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REVIEW ARTICLE

Implantation Techniques

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ABSTRACT

Decades of research and improving technology have taken *in vitro* fertilization techniques to a highly sophisticated level. Attention in the later twentieth century was increasingly paid to the endocrine stimulation of follicle growth and oocyte maturation. Hormonal stimulation became more complex. Methods for ovarian hyperstimulation became complex. GnRH agonists and antagonists came into play to provide a better control over FSH and LH concentrations during follicular and luteal phases of stimulated cycles including the correction of premature LH surges. A lot of stress is being paid nowadays on natural remedies to replace the high tech treatment modalities and so also evolved the so called natural cycle *in vitro* fertilization, the drug free or minimal drug fertility treatment. There are opinions worldwide of experts that the methods that are currently being used for ovulation and to stimulate growth of the follicle in infertile women are too expensive in addition to being extreme measures. Not only are they costly but the effect on the human body has given rise for the need for a more nature-friendly method. Alternative approaches are therefore being sought including minimal stimulation IVF, natural cycle IVF and maturing human oocytes *in vitro* to prepare them for fertilization *in vitro*. Each of these approaches avoids the use of large doses of human menopausal gonadotrophin (HMG) that have become essential for routine IVF. This article reviews the available literature on these methods and also their fallacies, if any.

Keywords: Infertility, Hormonal stimulation, Natural cycle, Minimum stimulation IVF.

INTRODUCTION

John Rock was the first to extract an intact fertilized egg.[1] The first pregnancy achieved through *in vitro* human fertilization of a human oocyte was reported in The Lancet from the Monash University team in 1973, although it lasted only a few days and would today be called a biochemical pregnancy. In 1977, Patrick Steptoe and Robert Edwards successfully carried out a pioneering conception which resulted in the birth of the world's first baby to be conceived by IVF, Louise Brown on 25 July 1978, in Oldham General Hospital, Greater Manchester, UK[2-4] followed by Courtney Cross on 16 October 1978 and Alastair MacDonald on 14 January 1979. This was then followed by the birth of Candice Reed in Melbourne in 1980. It was the subsequent use of stimulated cycles with clomiphene citrate and the use of human chorionic gonadotrophin (hCG) to control and time oocyte maturation, thus controlling the time of collection, that converted IVF from a research tool to a clinical treatment. Decades of research and improving technology have taken *in vitro* fertilization techniques to a highly sophisticated level.

Recent times have been witnessing a new revolution globally, the "Green Revolution". A lot of emphasis is being paid on ecological reserves and preservation of the natural habitat. It seems only natural that this change comes not only in the flora and fauna but also in the field of human sciences. A lot of stress is being paid now-a-days on natural remedies to replace the high-tech treatment modalities and also evolved the so called natural cycle *in vitro* fertilization, the drug free or minimal drug fertility treatment. Sounds like a contradiction but there is actually a "natural" version of the very sophisticated *in vitro* fertilization. Why this shift? A question most scientists would ask. There are opinions worldwide of experts that the methods that are currently being used for ovulation and to stimulate growth of the follicle in infertile women are too expensive in addition to being extreme measures. This close scrutiny of the fallbacks of the routine IVF has arisen because of the need for administration of high doses of recombinant hormones, gonadotrophin releasing hormones (GnRH) agonists and antagonists. Not only are they costly but also the effect on the human body has given rise for the need for a more nature friendly method. The cost of these hormones and the complexities of their use in ovarian stimulation are the primary objections, although simpler laboratory techniques may also be introduced based on IVF practice in animals. Alternative approaches are therefore being sought, including minimal stimulation IVF, natural cycle IVF and maturing human oocytes *in vitro* to prepare them for fertilization *in vitro*. Each of these approaches avoids the use of large doses of human menopausal gonadotrophin (hMG) that have become essential for routine IVF.

ENDOCRINE STIMULATION OF OVARIAN AND OOCYTE MATURATION

Attention in the later twentieth century was increasingly paid to the endocrine stimulation of follicle growth and oocyte

maturation in humans and in adults of various animal species. Gemzell et al[5] induced ovulation in amenorrheic women by initially administering FSH preparations extracted from human pituitary glands, followed a few days later by an injection of hCG to induce ovulation. Pregnancies were established, but many were multiple, including octuplets, since numbers of ovulated eggs could not be controlled as shown previously in mice.[6] Samples of human urinary menopausal gonadotrophins (hMG) were now given to oligomenorrheic women to stimulate the growth of several ovarian follicles, followed by hCG to induce ovulation.[7] Methods improved further when Donini and Lunenfeld[8] prepared highly purified, FSH-rich preparations of hMG that could be obtained commercially (named Pergonal), and used it with hCG to stimulate ovulation in oligomenorrheic and amenorrheic women. Two distinct means of obtaining human eggs at metaphase II and with an extruded first polar body were now available. They could be aspirated from growing Graafian follicles and matured *in vitro* for 37 hours as just described, or gained by administering modest doses of hMG and hCG to patients in order to stimulate several follicles to mature and then aspirating their preovulatory oocytes at 36 hours post-hCG, just before the follicles were about to ovulate. Ripe oocytes had to be aspirated an hour or so before the follicles ruptured for ovulation, otherwise they could have been lost in the peritoneal cavity. While pondering on whether to choose hormonal stimulation or maturation *in vitro* as the best approach to human IVF, Chang[9] reported that he had matured rabbit oocytes *in vitro* and then transferred them to oviducts of mated female recipients to see if the embryos developed normally. Some were found to be abnormal. This discovery militated against choosing mature human oocytes *in vitro* for IVF, especially since there had been no reports of anomalies among children born to amenorrheic mothers after stimulation by hMG/hCG. The decision was, therefore, taken to use the endocrine method for human IVF.

Hormonal stimulation becomes more complex as methods for ovarian hyperstimulation become complex. Many clinicians prefer to use contraceptives to stabilize the menstrual cycle before injecting hMG. GnRH agonists and antagonists came into play to provide a better control over FSH and LH concentrations during follicular and luteal phases of stimulated cycles, including the correction of premature LH surges.[10,11] Recombinant FSH and LH were introduced to avoid the use of urinary products which were claimed to be less specific in their actions and difficult to obtain in amounts sufficient to satisfy demand.[12,13] Occasional cases of hyperstimulation and polycystic ovaries arose during this treatment.

Recombinant hCG was introduced later and was very effective in providing luteal phase support in hyperstimulated cycles and to induce ovulation in treatment cycles.[14] Pregnancy rates nevertheless remained very low at 15 to 20%, whatever form of stimulation was employed. Low pregnancy rates led to three and more embryos being replaced in some clinics, resulting in numerous multiple pregnancies, many of them twins, triplets and, often, more. It still remains uncertain if the use of recombinant gonadotrophins has offered better pregnancy rates than those gained with urinary preparations, despite their very high cost. Insulin was now recognized as an important factor in hyperstimulation, and Metformin is being used to reduce aromatase activity and steroidogenesis, and so reduce the risks of polycystic ovarian syndrome. It might also serve to replace FSH, when given at 8-hour intervals from before the onset of stimulation, continuing until the first beta-hCG assay for pregnancy. Claims have been made for its effectiveness and various investigators remain unconvinced about its value in controlling polycystic ovaries.[15-17] Metformin may have other advantages in lowering concentrations of endothelial and coagulation markers, including soluble adhesion molecule and soluble intercellular adhesion molecule.

Pregnancy rates improved and multiple pregnancies declined as new methods identified the best-quality embryos for transfer. This was achieved by timing the first and second cleavage divisions *in vitro* and transferring one or two of the fastest growing embryos. This led to pregnancy rates of 12.9% when slow-growing embryos were transferred *vs* 42.3% with fast-growing embryos.[18] High-quality embryos could also be identified by scoring them for fragmenting blastomeres or multiple nuclei within blastomeres. New culture media also improved embryonic growth to blastocysts, and microarrays are currently entering clinical practice embryos to enable selection for desirable genes. Further developments improved the practice of IVF and preimplantation genetic diagnosis during the 1990s. Intracytoplasmic sperm injection (ICSI) was introduced by Palermo et al[19] and spread worldwide. Offering a simple method of replacing normal fertilization by injecting a single spermatozoon into a mature oocyte, it replaced the need to inseminate oocytes with hundreds or more spermatozoa. Its benefits included treatments for severe male infertility when very few testicular spermatozoa were available. It also avoided the attachment of many spermatozoa to the zona pellucida after insemination *in vitro*, which raised problems for preimplantation genetic diagnosis.

Another novelty raised pregnancy rates to still higher levels. Involving the use of FISH (fluorescent *in situ* hybridization), chromosomal complements in fertilized eggs could be identified and those that were heteroploid or polyploid carried translocations or other cytogenetic anomalies could be discarded.[20] Chromosomal anomalies were found to arise in as many as 50% of all human embryos growing *in vitro*, and pregnancy rates reached as high as 50% or more when one or two embryos diagnosed as normal were replaced. This considerable improvement above previous success rates occurred just as doubts began to emerge about the cost and efficacy of natural IVF cycle.

THE NATURAL WAY, IS IT REALLY BETTER?

Has the time now arrived to abandon routine IVF in favor of minimal stimulation IVF, natural cycle IVF or IVM? Can improvements be achieved in routine IVF? In fact, several possibilities of improving routine IVF that were known in the

earliest days of routine persist today. For example, the fact that LHRH has pulsatile release during natural cycles, as shown by Knobil[21] working with rhesus monkeys, has simply been overlooked together with any deleterious effects that may have been induced in IVF clinics administering large doses of GnRH agonists and antagonists, or using stimuli leading to high concentrations of estrogens in their patients. Three successive events occurring during natural menstrual cycles are controlled by timing the actions of different gonadotrophins, steroids and other regulatory factors.[22] The first step in follicular recruitment is decided by FSH dominance, and the last step in follicular maturation involves LH dominance. The former is difficult to detect, so its exact timing in stimulated cycles has been largely overlooked.

Substituting the first day of menses as a satisfactory measure of the onset of a new cycle thus has a weak endocrinological basis. Le Nestour et al[22] also suggest that the exact timing of the LH surge leading to ovulation cannot be predicted prospectively during the natural cycle, although it was tediously achieved, for example during Leslie Brown's menstrual cycle that led to the birth of her daughter. Timing this phase is usually achieved by triggering ovulation through an injection of hCG, but this can be easily mistimed and may shorten the final part of the follicular phase. Likewise, the incorrect timing of GnRH antagonists may result in falling concentrations of estrogens, which disturbs menstrual rhythms.

A better approach controlling the correct concentrations of FSH and LH is achieved by administering controlled concentrations of estradiol 3 days before menstruation is expected. Le Nestour et al[22] stress the significant role of gonadotrophin surge attenuating factor (GnSAF) in co-ordinating the menstrual cycle. It controls LH and its mid-cycle surge by antagonizing the surge-promoting activity of estradiol, and further refinements of this hypothesis may improve results with both routine and minimal stimulation IVF. Other major developments may yet be introduced to control menstrual rhythms and improve routine IVF. Minimal stimulation IVF and natural cycle IVF to a lesser degree could replace routine IV inhibin, activin and follistatin in many IVF clinics. In a sense, both represent steps in the development of routine IVF. Their techniques are virtually identical, and highly sensitive methods are now available to detect the onset of the LH surge.[23]

Novel techniques, such as these have stimulated some clinics to abandon the use of large doses of urinary or recombinant gonadotrophins in normal or poor responders in favor of tests on minimal stimulation IVF and natural cycle IVF. Caution was long overdue as evidence accumulated on the delicacy of feedback systems, such as the systemic roles of hypothalamic GnRH, the ovarian steroids estradiol and progesterone, and the gonadal peptides inhibin, activin and follistatin on the secretions of pituitary gonadotrophins.[22] Estradiol accordingly has a major role in determining concentrations of FSH at the luteal-follicular transition, and the need for care in stimulating patients became increasingly clear. The need for change was first mooted by an appeal to reduce doses of gonadotrophins and pay close attention to doses given to patients with specific defects in their menstrual cycles.[24] Minimal stimulation IVF is obviously very close to routine IVF, and differs mainly in its utilization of smaller doses of hormones. It has a major advantage of avoiding the production of high estrogens that may damage maturing oocytes and even distort chromosomal segregation. Used in the earliest days of routine IVF it fell out of favor as treatments involved increasing doses of urinary or recombinant gonadotrophins in the early 1990s.[25]

PROTOCOLS FOR NATURAL IVF

While almost every IVF program uses powerful hormones and medications to stimulate the ovaries, the natural IVF cycle is used specifically for women who cannot or do not want to take ovulation induction agents (especially those treated for breast cancer). Natural IVF takes advantage of a woman's own cycle. In between the customary IVF protocols and natural IVF is the minimum stimulation IVF wherein only the oral clomiphene citrate is used to stimulate the ovaries. Several different drugs and protocols were developed over the years, however, the most important issue is still to individualize the drugs and the protocol used. Although the first IVF baby was the result of the natural cycle, this protocol was soon deserted due to a higher cancellation rate compared with conventional ovarian stimulation. This occurred while comparing women with adequate response to gonadotropins. There are no clear advantages of one single protocol in improving the outcomes of low responders in IVF. Once the physician and the couple feel comfortable with the diagnosis of a low responder then the cost and simplicity of a protocol become more relevant in the management of these patients. The natural cycle is associated with a significantly cheaper cost and spares the patient from the use of gonadotropins. However, it is likely to result in a higher cancellation rate. In a randomized controlled trial involving 129 poor responders, according to a prior IVF cycle, the natural cycle was compared with micro dose GnRH agonist flare cycles. A total of 114 natural cycles were compared with 101 cycles involving the flare protocol. Both groups had a similar pregnancy rate per cycle and per transfer. However, there was a significant higher implantation rate among the natural cycle group (14.9% *vs* 5.5%).[26] The authors concluded that in poor responders the natural cycle is as effective as controlled ovarian hyperstimulation. The natural cycle, as are the minimum stimulation protocols, is an attractive alternative in poor responders due to lower costs and similar pregnancy rates compared with the standard controlled ovarian hyperstimulation regimens.[27] In 1996 Edwards et al[24] were the first to express concern with regard to contemporary ovarian stimulation approaches for IVF and called for the use of milder stimulation protocols. The aim of mild stimulation is to achieve cost-effective, patient friendly regimens and to optimize the balance between the outcome and risks of treatment. In mild ovarian stimulation the ovaries are stimulated with either gonadotropins and/or other compounds with the intent to limit

the number of oocytes obtained for IVF to fewer than seven. The administration of low doses of exogenous gonadotropins for a short duration and/or oral compounds in GnRH antagonist cotreated cycles, for ovarian stimulation for IVF, aiming to limit the number of oocytes.[28] The protocols for mild stimulation include GnRh antagonists, the natural cycle, clomiphene citrate, aromatase inhibitors, late start gonadotropin treatment, late follicular phase LH/hCG, short GnRH agonist. The use of GnRH antagonist during the mid to late stimulation phase allows for the initiation of IVF treatment in a normal menstrual cycle with an undisturbed recruitment of a cohort of follicles during the early follicular phase. This approach enables the endogenous intercycle FSH rise to be utilized rather than suppressed resulting in a reduction of the medication needed. In a meta-analysis of 27 IVF studies, GnRH antagonist was compared with the long agonist protocol. It was observed that there was a significant reduction in the number of days of GnRH analog and gonadotropin administration, amount of gonadotropin used in addition to a reduction in the incidence of severe OHSS.[29] Several factors affect the pregnancy success of IVF in natural cycles of which one is the accurate timing of hCG administration. The criteria used for hCG administration are serum estradiol levels between 0.4 and 1.1 mmol/L, follicle size of 15 to 20 mm endometrial thickness of 7 mm, a negative LH test or 17 to 18 days before the anticipated menstruation. Various protocols are mentioned to induce final oocyte maturation, of which one includes follicle diameter >17 mm and endometrial thickness > 7 mm and E2 levels are not measured. Another protocol by Paulson et al[30] advises hCG administration when dominant follicle attains a size of 16 to 20 mm or E2 levels indicate satisfactory follicular development, i.e. the level should be more than 1.1 to 0.73 nmol/L. Another protocol (Maribor center, Slovenia)[31] advocates hCG when dominant follicle is more than 15 mm in size and estradiol levels indicate satisfactory follicular development, i.e. > 0.49 nmol/L.

Paulson et al[32] after three cycles reported a cumulative pregnancy rate of 43.0%, after five cycles Aboulghar et al[33] reported 41.7%, Nargund et al[34] after six cycles gave a rate of 46%.

However, natural cycle IVF results are hampered by high cancellation rates due to premature LH rises, premature ovulation or reduced chances for successful oocyte retrieval. Various strategies have been suggested by various workers to improve results. The use of hCG for final oocyte maturation,[35] use of indomethacin to postpone follicular rupture.[36] The occurrence of a premature LH rise is prevented by the use of GnRH antagonist during the late follicular phase. The ongoing growth of the dominant follicle is supported by the addition of exogenous gonadotropins. In most studies, GnRH antagonist and gonadotropins (75-300 IU/day) are initiated at a follicular diameter of 12 to 17 mm.

DRAWBACKS

The significant drawbacks of this method have limited its use. For example, in approximately 20% of natural cycles, no egg is retrieved at the time of surgery. An additional 20% of cycles result in no fertilization, and therefore no embryo. Only half of patients have an embryo to transfer back to the uterus. The resultant pregnancy rate after transfer is only 10%, a percentage which is not higher than non-IVF therapy with ovarian stimulation and intrauterine insemination (IUI). Unfortunately, 10 to 25% of patients who successfully achieve a pregnancy with this therapy go on to miscarry. Committing patients to this approach expose almost 95% of women to the rigors of an IVF cycle monitoring, the surgical egg retrieval and laboratory techniques but with a very low likelihood of success. Minimal stimulation IVF utilizes lower doses of fertility drugs to try to stimulate two to four mature eggs to mature, and then proceeds in the same way to attempt egg retrieval, fertilization and embryo transfer. This attempt to have it both ways, more patient friendly than conventional IVF but better success than natural cycle really is an admission of the multiple benefits of multiple egg production to IVF success, but without realizing that success. Although these methods are described as patient friendly, proponents often advise that patients undergo multiple cycles—with their repeat operation, anesthesia and cost of egg retrieval, in order to achieve a successful pregnancy. Repeating a procedure with a 7 to 10% success rates four or five times is not the same as doing a single procedure with 40 to 50% chance of delivery in one cycle. In addition, with one egg there is no possibility of additional embryos to cryopreserve for future use, embryos which retain their potential for pregnancy even if the mother experiences a further decline in fertility over time.[37]

CONCLUSION

Pregnancy is a blessing. It is a complex process where the reproductive cells in the body join together to form a miracle, a new life. Since the introduction of IVF treatments, natural cycle IVF has been largely replaced by IVF with ovarian stimulation. However, natural cycle IVF has several advantages. It is associated with a close to zero multiple pregnancy rate, and a zero risk of ovarian hyperstimulation syndrome. Satisfactory results are obtained with the natural IVF/ICSI cycles regardless of the cause of infertility and day of embryo transfer. Also a higher pregnancy rate is expected with closer cycle monitoring. The cumulative pregnancy rate in three to four natural cycles is comparable with the pregnancy rate in selective one embryo transfer in stimulated IVF/ICSI cycle, in a selected group of patients. The natural cycle is an option for patients where only one or two oocytes are obtained in a stimulated cycle (poor responders) and proper timing for hCG administration in women under 38 years.

Per cycle, natural cycle IVF is less time consuming, physically and emotionally less demanding for patients, and cheaper than stimulated IVF, but also less effective. Efficacy of natural cycle IVF is hampered by high cancellation rates because of premature LH rise and premature ovulations. It is concluded that natural cycle IVF is a low-risk, low-cost and patient friendly procedure. However, a randomized controlled

trial comparing natural cycle IVF with current standard treatment strategies is warranted.

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