological specimen from the base medium; or

(ii) dipping the loop into a base medium to form a film of the base medium on the loop and depositing the biological specimen via pipette directly into the loop;

wherein the base medium comprises a cryoprotectant; and

b) placing the loop directly into a freezing material, wherein the biological specimen is directly exposed to the freezing material, thereby undergoing vitrification and further wherein the biological specimen will be viable after the biological specimen is thawed."

(c) The claims on the Second Auxiliary Request are identical with the claims of the First Auxiliary Request except that in claim 1 the expression "... a transfer instrument which is a loop wherein the transfer instrument is not an electron microscopy grid" is replaced by "... an open loop".

VIII. The arguments of the Appellants as far as relevant for the outcome of the decision may be summarised as follows:

The Appellants argued that the invention could be carried out, even with a paddle and in the absence of a viscosity increasing agent. The experiments provided by Respondent II were not relevant as they were conducted in the absence of cryoprotectants and of the biological specimens defined in the present claims. Furthermore, these experiments emphasised a high immersion speed whereas no such high speed was required according to the invention.

The Appellants gave reasons why they considered the amendments in the claims to satisfy the requirements of Article 123(2) EPC.

The Appellants considered the priority claimed to be valid and document (E1) not to form part of the prior art, so that the subject-matter of the claims was novel.

Document (E2) was the closest prior art. The problem to be solved was the provision of alternative methods of vitrification of biological samples which preserved the viability of the sample and allowed easy handling of the sample.

This problem was solved as a loop or a paddle allowed the formation of a thin film of the base material held by adhesive forces. Document (E2) gave no incentive to use another transfer instrument.

The skilled person in the art was an embryologist. He would never have consulted any of the documents (E3) to (E5) from the field of crystallography (as was evident from documents (E13) and (E14)). He would also not have used the loops that were known for spreading bacterial cultures (such as those disclosed in documents (E6) and (E7)), inter alia because these loops were not used to form thin films.

IX. The arguments of Respondent II as far as relevant for the outcome of the decision may be summarised as follows:

The alleged invention was not sufficiently disclosed as the desired effect was only demonstrated for the loop and in the presence of a viscosity increasing agent. Respondent II provided experimental evidence to show that the method claimed could not be performed using a

paddle and only worked for certain kinds of loops. It considered that the alternative (ii) of step a) as defined in claim 1 of the Main Request had no basis in the application as filed as far as a paddle was used as the transfer instrument. It did not maintain its novelty objections for the claims presently on file (see point VII above).

Respondent II also considered document (E2) as the closest prior art. When trying to solve the problem of rendering the handling of the sample easier, the person skilled in the art would have considered neighbouring fields. Thus he would have used the loops disclosed in documents (E3) to (E7). Hence, the subject-matter of the claims was not inventive.

X. The Appellants requested that the decision under appeal be set aside and the patent be maintained on the basis of

- claims 1 to 11 of the Main Request,
- claims 1 to 11 of the First Auxiliary Request, or
- claims 1 to 11 of the Second Auxiliary Request,

all submitted during the oral proceedings before the Board.

XI. Respondent I did not file any observations or requests during the appeal proceedings, nor was it represented at the oral proceedings before the Board to which it was duly summoned. In accordance with Rule 115(2) EPC the oral proceedings was continued without this party.

XII. At the end of the oral proceedings the decision of the Board was announced.

Reasons for the Decision

1. The appeal is admissible.

2. Article 123 EPC

2.1 Article 123(2) EPC

2.1.1 It was under dispute whether or not alternative (ii) of step a) as defined in amended claim 1 of the Main Request was based on the application as filed as far as a paddle was used as the transfer instrument (see under point IX above).

This alternative process step is disclosed on page 10, lines 9 to 12 of the application as filed as far as a loop is used as the transfer instrument.
As the Appellants pointed out, there is also a more general disclosure in the application as filed, namely

- on page 9, lines 28 to 30, which mentions that the "biological specimens ... are preferably transferred to a base medium."; and
- on page 10, lines 18 to 20, which discloses "... pipetting the biological specimen onto the transfer instrument ...".

Hence, the application as filed discloses a process step comprising "placing the biological specimen in a base medium and pipetting the biological specimen onto the transfer instrument", namely alternative (ii) of step a) as defined in claim 1 of the Main Request.

2.1.2 The remaining features of claim 1 of the Main Request are based on claim 1 as originally filed, where the selection of the biological specimens and of the transfer instruments are disclosed in original claims 2 and 3, respectively. The requirement that "the transfer instrument is not an electron microscopy grid" is based on page 6, lines 9 to 11. Alternative (i) of step a) is disclosed on page 10, lines 6-9, that the base medium is to comprise a cryoprotectant on page 9, line 30 to page 10, line 3 of the application as filed.

2.1.3 Claim 1 of the First Auxiliary Request is based on claims 1 to 3 as originally filed in combination with page 6, lines 9 to 11, page 9, line 30 to page 10, line 3 and page 10, lines 6 to 12 of the application as originally filed.

Claim 1 of the Second Auxiliary Request is based on claims 1 to 3 as originally filed in combination with page 8, line 18, page 9, line 30 to page 10, line 3 and page 10, lines 6 to 12 of the application as originally filed.

2.1.4 The wording of claims 2 to 11 is identical for the Main Request and for the First and Second Auxiliary Requests; these claims are based on claims 5 to 10 and 12 to 15 as originally filed, respectively.

2.1.5 Hence, the amendments in the claims do not contravene the requirements of Article 123(2) EPC.

2.2 Article 123(3) EPC

The present claims differ from the claims as granted in that in the former the only independent claim has been restricted by specifying the type of biological specimens, the type of transfer instrument(s) and the way the specimen is placed thereon, and by requiring a cryoprotectant to be present in the base medium.

All these amendments limit the scope of protection with respect to that of the claims as granted, and thus do not contravene the requirements of Article 123(3) EPC.

3. Article 100(b) EPC

In opposition appeal proceedings each party bears the burden of proof for the facts it relies upon (see T 270/90, O.J. EPO 1993, 725, in particular point 2.1, the bottom paragraph on page 726).

As Respondent II argued that the patent in suit did not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art, the onus was on Respondent II to show that this was indeed the case. Respondent II filed experimental evidence in this respect with its letters dated 22 and 25 January 2010.

In these experiments, cells suspended in a culture medium were transferred to liquid nitrogen at different immersion speeds using paddles and loops of different forms and sizes. Respondent II observed that the biological samples did not remain on the respective transfer instrument during vitrification if the

immersion speed was high, namely about 1 m/s, while it remained on the transfer instrument at immersion speeds of about 0.5 m/s. It argued that a high immersion speed was essential in order to ensure that the cells remained viable once thawed.

However, the immersion speed is not a feature of the present claims, nor has Respondent II demonstrated that an immersion speed of at least 1 m/s was essential, nor that the addition of a viscosifying agent was necessary in order to carry out the method claimed.

Hence, the experiments provided by Respondent II do not support its objections under Article 100(b) EPC. Consequently, no grounds under this Article prejudice the maintenance of the patent.

Main Request and First Auxiliary Request

4. Novelty

It was not contested that the subject-matter of the claims on file was novel. The Board is of the view that the subject-matter of the claims of these requests are novel. However, in view of the outcome of this decision as regards the Main and First Auxiliary Requests it is not necessary to give detailed reasons.

5. Inventive step

5.1 The closest prior art

Document (E2) is the only prepublished piece of prior art disclosing the vitrification of cells by extremely rapid cooling. In common with the patent in suit, document (E2) seeks to keep as many of the cells as possible viable after warming up (see paragraph [0002] of the patent in suit and the abstract of document (E2)). Hence, the Board agrees with the parties that this document is the closest prior art.

This document discloses the cryopreservation of bovine oocytes by

(a) suspending the oocytes in ethylene glycol solutions, placing these suspensions into plastic straws or onto electron microscopy grids, and

(b) plunging the straws or grids directly into liquid nitrogen (see the abstract).

5.2 The problem to be solved

5.2.1 In claim 1 of the Main Request and of the First Auxiliary Request it is specified that the transfer instrument is a loop (or paddle) but not an electron microscopy grid (see under points VII. a) and b) above). The Appellants considered the subject-matter of the claims of the Main Request and of the First Auxiliary Request to provide an easier handling of the samples and to improve the viability of the vitrified cells after thawing. The Board is satisfied that the problem of improving the handling is solved, as handles attached to the loops and paddles make it easier to

transfer the samples into the freezing material than by means of the open pulled straws or the electron microscopy grids used in document (E2).

5.2.2 There is no evidence showing that a more ambitious problem was solved. The comparative tests of the patent in suit show that the viability of the vitrified cells after thawing is improved if an **open** loop is used as the transfer instrument instead of an open pulled straw. However, the definition of the term "loop" in the patent in suit is not limited to an open one but may include loops which are "modified in any way known in the art to help retain the biological specimen in place, including the placement of extra polymeric mesh or wire grids within the loop ..." (see page 5, lines 56 to 58). These modifications may possibly affect the freezing rate of the sample and thereby the viability of the cells after thawing. Therefore, the comparative tests cannot show that the viability of the cells is improved over the whole breadth of the claims of the Main Request and of the First Auxiliary Request.

5.3 The solution

The present claims exclude that the transfer instrument is an electron microscopy grid. The loops and paddles used as the transfer instruments may, however, **contain** "wire grids" (see point 5.2.2 above) such as electron microscopy grids.

Document (E2) discloses that the electron microscope copper grids have a diameter of 3.05 mm and were "handled with a watchmaker's forceps" (see the bottom paragraph in the left column on page 1061).

The Appellants' argument that the person skilled in the art of embryology would not have been aware of documents (E3) to (E5) was based on point 6 of both affidavits (E13) and (E14). These affidavits do, however, not take into account that the problem to improve the handling is not a problem specific to embryology but a mechanical one. When solving this mechanical problem, the person skilled in the art would have consulted the art in neighbouring fields where the same problem could occur and might have been solved when vitrifying biological samples. Consequently, he would have been aware of document (E3). This document deals with cryocrystallography and discloses flash cooling of macromolecular crystals such as proteins in a loop (see Figure 4 on page 194 and page 195, the first two sentences under the heading "Flash Cooling"; see page 185, line 9 and the examples on page 190 which all concern proteins), optionally in the presence of a cryoprotective additive (see the chapter "Cryoprotectant Solvents" starting on page 186). Document (E3) mentions that the introduction of "a loop-mounting technique was a major advance ..." as compared to glass capillary tubes, fine glass fibres or small glass spatulas (see page 191, the first two sentences under the heading "Crystal-Mounting Tools and Techniques"). Therefore it was obvious to the expert to improve the handling of the samples on an electron microscopy grid by attaching the grid to a loop as disclosed in document (E3).

Hence, the subject-matter of claim 1 of the Main Request and of the First Auxiliary Request does not involve an inventive step.

As the Board can only decide on a request as a whole, both requests are rejected.

Second Auxiliary Request

6. Priority

It was not contested that the subject-matter of the claims of this request enjoys the priority claimed.

The Board found the priority to be valid. For instance the subject-matter of claim 1 is disclosed in the priority document

- on page 2 of 5, in the paragraph entitled "I Overview" and in the section entitled "II. Detailed description of the process" in the first two paragraphs and the first three lines of the third paragraph;
- on page 3 of 5, on lines 3 to 5 ("blastocysts"; "freezing material") and in the table ("morulas");
- on page 4 of 5, in lines 15 to 22 and in drawing 1/I on page 5 of 5 (step a) alternative (ii)).

7. Novelty

It was not contested that the subject-matter of the claims is novel. The only novelty objection raised in the appeal proceedings was based on the journal article (E1). This document was published after the present priority date. The priority being validly claimed, document (E1) does not form part of the state of the art. The Board is also satisfied that none of the other

prior art documents cited discloses the subject-matter of the present claims.

Hence, the subject-matter of the claims of the Second Auxiliary Request is novel.

8. Inventive step

8.1 Document (E2) is the closest prior art (see point 5.1 above).

8.2 The problem to be solved

The comparative tests described in the patent in suit show that the open loop technique (used in the patent in suit) as compared to the open pulled straw (OPS; used in (E2)) yields a higher percentage of viable cells after thawing.

Hence, the problem to be solved can be considered as to provide a vitrification technique for cells which improves the viability of these cells once thawed.

Said comparative tests show that this problem was solved in view of document (E2) as the closest prior art.

8.3 Is the solution obvious?

Document (E2) as such gives no indication that an open loop could be used to solve this problem. The other documents of the prior art do not address the problem to improve the viability. Documents (E3) to (E5) relate to crystallographic methods where the viability of a

cell is of no importance. Documents (E6) and (E7) neither address the viability of cells nor relate to any sort of conservation at low temperatures. Therefore the person skilled in the art would not have taken these documents into account when trying to solve the problem.

Hence, the subject-matter of claim 1 of the Second Auxiliary Request is based on an inventive step. The same applies to the subject-matter of dependent claims 2 to 11 of said request.

9. The Board is satisfied that no other deficiencies prejudice the maintenance of the patent on the basis of the claims of the Second Auxiliary Request.

10. Remittal

The claims of the Second Auxiliary Request contain various amendments with respect to the claims as granted. In order to ensure that the description be properly adapted under Rule 42(1)(c) EPC to the claims thus amended, the Board exercises its discretion under Article 111(1) EPC by remitting the case to the department of first instance.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to maintain the patent with the following claims and a description to be adapted:

   Claims 1 to 11 of the Second Auxiliary Request submitted during the oral proceedings of 23 March 2010.

The Registrar: The Chairman:

B. Atienza Vivancos P. Ranguis