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Datasheet for the decision of 19 June 2015

Case Number: T 0305/12 - 3.3.08

Application Number: 04783425.4

Publication Number: 1670902

IPC: C12N5/073

Language of the proceedings: EN

Title of invention:
METHOD FOR ENHANCING IN VITRO EMBRYO DEVELOPMENT BY SUPPLEMENTING CULTURE MEDIUM WITH ILOPROST

Applicant:
THE BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM

Headword:
Iloprost/THE BOARD OF REGENTS UNIVERSITY OF TEXAS

Relevant legal provisions:
EPC Art. 54, 56, 83

Keyword:
Main, first and third request - novelty (no)
Second, fourth and fifth request - not admitted
Sixth auxiliary request - requirements of the EPC met

Decisions cited:
T 0646/09

Catchword:

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Case Number: T 0305/12 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 19 June 2015

Appellant: (Applicant)	THE BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM 201 West 7th Street Austin, TX 78701 (US)
Representative:	Kenrick, Mark Lloyd Marks & Clerk LLP 1 New York Street Manchester, M1 4HD (GB)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 29 September 2011 refusing European patent application No. 04783425.4 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman	M. Wieser
Members:	B. Stolz
	D. Rogers

Summary of Facts and Submissions

I. The appeal lies against the decision of the examining division dated 29 September 2011 whereby European patent application No. 04 783 425.4 was refused. This decision was taken after the applicant did not approve the text of the sixth auxiliary request which was filed at oral proceedings held on 6 July 2010.

II. The main request in the appeal proceedings corresponds to the main request before the examining division, auxiliary requests 1 to 5 are newly filed, and auxiliary request 6 corresponds to auxiliary request 6 found allowable by the examining division (cf. point 43 of the minutes of the oral proceedings before the examining division).

III. The applicant (appellant) was summoned to oral proceedings. A communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), annexed to the summons, informed it of the preliminary non-binding opinion of the board on some of the issues of the appeal proceedings.

With this communication, the board introduced two new documents (D16 and D17) into the proceedings and informed the appellant why it considered these documents to be relevant for the assessment of patentability under Articles 54, 83 and 84 EPC.

IV. With letter dated 21 April 2015, the appellant informed the board that it was not attending the oral proceedings and that "*A decision on the papers currently on file is expected ...*".

V. Oral proceedings were held on 19 June 2015, in the absence of the appellant.

VI. Independent claims 1, 9 and 10 of the main request read:

"1. A method of enhancing *in vitro* development of a mammalian embryo comprising supplementing culture medium with Iloprost in an amount effective to promote complete hatching of the embryo.

9. A method of increasing the in vivo implantation potential of an *in vitro* fertilization embryo comprising: enhancing in vitro development of an embryo according to the method of claim 1, such that hatching potential of the embryo is enhanced and in vivo implantation potential is increased.

10. An improved cell culture medium for *in vitro* early development of a mammalian embryo wherein the improvement comprises a supplemental amount of iloprost effective to promote complete hatching of said embryo *in vitro*."

Dependent claim 15 of the main request reads:

"15. The medium of claim 10 wherein the amount of iloprost is between 0.1 µM and 10 µM".

Dependent claims 2 to 8 define specific embodiments of the method of claim 1. Dependent claims 11 to 14 and 16 to 20 define specific embodiments of the cell culture medium of claim 10.

VII. Independent claims 1 and 10 of the first auxiliary request are identical with claims 1 and 10 of the main request.

VIII. Claims 1 and 10 of the third auxiliary request read:

"1. A method of enhancing *in vitro* development of an embryo comprising supplementing culture medium with Iloprost in an amount effective to promote complete hatching of the embryo.

10. An improved cell culture medium for *in vitro* early development of an embryo wherein the improvement comprises a supplemental amount of iloprost effective to promote complete hatching of said embryo *in vitro*."

IX. Claim 1 of the sixth auxiliary request reads:

"1. A method of enhancing *in vitro* development of a mouse embryo comprising supplementing culture medium during the morulae to early blastocyst stage development of the embryo with Iloprost in an amount effective to promote complete hatching of the embryo."

X. The following documents are cited in this decision:

D16: Spinks et al., 1988, "*Antagonists of embryo-derived platelet-activating factor act by inhibiting the ability of the mouse embryo to implant*", J. Reprod. Fert. 84, 89-98,

D17: Spinks et al., 1990, "*Antagonists of embryo-derived platelet-activating factor prevent implantation of mouse embryos*", J. Reprod. Fert. 88, 241-248.

XI. In the written procedure the appellant neither commented on the novelty objection raised by the board in view of document D17 nor on the question whether the results reported in the patent application were in contradiction with the results reported in documents D16 and D17.

XII. The appellant requests that the decision under appeal be set aside and a patent be granted on the basis of the main request, or on the basis of the First to Sixth auxiliary request.

Reasons for the Decision

1. The main request, the first, the third and the sixth auxiliary request were submitted with the grounds of appeal. They are admitted into the proceedings.

2. In an appeal from a decision of an examining division in which a European patent application was refused the board of appeal has the power to examine whether the application or the invention to which it relates meets the requirements of the EPC. The same is true for requirements the examining division did not take into consideration in the examination proceedings or which it regarded as having been met. If there is reason to believe that such a requirement has not been met, the board shall introduce this ground into the proceedings (Headnote, decision G 10/93 (OJ EPO 1995, 172)).

3. When preparing the annex attached to the summons to oral proceedings, the board became aware of documents D16 and D17. The board informed the appellant of the two documents and why it considered them highly relevant for the assessment of novelty of claim 10 of

the main request and for the assessment of the compliance of the sixth auxiliary request with the requirements of Articles 83 and 84 EPC. With its letter informing the board that it would not attend the oral proceedings, the appellant did not address these objections.

Novelty (Article 54 EPC)

Main request and First auxiliary request

4. Claim 10 of the main and the first auxiliary request is directed to a cell culture medium for in vitro early development of a mammalian embryo comprising Iloprost in an amount effective to promote complete hatching of said embryo in vitro. The claim is a product claim which requires the product to be suitable for the in vitro early development of embryos. According to page 10 of the patent application as filed, effects of Iloprost on mouse embryo development were statistically significant at 0.1 µM or higher, and maximum augmentation occurred at 1 µM (lines 12-13). According to claim 15, which directly depends on claim 10, exemplary effective concentrations of Iloprost are between 0.1 µM and 10 µM.

5. Document D17 discloses effects of culture media comprising Iloprost on mouse trophoblast outgrowth in vitro. According to Table 4, Blastocyst outgrowth media comprising MEM/FCS and Iloprost at concentrations of 10 µg/ml, 1 µg/ml and further dilutions down to 10^{-5} µg/ml were tested (based on a molecular mass of Iloprost of 360 g/mol, this corresponds to concentrations ranging from about 27 µM to 27 pM); the concentration of 1 µg/ ml corresponds to 2.7 µM). These media were used for blastocyst outgrowth experiments (cf. page 243,

second full paragraph) and are thus suitable for the purpose of claim 1. The Iloprost concentration of 2.7 µM lies within the concentration range claimed to be effective for the development of embryos (cf. claim 15 and page 10, lines 12 to 13). The medium disclosed in document D17 therefore falls within the scope of claim 10.

6. Thus, the main request and the first auxiliary request lack novelty (Article 54(2) EPC).

Third auxiliary request

7. The cell culture medium of claim 10 of the third auxiliary request differs from the culture medium of claim 10 of the previous requests in that it is not only suitable for the in vitro development of a mammalian embryo but of any embryo.

8. For the reasons given in point 6 above, the cell culture medium disclosed in document D17 falls within the scope of this claim. Thus, the third auxiliary request lacks novelty (Article 54 EPC).

9. In view of the lack of novelty of claim 10 of the main, first auxiliary and third auxiliary requests, the board deems it not necessary to review compliance of these requests with the requirements of Article 123(2) EPC.

Admissibility of the second, fourth and fifth auxiliary requests

10. The appellant did not submit written sets of claims as the second, fourth and fifth auxiliary request. Instead, it phrased the requests submitted with its grounds of appeal as follows:

Second auxiliary request: "*By way of further amendments to the First Auxiliary Request, the Applicant deletes one or more claims 11, 12 and 13 of the First Auxiliary Request should the Board of Appeal consider any of these claims to be objectionable pursuant to Article 123(2) EPC.*"

Fourth auxiliary request: "*The Third Auxiliary Request is based upon the Main Request. However, the Applicant requests consideration of the Third Auxiliary Request: (i) in combination with the amendments made in the First Auxiliary Request; and(ii) in combination with the amendments made in the Second Auxiliary Request.*"

Fifth auxiliary request: "*For each of the First to Fourth Auxiliary Requests, the Appellant requests consideration of the allowability of:*
(i) only the method claims (i.e. claims 1 to 9 of the First Auxiliary Request); (ii) only the cell culture medium claims (i.e. claims 10 to 13 of the First Auxiliary Request)."

11. In essence, the appellant leaves it to the board to put a corresponding request in writing taking into consideration all of the reasons it may have for not allowing the higher ranking requests.

As stated in decision T 646/09 of 21 March 2014, "... *it is a fundamental principle of European patent law that the applicant is responsible for defining the subject-matter for which protection is sought by formulating appropriate requests (T 382/96, point 5.2; and T 446/00). This principle is enshrined in Article 113(2) EPC, which provides that the European Patent Office shall only consider and decide upon a European*

patent application in the text submitted to it, or agreed, by the applicant. The applicant cannot shift the responsibility to formulate requests to the EPO, in this case a board of appeal."

The board fully agrees with this reasoning and decides not to admit the second, fourth and fifth auxiliary request into the proceedings.

Sixth auxiliary request

12. Claims 1 to 5 of the sixth auxiliary request are identical with claims 1 to 5 of the fifth auxiliary request underlying the decision under appeal. The examining division decided that claims 1 to 5 of the fifth auxiliary request before it met the requirements of Article 123(2) EPC, that their subject matter was disclosed in the priority document and that they did not comprise subject matter excluded from patentability under Article 53(a) EPC (cf. points 29 to 31 of the decision).

The board has no reason to deviate from this decision.

13. Article 83 EPC (in general) and Articles 54 and 56 EPC (with regard to the subject-matter of claims 1 to 5 of the then fifth auxiliary request) were not an issue in the decision under appeal. In its communication attached to the summons to oral proceedings, the board raised objections under Article 54 and 83 EPC *ex officio* in the context of the disclosure of documents D16 and D17. Exercising the powers given to it under Article 111(1) EPC, the board will examine all these issues.

14. In the annex to the summons to oral proceedings, under Article 83 EPC, the board raised the question whether the results disclosed in documents D16 and D17 were in contradiction with the results disclosed in the patent application.

15. The method of claim 1 requires Iloprost to be added in an amount sufficient to promote complete hatching of the mouse embryo.

16. The patent application discloses the collection of mouse two cell embryos 2 days post conception. The embryos were cultured for 48 hours in HTF medium followed by 48 hours in α-MEM complemented with Earle's salts and 2 mM glutamine. Various amounts of Iloprost were added for various amounts of time. After 96 hours of incubation, 95% of the embryos had become blastocysts (page 6, lines 22-34). Embryos were examined for the presence of the zona pellucida, whose complete absence at this time was used as an indicator of complete hatching.

Iloprost enhanced the complete hatching of mouse embryos in a concentration dependent manner (page 10, lines 11-14). The effects were statistically significant at 0.1 µM or higher (Figure 1). Maximum augmentation of complete embryo hatching occurred at 1 µM, where 81 ± 7% (mean ±S.D. n=3) of the experimental embryos hatched completely. Exposure to Iloprost during the first 24 hours of culture (during this period most of the two-cell embryos developed into eight cell embryos) did not enhance hatching (page 10, lines 22-30). On the other hand, exposure to Iloprost between 0-72 hours or 24-72 hours after harvest yielded the same rates of complete hatching as did 0-96 hours exposure (Figure 2). The two critical periods of

exposure to Iloprost that ensured its full effects were from 24 to 42 hours and from 42 to 72 hours after harvest, corresponding to the transformation from eight-cell embryos to morulae and from morulae to early blastocysts, respectively (table at the bottom of Figure 2).

17. Document D17 discloses the culturing of 2 cell mouse embryos in protein free HTF medium for 72 hours until they reached the early blastocyst stage (page 243, second full paragraph). At this time 60-80% of the embryos had developed to expanded blastocysts. These blastocysts were then cultured for another 72 hours in MEM/FCS medium supplemented with various amounts of Iloprost. Blastocyst outgrowth was monitored. At 1 µg/ml Iloprost (corresponding to 2.7 µM) blastocyst outgrowth and thus embryo development, was completely inhibited (Table 4, and page 244, last paragraph). Although Iloprost was present at a concentration claimed to be beneficial during early embryo development, it was only added after the embryos had reached the early blastocyst stage. Thus, this result is not in contradiction with the results of the patent application.

18. Document D16 assays the effects of inhibitors of Platelet Activating Factor on mouse embryo development in early pregnancy. The factor is shown to have a positive effect. Document D16 discloses experiments in which two cell mouse embryos were cultured for 72 hours in media comprising 0, 5 or 10 µg /ml (corresponding to 0, 13.5 and 27 µM), respectively, of Iloprost (page 91, second paragraph). The presence of Iloprost did not affect the development of 2-cell embryos to the blastocyst stage (page 94). Read in the context of document D16, the board understands this paragraph as

saying that Iloprost did not have an inhibitory effect on embryo development. According to page 95, third full paragraph, however, embryos cultured in the presence of 10 µg /ml (27 µM) Iloprost produced significantly fewer blastocysts after 72 hours of culture. This concentration is however not within the range of 0.1 µM to 10 µM described and claimed to be effective by the patent application. In view of the fact that the claim requires the presence of Iloprost in an amount effective to promote complete hatching, the method disclosed in document D16 falls outside the scope of claim 1.

19. The board comes to the conclusion that there is no contradiction between the results disclosed in the patent application and those of the prior art. Hence no objection under Article 83 EPC against the sixth auxiliary request arises.

20. The purpose of a method claim is an inherent technical feature of the claim (Case Law of the Boards of Appeal, 7th edition, 2013, I.C.6.3.1, p. 154).

The purpose of the methods disclosed in documents D16 and D17 was to establish whether Iloprost has an inhibitory effect on embryo development which is different from the purpose of claim 1.

21. None of the prior art documents on file discloses a method of enhancing in vitro development of a mouse embryo comprising the addition of Iloprost during the morula to early blastocyst stage in order to promote complete hatching.

Claims 1 to 5 of the sixth auxiliary request are therefore novel and meet the requirements of Article 54 EPC.

22. Document D4, representing the closest prior art, discloses that prostaglandin F2α was able to overcome an inhibition of in vitro mouse embryo development induced by indomethacin, and possibly has a stimulating effect on the hatching of mouse embryos in vitro. Prostaglandin PGE2 could not overcome the indomethacin induced inhibition.

23. Starting from document D4, the technical problem underlying the claimed invention is seen in the provision of an alternative method of improving mouse embryo development in vitro.

24. The solution proposed by claim 1 comprises the addition of effective amounts of Iloprost to a culture of mouse embryos during development from the morula to the early blastocyst stage.

25. The results presented on page 10 and in Figures 1 and 2 of the patent application show that the method of claim 1 solves the technical problem.

26. It remains to be established whether this solution involves an inventive step.

27. Document D4 demonstrates an effect of prostaglandins on early embryo development but does not provide any reason or motivation for the skilled person to try Iloprost as an alternative.

28. Document D7 discloses that prostacyclin PGI2 and prostaglandin PGE2 are major products expressed in

human fallopian tubes where early embryo development takes place. Therefore, it proposes an effect of prostacyclin on early embryo development in vitro (ultimate sentence of the document). It also describes Iloprost as a prostacyclin analogue. Document D8 describes an effect of a prostacyclin on embryo implantation in the mouse. Document D3 discloses an effect of prostaglandin PGE2 on the in vitro hatching rate of ovine embryos. Document D16 discloses an inhibitory effect of high concentrations of Iloprost on blastocyst outgrowth in mouse embryos in vitro.

However, none of these documents renders obvious a stimulating effect of Iloprost on in vitro development of mouse embryos when added during the morula to early blastocyst stage.

On the basis of document D4, either alone or in combination with any of the other documents on file, the skilled person would not have arrived at the claimed solution in an obvious way and with a reasonable expectation of success.

29. The sixth auxiliary request meets the requirements of Article 56 EPC.

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 grant a patent with the following claims and a description to be adapted:

   Claims 1 - 5 of the sixth auxiliary request filed under cover of a letter dated 27 January 2012.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated