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Systemic Review

Assisted hatching – should we keep doing it?

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Introduction

Assisted Zona Hatching (AZH) like most add-ons was introduced to In Vitro Fertilization and Embryo transfer (IVF/ET) in the early 1990s, when the live birth rate (LBR) in older women, age 35-39, was only 6%. This frustrating rate called for unusual measures including some add-ons like Assisted Hatching (AH) that were eagerly adopted, in conjunction with many theories, with the hope to increase the success rate. But today, due to various improvements in ovarian stimulation, culture media, and embryo selection, the LBR has tripled in 2019 in women aged 38-39 to about 19% (Human Fertilisation and Embryology Authority, http://www.hfea.gov.uk. [1]) and it is doubtful if any of the add-ons, which were never proven effective, including AH, is still needed in routine IVF. Despite hundreds of publications, and probably millions of AH procedures performed in over 30 years, no proof as to its efficacy in terms of LBR, was provided. In recent years all add-ons including AH came under crossfire in the professional and lay media for being redundant, costly, and inefficient [2,3]. Nevertheless, all add-ons including AH remain in very high usage.

Why assisted hatching?

Zona Pellucida is a glycoprotein produced by the growing oocyte that is responsible for sperm binding and acrosome reaction and in preventing polyspermy [4]. Before implantation, repeated expansion-contraction cycles help to thin out the zona [5,6], to facilitate embryo hatching and thereby implantation. Years before the ICSI (Intracytoplasmic Sperm Injection) era in the late 1980s, an intensive search to increase fertilization rate in male factor cases included a procedure named Partial Zona Dissection (PZD) that was used to allegedly facilitate the defective or low count sperm to fertilize the oocyte. This was later developed into AH in humans.

During the 1988 ASRM congress in Atlanta, at the hotel bar, I asked Jacques Cohen what message he would like to convey during the 1989 6th world IVF congress in Jerusalem, where I was in charge of the program. Enthusiastically he described his observation of a few patients that underwent PZD to treat low sperm count, in whom the pregnancy test of hCG (Human chorionic gonadotropin) turned positive one day earlier than non-PZD embryos [7]. His interpretation of the phenomena was that the embryos hatched earlier due to the breach in the zona. My question on why an embryo would choose to get out from an 'artificial window' if it supposedly possesses the key to the "front door" remained unanswered, but soon a new field in human IVF was born and named AH, along with the theory of a thick zona or zona hardening requiring AH emerged. The basis for the AH procedure is the presumed inability of the embryo to hatch out of the zona pellucida (ZP) due to zona hardening, a physiological change that increases the resistance to proteolytic digestion and hence the block to polyspermy [8]. It was suggested that zona hardening occurs because of in vitro culture conditions or maternal aging.

Investors recognized the opportunity before any proofs were provided; money poured in to develop hi-tech instruments capable of measuring the thickness of the ZP, giving AH a feel of a hi-tech procedure. Various physical methods, including laser photoablation capable of drilling or thinning out the supposedly thick or hard zona, were introduced. The procedure turned very popular [8,9] despite doubts about its efficacy. The risks include possible damage to the embryo or blastomeres

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affecting its implantation potential and a possible increase in the odds of monozygotic twining [10].

Methods, theories and indications for AH

The procedure is routine in many centers. Methods include mechanical, chemical and physical (Laser) disruption of the ZP. Mechanical partial zona dissection is done by a microneedle that is introduced through the perivitelline space piercing about 20% of the circumference of the embryo and then rubbing against the holding pipette until a window is created. The chemical digestion of the ZP by Ac Tyrode's solution [11,12] is done by expelling the solution on the zona until a focal hole is established. The procedure is not easy to standardize in terms of time and amount of solution, and hence the dimension of the hole created by different biologists is varied [5,13]. Laser-assisted hatching [14] is performed with different instrumentations that transmit pulses of laser beams to create a slit or thinning out or complete denudation of the zona. All these different methods were traditionally used on cleaving embryos, and recently mainly on blastocysts, resulting in different degrees of zona thinning, including total denudation of the zona.

Many theories were developed to explain why AH should work including zona hardening in older age or due to in-vitro conditions [5] or that hatched embryos tend to implant one day earlier [15] or that AH can enhance hormonal and metabolite exchange with the endometrium [16]. It was also suggested that IVF embryos are slower [17] and also, the rate of hatching and blastulation is lower than in nature [18] or that delayed embryonic development and advanced endometrium may potentially allow AH to improve the results [17,19]. But none of these theories were proven.

The initial indications for AH include primarily women of advanced age, poor quality embryos and poor prognosis, and the iatrogenic entity of so-called repeated implantation failures-(RIF) [3]. Like many add-ons, AH has no contraindication and is practically offered also to "normal responders" and every patient who is willing to bear the price. AH was also offered to treat embryos with thick zona or blastomeres fragmentation or even embryos after ICSI [20] which in itself is used in many cases without obvious indication. Again, the results didn't demonstrate any advantage in LBR. Overall AH was used more frequently in frozen rather than fresh ET and in the blastocyst stage compared to cleaving embryos. Results in a large group of 46,029 (43.7%) out of 105,450 undergoing AH and single ET, showed lower LBR in fresh-AH and no increase in LBR after thawing [21], indicating the invalidity of the concept.

Of note is that most studies are plagued by mixed methods for hatching, various indications, timing, size of holes and heterogeneous population which limits the ability to compare between studies and perform a meta-analysis. Today, most programs prefer laser hatching at the blastocysts stage which is simple to master and takes only minutes to perform.

Results of AH

Of note is that most studies are plagued by mixed methods for hatching, various indications, timing, size of holes and heterogeneous population which limits the ability to compare between studies and perform a meta-analysis. Today most hatchings are performed at the blastocysts stage which is simple to master and takes only minutes to perform.

After over 3 decades there is still no-proofs that AH provides an increase in LBR, which should have condemned the procedure as a failure. The published meta-analysis relies on underpowered studies, with low to very low-quality data 22], on heterogeneous indications, different age groups, different hole sizes; drilling, breaching, thinning, partial or complete denudation of the zona. Moreover, many studies mix methodology. Results often do not include LBR and, when they do, no differences were found.

It is beyond the scope of this opinion paper to describe the numerous studies that are in support of an increase in pregnancy rate with AH [23] or suggesting a decrease in LBR [24]; Practice Committee of the Society for Assisted Reproductive Technology), or the many inconclusive meta-analyses. One of the larger RCTs [25] has randomized patients under 39 years, with good prognosis, undergoing first or second IVF, with good-quality cleaving stage embryos. Pregnancy rate and LBR with laser- AH was 53% and 47% (n = 121) compared to 54% and 46% in patients without hatching (n = 82). The miscarriage rate was also similar, 13% versus 15% respectively. Hagemann et al (2009) have randomized 121 patients, aged under 38 and ZP thickness of >13microm treated with Ac. Tyrode's solution, in a double-blind, cross-over study. They found no differences in all the parameters tested, including pregnancy rate, LBR, and miscarriage rate between hatched and non-hatched patients. In a retrospective analysis of two registries from the USA and Japan, AH was associated with significantly lower LBR [21,24], however, these studies lacked basic information on the methods, indications, and timing of the procedure, which does not allow unbiased comparison.

The most important effort to evaluate the results of AH in IVF treatment was done by Cochrane's repeated reports, in 2003, 2006, 2009 (Das 2009) and 2012 [26], (Carney SK, et al. 2020) in which they have repeatedly claimed that LBR, the most important parameter, is either not reported or reported as not improved by AH. That should have been sufficient to put the overall use of AH to rest with the growing list of add-ons that are deemed inefficient [27-29] and use it for research purposes. However, the Cochran reviews have also suggested that in some subgroups pregnancy rate may be increased, which apparently served as a justification to keep using AH and even expanding its use for other indications, like ICSI cases [20] and frozen-thawed embryos that did not show positive results in numerous studies. Neither the above-mentioned Cochrane reports nor a large registry [21] showed a positive effect of hatching after freezing.

The last Cochran report [22] includes 39 RCTs (7249 women) that are reporting pregnancy rates and only 14 studies reporting LBR (834 Live birth) with low to very low quality of data and poor reporting of the study methods. The authors stated that the odds ratio for LBR was 1.09 (CI 0.92 - 1.29) they

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commented that "This analysis suggests that if the live birth rate in women not using assisted hatching is about 28%, the rate in those using assisted hatching will be between 27% and 34%.", with slightly increased multiple pregnancy rate (OR = 1.38).

Discussion

Breaching or thinning of the zona pellucida by mechanical dissection or by dissolving the zona with acid thyroid solution or shaving with laser instruments is used to allegedly increase the rate of implantation and LBR. However, despite more than 30 years of use and numerous studies and meta-analyses, no report has shown an increase in LBR, the most important parameter of success. On the contrary, some studies have even suggested a lower LBR after AH [21,24] So, without any proven clinical advantage and no direct evidence of zona hardening that impedes implantation, the question is - why are we still utilizing AH?

It is well known that the results of IVF are age-dependent and most IVF patients are older than 35 years. At the female age of 35, fertility tends to drop faster, at a rate of 15% per year and even faster after 40 years, compared to 1-4% per year before 35. [30]. Accordingly, analysis from the UK for 2006 (HFEA, http://www.hfea.gov.uk.) showed that LBR drops sharply every year between the age of 38 to 42 from 17.9%, to 15.7%, 12.5%, 9.5% and 6.6 % respectively. The success rate drops primarily because the percentage of euploidy embryos drops sharply from 55.4% to 44.8, 32.4%, 18.2%, 8.5 at age <35, 35-37, 38-40, 41-42 and after 42, respectively [31], a problem which cannot be mended by any add-on, including AH. For example, at age 38-40 only one in 3 embryos is euploid, so it is not surprising that the success drops sharply and that in large series like all European countries the LBR for all ages is slightly above 20% [32].

The discussion on AH is part of a larger discussion on 'why are we using any add-ons?'. Results of IVF were considered far from perfect and perceived so even today. Searching for the holy grail to increase the seemingly low success rate is a continuous endeavor. In the 1990s when most add-ons, including AH, were introduced, the success rate then was extremely low but today, it reaches a reasonable rate. Statistically, it is known that only about 1 in 20 oocytes (5%) will eventually become a baby. Nevertheless, the reported LBR has increased over the last 3 decades (from 1991 to 2019) by 3 folds, from disturbing 6% to 19% in women aged 38-39 years (HFEA Authority, http://www.hfea.gov.uk), which is similar to the level of the natural fecundity in fertile population. The better pregnancy rate is mainly due to better laboratory work, and selection of the best embryos, than to any add-ons that are applied [33]. So maybe it's time to coordinate the expectation for success to the published reality and drop all the unproven add-ons?

Furthermore, add-ons aim at treating what is possible and not what is needed. When faced with the frustration that is associated with IVF failures, physicians feel obliged to offer all kinds of, albeit unproven, changes to encourage the patients to keep trying and deter them from abandoning the program. In most failures the etiology is not known hence the addition of add-ons does not treat a known cause. For example, freezing of all embryos in normal or poor responders cannot lead to better embryos and better results [28], just as treating the endometrium receptivity by ERA test or endometrial scratching cannot solve the age-related embryo's aneuploidy [3,29]. Similarly, AH is not expected to improve LBR by creating a slit in the zona to bypass an alleged zona hardening if aneuploidy is the main cause for failures. Since most patients present to IVF clinics after the age of 35, it stands to reason that AH, like all other add-ons, is offered more to women in advanced age in whom the so-called RIF -due to embryonic aneuploidy- is more common.

A recent study [34] has demonstrated in a large group of women (n=4429), at a mean age of 35.4 years, that the transfer of a single tested euploidy blastocyst over 3 cycles, reached 92.6% LBR. (LBR of 64.8%, 54.4%, 54.1% in each cycle). This almost perfect result, in selected good-prognosis women, indicates that no add-on including AH should be used or can significantly improve the results in the first few cycles cause "it's almost all dependent on the quality of the embryos" [29].

Admittedly, while AH provides no increase in LBR, the associated risks are limited, and the potential risks of increased twins can be controlled by a policy of single embryo transfer while monozygotic twins, despite the concerns, do not seem to be significant [7,9,35,36]. Also, there are no reports of an increase in chromosomal aberrations or malformations after Laser AH despite the fact that the wavelength is close to the absorption peak of DNA [37]. This apparent low risk might explain why despite the lack of efficacy, IVF programs are reluctant to relinquish from their toolbox, this seemingly hi-tech solution which is a significant income generator.

Finally, a pledge was published by ten leading professional groups for cultural changes towards more transparency on the experimental status of add-ons, their effectiveness and safety and to avoid extra charges. (*https://www.hfea.gov.uk/treatments/ treatment-add-ons; releases/2019-news-and-press-releases/fertility-regulator-calls-for-clinics-to-be-more-open-about-treatment-add-ons/*). Reviewing the text that is attached to the description of add-ons on the website of some leading universities gives the impression that some add-ons are a panacea with no doubts or downside. These texts should be attenuated to present reality [38]. National and international societies should independently evaluate the evidence and suggest which add-ons can be used under experimental registered protocols or strictly limited indications.

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