

Assisted hatching as a technique for use in human in vitro fertilization and embryo transfer is long overdue for careful and appropriate study

Michael J Tucker, Ph.D.

Assisted hatching (AH) as an adjunct to IVF-ET has been around for a long time (Cohen et al 1990); and as Venkat et al (2008) rightly pointed out in their recent paper, it has only been minimally studied, especially in a randomized manner. While their efforts to raise consciousness on this subject are appreciated by ourselves, we were disappointed that their own study was merely a retrospective review of five year old data, which ultimately just suggested inconclusively that AH was not beneficial following embryo thawing and transfer (FET). We felt it worth responding to this paper both to point out some shortcomings of the Venkat study; and to make some proposals moving forward, with the hope that we might stimulate more definitive study of this subject.

We would initially like to make it clear that we are advocates of the use of laser systems for zona pellucida (ZP) ablation, in particular for embryo biopsy procedures where this hands-free technology enables a much faster, and more convenient approach to opening the ZP than either chemical, or mechanical means (Han et al 2003). We suspect that with regard to this procedure that the laser has become the tool of choice.

On the other hand, assisted hatching, irrespective of the technical approach, remains a procedure of largely unproven worth regardless of how theoretically appealing it might seem to apply to poorer prognosis IVF-ET cases. The temptation to apply AH to repeat failure patients that may well include older patients, those with elevated basal FSH, cases of consistently poor embryo morphology and the like, is very real. But in so doing we continue to operate less as a science and more as a therapeutic art! That said, we have attempted to dispel some of these possible myths. For example, it is doubtful that an elevated basal FSH by itself is sufficient grounds to warrant application of AH (Assemi et al 2006); but although this was analyzed retrospectively in this particular study, it does, nevertheless, prompt future study of this preliminary conclusion in our clinics.

There does exist a little more circumstantial evidence to justify application of AH to poorer quality embryos. Morphologically challenged embryos consistently develop more slowly in vitro (Tucker & Liebermann 2003). Also in a non-randomized, but controlled study of the application of AH to fresh blastocyst transfers, it was shown that blastocysts that formed on day-6 benefited from AH compared to control unmolested blastocysts (Tucker 1999). Further, in vitro observation of over 300 research embryos left in culture up till day-9 of development showed a distinct reduction in both blastocyst formation, and hatching relative to increased age of the patient from whom the embryos were donated (Porter et al 2002). Additionally, hatching occurred less often the more slowly the embryo took to reach the blastocyst stage. Obviously a major caveat of this study is that it was an in vitro observational analysis, rather than a true in vivo implantation study; but it does profoundly implicate the role of a compromised ZP to restrict blastocyst hatching both in older women, and in couples whose embryos develop more slowly. This ties in well with the aforementioned study of the benefit of AH for more slowly

developing blastocysts (Tucker 1999). Consequently, we routinely apply AH using the laser in women 38yrs of age and older, and those consenting couples that present with poorly developing embryos.

Clearly both of these patient groups might benefit from better prospectively randomized controlled study of the impact of AH. So why is this not done? One cynical stance is that if AH were to prove ineffectual, then this would mean that a fee generating procedure would be dropped from the IVF laboratory. Another reason is that undertaking such a well-controlled study can be very painstaking. For example, in the case of a extremely well-undertaken study of AH it may take a very long time to recruit sufficient patients, as was the case where AH was applied to good prognosis IVF patients which took over three and a half years to complete (Sagoskin et al 2007). On the face of it, this study was a waste of time, because no benefit was observed from the use of AH. The converse of this is that it was good science reporting a null outcome, the real benefit of which was to indicate that if AH was carried out appropriately, then it was in no way deleterious to the embryos to which it was applied, nor to their eventual ability to implant.

And therein lies the rub... Applying AH appropriately is the key point here; and we would like to comment on the differing technologies available in passing, but more specifically the most user-friendly laser systems that can be utilized. While acidified Tyrode's medium became for a while these authors' approach of choice to ablate the ZP both for embryo biopsy and AH, we have since changed to the more convenient and quicker laser for both procedures, due to clear benefits in terms of embryonic development post-biopsy when the latter was applied versus the acidified medium approach (Han et al 2003). Mechanical opening of the ZP is possible, and avoids the problems encountered with potentially lingering chemicals, but technically it can be demanding, and inconsistent unless undertaken by very skilled hands. Mechanical dissection of the ZP is also not sufficiently subtle to enable partial ablation of the ZP, unlike the acidified medium approach or the laser. This brings us to our first concern with the Venkat paper which applied a large area ZP thinning approach to AH, which in our opinion is not effective (e.g., Tucker et al 1993) for AH, and more worryingly may in fact be harmful to the embryo.

When it comes to ZP ablation, it is important to be cognizant of the potential harm that any approach to AH might be imposing on the embryo be it mechanical, chemical, or in this case using the laser. Not all lasers that have been developed for micromanipulation of the human embryo are equal. The greatest danger of pointing a laser in the general direction of an embryo is thermal damage. Therefore to know the thermal characteristics of a laser beam is essential to minimize any potential harm. Lasers of wavelength 1480–1450nm are used for penetration of the embryo's ZP; and laser pulses of duration 100µsec to 20millisec have been used. Powers of 300mW and 47mW have been reported with different lasers. If we compare the heating in neighboring tissue caused by the laser pulse, this enables us to estimate potential thermal damage to the embryo caused by the laser.

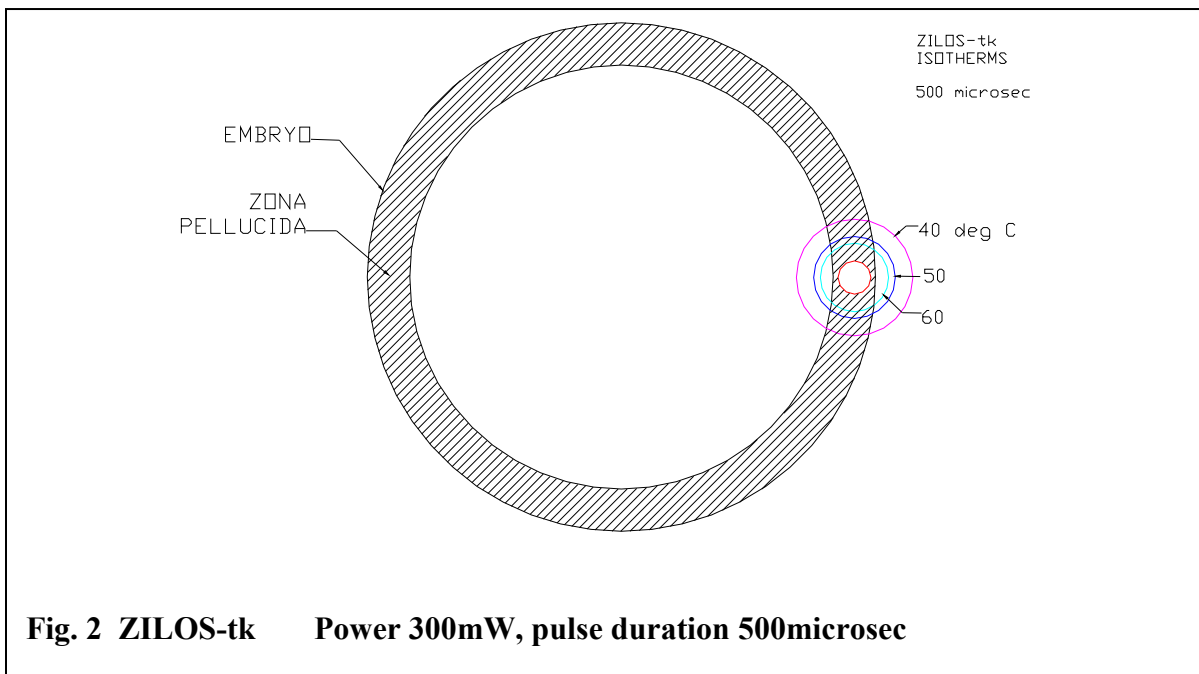
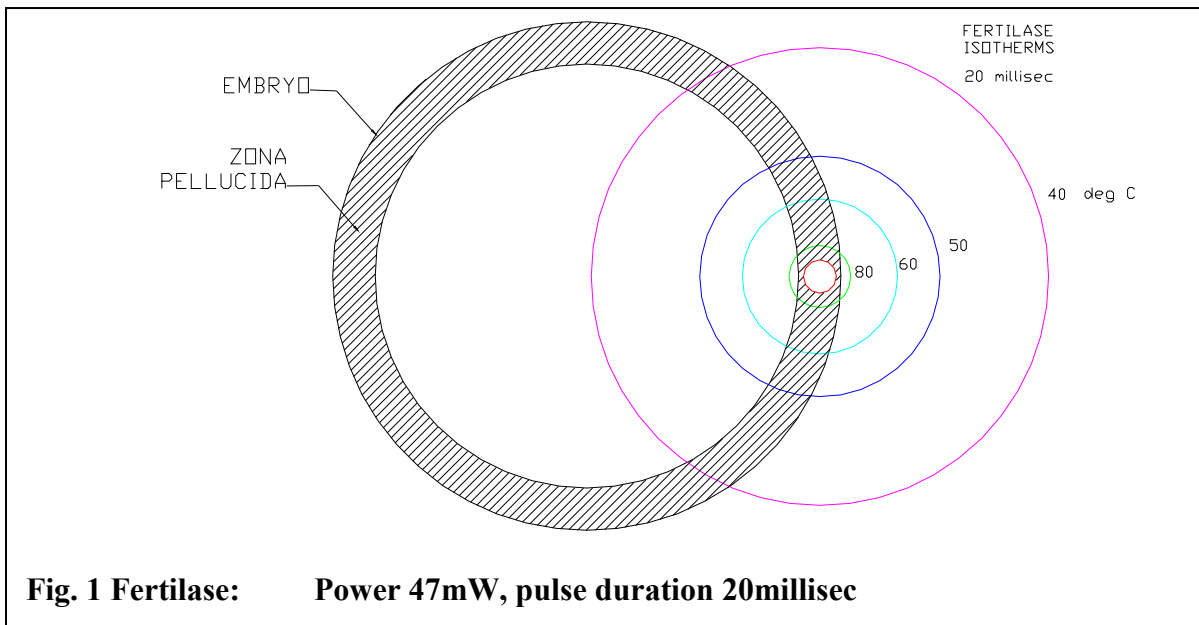
The theory of pulsed heating has been described in detail (Douglas-Hamilton & Conia 2001; Tadir & Douglas-Hamilton 2007), and can be used to compute the maximum temperature reached at any given distance from the laser beam axis. An equivalent way of expressing this is to calculate the radius at which a given maximum temperature is reached during the pulse. The circle at that radius describes the isothermal region, or isotherm for short, corresponding to that temperature.

If we look at the two laser systems as applied in two different studies, we can get a better idea how each laser system might have very different effects on the clinical outcomes. In the case of the system used by Sagoskin et al (2007), the laser power was 300mW with a pulse-duration of 500 μ sec [ZILOS-tk]. In the case of the Venkat study, the laser power was 47mW with a pulse-duration of 20millisec [Fertilase].

Therefore it can be seen for the above two laser pulses applied to the embryos the following isotherm radii, in microns, were generated:

Isotherm Temperature	Laser 300mW Pulse 500μsec	Laser 47mW Pulse 20msec
140°C	7.8 μ m	0 μ m
100	10.5	1.2
80	12.6	14.6
60	16.1	36.6
50	19.3	56.7
40	27.5	108.0

These two differing cases are shown in sketches below (Figures 1. & 2.). The embryo is estimated to be 120 μ m in diameter, and the isotherms are scaled accordingly. The contents of the embryo are not shown, but clearly one would like to disturb them thermally as little as possible.



Although the drilled-hole diameter is comparable, the volume heated in the interior of the embryo is much higher, and the heating lasts 40 times longer in the case of the Fertilase system. From this simple representation it is easy to conclude that when the Fertilase system is applied, even if not for full opening of the ZP, nevertheless the embryo may be in significant danger of thermal shock. This would be further amplified in an approach such as used by Venkat et al (2008), where 25% of the entire ZP was thinned extensively.

While we are grateful to Venkat et al for reporting their outcomes from the long term use of AH as they applied it to thawed embryos, we would like to question not only the validity of their results, but in addition wanted to point out the potential harm that they were applying to the thawed embryos for the duration of their study. We routinely apply AH with the Zilos-tk laser (more recently using just a 300microsec pulse-duration) to all of our thawed embryos. This is most commonly applied to cryopreserved blastocysts (e.g., Liebermann & Tucker 2006), as they pass through the sucrose dilution steps, during which time the blastocyst is usually collapsed. For AH of fresh day-6 blastocysts, we expose the embryo briefly (~1min) to 0.2M sucrose solution to collapse it partially, which allows safe laser ablation of the ZP at a site away from the inner cell mass (Sagoskin et al 2002). While we feel that this technology as we apply it for AH is at worst simply benign, it is essential that we undertake future studies to see if it is truly beneficial in the patients where we think it is helpful. This seems precisely to be the message that has been sent out by the SART/ASRM practice committees (2008) with reference to the use of AH, where it is not seen to be universally beneficial, but perhaps clinically useful in well-defined patient sub-groups. It remains for us to define such sub-groups more effectively.

References:

Assemi A, Richter KS, Tucker MJ, Sagoskin AW. Does assisted hatching benefit patients with an elevated basal follicle stimulating hormone (FSH)? *Fertil Steril* 2006;86:2S; P-363, ppS333.

Cohen J, Elsner C, Kort H, Malter H, Massey J, Mayer MP & Weimer K. Impairment of the hatching process following IVF in the human and improvement of implantation by assisted hatching using micromanipulation. *Hum Reprod* 1990;5:7-13.

Douglas-Hamilton DH & Conia J. Thermal effects in laser-assisted pre-embryo zona drilling. *J Biomedical Optics* 2001;6:205-13.

Han T, Graham J, Tucker MJ, Davis A, Sagoskin AW. Laser-assisted human embryo biopsy on the third day of development for preimplantation genetic diagnosis: two successful case reports. *Fertil Steril* 2003;80:453-5.

Liebermann J & Tucker MJ. Comparison of vitrification and conventional cryopreservation of day 5 and day 6 blastocysts during clinical application. *Fertil Steril* 2006;86:20-6.

Porter R, Tucker M, Graham J & Sills ES. Advanced embryo development observed during extended in vitro culture: observations of formation and hatching patterns in non-transferred human blastocysts. *Human Fertility* 2002;5:215-20.

The Practice Committees of SART & ASRM. The role of assisted hatching in in vitro fertilization: A review of the literature. A committee opinion. *Fertil Steril* 2008;90:S196-8.

Sagoskin AW, Han T, Graham JR, Levy MJ, Stillman RJ, Tucker MJ. Healthy twin delivery after day 7 blastocyst transfer coupled with assisted hatching. *Fertil Steril* 2002;77:615-7.

Sagoskin AW, Levy MJ, Tucker MJ, Richter KS & Widra EA. Laser assisted hatching in good prognosis patients undergoing in vitro fertilization-embryo transfer: a randomized controlled trial. *Fertil Steril* 2007;87:283-7.

Tadir Y & Douglas-Hamilton DH. Laser effects in the manipulation of human eggs and embryos for in vitro fertilization. *Methods in Cell Biol* 2007;82:409-31.

Tucker M. Relevance of assisted hatching with blastocyst stage transfer. Proceedings First World Congress on Controversies in Obstetrics, Gynecology & Infertility, Prague, Czech Republic, Oct 28-31, 1999; Monduzzi, Bologna, Italy, pp49-52.

Tucker MJ, Luecke NM, Wiker SR & Wright G. Chemical removal of the outside of the zona pellucida of day-three human embryos has no impact on implantation rate. *J Assist Reprod Genetics* 1993;10:187-91.

Tucker MJ & Liebermann J. Morphological scoring of human embryos and its relevance to blastocyst transfer. In: Patrizio P, Tucker MJ, Guelman V, eds. *A Color Atlas for Human Assisted Reproduction*. Philadelphia, PA: Lippincott, Williams & Wilkins, 2003:99-108.

Venkat G, Thornhill A, Wensvoort S & Craft I. Does laser assisted hatching using partial zona thinning (LAH) improve outcome in frozen embryo transfer (FET) cycles? *J Clin Embryol* 2008;11:17-30.