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20.1 Introduction

Human IVM was first reported more than 50 years ago [1]; however, with the introduction of gonadotrophins to stimulate multi-follicular growth [2], research into IVM treatment became less popular. This introduction of ovarian hyperstimulation also introduced the side effect of OHSS [3], which is a significant clinical consequence of gonadotrophin stimulation, resulting in patient discomfort in the mild stages and significant morbidity or mortality in the major forms of the condition [4]. Even though IVM provided a viable alternative to avoid ovarian hyperstimulation syndrome (OHSS), stimulated IVF treatment expanded worldwide, and it wasn't until 1991 when the first live birth was recorded after IVM following collection by ovary biopsy [5]. Following this, in 1994 IVM success was achieved following a transvaginal oocyte aspiration (TVOA) [6], and its reported use in the literature has continued at a steady pace ever since. IVM treatment has now expanded to treat a range of conditions including gamete donation and follicle-stimulating hormone (FSH)-resistant ovaries and for fertility preservation. However, the predominant patient cohort who are suitable for IVM treatment are those diagnosed with polycystic ovarian morphology (PCOM) and/or polycystic ovary syndrome (PCOS). Additionally and most importantly, IVM is currently the only treatment option which completely eliminates the risk of OHSS [7].

This chapter will outline the use of IVM as an effective treatment method for patients with PCOS and PCOM, as well as summarise the different protocols for treatment regimes, hormonal priming and culture conditions with resulting reproductive

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outcomes. It will also address the limited current evidence available for the outcomes of children born following IVM and where IVM research is heading in the future.

20.2 IVM in the Treatment of Women with PCOM/PCOS

The defining feature for increased IVM success rates is an increased antral follicle count (AFC) [8]. Only PCOS patients with PCOM (more than 12 antral follicles) and PCOS patients with a high antral follicle (although not so high to be classified as PCOM) count could have best benefit from IVM. As the AFC is a determining factor for IVM treatment, patients with fewer than five antral follicles should not be considered for this treatment [9]. Considering women with PCOM/PCOS who typically have a very high AFC, they therefore respond better to IVM treatment and have more oocytes collected from IVM cycles than patients without the condition. While those patients with PCOM may still benefit from IVM treatment, a recent meta-analysis showed that implantation and clinical pregnancy rates are highest in patients with PCOS [10].

20.3 Treatment Regimes and Hormonal Priming

In theory, IVM requires no exogenous gonadotrophin administration as the immature oocytes complete their final stages of maturation under the influence of suitable culture conditions, tailored to mimic the intra-follicular environment. However, hormonal priming using follicle-stimulating hormone (FSH) or human menopausal gonadotrophin (hMG) and/or human chorionic gonadotrophin (hCG) is often used to ‘prime’ the follicles prior to oocyte aspiration. The results of these protocols are contradictory and difficult to evaluate, due to differences in priming and culture conditions. FSH priming plays an important role in increasing follicular growth and contributes to higher rates of oocytes collected, increased maturation [9], fertilisation, embryo development and implantation [9], and the highest rates of IVM success have been achieved with 3–5 days of FSH priming without hCG triggering when the dominant follicle is less than 12 mm [11]. Protocols for hCG priming achieve the best result with a 38 h interval from 10,000 IU hCG triggering to oocyte collection [12] when the dominant follicle is less than 14 mm [13].

The predominant controversial issue is the use of hCG or gonadotrophin-releasing hormone (GnRH) agonist triggers in IVM protocols, as they induce nuclear maturation *in vivo* [14] and, hence, are at odds with the core concept of IVM, where maturation by definition takes place *in vitro*. Additionally, as hCG can induce oocyte maturation *in vivo* in follicles greater than 9 mm [15], this methodology is logistically problematic, as oocytes are at varying stages of development when they are collected. This in turn leads to multiple insemination times for the same patient and subsequent variations in embryo culture stages. Recently, an effort to change the clinical definition of IVM has been suggested to categorise the different protocols currently employed around the world [16]. The authors suggested four definitions of IVM treatment protocols: (1) IVM without triggering, (2) natural cycle IVF with early triggering combined with IVM, (3) IVM with short gonadotrophin stimulation and (4) modified natural cycle IVF with early triggering combined with IVM. However, these definitions are confusing and still allow for a category of

patients receiving both FSH priming and hCG triggering which others have suggested be termed ‘truncated IVF’ [17, 18].

More widely accepted definitions have been recommended to include three treatment groups, ‘truncated IVF’ (where priming includes both FSH and hCG) and ‘hCG-primed IVM’ (where there is no FSH administered and patients receive an hCG or agonist trigger), and the definition of ‘IVM’ is suggested to be reserved for those cycles with or without FSH/FSH-analogue priming, without the use of ‘gonadotrophins that are intended to trigger oocyte maturation *in vivo*, such as hCG or GnRH agonists’ [18]. This is a necessary development for IVM both clinically and for research purposes, to avoid comparisons between outcomes of what some consider to be true IVM vs an abridged version of standard, stimulated IVF treatment. This will aid to avoid confusion for clinicians, patients and health professionals when IVM is discussed.

20.4 Immature Oocyte Collection

Similarly to priming protocols, procedures for immature oocyte collection in IVM cycles show considerable variation. They are mostly centred around a standard TVOA procedure with modifications that enable the collection of oocytes from small follicles. Clinicians may use a double or single lumen needle, depending on whether the protocol employs follicular flushing. Some clinics have reported flushing each follicle up to three times [19], and others do not employ follicular flushing [20]. Follicular flushing solutions include HEPES [21] or Hartmann’s supplemented with heparin [19]. Additionally, the aspiration pressure used in TVOA for IVM has been reported in varying ranges, from 7 kpa (52.5 mmHg) [22] to 200 mmHg [21].

Such as in standard IVF, germinal vesicle (GV) stage oocytes can be identified by sight [11, 19], which involves additional training of the embryologist, or by filtering follicular aspirates through a mesh cell strainer [20, 23]. Figure 20.1 represents a GV stage oocyte located during an IVM oocyte collection. Once the cumulus-oocyte complex is removed from the follicle, there is a significant drop in the amount of gap junction communication between the cumulus cells and the oocyte and a dramatic decrease in cyclic adenosine monophosphate (cAMP)

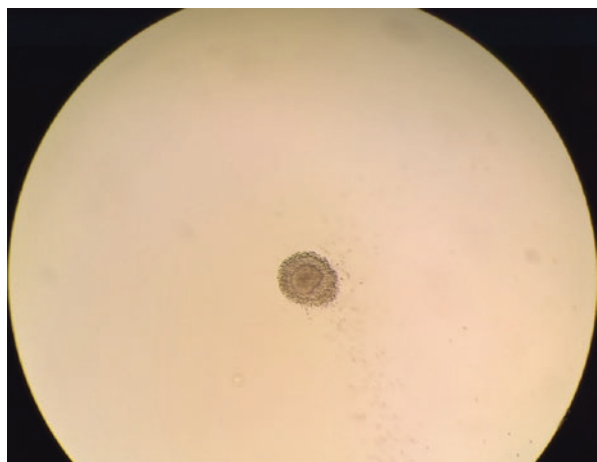


Fig. 20.1 A GV stage oocyte surrounded by compact cumulus oophorus, following follicular aspiration (800× magnification, SXZ12 stereo microscope, Olympus)

activity [24]. Following removal from the follicle, some oocytes may undergo spontaneous nuclear maturation, evidence by the progression to metaphase II (MII) status [25]. However, this does not necessarily correlate to cytoplasmic maturity, whereby the oocyte gains the capacity for activation and the resumption of meiosis, leading to successful fertilisation and ongoing embryo development [26]. This needs to be managed effectively in order to prevent spontaneous maturation and asynchrony between nuclear and cytoplasmic maturation, which can be detrimental to embryo growth, and this is predominantly managed through the development of specialised IVM culture systems.

20.5 Oocyte Maturation and Culture

IVM culture differs depending on the base media, culture timing and hormone and serum concentrations. A range of culture media have been formulated for use in IVM. The two mostly widely used commercially available IVM base media are Sage (CooperSurgical, USA) and MediCult (Origio, Denmark), which have shown similar success rates [27]. Media specifically formulated for blastocyst culture have been used successfully [11, 28] and have comparable success rates compared to Sage IVM media [29].

Unlike priming protocols, hormonal additives are common components for culture media in human IVM. Luteinising hormone (LH) and hCG are important mediators of oocyte maturation as they act on a common granulosa cell receptor to induce the intracellular rise in cAMP activity within the oocyte [30]. The cAMP cascade then promotes the resumption of meiosis and germinal vesicle breakdown [26]. Recombinant LH or hCG are added in concentrations from 0.1 IU/mL [23] to 0.75 IU/mL [31]. FSH is used to promote cumulus-oocyte complex (COC) expansion and a subsequent rise in cAMP activity leading to increased oocyte maturation [32, 33]. The concentration of FSH added is relatively consistent across IVM protocols at 0.075 IU/mL [23, 34] or 0.1 IU/mL [11, 19].

20.5.1 Other Culture Media Additives

Other additives have been suggested to be beneficial to IVM culture media by aiding in either maturation, fertilisation or embryo development. These include insulin-like growth factor (IGF-I) [35, 36], epidermal growth factor (EGF) [35, 37, 38], meiosis-activating sterols (MAS) [39, 40] and activin [41]; however, these are rarely used in routine culture.

20.5.2 Serum or Follicular Fluid in Culture

Human follicular fluid (HFF), inactivated autologous patient serum (maternal serum) or foetal bovine serum (FBS) are the three protein additives used most commonly in human IVM. HFF supplementation has been used in concentrations

ranging from 30% [42] up to 70% [21]. Heat-inactivated FBS has also been used at either 10% [43] or 20% [14, 44]. Maternal serum is the most common protein supplement, used in concentrations of 10% [23, 45] or 20% [22, 46]. The use of human serum in IVM culture media may provide a number of nutrients and growth factors that are involved in the maturation process. There are, however, a number of negative aspects associated with using serum in embryo culture including contributing to ammonia formation, which can be damaging to embryo development through mitochondrial disruption [47], and the interruption of the aromatisation process involved in the conversion of androgens to oestrogens in granulosa cells [48].

Additionally, concentrations of amino acids, lipids, hormones, antibodies and other immunological mediators vary between women according to diet, genetics and infection status, such that serum can be highly variable in its potency and toxicity. Additionally, there is the risk of contributing unknown contaminants (microbial and otherwise) to the cultured embryos when using serum or HFF preparations. However, it is not clear whether the negative effects of serum relevant to embryo culture also relate to in oocyte maturation culture. In most circumstances, oocytes are only exposed to maternal serum for the first 24–48 h of in vitro culture, depending on the protocol employed, and are then moved into commercial embryo culture media prior to insemination. Regardless, more research is needed into the effects of protein additives to IVM culture.

Recent research has investigated the benefits of supplementing IVM culture media with cAMP modulators. These include cilostamide and forskolin, which are designed to prevent the loss of gap cell junctions following removal of the oocyte from the follicle and the subsequent decrease in cAMP activity [49]. cAMP modulators may also prove beneficial during a short pre-IVM period, immediately after removal from the follicle, known as simulated physiological oocyte maturation (SPOM) [50], although in humans, the benefits of this pretreatment are inhibited by heparin, which is most often used during the collection procedure [51]. Initial testing of another cAMP modulator 3-isobutyl-1-methylxanthine (IBMX) in human participants was shown to be a safe additive, in terms of the incidence of embryo chromosomal aneuploidy rates [52]. Further large-scale trials into its effect on human IVM success rates are required.

20.5.3 Culture Timing

Similarly to priming protocols and culture media contents, there are considerable variations in oocyte maturation culture time reported in the literature. Following the oocyte collection procedure and transport to the embryology laboratory, oocytes are placed into maturation culture for at least 24 h [19]; however, culture periods of up to 40 h have also been reported [53]. Additionally, if a hCG trigger is used, there will be variations in culture timings such as at 24 and 30 h [54] for the same oocyte cohort. At the initiation of maturation culture in non-hCG-primed cycles, the GV stage oocytes will have a tightly compacted cumulus oophorus (Figs. 20.2, 20.3 and 20.4), and after maturation culture, expansion of the coronal cells should be visible (Figs. 20.5, 20.6 and 20.7).

Fig. 20.2 Compact coronal cells surrounding GV stage oocyte following oocyte collection



Fig. 20.3 Compact coronal cells surrounding GV stage oocyte following oocyte collection



Fig. 20.4 Compact coronal cells surrounding GV stage oocyte following oocyte collection



Fig. 20.5 Expanded coronal cells following 24 h in maturation culture

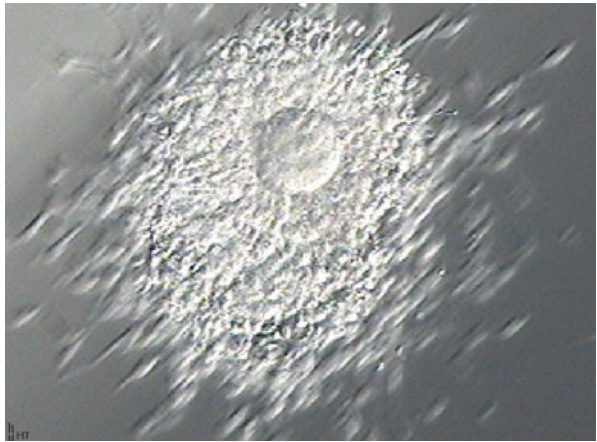


Fig. 20.6 Expanded coronal cells following 24 h in maturation culture

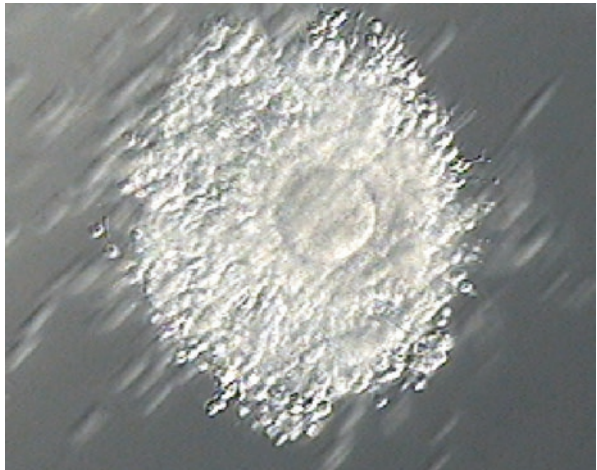
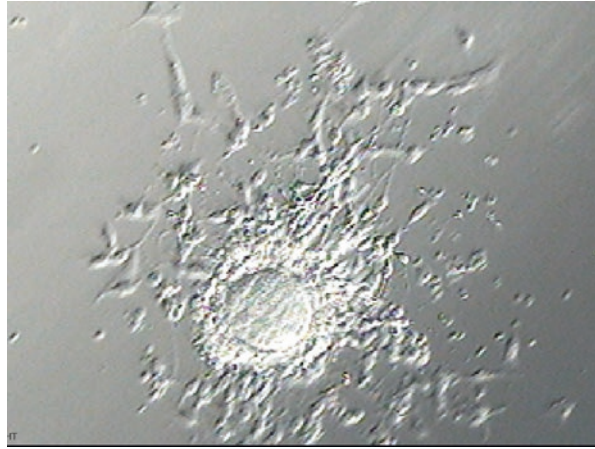


Fig. 20.7 Expanded coronal cells following 24 h in maturation culture



20.6 Fertilisation, Embryo Culture

IVM fertilisation is predominantly performed using intracytoplasmic sperm injection (ICSI) as fertilisation and implantation rates were originally reported as significantly lower than IVF-inseminated oocytes following IVM [23]. However, following a small, sibling oocyte study, no significant differences were seen between those fertilised by ICSI and IVF in outcomes of fertilisation, blastocyst development or implantation rates [28]. When using IVF as a fertilisation technique, it is important to consider that there is a potential conflict in the timing at which maturation/fertilisation check is performed between ICSI and IVF. IVF oocytes are allowed an additional 16–20 h prior to denudation and, therefore, additional time to undergo spontaneous and late maturation which may skew fertilisation results if they are calculated per oocyte matured.

Following fertilisation, embryo culture practices generally do not differ from standard IVF, and culture timing should reflect what is normally performed in any treating clinic. The majority of reports of clinical IVM culture perform 3 days of culture and transfer or freezing of cleavage stage embryos [22, 23, 44]; however, as with standard IVF treatment, blastocyst culture in IVM has become more prevalent [55]. Blastocyst culture may also be preferable in IVM as embryo development may be impaired during the early cleavage stages, with higher rates embryo arrest reported during the second and third cell cycles; however, embryo arrest during compaction and blastulation as well as kinetic time points were no different from standard ICSI treatment [56]. Blastocyst culture would therefore enable the deselection of embryos which may appear to be of suitable quality at the cleavage stage but fail to progress further.

20.7 Transfer/Cryopreservation

Endometrial priming is required if IVM-derived embryos are intended to be transferred in fresh cycles, and this is achieved using oestrogen and progesterone supplementation. This methodology shown to be beneficial when administration begins at the mid-follicular timing of the cycle [57] with at least 6 days of oestrogen necessary for endometrial receptivity [58]. High rates of implantation and live birth can be achieved by oestrogen supplementation 2 days prior and progesterone supplementation commencing on the day of oocyte collection, extending to the day of pregnancy test [11]. However, significantly higher rates of miscarriage and early pregnancy loss were seen following transfers in fresh IVM cycles compared to fresh IVF cycles, and this is not evident following frozen transfers [19]. Therefore, a freeze-all approach may provide the best outcomes for patients following IVM treatment.

20.8 Reproductive Outcomes

A recent systematic review and meta-analysis of treatment strategies for PCOS was inconclusive in regard to IVM as no RCTs were identified [59]. Success rates from non-RCT publications vary considerably in reported outcomes, and the results are difficult to compare due to the differences in treatment protocols. Reports of implantation rates for IVM range from 0% [9] to 34.5% [23] for cycles with no hormonal priming, 21.6% [9] to 47.7% [11] for cycles with recombinant FSH priming only, 8.9% [22] to 26.8% [60] for cycles with hCG priming only and 9.7% [46] for cycles with both FSH and hCG administered.

One of the primary reasons for the limited use of IVM around the world is that traditionally it is significantly less successful than standard IVF. There are only three reports of clinical IVM outcomes which include an IVF control group, all of which report lower live birth rates in PCOM/PCOS patients from fresh cycles [28, 34, 61]. In addition, miscarriage rates following fresh IVM cycles are significantly higher than in IVF and ICSI cycles [62], although this may be influenced by PCOS status and not the IVM procedure itself and/or insufficient endometrial conditions following fresh cycles, as this is not seen following frozen embryo transfer cycles [28]. The small size of these studies as well as the large variation in results further highlights the need for a large-scale, randomised clinical trial for IVM treatment.

20.9 Birth Outcomes

It is estimated that more than 3000 births have been achieved worldwide following IVM treatment, and although there is very limited research on the outcomes of these children, that which is available demonstrates a very positive outlook. Only six

Table 20.1 Neonatal outcomes of live births following IVM treatment

Reference	Singleton	Twins	Congenital birth defects (%)	Mean birthweight grammes (twins)	Preterm birth <37 weeks (twins)	IVF control group
[63]	24	4	2 (7.1)	3252 ± 516 (2361 ± 304)	1 2 (one set)	No
[64]	46	2	1 (2.1)	3720	1 2 (one set)	No
[65]	40	6	0 (0)	3550 ± 441 (2622 ± 194)	2 (4)	No
[66]	153	43	10, 2 from twin births (5.1)	3269 ± 616 (2311 ± 577)	26 (15)	Yes
[67]	34	4	Not reported	3119.5 ± 871	Not reported	No
[19]	33	0	1 (3.0)	3364 ± 590 (N/A)	2 (N/A)	Yes

publications to date have reported on neonatal outcomes from IVM births. The reported incidence rates of congenital birth defects include 0% [11, 65], 2.1% [64], 7.1% [63], 5.1% [66] and 3% [19] (Table 20.1). Additionally, Walls et al. and Fadini et al. included IVF controls and found no difference in congenital birth defects between the two treatments. However, the number of live births included within these data sets was small, with some including multiple births, and therefore, further large-scale studies are needed to determine the true impact of IVM treatment on congenital malformation.

Other measurements of neonatal health including Apgar scores have been reported in multiple studies and are within normal ranges [65] or show no significant difference to controls for singleton live births [66–68]. The incidence of adverse outcomes is often confounded by multiple births as many IVF centres worldwide still routinely transfer multiple embryos, though evidence suggests this is not best practice [69]. Regardless of multiple birth outcomes, the incidence of congenital birth defects, preterm birth and low birth weight, which are often associated with ART treatments (especially in PCOS patients), is low following IVM treatment.

Such as for birth outcomes, long-term outcomes from children born following IVM are unknown, and there are currently no long-term data on children born from IVM. However, the limited reports of the follow-up to children born from this technique do not demonstrate any adverse outcomes. The first study published on the development of children following IVM reported follow-up at 6, 12 and 24 months after birth and found that physical growth at all stages as well as neurological and neuropsychological outcomes were normal [65]. There were minor developmental delays in 8/43 children at 12 months (19%), but this decreased to 3% at 24 months. Their findings did not include IVF controls; however, they were within normal ranges for the general population.

Very few reports of early childhood outcomes are available employing standard IVF controls for IVM treatment. However, in one such study, it was reported that no differences in height or weight were found in 6 and 24 months of age in IVM infants

compared to standard IVF controls [68]. Additionally, there was no difference in mental developmental index and psychomotor scores between the two groups according to the Bayley Scales of Infant Development. Following these reports, in a cohort of French children, female infants born from IVM treatment displayed increased mean weight, height, body mass index (BMI) and head circumference at birth compared to those born following standard IVF treatment with ICSI. These outcomes remained significantly higher than the control group after 2 years of follow-up [67]. It is yet to be determined whether these findings are related to underlying infertility and PCOS rather than the IVM procedure itself. It appears that growth and development of IVM children falls within normal limits; however, further research is crucial to determine outcomes into early childhood, adolescence and adulthood.

20.10 The Risks of Aneuploidy and Epigenetic Variation

There is very little evidence of the effects of IVM on the risk of embryo chromosomal aneuploidy. Two case-control studies utilising fluorescence in situ hybridisation (FISH) reported no difference in the incidence of chromosomal abnormality between IVF- and IVM-derived embryos [70, 71]. Only one study has reported on the use of array comparative genomic hybridisation (aCGH) with IVM-derived embryos. This study involved the addition of the phosphodiesterase inhibitor IBMX into the culture media, and rates of aneuploidy were found to be similar to the researchers' previously published data from standard IVF treatments [72]. The limited data available in regard to aneuploidy and IVM highlights the need for more research in this area.

Similar to the risks of aneuploidy, concerns have been raised about the possible interference of IVM with epigenetic mechanisms and in particular with genomic imprinting. A systemic review of the risks of imprinting defects following oocyte culture in animal studies shows reassuring evidence of correct imprinted DNA methylation while highlighting the need for further research [73]. Additionally, research into the impacts of IVM on epigenetic variation in human oocytes is limited, and there is currently no information available on a genome-wide scale. Instead, researchers have focused on the analysis of selected imprinting genes and their error rates following IVM treatment. In one study of IVM, it was found that for the selected genes LIT1, SNRPN, PEG3 and GTL2, there were no significant increases in imprinting mutations [74]. In a more recent study, researchers compared six imprinting, five tumour suppressors, two pluripotency and two metabolic genes from cord blood and chorionic villus samples. Two repetitive elements were included to detect genome-wide DNA methylation changes in both, to detect allele methylation errors and found no difference in epigenetic changes between samples from 11 IVM and 19 IVF control neonates [75]. Therefore, while there is a clear need for further research, the limited data available so far is reassuring with respect to the continued use of IVM as a treatment for infertility.

Conclusions

With significant improvements to success rates in recent years, IVM may be considered a valuable treatment option for ART clinics. This is especially important for patients with PCOS who are at a significantly higher risk of developing OHSS. There have been significant milestones made in animal models from the investigation of oocyte-secreted factors growth-differentiation factor nine (GDF9) and bone morphogenic protein 15 (BMP15). These form part of the transforming growth factor β (TGF β) superfamily and are necessary components for functional fertility [76, 77]. These factors produced by the oocyte act through paracrine signalling as regulators of granulosa/cumulus cell expansion and differentiation [78, 79]. The addition of recombinant forms of these factors to IVM culture media and/or other additives such as cAMP modulators may help improve success rates even further.

However, there still remains a clear need for large-scale randomised controlled studies to validate IVM success compared to standard IVF. Additionally, further research into the long-term outcomes of children born following IVM is necessary, even though initial assessments of children born from the technique show promising results. Finally, in order for IVM to become a more widely accepted treatment method worldwide, there needs to be a more standardised approach to protocols which will enable clinics to more easily implement this important treatment option.

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