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Publications survey

OCTAX Laser Shot™ related scientific publications

A review of pre-clinical, clinical and follow-up studies

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1. Introduction and Document Details

About this document

OCTAX Laser Shot™: application and technical background

The OCTAX Laser Shot™ is a 1.48 um infrared diode laser system for microsurgery on a cellular level which works on semiconductor basis. It is focused onto the target, typically the zona pellucida of mammalian cleavage stage embryos through the microscope optics. Its intended use is to tangentially ablate a small section of the zona pellucida or thin the zona pellucida over a certain area to facilitate the hatching process of the embryo (assisted hatching). Assisted hatching has to be applied to cleavage stage embryos straight before embryo transfer on day two or three of embryo culture. It is a selected patient collective with otherwise poor prognosis of achieving a pregnancy which is thought to benefit from assisted hatching: patients of advanced maternal age, with a history of several failed IVF cycles, undergoing transfer of frozen-thawed embryos or showing oocytes with abnormal zona pellucida characteristics.

The optical and mechanical design of the OCTAX laser device is closely related to the first commercial 1.48 um infrared diode laser (Fertilase) developed by the same team lead by Dr. Klaus Rink. The OCTAX Laser Shot™ can be considered, with respect to the Fertilase, a second generation design in which duration and triggering of the laser pulse are software controlled whereas the optical and laser characteristics remain virtually unchanged, leveraging the extensive understanding and success of the Fertilase which is manifested in a large number of scientific publications and installations worldwide. Reference letters from some authors quoted in this document who are using both systems in parallel confirm the equivalence or the Fertilase and the OCTAX Laser Shot™ systems with respect to safety, effectiveness and treatment outcome.

The present document surveys basic, pre-clinical, clinical and follow-up studies on the safety and effectiveness of the OCTAX Laser Shot™ device and its predecessor, the Fertilase system. In doing so, it focuses on original publications only, i.e. book chapters and review articles summarizing previously published results will not be included.

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History

The OCTAX Laser Shot™, introduced to the market in October 2000, is the 2nd generation 1.48 um infrared diode laser developed at OCTAX Microscience GmbH by a team led by Dr. Klaus Rink. The development was based on extensive experience with the first commercial 1.48 um infrared diode laser (Fertilase), introduced to the market 1996 by MTM Medical Technologies Montreux SA, director research and development at that time: Dr. Klaus Rink (Physicist).

Document preparation

The document has been specifically designed to emphasize the clinical safety of the OCTAX Laser Shot™ device. The emphasis is on summarizing scientific key publications which are applicable to all 1.48 um infrared diode lasers for use in ART. Most of the basic studies were done on the Fertilase concept representing the predecessor of OCTAX Laser Shot™. Both systems, Fertilase and OCTAX Laser Shot, are sharing the energy range, the underlying optics, the laser beam characteristics and the laser-target interaction principle. Hence, there is substantial evidence that the pre-clinical and clinical results presented here are valid for the OCTAX Laser Shot™ system.

This document has been prepared by Dr. Klaus Rink and Dr. Thomas Schimming from OCTAX Microscience GmbH, assisted by Dr. Paul Gassner from MTG Medical Technology Vertriebs-GmbH. Dr. Rink has 15 years of experience with medical laser applications. Together with his research group, he adapted the 1.48 um laser technology to ART requirements, developing the only concept which now – under different brands – is commonly accepted for ART applications. Dr. Rink has been a co-author of all fundamental research papers published by the Swiss Federal Institute of Technology Lausanne, Switzerland and the CHUV Cantonal Hospital Lausanne, Switzerland, prior to the first commercialization of a 1.48 um Laser for use in ART. Beyond that, as a physicist, he has extensive knowledge of laser technology beyond the 1.48 um semiconductor laser used in the OCTAX Laser Shot™ and its predecessors (commercial and research).

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2. Contents and Scope

Overview of the literature available on the OCTAX Laser Shot™ system and its underlying technology.

Presentation of relevant literature

Each article will be presented following a fixed structure according to the guidance document of the FDA (U.S. Food and Drug Administration). Main information and results will be extracted and related to the claims for OCTAX Laser Shot™. Articles will be arranged according to the year of publication.

The presentations have been subdivided into the following categories:

Pre-clinical (basic) studies: fundamental investigations on laser effects, efficiency and safety

Clinical studies: investigations on the safety and efficacy of clinical laser application on human material in ART

Follow-up studies: investigations on the long term effects of laser treatment in ART

Pre-clinical (basic) studies

In this part, the available relevant publications covering the aspect of animal testing of the underlying laser technology are surveyed. It should be emphasized that a large majority of the scientific material published on this technology was authored and co-authored by a group involving Dr. Klaus Rink, the lead scientist responsible for the design of the OCTAX Laser Shot™ system. Therefore it is safe to state that the OCTAX Laser Shot™ system is based on extensive experience with the employed laser technology established over the last 15 years.

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Reference 1: **Rink K, Delacrétaz G, Salathé RP, Senn A, Nocera D, Germond M, Fakan S (1994)** ***1.48 um Diode Laser Microdissection of the Zona Pellucida of Mouse Zygotes*****. SPIE Vol. 2134A Laser-Tissue Interaction V,412-422.**

Objective(s): To determine the effectiveness and reproducibility of the 1.48 um laser in drilling holes in mouse zona pellucida; to investigate the morphology of laser drilled holes; to reveal the impact of laser exposure on embryonic development up to the blastocyst stage

Study protocol: 1.48 um laser beam (max. emission power 120 mW) coupled to an inverted microscope, combined with a 670 nm diode laser aiming beam; laser drilling of the mouse zona pellucida was studied in various laser power (up to 70 mW post objective) and pulse time (5-90 ms) combinations; the procedure was monitored using a CCD camera.

Groups, group sizes: group 1, control (n=150);group 2, laser exposed (n=150)

Endpoints: Hole diameter as a function of irradiation time; ultra structure (SEM) of the holes drilled by the laser; blastocyst formation rate in both groups

Follow-up: none

Results: By only one laser pulse, holes of controlled size can be drilled with very good reproducibility (< 1 um); there is a linear relationship between pulse length and hole size at a given laser power; at a pulse time of 90 ms, thermal convection of the medium near the drilling site was observed; scanning electron microscopy of the drilled holes could not detect any evidence of thermal damage; concerning post-treatment development, drilled embryos started hatching one day earlier, but no difference could be observed in blastocyst rate (70 %) between treated cells and the control group.

Relevance of results for the clinical safety of OCTAX Laser Shot™: The technology underlying the OCTAX Laser Shot™ system is effective in drilling holes in the zona pellucida with high reproducibility. As laser power is fixed, hole size is a strict function of pulse time. Consequently, setting the physical preconditions for laser application is safe, precise and easy. Under given conditions (temperature of the heated stage, laser application through a special objective), the laser will steadily maintain the selected hole size. Extremely long pulse times as tested in this experiment are irrelevant for clinical application.

Scanning electron microscopy revealed that the holes drilled by using an early version of the OCTAX technology show clear-cut walls with no thermal damage evident. It may be concluded that the thermal effect of laser drilling is limited to the direct vicinity of the hole and will not affect the oocyte (or a blastomere in embryos) if the drilling site is chosen carefully with a maximum distance to cellular structures.

The developmental potential of the zygote up to the blastocyst stage was not compromised by the application of the basic OCTAX laser technology. It can be concluded that the laser impact does not cause severe physiological or genetic damage leading to immediate changes/delay in early embryonic development.

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Reference 2: **Germond M, Nocera D, Senn A, Rink K, Delacrétaz G, Fakan S (1995)** ***Microdissection of mouse and human zona pellucida using a 1.48-um diode laser beam: efficacy and safety of the procedure*****. Fertil Steril 64(3),604-611.**

Objective(s): To investigate the efficacy and safety of a 1.48 um continuous wave diode laser for zona pellucida (ZP) microdissection; to evaluate in vitro and in vivo mouse embryo development and long-term genetic and reproductive effects after laser treatment.

Study protocol: Collaborative study between three Swiss academic centers; fresh mouse oocytes and zygotes as well as aged surplus human oocytes were submitted to 1.48 um laser ZP drilling at varying pulse times (6 to 100 ms). The hole characteristics and possible laser-induced structural alterations of the neighboring cytoplasm were investigated with scanning and transmission electron microscopy. After drilling, part of the mouse zygotes were cultured in vitro for 5 to 6 days. Treated and control embryos were transferred into foster mothers. Pregnancies were allowed to proceed to term. Development of the offspring was followed over several months and their reproductive ability was tested by various cross-matings.

Groups, group sizes:

In vitro culture: mouse control 1 (n=68): standard incubation conditions without any manipulation; mouse control 2 (n=84): standard incubation conditions after manipulation identical to the laser treated group, but without laser drilling; mouse experimental group (n=90): standard incubation conditions after laser drilling and the respective manipulations;

In vivo development after transfer: mouse control 1 (n=106): embryo transfer after standard incubation conditions without any manipulation; mouse experimental group (n=98): embryo transfer after laser drilling and the respective manipulations;

Endpoints: For in-vitro culture: hole characteristics under light, scanning and transmission electron microscopy; blastocyst formation rate; for in-vivo development: live-birth rate, abnormalities and reproductive capacity of the offspring

Follow-up: 35 newborn mice after embryo transfer were followed up over a period of 6 months

Results: The 1.48 um laser achieved a rapid, precise and easily controlled thermolysis of the ZP. Under light and scanning electron microscopy, the drilled hole appeared clearly delimited. Transmission electron microscopy on ultra-thin sections of 20 oocytes and some zygotes did not show ultra-structural alterations of the peripheral cytoplasm at the laser impact site. Hole series with increasing pulse lengths were drilled in mouse and human oocytes. Hole size was an exact function of pulse length in both species with an extremely precise hole-to-hole reproducibility in three repetitive experiments.

Mouse blastocyst formation rate in vitro was comparable among groups (control 1: 75.0 %, control 2: 66.6 %, experimental: 70.2 %). There were no statistical differences among groups regarding live-birth rate (control 1: 48.1 %; experimental: 42.9 %; Chi Square test). After laser treatment, no malformations were detected in 35 newborns followed over a period of 6 months. Laser treated offspring was cross-mated over two generations without any abnormalities in sex proportion, litter sizes or phenotype (exact data not shown).

Relevance of results for the clinical safety of OCTAX Laser Shot™:

All conclusions drawn from reference 1 are confirmed by the extended experiments presented here. Mouse and human oocytes seem to behave similarly under laser treatment.

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This underlines that fundamental experiments on mouse oocytes are valid for human application. Moreover, transmission electron microscopy on laser treated oocytes and zygotes could show that the applied technology (underlying the OCTAX Laser Shot system) is safe in that it has no effects on cytoplasmic ultrastructure (organelles etc.) even in close vicinity of the laser impact site. This is confirmed by the finding that laser treated zygotes maintain their full developmental capacity in vivo compared to control cells. The lack of any malformations or abnormalities regarding fertility, sex proportion and phenotype over 3 generations after laser treatment implies that the OCTAX technology is safe with respect to mutagenic, physiologic or metabolic effects. Laser pulse times used in these fundamental experiments exceed the pulse times recommended for use with the OCTAX Laser Shot™, establishing a certain factor of safety.

Reference 3: **Rink K, Descloux L, Delacrétaz G (1995)** ***Zona pellucida drilling by a 1.48 um laser: influence on the biomechanics of the hatching process.*** **SPIE 2624,26-32.**

Objective(s): Comparative biomechanical study of the hatching process in mouse blastocysts after 1.48 um laser drilling at the zygote stage

Study protocol: A 1.48 um laser was used to drill the zona pellucida (ZP) by one 15 ms laser pulse. Embryo development was monitored daily, determining outer ZP diameter and ZP thickness from digitized images.

Groups, group sizes: Control group: zygotes cultured in vitro without laser treatment; experimental group: zygotes cultured in vitro after laser drilling of the ZP (group sizes not indicated).

Endpoints: Culture time until hatching, ZP width (um), ZP diameter (um)

Follow-up: none

Results: In control embryos, the ZP volume increased by a factor of 1.8 during expansion of the blastocyst with a concomitant decrease of its thickness. In the laser treated group, neither an increase in ZP volume nor a prominent thinning of the ZP could be observed. Hatching through the drilled hole started one day earlier in the experimental group.

Relevance of results for the clinical safety of OCTAX Laser Shot™:

Assisted hatching using the underlying technology of the OCTAX Laser Shot system is effective (even if applied to zygotes instead of cleavage stage embryos as usual). This could be shown by the altered hatching behavior and the earlier time point of hatching in the experimental group.

Reference 4: **Germond M, Nocera D, Senn A, Rink K, Delacrétaz G, Pedrazzini T, Hornung JP (1996)** ***Improved fertilization and implantation rates after non-touch zona pellucida microdrilling of mouse oocytes with a 1.48 um diode laser beam.*** **Hum Reprod 2(5),1043-1048.**

Objective(s): To investigate the safety of microdrilling the zona pellucida of mouse oocytes with a 1.48 um diode laser by determining fertilization ability in vitro and development in vivo; to perform anatomical and immunohistochemical examinations on the offspring after laser treatment; to test the breeding ability of the progeny.

Study protocol: Oocytes from three groups with and without laser treatment were inseminated in vitro with suboptimal sperm concentrations and checked for fertilization; two-

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cell embryos from each group were transferred into foster mothers; the percentage birth rates were calculated; part of the progeny was bred over two subsequent generations, offspring of all study groups and from spontaneous breeding was submitted to anatomical and immunohistochemical investigations at an age of 15 weeks.

Groups, group sizes: Control 1: in-vitro fertilization of intact oocyte-cumulus complexes (n=1,785; 80 embryos transferred); control 2: in-vitro fertilization of denuded metaphase II oocytes (i.e., without cumulus cells; n=1,739; 128 embryos transferred), no laser treatment; experimental group: in-vitro fertilization of denuded, 1.48 um laser-treated metaphase II oocytes (n=1,760; 96 embryos transferred)

Endpoints: fertilization rates (%), pregnancies / transfer (%), numbers of offspring / embryos transferred (%), sex ratio male / female (%), litter sizes in the F2 generation, anatomical and immunohistochemical findings in laser treated offspring and subsequent generations

Follow-up: laser-treated offspring was investigated (see above) and followed up to the F2 generation

Results: Cumulus removal induced a significant drop in fertilization rate ($p < 0.001$; Chi square test) which could be (over-) compensated for by laser drilling of the zona pellucida which almost doubled fertilization rate ($p < 0.001$; Chi square test). Polyploidy due to several sperm entering the oocyte was not routinely observed.

Pregnancy rate per transfer was highest in control 1 (80.0 %), but comparable in control 2 (62.5 %) and the experimental group (66.7 %). Numbers of offspring were significantly reduced in control 2 (15.6 %) compared to control 1 (32.5 %) and the laser treated group (29.2 %; $p < 0.0001$). Sex ratios were similar among groups in both the F1 and the F2 generation and close to 50 %. Litter sizes indicating reproductive ability were similar as well (8.5, 7.5 and 8.0, respectively).

Anatomical examination of laser-treated offspring revealed no morphological alterations of the digestive tract, the urinary and reproductive tract and the brain and spinal cord structure as compared to control groups and offspring derived from spontaneous matings.

Immunohistochemical evaluation of the neuronal system confirmed that no apparent modifications of the expression of specific markers (neurotransmitters, receptors, enzymes, related proteins) were present in the experimental animals compared to controls.

Relevance of results for the clinical safety of OCTAX Laser Shot™:

Fertilization rate of denuded oocytes treated with the technology underlying the OCTAX Laser Shot system was equal to the rate obtained under physiological conditions with non-denuded control cells. This indicates that laser treatment does not compromise the physiological processes involved in the fertilization cascade of the oocyte. Cumulus removal by the enzyme hyaluronidase seems to alter zona pellucida hardness, leading to a significant decrease in offspring numbers in control group 2. This could be compensated for by laser treatment of the zygote in the experimental group, being effective as assisted hatching in the blastocyst stage after embryo transfer: the proportion of offspring per number of transferred embryos was back to normal values (represented by control group 1) after treatment with OCTAX laser technology.

The absence of both altered sex ratio/decreased fertility and anatomical or immunohistochemical abnormalities of the neuronal system suggests that laser treatment has no long-term mutagenic or developmental effects on offspring.

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Reference 5: **Montag M, van der Ven K, Delacrétaz G, Rink K, van der Ven H (1998)** ***Laser-assisted microdissection of the zona pellucida facilitates polar body biopsy.*** **Fertil Steril 69(3),539-542.**

Objective(s): To investigate whether polar body biopsy can be performed after laser microdissection of the zona pellucida (ZP)

Study protocol: A hole was drilled in the ZP of mouse zygotes using a 1.48 um noncontact diode laser (Fertilase) at pulse lengths of 12-16 ms. A microneedle was inserted and the polar body was aspirated. Control groups received laser treatment alone or no treatment.

Groups, group sizes: 154 zygotes were randomly assigned to group 1 (laser drilling and polar body biopsy; n=52), group 2 (laser drilling only; n=52) or group 3 (not treated; n=50)

Endpoints: Efficacy of polar body biopsy after laser drilling of the ZP; cleavage, blastocyst formation and hatching rates

Follow-up: none

Results: The area of the zygotes located close to the polar body was microdissected precisely by a single laser pulse. No damage to the polar bodies or zygotes was observed during the laser drilling and biopsy procedure. Polar bodies always maintained their cellular integrity. Cleavage (92, 87 and 94 %, respectively) and blastocyst formation rates (88, 93 and 89 %, respectively) were comparable among groups. In the untreated control group, blastocysts hatched one day later and at a significantly lower rate than in the laser treated groups (85, 84 and 62 %, respectively; $p < 0.05$).

Relevance of results for the clinical safety of OCTAX Laser Shot™:

Laser microdissection of the zona pellucida using the Fertilase (and OCTAX) technology is safe and reproducible. The cellular integrity of the polar bodies to be biopsied was fully maintained after applying the laser in close vicinity (pulse lengths 12-16 ms, exceeding the regular values needed with the OCTAX system), followed by the mechanical stress of aspiration. For assisted hatching, the laser would be applied identically, omitting both close proximity to the cells and mechanical stress. This makes assisted laser hatching even safer. Laser application does not compromise early embryonic development as shown by the normal cleavage and blastocyst formation rates of treated zygotes. The increased hatching rate of laser treated embryos and the early time point of hatching shows that laser drilling of the ZP is effective in facilitating the hatching process.

Clinical studies

For use of the 1,48 um laser technology in assisted hatching, the worldwide experience is documented in the following publications without any claim of completeness. All these publications have been established with the Fertilase laser, the predecessor of the OCTAX Laser Shot™.

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Due to technological similarity of all currently worldwide available 1,48 um lasers used in assisted hatching to the initial setup tested in the basic studies quoted above, these results also apply to the OCTAX Laser Shot™.

Reference 6: **Boada M, Carrera M, De La Iglesia C, Sandalinas M, Barri PN, Veiga A (1998)** ***Successful Use of a Laser for Human Embryo Biopsy in Preimplantation Genetic Diagnosis: Report of Two Cases.*** **J Assist Reprod Genet 15(5):301-306.**

Objective(s): To perform blastomere biopsy and PGD on human embryos using a 1.48-um diode noncontact laser for zona drilling

Study protocol: PGD for hemophilia was performed on 13 cleavage stage embryos from 2 patients. The Fertilase microsurgical laser was used to drill the zona pellucida, applying two to four consecutive shots of 8-22 ms pulse length

Groups, group sizes: none (case report)

Endpoints: blastocyst formation rate, implantation rate

Follow-up: none

Results: A well defined hole for blastomere biopsy was obtained in a minimum time, resulting in 100 % biopsy efficiency. Non-transferred embryos were submitted to extended culture, resulting in a blastocyst rate of 66.7 % in embryos lacking chromosomal abnormalities. In one of the patients, an ongoing singleton pregnancy could be achieved which at the time of publication had progressed to week 19. Implantation rate was 33.3 %.

Relevance of results for the clinical safety of OCTAX Laser Shot™:

This is one of the pioneering publications on the use of the Fertilase (corresponding to the OCTAX) technology for blastomere biopsy. The zona drilling procedure for blastomere biopsy closely resembles the laser assisted hatching process. This publication could show that applying the laser is easy and reproducible. The high blastocyst and implantation rates as well as the ongoing pregnancy indicate that laser treatment of cleavage stage embryos – even if using pulse lengths up to 22 ms – is safe and has no adverse effect on early embryonic development and implantation potential.

Reference 7: **Montag M, van der Ven H (1999)** ***Laser-Assisted Hatching in Assisted Reproduction*****. Croatian Medical Journal 40(3):398-403.**

Objective(s): To investigate the effect of laser assisted hatching on mouse zygotes and blastocysts; to evaluate a potential benefit of assisted hatching in patients of advanced maternal age

Study protocol: Openings of different sizes were drilled in the zona pellucida (ZP) of mouse cleavage stage embryos, using laser pulse times of 4-10 ms (Fertilase); development was monitored up to the blastocyst stage; in a second approach, two separate openings were drilled; a third experiment involved the zona dissection at the blastocyst stage. The efficacy of laser assisted hatching and the hatching behavior was observed in all three approaches.

Assisted hatching was offered to patients aged over 35 years who were retrospectively matched with controls receiving no assisted hatching.

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Groups, group sizes:

Mouse experiments: group 1, zygotes with a 15 um laser drilled opening in the ZP (n=52); group 2, zygotes with a 5 um opening (n=48); group 3, control, no laser drilling, n=50

Human study: group 1, laser assisted hatching (n=24); group 2, control, no assisted hatching (n=24)

Endpoints:

Mouse experiments: blastocyst formation rate, hatching rate, hatching behavior

Human study: clinical pregnancy rate (%), implantation rate (%)

Follow-up: none

Results:

Mouse experiments: Laser assisted hatching involving the use of Fertilase did not interfere with embryonic development, as all three groups showed similar blastocyst formation rates (81, 83 and 84 %, respectively). Blastocysts developing from laser-drilled zygotes with a larger ZP opening showed a significantly higher hatching rate on day 6 compared to the other groups (90, 50 and 69 %, respectively). There was no difference between groups 2 (small opening) and 3 (no drilling). Two large openings in the ZP resulted in hatching failure in 100 % of treated zygotes: both openings were used for escaping from the ZP and the embryos were trapped within the ZP. Laser drilling at the blastocyst stage resulted in a collapse of the embryos with delayed recovery after 4 hours and delayed hatching.

Human study: Study groups were comparable with respect to baseline parameters (age, number of previous treatment cycles etc.). There was a slight, non-significant trend towards a higher pregnancy rate in group 1 (33.3 % vs. 12.5 %). However, implantation rate was significantly elevated after laser treatment (22.0 % vs. 5.3 %; $p < 0.01$, Chi-square test), resulting in five twin pregnancies.

Relevance of results for the clinical safety of OCTAX Laser Shot™:

The use of the Fertilase (OCTAX) technology for assisted hatching is safe and efficient as far as a single opening of appropriate size is drilled and the procedure is performed on zygotes or cleavage stage embryos. Assisted hatching in human embryos does not compromise early embryonic development and implantation potential, but rather seems to have a beneficial effect on implantation and pregnancy rates in selected patient groups.

Reference 8: **Blake DA, Forsberg AS, Johansson BR, Wikland M (2001)** ***Laser zona pellucida thinning – an alternative approach to assisted hatching.*** **Hum Reprod 16(9):1959-1964.**

Objective(s): To assess the efficacy of zona thinning in human embryos and to study the hatching characteristics of these embryos.

Study protocol: In a prospective study, partial zona thinning was performed on surplus embryos using the Fertilase system: up to six laser ablations involving 50-80 % of the zona thickness over a total length of approx. 80 um were made; variation of zona thickness and degree of zona expansion was assessed; SEM was performed on hatching blastocysts to identify the site of hatching; the hatching rate was compared to a non-treated control group.

Groups, group sizes: Group 1, laser thinned: 110 embryos from 49 patients; group 2, control (sham laser treatment at 100 um distance): 42 embryos from 23 patients

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Endpoints: Variation of zona thickness (%), blastocyst formation rate (%), proportions of blastocysts initiating hatching and completing the hatching process; ultrastructure of the hatching site

Follow-up: none

Results: Variation of zona thickness was comparable between groups prior to laser thinning, but significantly elevated in the treated group after laser thinning (27.3 vs. 11.7 %; $p < 0.0001$; Student´s t-test). There was no significant difference in blastocyst formation rate (group 1: 51.0 %; group 2: 43.0 %). Significantly more blastocysts initiated hatching in group 1 (68.0 vs. 33.0 %; $p = 0.009$). None of the control blastocysts completed hatching within 7 days of culture compared to 50 % in the laser treated group. SEM analysis of 16 laser treated embryos revealed 100 % hatching through the thinned area.

Relevance of results for the clinical safety of OCTAX Laser Shot™:

The Fertilase system (corresponding to the OCTAX technology) is effective in performing zona thinning to assist the hatching process as shown by an increase in the variation of zona thickness and a 100 % hatching rate at the laser impact site. Zona thinning does not compromise early embryonic development. The increased proportions of blastocysts initiating and completing hatching in the laser treated group indicate that zona thinning facilitates the hatching process considerably.

Reference 9: **Hsieh Y-Y, Huang C-C, Cheng T-C, Chang C-C, Tsai H-D, Lee M-S (2002)** ***Laser-assisted hatching of embryos is better than the chemical method for enhancing the pregnancy rate in women with advanced age.*** **Fertil Steril 78(1):179-182.**

Objective(s): To compare the efficiency of laser and chemically assisted hatching

Study protocol: In this prospective study, two groups of patients aged Ó38 years received either laser-assisted hatching (Fertilase, 1.48 um, pulse time 5-10 ms) or chemical-assisted hatching (acid Tyrode´s). Hatching was performed on day 3 (cleavage stage) embryos.

Groups, group sizes: group 1, laser-assisted hatching (85 patients, 346 embryos); group 2, chemical-assisted hatching (56 patients, 255 embryos)

Endpoints: Implantation rate (%), clinical pregnancy rate (%), delivery rate (%)

Follow-up: Pregnancies were followed up to term

Results: Baseline parameters (oocyte numbers, fertilization rates, embryo quality, number of embryos per transfer, miscarriage rate, multiple pregnancies) were comparable between groups. Implantation rate (8.2 vs. 3.8 %; $p=0.032$), clinical pregnancy rate (31.8 vs. 16.1 %; $p=0.037$) and delivery rate (24.7 vs. 10.7 %; $p=0.039$) was significantly increased in the laser treated group (Chi-square test).

Relevance of results for the clinical safety of OCTAX Laser Shot™: Laser-assisted hatching is superior to the application of acid Tyrode´s with respect to toxic effects, ease of handling and reproducibility. This resulted in improved treatment outcome as compared to chemical-assisted hatching. Both the clinical pregnancy rate after day 3 embryo transfer and the delivery rate in women of advanced age were in the upper range after laser treatment, suggesting that laser assisted hatching using the Fertilase (OCTAX) technology is safe and might be beneficial if applied to selected patient collectives.

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Reference 10: **Joris H, De Vos A, Janssens R, Devroey P, Liebaers I, Van Steirteghem A (2003)** ***Comparison of the results of human embryo biopsy and outcome of PGD after zona drilling using acid Tyrode medium or a laser.*** **Hum Reprod 18(9):1896-1902.**

Objective(s): To compare the results of embryo biopsy and the outcome of PGD after zona drilling using acid Tyrode medium or a 1.48 um laser (Fertilase).

Study protocol: Zona drilling for blastomere biopsy and PGD was performed by acid Tyrode medium for a period of 7 months and subsequently by laser for another 7 months. The laser was applied in two to three pulses of 5-8 ms each. Results from both time intervals were analyzed retrospectively.

Groups, group sizes: Group 1, zona drilling with acid Tyrode medium (59 treatment cycles, 356 biopsied embryos); group 2, laser drilling of the zona (69 treatment cycles, 402 biopsied embryos); group 3, control: all regular ICSI cycles (i.e., without zona drilling and biopsy) during the respective study periods (n=685 during the acid Tyrode treatment period; n=593 during the laser treatment period)

Endpoints: Proportions of intact blastomeres after biopsy (%), embryo development over time before and after biopsy, embryo quality before and after biopsy, implantation rate (%), biochemical pregnancy rate (%), percentage deliveries per embryo transfer.

Follow-up: Pregnancies in both groups were followed up to delivery.

Results: Significantly more intact blastomeres were obtained in group 2 (95.2 vs. 98.3 %, p=0.02; t-test). There was no cell lysed by laser-assisted zona drilling, whereas 19 cells were lysed due to acid Tyrode´s exposure. Progress of embryo development was similar between groups, however, more embryos compacted earlier in group 2. Embryo quality was not affected by one of the drilling methods. Implantation rate (18.9 vs. 14.9 %), biochemical pregnancy rate (37.5 vs. 35.5 %) and delivery rate (31.3 vs. 25.0 %) was comparable between groups. No differences between either treatment group and the background control of regular ICSI cycles could be shown with respect to implantation, biochemical pregnancy and delivery rates.

Relevance of results for the clinical safety of OCTAX Laser Shot™: The procedures of zona drilling for blastomere biopsy and assisted hatching are fundamentally identical, as every zona opening for blastomere biopsy will facilitate hatching of the blastocyst. Under this aspect, the present study confirms the safety of the Fertilase (OCTAX) technology regarding cell integrity, embryo quality and implantation potential. Identical implantation, pregnancy and delivery rates after transfer of laser treated embryos and embryos after “regular” ICSI (no laser) imply that laser drilling does not affect embryonic and fetal development. It can be concluded that laser treatment does not interfere with the underlying genetic and ontogenetic processes.

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Follow-up studies

The following publication is the only relevant follow-up study on long-term effects of assisted hatching involving a considerably high number of cases. The study was performed using Fertilase. For the reasons mentioned above, there is substantial evidence that its results are fully applicable to the OCTAX Laser Shot™ system.

Reference 11: **Kanyó K, Konc J (2003)** ***A follow-up study of children born after diode laser assisted hatching.*** **Eur J Obstet Gynecol Reprod Biol 110:176-180.**

Objective(s): To evaluate the safety of 1.48 um laser assisted hatching, compiling data on karyotypes, deliveries, congenital malformations and growth parameters.

Study protocol: The study enclosed 134 children born after laser assisted hatching (LAH) using Fertilase. Indications for LAH were maternal age > 35 years, > 3 failed IVF cycles, or both. For laser drilling, two to three laser pulses of 10-15 ms each were applied to the zona pellucida of 2-6 cell stage embryos on the second day after oocyte retrieval. Embryo transfer was performed one day later. Follow-up comprised prenatal karyotyping in 56.7 % of the babies and the completion of questionnaires at delivery and after 3, 6 and 12 months.

Groups, group sizes: Group 1, LAH performed, maternal age > 35 years and/or >3 failed IVF attempts: 384 treatment cycles, 134 children born; group 2, internal control, LAH not performed, maternal age Ò 35 years, 219 treatment cycles; group 3, external control for follow-up, 894 children born after spontaneous conception.

Endpoints: Implantation, pregnancy, miscarriage and delivery rate for groups 1 to 3; karyotype; occurrence of minor and major congenital malformations

Follow-up: Children were followed up over a period of 1 year post delivery

Results: Treatment outcome: Group 1 showed no significant differences in implantation rate (14.0 %), pregnancy rate (30.9 %) and miscarriage rate (19.3 %) as compared to group 2 (15.5 %, 30.1 % and 13.6 %, respectively). Follow-up: two structural chromosome aberrations were diagnosed in the LAH treated groups by karyotyping, but both of them were detected in the parents as well. The rates of minor (10.4 vs. 11.1 %) and major congenital malformations (2.2 vs. 3.0 %) were comparable between groups 1 and 3. The follow-up examinations at 3, 6 and 12 months revealed no additional anomalies.

Relevance of results for the clinical safety of OCTAX Laser Shot™: This is a unique study with respect to the follow-up period after Fertilase treatment (the underlying technology of the OCTAX™ Laser Shot system is identical). Using this technology to perform LAH on human embryos was shown to have no adverse effects on implantation, pregnancy and miscarriage rates. Treatment outcome in terms of implantation and pregnancies equaled the results of an internal control group with better chances of treatment success due to lower maternal age and fortunate treatment history. This suggests that selected patient groups may benefit from LAH. There were no chromosomal abnormalities which could be attributed to laser treatment. No increase in minor or major congenital abnormalities could be detected after LAH in comparison with a large control collective. According to these findings, Fertilase (or OCTAX) treatment for LAH is non-mutagenic and non-teratogenic.

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Summary

The multitude of results from selected scientific papers presented here can be considered to be valid for the documentation of the safety and effectiveness of the OCTAX Laser Shot™ device.

The scientific results presented in this literature survey provide sufficient evidence that the OCTAX Laser Shot™ device, if applied appropriately

- is effective in drilling clear-cut holes in the zona pellucida of oocytes, zygotes and embryos in a highly reproducible way. The hole size can be precisely selected by varying the pulse length of laser irradiation.
- is effective in facilitating the hatching process of embryos at the blastocyst stage after both conventional laser assisted hatching and laser assisted zona thinning
- does not harm the treated cells/embryos with respect to detectable thermal effects leading to ultrastructural or histological changes in close vicinity of the laser impact site
- does not affect or impair short-term or long-term embryonic development in vitro and in vivo
- has no negative effect on the implantation process of the treated embryos and on pregnancy rate of patients opting for assisted hatching
- has no adverse effects on fetal development nor teratogenic effects
- has no mutagenic effects
- does not lead to an increase of minor or major malformations in treated offspring
- has no negative effects on the reproductive potential of laser treated offspring (as tested in animal studies)

In summary, this document provides reasonable assurance that there are no specific safety risks if the OCTAX Laser Shot™ device is used appropriately to treat the zona pellucida of human embryos in order to facilitate the hatching process.

The full articles summarized in this document can be provided upon request.